



# Abstract Book

Keynote Lectures, Oral and Poster Presentations

The 30<sup>th</sup> World Buiatrics Congress  
August 28 to September 1, 2018  
Sapporo, Japan



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# **ABSTRACT BOOK**

The 30th World Buiatrics Congress

August 28 to September 1, 2018

Sapporo, Japan

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## LIST OF THE BIENNIAL “WORLD BUIATRICAL CONGRESSES”

	Year	Venue		Year	Venue
1	1960	Hannover (Germany)	16	1990	San Salvador da Bahia (Brazil)
2	1962	Vienna (Austria)	17	1992	St Paul (USA)
3	1964	Copenhagen (Denmark)	18	1994	Bologna (Italy)
4	1966	Zurich (Switzerland)	19	1996	Edinburgh (Scotland)
5	1968	Opatija (Yugoslavia)	20	1998	Sydney (Australia)
6	1970	London (UK)	21	2000	Punta del Este (Uruguay)
7	1972	Philadelphia (USA)	22	2002	Hannover (Germany)
8	1974	Milan (Italy)	23	2004	Quebec (Canada)
9	1976	Nice (France)	24	2006	Nice (France)
10	1978	Mexico City (Mexico)	25	2008	Budapest (Hungary)
11	1980	Tel Aviv (Israel)	26	2010	Santiago (Chile)
12	1982	Amsterdam (The Netherlands)	27	2012	Lisbon (Portugal)
13	1984	Durban (South Africa)	28	2014	Cairns (Australia)
14	1986	Dublin (Ireland)	29	2016	Dublin (Ireland)
15	1988	Palma de Mallorca (Spain)	<b>30</b>	<b>2018</b>	<b>Sapporo (Japan)</b>

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## PREFACE

The World Buiatrics Congress (WBC) has been held biennially in different countries in the world since the first congress was held in Hannover in 1960.

It is a great honor and pleasure for Japan to organize the 30th WBC in Sapporo in 2018. This is the first WBC to be held in Asia, the world economic growth center and a promising region for further development of the animal industry.

Approximately 1.5 billion cattle and buffaloes and 2.2 billion goats and sheep are being raised in the world, and their products, particularly milk and meat, are imperative sources of protein for human beings. The development of the animal industry has been supported by advances in bovine medicine and bovine and other ruminant health management.

The WBC provides a unique opportunity for practitioners, researchers, consultants, students, and other specialists to share their knowledge and experiences in relevant fields.

We are very pleased to have received 756 abstracts from all over the world for presentation at WBC2018. We are also proud that a far greater number of abstracts than ever before have been submitted from colleagues in Asia. After careful review by the National and International Scientific Committee, 333 papers have been selected for oral presentation and another 322 abstracts have been chosen for poster presentation.

The abstracts have been published in the form in which they were submitted by the authors. The authors are responsible for the contents of the abstracts.

We wish to extend our sincere thanks to all the authors who submitted their abstracts as well as to the members of the National and International Scientific Committee for their reviews.

We hope that this abstract book will be utilized as a reference source.

**Motoshi Tajima**

President of the Organizing Committee

**Toshihiko Nakao**

President of the Scientific Committee



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# KEYNOTE LECTURES

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## **Control of Infectious Diseases**

- K01 BVD
- K02 Infectious Diseases: Virology
- K03 Infectious Diseases: Bacteriology
- K04 Immunology and Vaccines
- K05 Infectious Diseases: Parasitology
- K06 Tropical Animal Diseases
- K07 Epidemiology

## **Antimicrobial Resistance**

- K08-09 Anti-Microbial Resistance

## **Production Medicine**

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## Control of Infectious Diseases

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K01

### “The great challenge of BVD eradication – can we do it together?”

\*Joe Brownlie<sup>1,2</sup>

<sup>1</sup>Royal Veterinary College, <sup>2</sup>University of Bristol, School of Veterinary Science

One of the great challenges, and great rewards, for the veterinary practitioner is to eradicate an infectious disease. This may be at the farm, local, regional, national or even global level. Globally, only rinderpest has been eradicated whilst nationally a small number of diseases have been eliminated. Bovine virus diarrhoea (BVD) is a fine example where local and national strategies in many European countries have either succeeded in, or currently proceeding towards, eradication.

In this lecture, I intend to present the rationale for selecting BVD as a prime candidate for national control. To do this, there is a need to understand its complex pathogenesis in order to choose the best options for diagnosis and control.

BVD virus has a particular tropism for lymphoid, endocrine, neurological and gonadal tissues that, in turn, causes the pathology and functional consequences for these tissues. Most marked is the immunosuppression that potentiates other viral and bacterial infections, notably in the respiratory and enteric tracts.

An unusual but striking part of the pathogenesis is the passage of the virus across the placenta to the growing foetus. If this occurs in the early stages of gestation, the virus can establish a persistent infection (PI) that lasts throughout the foetal life and into neonatal and adult life. These PI become the major reservoirs of the virus. It is the search for PI animals that becomes the major objective for control. There are still some unknown and novel aspects of the pathogenesis that will be discussed in this lecture; some that have been uncovered by our deeper knowledge of the molecular basis of the virus and the variation in its virulence.

There are a number of routes to control and eradication; some with detection of PI animals alone and some with a period of vaccination with PI searches. These need to be understood before strategies are developed.

It is hoped that the welfare, economic and professional reasons discussed during this lecture will allow the practitioner to be fully cognisant of the way forward to control and eradicate this overwhelming infectious disease within our livestock herds. As the poster says “We can do it”!

---

K02

### Bovine leukemia virus: A major silent threat to proper immune responses in cattle

\*Paul Coussens Meredith Frie Monika Dziuba Ashley Greenlick

Department of Animal Science, Michigan State University

Bovine leukemia virus (BLV) is a deltaretrovirus, similar in structure to human T cell leukemia virus, type I. BLV specifically targets B cells of the immune system, but has also been found in other cell types, such as mammary epithelial cells. BLV is prevalent in the US dairy industry with over 83% of herds and 40% of all cows positive for infection. Although persistent lymphocytosis is common in BLV infected cows, less than 5% of infected cows will develop BLV associated leukemia or lymphoma. New research clearly demonstrates that BLV infected cows produce less milk and have shorter life spans than BLV negative herd mates, costing the US dairy industry over \$500 million annually. BLV infection has significant effects on many aspects of bovine immunity. We highlight our recent work demonstrating the deleterious effects of BLV infection on immune responses to routine vaccinations and to novel antigens, such as keyhole limpet hemocyanin (KLH). We present data demonstrating that BLV infection reduces overall antigen specific IgM in bovine plasma prior to and following inoculation. Reduced IgM reactive against specific booster vaccine components was observed on day 0 of a recent study and continued throughout a 60-day period. Similarly, KLH antigen-specific IgM is initially lower in plasma of BLV infected cows, relative to uninfected cows. This continues throughout the primary response. Further, we demonstrate that BLV infection generally reduces total plasma and possibly milk IgM levels (natural IgM and antigen-specific). Reduced IgM levels observed in plasma of BLV infected cows were consistent with reduced expression of IGJ mRNA in sorted B cells. As the J chain is essential for assembly of secreted pentameric IgM, reduced IGJ gene expression could partially account for BLV induced reduction in total plasma IgM. Recent studies examining the effect of BLV on mucosal immunity, such as reduced antigen-specific and total IgM will also be presented.

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K03

### Control of epizootic mycobacterial diseases: paratuberculosis and tuberculosis

\*Richard Whittington

The University of Sydney

There are currently two global epizootics affecting ruminant livestock caused by mycobacteria: tuberculosis and paratuberculosis. The causative organisms, *Mycobacterium bovis* and *M. avium* subsp. *paratuberculosis*, respectively, are not host specific and both can infect humans. Both diseases tend to have long incubation periods, spread slowly, may have wildlife reservoirs, and are difficult to diagnose. Consequently their true prevalence and impact is often underestimated in both developed and developing countries. For example, only about 30% of *M. bovis* infected cattle can be detected by abattoir surveillance, while less than 20% of *M. avium* subsp. *paratuberculosis*



infected cattle can be detected using ELISA tests on blood samples. These factors make the control of both diseases very challenging. Tuberculosis and paratuberculosis have increased in prevalence and expanded in geographic and host ranges over about 100 years, despite control efforts. There are very few examples of country-wide eradication of either disease. This is due to a long co-evolutionary period that enabled bacterial adaptation to ruminant hosts, the technical limits of diagnostic tests, the lack of practical or cost effective antimicrobial therapeutic regimens, the absence of protective vaccines that prevent infection or transmission, and the multicomponent on-farm control strategies that are necessary but often associated with poor compliance. Furthermore, international protocols for live animal translocation are technically inadequate, inconsistently applied and poorly coordinated, especially for paratuberculosis. In addition to the direct economic effects of bovine tuberculosis in livestock, animal health authorities in many countries mandate control of *M. bovis* to protect public health because there is an indisputable causation of human disease. However, beyond the production losses in livestock, the situation with *M. avium* subsp. *paratuberculosis* is unclear. While public health authorities accept that *M. avium* subsp. *paratuberculosis* can infect people, the infection may not cause Crohn's disease, the condition with which *M. avium* subsp. *paratuberculosis* is most often linked. Consequently there is reliance on animal health authorities to "do the right thing" and reduce exposure of humans to *M. avium* subsp. *paratuberculosis* by controlling paratuberculosis in livestock. These contrasting positions on the potential zoonotic role of two similar bacterial species has resulted in a dichotomy of control approaches. Bovine tuberculosis is well understood and so control programs are easy to justify and resources can be deployed over the long term. Paratuberculosis on the other hand is controlled in an inconsistent manner, in programs that differ substantially between countries and over time as resources wax and wane. An international collaboration was recently convened to review control programs for paratuberculosis and the responses from more than 20 countries on five continents (Europe, Asia, North America, South America and Australia/New Zealand) will be presented. There is a need for harmonisation of these programs, and a far greater role for organisations such as OIE.

K04

### Immunology of bovine respiratory syncytial virus (bRSV) in calves

\*Geraldine Taylor

The Pirbright Institute

Bovine respiratory syncytial virus (bRSV) is a major cause of respiratory disease in young calves, resulting in considerable morbidity and losses approaching \$1 billion per year. BRSV is an enveloped, non-segmented, negative-strand RNA virus belonging to the Pneumovirus genus, within the subfamily *Pneumovirinae* of the family *Paramyxoviridae*. BRSV is genetically and antigenically closely related to human (h)RSV, which is a major cause of respiratory disease in young infants, and the epidemiology and pathogenesis of infection with these viruses are similar. The viruses are host-specific, causing annual winter outbreaks of disease ranging from a mild upper respiratory

tract infection to severe bronchiolitis and pneumonia, with the peak incidence of severe disease in individuals less than 6 months of age. Although bRSV vaccines have been commercially available for decades, there is a need for ones with greater efficacy. The development of effective bRSV and hRSV vaccines face similar challenges, such as the need to vaccinate at an early age in the presence of maternally-derived serum antibodies, the failure of natural infection to prevent reinfection, and a history of vaccine-enhanced respiratory disease (ERD), which suggests that the immune response can contribute to the pathogenesis of disease.

Studies on the role of the innate and adaptive immune response in the pathogenesis of disease and protection against RSV infection have shown that although bRSV has evolved several mechanisms to modulate components of the innate immune response, innate immune responses contribute to the pathogenesis of disease. Thus, replication of bRSV in ciliated airway epithelial cells induces a number of pro-inflammatory chemokines and cytokines, which are responsible for recruiting neutrophils to the airways. An exaggerated neutrophil response during severe bRSV infection leads to the development of neutrophil extracellular traps (NETs) and airway obstruction. Neutralizing antibodies specific for the fusion (F) and attachment (G) glycoproteins are important in mediating protection. The most potently neutralizing RSV antibodies identified so far target the pre-fusion (pre-F) form of the F protein. However, pre-F is metastable and spontaneously undergoes structural rearrangements to the post-fusion (post-F) form, which does not present epitopes for many potently neutralizing antibodies. Studies on the role of T cells in protection against bRSV infection have highlighted the importance of CD8<sup>+</sup> T cells in virus clearance from both the upper and lower respiratory tract of calves. In contrast, ERD has been associated with RSV vaccines that induce low levels of neutralizing antibodies; prime a Th2-biased immune response; and do not induce a mucosal immunity or prime CD8<sup>+</sup> T cells. These findings suggest that a safe and effective bRSV vaccine would be either a live, attenuated virus; a virus-vectored vaccine; or a subunit vaccine composed of a stable form of pre-F delivered with an adjuvant that primes a Th1-biased immune response.

K05

### Tick borne diseases of cattle: understanding pathogen transmission for the development of control strategies.

\*Massaro Ueti

USDA-ARS-Animal Disease Research Unit, Pullman, Washington

Tick borne diseases pose a significant challenge to the livestock industry. A majority of cattle population live in endemic areas and are at constant risk of pathogen infection transmitted by tick vectors. This is illustrated by bovine babesiosis and anaplasmosis. These diseases cause significant economic losses to livestock due to morbidity and mortality. Animals that survive acute infection remain infected for life and are reservoirs for tick transmission. Parasites are acquired when tick vectors ingest infected blood during their feeding. Within the tick midgut the parasites undergo the first round of replication. After midgut infection, parasites are released into the tick hemocoel and in-



fect multiple organs. Babesia infects the ovary and transmit transovarially to the next tick generation that transmits to cattle. When Anaplasma is released into the tick hemolymph, the bacteria infect tick salivary glands and during subsequent feeding on cattle, bacteria are inoculated via tick saliva. Both pathogens infect red blood cells. During the initial replication cycle, pathogens cause acute disease characterized by high fever, decrease in the level of red blood cells, ataxia, and, in some case, death. Innate and humoral immune responses play important roles in the control of acute disease. The animals become chronically infected for life without overt sign of the disease. Understanding pathogen transmission by tick vectors and infectivity of mammalian hosts are critical for the development of tick borne disease control strategies.

K06

### **Foot-and-Mouth Disease (FMD): why understanding the risks and access to efficacious vaccines are key to progressive control of the disease - from herd level to global scale**

\*Keith John Sumption<sup>1</sup> Cornelius Van Maanen<sup>1</sup> Christianus Bartels<sup>1</sup> Donald King<sup>2</sup> Nicholas Lyons<sup>1,2</sup>

<sup>1</sup>European Commission for the Control of Foot-and-Mouth Disease (EuFMD), FAO of the UN, Rome, Italy, <sup>2</sup>The Pirbright Institute, Ash Road, Pirbright, Surrey, United Kingdom

Foot-and-Mouth Disease (FMD) remains an enormously important disease constraint to cattle productivity in most of Africa, South and East Asia, and across the middle-east; only in the Americas, Europe and Australasia have programmes managed to achieve national freedom from infection. The Global Strategy of FAO and OIE, launched in 2012, provided a global road-map for progressive control of FMD, in which countries would develop and implement national risk based strategic plans, with the aim of efficient use of public and private resources to first address the clinical impacts of FMD in the most affected sectors, before moving onto strategies for controlling virus circulation, a prerequisite for eventual area wide elimination.

This paper will review progress made and lessons learnt in the development of risk based control programmes; and in particular will consider how access to efficacious vaccines is a critical element of control programmes, and what is being done to improve access to information for vaccine selection, to assist veterinarians to herd to regional level.

The paper will also cover the importance of field epidemiology to identify risks of FMD, and how this information can assist development and evaluation of FMD control at herd, community and national scales. Despite decades of national vaccination programmes, efforts to quantify the benefits or impacts of programmes are in their infancy. Novel FMDV surveillance methods such as bulk-milk monitoring have potential to offer a cost-effective surveillance at different levels. Tools and methods for practitioners to investigate vaccine effectiveness in the field are now available, and as practitioners generate their own assessments, their influence and pressure to improve vaccine quality will grow, and the confidence needed in vaccination programmes should rise.

Internationally, FMD free countries will remain at high risk until virus circulation is brought under control in the great cattle populations in South Asia and Africa. A global, co-ordinated effort to increase the supply of effective vaccines is essential, and the paper will review efforts being made to increase the choice and quality of vaccines available to both practitioners and national risk-managers.

K07

### **Tools and Approaches for Problem Solving at the Herd, Regional, National and International level**

\*Mark Stevenson<sup>1</sup> Jaimie Hunnam<sup>2</sup> Sally Salmon<sup>2</sup>

<sup>1</sup>Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3010, Australia, <sup>2</sup>Department of Economic Development, Jobs, Transport and Resources, Attwood, Victoria 3049, Australia

Of all the species disciplines, cattle production medicine has a long and successful track record of using epidemiological principles to monitor and identify shortfalls in productivity. In the mid to late 1980s and early 1990s herd health software made it possible for herd managers and veterinarians to record individual animal lifetime event details and produce standardised measures of herd performance. This ability provided bovine clinicians with unprecedented opportunities to apply the principles of epidemiology to herd problem solving.

While herd health software packages allow shortfalls in performance to be detected in single herds, their ability to aggregate data across multiple herds for benchmarking has, for the most part, been limited. In this paper, we argue that cloud-based approaches (that is, the practice of using a network of remote servers hosted on the Internet to store, manage, and analyse data) can assist not only with herd benchmarking analyses but also facilitate the identification and management of animal health issues operating at the regional, national and international level.

At the regional level, summary measures of the frequency of disease at the herd level and aggregation of that information at the regional level allows locations with higher than expected frequencies of disease to be detected. Once detected, investigations can be initiated to identify their underlying cause.

For countries where infectious diseases such as foot-and-mouth disease are not endemic (such as Australia and New Zealand) considerable time and effort is spent by animal health authorities on foreign animal disease (FAD) outbreak preparedness. An important component of FAD preparedness is knowing not only where farm enterprises are located but exactly how many susceptible animals are present at each location. While most developed countries have systems in place to record the location of farm enterprises and the movement of animals from one farm location to another, the systems in place to update the counts of susceptible species at each farm location are highly variable both within and between countries. Opening a channel of communication between cloud-based herd data and spatial farm databases would be a logical way to resolve these issues that would seriously impede an effective FAD response.

At the international level, the World Animal Health Information



System (WAHIS) managed by the World Organization for Animal Health (OIE) records details of significant animal health events by its member countries. In the coming years it is likely that OIE member countries will be required to provide detailed quantitative evidence to support claims of disease freedom, as opposed to the current system where an absence of case event reports implies disease freedom. We proposed that recording of details of individual herd animal health investigations by either government or private veterinarians and aggregating the results of those investigations up to the national level (using a cloud-based approach) would address the need for quantitative evidence to (at least partially) substantiate claims for disease freedom.

In proposing a system to allow herd-level data to be used for regional, national and international reporting it is implicit that individuals and groups of individuals that contribute to the system are willing to share detailed information about their enterprise. A level of trust is required here – industry participants will need to trust system administrators to use the information supplied to them only for the purpose it was intended. System administrators will need to trust industry participants that the information they provide is an accurate reflection of the current animal health status on their enterprise. Few technical issues remain for cloud-based data management and reporting to become a reality; we expect that issues related to privacy and data sharing will be the greatest obstacle to realizing the individual herd level, regional level, national level and international level reporting requirements proposed in this paper.

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## Antimicrobial Resistance

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K08

### Origin of biofilm-related infections and therapeutic perspectives

\*Christophe Beloin

Institut Pasteur

Nowadays it is well accepted that, in most environments, microorganisms can switch from a free-living state to a sessile mode of life to form biofilms. Besides their ecological roles in nature, biofilms are also recognized as major threats when developing in industrial and especially medical settings. In these contexts, they are a source of contamination and infection that are extremely difficult to eradicate. Biofilms can be depicted as highly structured environments composed of communities of self-producing extracellular matrix microorganisms interacting with each other and with biotic or abiotic surfaces. As such they differ from planktonic populations and display original properties among which a high tolerance towards various physical and chemical hazards including antimicrobial agents.

With the increasing use of medical devices in modern medicine the burden of biofilm-related is predicted to raise in the future. Because antibiotics have a low efficacy against biofilms and biofilm-related infections there is a dire need to develop novel anti-biofilm strategies. There are multiple steps during biofilm development that can be targeted with new anti-biofilm molecules including mature biofilms containing a high frequency of a sub-population of bacteria called persisters. These persisters are today considered as the principal mechanism of biofilm antibiotic tolerance and are thought to be responsible for the recurrence of biofilm-associated infections.

During this lecture I will present the main biofilm properties and the therapeutic strategies that have been developed these last years to mitigate biofilm formation and eradicate biofilms. I will also highlight the existence of biofilm-associated infections in animals and discuss whether development of novel strategies to fight biofilm-associated infections in human can be transfer to veterinary medicine.

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K09

### Antimicrobial use and antimicrobial resistance in dairy cows

\*Theo Lam

GD Animal Health and Utrecht University

Antimicrobials are crucial to cure bacterial diseases, both in humans and in animals. During the past decade, however, antimicrobial usage in the livestock industry became a political issue in several countries worldwide. This was due to both, the relatively high antimicrobial usage in the animal industry, as well as a number of incidents with methicilline resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase forming bacteria (ESBL) in animals. Both of these were also found in dairy cattle.



Although antimicrobial usage in dairy cows is relatively limited, there is a relation with antimicrobial resistance and prudent use is also in this sector of utmost importance. In many countries antimicrobial usage in dairy cows can be decreased and improved. The use of blanket dry cow treatment for instance, is preventive rather than curative use of antimicrobials, and can in most situations be replaced by selective dry cow treatment. Also the added value of (extended) antimicrobial treatment of (sub)clinical mastitis can be questioned as well as the use of zero-withdrawal products that have been described to be related to the occurrence of development of ESBL's.

In this presentation antimicrobial use and antimicrobial resistance, directly or indirectly related to antimicrobial use in dairy cattle will be discussed. Based on experiences from the Netherlands and some other countries possibilities to limit antimicrobial use and resistance at the level of the individual animal and the dairy herd will be discussed as will be approaches at the level of a region or country.

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## Production Medicine

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K10

### **Bovine behavior and welfare in production medicine**

\*Ed Pajor

University of Calgary

The welfare of beef cattle in an important issues to producer, consumers, veterinarians and others. In 2008 members of the OIE agreed to a definition of animal welfare and included it in the Terrestrial Animal Health Code. One of the key animal welfare frameworks is the three conceptions model, biological functioning, affective states, and natural behaviour. Traditionally, veterinarians have considered good animal welfare to be synonymous with good biological functioning which includes health and productivity. Recent scientific advances have increased awareness about the importance of issues such as an animal's ability to feel pain, experience distress, or perform highly motivated behaviours. A wide variety of issues animal welfare issues exist in the beef industry. These include management practices that are done to the animal such as painful procedures, handling, unidentified lameness, weaning, transportation, and slaughter. Other issues include arise from the environmental and housing conditions. Finally, approaches to manage growth through the use of technologies (antibiotics, ionophores, Beta-Agonists, and hormonal treatments and implants) improve health and growth but raise a variety of animal welfare and, in some cases human health concerns. The first step in using animal behaviour in production medicine is to understand and be able to identify what is normal behaviour. By understanding normal behaviour, illness behaviours, painful behaviours, and abnormal behaviours can be identified and appropriate treatment provided. New technological advances are automating the detection of such behaviours in some production systems. Animal welfare assessments are being implemented or developed for various sectors of the cattle industries and veterinarians are expected to play a key role in these assessments. Veterinarians need to promote not just health but animal welfare and need to provide their clients with information, evaluations, and practical solutions to on farm issues. Public concerns regarding animal welfare and the demands on producers are not expected to decrease in the future. Furthermore, additional scientific discoveries regarding animal welfare will require the industry to address management practices that today may not be on the radar. Finally, concerns for the bovine industries are expanding beyond traditional production medicine to include topics such as one health, one welfare, and sustainability. There is no question that veterinarians will be expected to provide leadership in these areas. Will you be prepared?





K11

## Veterinary Herd Health & Productivity Management Advice on Dairy Farms

### A Necessity

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Veterinary herd health & productivity management programs (VHHPM) on dairy farms are not new. Set apart the mastitis control programs or herd fertility schemes from the 70s, these programs were initiated in the 80s already. However, this does not mean that they are amply being implemented worldwide. One of the reasons is that in some countries veterinarians do not want to be involved in this type of veterinary activity or have never been trained in this domain. In other countries, farmers appear to be insensitive for this type of advisory program, possibly due to misinformation –by practitioners? — or their own poor training in the field.

Since the 80s, the development of VHHPM has continued, not in the least because technology has progressed (computers, tablets, smartphones, social media) which facilitates the rapid communication between, for example, farmers and veterinarians. Moreover, one can detect a slow shift or expansion from classic VHHPM to a more pronounced routine monitoring of the herd, and further to risk factor identification for purposes of control and prevention. Paramount in veterinary advisory programs is the proper training of the practitioners in professional communication, professional advisory skills, organization and management skills.

The last development of veterinary advisory programs regards quality risk management. The latter incorporates the routine monitoring of relevant parameters in animals, their environment and management, and farm data. Also, incorporated in quality risk management (QRM) is the protocol-like assessment of strong points and weak points in farm management, of risk factors for animal health, productivity, cow comfort, public health and food safety, as well as their proper management. Sometimes this approach is also called 'One Health'. Quality in this context regards the different, forenamed domains and their parameters during the production process on the farm. QRM is much more organized, formalized and protocolled than VHHPM.

The formal integration of the classic VHHPM and this new QRM provides the farmer with an up-to-date advisory program in an economic sense, to best advise the farmer in his decision-making on an operational level, tactical level and strategic level. Last but not least, VHHPM/QRM contributes largely to achieving goals of sustainability on the dairy farm.

This proves once again that VHHPM/QRM is and should be very dynamic, flexible and adaptive to changing situations, if only the veterinarian is capable in following these changes, or preferably, be proactive, and adjust his/her VHHPM/QRM to the current situation.

Several of the forenamed issues are addressed in this paper, to give an overview of the situation where we stand today and to point out some necessities and constraints that we should deal with when we aim to keep on going. One of the latter issues regard the system of milk production and distribution it-

self: should it be local/regional/national or should it be export-focused mainly? How sustainable is this? Which are the consequences locally and internationally for farmers and for consumers?

K12

## Strategies to reduce dairy lameness by improving claw health. Is zero lameness a reachable goal?

\*Christer Bergsten

Swedish University of Agricultural Sciences

Lameness is a serious welfare issue and also affects dairy cow production, fertility, and longevity, with major economic consequences. Delayed treatment of lameness can often prolong recovery time and the possibility of complete recovery, so there are many incentives for farmers to diagnose and treat lame cows rapidly.

Severe lameness can appear suddenly, but can also be a slowly developing chronic condition. Farmers are less likely to recognize lameness than researchers, mainly because of different perceptions of lameness. Sole ulcers are strongly correlated with gait score (a numerical system), while an arched back shows a good correlation with lameness. A rapid lameness screening method based on standing with an arched back would be valuable to farmers. Research on lameness detection is also working to develop automatic systems to identify lame cows for early treatment.

Lame dairy cows almost always have at least one of many different claw disorders and/or leg lesions. The most common claw disorders causing lameness are sole ulcer and white line disease, affecting the claw horn, and digital dermatitis and foot rot, affecting the digital and interdigital skin. Different disorders must be treated correctly and a program must be devised to eliminate, reduce, or control the problem in the short-term and long-term. All programs must be based on management measures, because dairy managers/staff are responsible for decision making and performance on farms. However, the human element is often the greatest barrier to management changes.

A whole herd investigation with functional trimming should be the first step in a maintenance/preventive footcare/trimming program. This enables detection and treatment of disorders and prevention of new lesions at the same time. If there is a lameness problem in the herd, it is advisable to check all cows in the herd at or around the same time. It is very important to keep records of all trimmings and claw care events, so that each individual cow can be tracked back in time in order to make the right decisions. As herds become larger, a system for recording claw lesions is needed. Infectious disease problems must rely upon good biosecurity, e.g., a five-point plan is now available for digital dermatitis. Feeding/nutrition is a direct and indirect factor in claw diseases, through supporting natural disease resistance and through manure contamination, but the importance of poor body condition as a cause or effect of lameness is uncertain. A grazing system is considered best from an animal welfare point of view, but management plays a significant role. Lameness problems can arise in all kinds of systems, as exposure of feet and legs to hard, abrasive and unhygienic floors is directly proportional to incidence of claw disorders and



lameness. It is important to make good long-term strategic investments in new dairy barns as regards cow comfort and flooring. There are many options to improve comfort and hygiene in cubicles/stalls and walking areas, which will benefit the feet and legs of dairy cattle and reduce the risk of lameness. Improving genetics by breeding is a relatively long-term strategy to reduce claw disorders and lameness.

For long-living, high-producing dairy cows, both short-term and long-term strategies for healthy feet should be adopted. Short-term strategies include maintenance foot trimming, biosecurity, and nutrition, while longer-term strategies include management, housing, and genetics. By preventing claw disorders and detecting and treating lesions before lameness becomes apparent, zero lameness in the dairy herd can be achieved.

K13

### **Effects of Metabolic Disorders on Dairy Cow Health and Reproductive Performance**

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Dairy cattle experience a sudden outflow of calcium and energy at the onset of lactation that leaves them vulnerable to metabolic disorders. Maintaining calcium homeostasis is the cow's first major metabolic challenge after calving. Hypocalcemia may cause overt clinical signs of recumbency or may be subclinical. The subclinical form of hypocalcemia affects more than half of cows in their second or greater lactation and is more economically important than clinical milk fever. Subclinical hypocalcemia is associated with immune suppression, altered uterine involution, increased risk for secondary diseases, reduced potential milk yield, and impaired first service conception. The negative impact of subclinical hypocalcemia is greatest when the hypocalcemia persists for three or more days after calving.

Ketosis may be practically defined as hyperketonemia. This is possible because cowside tests are available for blood beta-hydroxybutyrate determination and milk-based tests are available to predict blood beta-hydroxybutyrate. Milk ketones alone are not well correlated with blood beta-hydroxybutyrate because the udder may selectively metabolize or excrete the ketone bodies it takes up from the bloodstream. Hyperketonemia has been associated with higher risk for displaced abomasum, increased risk for removal from the herd in early lactation, and reduced milk yield. Associations between hyperketonemia and fertility have been inconsistent. Early detection and prompt treatment of hyperketonemia reduces the risk for displaced abomasum and herd removal, increases milk yield, and improves first service conception.

Protocols have been developed to monitor subclinical hypocalcemia and hyperketonemia in dairy herds. Such monitoring programs can provide extremely valuable information about herd management and feeding. However, monitoring programs are hindered by difficulties in determining precise cutpoints for blood calcium or beta-hydroxybutyrate. Additionally, days in milk at sampling and parity may confound the herd results. Future herd-based monitoring approaches could evaluate multiple

cutpoints (with corresponding goals for each cutpoint) and adjust for confounding factors.

Other significant metabolic diseases of dairy cattle include hypomagnesemia and hypophosphatemia. Dairy cattle consuming primarily pasture are at risk for hypomagnesemia because of the practical difficulties in supplementing magnesium when little to no supplemental concentrates are fed. Hypomagnesemia may have clinical and subclinical manifestations and is sporadic enough that herd-based monitoring systems are not well developed. Hypophosphatemia is also a sporadic problem and is most commonly secondary to clinical hypocalcemia. The contribution of hypophosphatemia to continued recumbency is not well understood.

K14

### **Mastitis control and milk quality**

\*Peter Edmondson

Udderwise Ltd

Mastitis in the developing world has changed significantly over the last number of years. The incidence of contagious mastitis pathogens has reduced. The national average somatic cell count (SCC) for many countries is below 200,000.

Environmental mastitis remains a problem. Herd sizes are increasing, milk yields are rising, housing can be suboptimal, availability of skilled milkers is becoming more difficult and climatic conditions when cows are at pasture are changing in some countries. All these can increase the risk of clinical mastitis due to environmental pathogens.

There is pressure to reduce clinical mastitis levels to improve animal welfare, decrease antibiotic usage, maximize production and increase longevity. Many herds are moving to selective dry cow therapy in response to responsible use of antibiotics and consumer pressure. There is an increase in use of internal teat sealants which reduce dry period infections and clinical mastitis in the following lactation.

The number of automated milking systems (AMS) continues to increase. *Streptococcus agalactiae* is a re-emerging pathogen associated with AMS use. Prevalence of *Mycoplasma* and *Prototheca* is increasing in some of the large dairy herds in the US and other countries.

In developing countries, where many cows are hand milked, milk production is expanding. Seasonal rainfall can result in outbreaks of clinical mastitis. SCC and total bacterial counts (TBC) are generally higher than in the developed world, and in some areas cannot be measured. Some farms machine milk but adequate machine maintenance can prove difficult. Advisory support services and laboratory facilities to carry out basic bacteriology and individual cell count testing are often limited.

All of the above present significant challenges and opportunities for mastitis control for all countries throughout the world. This paper will look at ways to minimize mastitis and improve milk quality to meet future challenges.



K15

## Managing Clinical Mastitis To Minimize Antibiotic Usage

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Antibiotics are an essential tool for combatting bacterial diseases and their use has contributed to increased welfare of both human beings and animals. Use of antimicrobials in animal agriculture is increasingly scrutinized and it is important that veterinarians are engaged with farmers to ensure that antibiotics are used in a manner that minimizes unnecessary use while maximizing animal well-being and farm profitability. In most countries, mastitis is the most common bacterial disease of adult dairy cows and it is often treated by farm workers with minimal veterinary supervision. In surveys, treatment of mastitis has accounted for the majority of antimicrobials given to dairy cows and most cases are treated symptomatically, without knowledge of etiology. Symptomatic treatment is difficult to justify as the distribution of mastitis pathogens has shifted from primarily contagious to opportunistic and the prevalence of pathogens often varies among farms based on differences in housing and management. On many modern dairy farms, a large proportion of cases are microbiologically negative when detected, or are caused by pathogens that have high rates of spontaneous cure. Other cases are caused by bacteria that are intrinsically resistant to available antimicrobials or occur in cows with characteristics that greatly reduce the probability that antimicrobial therapy can result in successful bacterial clearance. Use of antimicrobials for treatment of clinical mastitis can be justified for cases caused by most Gram-positive organisms, but evidence of improved outcomes based on antimicrobial therapy for many other pathogens is lacking. When antimicrobials are routinely used to treat mastitis without knowledge of etiology, approximately 35-60% of antimicrobial treatments will be of no-benefit to the cow. In an era when use of antimicrobials on farms is increasingly controversial, this is difficult to justify. To develop appropriate treatment protocols, veterinarians should review the spectrum of action of approved drugs and implement protocols that include options for managing cases that will not benefit from antimicrobial use. When possible, veterinarians should encourage farmers to use culture-based treatment protocols and review the medical history of the cow before administration of antimicrobial treatments. When culture-based protocols are not feasible, veterinarians should recommend use of approved narrow-spectrum intramammary antibiotics for short durations. There is considerable opportunity for veterinarians to promote responsible use of antimicrobials by increasing engagement with farmers in development of mastitis treatment protocols. Appropriate use of antibiotics for treatment of mastitis is based on assessment of the etiology, the medical history of the cow and application of sound therapeutic principles to select approved antibiotics. The purpose of this paper is to present research based principles that can help veterinarians work with farmers to ensure responsible and justifiable usage of antibiotics for treatment of clinical mastitis.

K16

## Intensive care of the newborn dairy calf – knowledge into practice

### Perinatal calf care

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The perinatal period (0-48h) is the most hazardous in the life of all eutherians; calving compresses mortality. Some 75% of calves which die in the perinatal period are alive at the start of calving and some 75% of calves which die within one hour of birth have >50% of their lungs inflated, so much of this loss is preventable through better newborn calf care. Internationally, calving assistance rates are high (~40% of heifers, ~20% of cows). Calving assistance, if improperly administered, can compromise calf health, sometimes fatally. Yet, most producers receive no formal training in either calving assistance or newborn calf management and can become inured to calf mortality by the anaesthesia of familiarity (bad becoming normal - “farm blindness”). Despite this, veterinary calving contact is decreasing (<5% of dairy calvings are veterinary-assisted). Hence, practitioners can play a critical role in calving and newborn calf care education and training. This involves producer training in calving/perinate care and trouble-shooting calving problem/high calf mortality herds. As dairy herd sizes continue to grow internationally the role of the veterinary practitioner will continue to evolve from an individual reactive response to part of a team-based, SOP-aligned proactive process involving intra-farm communication across different farm staff job titles. Veterinarians should also be advocates for welfare initiatives and genetic changes to reduce dystocia and calf mortality. The management of the cow on the point of calving, of the calf immediately after birth and the investigation of calf losses have all seen new developments recently; these, and current human perinatal care guidelines, are reviewed from the perspective of veterinary practitioner clinical utility. One of the major problems encountered at calving is the dystocia-perinatal mortality dyad. Recent estimates of the prevalence of these periparturient problems are 30% for assisted calvings, 7% for dystocia and 5% for all-cause perinatal mortality. These average national statistics obscure the fact that herd-level data follow a right skewed distribution where most herds have none or minimal losses but some herds have high losses. This is the ‘problem herd problem’ paradigm where veterinary practitioners can have an important impact. Calves born following dystocia, bradytocia, premature calving and maldisposition are at increased risk of neonatal co-morbidity or mortality due to pre- or parturient metabolic stress (hypoxia, hypercapnia, acidaemia and ischaemia), encephalopathy, RDS and/or infection. In order to determine the need for care in ‘at-risk’ calves, the fetus/newborn calf must be assessed before, during and immediately after birth (perinatal triage). The urgency of providing critical care is underlined by the fact that almost two-thirds of perinatal calf mortality occurs within an hour of birth and some 95% of this loss occurs within five minutes of birth; hence the “Survive to 5” program. The human emergency medicine concept of the ‘golden hour’ (the period during which there is the highest likelihood that prompt medical treatment will prevent death) can be applied to the intensive care of newborn calves. Numerous calf vitality scoring systems have been developed, based on the original human Apgar scoring system, but none are widely used in farm practice. Practical indicators which can be used imme-



diately after birth to indicate an immediate need for intervention include abnormal calf breathing, poor reflex responses and poor muscle tone. A decision tree algorithm for active management of normal and compromised newborn calves is the basis of a perinatal point-of-care SOP. The building blocks of a modern step care model are good calving supervision, perinatal triage, calf resuscitation and critical aftercare. Perinatal management of all newborn calves should include – establishment of a patent airway, establishment of a normal breathing pattern, establishment of normal circulatory function, prevention of anaemia, prevention of prolonged metabolic acidosis, pain relief as necessary, prevention of hypothermia, prevention of umbilical infection, prevention of failure of passive transfer (FPT), and prevention of *Mycobacterium avium* subspp. *paratuberculosis* (MAP) transmission and calf identification. Early warning score systems, intervention thresholds and escalation protocols are integral to this calf care model.

vine conceptus EMT could be regulated through the spatiotemporal expression of FST, activin A and OVOL2, and suggest that the construction of uterine environment through dynamic changes in gene expression at conceptus and endometrium must occur concurrently during the peri-implantation period, if a pregnancy is to be established. We propose that cell adhesion molecules allow the close proximity between trophoblast and the uterine epithelium, but EMT allows the adhesion of these cell types.

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K17

### **Conceptus implantation to the maternal endometrium: cellular mechanisms associated with the adhesion of two cell types.**

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In most mammals, embryo implantation to the uterine endometrium is required for pregnancy establishment. Using the *in vitro* co-culture system with bovine trophoblast CT-1 cells and endometrial epithelial cells (EECs), we previously demonstrated that CT-1 and EECs treated with uterine flushings (UFs) obtained from pregnant day 17, 20 or 22 (P17, P20, P22 respectively; day of conceptus attachment to EECs is initiated on days 19-19.5) could mimic the gene expression *in utero* on day 17, 20, or 22, respectively. We then found that molecules related to epithelial-mesenchymal transition (EMT), ZEB1, SNAI2, N-cadherin (CDH2) and vimentin (VIM), were required for the progression of conceptus implantation to placentation in the ruminants. EMT-related transcripts as well as the epithelial marker cytokeratin were present in the bovine trophoblast, but surprisingly these changes were noted only after the conceptus-endometrium attachment. To characterize EMT inducing factors, we initially undertook iTRAQ analysis with UF samples obtained from pregnant animals on day 17 (pre-attachment) and day 20 (post-attachment), demonstrating that follistatin (FST), an inhibitor of activin A, increased in P20 UF samples. We also found that FST decreased in P22 conceptuses, whereas activin A increased in P20 UF samples and endometria, and further increased on P22. In addition, phosphorylated SMAD2 increased in P22 conceptuses. In bovine trophoblast cells, the treatment with P22 UF samples or activin A up-regulated EMT marker expressions, which were inhibited by FST. Global expression analysis further identified that a transcription factor *OVOL2*, essential for the process of mesenchymal-epithelial transition (MET) in various cancers, decreased following CT-1 cells attachment to the EECs, allowing increases in ZEB1, followed by CDH2 and VIM up-regulation. These results indicated that the initiation of bo-



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## Technological Development

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K18

### Orthopaedic ultrasonography in cattle

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Diagnostic imaging is one of the most dynamically evolving fields in veterinary medicine. The availability of high quality portable ultrasound machines has made application of this imaging modality part of everyday bovine practice. The 5- to 7.5-MHz linear transducers commonly used for pregnancy diagnosis are suitable for the diagnosis of various musculoskeletal disorders, and thus there has been a steady increase in the use of ultrasonography as part of the clinical examination of cattle with locomotor disorders. Ultrasonography has increasingly replaced radiography for the diagnosis of a number of musculoskeletal disorders including pelvic fracture and early detection of sequestrum formation. A 3.5-MHz transducer is required for deeper structures such as the hip joint in a cow.

Ultrasonographic images of musculoskeletal lesions such as fractures, sequestra and osteomyelitis are highly consistent and have characteristic features, independent of the location in the body. In many cases, this allows for a definitive diagnosis and facilitates a prognosis. Ultrasonography has traditionally been used to diagnose soft tissue changes such as haematomas, seromas, septic arthritis and tendon lesions. Lesions can be detected readily even by less experienced examiners, and learning is quick because of continual improvements in the quality of the equipment. It must be remembered that a clinical lameness examination and in-depth knowledge of the anatomy of the region are still prerequisites when using any imaging modality.

A comprehensive ultrasonographic examination requires the examiner to use guide or indicator structures for each region as well as reference values derived from healthy animals. The use of guide structures simplifies the examination because only a few images of a region are needed to put the pieces of the puzzle together and arrive at a diagnosis. Research projects are an ideal method for determining the best indicator structures for each region, which make examination of a region easier for practitioners. Panoramic views and 3-D imaging are fascinating advancements in ultrasonography that have been recently introduced into clinical veterinary medicine. Panoramic views can consist of multiple single images or a complete overview of a region such as a septic stifle joint, a muscle rupture or a large hematoma. A 3-D view delivers intriguing insights into the detailed anatomy of a region of interest and its pathologically altered structures. The future of (orthopaedic) ultrasonography has only just begun.

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K19

### Teat surgery in dairy cattle

\*Adrian Steiner

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Teat surgeries are among the most frequently demanded surgical interventions in the field of dairy production. Undisturbed teat function is of utmost importance for dairy cows, as premature slaughter or drying-off of the affected quarter are the only valuable options if repair is not successful. The success rate of surgical interventions in the area of the teat depends on aseptic conditions and meticulous surgical technique. Such interventions should, therefore, only be performed, if conditions concerning hygiene in the barn or surgery theater allow to do so. This presentation deals with the following topics: Anatomy; diagnostic tools and procedures; preparation of the cow and the surgical field; teat anaesthesia; decision making, repair and short- and longterm prognosis of the following teat pathologies that require surgical management: teat lacerations, teat fistula, milk outflow disturbances and congenital disorders.

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## Water Buffalo

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K20

### Management of Production and Health of Water Buffaloes

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#### Background

About 200 million water buffaloes are distributed in nearly 50 countries covering all five continents of the World. In 2017, the total global milk production was estimated to 800.3 million tonnes, where buffaloes share was 83.4 million tonnes. World's total meat production from buffaloes in 2016 was estimated to 4.18 million tonnes. In many Asian countries, buffaloes remain the major source of agricultural draft power. Data relevant to buffalo production and health management focussing on sustainable increase in the production of milk and meat is summarized in this article.

#### Breeding and Reproduction

Buffalo breeding is mostly in hands of smallholders. Animal identification and recording of performance and pedigree data are generally lacking. Without a handful of exceptions, artificial insemination (AI) services if any are in hands of public organisations. Extension of assisted reproductive techniques, including AI and embryo transfer are badly limited by poor oestrus detection and poor ovarian response to hormonal stimulation. The latter was attributed to fewer ovarian follicles than in cattle. AI at induced oestrus and or ovulation resulted conception rates ranging from 30 to 50% using frozen semen with or without prior sexing. The whole genome of buffalo has already been sequenced, which is a large step forward to the development of genomic tools for marker assisted breeding and genomic breeding value estimation provided a large set of performance and pedigree data are available, representing the local population. The expansion of AI services along with early pregnancy diagnosis using immunoassays and or transrectal ultrasonography are expected to help in rapid dissemination of desired genetic merit.

#### Feeds and Feeding

Buffalo feeding practices around the World have been adapted from that of cattle. Asian buffaloes are fed mostly with crop residues and milling byproducts with limited access to cut-and-carry green grasses. On the other hand, in Europe and Brazil, farmers practice intensive feeding and grazing on pastures of variable qualities. Buffaloes are better converters of poor quality roughages than cattle with significantly low level of greenhouse gas (GHG) emission. Research results strongly support that improving nutrition not only increases milk and meat production but also enhance reproduction by reducing the age at first calving, increasing conception rates and braking the trend of seasonal breeding, which often coincides with the environmental conditions and the availability of feeds. Total mixed ration (TMR) based on crop residues and treatment with en-

zymes and yeasts are potential R & D areas to improve the digestibility of fibrous crop residues and tropical grasses.

#### Health and Management

Housing at smallholders farming limits to providing shade with variable provisions for ventilation and often with compromised sanitation, which increases the risk of mastitis. However, technology driven buffalo production has been increasing over the last decades involving well-designed houses, facilities for wallowing, mist spray to cool buffaloes, machine milking and rapid cooling and storing facilities for the milk. Foot and mouth disease, anthrax, haemorrhagic septicaemia and mastitis have been recorded as common health issues in buffaloes. Vaccines developed for cattle against common diseases are generally effective in buffaloes as well; however, the proportion of immunised animals remained very low in smallholders' production system, perhaps because of poor accessibility. On farm mastitis diagnosis depends on the examination of the animal, udder and milk. California mastitis has not been useful; however, somatic cell count (SCC) and bacterial culture of milk significantly improve the accuracy of diagnosis, especially of subclinical mastitis. Poor housing and sanitation and overcrowding of animals remain major risk factors for mastitis. Calf mortality is high, often exceeds 40% and the major courses are gastroenteritis and parasite infections.

#### Conclusions

Buffaloes are important animals that provide protein and subsistence income to millions of smallholders and draft power to crop production and rural transports. Investment on recording of performance and pedigree data, application of genomic tools for genetic evaluation and selection of breeding animals, extension of AI services with frozen semen and the optimisation of feeding for sustainable production increase while reducing GHG emission remain future research challenges to sustainable growth increase in the buffalo industry.

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## Insight into Veterinary Science in the Near Future

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K21

### Future perspectives for farm animal internal medicine

#### Towards a new balance between disciplines within veterinary medicine

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The professionalization of the cattle industry came along with a change of expectations from veterinarians working in production animal medicine. The expected area of expertise has broadened well beyond the core discipline of internal medicine to areas such as nutrition, housing, epidemiology and others that have been recognized as similarly important for health and productivity. Simultaneously farm animal internal medicine has become a highly specialized discipline which increasingly includes advanced technology in diagnosis. These developments have understandably shifted the balance between disciplines considered as core disciplines of the production animal veterinarian away from the formerly pre-eminent field of internal medicine.

Despite of these developments internal medicine remains a core competence of the farm animal practitioner that cannot be provided by any other profession. The quality of skills and knowledge in internal medicine conveyed with undergraduate and graduate education however are the solid foundation of the education of the farm animal veterinarian and will be essential for the success in the profession.

Specialists in farm animal internal medicine have among others the responsibility to warrant the quality of clinical and theoretical education in internal medicine, to maintain a pool of expertise allowing to serve the community and the profession for second opinion, to contribute to the monitoring of cattle health on an regional level and beyond and not least to advance the discipline to improve health, diagnostics and treatment in buiatrics.

Supporting this discipline in keeping up with these responsibilities will require commitment and financial support from industry government and academia alike. This commitment will be critical to sustain the quality of student education and veterinary care and should be seen as investment into the future of farm animal health and the success of production animal medicine.

K22

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### Predictive Modeling for Large Data Sets and One Health

\*Doerte Doepfer

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Associations between (risk) factors and health or production outcomes are often modeled using parameter estimation mod-

els and prediction models. Prediction modeling relates to the process of “Statistical Learning” and a multitude of machine learning algorithms and Artificial Intelligence. There are profound consequences for how humans, animals, plants and the environment function today, given the decisions influenced by prediction models applied to large data sets.

With the advent of increasing data volumes, freeware, pipelines and computing capacity, a process termed “democratization” of for example deep learning modeling has been initiated that promotes access for everyone to state-of-the-art deep learning and machine learning approaches including data mining, dimension reduction and parameter shrinkage. It is not surprising that large data sets reach across species into prediction modeling of One Health outcomes connected to environmental, plant, animal, human and “Cyber Health”. These are fascinating times for applications of predictive modeling and data science!

The presentation will provide examples of machine learning approaches, strategic stepwise decision-making processes for pre-processing of data, modeling choices and Full Model Selection tools for machine learning and optimized performance evaluation aimed at One Health outcomes. The systematic optimization of prediction models in the face of large data sets is necessary to result in better decisions guided by prediction models.

“Big Data Science” continues to evolve around economics, physics, social and biological sciences such as the -omics disciplines, to name a few. Forecasting from multinomial outcomes, time series, text analysis, web-scraping, social media, computer recognition of visual, audio, printed and handwritten inputs today is available to an exponentially increasing often “self-taught” community. This makes tools for optimization of prediction models essential for their rational and ethical application.

The speed of developments in the “remote-expert” sphere, where everyone has the voice of an expert, has no equal in history. At the same time, ethics of machine learning and Artificial Intelligence are not clearly defined. One Health efforts will therefore need to include topics such as “Cyber Health” in the very near future.

There is a big need to train many more biological and agricultural scientists, including veterinarians, to meet the demands of current and future modeling approaches and data sciences. The selection for animal, plant and human “Cyber Talent”, those are individuals and entities able to function well within a world guided by Artificial Intelligence, will start at a very young age. Talent for this type of logic will be selected using different didactic and scholarly algorithms likely originating from the “remote-expert” realm. Given the genetic manipulation techniques available, the process of selection for “Cyber Talent” could profoundly affect the genetics of all elements involved. At the same time that evolution of Artificial Intelligence happens, algorithms adding compassion and kindness to the selection of outcomes and decisions derived from predictions should be prioritized to improve “One Cyber Health”.



K23

## E-learning and continuing education

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The internet is a disruptive technology that is changing the way education is delivered. For example, the immediate access to knowledge is transforming the veterinary curriculum from a knowledge-based focus to a competency-based focus. This change will not negate the need for “walking around knowledge”, such as what constitutes an abnormal rectal temperature or heart rate for a beef calf. However, the increased access to knowledge will drive changes in learning practices. The main advantage of electronic-learning (E-learning) is its ability to deliver state of the art material at relatively low cost to a large number of learners in an asynchronous manner. This is particularly advantageous to cattle veterinarians in small clinical practices where it can be challenging and expensive to attend continuing education conferences on a frequent basis. The likely immediate application of Massively Open Online Courses (MOOCs) and E-learning for bovine veterinarians is the delivery of post-graduate certificate programs and “bundles” of certificates that are sufficiently focused for a Masters degree in a discipline relevant to the cattle industry. These certificates will need to provide a skill set that is valued by the industry, and it is likely that such certificates will become an important part of the last two years of the clinical training for veterinary students. For example, students interested in dairy practice could elect to take a certificate program in dairy cow nutrition, cattle practice economics, or antimicrobial stewardship. Students completing relevant and high quality certificate programs will be more competitive in the market place and command a larger salary in their first few years of practice. E-learning cannot satisfactorily replicate experiential learning related to mastery of technical skills (such as corrective foot trimming, embryo transfer, breeding soundness examination, etc), and these will remain a focus of structured wet laboratories at continuing education conferences. However, technical skill mastery would benefit from a hybrid (blended or mixed-mode) instructional model, where an on-site structured experiential learning experience, often over one or more weekends, is supplemented with asynchronous on-line education. Blended courses also provide the opportunity for networking opportunities and other valuable interactions that occur in face-to-face education, including the strengthening of important soft skills related to effective communication, empathy, and resilience.



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# ORAL PRESENTATIONS

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Anti-Microbial Resistance [AR]  
Bovine Welfare and Cattle Comfort [WE]  
Buffaloes, Camelids and Wild Ruminants [BU]  
BVD [BV]  
Clinical Genetics [CG]  
Diagnostic Imaging [DI]  
E-learning and Continuing Education [EL]  
Emerging Diseases [ED]  
Epidemiology [EP]  
Herd Health Management [HH]  
Hoof Health and Lameness [HL]  
Immunology and Vaccines [IV]  
Infectious Diseases: Bacteriology [BC]  
Infectious Diseases: Parasitology [PA]  
Infectious Diseases: Virology [VR]  
Internal Medicine [IM]  
Japanese Black Cattle (Wagyu) [JB]  
Nutrition and Metabolic Diseases [NU]  
Organic and Sustainable Production Systems [SP]  
Public Health and Food Safety [PH]  
Reproduction [RE]  
Reproductive Technology [RT]  
Small Ruminants [SR]  
Surgery [SU]  
Therapeutics and Pharmacology [TP]  
Toxicology [TX]  
Tropical Animal Diseases [TD]  
Udder Health and Milk Quality [UH]  
Young Stock [YS]  
Miscellaneous Topics [MT]



AR-01

## “It’s cheaper than a dead cow”: Understanding veterinary medicine use on UK dairy farms through qualitative methodology

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**Objectives:** Understanding the way in which prescription veterinary medicines are used by dairy farmers is an important but complex topic. While measuring use quantitatively is instrumental to understanding use, it is also necessary to investigate and understand qualitative aspects of veterinary medicine use behaviour. The values, perceptions and behaviours surrounding the use of veterinary medicines by farmers is an area needing substantial further research. Farmers have been identified to regularly exceed prescribing guidelines for treatment duration of clinical mastitis because they feel insecure about how to treat mastitis effectively, and studies have shown that both overdosing and under-dosing of medications is common. It is therefore likely that other veterinary prescription medicines left on farms are not used according to prescribing guidelines, despite assumptions that this is the case. Similarly, research in human medicine has shown that a variety of social and cultural influences and beliefs affect compliance. Understanding the reasons behind these effects requires qualitative methodology to investigate and to inform future policy interventions.

**Materials and Methods:** Twenty United Kingdom (UK) dairy farmers were recruited through their veterinarians. Semi-structured, in-depth face-to-face interviews were conducted to investigate their attitudes, beliefs and values in relation to the storage, recording and actual use of veterinary medicines. Farms and farmers were purposively selected to be heterogeneous and reflect the different types of dairy farms present in the UK. In addition, three dairy farms in South West England were recruited for a 12-month intensive participant observation study. Approximately five hours each month was spent on each of these three farms, with the researcher observing and participating in all aspects of dairy cow management, particularly focussing on the storage, recording and use of veterinary medicines. Ethnographic narrative field notes and interview transcripts were analysed using an inductive, thematic approach.

**Results:** Several important themes were identified, falling broadly into the categories of trust (in the farm staff, the veterinarian and the medicine), risk (of using versus not using a particular medicine), past experience and the concept of medicine effectiveness being specific to “this farm”. Additionally, ethnographic work identified social conflict and a struggle for power and agency relating to medicine use between key decision makers on-farm. These tensions were observed to drive both coercive and subversive medicine use behaviours. It was apparent that licensed dosing regimens were regularly exceeded for various reasons. Risk-averse behaviour was commonly documented:

*“...you know the protocol says standard mastitis treatment is three days, but we actually find we need to do five or we get them coming back round the week afterwards with the same thing again...”*

The key factors influencing decisions about medicines were: advice from the farm’s veterinary surgeon, advice from peers

and personal anecdotal experience from perceived past successes or failures. Availability, cost and withdrawal periods of medicines also influenced the choice of medicines used in different situations. There was strong awareness amongst farmers that their access to certain antimicrobials may soon be limited, although there was varying understanding of the antimicrobials involved and the full reasons for this:

*“I’m using it while I can ‘cause they’re taking it away soon aren’t they?”* – Dairy farmer discussing the use of injectable ceftiofur

**Discussion:** This study provides a more in-depth understanding of the ways in which medicines are used and the decision making process occurring on UK dairy farms. Veterinary medicine use is complex and decisions are not always based on evidence, best practice or veterinary advice. The effect of social conflict between farm staff on treatment decisions should be recognised by veterinarians and policy makers. The data suggest that a ‘one-size-fits-all’ approach to policy making - particularly policy regulations pertaining to the use of antimicrobials - may not be appropriate. While the veterinary surgeon is an important source of advice and knowledge, there are several other influencing factors of various weightings that influence the way veterinary medicines or antimicrobials are being used on dairy farms.

AR-02

## What can we learn from farmers experiences and attitudes to Selective Dry Cow Therapy?

### A survey of 496 UK Dairy Farmers

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**Introduction:** Selective Dry Cow Therapy (SDCT) is a relatively new development for the Dairy Industry in the UK. The drive for SDCT has been from the processors and retailers as part of their commitment to reducing the use of antimicrobials and risk of antimicrobial resistance. Arla hosted a major initiative in 2015 in the UK where 40% of its producers (1750 farmers) were trained in the use of SDCT. Parallel training of 300 vets was delivered.

**Method:** An electronic convenience survey of 496 farmers was distributed through veterinary practices, processor websites and twitter in October 2016. 496 farmer responses were received. The objective of the survey was to establish the importance, beliefs and attitudes of the farmers to SDCT together with their experiences and future requirements. The survey methodology allows for analysis of the data based on the farmer attitude (belief and importance) and revealed different results for each group (Proactivists, Unconcerned, Deniers, Disillusionists) based on the work by Ritter(2016)

**Results:** The survey revealed that 82% of farmers believed that reducing antibiotic usage would be good for their livestock but 54% feared that SDCT would result in death or mastitis without antibiotic use. Significant concerns existed regarding cell counts not being controlled effectively (42%), high cell counts developing (39%) and risk of more mastitis in following



lactation (33%) or severe mastitis occurring (24%)

82% of farmers believed it would be good for their livestock to reduce antimicrobial use. Approximately 50% of the farmers classified themselves as firm believers and would recommend SDCT to other farmers (Proactivists), 18% agreed it was a good idea but low on their priority lists (Unconcerned) and 15% did not believe it worked or had bad experiences before (Disillusionists). Only 1.5% did not believe in SDCT (Deniers).

Division of the farmers into 3 groups-Proactivists, Unconcerned and Disillusionists revealed that the requirements to engage each group differed. The Proactivists were driven by more on farm training on SDCT (30%) and help from their vet (13%). The Unconcerned group needed more convincing that the rewards outweighed the benefits (42%) and more evidence that SDCT worked (37%). Only 6% of the Disillusionists group were motivated by on farm training.

However, the Net Promoter Score revealed there were more detractors (51%) than promoters (26%) indicating that without external support and encouragement the program may fail if relying on farmer recommendation alone.

**Summary:** Most of the farmers surveyed appear to understand the importance to reduce antibiotic use for the benefit of animals and people. However only approximately half the respondents surveyed classified themselves as firm believers of SDCT and would recommend the procedure to another farmer. However more detailed analysis revealed that of this proactive group only half of these would recommend SDCT to another farmer as evaluated using the Net Promoter Score. Only 25% of farmers surveyed would be classified as being true promoters of SDCT using the Net Promoter methodology. The lack of belief or faith in SDCT is a crucial area to address for the UK SDCT program to progress within both the veterinary and farming communities.

Further progress has been achieved in the UK by widespread training of veterinarians, the development of training videos on infusion techniques and more widespread communications to farmers regarding the benefits of SDCT. The RESET Model (Rules, Education, Social Norms, Economics and Tools) will need to be applied to ensure full adoption of SDCT. A central part of the success in the Netherlands was the adoption of this model in combination with banning blanket dry cow therapy. (Lam 2017)

Challenges that do exist is how to engage the Unconcerned and Disillusionists farmers who typically fail to attend training of education programs and as such run the risk of experiencing adverse events through SDCT which further confirm their beliefs.

**References:** Lam. T. J. G. M. et al (2017) The RESET Mindset Model applied on decreasing antibiotic usage in dairy cattle in the Netherlands Ir Vet J. 2017; 70: 5

Ritter.C et al (2016) Dairy Farmers' perceptions toward the implementation of on-farm Johne's disease prevention and control strategies. J. Dairy.Sci 99:1-12

AR-03

### Characterization of changes in the ileal microbiome of cattle after a single-dose administration of a macrolide antibiotic.

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**Objectives:** The gastrointestinal bacterial population provides important metabolic functions for the host and bacterial dysbiosis can adversely affect metabolism, productivity, and overall health. The effect of systemic antimicrobial administration on the bovine enteric microbiome has not been thoroughly examined but has the potential to result in intestinal barrier dysfunction and the development of antimicrobial resistance in treated animals. The aim of this project was to evaluate the changes in bacterial diversity of the enteric microbiome in cattle after the administration of a single dose of an extended-duration, commercially-available parenteral macrolide antibiotic.

**Materials and Methods:** Six crossbred dairy calves were surgically fitted with indwelling ileal cannulas. Following a post-op recovery period, luminal contents and epimural surface biopsies were collected for analysis of the associated bacterial populations on Days 0 (before drug administration), 3, 8, 15, and 35. On Day 0, all calves were given a subcutaneous dose of gamithromycin according to label instructions. Biopsy samples were collected using video-endoscopy via the indwelling catheter. The bacterial populations present, and their relative abundance, were assessed using bacterial amplicon pyrosequencing.

**Results:** Significant differences were observed in the relative bacterial populations between the luminal contents and epimural surface biopsies. In each case, the primary phyla represented were the Firmicutes, Bacteroidetes, Spirochaetes, and Proteobacteria but their relative proportions differed between sample locations. Long-term dysbiosis following macrolide administration was not observed in this study; though changes at the phylum level were apparent in some individual calves, baseline microbial populations were largely reestablished before the end of the established drug withdrawal time (35 days).

**Conclusions:** Significant difference exist between the microbial populations of the small intestinal epimural surface and the gastrointestinal contents. Systemic antibiotic administration may result in short-term disruption of the ileal microbiome. However, the composition of the bacterial populations of both the epimural surface and the luminal contents largely return to baseline levels within the established withdrawal period.

AR-04

### Antimicrobials use and Extended Spectrum Beta-Lactamase Producing *Escherichia coli* (ESBLs producing *E. coli*) in small holder dairy farms in Chiang Mai, Thailand.

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Dairy farming in Thailand was established in 1962 in Saraburi province and then distributed to all over Thailand. Chiang Mai



province, Northern Thailand, is one of the highly density area of dairy farming. In 2015, from the report of Department of Live-stock Development, there were 40,187 dairy cattle with 22,645 lactating cows from 1,070 farms. Most farms are classified as small holder dairy farms (< 20 lactating cows). The objectives of this study were to gain insight into the usage of antimicrobials and the presence of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* (ESBLs producing *E. coli*) in small holder dairy farms in Chiang Mai, Thailand. Bulk tank milk samples were collected from 130 farms in Mae-On district, Chiang Mai province from March to August 2015. All farms were interviewed for their antimicrobial use for their animals using questionnaire. The average number of milking cows was 19 animals and the average of milk production was 172 kg per farm. Farm owners reported that mastitis was the most common health problem in small holder dairy farms. Only 18% of the farmers sent milk samples to identify mastitis pathogens before treatment. The most common intramammary infused antimicrobial agents used to treat bovine mastitis were ampicillin combined with cloxacillin (87%) and cephapirin (82%). When the animals are sick, 87% of the farmers treated the animal by themselves, 78% called para-veterinarians and 49% called veterinarians for treating their animals. In bulk tank milk of small holder dairy farms, we found ESBLs producing *E. coli* in 3.8% of samples. The result from this study indicated that antimicrobial use is still necessary for treatment of sick animals in small holder dairy farms. However, the inappropriate use of antimicrobial can be the factor of developing the antimicrobial resistance. The farmer should be educated on prudent drugs use and the importance of antimicrobial resistance to prevent the development of resistant bacteria. Moreover, there should be the good collaboration between farmers, dairy cooperative, veterinarian, and veterinary authorities to control the use of antimicrobials in dairy farms.

AR-05

### Characterization of resistome-virulome in metritic cows that failed or attained cure after treatment with $\beta$ -lactam antimicrobials

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Puerperal metritis is a costly and prevalent postpartum disease that threatens dairy cows' welfare and survivability requiring treatment with antimicrobials. In the US,  $\beta$ -lactam antimicrobials are the main therapeutic options for treating cows with metritis, but ~35% of the cows fail to respond to treatment. The indiscriminate use of  $\beta$ -lactams to treat metritis and other cattle diseases have been associated with antimicrobial resistance genes (ARG) dissemination. Additionally, the odds of developing metritis increases with the presence of specific virulence factor genes (VFG) such as *fimH* from *E. coli*. Recently, whole genome shotgun sequencing (WGS) became the tool to characterize the dynamics of the collection of all ARG (resistome) and VFG (virulome). We hypothesize that cows that failed to cure of metritis have a distinct resistome-virulome than cows that attained metritis cure.

**Objectives:** We WGS to characterize the resistome-virulome in

uterine samples of cows that attained or failed to cure metritis after treatment with  $\beta$ -lactam antimicrobials. We also describe the uterine microbiome and performed a networking analysis to determine co-occurrence of microbes, ARG, and VFG.

**Materials and methods:** A total 24 primiparous Holstein dairy cows diagnosed with metritis were either treated with ceftiofur hydrochloride ( $n = 12$ ) or ampicillin tri-hydrate ( $n = 12$ ), two common  $\beta$ -lactam used to treat metritis. Uterine swabs were collected for each cow at the time of metritis diagnosis (D1) before antimicrobial treatment and five days later (D6) one day after treatments finished. Samples were sequenced using HiSeq 2500 platform. We used Diamond blastx function for aligning the clean fragments against the CARD, VFDB and NCBI-nr databases for the identification of ARG, VFG, and other proteins, respectively. We used Kraken program for assigning the clean data taxonomic labels from NCBI nr database.

**Results:** Half of the cows (12/24) cured after the five-day treatment (7 from ampicillin and 5 from ceftiofur). The WGS revealed that reads aligned to 131 AMR and 377 VFG composing the resistome and virulome of uterine samples, respectively. We also had reads aligning to 830 microbial taxa types. The resistome of uterine samples were dominated by tetracycline resistance genes (79.6% of all read assigned). The most prevalent ARG were *tetQ*, *tetB*, and *tetO*. However, no differences in AGR, antimicrobial resistance class, and antimicrobial resistance mechanism between cows that failed or attained cure for metritis were identified in the current study. Treatment with  $\beta$ -lactam increased the relative abundance of ARG. No differences were identified for type of  $\beta$ -lactam antimicrobial. The virulome had as the most prevalent VFG elongation factor tu (*tuf*), putative enolase of *Streptococcus* sp. (SSU98\_1513), and antigen-O of *Yersinia* sp. (YPA\_2597). A spearman correlation followed by networking analysis for microbes and VFG indicated that cows that failed to cure from the disease have a greater richness of VFG for the same microbial taxa than the herd-mates that attained cure after treatment with  $\beta$ -lactam antimicrobials.

**Conclusions:** Uterine resistome was dominated by tetracyclines resistant genes, but no differences in AGR relative abundance, richness and diversity, and antimicrobial class and mechanisms were found between cows that failed or attained of metritis after treatment of  $\beta$ -lactams. Uterine virulome revealed that cows that failing to cure from the disease have a greater richness of VFG for the same microbial taxa than the herd-mates that attained cure after treatment with  $\beta$ -lactams. Taken together, the data suggests that the virulence of the strain of microbes are pivotal for the metritis prognosis after treatment with  $\beta$ -lactam antimicrobials.

AR-06

### $\beta$ -lactamase-encoding gene identification by microarrays in phenotypically resistant pathogenic *Escherichia coli* from young calves in Wallonia, Belgium.

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**Objectives:** Since 2012, a decrease of  $\beta$ -lactam resistance of pathogenic *Escherichia (E.) coli* from calves has been observed at ARSLA, the Regional Veterinary Diagnostics laboratory in Wallonia, Belgium. This may be a consequence of the recommendation for a prior antibiotic sensitivity test of bovine pathogenic *E. coli* for human critical antibiotics, like 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins. The most frequent  $\beta$ -lactam resistance mechanism is the production of a  $\beta$ -lactamase enzyme that inactivates the antibiotic. Actual classification(s) of  $\beta$ -lactamases (BLA) is highly complex, but four groups can be summarized: classical BLA (BLA<sub>C</sub>), extended cephalosporinases (BLA<sub>AmpC</sub>), extended spectrum BLA (BLA<sub>ESBL</sub>) and carbapenemases (BLA<sub>CPE</sub>). The aim of this study was to identify the resistance genes coding for  $\beta$ -lactamases in pathogenic *E. coli* from calves with a resistance phenotype at the disk diffusion assay.

**Materials and methods:** From October 2017 to March 2018, pathogenic *E. coli* with BLA resistance phenotypes will be collected at ARSLA from calves with clinical and/or necropsy diagnosis of enteritis or septicemia. After an initial growth on Columbia blood and Gassner agar plates, three colonies from diarrheic feces or intestinal contents are transferred onto Minca and Ehly agar plates. One isolate positive at the agglutination for the F5, F17 or CS31A surface antigens and/or one isolate producing an enterohaemolysin are subsequently tested by the disk diffusion assay. The samples from blood and internal organs are also inoculated onto Columbia blood and Gassner agar plates. When a pure culture is obtained one colony is tested by the disk diffusion assay. The disk diffusion assay is performed with 16 antibiotics including 8  $\beta$ -lactams (amoxicillin, amoxicillin + clavulanic acid, cefoxitin, cefotaxime, cefotaxime + clavulanic acid, ceftiofur, cefquinome and meropenem). The results are read with a Sirscan (I2A). A total of 500 colonies with a  $\beta$ -lactam resistance profile will be stored at -80°C in peptone broth with 40% glycerol and 120 of them will be chosen for the micro-array assay (Check-MDR ICT 103XL, Check-points).

**Results:** Right now (December 2017), 61 *E. coli* with a BLA<sub>C</sub>, 2 with a BLA<sub>AmpC</sub>, 12 with a BLA<sub>ESBL</sub>, but none with a BLA<sub>CPE</sub> resistance profile have already been collected. One additional isolate simultaneously presents a BLA<sub>AmpC</sub> and a BLA<sub>ESBL</sub> profile. So far, *E. coli* with an intermediate result for some of the 8  $\beta$ -lactams tested have been included in the resistant isolates. The choice of the 120 isolates for the micro-array assay will of course be based on their resistance profiles. However they will include a higher proportion of BLA<sub>AmpC</sub> and BLA<sub>ESBL</sub>, than BLA<sub>C</sub>, since 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins are critical antibiotics in human medicine.

**Conclusion:** According to the results of the micro-array assay, specific PCR will be designed to test the whole *E. coli* collection. The comparison between the results of the disk diffusion assay and the resistance gene identification will link the  $\beta$ -lactam resistance phenotypes to the prevalence of the different  $\beta$ -lactamase-encoding gene families. Moreover their evolution in time will be followed since a second study is already planned in 2018-2019.

AR-07

### Antibiogram of *klebsiella* species isolated from bovine mastitis in China

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Bovine mastitis is an important disease of dairy industry and causes huge economic losses worldwide. The veterinarian usually used antibiotics to treat clinical mastitis in dairy farms, thus, monitoring of antimicrobial resistance in bacteria has clinical significance. Therefore, the objective of the study was to investigate the antimicrobial resistance profiles of *klebsiella* species (n = 130) that isolated from 1278 milk samples of bovine mastitis collected from 45 large Chinese dairy farms in 10 provinces in China. The minimal inhibitory concentrations (MICs) of 9 antibiotics included amoxicillin and clavulanate potassium (ACP), cefquinome (EFQ), ceftiofur (EFT), enrofloxacin (ENR), imipenem (IMI), kanamycin (KAN), penicillin (PEN), polymyxin B (POL) and tetracycline (TET) which were mostly used for veterinary treatment, were determined according to the standard broth microdilution method. The antimicrobial resistance was evaluated by MIC breakpoints for veterinary pathogens issued by clinical laboratory standard institution (CLSI). Results of the current data showed that the resistance proportion for PEN 100%, ACP 37.69%, TET 31.54%, EFT 20.77%, KAN 14.62%, EFQ 10.00%, ENR 1.54% and IMI 0.77%, respectively. As finding of polymyxin B (POL) confirmed that 23.85% were non wild type (NTP) and 76.15% were wild type (WT) isolates. Furthermore, 81 isolates were resistant to two or more antimicrobials carrying TET-PEN, PEN-POL and ACP-PEN resistance patterns which were observed in 16 (19.28%), 7 (8.43%) and 7 (8.43%) isolates, respectively. This data indicated that most isolates were multidrug resistant and the penicillin is invalid for all *klebsiella* species isolated from bovine mastitis in China. This study provides guiding lines for antibiotic therapy of udder infection due to *klebsiella* species.

**Keywords:** antimicrobial resistance, *klebsiella* species, bovine mastitis, MIC

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AR-08

### Genetic characterization of multi-drug resistant *Mannheimia haemolytica* isolated from high-risk stocker cattle

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**Objectives:** Antimicrobial resistance in *Mannheimia haemolyti-*



*ca* (*Mh*) is becoming a serious concern as diagnostic labs are reporting an increased prevalence of multi-drug resistant isolates. Documenting the resistance genes present in this pathogen, and the role a gene may or may not play in a resistant phenotype is an important step in understanding the epidemiology of multi-drug resistant *Mh*. Therefore, our objectives were to identify, quantify, and map the resistance genes present in *Mh* isolates collected from calves prior to and following treatment with the antimicrobial drug tulathromycin, and to assess the concordance between genotype and resistance phenotype.

**Materials and Methods:** Deep nasopharyngeal swabs (NPS) were collected from 169 bull and steer calves at arrival to a stocker facility. Calves were processed following standard industry protocol and received a metaphylactic dose of the drug tulathromycin. A second NPS was collected 10 to 14 days later at revaccination. For samples culture positive for *Mh*, a maximum of three colonies from each sample were subcultured and submitted for antimicrobial susceptibility testing. DNA was extracted and whole genome sequencing was performed on isolates from calves *Mh* positive at both time points. Phylogenetic trees and SNP matrices were constructed to illustrate the phylogenetic relationships between these "matched" isolates. The sequences were then BLASTed against resistance genes documented in the Comprehensive Antibiotic Resistance Database (CARD) and the Microbial Ecology Group Resistance Database (MEGARes).

**Results:** There were 22 calves culture positive at both time points, and a total of 50 isolates with unique susceptibility profiles; 27 from arrival samples and 23 from revaccination. Of all arrival isolates, 15 (55.6%) were susceptible to all antimicrobials tested, 10 (37%) were intermediate or resistant to one class of antimicrobial, and two (7.4%) were intermediate or resistant to two classes of antimicrobials. Of the revaccination isolates, none (0%) were susceptible to all antimicrobials tested, 11 (47.8%) were resistant or intermediate to three classes of antimicrobials, and 12 (52.2%) were resistant or intermediate to four classes of antimicrobials. Phylogenetic analysis of the sequences of these 50 isolates revealed five genetically distinct groups. All of the isolates collected at revaccination belonged to one group and are clonal; one arrival isolate is included with this group as well. The remainder of the arrival isolates comprise four distinct clonal groups. There were a total of 17 resistance associated genes isolated from all 50 isolates, representing eight different classes of antimicrobials. In the isolates from revaccination, many of these genes were located very closely together on the same contig within each genome. Point mutations resulting in amino acid changes were identified in the genes *gyrA* and *parC*, which are involved in fluoroquinolone susceptibility. In all isolates resistant to fluoroquinolones, there was an amino acid change from S to F at amino acid 83 and from D to N at amino acid 87 in *gyrA*. In *parC*, there was a change from E to K at amino acid 84. Concordance between genotype and resistance phenotype was 94% for *floR* and florfenicol susceptibility, and 100% for *tetH* and tetracycline. For the macrolides, to be concordant, an isolate must be susceptible to a given macrolide and negative for all macrolide resistance genes or, resistant/intermediate and positive for any one macrolide resistance gene. For tilimicosin, concordance was 80%, for gamithromycin 96%, and for tulathromycin, 90%.

**Conclusion:** All of the isolates from revaccination were multi-drug resistant, whereas only 7.4% of the arrival isolates could be considered multi-drug resistant. Along with this, there was an increase in the number of resistance genes present in a given isolate between arrival and revaccination. There was addi-

tionally a decrease in the genetic diversity of the isolates from arrival to revaccination, as there was a decrease from five clonal groups to one. Furthermore, we found that the presence or absence of a resistance gene is not always the best indicator of phenotypic resistance. It is important that we continue to study the role of antimicrobial resistance genes in the *Mh* genome on phenotypic resistance, and to assess the role of antimicrobial exposure to the development of multi-drug resistance.

AR-09

## "Antibiotics Anonymous"- Lessons learnt measuring antimicrobial use with UK dairy farmers

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### Objectives:

- Quantify antimicrobial use (AMU) of a select group of UK dairy farms participating in Farmer Action Groups (n=30)
- Understand assumptions and limitations of measuring AMU at prescription level and farm level
- Collect farmers' thoughts and ideas on different ways of measuring AMU

**Materials and Methods:** The Farmer Action Group (FAG) project brought together 5 groups of dairy farmers across South West England to share best practice about reducing antimicrobial use. Medicine reviews were carried out for each participant to measure and monitor use of antimicrobials over two years. One aim was for participants to reduce overall use and especially critically important antimicrobials (CIAs) by improving herd health.

Medicine reviews were based on veterinary prescription data and covered 2 sequential 12-month periods. The first review was based on the 12 months before each participant hosted the Farmer Action Group and the second on the subsequent 12 months. Antimicrobial use was measured in mg/kg, mg/1000 L of milk produced, grams of antibiotic/cow/year from intramammary tubes, Animal Daily Doses or DDDfarm and Cow Calculated Courses. Brief costings were done also to evaluate pence/L expenditure on antimicrobials as well as expenditure on other veterinary medicines (e.g. vaccines, anti-inflammatories, anthelmintics)

Prescription data was received from 16 veterinary practices in a variety of formats. AMU was collated and medicine costs were calculated. The data were presented in a report for discussion with the relevant participant and their Farmer Action Group.

Discussions at each FAG meeting covered the host farm's medicine review (with the farmer's consent). A specific meeting for each FAG was used to discuss the metrics used in the reports. All farms were benchmarked to stimulate discussion, which was recorded and transcribed.

**Results:** Medicine reviews were conducted for 30 participants. Farms varied in calving pattern (10 block calving herds, 3 seasonal herds and 17 all-year-round calving herds), size (68-590 milking cows) and location in South West England (n=6188 to-



tal cattle included). Two farms were organic and 2 had robots. Yields varied from 3700 L/cow/year to 11795 L/cow/year.

Some farmers preferred measuring AMU against a production parameter (i.e. mg/1000 L milk); others felt this could be misleading to the public, as it could be mistaken for antibiotics in milk, and there were concerns that high-yielding herds should not be justified in using more antimicrobials. Farmers that knew how the Dutch were measuring AMU preferred the daily dose metrics and were impressed with the collaboration between the farmers and veterinarians on responsible use. Some participants were concerned with using mg/kg because of the risk of driving use towards CIAs with lower dosages. Participants also preferred having AMU split between adults and youngstock as it highlighted any overuse. Participants also preferred an adult cow weight of 600 kg to the 425 kg used by Population Correction Unit metrics.

At the start of the project, AMU ranged widely (1.9-65.3 mg/kg, median=20.8 mg/kg) as did Animal Daily Doses (0.9-9.3, median=2.4). Antimicrobial use measured in mg/1000L of milk was also variable (186.5-3208 mg/1000 L milk, median=804.5 mg/1000L milk). The majority of farms used at least 1 CIA at the start of the project. Medicine reviews and group discussions helped raise awareness around multiple issues concerning AMU and offered many practical ways for farmers to reduce use.

**Conclusions:** The medicine review process highlighted CIAs to dairy farmers and has been instrumental in empowering farmers to discuss the antimicrobials prescribed and used on their farms with their veterinarian. Compiling medicine reviews required veterinary prescription data, as on-farm medicine records are inconsistent. However, on-farm records are essential to increase detail in the metrics, such as using farm-specific course lengths versus data sheet values. There are many ways to measure AMU, and the farmers were aware of the limitations of using mass metrics as opposed to daily dose metrics. All farmers agreed on the benefit of benchmarking and were keen to be compared.

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AR-10

### Potential risks for antimicrobial resistance (AMR) from inappropriate treatments for viral diseases by para-veterinary professionals in developing countries

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The University of Sydney

**Objective:** Although antibiotics have revolutionised how we treat humans and animals, the effectiveness and sustainability of antibiotics is being undermined by misuse, with antibiotic resistance rapidly emerging as one of the greatest global health challenges we face. Managing antimicrobial resistance (AMR) from poor prescribing behaviours of para-veterinary professionals requires identification of inappropriate treatment risks, as is suspected to occur commonly in developing countries when animals with viral diseases such as Foot-and-Mouth Disease (FMD) are treated with antibiotics.

**Materials and methods:** Cross-sectional survey questionnaires of Village Animal Health Workers (VAHWs) were con-

ducted in 19 provinces in 2008 (n = 445) and two provinces in 2015 (n = 80) in Cambodia. Both surveys consisted of questions regarding training, income, services provided and treatment and reporting behaviours. Interviews were conducted individually and face-to-face in Khmer language. Qualitative descriptive statistics was conducted on the 2008 data while qualitative descriptive statistics and multivariable modelling with logistic regression was conducted on the 2015 data.

**Results:** The majority of VAHWs surveyed had been in this role for at least 5 years (57% in 2008 and 86.5% in 2015). In both data sets initial training was provided to most by either Non-Government Organisations, Provincial Veterinarians or the General Directorate of Animal Health and Production. In 2015, most VAHWs stated they had received training on health (85%) and husbandry practices (70%) and these were the two areas that most expressed the need for ongoing training. Inappropriate use of antimicrobials in 2015 was seen in 89% of VAHWs who reported using antibiotics to treat Foot-and-Mouth Disease (FMD), 83% who report using antibiotics to treat sick pigs regardless of illness and 35.4% who report treating chicken with antibiotics regardless of illness. This has increased from 2008 when 74% of VAHWs reported using antibiotics to treat FMD cases in cattle. Univariable analysis of antibiotics use in 2015 for FMD, pigs and poultry found only frequency of disease reporting to be associated with use of antibiotics for FMD in cattle ( $P = 0.067$ ), and duration of employment associated with use of antibiotics in sick poultry ( $P = 0.017$ ). Four variables were found to be significant in the univariable analysis ( $P < 0.20$ ) of antibiotic use for sick pigs. The final model found associations with training conducted by provincial veterinarians ( $P = 0.007$ ) and animal health training ( $P = 0.04$ ) to be associated with the use of antibiotics in sick pigs.

**Conclusions:** The inappropriate use of antimicrobials within the veterinary and food producing industries requires increasing scrutiny as the risks of antimicrobial resistance and residues increases. Para-veterinary professionals, VAHWs, are responsible for providing the majority of veterinary assistance to smallholder farmers in Cambodia, although these individuals undergo only rudimentary training. With minimal supervision and monitoring of the field activities of VAHWs, unregulated access to antimicrobials from markets and low input from government services, behaviours focused on treating disease with expensive and usually unnecessary antibiotics appears common. Further training for and supervision of para-veterinary professionals is required to change the current treatment behaviours and reduce the risk of increased AMR within smallholder livestock and their products.

The inappropriate use of antimicrobials threatens public health and impacts negatively on smallholder farmer incomes and national economies, due to the high costs of animal treatments. The results from this study indicate that VAHWs using antibiotics to treat FMD affected animals were more likely to wait until animals were sick or dying to report outbreaks rather than report disease immediately, supporting previous assumptions that VAHWs use antibiotic treatments to generate income. Work is urgently required between the many national veterinary authorities and para-veterinary professionals in developing nations to address the AMR issue and ensure a more sustainable business model can be developed that directs their focus away from inappropriate treatments and towards more preventative health interventions.



AR-11

### Association between antimicrobial use and resistance in non-*aureus* staphylococci isolated from dairy herds

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**Objectives:** There is an intense pressure for reducing antimicrobial use in food-producing animals. For the dairy industry, recent efforts are directed towards the use of selective dry-cow therapy. In this study, we determined the association between AMU and antimicrobial resistance (AMR) in non-*aureus* staphylococci (NAS) isolated from Canadian dairy herds. Distinct types of dry cow therapy, antimicrobials and routes of administration were contrasted. Specific objectives were to: 1) study herd-level associations between AMU and prevalence of AMR; 2) verify whether these associations depended on the antimicrobial of interest and route of administration; and 3) study relationships between type of dry cow therapy and prevalence of AMR.

**Materials and methods:** A total of 89 Canadian dairy herds were followed for 2 years. Farm personnel were instructed by veterinarians and technicians to dispose all empty drug containers into 40-L receptacles, placed strategically on the farms. Antimicrobials were classified according to their route of administration into systemic, intramammary or intrauterine. AMU was defined as number of ADDs (antimicrobial daily doses) per cow/year. Milk samples from lactating cows were collected systematically. A total of 1,702 NAS isolates were randomly selected and their phenotypic AMR profile determined (micro-broth dilution method) against a panel of 23 antimicrobials, following standardized guidelines. Multi-drug resistance (MDR) was defined as resistance against 2 or more drug classes. Whole-genome sequencing (MiSeq platform) was performed on a subset of 405 isolates. Presence of AMR genetic determinants, e.g. resistance genes, was determined using data from 4 databases. All analyses were done at the herd-level using generalized linear models in R.

**Results:** The prevalence of MDR NAS isolates increased with increasing total AMU. The relative risk of MDR per increase of 1 ADD per cow/year was 1.09 (95% confidence interval (CI) 1.00 - 1.18). Only systemic AMU was associated with the prevalence of MDR NAS, whereas intramammary or intrauterine use were not. Prevalence of MDR NAS was 1.28 times higher with an increase of 1 ADD per cow/year administered systemically. Hence, herds that administered 2 ADDs per cow/year systemically had, on average, 28% more MDR NAS isolates than herds that administered 1 ADD per cow/year. Systemic use of penicillins, macrolides and 3<sup>rd</sup> generation cephalosporins was associated with the prevalence of resistance against that specific drug class (separate analysis for each). Effects did not depend on intramammary or intrauterine use of the same antimicrobials, which were not associated with prevalence of resistance in NAS isolates. Estimated relative risks associated with an increase of 1 systemic ADD per animal-year were 1.55, 1.36 and 4.81 for penicillins, 3<sup>rd</sup> generation cephalosporins and macrolides, respectively. Selective dry cow therapy was not associated with prevalence of MDR, nor with prevalence of drug-specific resistance in NAS. Systemic use of macrolides was associated with prevalence of *erm* genes. Estimated relative risk associated with an increase of 1 ADD of macrolides per

cow/year was 10.6 (95% CI 2.8 – 40.6). Prevalence of *tet* genes was 2.4 times higher following an increase of 1 ADD of tetracyclines per cow/year administered systemically (95% CI 1.4 – 4.1). No association between drug-specific AMU and prevalence of *blaZ*, *mphC* or *msrA* was identified, irrespective of route of administration.

**Conclusions:** Increased prevalence of AMR in NAS resulting from increased use of antimicrobials in dairy herds was only present when antimicrobials were administered systemically. Hence, based on our results, the impact of decreased use of antimicrobials on AMR in NAS following successful implementation of selective dry cow therapy is probably going to be minimal due to a lack of association between AMR and antimicrobials administered intramammarily. Moreover, our study identified 3 drug classes that may pose an additional threat when studying drug-specific associations: penicillins, 3<sup>rd</sup> generation cephalosporins and macrolides. This summary of evidence is crucial and necessary for discussion and implementation of strategies aimed to mitigate the emergence of AMR in the dairy industry.

AR-12

### A comparative analysis of antimicrobial use in dairy cows in New Zealand and in the UK

#### Contrasting and interpreting similar dairy antimicrobial use data across different countries and farm production systems

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**Objectives:** Antimicrobial resistance (AMR) is recognized as one of the greatest risks to human health globally (O'Neill, 2015). There have been links made between antimicrobial use (AMU) and AMR in agriculture (Chantziaras et al. 2014); and, by volume, the majority of AMU globally is in agriculture. Therefore, it is important for the agricultural sector to both monitor and manage AMU.

Gathering and comparing AMU data across countries is challenging, not least because of the different approaches to gathering, recording and then calculating AMU.

This paper compares recently gathered data on AMU on dairy cattle in the UK and in New Zealand, where the methodology and calculations are consistent and comparable between the two countries; and highlights both the limitations of comparison, and opportunities for improvement in collaboration in comparing AMU data.

**Materials and Methods:** Reliable dairy AMU data, using sales as a proxy for use, and measured in mg per population correction unit (mg/PCU), were available from recent, large scale nationwide studies in both New Zealand and the UK. The studies reflected similar 12-month periods (2015/2016 and 2016 respectively), and were both gathered using AM sales data from veterinary clinics and animal denominator data from farms.

The data in each case represented an annual AMU figure. Mg/PCU is calculated according to the amount of active ingredient (mgs), using a denominator of liveweight (kg).





The data also identified actives, route of administration, farm size, ownership structure and geography as other variables. For both datasets, youngstock were removed from the denominator.

There were a total of 358 dairy farms and 81,640 dairy cows represented from the UK, and 1462 dairy farms and 623,430 dairy cows from New Zealand. The UK data was collated from 4 large veterinary clinics and the New Zealand data from 6 veterinary clinics.

**Results:** Mean AM use (sales) in New Zealand and the UK was 8.54 and 22.11 mg/PCU, respectively. At a farm level, the range in the UK was 0.36 to 97.79mg/PCU; whilst at a clinic level, the range in NZ was 4.72 to 11.91.

For both countries, the greatest route (by weight) used was by injection (78.1%, 56.3%, UK and NZ respectively). In both countries, this was followed by either intramammary lactating cow therapy (6.5%, 8.9%), or by DCT (6.0%, 33.4%).

Beta lactams made up the bulk of antimicrobial actives in both countries, totaling 42.8% and 77.6% of mg (UK and NZ respectively). The second highest actives in the UK were aminoglycosides (20.9%), and in NZ were macrolides (9.0%).

**Conclusion:** These data highlight both similarities and differences in AMU between the UK and NZ. The patterns of use (route and actives) are quite similar, although the volumes vary. Both of these studies are the first extensive, national and comparable sets of use data for both countries.

The UK data likely overestimates AMU. Firstly, because it uses a mean liveweight of 425kg, which likely underestimates actual liveweight. It also uses as a denominator adult milking cows, whereas the NZ data uses all cows and R2s overwintered. The UK data also includes AMs used in footbaths. Both sets of data use sales as a proxy for use. There is a degree of disconnect between these parameters, which is difficult to estimate.

These data also do not take into account other factors such as disease risk, housing or production. Although the data for NZ tends to suggest a lower overall AMU, correction for production may smooth this to some extent. It is likely that the mean UK annual milk production is approximately 60% higher than in NZ. The management systems across both countries are similar, but a far greater proportion of NZ dairy farms are seasonally calving, and pastorally based, with limited housing. These factors may also have an influence.

In summary, comparison of consistent and reliable data across countries is the first step in understanding and sharing opportunities for improving (reducing and refining) AMU in the agricultural sector. Veterinarians are well placed to coordinate and deliver this.

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AR-13

## The use of an antimicrobial practice sales report to identify prescribing patterns and as an active tool in herd health management

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Origin Animal Health

**Objectives:** Concerns regarding the development of antimicrobial resistance has increased the awareness of the requirement for increased AntiMicrobial Stewardship (AMS) based on sound principles of the appropriate medicine at the appropriate time.

The aim was to develop a tool to allow reporting and benchmarking of antimicrobial use at either a farm unit level or a macro level. This tool can then be used to identify trends in prescribing patterns on farms, between farms and at a national level. AMS can then be applied on individual units and at a business level to ensure the medicines at our disposal are being used responsibly.

**Materials and Methods:** It is well reported that medicine use recording is variable in on-farm records, with under reporting frequently identified. A proportion of farms also use non-electronic methods to record use such as paper record books or treatment diaries. This makes the mass reporting using farm records both technically difficult and unreliable. As such, a combination of practice sales data and prescription records can be used as a proxy for antimicrobial use.

A tool was developed by Origin Animal Health using practice sales records automatically uploaded from the Client Management Software (CMS), manual entry of medicine prescription records and basic farm information. This data is then accessible at two levels: an individual farm report, or macro-analysis.

The individual farm report visually displays total antimicrobial sales over the past 12 months in three different metrics as defined by the European Surveillance of Veterinary Consumption Group (ESVAC); mg per Population Corrected Unit (PCU), Defined Daily Dose for animals (DDDvet) and Defined Course Dose for animals (DCDvet). This is then represented against the previous 12 months' sales for the farm, as well as the mean over all farms for these metrics and, where relevant, UK sector-specific targets.

The antimicrobial use is then further broken down to provide management data for the farmer and veterinary advisor in these different areas; antimicrobial class, with those designated High Priority – Critically Important Antimicrobials (HP-CIA) highlighted in red, route of administration, individual product names. For all of these a comparison with the previous year is included to identify trends.

A selective dry cow indicator is also included where the number of antibiotic dry cow tubes sold per cow is graphically compared with the number of teat seal tubes sold, for this year, the previous year, and the group average.

Finally there is a distribution chart of all farms data in mg/PCU with the farm for which the report is produced highlighted as a benchmarking tool.

Collation of this data allows macro-level analysis including trends in sales of different antimicrobial classes. It is also possible to compare between different farming systems and categorisations. This may include but is not limited to milking frequency, production level, and somatic cell count level.

**Results:** The farm level report produced is available in a number of ways – regular (monthly or quarterly) reports for close monitoring of trends, bespoke one-off reports for snapshot analysis or incorporation into herd health planning.

The use of the reports stimulates discussions around the AMS on farm – benchmarking total usage against other farms; indicating the socio-political aspects surrounding the use of HP-CIAs; highlighting the common requirements for treatment and



discussing both disease reduction and appropriate therapeutics considering non-antibiotic approaches; discussing the implications of selective dry cow therapy.

Macro-reporting can be produced for within practice use, or for external stakeholders such as milk producers assuming data confidentiality is respected. These can be then used to advise and inform prescribers and help formulate policy. The results of the macro-reporting will be discussed in the presentation.

The report is currently available for dairy farms with beef and small ruminant models in development.

**Conclusions:** A bespoke reporting tool using farm records allows the integration of AMS reports into active herd health planning as well as macro-reporting to monitor ongoing trends together with reporting to external stakeholders.

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#### AR-14

### Antimicrobial use in Canadian beef cattle: extent, indications, and risk factors for treatment.

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The majority of Canadian feedlot cattle placement occurs in the four western Canadian provinces, and antimicrobial drugs (AMDs) are used in Canadian feedlot cattle production systems to support and maintain health and as therapy for illness. Antimicrobial use (AMU) in food-producing animals is under increasing scrutiny due to the potential for promotion of antimicrobial resistance. Historically, comprehensive data related to the types of AMDs used, extent of use, the most common indications for use, and the demographics of the cattle populations receiving antimicrobials in Canada have been limited. Antimicrobial stewardship is a priority for the Canadian Beef Industry, and a thorough understanding of these baseline factors is important for meaningful assessment of the associated public health risk and to inform stewardship activities.

The objectives of this study are 1) to provide robust AMU estimates for Canadian feedlot cattle, 2) to interpret the AMU data and relate it to Canadian feedlot production practices, 3) to validate Population Corrected Unit estimates for Canadian feedlot cattle, and 4) to assess how a standardized collection method/system might be applied nationally (including logistics and meaningful inputs).

Detailed data regarding AMU in 2,615,089 cattle from 36 feedlots in western Canada were collected over 4 years (2008-2012). Of these cattle, the majority entering the feedlots were male (63%; 1,643,528), were considered low risk for developing bovine respiratory disease (BRD; 61%; 1,593,450), were identified as yearlings at the time of feedlot entry (55%; 1,434,589), and arrived during the fall or winter (62%;

1,616,691). Descriptive AMU data (both parenteral and in-feed) from this comprehensive dataset will be presented, including use trends over the four years, use based on the category of public health importance (as defined by Health Canada) and use by antimicrobial class (e.g., quinolones, macrolides, tetracyclines.). As well, AMU comparisons will be made between sexes, between geographical regions (provinces) and between seasons.

The comprehensive nature of this project allows for robust and high-resolution summarization, which is different from many other datasets. For example, over the four years of this study in these 36 feedlots, 70% of animals were administered parenteral AMDs for BRD metaphylaxis early in the feeding period, 6% for BRD therapy, and 4% for therapy for other reasons. These AMU indications will be stratified further by animal classification at feedlot arrival for risk of developing BRD (high or low), and by antimicrobial class; to allow detailed insight into AMU practices in Canadian beef production and antimicrobial exposure in Canadian feedlot cattle.

This presentation will also include a brief discussion of the importance of access to these types of data if the Canadian Beef Industry is to be proactive in the fight against antimicrobial resistance, is to continue to be a leader in antimicrobial stewardship, and is to be transparent related to access to global markets.

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#### AR-15

### Feeding preweaned calves milk containing drug residues ("waste milk"): impacts on the functional profile of the fecal microbiota

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**Objective:** While the practice of feeding drug residue-containing milk (waste milk) to calves is common worldwide, there is concern that it could potentially select for bacteria that are resistant to antimicrobial drugs that are of great relevance to human and animal health. Currently no information is currently available on the impact of this practice on the functional profile of the fecal microbiota. The objective of this study was to characterize the functional profile of the fecal microbiota of preweaned dairy calves fed raw milk with residual concentrations of antimicrobials commonly found in waste milk from birth to weaning.

**Material and Methods:** At birth, thirty calves were randomly assigned to a controlled feeding trial where: 14 calves were fed milk with no drug residues (NR), and 15 calves were fed milk with drug residues (DR) by adding ceftiofur, penicillin, ampicillin, and oxytetracycline. Fecal samples collected from each calf once a week starting at birth, prior to the first feeding in the trial (pre-treatment), until 6 weeks of age. Shotgun sequencing of the microbiota was conducted using the Illumina platform.

**Results:** Milk drug residues resulted in a significant difference in relative abundance of microbial cell functions, especially with genes linked with stress response, regulation and cell signal-



ing, and nitrogen metabolism. These changes could directly impact selection and dissemination of virulence and antimicrobial resistance in the microbiota. Drug residues also impacted selection and distribution of genetic functions in Resistance to Antibiotics and Toxic Compounds (RACT), resulting in a transition to a microbial RACT function more similar over different sampling time points in DR when compared to NR calves. Our data also identified a strong association between age in weeks and abundance of RACT, independent of treatment group. Regardless of treatment group, the age of calves in weeks revealed a significant shift in abundance of RACT function, including increases in functions related to resistance to vancomycin and fluoroquinolones as calves became older.

**Conclusion:** Findings from this study support the hypothesis that drug residues, even at very low concentrations, impact the gut microbiota of calves and result in changes in the functional profile of microbial populations.

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AR-16

### Waste milk from dairy farms in Central California: distribution of antimicrobial drug residues and farm practices

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**Objectives:** The aims of this cross-sectional study were to: 1) identify and measure concentrations of antimicrobial residues of WM samples from dairy farms in Central California, 2) identify management practices associated with the occurrence of specific antimicrobial residues in WM, and 3) characterize the antimicrobial resistance patterns of *E. coli* cultured from WM samples.

**Material and Methods:** Raw bulk tank WM samples were collected from 25 dairy farms located in California's Central Valley. A questionnaire was used to collect information about farm management practices. WM samples were analyzed for a multi-drug residue panel using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Bacteria were cultured and antimicrobial resistance was assessed using standard techniques; milk quality parameters (fat, protein, lactose, solids non-fat, somatic cell count, coliform count, and standard plate count) also were measured.

**Results:** Of the 25 samples collected, 15 (60%) contained detectable concentrations of at least one of the 26 antimicrobials tested. Eleven (44%), 4 (16%), and 1 (4%) sample had detectable levels of  $\beta$ -lactam, tetracycline, and sulfonamide residues, respectively. Of the drug residue positive samples, 5 (33%) had  $\beta$ -lactams and 2 (13%) had tetracycline residues above the tolerance/safe limits established by the FDA. No samples had sulfonamide residues above the FDA tolerance/safe limit. The most prevalent drug residues were ceftiofur ( $n = 7$  (28%); mean  $\pm$  SE =  $251 \pm 177.0$  ng/ml), oxytetracycline ( $n = 4$  (16%);  $16378 \pm 308.7$  ng/ml) and cephalosporin ( $n = 3$  (12%);  $39 \pm 24.5$  ng/ml). Of these, ceftiofur (88%) and cephalosporin (40%) also were the most commonly used to treat mastitis based on questionnaire answers. No significant association was identified between

farm characteristics, management practices and presence of drug residues in WM. *Streptococcus spp.* ( $n = 21$  (84%);  $51,010 \pm 19,631$  cfu/ml) was the most common bacterium cultured from WM samples, followed by *Staphylococcus spp.* ( $n = 20$  (80%);  $8,625 \pm 4,939$  cfu/ml), *Escherichia coli* ( $n = 10$  (40%);  $5,870 \pm 2,478$  cfu/ml), non-fermentative gram-negative organisms ( $n = 8$  (32%);  $9,625 \pm 3,087$  cfu/ml), *Staphylococcus aureus* ( $n = 5$  (20%);  $121,400 \pm 119,652$  cfu/ml), *Bacillus spp.* ( $n = 5$  (20%);  $860 \pm 563$  cfu/ml), and *Klebsiella spp.* ( $n = 4$  (16%);  $78,750 \pm 73,771$  cfu/ml). *Mycoplasma spp.* was cultured from two WM samples (8%). Two of the 10 *E. coli* isolates were resistant to three or more classes of antimicrobials. In this study, 20% of farms did not pasteurize WM prior to feeding to calves.

**Conclusions:** WM fed to calves on dairies in California's Central Valley contains residues of antimicrobial drugs important to veterinary medicine. The presence of drug residues in WM at concentrations that increase selection of resistant bacteria indicate the need for additional studies targeting on-farm milk treatments to degrade drug residues before feeding to calves. The presence of multidrug resistant *E. coli* in WM urges the need for on-farm practices, such as pasteurization, that reduce calf exposure to resistant bacteria.

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AR-17

### Use of on-farm bacterial culture and decision support tools to reduce antimicrobial usage in cases of mild to moderate clinical mastitis in cows

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**Objectives:** The aim of the study was to assess antimicrobial usage for clinical mastitis following implementation of a novel on-farm culture system and selective therapy based on culture results.

**Materials and methods:** Milk samples were collected from 506 quarters with mild to moderate clinical mastitis from 7 farms for on-farm microbiology culture. The culture system was a plate which included 4 quadrants (a 5% blood agar, a Gram positive selective media, a Gram negative selective media, and a yeast/fungi selective media; Check-Up Farm Medix, Auckland, New Zealand). Within sequential pairs of cows, half of these quarters were assigned to be treated without regard to the culture results (Blanket treatment group), while the culture results were used to decide the treatment outcome for the remaining quarters (Selective treatment group), i.e. no treatment for quarters from which no bacteria or gram-negative organisms were isolated, intramammary infusion of cloxacillin for quarters from which *Staphylococcus aureus* was isolated, and intramammary or parenteral narrow-spectrum penicillin for quarters from which *Streptococcus spp.*, coagulase-negative *Staphylococcus spp.* or other bacteria were recovered. Milk samples were also submitted for routine laboratory-based microbiology culture. On-farm culture results and mastitis treatment records were recovered and the natural log of the sum of antimicrobial doses/cow was compared between treatment groups. The level of agreement between the on-farm and laboratory-based culture techniques was compared with  $c^2$  analysis.

**Results:** Compliance with the treatment protocols was higher



amongst quarters assigned to the Selective (199/233; 85.4%), compared with the Blanket (171/249; 68.7%) treatment group ( $p < 0.001$ ). Quarters assigned to the Selective group had a lower mean Ln dose (1.00 (SEM 0.03)) than those assigned to the Blanket (1.22 (SEM 0.03)) group ( $p = 0.005$ ). There was no difference between treatment groups in the hazard that cows would be re-diagnosed with clinical mastitis within 60 days of enrolment (hazard ratio (Selective relative to Blanket) = 0.82 (95% CI=0.39-1.69);  $p = 0.59$ ). *Streptococcus* spp. (61%) were the most common isolates followed by *S. aureus* (13%) and no growth (12%). The agreement between on-farm culture and laboratory testing was 188/331 (56.8%).

**Conclusions:** Use of on-farm culture with Selective therapy based on culture results resulted in approximately a 20% reduction in antimicrobial usage.

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AR-18

### Non-antibiotic topical treatment of interdigital phlegmon (foot rot) with salicylic acid

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**Objectives:** Foot rot is a common cause of lameness in dairy and beef cattle. Cows with foot rot are typically acutely lame, with swelling of the foot, fever and a malodorous interdigital cleft [1]. The infection involves *Fusobacterium (F.) necrophorum* in a mixed flora [2]. Recommended treatment in Sweden is an intramuscular penicillin injection for three days, which is used in 90 per cent of systemic treatments. An alternative topical treatment of foot rot by farmers requires early detection and prompt cleansing, disinfection and bandaging. Topical application of salicylic acid is common practice in treating digital dermatitis in Scandinavia [3] and a similar treatment could be an alternative also to treat foot rot. An immediate non-antibiotic topical treatment aims to reduce antibiotic consumption, milk discards, development of resistance, and treatment costs. However, the treatment effect of salicylic acid has not been scientifically evaluated. Thus, in this study we investigated whether topical treatment with salicylic acid has beneficial therapeutic effects on foot rot in dairy cows under field conditions.

**Materials and Methods:** Cows with foot rot were identified by farmers based on any degree of lameness, affected general condition, increased temperature, and swelling of the foot, and brought to a trimming chute. Farmers followed a treatment protocol recording cow ID, date and symptoms. The affected foot was immobilised, the circumference around the coronet was measured, the interdigital space was washed and inspected, and the site of the lesion was described [4]. A swab was taken from the interdigital skin lesion of the five first cases from each participating farm and sent to the laboratory for bacteriological examination and antibiotic resistance testing of obtained *F. necrophorum* isolates. The topical treatment comprised applying a tablespoon of dry 100% concentrated salicylic acid powder to the interdigital lesion site and covering with an elastic bandage. The disease parameters (except circumference) were

again assessed by the farmer on day 1-2 and again when the bandage was removed after 3-5 days. The protocol data were analysed statistically with paired t-test and Kolmogorov-Smirnov test.

**Results:** Of 109 cases from 24 herds seven were excluded due to early systemic penicillin treatment because they were considered too severely affected by the infection and two cases because the protocol was missing. *Fusobacterium necrophorum* was isolated from 30 of 60 samples, while in remaining samples no or a mixed flora was found. All 30 isolates tested for resistance were susceptible to penicillin (MIC 0.016-0.06 mg/L). The proportion of lame animals and with affected general condition were significantly lower day 3-5 than on day 0. The temperature was significantly lower ( $P < 0.001$ ) on day 1-2 and day 3-5 and the coronet circumference were significantly lower ( $P < 0.04$ ) on day 3-5 compared with day 0.

**Conclusions:** Treatment with salicylic acid locally in the interdigital space, of early detected non-complicated foot rot, gave a satisfactory treatment result. Within three to five days, treated cows responded with an improved general condition, reduced lameness, lower body temperature, and decreased coronary swelling, compared with day 0 when the treatment started. Salicylic acid is thus an attractive alternative to antibiotics in the treatment of foot rot. The advantages of salicylic acid are less treatment cost, immediate treatment, no withdrawal time and no selection for antibiotic resistance. However, the cow has to be managed in a trimming chute or correspondingly to facilitate bandaging.

**Acknowledgements:** The study was funded by the Swedish Farmers' Foundation for Agricultural Research.

The study was ethically approved for the use of animals in research Swedish Board of agriculture no C 131/13.

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WE-01

## Pain relief in Australian Cattle; Where to from here?

### Multimodal and extended pain relief in cattle.

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**Objectives:** Cattle regularly experience painful husbandry procedures associated with routine management practices. While the use of anaesthesia and analgesia is mandated in some cases, more often these procedures are performed without pain relief. There is growing concern that consumer confidence in animal products is under threat from such activities increasing the need for pain relief during such procedures. In Australia the adoption of a topical 'spray-on' anaesthetic (TA) formulation has led the way in terms of providing an easily applied treatment for use in lambs and calves undergoing surgical husbandry procedures. Furthermore, multi-modal pain management, through the combined use of nonsteroidal anti-inflammatory drugs (NSAID's) with TA has shown significant benefit by reducing pain in lambs and calves following castration, tail-docking and dehorning. But do these once-only applications of pain relief, usually applied immediately after the procedure, go far enough in addressing the long-term pain associated with these procedures? Long-term pain relief is being investigated utilising a long acting injectable formulation of a NSAID. In addition an alternative method of self-medication is being investigated to provide pain relief over an extended period of time.

**Methods:** Several formulations of long acting NSAIDs are currently being trialled with the aim of providing a 4 to 5 day therapeutic effect from a once only dose. In addition, alternative methods of providing NSAIDS via self-medication in supplements is being investigated so that animals can access pain relief over an extended period of time.

**Results:** Preliminary results using sheep models indicate success in providing the desired outcome of extended analgesia from a once only dose. Full results will be available in early 2018. Pilot studies into self-medication via supplements are due to begin in early 2018 with results due by early May 2018.

**Conclusion:** New forms of extended pain relief will be developed to increase the options for providing a multimodal approach to analgesia over an extended period of time to address the welfare concerns associated with painful procedures.

WE-02

## The effect of topical anaesthetic and meloxicam, alone and in combination, on pain and haemorrhage following spaying of female beef cattle

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**Objectives:** The objectives were to evaluate the effect of a topical anaesthetic (TA) containing adrenaline, delivered via a modified ovariator, and meloxicam (MEL) delivered subcutaneously, alone and in combination on body weight (BW), packed cell volume (PCV), total plasma protein (TPP) and behaviour of heifers and cows in the acute period following spaying via the Willis Dropped Ovary Technique (WDOT).

**Materials and methods:** Seventy-five heifers and 17 cows were randomly allocated to 1 of 5 treatment groups: (1) Control / no spay (CON, n = 15 heifers and 4 cows); (2) WDOT spay (S, n = 15 heifers and 4 cows); (3) WDOT spay with peri-operative delivery of TA (STA, n = 15 heifers and 3 cows); WDOT spay with peri-operative delivery of MEL (SMEL, n = 15 heifers and 3 cows); and WDOT spay with peri-operative delivery of TA and MEL (STAMEL, n = 15 heifers and 3 cows). All animals were weighed immediately prior to treatment and at 24 hours following treatment. Blood samples were collected from all animals immediately prior to treatment and at 24 hours following treatment. Blood samples were analysed for packed cell volume (PCV) and total plasma protein (TPP). All animals were filmed for 8 hours following treatment and for 1 hour at 24 hours following treatment for analysis of animal behaviour. A post-mortem was conducted on two animals that died following treatment. Change in body weight (BW), PCV and TPP from before treatment to 24 hours following treatment was calculated and statistically analysed. Behavioural data is currently being analysed.

**Results:** One S animal and one STA animal died at approximately 4 hours and 44 hours following treatment, respectively. A post-mortem confirmed that significant internal haemorrhage was the cause of death for both of these animals. There were no significant effects of treatment on change in BW ( $P = 0.810$ ), PCV ( $P = 0.284$ ) or TPP ( $P = 0.893$ ). There were two animals that had decreased PCV outlying the normal range of the data. The animal that died 44 hours following treatment was one of these animals.

**Conclusions:** Packed cell volume appears to decrease in cases of moderate to severe internal haemorrhage following WDOT spaying of female cattle. For these cases, TA and MEL does not appear to have any effect on haemorrhage. The effect of TA and MEL on pain is yet to be evaluated through analysis of post-operative behaviour.

WE-03

## Effects of Post-Partum Pain Relief on Behaviour in Calves Born With and Without Assistance

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**Objectives:** Dystocia is likely to be a painful event for calves and research indicates that veterinary surgeons in practice sometimes provide analgesia to calves born to a difficult calving; there is however a lack of research investigating the possible benefits of providing analgesia to newborn calves. We investigated the effects of administration of a non-steroidal



anti-inflammatory drug (ketoprofen) in calves in the immediate postpartum period as assessed by behavioural time budget analysis in the first 48 hours postpartum.

**Materials and Methods:** A single 700 cow dairy herd in Scotland was recruited for the study; all calves included were purebred Holstein heifer calves in the same management system. Seventy six calves were attributed to either an assisted calving group or an unassisted calving group using an established calving scoring system. Calves within each of these two groups were randomly allocated to either a placebo group (saline injection) or a treatment group (ketoprofen injection) in a 2x2 design. Closed circuit television (CCTV) cameras were set up in the calf accommodation and calves were continuously filmed for 48 hours postpartum. Video footage was analysed using scan sampling. Behavioural time budgets for lying behaviours, active behaviours and secondary behaviours (such as social and feeding behaviours) were established for four time blocks (0-12 hours, 12-24 hours, 24-36 hours and 36-48 hours postpartum). Time budget comparisons were made between each group of calves.

**Results:** Data analyses indicate significant differences between groups. Calves in the placebo group spent more time engaging in lying behaviour than calves in the treatment group for both the unassisted and the mild assistance groups ( $p < 0.001$ ). Calves in the unassisted treatment group spent less time in lateral recumbency than calves in the other three groups ( $p = 0.009$ ), suggesting that even a normal birth is painful for calves.

Play behaviour was exhibited most often in the 12 to 24 hour time period and declined in all calves as time progressed ( $p = 0.002$ ). Calves in both treatment groups spent more time engaging in play behaviour than control calves ( $p = 0.041$ ); as play is a luxury behaviour for calves, this is suggestive that analgesia at birth provides a positive benefit to calves. Although the interaction between intervention and time period was not significant for play behaviour, calves in the combined treatment groups spent numerically more time engaging in play than calves in the placebo groups. This suggests that the positive benefits of providing analgesia in the immediate postpartum period may be sustained for up to 48 hours.

**Conclusions:** The differences observed are suggestive of improved calf welfare if analgesia is provided at, or shortly after, the time of birth. Effects were observed in calves born to unassisted calving as well as assisted calving, suggesting that there is a welfare benefit of providing analgesia to all calves at birth.

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WE-04

### Flunixin transdermal reduces pain in cattle

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**Objectives:** Flunixin has anti-inflammatory, anti-pyretic and analgesic effects, and is commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature. A 50 mg/ml flunixin transdermal formulation was developed by MSD Animal Health and is now the first NSAID registered to be administered as a pour-on. Two studies were conducted to determine the effectiveness of flu-

nixin transdermal to control and reduce pain in cattle affected with lameness, under experimental field conditions in United States (US) and natural field conditions in Europe (EU).

**Materials and methods:** Two different studies, an experimental one in US with a challenge of *Fusobacterium necrophorum* inducing foot rot, a natural one in EU with natural field infectious lameness, were performed to evaluate flunixin transdermal to manage pain in cattle.

In the experimental US study, calves were challenged by subcutaneous injection of *F. necrophorum* into the interdigital space of the right front foot using a method validated to induce pain representative of foot rot. Enrollment into the study required that calves demonstrate signs of pain associated with foot rot (moderate to severe lameness, small to large lesions, and moderate to severe swelling). A total of 60 animals, 8 months old, were enrolled on two study sites. A real-time gait analysis system was used to evaluate the maximum total force and contact area on a pressure mat for the right front foot before the induction of foot rot, at enrollment, and at six hours after treatment. Lameness severity was also scored six hours after treatment.

In the natural EU study, calves were monitored for evidence of pain and enrolled when showing moderate to severe lameness only in one limb (caused by interdigital dermatitis, phlegmona interdigitalis or dermatitis digitalis), and mild to very severe lesions. A total of 65 animals, aged from 2 to 9 years, were enrolled in France and Spain. Animals were then observed for signs of pain at 6 hours after treatment initiation and daily thereafter for 3 consecutive days.

In both studies, at day 0, animals were treated once either with flunixin transdermal (Finadyne® Transdermal, 3.3 mg/kg flunixin; MSD Animal Health), or with a negative control, saline with red dye, both administered topically along the dorsal midline. In the natural disease EU study, animals received also cefquinome (Cobactan® 2.5%, MSD Animal Health) administered intramuscularly, on days 0, 1 and 2. In the US experimental study, no antibiotic was administered.

**Results:** In the experimental US study, at six hours after treatment with flunixin transdermal, the success rate based on lameness score improvement was 100% on site 1 and 93.33% on site 2. The mean change in maximum force between enrollment and six hours after treatment was significantly greater in animals treated with flunixin transdermal (43.08 kg-force on site 1 and 34.32 kg-force on site 2) than in animals untreated (-4.14 kg-force on site 1 and -0.54 kg-force on site 2) at six hours after treatment ( $p < 0.0001$ ). The mean change in contact area between enrollment and six hours after treatment was also significantly greater in animals treated with flunixin transdermal (16.76 cm<sup>2</sup> on site 1 and 16.38 cm<sup>2</sup> on site 2) than in animals untreated -2.70 cm<sup>2</sup> on site 1 and -0.96 cm<sup>2</sup> on site 2) ( $p < 0.0001$ ).

In the natural disease EU study, the success rate based on lameness score improvement at six hours after treatment was 66.67% with flunixin transdermal, and was significantly greater compared to 28.57% with negative control ( $p = 0.0174$ ). Over time, a significant decrease in the severity of lameness and lesions was observed in animals treated with flunixin transdermal compared to negative control animals, at each time point after treatment, 6 hours, day 1, day 2 and day 3.

**Conclusions:** The reduction of lameness score was defined as the primary criterion to demonstrate effectiveness in the control



and relief of pain. In both studies, the experimental US study and the EU natural disease study, flunixin demonstrated under different conditions a reduction of clinical signs of pain associated to lameness. The single transdermal application of flunixin is a safe and effective therapy in the relief of pain in cattle.

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WE-05

### Use of topical anaesthesia to control pain during and after trimming hoof lesions in dairy cows.

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Treatment of hoof lesions such as sole ulcers in cattle frequently involve trimming of healthy and inflamed tissue (corium). Trimming may cause acute and severe pain resulting in violent reaction with risk for humans' safety as well as potentially affecting the animal's immediate welfare. Specific behaviours are easily identified when pain is caused. Due to technical and practical difficulties and because the procedure is generally carried out by a non-veterinarian, pain management is seldom used during hoof trimming in dairy farms worldwide.

Tri-Solfen® is a "spray and stay" topical anaesthetic formulation containing lidocaine for rapid onset wound anaesthesia, bupivacaine as a long-acting local anaesthetic to provide prolonged duration of effect, adrenalin to concentrate the anaesthetic effect at the wound site and reduce haemorrhage, and cetrimide to provide wound antiseptics, formulated in a viscous gel base. It can be easily applied by non-specialized personnel as a once-only application. Efficacy and welfare studies in mulesing, castration and tail docking in lambs and castration in calves, have shown that Tri-Solfen assists in the immediate alleviation of pain with prolonged effect, reducing both pain related behaviour, and wound pain responses from within 1 minute up to and including 24 hours post procedures.

Our study was designed to determine the effect of Tri-Solfen, applied during the procedure, in attenuating pain during and after trimming bleeding hoof lesions in dairy cows. In treated cows, the gel was applied after removing superficial horn and necrotic tissue and when corium was exposed.

Cows selected for hoof trimming at drying off were graded for lameness (1-non lame to 5-severely lame). The hoofs of those with lameness score above or equal to 3 were carefully examined for lesions by an experienced veterinarian. Lesion site, type and size were registered. Animals with horn lesions requiring trimming were randomly distributed to two groups: C – usual trimming with no pain control; T – trimming with Tri-Solfen being applied once live corium was exposed and on completion of trimming. Tri-Solfen application was repeated if deemed necessary (e.g. extensive or deep lesions) with the total quantity being registered. Trimming was resumed one minute after each application.

Lameness was scored on the way to the chute, when leaving the chute and three days after trimming. Reaction to trimming was scored (1 to 3) based on the display of the following pain related behaviours: kicking, efforts to release limb and vocalization. A digital algometer was also applied on the lesion area before, during and after trimming. Behaviour observation was done by two persons blinded to treatment.

Across several months this study was repeated in three large dairy farms. In total 28 lame cows were trimmed with Tri-Solfen and 22 were used as control. Two types of statistical analysis tests were used: non-parametric tests and analysis of variance. Treatment significantly influenced reaction to trimming and lameness score after trimming ( $p < 0.01$ ), with better results for those animals treated with Tri-Solfen. Algometer values tended to be higher after application of Tri-Solfen. No differences were shown for lameness after three days. Differences between groups were not influenced by the limb (front or hind) or hoof (outer or inner) affected. Algometry also showed increased pressure threshold in two interdigital skin lesions after application of Tri-Solfen.

In conclusion, this study shows that a topical gel with lidocaine and bupivacaine can reduce pain when trimming severe and extensive hoof lesions, improving dairy cow welfare and eventually trimmer safety.

With thank Animal Ethics, Australia for providing the product for the trials.

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WE-06

### Effect of the administration of flunixin transdermal alone or in combination with an antibiotic on health and performance of newly received beef cattle

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**OBJECTIVES:** Bovine respiratory disease (BRD) is the most common health problem in intensive beef cattle production systems. BRD is a multifactorial syndrome exacerbated by stressful conditions such as transportation, weaning and weather. BRD morbidity and mortality is commonly managed through the administration of approved antibiotics. The objective of the present study was to evaluate the effect of administration of antimicrobials or NSAID's at arrival on health and performance of newly received beef cattle.

**MATERIALS AND METHODS:** A total of 318 Charolaise bulls with an average body weight of 430 kg were transported from France to an Italian fattening farm. Upon arrival, animals were treated for parasites, vaccinated, weighed, and their temperature was collected. Animals were randomly assigned to one of four different treatment groups: 1) Control (CON; n=81); animals did not receive antibiotics or anti-inflammatories. 2) FTD (n=80); animals received flunixin transdermal (Finadyne transdermal, MSD Animal Health). 3) AB (n=79); animals received



tildipirosin (Zuprevo, MSD Animal Health). 4) AB+FTD (n=78); animals received tildipirosin and flunixin transdermal. After treatment, animals were placed in 4 different pens according to treatment group and monitored for a 40 day adaptation period. Animal health was monitored daily during the adaptation period by the staff veterinarian who was blinded to treatment. Nasal swabs from each animal affected with BRD were taken for microbiological identification. Blood samples from 10 animals per treatment group were randomly collected on day 0, day 17 and day 40 post-arrival to evaluate: serological screening for viruses and bacterial pathogens, non-specific immunity (serum bactericidal activity and  $\gamma$ -interferon), antioxidant status (Kit Radicaux Libres – KRL), and inflammatory status (haptoglobin, interleukin 6, and lipopolysaccharide-binding protein - LBP). Individual body weight on arrival and on day 40 was used to determine average daily gain (ADG). Statistical analysis was performed using SAS 9.4.

**RESULTS:** Upon arrival, 18.5% of the animals had shipping fever (rectal temperature  $>40^{\circ}\text{C}$ ) and no differences were found among treatments groups. During the 40 day adaptation period, the incidence of BRD tended to be greater in animals in CON group (18.5%) compared to animals in FTD group (15%), AB group (12.7%) ( $P<0.1$ ) or AB+FTD group (11.5%) ( $P<0.1$ ) BRD relapses were low across all treatment groups without statistical differences. No mortalities were observed during the study. Serum evaluation showed that cattle at arrival were not previously exposed or vaccinated to BHV-1, BVDV or *M bovis*. Animals receiving AB+FTD showed a greater response to vaccination for BHV-1 and BVDV compared to CON ( $P<0.05$ ). The main bacteria isolated from 44 animals diagnosed with BRD were *P multocida* and *M haemolytica* and no differences among treatment groups were observed. Animals receiving AB+FTD tended to have greater levels of serum bactericidal activity ( $P<0.1$ ) compared to CON and FTD treated animals at day 17 post-arrival. Similarly, animals receiving AB+FTD tended to have greater KRL ( $P<0.1$ ) compared to CON and FTD groups at day 40 post-arrival. Animals receiving AB+FTD had better levels of LBP compared to animals in CON ( $P<0.05$ ) or FTD groups ( $P<0.1$ ). ADG was significantly greater ( $P<0.05$ ) in animals treated with AB+FTD (1.60 kg/d) and AB alone (1.59 kg/d) as compared to CON (1.40 kg/d). Animals treated with FTD alone (1.53 kg/d) showed numerical improvement in ADG as compared to CON (1.40 kg/d).

**CONCLUSIONS:** Under the present study conditions, the administration of tildipirosin alone or in combination with flunixin transdermal at arrival tended to reduce BRD morbidity, improved immune response, antioxidant body defenses and pro-inflammatory status, and ADG compared to controls. FTD alone had no effect on the improvement of the immune response compared to controls. However, further research should be conducted to evaluate the stand alone benefits of FTD on animal health, performance and wellbeing, as a numerical improvement in ADG has been measured compared to controls and how FTD can contribute to the judicious use of antimicrobial in cattle upon arrival to a fattening farm.

WE-07

### Dairy cattle welfare assessment – an innovative approach for cattle in pasture in the Azores Island

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Animal welfare is a multidimensional concept, defined as a state of complete mental and physical health where the animal is in harmony with its environment. Sustainability, animal welfare and environmental concerns have increased consumers' interest in knowing how, where and by whom food is produced and handled from "farm to fork". The "Milk from Happy Cows" programme in the Azores Island (Portugal) aims at providing certification of good practices and high animal welfare. This is a way to help save milk producers from regions where competitiveness for low prices is almost impossible.

To achieve this purpose an innovative welfare assessment protocol was developed. This was based on the Welfare Quality® protocol, but adapted to systems with permanent grazing. In this paper we will go through the process of selecting adequate indicators and of protocol application in 33 dairy farms. Finally the way used to grade these farms is discussed.

In 2004 the Welfare Quality® project developed an integrated standardized methodology for the assessment of welfare of intensively kept dairy cows. Four main areas of concern, further split into twelve criteria each of which corresponded to a key welfare dimension, were proposed: 1) Good feeding (appropriate nutrition, absence of prolonged thirst); 2) Good housing (comfort around resting, thermal comfort, ease of movement); 3) Good health (absence of injuries, absence of disease, absence of pain and pain induced by management procedures); 4) Appropriate behaviour (expression of social behaviour, expression of other behaviours, good human-animal relationship, positive emotional state). When a criterion was not satisfactory addressed by an animal-based indicator, environment or management measures should be used.

The WQ® protocol was intended for one particular production system but adaptations have been tested for other conditions. We altered the WQ protocol so as to apply it to farms in the Azores Islands where cows are kept outside all year round. Several indicators were removed while others were added. In summary, cows are first assessed at pasture (e.g. flight distance, isolation, thermal comfort, ease of movements), then on the path to the milking parlour (e.g. lameness, human-animal relationship), at the waiting pen (e.g. BCS, agonistic behavior, lesions) and finally when being milked (dirtiness, injuries, teat lesions, hoof condition). At the end a survey is applied seeking to collect further indicators such as SCC, mortality and culling prevalence, pain management at disbudding and many more.

Calves and heifers are also assessed mainly using animal based indicators such as BCS, dirtiness, prevalence of coughing, diarrhea or injuries.

Threshold for the different indicators were established based on the Azores average farms' prevalence or on previously defined rules for the "Milk from Happy Cows" programme. Hence, each indicator could be graded as GREEN (threshold fully attained), YELLOW (close to threshold), RED (distant from threshold). The overall assessment resulted from the number of indicators in each grade and certification was awarded to farms with no RED indicator or no more than two YELLOW indicators. Farms with one or two YELLOWS were given a period





of time to resolve them. Some constraints encountered are discussed in this paper, namely on how to achieve threshold values through benchmarking and how to discriminate critical indicators (e.g. mortality) from more prevalent but less significant measures (e.g. overgrown claws). Although some flexibility may be introduced, this method also has the disadvantage of not distinguishing farms that have one RED from those having several "REDS" or many YELLOWS. All would fail to reach acceptance, but some would have more serious welfare issues.

The first assessment of the 33 farms of the programme resulted in 79 YELLOWS and 9 REDS in total. This was a way of identifying the major problems and to quickly address the most important welfare problems. Most farms at a second audit had already resolved most of the cows' issues and were already addressing the most complicated ones detected at the rearing department.

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WE-08

### Response of Bunaji and Friesian cows to heat stress in Bauchi, Nigeria

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**Objectives:** This study was aimed at determining the effect of heat stress on vital and haematological parameters of Bunaji and Friesian cows in Bauchi, Nigeria

**Methodology:** Five cows each of Bunaji and Friesian breeds aged five years belonging to the Dairy Research Center of the Abubakar Tafawa Balewa University, Bauchi, Nigeria were used in this study. Two hundred and forty (240) blood samples were collected for haematological evaluation using automated haematological analyzer (SYSMEX, XP-300). Measurements were taken weekly during morning (7:00 am) and afternoon (1:00 pm) for three months spanning from March to May, 2017. Vital parameters including body temperature, respiratory rate and pulse rate were measured using digital clinical thermometer (D) and an infrared thermometer (I). Ambient temperature, relative humidity and wind speed were obtained from the National Meteorological Center, Bauchi, Nigeria. The Statistical Package for Social Sciences (SPSS) was used to analyze the data collected.

**Results:** Periods of the day and months coefficients of adaptability were estimated at 2.35 and 2.54 for Friesians and 1.92 and 2.13 for Bunaji for the morning and afternoon sampling respectively. Friesians vital and haematological parameters were significantly ( $P < 0.05$ ) higher than Bunaji breeds. All afternoon vital parameters were observed to be significantly ( $P < 0.05$ ) higher than that of the morning in the two breeds studied. Afternoon Red Blood Cell (RBC), Packed Cell Volume (PCV) and Haemoglobin (Hb) of Bunaji were significantly ( $P < 0.05$ ) higher, while in the Friesian, White Blood Cells (WBC) was the only parameter that was significantly ( $P < 0.05$ ) higher than in the

morning. All the vital parameters in the month of May were observed to be significantly ( $P < 0.05$ ) higher in both breeds except Vaginal Temperature Digital (VTD) and Skin Temperature (ST) of Friesians. The Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelets (PLTS) in April and WBC in May of the Friesians were significantly ( $P < 0.05$ ) different, while RBC, PCV and Hb of Bunaji differed significantly ( $P < 0.01$ ) in March. Bunaji RBC, PCV and Hb showed highly significant ( $P < 0.01$ ) months and periods of the day interaction effects.

**Conclusion:** This study revealed that breeds, months and periods of the day affects all the vital parameters and some of the haematological parameters of Friesians and Bunaji cows in Nigeria, which is probably their mechanism of adaptation to the harsh environment the cattle were exposed to, this will invariably be responsible for the poor reproductive performance of such cattle during the season of critical period.

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WE-09

### How well are our dairies during the summer? Summer to Winter (S:W) Ratios of Somatic Cell Score and milk yield in USA

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The Summer to Winter (S:W) ratio of performance variables such as milk production and somatic cell score was previously developed as a metric to estimate on-farm heat stress and the effectiveness of cooling strategies on dairy farms in Israel. The closer the ratio is to one, the less heat stress is experienced by cows within the herd. We aimed to test the usefulness of this metric in the USA. We used S:W ratios to compare ECM and SCS in summer (numerator) and winter (denominator) using test day herd averages within each southeast (SE) state or U.S. region. Performance data from Dairy Herd Improvement Association (DHIA) from 2007 to 2016 were obtained for all DHIA herds processing records through DRMS (Dairy Records Management Systems, Raleigh, NC). The GLM procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) was used to compare test day ECM and test day SCS summer and winter averages and ratios for herds within each SE state ( $n=6$ ) or U.S region ( $n=7$ ). The model included herds with more than six test days per year and excluded herds with  $>16$  test days per year. Year and mean DIM were included as covariates. The highest ECM S:W ratio was for the Pacific Northwest ( $1.03 \pm 0.0235^a$ ) and the lowest for the Southern Plains ( $0.87 \pm 0.0019^e$ ), indicating a larger decrease in ECM in the summer than the winter for the Southern Plains region. The test day SCS S:W ratio for the Southern Plains ( $1.09 \pm 0.0038^b$ ) showed the most seasonal difference and the Northern Plains showed the least ( $1.00 \pm 0.0040^e$ ). Year and DIM were significant for ECM and SCS ( $p < 0.01$ ). For the Southeast states, highest ECM S:W ratio was VA ( $0.92 \pm 0.0017^a$ ), and lowest for MS ( $0.80 \pm 0.0074^d$ ), indi-



cating that cows in VA herds experience less heat stress. Somatic cell score S:W ratios indicate that herds in MS ( $1.10 \pm 0.0135^{ab}$ ) and FL ( $1.10 \pm 0.0126^a$ ) experience the most heat stress in the summer. Year was significant for ECM and SCS ( $p < 0.01$ ), but DIM was not significant. The S:W ratios could be beneficial for producers and consultants as a tool to assess heat stress and animal welfare. It may also be useful to encourage improvements for heat abatement such as assessing the efficiency of cooling strategies.

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WE-10

### Welfare status of bobby calves at an abattoir in Victoria, Australia.

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**Background:** In Australia, a large proportion of male calves born on dairy farms are consigned to the abattoir for slaughter between 5 and 30 days of age. Management of these calves on farms, during transport and in lairage is crucial as they are very young and may not be capable of coping with the stresses of transport. The welfare of these calves has been studied in Australia under experimental conditions, and minimal adverse effects on health and welfare were reported. However, it is important to monitor the welfare of calves under commercial conditions to determine whether studies conducted under experimental conditions are reflective of industry practice.

**Methods:** Blood samples from young calves ( $n=494$ ) were collected immediately after the slaughter at a commercial abattoir in Victoria. For the identification of each calf, a portable stick reader (Allflex® RS 420) was used to scan the radio-frequency identification ear tag (RFID) instantly and then later transferred to a spread sheet. Based on the google map approximate distance travelled by each calf from the property of origin to the abattoir was calculated. Calf welfare status was determined by measuring metabolic indicators (glucose, beta-hydroxybutyrate and urea), hydration state (PCV), passive immunity (gamma-glutamyl transferase activity) and indicators of muscular exhaustion and bruising (lactate and plasma creatinine kinase). Packed cell volume was estimated within 6 h of blood collection by using micro-haematocrit technique. Plasma was analyzed for concentrations of glucose, beta-hydroxybutyrate (BHB), urea, total plasma protein (TPP), gamma-glutamyl transferase (GGT), creatine kinase (CK) and lactate using a multi-channel analyzer (COBAS INTEGRA® 400 plus, Roche) following manufacturer instructions.

**Results:** Hypoglycaemia (blood glucose  $< 2.8$  mg/dl) was recorded in only 2.5% of calves although the mean glucose level (4.60 mmol/L) was towards the lower limit of the reference range. High concentrations of BHB (BHB  $> 0.11$  mmol/L), indicative of lipolysis, was evident in 90.2% of the calves. The mean

BHB concentration was 0.24 mmol/L. However, the bobby calves after transportation to the abattoir did not suffer severe negative energy balance as indicated by a low plasma urea concentration, a parameter indicative of amino acid catabolism during a severe shortfall of energy. The mean urea level was 4.95 mmol/L which is within the normal reference range and only 1.88% calves exceeded the maximum reference range (urea  $> 15.7$  mmol/L). There was evidence of failure of passive transfer (GGT  $< 75$  IU/L) in 23% of the calves. Based on PCV, 27.5% of the calves were dehydrated. The major effect in bobby calves after transport was seen in blood CK and lactate concentrations, as more than 95% of the calves exceeded the maximum physiological range (CK  $> 125$  IU/L and lactate  $> 1.89$  mmol/L). The mean values of CK and lactate were 384.20 IU/L and 5.20 mmol/L respectively. The effect of journey distance on blood glucose, PCV and CK concentrations had been assessed. No significant correlation was observed between journey distance and blood glucose ( $r = 0.028$ ,  $P = 0.575$ ). Similarly, no correlation had been found between distance of journey and PCV ( $r = -0.006$ ,  $P = 0.899$ ) and between journey distance and plasma CK ( $r = 0.004$ ,  $P = 0.937$ ). Little or no difference was observed in the plasma biochemical indicators of bobby calves transported from different dairy regions, between seasons, breeds or gender.

**Conclusion:** The findings from this single abattoir study indicate that the energy level of bobby calves may be somewhat compromised before slaughter. Approximately one-third of calves suffered from dehydration and one-fourth of calves had failure of passive transfer which indicates further research is needed to find out the causes of dehydration and the farm level risk factors of failure of passive transfer. The higher plasma CK and lactate level highlighted that careful management is needed during handling and transportation of bobby calves. Further research would be useful to find out the key risk factors responsible for elevated CK and lactate. A wider scale study with periodic monitoring would be beneficial to determine how the indices which reflect energy status vary at different time points between farm and abattoir. This would give a greater understanding of the possible risk factors of hypoglycaemia in these animals.

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WE-11

### Recumbency at Slaughter Premises: A Case-Control Study

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**Objectives:** Dairy cattle becoming recumbent either during transport to slaughter or whilst waiting to be slaughtered is a significant welfare and economic issue even though it only affects a small proportion of cull cattle every year. The aim of this study was to use a case-control methodology to better understand the causes of recumbency in dairy cattle at abattoirs in New Zealand

**Materials and methods:** The study was undertaken between March 2013 and May 2014, at a convenience sample of 5 slaughter premises across New Zealand. At these premises all dairy cattle which arrived recumbent, but alive, or became recumbent between arrival and slaughter were eligible for the



study. Three controls were selected per case: (1) an ambulatory from the same farm as the case; (2) normal dairy cow from a group with no recumbency that arrive prior to the case; and (3) normal dairy cow from a group with no recumbency that arrive prior to the case.

A questionnaire was provided for case and control cows which were filled in by veterinary staff at the slaughter premises. This provided information about individual cow risk factors – such as age, body condition (BCS), and breed; and transport factors – such as distance from farm to slaughter and whether the cow had been kept overnight at the premises or slaughtered on arrival. Questionnaires were designed to be filled in for all cases and all controls.

In addition all cases and control 1 and 3 cows were blood sampled and tested for beta-hydroxy butyrate, non-esterified fatty acid, magnesium, phosphate (P), and calcium (Ca) as well as creatinine kinase (CK) activity.

Logistic regression was used to identify the effect of parameters on the odds of being a case or a control. For the biochemistry data, the results were categorised into low, normal or high based on reference ranges, and the data used in a multivariable logistic regression with backwards elimination. For the other data, such as age category, BCS, and distance travelled a univariable logistic regression was used. In all cases two models were created – one for case vs control 1 and one with case vs control 2+3.

**Results:** Compared to control 1 cows, case cows had significantly greater odds of having increased CK activity (Odds ratio (OR) = 4.3), and decreased Ca and P concentrations (OR 5.4 and 5.1, respectively). The same biochemical changes were seen when case cows were compared to control 3 cows with similar odds ratios. No evidence was found of an association between hypomagnesaemia and recumbency or ketosis and recumbency

The only univariable logistic regression which found a significant effect was that which evaluated distance travelled. The odds of recumbency increased by 1.267 times for every 100 km increase in distance travelled. This meant that the odds of a cow being recumbent rather than ambulatory when it had travelled the longest distance seen in this study (825 km) were >10 times that of the cow that travelled the shortest distance (1.5 km).

**Conclusions:** This study has found that the principal problem causing recumbency in this population of dairy cattle was hypocalcaemia, as although CK was the most common biochemical abnormality (recorded in 68/71 cases), all but 12 of those also had decreased serum Ca concentrations. Furthermore of the 39 cases with decreased P concentrations (<1.1 mmol/L), only 5 had normal Ca concentrations. This finding is consistent with other reports of transport recumbency, and consideration should be given to improving Ca status of cull dairy cattle before slaughter. However, reducing the distance travelled by cull cows is likely to be significantly more effective at reducing the proportion of recumbent cows at slaughter, because it is easier to ensure that the recommendations are being adhered to.

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WE-12

### The risk of iatrogenic sciatic nerve damage in dairy cattle when injecting into the dorsal gluteal region

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**Objectives:** Despite damage to the sciatic nerve in dairy cattle being highlighted in many clinical textbooks there is little evidence on either the incidence or aetiology. Whilst the neck is the recommended site for intramuscular injection, the dorsal gluteal region is commonly used by veterinarians and farmers. With a reduction in gluteal muscle mass in certain dairy breeds and the use of large injection volumes it was hypothesized that iatrogenic nerve damage resulting from intramuscular injection in dairy breeds could be a significant cause of sciatic nerve damage and subsequent pain. This study aimed to describe, in detail, the position of the sciatic nerve in dairy cattle and to evaluate the risk of damaging the sciatic nerve when injecting commonly used products into the dorsal gluteal region.

**Materials and methods:** The sciatic nerve position was measured and described in detail in four adult dairy cattle cadavers. Fifty-four participants (veterinarians, veterinary students and farmers) injected into the dorsal gluteal region of these cadavers and their injection sites were recorded. Measurements of injection site location and sciatic nerve location were combined to indicate needle position in relation to the nerve. Intra-participant variability was assessed to further evaluate variation of needle placement by the same veterinarian.

**Results:** Seventy per cent of participants confirmed that they use the dorsal gluteal region as their most common site for intramuscular injection. In addition, 69% of injections were located in close proximity (less than 5 cm) to the sciatic nerve. The sciatic nerve is therefore considered to be at considerable risk of iatrogenic damage when injecting in the dorsal gluteal region. The average intra-participant variability was measured at approximately 32 cm<sup>2</sup>, representing the surface area in which 10 injections on the same site would occur, given by the same person.

**Conclusions:** This presentation will visualize and describe the shape and location of the sciatic nerve in dairy cattle and will demonstrate injection sites used by study participants and their distance from the sciatic nerve. Based on these results a safer position for gluteal intramuscular injection is proposed.

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WE-13

### Long-term effects of disbudding – the relation of horn status and HPA responsiveness as measured by repeated ACTH challenges in fattening bulls

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**Objectives:** According to recent studies, 75% of the European farmers that dehorn their cattle use the method of disbudding in young calves. Disbudding itself has been identified as a painful stressor that activates the hypothalamo-pituitary-adrenal (HPA) axis on a short term, but no study yet investigated



possible HPA function-related long-term effects of disbudding. In cattle, enduring exposure to stressors can increase or lower the responsiveness of the adrenals to adrenocorticotropic hormone (ACTH). One approach to evaluate this responsiveness is the stimulation of the adrenal glands by injection of synthetic ACTH in order to quantify the release of cortisol from the adrenal cortex, namely an ACTH challenge. The study aimed at investigating the possible long-term effects of disbudding by repeated ACTH challenges in fattening bulls, by comparing bulls with horns (H) to disbudded bulls (D) a) kept in groups composed exclusively of bulls with horns or exclusively of disbudded bulls and b) kept in mixed groups (M, composed of 50% H, 50% D).

**Materials and methods:** The study was conducted on 77 fattening bulls raised under similar conditions from autumn 2015 to autumn 2016 at Agroscope Posieux, Switzerland. The first ACTH challenge (C1) was performed at the age of six weeks. Saliva was sampled at times -60, -30, +30, +60, +90, +120 and +150 minutes before and after the intravenous injection of 0.5 IU/kg metabolic body weight (BW) ACTH. Salivary cortisol concentration was analyzed by a direct ELISA. Cortisol concentrations were described by the baseline (BL) values, the peak values and the area over baseline (AOB; total increase of cortisol after injection corrected for the second BL). After C1, the animals were allocated into three treatment groups, balanced for the AOB at C1: exclusively with horns (H; n=26), exclusively disbudded (D; n=27) and mixed (50% H, 50% D; n=24). Disbudding of the concerned bulls was performed at the age of seven weeks under anesthesia and locally and systemically administered analgesia. The second ACTH challenge (C2) was performed at the age of 11 months by using the same sampling schedule and an ACTH dose of 1 IU/kg metabolic BW. Linear mixed effects model were used for statistical analysis.

**Results:** At each challenge, the salivary cortisol concentration significantly raised after ACTH injection ( $P < 0.001$ ) and remained at +150 min still higher than the BL ( $P < 0.001$ ). At C1, no significant difference in AOB was revealed between treatments ( $P = 0.96$ ). The AOB increased between C1 and C2 (+7.6 ng/mL;  $P < 0.001$ ). At C2, bulls of treatment H had a markedly higher AOB (+9.9 ng/mL;  $P < 0.01$ ) and peak concentration (+ 2.9 ng/mL;  $P < 0.01$ ) than bulls of treatment D. Within the M treatment at C2, disbudded bulls (D) had higher AOBs than bulls with horns (H; +4.1 ng/mL,  $P > 0.1$ ). The BL cortisol concentrations before ACTH injection did not differ between treatments neither at C1 nor at C2.

**Conclusions:** The study confirms the feasibility of accessing saliva cortisol during an ACTH challenge with bulls. The increase in AOB between C1 and C2 can be explained by the previously reported dose-response effect of injected ACTH on cortisol secretion. Further, as the results indicate that the adrenal responsiveness differed by horn status, the horn status should be taken into account when performing ACTH challenges in cattle. However, as the marked difference in AOB at C2 between the bulls of treatment H and D is difficult to interpret, further investigation (i.e. hair cortisol concentration as further indicator of long-term adrenal cortex activity as well as analysis of social behavior) is needed in order to interpret the results.

**Comments:** The authors are grateful for the financial support of the foundation Sur-La-Croix, Switzerland.

WE-14

## An epidemiological survey of the health and welfare of cows in shelters (gaushalas) in India

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Gaushalas (cow shelters) are traditional retirement homes for sheltering old, infertile, diseased or abandoned cows until they die from natural causes. These Indian institutions exist because the Hindu majority revere the cow as a holy animal, and therefore cow slaughter is illegal in most Indian states. Local individuals, communities, and governments subsequently create shelters for these cows, whereas other regions would typically slaughter them.

The goal of the present study was to develop a tool to assess the welfare of cows in gaushalas based on animal and resource measures and information provided by the gaushala managers.

The present cross-sectional study involved field visits to 54 gaushalas in 6 Indian states that represent the predominant gaushala populated states of India: Rajasthan, Gujarat, Maharashtra, Punjab, Haryana and Himachal Pradesh. A total of 1620 cows were clinically examined individually for 38 health and welfare parameters. 31 resource-based parameters were recorded in each gaushala. Interviews with the managers of each of the 54 facilities took place, and a questionnaire documenting their knowledge, attitudes, resources available and the practices followed in the gaushala was completed.

Most cows were non-lactating (87.96%), which reflected the non-commercial purpose of gaushalas. Even though 63% of the gaushalas had lactating cows, they sold milk to generate resources for the cow's care. The mean age of the cows was 10.9 years, which confirmed that gaushalas sheltered aged cows. The mean number of cows per gaushala was 578, and the reported mean mortality rate was 19.8%. 96% of gaushalas vaccinated cows against foot and mouth disease (FMD), haemorrhagic septicaemia and black quarter disease. Most gaushala vaccination (80%) was carried out twice a year. Screening for tuberculosis or brucellosis did not take place for the majority of the cows (98%). The administration of ectoparasiticide and endoparasiticide drugs to cows occurred on a routine basis (89 and 93%, respectively). In 72% of gaushalas, veterinarians visited in emergencies while 22.22% had in-house veterinarians to treat the cows. Most gaushalas disposed of carcasses by deep burial within the gaushala premises (54%) or through municipality contractors (41%). A few (6%) disposed of the carcasses in open areas outside of the gaushalas. The introduction of new animals involved biosecurity measures in 46% of gaushalas, and 70% provided isolation rooms for diseased cows. Disease outbreaks were reported in 41% of the gaushalas in the last five years, primarily of FMD. The disposal of dung as organic manure on nearby farms, or for fertilising the gaushala's pastures occurred in 72% of cases, and only 13% utilised it for biogas production. Mean cow body condition score (a 1 - 5 scale from thin to fat) was acceptable at 2.7, where most places fed wheat straw, agricultural waste and greens, and 86% fed supplementary concentrates. Most gaushalas (59%) did not provide access to pastures, and 13% had no access to yards.



The study found concerning public health and disease surveillance issues in the gaushalas. These are potential risks to the personnel working in, and to the general public residing near the gaushalas. The welfare assessment protocol developed through this study aims to address welfare issues in gaushalas and provide feedback to the stakeholders to assist them in improving their facilities and management practices. This protocol will also inform the development of guidelines for welfare assessment and improve the husbandry of cows in gaushalas.

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WE-15

## Changes in mortality rates in dairy cattle farms after switching from a milking parlor to automatic milking

### Milking robot and mortality rates

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The aim of the study was to determine the effect of switching from a milking parlor to automatic milking on the mortality rates of lactating cows. For this purpose 23 farms were selected from the region of Galicia (NW Spain). In these farms, during the study period, no change was made in the facilities except the modification of the milking system.

For the study, culling records were provided by the Dairy Herd Improvement Program of the region. The reasons for losses were coded as follows:

(1) Death/urgent slaughter: discarded because the animal was found lying down or dead or animal sent to emergency slaughter (accident or severe illness), (2) Lack of productivity: animals are discarded because of low production, (3) Mastitis: animals are discarded because of udder problems (mastitis, loss of quarters of the udder, sagging udder), (4) Infertility: animals are discarded because of reproductive problems (such as abortions, metritis, infertility, sterility, mummified fetuses), (5) Others: animals are discarded either for some reasons which are not included in the classification above or for multiple causes and (6) Lameness: animals are discarded because of musculo-skeletal problems (such as lameness, hoof infection).

Cox models for survival analysis were fitted to estimate the risk of culling (overall and for a variety of reasons, including death/urgent slaughter, unproductivity, mastitis, infertility and lameness) in the two years prior to switching from a milking parlor to automatic milking systems (period 1) with respect to the year 1 (period 2) and the years 2 and 3 (period 2) after the change. Besides, parity number was included as control variable. Robust standard errors were calculated using the robust variance estimator method to control within-herd cluster effects.

Thereby, seven models were fitted to assess the risk of culling of lactating cows: one considering all losses and one for each of the six causes reflected.

The results indicated that the mortality rates were 1.311, 1.981 and 1.903 losses per 1000-animals and day at risk during the periods 1, 2 and 3, respectively.

Cox regression indicated that the overall risk of culling was

1.523 ( $p < 0.001$ ) and 1.594 ( $p < 0.001$ ) times higher in the periods 2 and 3, respectively, than in the period 1, after controlling for parity and herd cluster effects. However, the risk of death/urgent slaughter was 0.792 ( $p = 0.032$ ) and 0.835 ( $p = 0.045$ ) times lower when comparing periods 2 and 3 with period 1. Being culled by unproductivity was 2.150 ( $p < 0.001$ ) and 2.621 ( $p < 0.001$ ) times more likely during the periods 2 and 3 than during the period 1, whereas the risk of being culled by mastitis was 2.525 ( $p < 0.001$ ) and 2.939 ( $p < 0.001$ ) times higher in the periods 2 and 3 than in the period 1. The change in the milking system also increased the risk of culling due to infertility by 2.522 ( $p < 0.001$ ) and 2.316 ( $p < 0.001$ ) times (again, comparing periods 2 and 3 with period 1). As regards other causes of culling, the risk of loss by this reason increased 1,464 ( $p < 0.001$ ) times in period 2 in comparison to period 1; there were no significant differences between periods 3 and 1. Finally, the risk of culling due to lameness increased by 1,653 ( $p < 0.012$ ) and 2.281 ( $p < 0.001$ ) times during the periods 2 and 3, respectively, considering the period 1 as a reference. The higher risk of culling after switching from a milking parlor to automatic milking could be related to the fact that automatic systems limit the number of cows per unit (robot) in order to maximize the number of milkings per cow and its productivity. This could change the culling decisions in the farm with respect to the conventional milking systems, in which some cows with fewer production, higher somatic cell counts or worse reproductive performance would remain more time in the herd. In addition, lame cows in conventional systems could be forced to milking, whereas in automatic systems this condition implies fewer or no visits to the robot, decreasing productivity. For the particular case of death/urgent slaughter, these reasons are often related to post-partum diseases such as acute metritis, abomasal displacement, acidosis or ketosis. The different schedule of concentrate distribution in automatic systems (several times a day) could contribute reducing the risk of post-partum metabolic diseases. The increase of losses due to other causes in period 2 could be related to cows that did not adapt to the automatic system.

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WE-16

## Locomotion characteristics of dairy cows walking on pasture and the effect of artificial flooring systems on locomotion comfort

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This study examined the characteristics of locomotion behavior on pasture (gold standard with optimal locomotion comfort) and compared it with those of cows walking on mastic asphalt and solid rubber mats. Our hypothesis was that gait variables were significantly different between pasture versus mastic asphalt and of mastic asphalt versus rubber.

**Materials and Methods:** The study protocol was approved by the animal experimentation committee of the canton of Bern, Switzerland (permission # 25162). Twenty four dairy cows kept in a tie-stall facility, allowed daily access to pasture during the



grazing season (April to October) and weekly access to an outside pen during the winter feeding season (November to March) were enrolled in this experimental trial. Three flooring types were tested in the same artificial passageway (1.5 m wide x 15 m long): mastic asphalt, solid rubber (Type Kura G<sup>®</sup>; called now Kura Flex<sup>®</sup>, Gummiwerke Kraiburg, DeLaval) and pasture (gold standard). The pedogram was measured using two stand-alone 3D accelerometers (400 Hz), which were fitted at the level of the metatarsus to both hind limbs. The extracted pedogram variables included temporal events (kinematic outcome = gait cycle, stance phase and swing phase duration) and peaks (kinetic outcome = foot load, toe-off). The cows were further video-recorded to calculate walking speed and stride length. Locomotion score (LS) according to Flower and Weary (2006) (3) was performed on asphalt floor to enroll only non-lame cows (LS <3). For comparison between different floor types, repeated measures analysis of variance was performed with cow as a subjective variable, session time of measurement as within factor variable and flooring type as a fixed effect.

**Results:** The results of this study showed that the rubber flooring - as compared to mastic asphalt - does not reveal a significant improvement of the evaluated variables of locomotion comfort that are evident in dairy cows on pasture

**Conclusions:** Using cow pedogram analyses and video-recordings allows to differentiate between floors with minor and such with good locomotion comfort. The locomotion comfort variables detected in cows walking on pasture were significantly different from those of cows walking on mastic asphalt or solid rubber. This suggests that regular pasturing is an important management procedure to improve locomotion comfort of dairy cows.

quarter and one over the central ligament in the area of the rear quarters) were taken by either one of three trained veterinarians. Thereafter, these scans (n=1'035) were blinded and scored to evaluate the newly proposed objective SS of udder edema. The following definitions were used: Grade 0 = normal (no signs of edema present); grade 1 = slight edema: Front quarter (2-4 distinct edema lines and/or less than 1cm deep, as measured from the probe-skin interface) and rear quarter (2-5 distinct edema lines and/or less than 2cm deep); grade 2 = moderate edema: Front quarter (5-8 distinct edema lines and/or 1 to 2cm deep) and rear quarter (6-8 distinct edema lines and/or 2 to 3cm deep); grade 3 = severe edema: Front quarter (more than 8 edema lines and/or more than 2cm deep) and rear quarter (more than 8 distinct edema lines and/or more than 3cm deep). Inter- and Intraobserver agreements were determined by the adjusted kappa test.

**Results:** Inter-observer agreement for objective ultrasound scoring among three trained veterinarians was estimated as "almost perfect" ( $\kappa=0.81$ ). Intra-observer agreement was very high ( $\kappa=0.90$  and  $\kappa=0.85$  for two different observers, respectively).

**Conclusions:** The newly developed SS allows the description of udder edema appearance in dairy cattle at cow shows, as an indirect measure of udder fill. The high inter- and intraobserver agreement suggests that the proposed SS might be a very useful tool in the future for objective identification of cows at dairy cow shows with "over-bagged" udders as a consequence of PMI.

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WE-17

## Development and validation of a sonographic edema scoring system for show cows

**Key words:** udder, edema, scoring system, ultrasound, dairy show

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**Objectives:** "Over-bagged" udders are commonly seen at dairy cow shows all over the world. "Over-bagging" has an important negative impact on cow's well-being. Currently, udder filling is determined visually and rating is, therefore, very subjective. Edema occurs at defined udder locations in the course of prolonged milking intervals (PMI). The objectives of this study were to develop and validate a sono score (SS) of udder edema, occurring in show cows after PMI.

**Materials and Methods:** 319 cows at 4 highly competitive Swiss dairy cow shows between September 2016 and April 2017 were examined. Ultrasound scans (MyLabOne with a rectal probe of 10MHz) at defined positions (one at each front



BU-01

### Stress responses of alpacas using different methods of restraint and shearing

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**Objectives:** In contrast to sheep there is little information available on stress response associated with shearing in alpacas. Different methods of restraint and shearing may result in different amount of stress responses and may have different impact on animal behaviour and welfare. Various clinical, laboratory and behavioural parameters can be used to assess the impact of the manipulations during shearing. The aims of the study were to assess and compare different methods of restraint and shearing of alpacas according to the stress response of the animals and to develop recommendation based on the results.

**Material and methods:** The study consisted of two parts to allow separate assessment of restraint alone in contrast to restraint and shearing. Animals were restrained but not shorn in part 1 of the study; in part 2 animals were restrained and shorn. The restraint of the animals was performed in three different ways: 1. In standing position, 2. On a mattress on the ground using ropes on front and hind limbs, and 3. On a shearing table using ropes on front and hind limbs.

Heart rate, respiratory rate and body temperature were measured. Saliva for cortisol analysis was obtained before and at 20, 40 and 60 minutes. Faeces for faecal cortisol metabolite analysis were obtained at the start of the manipulation and 33 hours later.

Part 1: The 3 restraint methods were applied on 15 alpacas. Every animal was restrained using all 3 methods in a random order applying a Latin square design.

Part 2: Each restraint method was also applied in 15 animals following a Latin square design. Since the animals were restrained and shorn 45 animals in total were used. Duration of shearing and injuries were recorded.

**Results:** All 15 animals of part 1 could be restrained without any problems or injuries. In part 2, one alpaca which was supposed to be shorn in standing position could not be restrained for shearing without putting the animal and personnel in danger. The duration of shearing using different methods was not significantly different. The number of superficial injuries did also not differ between the groups (standing 7 of 14; mattress 3 of 15; shearing table 4 of 15 animals).

Part 1: Animals which have been restrained in standing position showed non-significant fluctuations of heart rate, respiratory rate and body temperature. With the exceptions of heart rate in animals restrained on the tilt table (t5 and t10 min) and the respiratory rate at t20 min in animals restrained on the on the ground restraint did not result in significant changes over time. There were no differences at any time point comparing the restraint methods. Animals in all groups showed significant increases of salivary cortisol concentrations 20, 40 and 60 minutes after start of the restrain without differences between the groups. The concentration of faecal cortisol metabolites were

significantly increased after 33 h which was also irrespective of the restraint method.

Part 2: Significant differences were found in animals restrained on the ground (respiratory rate) and on the shearing table (heart and respiratory rate). No differences were found at any time point comparing the shearing methods. The saliva cortisol concentrations tended to increase in all animals irrespective of method. However, cortisol concentrations varied very extensively between individual animals. The increase over time is significant only in animals, which had been shorn on the ground. Comparing the methods no differences could be detected. Concentrations of FCM were increased in all groups 33 h after shearing but also varied in wide ranges and did also not differ between the groups.

**Conclusion:** The main results of the study are that all restraint and shearing methods provoked a stress response with very high variability between the animals. If the animals were restrained in the standing position they tolerated this better than either recumbency on the ground or on the shearing table. In conclusion the standing position is the first choice for animals which appear to be tolerating the gathering, restraint and shearing well. However, in animals that cannot be restrained adequately in standing position the risk of injuries for the animal (and potentially for the assisting persons and the shearer) increases so they should preferably be shorn restrained either on a mattress on the ground or on a tilt table.

BU-02

### *Mycobacterium avium* spp. *paratuberculosis* in new world camelids in Austria

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**Objectives:** *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD) or paratuberculosis. The primary hosts are domestic ruminants but MAP can also be found in non-domestic ruminants. MAP has been diagnosed in Austria in cattle, a sero-prevalence of 19.05% MAP-positive cattle herds was determined (Baumgartner et al., 2005) and in a recent survey 7.6% of cattle farms were detected MAP-positive in the Austrian province of Tyrol (Khol et al., 2015). Additional to MAP infections in ruminants, there is a small number of individual case reports of JD in llamas and alpacas. These animals often not only have close contact to their owners, but in a substantial number of herds in Austria, the camelids are kept together with ruminants or they have indirect contact by sharing the pasture. Therefore, the aim of the present study was to evaluate the prevalence of MAP in new world camelids (NWC) in Austria. It was aimed to assess the risk for spreading of MAP within the NWC population but also between NWC and domestic or wild ruminants and to evaluate the possible role of NWC as a host for MAP.

**Material and methods:** A convenience sample of 445 animals originating from 78 farms was enrolled in the study. The sample size represents approximately 10% of the Austrian NWC population and the sampling was performed in all regions of Austria.



Of the sampled animals 184 (41.35%) were llamas and 261 (58.65%) alpacas, respectively. 443 blood samples for MAP-ELISA and 399 faecal samples for qPCR and culture for MAP have been collected.

The ELISA for the detection of specific Ab against MAP was performed using the ID Screen® *Mycobacterium avium* Indirect ELISA (IDvet, Montpellier, France). This multi-species ELISA is designed for the detection of anti-*Mycobacterium avium* Ab in swine and ruminants. The same antigen as in the ID Screen® Paratuberculosis for the detection of MAP in cattle is used in this kit but a different conjugate showing a high binding affinity to camelids is included. The specificity reported by the company of the test is 100% with an estimated sensitivity of 50% to 60%. Bacteriological culture of MAP was carried out according to Whipple et al. (1999) and modified by Pavlik et al. (2000) using Herrold's egg yolk medium for a total time of 3 months. The cultures were evaluated for bacterial growth weekly, positive results were confirmed with PCR. Fecal samples were tested for the presence of MAP by qPCR detecting the IS900 and F57 genes according to Slana et al. (2008).

**Results:** All of the 399 animals tested for shedding of MAP were negative by faecal culture. Using qPCR 15 (3.8%) of the animals were MAP-positive and 384 (96.2%) negative, both in the IS900 and F57 protocol. Eleven of the qPCR-positive animals were llamas and four alpacas, respectively, leading to a MAP-prevalence of 7.2% in llamas and 1.6% in alpacas. Out of the 443 serum samples examined for specific Ab against MAP by ELISA, 6 (1.4%) were positive, 1 (0.2%) was questionable and 436 (98.4%) samples were negative. Two of the positive animals were llamas, and four alpacas, therefore 1.1% of the examined llamas and 1.5% of the alpacas were MAP-seropositive.

Of the 22 qPCR or ELISA positive animals 4 were 2 years old and 13 had an age of 5 years or older, indicating an increase of MAP-shedding and the production of specific Ab with age. Six of the positive animals were imported, but the comparison to the percentage of imported animals in the sample population did not indicate differences. Four of the MAP-positive animals were housed in close contact to sheep, goat or cattle, leading to the risk of interspecies transmission of the bacterium. The low number did not allow a risk assessment.

**Conclusions:** From the results of the presented study it can be concluded that MAP is present within the population of NWC in Austria to certain extent and might pose a possible risk for infection of other domestic and wild ruminants. Therefore testing of NWC for MAP should be considered in case of clinical symptoms of JD and in the course of control programs in ruminant livestock.

BU-03

## Pathological investigation of trachea and lungs of buffaloes in Gazipur district of Bangladesh

### Respiratory diseases of buffaloes

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**Objectives:** The buffalo is commonly known as an "Asian Animal", has attracted global concern. The buffalo is the dairy, draught and meat animal of Asia. In Bangladesh buffalo has been addressed and remained neglected species despite their important role in the national economy. The disease of the respiratory system, either acute or chronic, causes debility and death leading to great economic loss. This loss can be minimized by prevention and treatment of the diseases of respiratory system after proper diagnosis. In Bangladesh, few works have been performed on some aspects of buffalo diseases but no systemic works have been performed on buffalo respiratory system in Gazipur district. Therefore, the present investigation was undertaken to study the pathological lesions of trachea and lungs of buffaloes after slaughter.

**Materials and methods:** A total of 160 (trachea = 80, lungs = 80) samples were collected from buffaloes after slaughter from two slaughterhouses of Gazipur City Corporation, Gazipur, Bangladesh, during the period from July 2016 to March 2017. After slaughter, gross tissue changes were observed and recorded carefully by observation in naked eye. Besides, the samples were collected in 10% buffered neutral formalin for histopathology. All the samples were transferred to Histopathology Laboratory of the Department of Pathobiology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur for histopathology. The formalin-fixed tissues were trimmed, processed, sectioned and stained following standard procedure and photomicrography was taken with compound light microscope.

**Results:** A total of 80 respiratory tract (trachea and lung) buffaloes were examined and of them 09 trachea (out of 80) and 30 lungs (out of 80) were found to be apparently abnormal in naked eye. The occurrence of tracheal petechial haemorrhages was recorded 11.25%. The gross lesions of lungs were 37.5% in buffaloes. Grossly, five types of lung lesions were recorded such as haemorrhages and congestion in lungs (16.25%), hard nodule formation (7.5%), and cyst in lungs (6.25%), emphysematous lungs (5%) and thickened pleura (2.5%).

Histopathologically, different types of pneumonic lesions and some other lesions were found in the lungs. For morphological descriptions, the lesions were categorized into various types; however a single section showed intermixed of various lesions. The histopathological lesions of lungs were recorded 53.75%. The histopathological lesions or conditions were chronic interstitial pneumonia (3.75%), edema fluid (3.75%), broncho-pneumonia (3.75%), bronchitis (2.5%), bronchiolitis (2.5%), pneumonia-congested stage (8.75%), hydatid cyst (6.25%), fibrinous pneumonia (3.75%), healed nodule (7.5%), purulent broncho-pneumonia (1.25%), fibrino-purulent pneumonia (1.25%), subacute fibrinous pneumonia (1.25%), pulmonary adenomatosis (1.25%), pleuritis (2.5%) and hemosiderosis (3.75%).

**Conclusions:** The overall gross and histopathological lesions of lung were 37.5% and 53.75%, respectively. Though the buffalo is an important part of livestock in Bangladesh, there are no documented research studies so far. This investigation will create the scopes and opportunities of buffalo production in Bangladesh. The findings of this investigation will help the veterinarians to know the nature and type of common pathological lesions of respiratory system of buffaloes.





BU-04

### Clinical Investigation on Impact of Dry Therapy on Bovine Mastitis, Milk Somatic Cell Count and Milk Yield in Buffaloes

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Dry cow therapy, i.e. infusing quarters of cows with long acting antimicrobial at the end of lactation, has been widely studied and followed as a standard practice for the control of mastitis in dairy cows. But, in buffalo, that seems to possess some different teat anatomy and longer dry period, role of dry therapy in mastitis control needs to be ascertained. The present study was undertaken to evaluate the effect of blanket dry therapy on intramammary infection, milk somatic cell count, clinical mastitis, milk yield in buffaloes (*Bubalus bubalis*) at the university dairy farm. As a farm practice, the various buffaloes at dry off, irrespective of quarter health, were infused in all the quarters with "Cephrin dry cow" (cephalonium dihydrate 250 mg, MSD Animal Health). A day before dry therapy was infused; the quarter foremilk/ bucket milk samples were collected and analyzed for bacteriology (Microbial procedures of National Mastitis Council, USA) and somatic cell count (Somatic cell counter, Delta Instruments, The Netherlands). The animals were followed through dry period and until 03 months post-calving for occurrence of clinical mastitis if any. In this way, 69 animals (48 multiparous buffaloes infused with dry therapy and 21 heifers) that calved during the year were available for measurement of new intramammary infections and clinical mastitis. The post-calving quarter infection and milk SCC levels were assessed at 3-5 d of calving. The effect of dry therapy on clinical mastitis, and milk yield was judged by comparing the current lactation data with that of previous lactation when animals received no dry therapy. The effect of lactation on milk yield was balanced by estimating and applying the least square constants for different parities from previous herd records. The dry therapy could eliminate 26/31 (83.87%) existing quarter infections comprising of *Staphylococcus aureus* (7/9, 77.78%), coagulase negative staphylococci (17/20, 85.0%) and corynebacteria (2/2, 100%). The establishment of new intramammary infections in the eligible quarters (free from infection at dry off) constituted 10/161 (6.21%), all coagulase negative staphylococci, in dry treated quarters compared to 8/84 (9.52%), 02 *S. aureus* and 06 coagulase negative staphylococci, in heifers ( $p = 0.43$ , Fisher's exact test). The occurrence of clinical mastitis during the dry period and up to 45 days post calving was significantly reduced in dry treated buffaloes. No clinical mastitis case was reported in dry treated buffaloes up to 30 d post-calving as compare to 03 (08 quarters) in 67 buffaloes where they received no dry therapy in the previous lactation. One case (01 quarter) of mastitis occurred in the treatment group as compared to 07 (15 quarters) in the untreated control group up to 45 d post calving; a significant difference ( $p = 0.03$ , Fisher's exact test). The corresponding figures up to 03 months post calving were 03 (04 quarters) in dry treated buffaloes and 08 (17 quarters) in untreated control ( $p = 0.10$ ). The average bucket milk SCC in dry treated animals decreased significantly ( $p = 0.020$ ) from  $615 \times 10^3$  cells/ml at dry off to  $359 \times 10^3$  cells/ml at calving. The measure of first 30 days post-calving milk yield showed that the average milk yield of buffaloes enhanced from 283 Kg in the pre-

vious lactation to 327 Kg in the current lactation, following dry therapy, indicating more than 15% improvement in milk production ( $p = 0.002$ ; pair t-test). Thus, dry therapy in buffaloes appeared an effective tool for the prevention of mastitis, lowering milk cell count and improving milk production.

BU-05

### Factors Affecting the Efficacy of Timed Artificial Insemination Protocols in Nepalese Buffaloes

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**Objectives:** The study was designed to understand some factors affecting the efficacy of timed artificial insemination protocols in Nepalese buffaloes.

**Materials and Methods:** A total of 99 buffaloes were used in the study. Ovsynch protocol was applied during good breeding season ( $n=43$ ) and CIDR co-synch protocol was applied during transition ( $n=14$ ) and low ( $n=42$ ) seasons. Body condition score (BCS) was recorded prior to applying the protocols. Moreover 42 buffaloes, 14 in each season, were used for blood sampling to analyze nutritional parameters (glucose, total protein, cholesterol) and stress factor (cortisol). Transrectal ultrasonography was used to confirm ovulation and pregnancy.

**Results:** Ovsynch protocol during good season produced 51.1% pregnancy while CIDR co-synch protocol produced 50.0% and 42.9% pregnancy during transition and low season, respectively. Body condition score showed a significant ( $P<0.01$ ) effect on the pregnancy rate after treatment. Blood cholesterol and cortisol levels were different ( $p<0.05$ ) among three seasons. While comparing the blood nutritional parameters with pregnant buffaloes, levels of glucose and total protein were lower ( $p<0.05$ ) and cholesterol level tended to be lower ( $p=0.07$ ) in non-pregnant group.

**Conclusion:** In conclusion, CIDR protocol during transition and low breeding season is as effective as ovsynch protocol during good season in Nepalese buffaloes. However, the outcome depends largely on nutritional and stress factors.

**Key words:** Breeding season, Buffalo, Hormonal protocols, BCS, Pregnancy

BU-06

### Evaluation of cross reaction between alpha herpesvirus types 1 and 5 in buffalos herds at São Paulo state, Brazil

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meat rubbed with smoke flour was better than of Bali beef.

**Keywords:** Aging, Bali Beef, Buffalo, Ruminant, Smoke flour

The state of São Paulo includes the third largest population of buffaloes in Brazil and stands out for the quality of the genetic material; the region of Vale do Ribeira is considered to be the pole of dairy buffalo herds. Despite the economic importance, there is a lack of data on the health status of buffaloes herds, mainly for the epidemiology of viruses causing economic losses. The present work conducted a retrospective study on the  $\alpha$ -Herpesvirus bovine types 1 and 5 (BoHV-1 and BoHV-5) which are responsible of encephalitis and abortion in cattle and whose circulation is endemic in Brazil. 692 of buffaloes serum samples from the Murrah and Mediterranean breeds in region of Vale do Ribeira, São Paulo, Brazil were collected in the period from 2012 and 2013. Cross serum neutralization assay (VNT) against BoHV-1 and BoHV-5 was performed according Manual of Diagnostic Tests and Vaccines for Terrestrial Animals/OIE. Concentration of 100TCID<sub>50/50</sub> mL was used for each virus. After 24 hours of virus-serum incubation, MDBK cells at  $3 \times 10^5$  cells/mL were added and the samples were keep at 37°C with 5%CO<sub>2</sub> for 72 hours. The animals were considered reactors with a titers <sup>3</sup> log 0.3. Overall, the prevalence of sera reacted against BoHV-5 was 82% (568/692) while for BoHV-1 was 80.4% (556/692). The antibody titles varied from log 0.3 to 3.0, has being observed highest titration for BoHV-5. A cross-reactivity between BoHV-1 and BoHV-5 can be noted in the animals analyzed by virusneutralization assay and development of novel diagnostic tests are necessary to distinguish  $\alpha$ Herpesvirus species.

BU-07

### **Meat quality of buffalo meat and Bali beef rubbed with smoke flour and aged at different time**

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This research aims to look at the comparison of the quality of meat from buffalo meat and Bali beef after rubbed with smoke flour and aged at different times. This research uses the muscle *Longissimus dorsal* (LD) of three buffaloes and three cows. Before the aging, muscle LD of both types of these ruminants rubbed with smoke flour 2% of the weight of the sample (w/w). This study used a complete randomized design of factorial pattern 2x3x4 where the first factor is the type of ruminants (buffaloes and cows), the second types of smoke flour (oven, freeze dry, spray dry), and the third aging time factors (0, 7, 14 and 21 days). The observed parameter was water holding capacity (WHC), shear force value of cooked meat (CMSF), the rate of fat oxidation (TBA), and cooking loss (CL). The results showed that the Buffalo meat resulted in the lower WHC (26.59%), TBA (0.43 mg MDA/kg), CMSF (1.58 kg/cm<sup>2</sup>), and CL (17.04%). Type of smoke-dried flour gave the same result for all parameter. The spray dried smoke flour tended to lower of all parameter than of two other of type smoke flour. Aging time decreased WHC (26.57% at 21 days of aging), while TBA, CMSF, and CL were just the same. It could be concluded that quality of buffalo



BV-01

## Progress toward eradication of BVDV in Ireland

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**Objectives:** A national BVD eradication programme began in Ireland in 2013 with the goal of achieving eradication by the end of 2020. An update on progress to date, key challenges encountered and factors for success are presented.

**Materials and methods:** Following a voluntary programme in 2012, an industry-led national programme, supported by legislation, began on 1<sup>st</sup> January 2013. All calves born after this date were required to have a tissue sample collected using an approved tissue tag or supplementary tag. The programme is led by Animal Health Ireland ([www.animalhealthireland.ie](http://www.animalhealthireland.ie)), with technical input from a working group and programme decisions taken by a cross-industry BVD Implementation Group. Tissue samples are submitted to a network of designated private laboratories where they may be tested by antigen capture ELISA or RTPCR. The national reference laboratory provides a range of supports to the programme, including confirmatory testing of calves and testing of their dams. Results are transmitted to a programme database provided by the Irish Cattle Breeding Federation ([www.icbf.com](http://www.icbf.com)) which relays them to individual herd owners and to the database of the Department of Agriculture, Food and the Marine (DAFM). All calves born since the start of the programme must have a recorded negative to move. The database also assigns indirect statuses to dams, and herd-level statuses, based on results received.

**Results:** The annual prevalence of calves born persistently infected (PI) with BVDV has decreased in successive years, falling from 0.66% in 2013 to 0.10% in 2017. The database now holds a negative status for over 99% of approximately 6 million animals in 83,000 breeding herds, of which 86% have been assigned negative herd status (NHS). The retention of PIs by a minority of herdowners was a key challenge despite some financial supports for early removal, particularly in the first years of the programme. A number of programme enhancements were introduced in 2016 to address this, and have resulted in a significant improvement in the rate of removal of PIs. By the end of 2017 only 70 known PIs were alive nationally, with a number of counties containing no PIs. Key factors contributing to the success of the programme were considered to include: industry support, backed by cost-benefit analysis; clear and simple legislation; an effective network of designated laboratories; an effective programme database; provision of limited financial supports for removal of PIs; development of a national BVD model and epidemiological studies to inform decision making; automated restriction of herds retaining PI animals; issuing of biosecurity notifications to the neighbours of retaining herds; funded investigation of herds with PIs.

**Conclusions:** Substantial progress toward eradication has been made, with modelling indicating that eradication by the end of 2020 is feasible. An industry-led programme, based on consensus between stakeholders, can deliver real change.

BV-02

## Analysis of Fetal bovine Serum reveals the presence of emerging Hobi-like viruses in Argentinean livestock

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Fetal bovine serum (FBS) is widely used as a growth supplement in cell culture media and as a component of biological products used in animal and human health. HoBi-like viruses are a group of emerging pestiviruses that affect cattle, buffaloes and goats. Since its first identification in Brazilian FBS samples in 2004, HoBi-like viruses have been isolated in other European and Asian countries. HoBi-like virus infection seems to be clinically indistinguishable from that caused by BVDV, which includes respiratory disease, reproductive failures and persistently infected animals in herds. Though BVDV is endemic in Argentina, HoBi-like viruses had been neither detected nor isolated locally. In this sense, we have recently reported serological evidence of this agent in water buffalo herds (Pecora et al, 2017).

The first aim of this work was to evaluate the presence of traces of HoBi-like viruses in local FBS samples. Moreover, this work sought to indirectly determine which BVDV genotype prevails in Buenos Aires region.

Semi-elaborated FBS samples (n=112) were obtained prior to the irradiation stage, belonging to a laboratory that commercializes blood-derived products obtained from slaughterhouses located in Buenos Aires province. The samples were subjected to RNA extraction, followed by a reverse transcriptase reaction. Two polymerase chain reactions (PCR) were carried out to amplify 5' pestiviral untranslated region (5'UTR): one with the 324-326 primers (Vilcek et al, 1994) and another with the N2-R5 primers, which detect BVDV or HoBi-like viruses, respectively (Bauermann et al, 2014b). In the PCR-positive samples, amplification products were direct sequenced. Then phylogenetic analyses were performed using the neighbor-joining method based on the Kimura model. Since the FBS had not been irradiated, HoBi-like virus positive samples were subjected to passages in Marbin Darbin Bovine Kidney (MDBK) cells in order to isolate the viruses.

Lastly, the HoBi-like isolates were used as immunogens in guinea pigs to evaluate their immunogenicity. Further, the sera of the vaccinated guinea pigs were subjected to cross-neutralization against BVDV-1a, BVDV-1b, BVDV-2 and a reference strain of HoBi-like virus to study the serological profile.

The PCR performed with 324-326 primers amplified 42 FBS samples which were grouped in BVDV-1a (n=6), BVDV-1b (n=34) and BVDV-2 (n=2). The PCR carried out with N2-R5 primers amplified other 4 FBS samples, which belonged to the Hobi-like virus clade. After several cellular passages, the 4 HoBi-like viruses were successfully isolated. When using these viruses as immunogens, all the vaccinated guinea pigs developed neutralizing antibodies against the HoBi-like virus reference strain, ranging from 1/128 to 1/512. Antibody titers against BVDV-1a, BVDV-1b and BVDV-2 were lower in all the cases.

In conclusion, HoBi-like virus genome was detected in 4 non-irradiated FBS, which confirms that the virus is circulating in bo-



vine herds in the Buenos Aires region. The isolated viruses evoked a specific antibody response in guinea pigs. Future work will be performed to characterize these agents.

On the other hand, the high percentage of BVDV-1b strains found in this work (81% of the BVDV positive samples) reinforces our previous findings, which reported a high frequency of BVDV genotype 1b in Argentina (Pecora et al, 2014).

These results highlight the importance to perform standardized irradiation protocols on FBS batches previous commercialization, to avoid detrimental effects due to the contamination of biotechnological products with endemic or emerging pestiviruses.

#### BV-03

### Cross-reactivity of bovine viral diarrhea viruses 1 and 2 against HoBi-like viruses in field cattle sera collected in Japan between 2012 and 2017

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HoBi-like virus, also referred to as bovine viral diarrhea virus (BVDV)-3 or atypical bovine pestivirus, is one of the emerging pestiviruses. HoBi-like viruses have been isolated from fetal bovine sera of South American origin and cattle and/or water buffaloes in South America, Southeast Asia and Europe, but their prevalence in Japan remains unknown. To investigate the HoBi-like virus infections in Japan and evaluate the immune response induced by current BVDV vaccines, we detected antigens and neutralizing antibodies for BVDV-1, BVDV-2 and HoBi-like viruses in field cattle sera collected in 2012–2017. Neutralizing antibody titers against HoBi-like viruses ranged from <2 to 4,096 in 49 samples collected in 2012–2014. On comparing the titers against HoBi-like viruses by the cross-neutralization assays using sera collected in 2015–2017 with/without BVDV vaccination, cattle immunized with modified live and/or killed vaccines containing BVDV-1 and BVDV-2 demonstrated statistically higher titers [geometric mean titers (GMTs) of 30.2–42.2] than those immunized with modified live vaccine containing BVDV-1 (GMT of 5.8). We also sought to detect HoBi-like viruses by RT-PCR and virus isolation but could not detect any. In conclusion, there is no evidence of HoBi-like viruses in Japan. Cattle infected (naturally or vaccinated) with

BVDV-1 and BVDV-2 demonstrated cross-reactivity against HoBi-like viruses, suggesting that continuous vaccination with both BVDV-1 and BVDV-2 contributes to the control of HoBi-like viruses in Japan, though it is important to prevent their introduction.

#### BV-04

### Epidemiological survey of cattle persistently infected with bovine viral diarrhea virus in Hokkaido, Japan

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In Japan, the infection of bovine viral diarrhea virus (BVDV) in cattle has been designated as a notifiable disease since 1998. In Hokkaido, the number of notification of this disease has been increasing in recent years. To control this disease effectively by vaccination and elimination, epidemiological survey of persistently infected (PI) cattle was conducted.

**Materials and methods:** The information on the clinical symptoms of PI cattle, the age and vaccination history of the dams and presumed infection was investigated for the 765 PI cattle, which were diagnosed in Hokkaido from January 2011 to December 2015. The genotype of isolated 160 BVDVs was identified by phylogenetic tree analysis based on the nucleotide sequence of the 5' untranslated region.

**Result:** These isolates were classified as genotype 1 (BVDV-1; 100 isolates) and genotype 2 (BVDV-2; 60). BVDV-1 isolates were further divided into BVDV-1a (23), 1b (66) and 1c (11) subgenotypes, and all BVDV-2 isolates were grouped into BVDV-2a subgenotype (60). In the 765 cases, 84.6% (n=647) of cattle infected with BVDVs showed no clinical signs and the others showed typical clinical signs of BVDV infections, stunted growth (7.5%, n=57), diarrhea (6.1%, n=47) and respiratory ailments (5%, n=38). In the dams which gave birth to the PI cattle, 42.7% (n=327) were 24 months old or younger. The presumed infection sites were identified for the pregnant dams, which were not moved between 30 to 150 days of gestation, the possible period of *in utero* infection with BVDV. Hereby, the presumed infection sites were identified for 533 PI cattle of 227 farms. From this analysis, it was indicated that more than 5 PI cattle were detected in the 8 farms containing 2 large scale farms, 6 public ranches and facilities for pregnant cows.

**Discussion:** It was suggested that the elimination of PI cattle before the onset of mucosal disease has been in progress in Hokkaido since asymptomatic cattle were 84.6% among diagnosed PI cattle. However, it was also clarified that a large number of pregnant cows were infected with BVDV in places such as deposit facilities, where they were gathered from a lot of farms and BVDVs were spread in a wide area; suggesting that elimination of PI cattle before their movement to these facilities is necessary. On the other hand, it was found that BVDV-1 and BVDV-2 were similarly prevalent in Hokkaido as reported previously. For the control of BVDV infection, the vaccination with BVDV bivalent vaccine corresponding to the epidemic is strongly recommended especially for the heifers to provide sufficient immunity before their breeding because dams of PI cat-



tle are mostly 24 months old or younger.

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BV-05

### Extending the interval between primary BVD vaccination from 4 weeks to 6 months, markedly enhances amnestic response.

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**Objectives:** The primary objective was to demonstrate that the amnestic response given by a primary vaccination course with an inactivated cytopathogenic type 1 bovine viral diarrhoea virus (BVDv) vaccine (Bovilis<sup>®</sup> BVD, MSD Animal Health NZ) using an inter-vaccination interval of 6 months was non-inferior to a standard four week inter-vaccination interval. A secondary objective was to assess the effect of a liquid oral vitamin (A, C, D, and E) and mineral (chromium, iodine and selenium) supplement (VetLSD<sup>®</sup> - LSD Limited) on this amnestic response.

**Materials and methods:** The study population was a single group of 90, 16 -18 week old Friesian dairy calves, born to dams vaccinated against BVDv, in the spring of 2015 on a dairy farm considered free of BVDv, in Marlborough (New Zealand). The study was designed as a parallel-group, non-inferiority field trial. Eighty calves were randomised, in age-rank, into test (6-month inter-vaccination interval) and reference (4-week inter-vaccination interval) cohorts. Within cohort, half of the calves (n = 20), were supplemented with LSD at the same time as vaccination.

All calves were vaccinated twice intramuscularly with 2ml of the vaccine; the first (V1) at enrolment for the test cohort and 5 months later for reference cohort. The second (V2) was injected into all animals four weeks later.

Freedom from natural BVDv exposure was determined by quantifying BVDv antibodies using an ELISA (enzyme-linked immunosorbent assay, IDEXX Total Ab Test) in sera collected from all calves immediately prior to V1, and a further ten sentinel calves at the end of the study.

The primary outcome was serum BVDv neutralising antibody titre at 14 days after V2. These antibodies were quantified by VNT (virus neutralisation test, using heterologous type 1c "Trangie strain" of BVDv at the OIE BVDv reference laboratory, Elizabeth MacArthur Agriculture Institute in Australia).

The reciprocal of each V2 + 14 VNT titre was log-transformed and modelled as a function of cohort adjusted for LSD status using linear regression. A non-inferiority margin of 0.63 was set *a priori* for the lower limit of the one-sided 95% test/reference geometric mean ratio (GMR) confidence interval (CI). (Note a 0.63 GMR corresponds to a difference of about two thirds of a twofold dilution in a serial dilution assay.) The effect of LSD supplementation on the amnestic response, adjusted for vaccination interval, was also modelled using linear regression. Evidence of statistical significance was made when the probability of a test statistic (P-value) was 0.05 or less.

Animal ethics approval was granted for this study.

**Results:** There was no evidence of natural BVDv exposure throughout the study.

Two weeks after V2, the geometric mean titre (GMT) [95%CI] in the test (6-month) and reference (4-week) cohorts were 147 [104 to 209] and 71 [50 to 101], respectively. The test cohort GMT was significantly (GMR 2.07 [1.26 to 3.40],  $P_{t=2.92; df=78} = 0.005$ ) higher than for the reference. Given this finding of statistical superiority, obviously the lower one-sided GMR 95%CI of 1.37 was greater than the 0.63 non-inferiority margin.

LSD had no detectable effect on amnestic response (GMT supplemented 91 [64 129] vs. non-supplemented 91 [64 129]; GMR 0.78 [0.48 to 1.29];  $P_{t=0.97; df=78} = 0.333$ ).

**Conclusions:** LSD supplementation did not measurably affect the amnestic response. This (while not measured) may reflect the calves adequate vitamin status.

The amnestic response was significantly (2-fold) enhanced given a five month increase in inter-vaccination interval. This enhanced amnestic response, with increasing intervals, has also been observed with other vaccines (e.g. Albas 2006, Bernath 2004, Cooper 1976).

The enhanced amnestic response in this study was observed in a cohort of calves initially vaccinated at 16-18 weeks of age, when some had lingering maternally derived antibodies (MDA) against BVDv; whereas MDA was depleted in the 4-week cohort vaccinated at 37 weeks old.

The traditional 4-6 weeks interval between primary vaccinations is promulgated to gain sufficient immunity in the shortest time; immunologically it is not necessarily optimal. This is supported by this study, and moreover enables greater flexibility vaccination regimes when using this vaccine.

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BV-06

### A UK Practitioner's Experiences With BVD Incursion into Two Naïve Herds And The Subsequent Reproductive Performance Following Vaccination With A Double Deletion Live Vaccine .

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**Objectives:** 1.To assess the adequacy of routine BVD surveillance on two BVD-free herds that subsequently demonstrated BVD virus incursion;

2.To assess the reproductive performance comparing periods before and after vaccination with a double-deletion live vaccine ("Bovela",Boehringer Ingelheim Ltd.,Bracknell, England.).

**Materials and Methods:** Retrospective analysis of 3-monthly bulk tank milk samples tested for BVD antibody by indirect ELISA and annual cohort 10-12 month old heifer blood screening for serum BVD antibodies by a "Modified Method" ELISA (APHA,England) was used to determine when BVD infection first occurred in both herds.Then, using fertility data recorded on the dairy software programme Interherd-Plus (Agrisoft,UK), a comparison of reproductive parameters including conception rate, abortion rate and reabsorption rate (defined as the num-



ber of animals returning to visible oestrus 72 days or more after the previous service and confirmed not pregnant after having been confirmed pregnant between 28 and 42 days post-insemination, both by rectal ultrasound examination) was performed for the "Risk Period" (RP) before vaccination with the double-deletion live vaccine and the "Post-Vaccination Period" (PVP) afterwards.

**Results:** BVD incursion was first demonstrated in one herd (H) by antibody appearance in a routine abortion screening whilst in the other herd (J) it was detected by antibody presence in screening of a series of 5 reabsorptions in a 4 week period despite the BTM and cohort screening being antibody negative for both herds.

There was no significant difference in the conception rate in Herd H ( 33% and 35% for the RP and PVP respectively) nor in Herd J ( 40% and 47% respectively ); nor in the abortion rate ( 1 in the RP, 3 in the PVP in Herd H; nil cases in either period in Herd J).

There was significant evidence that the reabsorption rate was non-random when comparing the RP with the PVP in both herds ( 7 versus 1, Herd H; 11 versus 1 in Herd J;  $p < 0.05$ , Fisher's exact test).

**Conclusion:** Whilst BTM BVD Antibody screening is a useful tool in monitoring herd status, detection and screening of reabsorptions and/or abortions may be more useful in detecting BVD virus incursion into a naïve herd in a shorter time frame either by antibody testing of the affected dam and/or testing of aborted foetus (antibody and/or antigen ELISA and/or tissue PCR).

Additionally, the use of the double-deleted, modified live BVD vaccine may protect against and reduce the number of reabsorptions of confirmed pregnancies in such herds.

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## BV-07

### Outbreak of acute infection of bovine viral diarrhoea virus (BVDV) in a farm

#### Are persistently infected (PI) cattle produced by acute infection after birth?

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In a beef stocker operation farm, 86 cows acutely infected with bovine viral diarrhoea virus (BVDV) were detected, and 46 of which were BVDV positive in the peripheral blood at 2- to 3-week intervals. The detection rate was 2.1% over an 18-month period (86 positive/ 4,004 examinations). The present study estimated the suspected causes of this prevalence. **OUTLINE** of the farm: Every month, 50 to 100 beef calves were purchased from various markets in Japan. The calves arrived at the farm a few days to a few weeks old, and were confirmed as BVDV negative by the RT-PCR examination. For the first week they were fed in individual hatches, then moved to a calf stall with a robotic milk-feeding system. They were moved to pens following growth, and about eight months later the cattle were sold. **OCCURRENCE** of prevalence: On May 2016, many calves in

the calf stall exhibited respiratory disorder and diarrhoea, and responded poorly to medical treatment. All calves in the stall (161 calves) were examined for BVDV again. Sixteen BVDV-positive calves were detected. Nine of them converted to negative within 2 weeks, but seven were BVDV-positive after a further 2 or more weeks. All these seven calves died or were euthanized. By March 2017, 69 calves had been found to have acute BVDV infections, and 39 of them were recognized as BVDV positive 3-week interval. After that, until the end of August 2017, there were no BVDV positive cases among 1100 examinations. **RESULTS & DISCUSSION:** In 84 cases, BVDV-2 was identified, and the nucleotide homologies of their 5'-UTR were 97.5-100%. The amino acid homologies of a part of E2 antigen were 98.6-100%. The oldest of the infected came to the farm on January 2016, and the last PI cattle detection in the farm was March 2016. The viruses from these two cattle were identical. **CONCLUSION:** By the acute infection of BVDV in the herd, a persistent infection could be established continuously via an unknown transmission pathway.

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## BV-08

### The BVD Quickscan, a tool for determining the BVDV status of a dairy herd

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Bovine viral diarrhoea (BVD) is a disease that occurs worldwide. National and regional programs are in place in several countries to control or eradicate the disease. An important aspect in the epidemiology of bovine viral diarrhoea virus (BVDV) is the existence of persistently infected (PI) animals. Due to the continuous shedding of large amounts of virus PI animals are the most important source of spreading the virus and the main reason why herds remain infected. In the Netherlands BVD is endemic, therefore GD offers several voluntary programs to control the disease at the herd level. To help the dairy farmer decide which program is optimal for his herd the BVDV herd status can be determined at the start with a BVD Quickscan. The BVD Quickscan is a combination of three tests to determine whether BVDV has recently circulated or was present in the past or was not present in a herd.

The BVD Quickscan consists of (i) a BVDV antibody-detecting ELISA in bulk milk, (ii) a real time-PCR in bulk milk and (iii) a BVDV antibody-detecting ELISA on blood samples of five randomly selected calves at the age of 8-12 months (spot test). If BVDV antibodies are detected in the bulk milk this is indicative for a BVDV infection (recent or in the past). If BVD virus is detected in the bulk milk this is indicative for a PI animal in the lactating herd. The spot test in the young stock provides information about recent infections in the herd. If antibodies are detected, the herd most likely had a recent BVDV infection, most probably caused by the birth of a PI calf. Depending on the outcome of the BVD Quickscan a herd is advised to participate in the BVDV-free program, which is based on identification and removal of PI animals, the BVDV bulk milk program which is suitable for monitoring herds without BVDV antibodies in their bulk milk, or the BVDV young stock monitoring program for herds with antibodies in their bulk milk but without recent BVDV



infection in the herd.

In 2016 a BVD Quickscan was performed for almost 3000 herds. Of these, 94% of the BVD Quickskans came from herds with an unknown BVDV status. Results showed that 40% of herds had no indication for BVDV circulation in the past and present and therefore were advised to start with the BVDV bulk milk program. 18% of the herds had indication for recent virus circulation and were therefore advised to identify and remove PI animals and participate in the BVDV-free program. 42% of the herds only had an indication of older BVDV circulation and were therefore advised to participate in the BVDV young stock monitoring program.

The BVD Quickscan proved to be a practical tool to determine the BVDV status of a dairy herd. With this knowledge the farmer can choose the optimal BVDV control program for his herd that is economically most feasible.

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BV-09

### Comparison of two rapid antigen detection tests for Bovine Viral Diarrhea Virus

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**Objectives:** Bovine viral diarrhoea (BVD) is a significant and global economic disease of cattle. Detection of persistently infected (PI) animals is critical to the management of the disease and requires diagnostic tests which are proven to be reliable. A new immunochromatographic test (WITNESS® BVDV, Zoetis LLC) was licensed in the United States for the detection of Bovine Viral Diarrhoea Virus (BVDV) Erns antigen in bovine ear tissue samples. The objective of this study was to compare this test to an existing licensed point-of-care test (SNAP® BVDV Antigen Test, IDEXX Laboratories Inc.) and to demonstrate its suitability for PI detection and BVD control in North America.

**Material and methods:** Ear tissues were sampled from cattle of US origin which were primarily multiple, mixed breeds or Holstein to provide paired sets of 150 ear samples each. One set contained small ear notch punches of 3 mm in diameter. The second set contained large ear notches of at least 1 cm on at least two sides. The status of the samples was established by Polymerase Chain Reaction (PCR). The collections were subsequently provided randomized, blindly and in duplicate to the Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX for testing on the two point of care tests. Test kits evaluated were a rapid immunochromatographic test (Kit A: WITNESS BVDV, Zoetis) and an additional comparative rapid tests (Kit B: SNAP BVDV Antigen Test, IDEXX Laboratories Inc). The extraction methods and assay protocols were performed per kit specific directional inserts.

Sensitivity and specificity together with 95% Jeffreys' confidence interval were estimated for each of the two tests compared to the reference method using SAS (SAS Institute, Cary, NC).

**Results:** Classified by PCR, the panel consisted of 49 positive

samples and 101 negative samples each presented as large and small ear tissue. Both point of care test kits correctly identified all 49 positive samples and all 101 negative samples regardless of sample size. Thus, the observed diagnostic sensitivity was 100% (95% CI: [95%-100%]) with diagnostic specificity estimated to be 100% (95% CI: [97.5%-100%]) for both the small and large ear notches in comparison to PCR.

**Conclusion:** This study determined the diagnostic sensitivity and specificity of a new rapid test using positive and negative, large and small ear notch samples sourced from the United States of America. Regardless of sample size, diagnostic sensitivity and specificity were both observed to be 100% and similar to that obtained with an existing commercially available test and PCR. The assay performance demonstrates the suitability of the new test (WITNESS BVDV, Zoetis LLC) for PI detection and BVD control particularly when a timely result on the farm is required.

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BV-10

### Investigating the epidemiological and clinical effects of bovine viral diarrhoea virus infection in sheep and other non-bovine species in Australia

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**Objectives:** Bovine viral diarrhoea is a disease of cattle known to cause severe reproductive dysfunction and immunosuppression in infected animals. It has also, on many occasions, been reported that the BVD virus (BVDV) is able to cross species barriers infecting other non-bovine species such as sheep, goats, deer and alpacas. When developing control or eradication programs for BVDV, the ability of BVDV to infect non-bovine species is of great concern, particularly in areas where cattle regularly come into contact with other susceptible species.

In Australia, cattle and other livestock species, predominantly sheep, are often farmed in close proximity. Previously it has been reported that the predominant BVDV strain in Australia, BVDV-1c, can naturally transmit from cattle to sheep. As such it was considered important to understand the epidemiological and clinical effects associated with BVDV-1c infections in sheep and other commonly farmed Australian livestock species.

**Materials and Methods:** A total of five infection studies were undertaken, between 2014 and 2016 focusing on the clinical effects of BVDV-1c infections in sheep and alpaca, as well as the epidemiological importance of young BVDV-1c PI sheep in the spread of the virus to susceptible cattle and sheep. A further two opportunistic prevalence studies were undertaken using samples collected from South Australian sheep in 2016 and Northern Territory water buffalo from 1991-2003.

**Results and Conclusions:** This body of work indicates that acute BVDV-1c infections of pregnant ewes can lead to severe reproductive losses including absorptions, stillbirths and lambs lambs presenting with a wide range of pathological lesions;



such as anasarca, hydranencephaly and skeletal deformities. A viable, neonatal, BVDV-1c persistently infected (PI) lamb was also born during this study. Despite the severe lambing losses which develop following early to mid-gestational infection with BVDV-1c, results also suggest that sheep are unlikely to play a pivotal role in the spread and persistence of the virus in Australia. Furthermore, results also indicate that species such as alpaca and water buffalo are susceptible to infection with BVDV however further work should be undertaken in order to fully understand the role these and other non-bovine species play in the spread and persistence of the virus within Australia.

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BV-11

## A concurrent outbreak of BVD and Bovine Tuberculosis in a dairy herd

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**Summary:** An intensive Holstein dairy herd of experienced an outbreak of bovine tuberculosis (bTB), first detected by 25 adult cows reacting to the routine skin test in February 2016. Repeated skin testing in accordance with the statutory control programme detected a total of 129 reactors from the herd over a twelve month period, with 24 of the reactors showing visible lesions at post mortem.

During investigations to determine the source of the tuberculosis outbreak, and the possible explanation for the high prevalence, a Bovine Viral Diarrhoea Virus (BVD) persistently infected animal was discovered in the herd, with acute BVD infection in several adult cows indicating a high prevalence of BVD in the unvaccinated herd.

It is likely that the high prevalence and rapid spread of bTB in the herd was exacerbated by the presence of BVD, having an immunosuppressive effect, and possibly interfering with the sensitivity of the tuberculin skin test.

**Introduction:** Bovine tuberculosis is a growing problem in cattle herds in the South West of England, with approximately 15% of all cattle herds being defined as infected at any one time. Most outbreaks are of low within herd incidence and of short duration. However, some herds have high prevalence outbreaks, without explanation. This 120 cow dairy herd (with a total cattle population of 200 animals including young stock and replacements) experienced such an outbreak with a concurrent outbreak of BVD with devastating consequences on the health and productivity of the herd, resulting in over 70% of the original herd being culled through bTB infection.

**Clinical History:** A herd of 120 adult Holsteins was kept in an intensive housed system, milked through a guided robotic milking system, and producing approximately 12,000 litres per cow per year. The herd was replenished from homebred replacements with no purchases of any animals, making the herd reasonably biosecure. However, young stock had intermittent direct contact with neighbouring cattle of unknown disease status. For this reason, the herd (monitored BVD free) was vaccinated with killed BVD vaccine. However, for reasons of economy, vaccination was ceased in 2014, with all animals born after 2013 not receiving any vaccination.

The herd was routinely monitored for bTB under the UK statutory TB surveillance programme using the single comparative intradermal tuberculin test (SCITT). In February 2016, this routine test disclosed 25 reactors in the adult herd of 120 cows. At post mortem 9 of these had visible lesions suggesting advanced infection.

At the same time, routine monitoring of bulk milk samples were negative for BVD virus using PCR tests. However, in December 2016 (10 months after the start of the bTB outbreak) a positive bulk milk PC for BVD suggested the presence of BVD in the herd, and this was further investigated by blood samples looking for the source of the BVD virus.

**Results:** Over the 18 month course of the bTB outbreak, 129 animals were slaughtered as TB reactors, of which 42 had visible lesions at post mortem examination.

Just one persistently infected animal was discovered (a two year old maiden heifer that was kept in the milking herd), which was removed once discovered. The whole herd was vaccinated with live BVD vaccine in December 2017 as soon as acute BVD infection was discovered.

Other risks of disease spread were assessed, identified and managed to reduce the infection in the environment and minimise the risks of spread within the herd.

The herd tested clear of BVD in February 2017 and clear of TB in October 2017.

**Discussion:** This severe outbreak of bovine tuberculosis was probably fuelled by a concurrent outbreak of BVD, with acute BVD virus infection behaving as an immunosuppressive and increasing susceptibility to infection with mycobacterium bovis, and also moving latent infection to clinical disease as occurs with HIV infections in human populations infected with TB. There is also some suggestion that animals with acute BVD infection are less sensitive to the SCITT test routinely used to detect bTB.

The paper and presentation will discuss the importance of investigating disease outbreaks with a concern of concurrent infection interfering with the disease epidemiology and testing programme.





CG-01

### Novel findings of Bovine Familial Convulsions and Ataxia

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**Background:** Bovine familial convulsions and ataxia (BFCA) is considered a rare heritable condition. Cerebellar abiotrophy is the major distinguishing pathological finding. Animals present with clinical signs of cerebellar dysfunction and convulsions.

**Case series:** A newly purchased Black Angus bull sired 19 calves out of 200 calves produced in a herd for a single year. The females sired represented several different breeds. Seventeen of the nineteen calves showed clinical signs attributable to cerebellar dysfunction. The clinical signs became apparent at various ages. Abnormal calves were classified as presenting with generalized ataxia, hypermetria, a wide based stance, normal strength, normal mentation, ability to navigate their environment and occasional generalized or partial seizures. Clinical examinations, serological examination, gross necropsies, and brain histology were done to investigate this problem.

**Conclusions:** In this case series, a clear familial link from a single bull was made to all affected offspring. Evidence of a dominant inheritance pattern with incomplete penetrance is presented. Clinical signs and histopathologic findings consistent with previous reports of BFCA were observed. Seventeen of nineteen (89.5%) calves sired by a single bull were affected by BFCA. This is a much higher prevalence than previously reported. All nineteen calves had marked hind limb muscle hypertrophy which could not be explained by the parental phenotypes. This has not been reported previously. Desirable muscling characteristics may incentivize owners to retain carrier animals.

CG-02

### Genetically selected heifers differ in peripheral blood concentrations of IGF-1 and $\beta$ HBA

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Incidence of metabolic and reproductive diseases around parturition in cattle has an essential impact on animal welfare, usage of antibiotics and economics of dairy farming. In this experimental setup n=36 heifers, which had been selected via single nucleotide polymorphism typing for alternative paternal chro-

mosome 18 haplotypes associated with favourable (Q) or unfavourable (q) udder health were compared. Health status of Holstein Friesian heifers (18Q/18q) was supervised three weeks before, around calving until necropsy on d39 ± 4 after calving. Incidence of diseases was recorded and animals were treated according to good veterinary practice. Blood samples were taken two times per week and non-esterified fatty acid (NEFA), beta-hydroxybutyrate ( $\beta$ HBS), Insulin-like Growth Factor-1 (IGF-1), insulin and Growth Hormone (GH) concentrations were measured via established immunoassays. Milk yield was recorded and milk samples were taken weekly. Statistical analyses were performed with SAS studio, using Chi-squared test, unpaired t-test and mixed model procedures. At the beginning of the experiment, no differences between the two groups were detected concerning body weight, body condition score, health status including udder health and lameness ( $P>0.05$ ). Incidence of retained fetal membranes did not differ between the two groups ( $P>0.05$ ), but Q-heifers showed significantly less incidence of metritis compared to q-heifers ( $P<0.05$ ). Daily milk yield was comparable ( $P>0.05$ ). No differences concerning NEFA, GH and insulin concentrations were detected between the two groups, but  $\beta$ HBS was significantly lower in Q-heifers ( $P<0.05$ ) and IGF-1 was significantly higher in Q-heifers ( $0<0.01$ ), especially during the period after calving. In conclusion, genetic selection might offer a sustainable tool for breeding cows with reduced susceptibility towards periparturient illnesses. The underlying metabolic and immunological mechanisms still need to be elucidated by further investigations.

CG-03

### The Genomic Value of Dairy Breed Natural Service Sires

#### A Survey of Breeding Indices and the Relationship between Scrotal Circumference and Daughter Fertility

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**Objectives:** Natural service sires are an often-neglected part of pasture based dairy reproduction systems. Natural mating is generally an adjunct to artificial insemination in these herds and the calves that are produced are not usually retained as they are not considered to improve the genetic merit of the herd. However, advances in the estimation of genetic worth may change the way natural service sires are selected and used. Small samples of genetic material from bulls can be submitted for genomic analysis and used to estimate breeding indices, providing producers with an additional tool to manipulate the genetic merit of their herd by selection of artificial as well as natural services sires. The aim of this study was to conduct a survey of the genetic worth of pasture raised natural service dairy breed sires.

Given recent declines in herd fertility, daughter fertility is a highly sought-after genetic attribute. Previous research in the beef



industry suggests that there is a positive correlation between the scrotal circumference of a sire and daughter fertility. A secondary aim of this study was to quantify this association in the dairy industry between natural service sire scrotal circumference and daughter fertility breeding index.

**Materials and Methods:** Hair samples were obtained from Jersey ( $n = 84$ ) and Holstein ( $n = 124$ ) bulls from two dairy farms in north western Tasmania, Australia. Samples were submitted, along with details of each bull's pedigree, to Zoetis Australia for genomic evaluation (Clarifide). Both genetic material and pedigree information were used to allocate a set of Australian Breeding Values (ABV) to each bull. In addition, both groups of bulls were subject to six weekly scrotal circumference and body weight measurements from approximately 6 months to 18 months of age.

The Balanced Performance Index (BPI) and daughter fertility ABVs of both groups of bulls were plotted as frequency histograms. Two linear regression models were developed to quantify the association between scrotal circumference (as an explanatory variable) and daughter fertility breeding value as the outcome variable; one for Holstein-Friesians and the other for Jerseys.

**Results:** Frequency histograms of BPI and daughter fertility ABVs were normally distributed for both breeds. Individual bulls that recorded either high and low values for each of the breeding indices were investigated.

For Holstein-Friesians, a one centimetre increase in a sire's scrotal circumference was associated with a  $-0.4$  (95% CI  $-1.0$  to  $0.17$ ) unit change in daughter fertility breeding value ( $P=0.16$ ). For Jerseys, a one centimetre increase in a sire's scrotal circumference was associated with a  $+0.10$  (95% CI  $-0.27$  to  $+0.47$ ) unit change in daughter fertility breeding value ( $P=0.59$ ).

**Conclusions:** The study represents the first survey of genetic merit conducted in natural service sires and if genetic gain is sought via natural service, genomic testing provides producers with a tool to select bulls as well as cows.

In this study, based on data from 208 bulls, we were unable to identify a clearly defined association between scrotal circumference and daughter fertility. It must be considered that the relationship in the beef industry was measured as time to first service for heifers. This approach is quite different as the dairy ABV assessment for daughter fertility index involves measuring the percentage of daughters pregnant within six weeks of planned start of mating period. Whilst it could be argued the two are linked, it appears that there is a more complex relationship with scrotal circumference than that which was assessed in this study.

DI-01

## Prevalence of physal changes of the distal metatarsal growth plate in finishing bulls – a postmortem study

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**Objectives:** The aims of this study were to assess the prevalence of lesions of the distal metatarsal growth plate of finishing bulls in a regional abattoir and to describe the radiographic and histologic appearance of the lesions.

**Materials and Methods:** The hind feet of 102 slaughtered bulls (204 hind feet) were collected from a regional abattoir and examined radiographically. A grid was established to classify the changes and lesions. 14 feet from 7 different bulls were further examined using computed tomography and the growth plates from five different bulls were examined histologically. Additionally the hind claws of all the bulls were examined macroscopically.

**Results:** The bulls originated from 20 different farms that were managed using one of two programs aimed at improving feedlot management standards. The average age at slaughter was 409 days. Ten (4.9%) of the 204 examined growth plates (5 bulls) had no radiographic changes, 67 (32.8%) had mild changes (31 bulls), 109 (53.4%) had moderate changes (52 bulls) and 18 (8.8%) had severe changes (14 bulls). Only 10 bulls showed a different degree of radiographic changes between the left and the right growth plate. Significantly more severe changes occurred in the fourth metatarsal bone than in the third metatarsal bone. The most frequent radiographic changes were marginal osteophytes (lipping, 99.5%) and sclerosis of the adjacent bone (66.6%). Histologic changes included widening of the growth plate, focal cartilage tongues protruding into the metaphysis, circular arrangement of chondrocytes, calcification and clefts within the growth plate. The factors producer, management program, carcass weight and average daily weight gain were significantly related to the occurrence of radiographic changes. Claw lesions were mild to moderate and consisted primarily of sole hemorrhages and heel erosions.

**Conclusion:** The prevalence of osteochondral lesions in the growth plates of the third and fourth metatarsal bones of finishing bulls was high in the present study. It is possible that large daily weight gains combined with skeletal lesions resulting from inadequate housing systems contributed to this problem. Because this constitutes a potential animal welfare problem, gait and lameness assessments, which should include radiographic examination, are needed to determine the reasons for the high prevalence of metatarsal bone lesions in feedlots.



DI-02

### Diagnostic imaging of abomasal curd formation in preruminant calves by ultrasonography

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**Objectives:** Abomasal curd formation is a unique digestive system that occur in milk-fed pre-ruminant calves. Although curd formation has long been studied, a controversy exist as to the exact function of curd formation. Importantly, previous studies did not use real-time technique to confirm whether curd was formed in the abomasum of calves that were fed milk replacer, while clotting or non-clotting properties were determined by in vitro assays. Since some calves are born with low curd forming enzyme activity in the abomasum (Gregory, 2003), it has been suggested that in vivo curd formation is affected not only by the in vitro clotting properties of the milk replacer, but also by the abomasal luminal pH and clotting ability. Ultrasonography is a useful technique for visualizing the gastrointestinal organs and their contents. The aim of this study was to determine whether ultrasonography could be used to evaluate curd formation and apply for diagnostic imaging.

**Materials and Methods:** Fifteen Holstein-Friesian calves, 13 males and two females, 2-11 days old, with a mean body weight (BW) of 46±5 kg, were randomly assigned to one of three dietary treatments; clotting, non-clotting, and pH-dependent clotting, with clots forming at pH5.5 but not at pH6.5, milk replacers. Calves were fed milk replacers at 10% of BW daily at 12h intervals (08:00 and 20:00). Ultrasonography was performed 0, 0.5, 1, 2, 4, and 6h after morning feeding using a Super Eye SSD-500 (Aloka) with a 5.0 MHz linear transducer, a Sono Ace 600 (Medison) with 5.0 MHz linear transducer, or a Sono Ace 6000 C (Medison) with 4.0 MHz convex transducer. The transducer was placed transversely across the ventral midline and moved along the ventral midline from the xiphoid process to the penis or the corresponding area in female calves. Then, at the point where maximal abomasal area was observed, both paramedian region were examined by moving the transducer laterally. To test the abomasal ability to form curd, ultrasonographic evaluation of abomasal contents were further performed after feeding same clotting milk replacer to 29 preruminant calves (20 Holstein-Friesian, 9 males and 11 females with a mean birth BW of 43±5 kg, and 9 F1 hybrids of Holstein Friesian and Japanese black, 4 males and 5 females with a mean birth BW of 37±3 kg), 4-14 days old, kept in a large dairy farm.

**Results:** Ultrasonographic images of the abomasum in the calves fed clotting milk replacer showed curd as an echogenic image surrounded by a clear outline and whey as an anechoic image from 1 to 4 h after feeding. In the calves fed non-clotting milk replacer, the entire abomasum was filled with a uniform echogenic image, while no echogenic image with a clear outline corresponding to curd was observed during experiment. Several small curds were observed as echogenic images in the ventral portion of the abomasum in the calves fed pH-dependent milk replacer. Abomasal curd was absent in 8 of 29 calves at 2h after feeding clotting milk replacer.

**Conclusions:** Ultrasonography is a practical and useful technique for diagnosis of abomasal curd formation in preruminant calves and provides in vivo evidence to distinguish curd-con-

taining abomasum from one that does not contain curd.

DI-03

### Triple nostrils in a calf

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**Objectives:** Abnormalities of the nose and nostril are extremely rare in bovines. In humans, nasal deformities are mainly classified as proboscis lateralis (formation of a tube- or trunk-like appendage projecting from the surface of the face near the nose) or supernumerary nostrils (formation of accessory nostrils located near the two nostrils of the nose). The present report considers the etiology based on the computed tomographic (CT) finding.

**Materials and Methods:** A female Holstein calf presented with congenital formation of triple nostrils in the nose at birth. During a 1-month suckling period, the calf showed good weight gain, and normal eating and drinking behaviors. No respiratory signs were evident, including cough or dyspnea. Of the three nostrils, the right and left nostrils existed in the normal locations within the nose. The middle nostril was located right of center on the nose, in the neighborhood of the right nostril. This middle nostril was flat in the dorsal-ventral direction, and smaller than the left and right nostrils. The hair-bearing region was found just near the left edge of the middle nostril, as a region not normally seen on the nasal speculum. On the dorsal view, the bridge of the nose was running straight, with a sudden curve toward the left side.

**Results:** Under general anesthesia, the calf underwent CT using a helical scanner (Pronto SE, Hitachi Co. Ltd, Tokyo, Japan). Three-dimensional CT of the skull revealed that the left-sided nasal bone was greatly curved toward the left side at a third of the apex-caudal length of the nasal bone, compared with the gentle curvature toward the left side within the right-sided nasal bone. Coronal CT of the nasal cavity revealed that the nasal septum was abnormally curved along the transformed left-sided nasal bone, with severe protrusion toward the right-sided nasal cavity within the middle area of the nose. The abnormal curve of the nasal septum allowed constriction within the right nasal cavity. Of the three nostrils, the right and left nostrils were connected to the respective nasal cavities. The middle nostril ran obliquely from an opening within the nasal speculum toward the left side, and straight backward at the deeper site. The lumen stopped in a blind-ended structure approximately 4 cm from the opening within the nasal speculum. A bone-like structure was seen running within the nasal septum from 5-mm deep to the nasal speculum to the bending point of the nasal septum. The blind ending of the middle nostril was located near this bone-like structure.

**Conclusions:** Abnormality of the nose in the present case was characterized as: 1) presence of a hair-bearing region histologically resembling normal hair-bearing skin within the nasal speculum between the middle and left nostrils; 2) formation of a bone-like structure located from deep to the hair-bearing re-



gion of the nasal speculum to the nasal septum and separating the middle and left nostrils on CT; 3) abnormal curvature of the nasal septum resulting in a narrower right nasal cavity; and 4) deformation of the nasal bone. These features of the affected nose predict the following anomalous processes: 1) premature development, with formation of a pair of nostrils (resulting in the right and middle nostrils) within the right nasal bud during the course of development of the nasal placode; and 2) fusion of the right nasal bud with the left nasal bud resulting in the left nostril. These findings is identical with supernumerary nostrils, characterized by formation of accessory nostrils located near the two nostrils of the nose in humans.

EL-01

### Examples for E-learning tools to improve and widen farm animal veterinary education

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**Objectives:** Veterinary education is at a pivotal point in time where the implementation of e-learning technology is progressing fast and delivery of teaching at universities is challenged by increasing student numbers. This lecture will provide shareable examples of e-learning resources to encourage everyone involved in farm animal veterinary training to use and share teaching methods to create a sustainable future for veterinary education and improve animal health worldwide.

**Materials and methods:** Youtube videos were developed at the School of Veterinary Medicine and Science (University of Nottingham, UK) and used as a resource to demonstrate practical cattle handling and examination skills to support hands-on live animal practical classes. For postgraduate training and knowledge exchange Youtube 'Research explained' videos were developed, summarising recent research publications to inform students and the wider public.

Open Labyrinth (OL) was employed to create virtual scenarios which support the integration and assessment of team-based learning. Common clinical case scenarios were used to encourage students in groups to work through the decision-making process involved. Staff involved could review progress of each group throughout the session and could therefore target online feedback specifically to the area of concern noted from the digital feedback received.

**Results:** Youtube analytics demonstrated the videos received numerous views from a worldwide audience and were also well received and used on site by undergraduate students within the university. They enhanced the revision of practical skills assessed throughout the 5 years of the veterinary degree course. A brief overview of e-assessment used to assess these practical skills will be presented. The 'Research explained' videos received a significant number of views, expanding the impact of the research findings and engaging stakeholders and veterinary students in the research carried out at the School of Veterinary Medicine and Science.

OL provided an easy-access interactive group work environment which encouraged students to have in-depth discussions around clinical cases they were presented with. Currently off-site online access for this tool is developed to facilitate interaction with interested learners outside the university. The opportunities to develop OL to engage with other stakeholders, i.e. students at other veterinary/agricultural institutions, farmers, and researchers) are vast and collaborations with specialists in the medical sphere are ongoing to further this field to benefit veterinary education worldwide.

**Conclusions:** E-learning, using for example Youtube and OL, provides students worldwide with the opportunity to learn about animal health in an interactive way. In addition, it brings together learners and experts from university with learners and experts from other parts of the world. This will enhance the student learning experience by broadening their view on animal health outside their familiar environment. The ability for the student to access the material at any time in any place is putting the student in control of their learning and provides an open



and transparent environment to evaluate approaches, understand processes and develop their learning.

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EL-02

### Technical rescue of ruminants within Establishment of safer animal rescue capacity Erasmus+ KA2 project

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**Objectives:** Natural and anthropogenic disasters are common nowadays. Animal rescue is to transfer an animal from a place of danger to a safe place by appropriate methods. Animals are in distress in disasters, which may lead to dangerous, unpredictable situations especially with large ruminants. The safe technical rescue of animals is universal problem that requires the attention and involvement of a number of experts and institutions including veterinarians, rescue experts and volunteers. Training for such operations is none-existing in many countries. For this purpose it is essential to prepare and train rescue teams consisting of search and rescue personnel, veterinarians and often also volunteers that work together in coordinated manner.

**Materials and methods:** The project Establishment of safer animal rescue capacity was adopted to tackle these issues. It was coordinated by Aksaray AFAD (Aksaray Disaster and Emergency Management Directorate) and partnered by University of Ljubljana, Veterinary faculty - Slovenia, and Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine - Macedonia, University of Aksaray, Faculty of veterinary medicine and Harran University, Faculty of veterinary medicine - Turkey to contribute to promotion, further development of animal rescue and establishment of animal technical rescue training centre in Turkey. Within the scope of the project; training of search and rescue teams for rescuing ruminants and other animals was an important task.

**Results:** The first two weeks long workshop about safer animal rescue capacity was executed in Ljubljana, Slovenia and intended for future trainers of animal search and rescue teams. Trainees were acquainted with basic anatomy, physiology, nutrition, biosecurity, welfare, safety when handling ruminants and clinical examination techniques. Role of veterinarians, fire fighters and military in technical rescue was introduced. Practically they were performing approach, handling and clinical examination of ruminants. Rescue equipment for ruminants consisting of ropes, halters, nose pliers, slings, planks and canvas was introduced to them. Brochures about technical rescue were compiled for the future trainees to prepare them for safer animal rescuing in case of disasters. Different cases of technical rescue are studied and introduced in the book as well. The main output of the project was establishment of animal technical rescuing training base, fully equipped for technical rescuing and with the trained staff, in Aksaray, Turkey, which will be responsible for a certified training program on safe animal rescue,

where the partner institutions would be contributing its academic, operational and training aspects on a regular basis training of rescue teams and public for the whole Turkey. Within the project public demonstration of rescue, video spots for TV, Safer animal rescue awareness book, flyers and posters for gaining of public awareness about needs of animals and safer rescue in disasters were released. These activities of the project are not important just for rescuing animals but also to protect people who voluntarily help animals, as animals in distress cause people to rush in to help but this voluntary action may often lead to serious injuries in animals and voluntary rescuers.

**Conclusions:** All the goals of the project were successfully achieved, including increase of animal rescue capacity of governmental organisations through the trainings, improvement of animal welfare during rescuing, decrease of unwanted complications during rescue, reduction of economic losses and increase of public awareness on animal rescue.

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ED-01

## Two cases of embolic pneumonia secondary to udder cleft dermatitis in dairy cattle from the same farm

### A case report demonstrating two cases of a previously undescribed sequela of udder cleft dermatitis

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**OBJECTIVES:** A case report documenting previously undescribed aetiology of embolic pneumonia and sepsis associated with udder cleft dermatitis lesions in dairy cattle.

Udder cleft dermatitis (UCD) is a condition that is widely acknowledged by veterinary surgeons and farmers but is thought to be of little significance to production and not a cause of systemic illness. This report documents a newly recognised sequela of septic emboli occurring secondarily to udder cleft dermatitis lesions in dairy cattle. This severe and ultimately fatal association is being increasingly observed in disease surveillance centres in the UK. This report documents two cases seen in cattle from the same dairy farm.

**MATERIALS AND METHODS:** Two Holstein cows from the same farm were presented within eight months of each other with malaise, milk drop and dyspnoea. Both cows had active, or historic udder cleft dermatitis (UCD) lesions. Clinical examination and diagnostic tests led to an initial diagnosis of chronic suppurative pneumonia in both cows. The cases were poorly responsive to antibiotic treatment and died or were euthanised when presented with severe bleeding bilaterally from the nostrils, 18 and 16 days respectively, after presentation.

**RESULTS:** Post mortem examination of these cows revealed the presence of healing (cow 1) or active (cow 2) udder cleft dermatitis lesions with an area of fibrosis and thickened cutaneous and subcutaneous tissues of the adjacent body wall as well as multiple abscesses containing dark brown material. Haematogenous and lymphatic dissemination of the infection resulted in grossly similar abscessation of a precrucial lymph node and the right fore quarter of the udder in cow 1, as well as the presence of an endocarditis lesion on the semilunar heart valve. A large thrombus was found in the milk vein of cow 2. Both animals had pleuritis, several small abscesses throughout the lung lobes as well as larger abscesses containing dark brown malodorous material, similar to that seen in the abscesses in the ventral abdominal wall of each cow. Extensive emphysema and enlarged mediastinal lymph nodes were also found.

Bacterial culture of organisms isolated from the skin (pure growth) and lung (predominant organism in mixed growth) abscesses of cow 1 identified *Providencia stuartii*, a facultative anaerobic bacteria which is considered to be an opportunistic pathogen in this case.

**CONCLUSIONS:** In both cases the cause of the pathology seen was considered to be a primary UCD lesion which had resulted in deep cutaneous and subcutaneous infiltration before haematological spread to lymph nodes, udder, heart and lungs. Sepsis, due to infection with opportunistic pathogens was the diagnosed cause of deterioration (before euthanasia) or death in each case.

It is crucial that farm animal veterinarians and pathologists are made aware of this newly recognised aetiology of embolic pneumonia and sepsis secondary to UCD, of which numerous cases have now been reported to and diagnosed by surveillance centres across the UK, including the production animal pathology service at the University of Bristol, UK. Increased awareness will result in better recognition of such cases, and thus allow more appropriate and timely interventions to be made. Producers should also be aware that what may be considered a minor surface lesion on or adjacent to the udder can have severe consequences, and further research into this commonly recognised but poorly understood disease is indicated.

ED-02

## Salmonella Dublin in dairy calves: an outbreak investigation.

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**Objective:** The aim of this study was to describe an investigation, analysis and management of an outbreak of septicemia associated with *S. Dublin* in a large dairy farm.

**Materials and methods:** This study involved an etiologic investigation of a dairy farm salmonellosis outbreak using culture-based methods, molecular typing (pulsed field gel electrophoresis (PFGE), milk antibody levels, fecal and environmental surveillance. The outbreak occurred on a 700 milking-cow dairy farm in Ontario, Canada. Investigation was initiated after identifying *Salmonella* enteritis or sepsis in 5 calves. *Salmonella* surveillance included culture of fecal samples from 100 pre-weaned calves (35 calves had diarrhea or pneumonia or both), 100 heifers (20 heifers had pneumonia), 100 milking cows (25 cows had diarrhea, pneumonia, lameness or mastitis or a combination of them), and 12 calf barn environmental samples. Enrichment culture was performed using Hectoen and tetrathionate broth, followed by inoculation onto brilliant green sulphur agar. Molecular typing was performed by PFGE after XbaI digestion. A milk sample from bulk-tank was tested for the presence of *S. Dublin* antibodies by an ELISA test.

**Results:** During a period of 3 months (winter-spring season) the farm experienced an outbreak of calf septicemia. Five calves between 2 and 10 weeks of age died. Four calves were housed in individual pens in close proximity into the calf barn, while the remaining calf died in the barn for sick animals. Post-mortem examinations were consistent with septicemia in all cases. All calves had suppurative bronchopneumonia and three also had suppurative enteritis. *S. Dublin* was isolated in all cases from the lung tissues. All strains had identical phenotypic antimicrobial resistance (ampicillin, ceftiofur, kanamycin, sulphonamides and tetracycline) and susceptibility (spectinomycin and trimethoprim/sulfa). The PFGE pattern was indistinguishable among all isolates. *Salmonella* was isolated from fe-



cal samples from 3/25 (12%) milking diarrheic cows, but these were non-group-D *Salmonella*, and therefore not *S. Dublin*. *Salmonella* was not isolated from fecal samples of pre-weaned calves (0/100, 0%), heifers (0/100, 0%). All environmental samples were negative (0/12, 0%). The bulk tank milk sample tested negative for *S. Dublin* antibodies. Recommendations were made to prevent and control spreading of *S. Dublin* in the herd, focusing on prompt diagnosis and containment of disease in calves. Ongoing surveillance of death calves was instituted. No further cases had occurred at the time of last follow up (8 months).

**Conclusions:** It is speculated that the *S. Dublin* entered the farm through introduction of new animals without quarantine. The clustering of the mortalities, in conjunction with PFGE patterns is indicative of a single *S. Dublin* clone was the cause of this outbreak. Prompt surveillance response and institution of biosecurity measures might contribute to contain this host-adapted *Salmonella* serovar.

EP-01

## The seasonal outbreaks of Foot and Mouth Disease and its history in Iraq

### Annual FMD outbreaks in cow, buffalo, and small ruminants in Iraq

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**Objectives:** This study intended to explore the status of FMD in Iraq through assessment of FMD outbreaks that reported among cow, buffalo and small ruminants (sheep& goat) in 15 Iraqi governorates except Kurdistan region during 2011, 2012, 2013, 2014, 2015 and 2016.

**Materials and Methods:** The reported data regarding FMD cases collected from veterinary hospitals in different Iraqi governorates and analyzed.

**Results:** The results of this study revealed the annual FMD outbreaks in cow, buffalo, and small ruminants in various Iraqi governorates with variability in the numbers of the infected and dead animals. However, the total number of the infected animals was raised in 2016 in compare with 2015 due illegal importation of FMD infected cows at the end of 2015. The prevalence rates of FMD were 68.7%, 46.6% and 30.3% in cattle, buffalo, and small ruminants respectively in 2016, while this rate was 18.4%, 19.9% and 17.3% respectively in 2015. All infected animals showed typical FMD clinical signs. The current study revealed the recurrent appearance of FMD outbreaks in Iraq during 2011 consequence to the severe outbreaks that occurred during 2009-2010 and led to devastating economic loss in the livestock in Iraq. Moreover, this study found the several reasons for the reemergence cases and continuity of FMD as epidemic in 2010, first of all, was the delayed processing in releasing of vaccine with absence of management and coordination. In addition, the dynamic risk reason was a freely trade in live animals with much greater than meat because traditionally the people prefer consumption of meat from slaughter of live animals without proper dispose of carcasses. In this decay, new serotypes were reported in Iraq such as FMDV-A ASIA Iran05 BAR-08, FMDV-A ASIA Iran05 AFG-07, FMDV-O ME-SA PanAsia2 ANT-10 and FMDV-A Asia 1 Sindh-08 & FMDV-A Asia1 Iran05SIS-10 in 2009, 2010, 2011and 2013 respectively. There are probability that a new sub lineage of virus led to re-emerge of 2016 FMD outbreak in Iraq and to increase the number of infected animals. In conclusion, this study approved the reemergence and endemic nature of FMD in Iraqi livestock. Prompt procedures and a new future strategy need to being implemented including the isolation and identification the immunological serotypes and subtypes that are responsible for outbreaks to control the increasing emerging of FMD in Iraq.

EP-02

## On-farm mortality and related risk factors in Estonian dairy cows

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On-farm mortality (unassisted death and euthanasia) is an indicator of reduced animal health and welfare and is associated with unwanted financial losses for the farmer. The objective of this study was to identify risk factors associated with on-farm mortality in dairy cows. Individual cow survival data for a study period starting in January 1, 2013 and until December 31, 2015 was retrieved from the Estonian Agricultural Registers and Information Board. Cows calving during the study period in herds with more than 20 cow-years enrolled in the milk recording system were included. Animal- and herd-level data used for risk factor analyses was collected from the Estonian Livestock Performance Recording Ltd. Analyzes included data of 86,459 primiparous cows from 390 herds and 109,314 multiparous cows from 389 herds. The observation period for each cow started at the day of calving and ended at the next calving, at the day the cow left the herd (due to death, euthanasia, slaughter, selling) or at the end of the study period. Primiparous and multiparous cows were analyzed separately. Multivariable Weibull proportional hazard models with herd as random effects were composed for identifying associations between potential risk factors and on-farm mortality (composed outcome including on-farm unassisted death and euthanasia).

Mortality rate (MR) was lower in primiparous cows (MR = 5.09 per 100 animal-years, 95% CI 4.93; 5.26) compared to multiparous cows (MR = 8.28 per 100 animal-years, 95% CI 8.13; 8.44),  $p < 0.001$ . Early lactation period was associated with highest mortality hazard. In multiparous cows the mortality hazard was lowest at second parity, increasing after that being considerably higher after the fourth parity. Animal-level factors that were significantly associated with mortality in cows were Holstein breed, low/high (for primiparous/multiparous cows, respectively) relative milk yield breeding value, older age at first calving, male sex of the born calf (for multiparous cows), birth of twins/triplets (for multiparous cows), stillbirth (for primiparous cows), dystocia, low milk yield and high fat/protein ratio at first test-milking. Also, high milk somatic cell count at the last test-milking of the previous lactation and the first test-milking of the current lactation were associated with higher mortality hazard in multiparous and primiparous cows, respectively. Longer previous calving interval was associated with higher mortality hazard at the next lactation in multiparous cows. Herd-level risk factors associated with mortality were larger herd size and decrease of herd size from 2013 to 2015 with more than 15%. Herd performance indicators reflecting poorer performance e.g. lower herd average number of lactations, longer herd average interval from calving to insemination (for primiparous cows) and higher herd average number of inseminations per conception (for multiparous cows) were associated with higher mortality hazard of a cow.

This study indicates the importance of paying more attention to good health of the cows at dry-off and during the early lactation period and to ensure easy calving. Good herd management is a requisite to reduce mortality of cows.

EP-03

## Patterns and determinants of culling and mortality are unique to

### pasture-grazed, seasonal-calving dairy cows in New Zealand

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Non-production culling and mortality of dairy cows have important management and economic consequences for farmers, and are indicators of the health and welfare status of their herds. In order for farmers to achieve their goals for these outcomes, it is important that they have benchmarks against which to measure their own performance, and that animal health programs to control non-production culling and mortality are based on quantified risk factors. However, benchmarks and control programs may not be transferable between countries because of differences in management systems and cow genetics.

Hence, we described the incidences of culling, sale, and mortality, and their reported causes, and investigated their risk factors, in a recently-collected sample of pasture-based, seasonal-calving dairy herds from four regions of New Zealand. These data were from a previous observational and intervention study of herd reproductive performance. The descriptive study used electronic records collected between June 2009 and May 2011 of calving and animal removal events, and farmer-reported reasons for animal-removal. This final data set included records of 64,093 lactations from cows in 113 dairy herds.

We further investigated cow-level risk factors for culling and sale combined (hereafter defined as culling) and mortality among a subset of these herds that production-tested by use of electronic and on-farm paper records of diagnoses and treatments of disorders.

We used the first occurrence in a lactation of the most frequently-recorded, modifiable disorders, and, additionally, breed of cow, as putative risk factors. We estimated hazard ratios for culling (HR<sub>c</sub>) or mortality (HR<sub>m</sub>), or both combined, for risk factors by use of Cox proportional hazards models, with competing risks, shared frailties and time-dependent covariates, to account for the possibility of cow removal because of either culling or mortality, the clustering of cows within herd-seasons, and, changing values of covariates over time, respectively. An individual cow lactation was the unit of measurement. This final data set contained records from 65 herds, 123 herd-seasons and 70,964 cow lactations.

The first quartile and median herd-level incidence risk per parity (IR) of culling and mortality in mixed-age cows were 12.9 and 15.5, and 1.2 and 1.7%, respectively. However, the third and fourth quartiles were as great as 18.5 and 25.0, and 2.8 and 6.7%, respectively. The greatest incidence density of mortality occurred in cows aged 5 yr and older during the first two weeks following calving, and approximately 13 and 22 mo later, which coincided with subsequent prepartum periods. Reproductive failure was most the frequently reported cause of culling among mixed-age cows (42%), and production-related culling was uncommon (10.5%). Causes of mortality, however, were less clearly characterized.

In both primiparous (2 yr old) and multiparous (> 2 yr old) cows,





calving difficulty and treatment for a calving difficulty were each associated with increased HRc and HRm, and clinical mastitis was associated with increased HRc but not HRm. However, early and late uterine infection (< 14 d and  $\geq 14$  d post-calving, respectively) and metabolic disease were only associated with increased HRc and HRm in multiparous cows. Lameness was not associated with either outcome in either age group. Holstein-Friesian breed was associated with increased HRc but not HRm in multiparous cows, but breed was not associated with either outcome in primiparous cows.

The population incidences of culling, sale and mortality were less than reported from other countries with modern dairy industries.

However, their wide range and the infrequent culling for poor production indicate opportunities to reduce financial losses and improve dairy cow welfare. Furthermore, previously-recognised and novel risk factors for both culling and mortality mainly originated from maladaptation of cows during the transition period and some risk factors identified in other housing systems were not influential in New Zealand herds. These findings present an opportunity to control mortality and non-production culling by improving genetic merit for survival and targeting control measures over the condensed calving period in seasonal-calving herds, and that these solutions should be unique for this particular management system.

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EP-04

### Risk factors associated with mortality identified at arrival to a milk-fed veal facility

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**Objectives:** The veal industry experiences calf losses during the growing period which represent a challenge to animal welfare and profitability. Health status at arrival may be an important predictor of calf mortality. The objectives of this prospective cohort study were to describe the health status of calves arriving at a veal farm and determine the risk factors associated with early and late mortality.

**Materials and Methods:** Using a standardized health scoring system (Calf Health Scorer, University of Wisconsin-Madison, Madison, WI), calves were evaluated immediately at arrival to a commercial milk-fed veal facility in Ontario. Data on weight at arrival and the supplier of the calf were also recorded. The calves were followed until death or the end of their production cycle. Two Cox proportional hazard models were built to explore factors associated with early (< 21 d following arrival) and late mortality (> 21 d following arrival).

**Results:** A total of 4,825 calves were evaluated from November 2015 to September 2016. The overall mortality risk was 7%, with 42% of the deaths occurring in the first 21 days after arrival. An abnormal navel, an elevated level of dehydration, housing location within the farm, arriving in the summer and the presence of a sunken flank were associated with increased hazard of early mortality. Drover-derived calves and calves with a greater body weight at arrival had lower hazard of early mortality. Housing location within the farm, being derived from auc-

tion facilities and an abnormal navel, were associated with higher hazard of late mortality.

**Conclusions:** A significant number of calves are entering into the veal facility with identifiable health abnormalities representing a significant welfare concern. These results also demonstrate that risk factors for mortality can be identified at arrival which represents a potential opportunity to selectively intervene on these calves to reduce mortality. However, methods of preventing the development of these conditions prior to arrival needs to be explored and encouraged to improve the welfare of the calves entering the veal industry.

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EP-05

### Why leptospirosis continues to occur in workers of vaccinated dairy herds

#### Discovery of an unnoticed emerging zoonotic pathogen

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**Leptospira:** infection in dairy cattle and leptospirosis in dairy farm workers was common in New Zealand prior to the introduction of vaccination in the 1980's. Recent evidence of leptospirosis in dairy workers raised concerns about the level of protection from vaccination. A cross sectional study was therefore conducted from January – April 2016 to investigate reasons for potential vaccination failure.

Two hundred dairy farms were randomly selected from a population database. Twenty paired blood and urine samples were collected from adult cows of each of 200 farms (n=4000). Sera were tested by the Microscopic Agglutination Test for serovars Hardjobovis, Pomona, Copenhageni, Ballum and Tarassovi with a cut point equal to or greater than 1:48 being positive. Urine was tested using quantitative real-time PCR (qPCR) that amplify *gyrB* gene.

All but one herd had been vaccinated, 80% with a bivalent Hardjobovis/Pomona vaccine and 20% with a trivalent Hardjobovis/Pomona/Copenhageni vaccine. In total, 2.4% of cows and 27% of farms were urine qPCR positive. Overall 63% of cows were positive to one or more serovars, 44% for Hardjobovis, 28% for Pomona, 6% for Copenhageni, 3% for Ballum and 17% for Tarassovi. Of 94 PCR positive cows, 51 were serologically positive to Tarassovi which was the only serovar with a strong and positive association between MAT titre and the proportion of urine-PCR positive cows.

This paper demonstrated a high exposure to the previously unnoticed and emerging serovar Tarassovi which is likely to cause urine shedding of *Leptospira* in dairy cows in New Zealand. Exposed dairy farm workers notified with clinical leptospirosis were significantly more likely to test positive for Tarassovi than rural workers of other farms or abattoirs.



EP-06

### Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan

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**Objectives:** Leptospirosis is one of the most prevalent zoonoses caused by bacteria of the genus *Leptospira*. The most common causal species of bovine leptospirosis is *Leptospira* belonging to serovar Hardjo (*L. Hardjo*), and cattle are the maintenance host. Bovine leptospirosis is economically important due to reproductive losses and human leptospirosis cases caused by *L. Hardjo* relating to livestock occupations in Japan have not been reported, though it is common in New Zealand a dairy industrialized country, and might be neglected. Thus, it is important to control it on the aspects of both economic impacts on farming productivity and human public health. The objectives of this study were to investigate the sero-prevalence and risk factors for *L. Hardjo* infection of dairy herds in Yamagata Prefecture, the southern Tohoku, Japan.

**Materials and methods:** A cross-sectional study was designed to generate information on the herd level prevalence and the risk factors for *L. Hardjo* in Yamagata, the southern Tohoku, Japan. Bulk tank milk samples from 109 dairy herds were used to test the herd level sero-prevalence of *L. Hardjo* using a commercial ELISA kit, which detects both *L. interrogans* serovar Hardjo and *L. borgpetersenii* serovar Hardjo. A questionnaire survey was conducted at the sampled farms, and univariable and multivariable analyses were performed. For the univariable risk factor analysis at the herd level, comparisons between the herd level sero-positivity on each factor of the questionnaire were analyzed. The variables with a p-value < 0.2 in univariable analyses were investigated further for collinearity, and variables whose correlation coefficient were < 0.9 with any of the other variables, and variables that had biological plausibility were fed into a multivariable model as explanatory variables. For the multivariable analysis, a logistic regression was performed using a generalized linear model with binomial errors choosing the serological test results as the outcome variable. Step-wise model simplification was performed checking with a likelihood ratio test. Models with a biologically important variable which was removed by the model simplification were compared to the simplest model. To evaluate a model, goodness-of-fit tests by Hosmer-Lemeshow test and Akaike's Information Criteria (AIC) were used. Spatial clustering of *L. Hardjo* at the herd level was examined using spatial scan statistics.

**Results:** Seventy-one herds were found to be positive for *L. Hardjo*, and the apparent herd prevalence was 65.1% (95% CI: 56.2-74.1%). The risk factors for sero-positivity were larger herd size (OR = 1.08, 95% CI: 1.02-1.14,  $p < 0.05$ ) and cows with a history of staying in Hokkaido (OR = 15.79, 95% CI: 4.16-76.73,  $p < 0.01$ ), and a model with the lowest AIC contained presence of cats at the farm premises (OR = 0.33, 95% CI: 0.08-1.26,  $p = 0.10$ ) as a preventive factor though it was not statistically significant. The spatial scan statistic detected a most likely cluster (relative risk = 1.87, log likelihood ratio = 9.93, radius = 13.70 km,  $p < 0.01$ ) in the southern part of the study area where there are large herd sizes and farm density is high.

**Conclusions:** This study revealed that *L. Hardjo* is prevalent among large herds, particularly in the aggregated dairy production area in Yamagata Prefecture. The main source of disease introduction was purchases of cattle from and/or use of common grazing in Hokkaido. A further investigation into the epidemiology and economic burden in dairy herds in other parts of the country is urgently required to understand the potential public health impact as well as economic losses in the dairy industry. A coordinated control program for *L. Hardjo* in dairy herds should include vaccination and improvement of biosecurity and rodents control. Communication to farmers about the risk of infection should be started.

EP-07

### Meat inspection, variability depending on the veterinary inspector: a cross sectional study

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**OBJECTIVES:** According to legal regulations, all slaughtered in the European Union are subject to routine meat inspection at the slaughterhouses. A sufficiently high quality of meat inspection implies that the results of the inspection must not depend on the persons carrying out the examinations. Post-mortem pre-defined findings (lung and liver pathologies) obtained from the 7764 slaughter of pigs, cattle 20161 and 34062 sheep were analyzed by an Italian slaughterhouse. The data were collected following the post-mortem findings were routinely recorded by 17 official meat inspectors under real working conditions. The objective of the study at hand is the estimation of the amount of variation in these post-mortem findings attributed to the official meat inspectors.

**MATERIALS AND METHODS:** This Cross sectional study describes the prevalence of cases of diagnosed pathologies. The population under examination is given by pigs, sheep and cattle admitted to ordinary slaughter from 04/04/2011 to 30/04/2017 at an Italian plant, susceptible to exhibit lesions detectable by inspection. Exclusions: pigs with carcass weight <25 kg; sheep with c.w. <12 Kg; cattle aged <8 months. Inspection by definition is a census that can not suffer from selection bias. The post-mortem results derive from the records made at the slaughterhouse by 17 veterinaries who have observed 7764 pigs, 20161 cattle and 34062 sheep. The pigs have constituted 12,53%, the cattle 32,52% and the sheep 54,5% of the observations. Only hepatic and pulmonary pathologies detected during routine inspections are considered to be cases. Variables of outcome: liver pathologies, lung pathologies. Exposure variables: Time (06 years with 06 seasonal cycles to check seasonal variability). Veterinary inspector. Statistical analysis investigates the prevalence of cases of centrality and dispersion indices. There has been used STATA statistical software (Stata Corporation, College Station, Texas) in its version 14.0.

**RESULTS:** The individual contribution to the diagnoses of 17 veterinary inspectors has wide variability, the proportion of diagnosed cases by the various inspectors is on the average 19,34% for liver pathologies and 19,46% for lung pathologies, the median respectively 18,91% and 17,41%. As to the dispersion indices: the range ranges from 5% to 41% for both diseases.



es and SD respectively 10,92 for liver diseases and 10,36% for those of the lungs.

**CONCLUSIONS:** The inspection is conducted on the same legal basis throughout Europe, however many factors have an impact respect the inspection protocols. The sensitivity of post-mortem inspection is limited and it is influenced by personal aspects such as: training, skill, experience and application of the veterinary also depends on the degree of pathological alteration. The diagnostician capacity of the inspector is beside influenced by operative factors: the layout of the slaughter line, the speed of the line and the number of inspectors. Assuming the absence of "period effect" with regard to possible epidemiological variations of stock-farms, the study supported the hypothesis that the diagnostician method is exposed to considerable subjectivity, its amplitude confers external validity representing real conditions. The cross sectional model does not allow causal association between observed cases and variability of the diagnostician capacity of the inspector, however it generates evidences that justify further studies with models with greater intrinsic validity.

EP-08

### Antibody concentrations against gastrointestinal nematodes in adult beef cows from the prairie provinces of western Canada

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**Objective:** To describe the seroprevalence and quantity of serum anti-*Ostertagia* antibodies of adult beef cows from the western Canadian prairie provinces, using the SVANOVIR<sup>o</sup> *Ostertagia ostertagi*-antibody enzyme-linked immunosorbent assay (ELISA).

**Materials and methods:** Serum from 2,064 adult beef cows from 106 herds from Alberta, Saskatchewan, and Manitoba, were collected in the fall of 2014. Frozen (-80°C) serum samples were analyzed in duplicate for the presence of anti-*Ostertagia* antibodies using the SVANOVIR<sup>o</sup> *Ostertagia ostertagi*-Ab ELISA. Optical density values were standardized as an optical density ratio (ODR) using duplicate negative and positive control sera on each plate. Mean ODR were calculated for each cow. A cut-off point of 0.5 ODR was used to identify cows with high amounts of anti-*Ostertagia* antibodies.

**Results:** The mean cow ODR was 0.7 (Standard Deviation 0.3). Seventy-four percent (95% Confidence Interval 72-76) of cows had an ODR above the 0.5 ODR cut-off point suggesting a high amount of antibody.

**Conclusion:** This is the first study demonstrating the use of serology for determining anti-*Ostertagia* antibodies in adult beef cattle. Mean ODR in this study were similar to those seen in mature dairy cattle, but higher than mean ODR from young beef stock when compared to published literature. In this study, 74% of ODR were above 0.5, a value that correlated with reduced milk production in dairy cattle. The relationship between adult beef cow ODR and production indicators should be explored, for this test to be considered as a potential diagnostic tool.

**Funding:** Saskatchewan Agriculture and Food Grant, Beef

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EP-09

### The detection of the *Mycobacterium avium* subspecies *paratuberculosis* in environmental samples as a screening test to determine a herd's paratuberculosis status

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**Objectives:** Paratuberculosis (PTB), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is chronic enteritis that provokes infertility and a decrease in milk production. The amount of MAP in the feces (shedding level: SL) of MAP-shedding cattle progressively increases, and that of cattle with clinical symptoms is 10<sup>8</sup> CFU/g. Calves reared in an environment heavily contaminated with MAP by high-shedders are easily infected. Since, the prevention of MAP transmission requires the culling of low-shedders that have not yet become high-shedders. As the national PTB eradication surveillance in Japan, all targeted cattle are tested and culled at intervals of less than 5 years. To accelerate eradication, the test interval must be shortened because most cases occur between 3-5 years of age. However, more frequent testing of individual cattle is hampered by a shortage of labor. It has been reported that the detection of MAP in environmental samples by quantitative PCR (ENV-qPCR) is simple and cost-effective. This study evaluated the efficacy of ENV-qPCR as a screening test to determine a herd's PTB status.

**Materials and Methods:** Environmental sampling was conducted in 29 herds at the point of detection of MAP-shedding cattle. Ten environmental samples were collected with waste paper from the floor of a manure storage area of each herd. Each sampling point consisted of a square 15 cm by 15 cm. MAP DNA was detected by qPCR in pools of 5 environmental samples. Associations between the probability of MAP DNA detection (Pd) and SL, number of MAP-shedding cattle, herd size, and breed (beef or dairy) were statistically evaluated using logistic regression models.

**Results:** The sensitivity of ENV-qPCR was calculated to be 66% (19 of 29 herds). MAP DNA was detected in all 16 herds including  $\geq 7.0 \times 10^1$  CFU/g MAP-shedding cattle. The results obtained from our logistic regression model showed that Pd significantly increased with increasing SL of MAP-shedding cattle ( $P=0.022$ ), and that the SLs of MAP-shedding cattle associated with 10%, 50% and 90% Pd were  $1.2 \times 10^1$  CFU/g,  $3.1 \times 10^1$  CFU/g and  $7.8 \times 10^1$  CFU/g, respectively. On the other hand, the results obtained from the present logistic regression model showed no significant associations between Pd and number of MAP-shedding cattle ( $P=0.996$ ), herd size ( $P=0.547$ ), or breed ( $P=0.913$ ).

**Conclusion:** The SLs were founded to be lowly infectious, because they were lower than SL of cattle with clinical symptoms, and because  $3.0 \times 10^5$  CFU has been reported to be the lowest confirmed infectious dose for young calves. These results indicate that ENV-qPCR will make it possible to detect MAP in herds including lowly infectious cattle. The present results sug-



gest that repeated ENV-qPCR can contribute to the progress of the PTB eradication program in Japan. (The present study was published in Journal of the Japan Veterinary Medical Association, Sakakibara et al, 2017.)

#### EP-10

### Incidence of fecal excretion of *Mycobacterium avium* subsp. *paratuberculosis* in dairy cows before and after the enrolment in the Québec Voluntary Program

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**Introduction:** Paratuberculosis is a chronic and contagious enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). This disease of worldwide distribution is responsible for significant economic losses and the etiological agent has been linked to human Crohn's disease. Paratuberculosis control programs focus on reducing MAP transmission by implementing better management practices that target infection routes. In Québec, a Voluntary Paratuberculosis Prevention and Control Program (QVPPCP) was launched in 2007. Upon enrollment, producers complete a risk assessment questionnaire to evaluate the risk of MAP introduction and transmission. No sampling is performed the first year of enrollment. The following years, the veterinarian evaluates the application of the recommended measures and environmental samples are collected.

**Objectives:** The objectives of this cohort study were to: 1) describe changes of the incidence density rate of individual fecal excretion of MAP in 2 cohorts of cows – cows born before and after farm enrolment in the QVPPCP; 2) determine the impact of the risk score of within-herd transmission of MAP (measured by a risk assessment questionnaire) on the incidence of individual fecal excretion of MAP; and 3) evaluate the impact of calf rearing management practices on the incidence of individual fecal excretion of MAP.

**Materials and methods:** Eighteen MAP-positive herds enrolled in the QVPPCP were visited annually from 2011 to 2015. At each visit, individual fecal samples from all adult cows were collected. MAP was cultured using the MGIT Para TB culture media and the BACTEC 960 system. A risk assessment questionnaire was completed upon enrolment in the QVPPCP and at each visit. Cox proportional hazards models were used to estimate the hazard ratios (HR) for the exposure variables. Herd clustering was taken into account using robust standard errors.

**Results:** A total of 2158 cows were included (cohort born before  $n=919$ ; cohort born after  $n=1239$ ). The incidence and haz-

ard of fecal excretion were significantly lower for the cohort-after than the cohort-before (incidence rate ratio=0.38; 95% CI: 0.18-0.78 and HR=0.48; 95% CI: 0.23-0.98). The HR of fecal excretion for cows exposed to a high RAS was 2.20 times (95% CI: 1.21-3.99) that of cows exposed to a low RAS. Poor calving cow hygiene (HR=3.41; 95% CI: 1.40-8.31) and contact between pre-weaned heifers and adult cows or their feces were significantly associated with an increased hazard of fecal excretion of MAP (HR=2.66; 95% CI: 1.08-6.56).

**Conclusions:** Our results suggest that enrolment in the QVPPCP reduces the risk of individual MAP fecal excretion. Contact between calves and adult cows or their feces increases MAP transmission.

#### EP-11

### Serological detection of *Mycoplasma bovis* in dairy herds using calf blood samples collected from abattoirs

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*Mycoplasma bovis* is an important pathogen in dairy herds causing a variety of clinical signs in young calves and mastitis in the milking herd. Detection of the pathogen using routine microbiological techniques can be difficult and unreliable. Serological testing of milking cow blood can demonstrate exposure to the pathogen, however the practicalities of sample collection can be problematic. The interpretation of results can also be complicated. Serological testing of blood collected from calves slaughtered within the first weeks of life provide an indication of maternal serological status of the herds of origin. Sampling of blood from calves at abattoirs has substantial logistic advantages. Relatively large numbers of herds can be sampled efficiently and the herds of origin can subsequently be identified by reference to identification ear-tags applied to the calves prior to departure from the originating farm. Collection of blood samples from multiple calves immediately after slaughter is quick, easy and does not involve handling of individual live calves.

Blood samples were collected immediately after slaughter from 327 very young dairy calves processed at a regional abattoir in south eastern Australia. Dairy calves processed at this abattoir are predominantly aged between 5 and 30 days at slaughter. The 327 calves samples in this study originated from 166 dairy herds, identifiable using the Australian National Livestock Identification System. The number of calves sampled from individual herds ranged between 1 (88 herds) and 10 (1 herd). Serum samples were tested with an indirect ELISA to detect *Mycoplasma bovis* specific maternal antibodies passed to the calves through ingestion of colostrum. In herds in which more than one calf was sampled, not all calves were serologically positive from *Mycoplasma bovis* antibodies. Antibodies to *Mycoplasma bovis* were detected in at least one calf from 51 of the 166 herds tested.

Although the number of calves sampled from each individual herd was very low, the detection of antibodies in calf serum is a reliable indicator of the presence of antibodies in the milk of cows in that herd. As such, this study is likely to under-estimate



the occurrence of *Mycoplasma bovis* in local dairy herds.

This study confirms the diagnostic value of using culled calves as a source for serological surveillance of infectious disease in herds of origin. The results of this study suggest that *Mycoplasma bovis* may be more common as a pathogen in Australian dairy herds than previously reported.

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EP-12

### Searching for anthrax in the clouds

#### An anthrax early warning system for Victorian dairy farmers

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Anthrax is a world-wide disease caused by *Bacillus anthracis*, a resilient, spore-forming bacterium that primarily affects herbivores although is infamously zoonotic. Anthrax was first recorded in livestock in Australia in 1847; reportedly introduced in contaminated fishmeal fertilizer. It spread south following historic stock routes with recent major Australian outbreaks in 1997 (83 farms) and 2007 (10 farms) in the Goulburn-Valley district and 2017 (5 farms) in Swan Hill, Victoria. Today, many Victorian farmers resist prevention measures (primarily vaccination) as they are believed to decrease herd production and fertility. Profound social and media stigma associated with the infection also affects willingness to report suspect cases. If, as anecdotal evidence purports, disease incidence is associated with climatic conditions, meteorological data could predict the periods of greater risk offering more effective and efficient application of control measures and promote targeted compliance, especially if delivered through a trusted communication route.

This project modelled historic daily meteorological data from each shire in Victoria and New South Wales to predict high risk periods based on all confirmed regional diagnoses from 1958. The model parameters were then applied to current daily weather data in each shire to predict the odds of a case over the coming 30 days. Concurrent to the building of the model, the social impact of anthrax and farmer engagement with disease prediction was assessed. This was then used to guide the design of an optimal tool to best demonstrate the daily risk and improve outcomes.

**Predictive model:** Cumulative rainfall and mean maximum and minimum daily temperature data from each shire were summarised in incremental durations from 10, to 100 days preceding both the start of the shire-year risk period or the case-date respectively. Absolute values, deviations from the annual mean value and transformed values were tested in a generalised linear mixed effect model where shire was fitted as a random effect to account for climatic differences between shires. Model selection was led by the need to maximize the predictive performance of the model and was quantified using the area under a receiver operating characteristic (ROC) curve. Shires were given a spatial weighting based on frequency of previous cases and previously modelled geological risk.

Greater odds of disease were found in shires-years that had cooler, wetter springs (OR = 0.56 (95%CI 0.37-0.81) and OR = 0.99 (0.99-1.00) respectively) and warmer minimum temperatures in early summer as well as following 30-day periods of warmer than average minimum temperatures (OR = 1.55 (95% CI 1.27-1.91) and OR = 1.64 (95% CI 1.25-2.18) respectively).

**Communication route:** To determine the most effective platform to demonstrate the outcome of this predictive model, a sample of Victorian dairy farmers volunteered to participate in a Design Thinking process that included in-depth interviews and structured post-interview interpretation. Distinct themes that reflected findings from the composite interviews included geographically distinct perceptions of risk, a profound sense of social isolation following a diagnosis and an implied resistance to report fallen stock due to previous management of infected premises. Farmers cited public perceptions of bioterrorism coupled with a sense of powerlessness to control the disease as primary reasons for its elevated profile. Highest engagement with disease predictions was reported if information was received from more than one of their farm network and not simply through a web application. However, key farm network representatives preferred visual cues delivered through a web application hosted by official State Departments.

Preliminary release of the Anthrax Early Notification System web application to farm network representatives provides timely coverage of an overlooked issue in the industry, especially following the recent outbreak in Swan Hill, Victoria. It heralds a refocus by Victoria on how it engages with farming communities regarding anthrax in livestock and can inform the department of the future viability of lightweight, targeted disease forecasting models.

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EP-13

### DAGs - Directed Acyclic Graphs as a tool for Buiatricians to examine causal inference.

#### Appraising information – a key component of evidence-based advisory practice.

\*Mark Andrew Burgemeister

Thinking Cows Pty Ltd

One of the oldest questions to puzzle humankind is 'how do we know that **a** causes **b**'?

As practical cattle Veterinarians, working with animal carers to be trusted sources of evidence-based advice, we need to consider this point in detail. This is increasingly important as we move from individual animal to population level advice – and our business clients know this as they have large investments 'riding on our advice'!

As evidence-based Buiatricians interpret and generalise scientific information from journal papers, scientific communications and company information – 'how do we know or how could we predict that implementing **A** will cause **B** to occur?' How do we interpret the 'true measure of effect' in the results of a study presented to us? How do we assess our level of doubt and develop questions to prompt the answers we need?

In at least the last decade, epidemiology has made big advanc-



es in the examination of and accounting for bias and confounding in scientific studies - especially through the use of Directed Acyclic Graphs (DAGs). These visual figures allow examination of how various factors impinge on each other as causes move to effect – and it is important for Buiatricsians to have an appreciation of this as they develop their evidence-based conclusions.

Although they might seem complicated at first sight, this short presentation will strive to show how 'looking for a DAG at the start of a presentation or scientific communication' will reduce the risk of coming to spurious conclusions of cause and effect – and provide a powerful argument against 'p-value hunting'. Interrogation of evidence presented to Buiatricsians by seeking to 'understand the DAG' is a key component of time-efficient and scientifically robust critical appraisal of information.

HH-01

### **Construction of a framework for 'better evidence based recommendations' in a dairy herd**

\*Dorte Bay Lastein

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The provision of 'evidence based veterinary service' to support dairy herd management is complicated because each herd usually requires a specific solution (context-specific knowledge). The aim of this study was to construct a framework that can guide cattle veterinarians (and other dairy consultants) to structure and enhance their professional work and development of veterinary services to fit the needs of the increasingly automated and data-wise complex dairy industry of tomorrow.

The construction of the framework is inspired by Sackett et al.'s definition of "Evidence Based Medicine: what it is and what it isn't" (1996). Quantitative and qualitative studies of Danish cows, dairy farmers and veterinary health advisors were performed during a Ph.D. project from 2007-2012. I used semi-structured interviews to collect 12 herd veterinarians' views on motivation to collect data and give advice to dairy farmers and I observed the veterinarians' practical procedures during joint herd visits.

The concurrence of views and observations demonstrates that a major component of veterinary advisory service is a delicate balance between legal constraints and business aspects. The commercial (private business) Danish veterinary services are regulated by the veterinary authorities. However, laws change frequently according to demands of society (e.g. animal welfare and antimicrobial resistance) and national health treats (e.g. risk of foot and mouth disease). The demand for veterinary service is governed by farmers' needs (e.g. tradition, personal perceptions, and agricultural and economical conjunctures). But to run and develop their advisory business, herd health advisors with an academic and scientific training are expected to issue recommendations according to 'evidence based principles'. In a dairy context, evidence-based advice means a conglomerate between the advisor's personal experience and expertise (e.g. tacit knowledge and personal preferences), the farmer's demands (e.g. production optimization and/or personal lifeworld), cows' need (e.g. welfare), best general scientific knowledge (e.g. scientific literature on general biological associations) and best local knowledge (e.g. herd-specific analysis, simulation and trials). The 'better evidence based advice/recommendation' for any given herd-specific question can be issued if the entities described above are recognized and the potentials within each are explored and can be synthesized into meaningful recommendations.

The conclusion is that none of these entities should stand alone (e.g. neither experience nor simulation, neither needs nor number). The integration of evidence from different sources is what makes the advice better. In addition, we conclude that not all entities suit for all problems; the advisor must listen, use systematic procedures and learn when to work for stronger evidence (e.g. change from clinical towards epidemiological principles), but also sometimes work towards more meaningful human goals instead (e.g. change from production optimization to reducing farmers workload aiming at more time with the family). The advisor must learn and appreciate that change towards better 'evidence based recommendations on farm level' first of all happens in the minds of the



farmers – and themselves.

HH-02

### Veterinarian communication on herd health: a feasibility study of Motivational Interviewing and farmer change language

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**Introduction:** Motivating farmers to adopt veterinary advice remains a critical challenge to improving herd health and welfare. In the medical and psychological sciences, practitioners are increasingly employing an evidence-based communication methodology called Motivational Interviewing (MI) to promote client engagement in discussions on behaviour change (1). This paper presents the outcomes of a feasibility study of brief MI training for cattle veterinarians and their dairy clients.

**Objectives:** To examine (i) the effect of brief MI training on veterinarian use of MI consistent and MI inconsistent communication behaviours and (ii) the effect of brief MI training on farmer change language.

**Methods:** Practicing cattle veterinarians (n=14) attended brief MI training and recorded an audio file of advisory communication on 'any change for the benefit of herd health' before and after the experience. Anonymised audio files (n=31) were analysed in Noldus Observer 5.0 using the MI Treatment Integrity code (2) to capture veterinarian advisory communication behaviour and the Client Language Assessment in MI code (3) to capture farmer change language. Data were analysed using SPSS 21.0 to determine whether communication behaviour(s) altered significantly post-training.

**Findings:** A brief MI training experience led to a significant ( $p=0.001$ ) increase in veterinarian use of MI-consistent skills, a significant ( $p=0.009$ ) decrease in veterinarian use of MI-inconsistent skills and a significant ( $p=0.006$ ) increase in positive farmer change language.

**Discussion:** Using these data, critical insight will be provided on how veterinary communication styles influence farmer motivation and the efficacy of MI to enhance farmer engagement in discussions of herd health.

(1) Miller WR and Moyers TB (2017). Motivational Interviewing and the Clinical Science of Carl Rogers. *Journal of Consulting and Clinical Psychology*. 85 (8): 757-766.

(2) Moyers TB, Manuel JK and Ernst D (2014) *Motivational Interviewing Treatment Integrity (MITI) Coding Manual 4.2.1*. Unpublished manual.

(3) Miller WR, Moyers TB, Manuel JK, Christopher P and Amrhein P (2008) *Client Language Assessment in Motivational Interviewing (CLAMI)*. University of New Mexico; Centre on Alcoholism, Substance Abuse and Addictions (CASAA).

HH-03

### Evaluating key performance indicators to monitor beef herd production

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**Objectives:** Monitoring performance is crucial to any business, and whilst key performance indicators (KPIs) are used relatively widely in the dairy, pork and poultry sectors in England, traditionally use in the beef sector has been more limited. More recently, uptake of such tools in the beef industry has been increasing, partly due to increased environmental and financial pressures on the sector. The data and evidence behind these KPIs however is limited, in part due to practical limitations of collecting data sets of sufficient size, and of allowing for the many confounding variables affecting herd performance and overall farm success. The objectives of this project were:

- 1) To appraise farmer attitudes to monitoring herd performance through data collection and analysis.
- 2) To provide guidance for farmers and vets around the choice and use of performance indicators on farm.
- 3) To provide evidence on how selected performance indicators affect overall farm success.

#### Materials and methods:

**Objective 1:** Focus group discussions with a small group of beef farmers (both sucklers and grower/finishers), beef advisers and industry experts were used to evaluate attitudes towards monitoring herd performance through data collection and analysis. In order to incorporate a wider variety of opinion, a survey was distributed to beef farmers across the UK via Survey Monkey, and 143 responses were collected.

**Objective 2:** Through focus group discussion and analysis of herd performance data from a small group of farms, the practicalities around KPI use on farm were investigated (for example data collection, extraction of data from software, and data presentation methods). Performance indicators were also scored and ranked, allowing them to be structured into a 'toolkit'.

**Objective 3:** Data from a larger selection of farms over multiple years was used to investigate relationships between performance indicators in the toolkit and net margin using multiple regression. This was expanded on by developing a stochastic simulation model of a beef system in order to further define these relationships.

#### Results:

**Objective 1:** Farmers value their data and would often like to make more of the data they already have rather than collect more. Farmers also perceive data analysis to be slightly more challenging than data collection. Of those farmers that would like to record more, weight data was the most common type of data they would like to collect.

**Objective 2:** Due to the diversity of the beef industry, a KPI 'toolkit' was generated allowing farmers and advisers to select appropriate performance indicators according to herd targets and current performance. It is structured in a hierarchical fashion with comprehensive performance indicators at the top (those covering multiple aspects of production) and more specific indicators underneath. This provides a starting point for



discussion on farm, and structure for a decision making pathway where comprehensive KPIs are used to monitor overall performance and more specific performance indicators are used to investigate problems in more detail.

**Objective 3:** Using data from 56 suckler farms over 3 years, several performance indicators were identified as being significantly associated with net margin per cow bred, including age at first calving, cow to bull ratio, scanning percentage, pre-weaning mortality rate, daily liveweight gain up to weaning, stocking rate and cow size. Limitations on size and content of this data set led to a simulation model being developed to further investigate these relationships. It is anticipated that by the time of the conference this will have been used to further define the relationships between these (and various other) performance indicators and overall enterprise success.

**Conclusions:** Farmer and veterinary interest in performance indicators in the beef industry is increasing with increased financial and environmental pressure on the sector. The complex and confounding nature of the farm system makes cause and effect of input and output variables of the system challenging to study, leading to limited evidence around the relationship between performance indicators and overall farm success. This project is adding to the existing evidence, allowing farmers and vets to make more informed management decisions to improve beef production efficiency.

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HH-04

## Evaluation of Metrics for Benchmarking Antimicrobial Use in Dairy Cattle

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**OBJECTIVES:** Good herd health involves farmers and veterinarians working together to manage cow health and ensure cattle are well cared for. Herd health, when viewed through the lens of antimicrobial use (AMU), is particularly important to the livestock industries to encourage prudent use of antimicrobials (AMs). Several metrics for quantifying AMU are defined and in current use, although all give different interpretations. A useful metric should be understandable and relevant to the veterinarians and farmers who prescribe and use AMs, meaning that clear methods, assumptions (and possible biases), standardised values and exceptions should be published. Particularly relevant are assumptions around the number and weight of cattle at risk of treatment and definitions of dose rates and course lengths; incorrect assumptions can mean metrics over- or under-represent AMU. This work reviews, compares and makes recommendations about a selection of AMU metrics used across the dairy industry worldwide.

**MATERIALS AND METHODS:** Five types of metrics for AMU were described:

**1) Total mg** of active substance is simple to calculate and easy to understand. However, it ignores variation in dose rates

across AMs and differences between farms and veterinarians.

**2) Total mg/kg** improves on total mg by dividing the mass of medicine by the total weight of cattle at risk of treatment, therefore accounting for variation in cattle numbers and weights across farms.

**3) Daily dose metrics** divide the total mg of medicine by both total animal weight and an estimate of daily dose. These metrics help overcome the issue of variation in AM doses. As well as using actual or standard weights for animals at risk of treatment, daily dose metrics can use “*actual*” daily doses (farm-specific) or “*defined*” daily doses (recommended or standard doses).

**4) Course dose metrics** assign the number of courses an animal receives, incorporating daily dose and course length. As with daily dose metrics, if actual dosages, course lengths and cattle weights are used, these produce the most accurate course dose metric for each unit (farm, veterinarian, etc.).

**5) Cow Calculated Course (CCC)** is a metric conceived in the UK that uses course length and dosing regimen as per UK documentation as well as number of cattle on the holding. CCC splits out medicine use into youngstock and adult stock by assuming certain products are only used in certain groups.

To illustrate the different metrics, data on AM use for a 12-month period from farms enrolled in UK AMR research were compared.

**RESULTS:** Analysis of UK farms confirmed different rankings by various metrics for total AM use, although they were largely similar for use of highest priority critically important AMs (HP-CIAs). Further details can be found at <https://www.biorxiv.org/content/early/2017/09/15/186593>.

Each metric presented is in common use, although work in the UK and Netherlands suggests the need for specific metrics to be chosen and used consistently. Metrics need to be clearly explained so users understand the data, assumptions and biases behind the calculations. For a metric to be useful, it must be accurate and comparable at the unit level for benchmarking. This ideally means considering varying cattle numbers and weights as well as different management systems and not penalising use of medicines with higher mg/kg dose rates. All metrics also require use of accurate, representative and validated data.

**CONCLUSION:** Many metrics have been presented and none is perfect. Veterinarians and farmers may want to use a variety of metrics to compare AMU. The most elucidating metrics for the dairy industry are country-specific versions of daily dose and course metrics using actual (or country-estimated) cattle and youngstock weights with actual (or country-specific) treatment regimens for medicines currently licensed in that country. Country-level metrics for livestock also need clear assumptions for determining the number and type (specifically age) of animals at risk of treatment. The ideal metric would use doses and courses specific to the unit and use actual cattle numbers and weights.

To prevent a shift towards HP-CIAs to achieve an overall mg/kg target, there should always be a separate calculation for HP-CIAs. In the drive to keep herd health high and reduce AM resistance, it is necessary to recognise that, in some instances, using more mg of medicine (e.g. moving from fluoroquinolones to tetracyclines) may be beneficial.





HH-05

### Influence of calf characteristics to the calf mortality in calf rearing units in Finland

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**Objectives:** In Finland beef production is mostly based on dairy bull calves. Calves are commonly transported from the dairy farms to the calf rearing units at 2–4 weeks of age. After 5 months growth period weaned calves are transported to the beef finishing farms. Calves are dairy breeds and dairy breeds mixed with one of the meat breed. In calf rearing units bovine respiratory disease is a most common reason for treatments. Our study objective was to find the factors affecting mortality in Finnish calf rearing units.

**Materials and Methods:** A total of 60 calf rearing unit from different parts of Finland were randomly selected to the study. National cattle herd register data and transportation data from meat companies was used. Calves were transported to these selected calf rearing units between January and October 2016 (n= 20 919) and followed 180 days. Multivariable logistic regression model was used to evaluate risk factors for mortality: breed and three continuous variables; age at the time of transportation, size of the birth farm and calf mortality in the birth farm of the calf. A calf rearing unit was included as a random factor. Different breeds represented in the data were Holstein (n=9075), Ayrshire (n=8586), mixed breed Aberdeen Angus (n=388), mixed breed Limousin (n=752), mixed breed Blonde d'Aquitane (n=1354) and other breeds (n=564) calves. Weight of the calf at the time of transportation was excluded from the model due to correlation with age of calves.

**Results:** The overall calf mortality in the calf rearing units in Finland was 6.01% (1.70–22.33%) during 180 days rearing period. Odds for untimely death was higher for Ayrshire breed 7.2% OR: 1.93 (P<0.005), mixed breed Limousin 15.43% OR: 4.92 (P<0.005) and mixed breed Blonde 8.05% OR: 2.17 (P<0.005) compared to Holstein breed 4.06% (ref level OR=1). Odds for untimely death did not differ significantly between Holstein calves and mixed breed Angus 3.23% OR: 0.84 (P=0.473). Calves transported in older age have also lower risk of untimely death in calf rearing unit OR: 0.98 (P=0.006). Also a trend can be seen so that low calf mortality in the birth farm lowers the odds for untimely death in the calf rearing unit OR: 1.01 (P=0.056). Size of the herd where the calf was born seems not to affect the risk of death in calf rearing unit.

**Conclusions:** Holsteins and Ayrshires are the most common breeds in calf rearing units. For unidentified reason Ayrshire, mixed breed Limousin and mixed breed Blonde d'Aquitane calves seem to have significantly higher mortality compared to Holstein calves. Further studies should be carried out to study this phenomenon. Also calves transported from the dairy farm in older age are in lower risk of death in calf rearing unit.

HH-06

### Biosecurity Practices in Canadian Dairy Herds

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**Objectives:** Biosecurity practices aim to minimize the spread of diseases between animals within a farm and between farms. The Canadian dairy industry wants to implement a National Standard to achieve consistent biosecurity on dairy farms across the country. It is unclear how widely biosecurity practices are currently adopted. The objective of this study was to describe adoption of biosecurity practices in Canadian dairy herds.

**Materials and Methods:** A bilingual questionnaire was distributed online (Qualtrics, Utah, USA), by mail or over the phone to Canadian dairy farmers to assess, amongst others, biosecurity practices. Biosecurity practices included animal health management, animal addition and movement, sanitation, and human and equipment movement. Logistic and multinomial logistic regression models were used to identify associations between demographic variables (geographical region, herd size, and type of housing) and biosecurity practices. Statistical analyses were conducted using SAS Studio 3.6 (SAS Institute, Cary, USA).

**Results:** A total of 1,373 dairy producers across Canada participated in the study from March 1 to April 30, 2015. Herds were classified as very small (< 45 cows; 21%), small (45 to 65 cows; 23%), medium (65 to 100 cows; 25%), and large (> 100 cows; 31%). Respondents housed their milking cows in freestall (41%) and tiestall barns (59%).

**Animal health management.** If 93% of the respondents kept a health record, only 45% reviewed the occurrence of diseases in their herd at least once a year. Respondents reported having protocols in place for lameness (49%), mastitis (93%), retained placenta and metritis (73%), respiratory disease (56%), pink eye (15%), and calf diarrhea (54%), which varied by geographical region. Infectious diseases that most respondents were trying to prevent from entering their herd were bovine viral diarrhea (BVD; 58%), and Johne's disease (51%). Infectious diseases respondents wished to eliminate or control from their herd were *Staphylococcus aureus* mastitis (69%) and digital dermatitis (48%). Forty percent of the respondents reported having at least one lactating cow that died or was euthanized for an unknown reason, and 24% of them reported having a post-mortem performed.

**Animal addition and movement.** Forty-one percent of the respondents had a closed herd (no introduction or reintroduction of animals in the herd). In herds where animals were added, 75% inquired about the disease status of the herd the new animal originated from, but only 25% tested the new animal for diseases, mainly for contagious mastitis pathogens, *Neospora*, and bovine leucosis virus (BLV). Segregation and vaccination of a new animal was used, most of the time or always, by 24% and 40% of the respondents, respectively. Larger herd were more likely to use segregation and vaccination than smaller herd.

**Sanitation.** Eighty-three percent of the respondents ensured cow udders and lower legs were free of manure before calving, and 76% of them cleaned out, sanitized, and re-bedded the



calving pen after calving. They were both more common in smaller and tiestall operations than in larger and freestall operations, respectively. Sixty-four percent of the respondents never used the same equipment to handle both manure and cattle feed, and 39% of the respondents sending their animals to pasture prevented their animals to graze pasture where manure has been spread in the same growing season.

**Human and equipment movement.** There was very little control for farm access: 2% of the operation had a gated main entrance, 14% had biosecurity signage, and 4% locked their doors when staff were not working in the barn. Employees were required to use boots and coveralls designated for the farm in 53%, and 37% of the operations, respectively. Consultants and visitors were required to use boots and coveralls designated for the farm, most of the time or always, in 62%, and 33% of the operations, respectively. Thirty-seven percent of the operations shared farm vehicles or equipment with a neighbor's farm, and was more common on smaller operations than larger operations.

**Conclusions:** This study allowed us to identify biosecurity practices that were less adopted by Canadian dairy farmers, as well as farm characteristics associated with the adoption of the practices such as geographical region, herd size, and type of housing. This knowledge is important for building consistent biosecurity in the dairy industry.

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HH-07

### **Integrating the use of medicine audits into herd health planning to achieve the cessation of highest priority critically important antimicrobial use alongside improved production, health and welfare parameters in dairy cows**

**A study of seven commercial dairy farms which have achieved responsible and sustainable antimicrobial use through farmer engagement and herd health planning**

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**OBJECTIVES:** While veterinarians and farmers are aware of scientific, public and political concern regarding antimicrobial use in the dairy industry, particularly antimicrobials that are 'highest priority critically important to human health' (HP-CIAs), many influences may preclude changes in prescribing and on-farm antimicrobial use, including concerns of reduced animal health and welfare or production losses.

This study demonstrates how engaging farmers in proactive herd health management and using medicine auditing processes to inform farm-specific changes in antimicrobial prescribing alongside herd level husbandry changes can result in cessation of HP-CIA use at farm level without an associated reduction in animal health and welfare or a deterioration in production parameters.

**MATERIALS AND METHODS:** Farmers from seven dairy farms in South West England were engaged in an active pro-

cess of changing antimicrobial use on their farms through farmer education, herd health planning and farm-specific medicine auditing from 2010-2015. Prescribing data for analysis were collected from veterinary medicines sales records; health and production data were accessed via farm-recorded and milk recording data. Data were analysed for trends and 95% credible intervals were calculated to evaluate changes in antimicrobial use and health parameters.

**RESULTS:** The number of animal daily doses (ADD) of HP-CIAs prescribed to farms in 2010 accounted for 41% of doses of all antimicrobials, including 57% of intramammary preparations. The use of HP-CIAs was reduced until all HP-CIA use was ceased in 2015; the use of HP-CIAs in intramammary tubes ceased in 2014. The ADD of all antimicrobials prescribed to the study farms was lowest in 2015.

Production parameters, including 305-day yield, remained stable throughout the six years of the study. Many reproductive health parameters improved over the study period including calving index, calving to conception interval and 100-day in-calf rates. Clinical mastitis case rates decreased on five of the seven study farms, and clinical mastitis cure rates increased across six of the study farms. Mobility scores indicated that lameness rates on all study farms decreased over the study period while culling rates remained stable.

**CONCLUSION:** Utilising medicine auditing processes to guide herd health management changes - including antimicrobial prescribing and use - alongside a focus on improved animal husbandry made it possible to reduce overall consumption of antimicrobials on farms as well as cease use of HP-CIAs. Responsible and sustainable antimicrobial use changes can be made whilst maintaining and improving cow health, welfare and production. This study also demonstrates the need to consider different metrics when analysing antimicrobial use data, making sure to include dose-based metrics as well as those of total quantities in order to allow better representation of the direction and magnitude of changes in antimicrobial use.

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HH-08

### **Accuracy and agreement between human observed Body Condition Scores (BCS) and an automated BCS system**

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The Body Condition Score (BCS) of a dairy cow is an assessment of the proportion of body fat that the animal possesses. BCS change can be used to assess mobilization of energy reserves and thus has important implications for cow performance and management. Current methods for BCS assessment are subjective, time-consuming, require trained personnel, and may not be consistent over time. Automated systems offer significant potential to inform improved BCS management by



providing regular objective measures.

The BodyMat F (BMF) (Ingenera, Switzerland) consists of a laser that projects several lines onto the loin area of a cow. A 2D camera simultaneously captures an image of the area of the cow where the laser lines have been projected. This method of image capture allows for accurate depth analysis. These images are processed with a dedicated algorithm to estimate cow BCS based on the body shape of the imaged area.

The study reports an experiment to validate the BMF. The experimental farm used in the present study has a production system based on the intensive utilisation of grazed pasture. This study assessed 105 mostly Holstein Friesian cows with the BMF. For validation, a score derived by the BMF from a single scan was compared with the mean of two scores assigned by two trained human observers (gold standard).

The BMF initially used a 0-5 point scale which is predominant in France. Analysis indicated that the conversion initially used to transform the 1-5 scale used in Ireland where the experiment occurred was poor. This was evidenced by poor agreement statistics with the gold standard (CCC=0.27) but relatively strong Spearman's rank correlations. The Spearman's rho between the human observers was 0.71 and between the human mean and the BMF it was 0.65. The Spearman's rho results indicated the system output was co-varying with variation in human mean scores but was not in agreement on the exact scores as indicated by the concordance correlation coefficient. The conversion was recalibrated and the agreement analysis was then rerun. A CCC of 0.66 resulted which compared well to the human versus human observer agreement of 0.7. Only BMF scores based on single scans were matched to mean human observer score for this analysis. As regular repeated measures will be available when a BMF is installed on a commercial farm, averaging of multiple BMF score may improve the reliability of the BMF output further.

Overall, the BMF demonstrated an ability to accurately rank cows by BCS. Importantly from a management perspective, the output was in good agreement with the gold standard measure compared to the inter-human observer agreement. The regular and automated generation of cow BCS score by the BMF is thus likely to be of value for dairy farmers. If averaging of repeated measures can be applied to the output farmers receive, this will likely improve the usefulness of the tool further.

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HH-09

### **Characterising the relationship between rumination time and peripartum disease events in pastured Australian dairy cattle using an automated health monitoring system.**

\*Alexander J Crosbie<sup>12</sup>

<sup>1</sup>The University of Melbourne, <sup>2</sup>The Vet Group

Characterizing the relationship between rumination time and peripartum disease events in pastured Australian dairy cattle using an automated health monitoring system.

**Objectives:** The rapid pace of development in sensor technologies has provided the ability to remotely monitor the health and reproductive status of dairy cattle. While the efficacy of

these systems in identifying disease events has been demonstrated in housed dairy cattle, research in pastured herds such as those typically found in southern Australia is more limited. The objective of this study was to determine if significant relationships existed between sensor-derived measurements of rumination time and peripartum disease events in dairy cattle managed under Australian conditions.

**Materials and methods:** A prospective, observational cohort study was undertaken in which primiparous and multiparous Holstein cows (n=168) were subjected to physical examination by a veterinarian at 4-6 days in milk to determine the presence of post-partum health disorders. All cows were fitted with an automated remote monitoring device, the SCR HR-LD tag (SCR Engineers, Netanya, Israel) by the herd manager, and two-hourly records of rumination and activity recorded from 10 days prepartum to 21 days post-partum. Multivariate analysis was performed on these data to determine if there were significant changes in pre and post-partum rumination time in cows diagnosed with subclinical ketosis, metritis, endometritis, retained foetal membranes and left sided displacement of the abomasum.

**Results:** Significant declines in rumination time were present in the five days prior to diagnosis animals affected by left displaced abomasum, metritis and subclinical ketosis. No significant relationship was observed between two-hourly or daily rumination time in cows affected by retained foetal membranes or endometritis. Declines in prepartum rumination time were also noted in cows that subsequently developed left displaced abomasum and subclinical ketosis.

**Conclusions:** Preliminary findings indicate that these technologies may have applications in pasture-based dairy systems for the timely identification of peripartum health disorders, and may be a tool for improving the management of early lactation cows in seasonally calving dairy herds. Rumination time in pastured dairy herds appears to have a similar relationship to disease events as is observed in housed cattle consuming a substantially different diet. Further analyses are being undertaken to characterize the relationship between sensor measurements of activity and peripartum disease events.

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HH-10

### **Influence of pre-partum rumination times on the occurrence of diseases and level of production in dairy cows**

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**Objectives:** We monitored the rumination time before calving in multiparous dairy cows in order to examine its influence on the occurrence of clinical diseases, culling rate and performance in the subsequent lactation.

**Materials and methods:** The study was carried out on a commercial dairy farm and involved 159 Hungarian Holstein Friesian



cows. The rumination time was recorded using an automatic system (Hr-Tag, SCR Engineers Ltd., Netanya, Israel), which was placed on the cows approximately 2 weeks before calving and was removed 4 days after calving. The data were recorded in 2-hour intervals. The rumination times of the last 4 days pre-partum were summarized and the herd mean was calculated. Cows below the herd average were assigned to the Low rumination (L) group, and those above the herd average were assigned to the High rumination (H) group.

Data from the subsequent lactation were collected using the herd management software. These included lactation number, clinical diseases occurring in the first 30 days (retained placenta, milk fever, metritis, mastitis, left displacement of the abomasum), number of cows dead or sent to slaughter in the first 60 days, date of the first insemination and if it was successful, and 305 days corrected milk yield. Using these data, statistical analysis was performed.

**Results:** The mean rumination time of the herd before calving was 1967 min/96 h. Group L consisted of 70 cows and group H had 89 cows. Their mean rumination times were 1699 min/96 h and 2176 min/96 h, respectively. Lactation number had no influence on any of the parameters examined.

There was a significant difference in the occurrence of mastitis among groups ( $n=40$  and  $n=29$  in L and H, respectively). The L group also had 2.73 times greater chance (odds ratio, OR) for mastitis ( $P<0.01$ ). Rumination times had no significant influence on the other diseases, although every problem occurred more often in group L.

There was 3.10 times greater chance for cows in group L to die or be sent to slaughter in the first 60 days of lactation ( $n=7$  and  $n=3$  in L and H, respectively,  $P=0.11$ ). The pregnancy rate after the first insemination did not differ among groups, but cows in group L had their first insemination 6.49 days later than cows in group H ( $P=0.06$ ). The milk yield corrected for 305 days did not differ between the groups, but the number of cows completing the lactation was significantly higher in group H ( $n=77$ ) than in group L ( $n=44$ ) ( $P<0.01$ ). Cows in group L had 3.82 times greater chance to be removed from the herd before dry-off than cows in group H ( $P<0.01$ ).

**Conclusion:** Monitoring the rumination times before calving can be useful to determine which cows are at risk for diseases and may require extra attention in the periparturient period. It could also help identify cows at risk for slaughter in the subsequent lactation.

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HH-11

### Monitoring rumination time, chewing cycles and rumination bouts in indoor housed dairy cows by an ear-attached accelerometer

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**Objectives:** Rumination activity is considered as an important parameter for detecting animals suffering from metabolic diseases and indicates imbalances in the diet fed. Because of this, a valid automatic and real-time system for monitoring rumination activity is beneficial to facilitate a decision to check the animals' health or to adjust the feeding ration.

The objective of this study was to evaluate an ear-attached 3D-accelerometer (SMARTBOW, Smartbow GmbH, Weibern, Austria) for monitoring the rumination time, chewing cycles and rumination bouts in indoor housed dairy cows.

**Materials and Methods:** The study was conducted at the Teaching and Research Farm at the University of Veterinary Medicine Vienna, Austria. Ten dairy cows were equipped with ear tag based accelerometers. Animals were housed for approximately 3 weeks in a freestall pen equipped with feed bins (Insentec, Marknesse, The Netherlands) for monitoring feed intake. Animal behavior was recorded 24 hours per day with a digital video system. The agreement between the video recordings and direct animal observations was tested by two independent observers. Additionally, the inter- and intra-observer reliability was tested. The data of the analyzed videos were used as golden standard in this study and used for algorithm development and testing. Rumination time as well as jaw movements were analyzed on a subset of 10 randomly chosen video sequences of 1 hour, each per animal (i.e. 100 hours in total) with a professional video analyses software (Mangold Interact, Mangold International, Arnstorf, Germany). Based on the first 50 hours of classified videos, an algorithm to predict rumination time and jaw movements was developed. Based on the other half (i.e. 50 hours) of the classified video material the algorithm was tested.

**Results:** Inter- and intra-observer reliability as well as the comparison of direct against video observations revealed in high agreements for rumination time and chewing cycles with Pearson correlation coefficients of  $r>0.99$ . The rumination time, chewing cycles as well as rumination bouts detected by SMARTBOW were highly associated ( $r>0.99$ ) with the analyses of video recordings. Algorithm testing revealed in an underestimation of the average rumination time per 1-hour period by the SMARTBOW system of  $17.0 \pm 35.3$  s (i.e. -1.2%), compared with visual observations. The average number of chewing cycles and rumination bouts was overestimated by SMARTBOW by  $59.8 \pm 79.6$  (i.e. 3.7%) and by  $0.5 \pm 0.9$  (i.e. 1.6%), respectively compared with the video analyses.

**Conclusions:** In summary, the agreement between the SMARTBOW system with video analyses was excellent. From a clinical and practical point of view, the detected differences were negligible. Future studies are planned on testing the system under various field conditions and on evaluating the benefit of implementing rumination data into herd management decisions.

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HH-12

### Prediction of postpartum (subclinical) ketosis during the dry period using sensors.

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**Objectives:** The transition period is a key period for profitability of a dairy farm. Management of the dry- and the early lactation period needs to meet high standards. Especially the feeding requires attention. Not only the quality and energy content of the ration are of importance, but also the dry matter intake of the cows needs to be at a sufficient level. The problem is that there are not many ways to monitor feed intake on a commercial farm. However, if this is inadequate, problems may occur after parturition. One of the most evident problems is (subclinical) ketosis, a very important and widely underestimated problem in the dairy industry. Ketosis is not only a problem in itself, but also correlated with many other health issues like mastitis and reduced fertility. The aim of the present study was to determine if (subclinical) ketosis, in the first weeks postpartum, could be predicted by sensor data of activity, feeding- ruminating and lying time during the dry period. With these sensors a farmer can monitor the behavior of his cows 24/7. This provides information that cannot be obtained without these sensors because farmers cannot monitor their cows all the time.

**Materials & Methods:** Cows (n= 819) were studied at eight dairy farms in the Netherlands. Eating- and rumination time was measured from 6 weeks prepartum – 4 weeks postpartum with the aid of a neck sensor (Nedap, the Netherlands), which recorded automatically the time a cow was eating and chewing in secs per 15 min. The number and length of lying bouts was calculated. Another sensor fixed to one leg (Nedap, the Netherlands), measured the number of steps made, and time spent standing/lying, also in number/seconds per 15 min. Ketosis was determined by measuring the  $\beta$ -hydroxybutyrate (BHB) concentration in blood in the first and second week postpartum. For statistical analysis, an univariate general linear model was used.

**Results:** It appeared that dairy cows that developed (subclinical) ketosis in the first two weeks postpartum, spent less time (0.5 h per day) eating during the six weeks prepartum. So far it is not clear what causes this reduced eating time. Furthermore, a difference in prepartum number of lying bouts and pre- and postpartum number of steps was found, based on blood BHB concentration with a cut-off value on 1.2 mmol/L to identify cows at risk for ketosis. Increased eating time, ruminating time, number of meals, meal length and number of steps, and decreased lying time, number of lying bouts and lying bout length pre- and postpartum were associated with lower postpartum blood BHB concentrations. Eating time pre- and postpartum (from -6 to +2 weeks relative to calving) and ruminating time during close-up had the strongest relation to blood BHB concentration in week 2 postpartum.

**Conclusions:** It appeared to be possible to predict BHB levels postpartum based on prepartum sensor data.

Kyoritsu Seiyaku Corporation

**Objective:** New dairy management technologies and medicines have been developed to reduce the mastitis damages; however mastitis has still been one of the major causes of economical loss on dairy management. We observed the efficacy of a polyvalent mastitis vaccine in a large scale farm with more than 20,000 ton milk production on reducing the damage caused by mastitis.

**Materials and Methods:** The farm is located in Tokachi area in Hokkaido, Japan. 2,100 milking cows are raised and are expecting to produce about 25,000t milk for 2017. Average daily mastitis treatment case (including washouts after treatment) is 2% to 3% among the total milking cows. Mastitis causing bacteria is isolated on every case, and *S.aureus*, Coliforms, Streptococci and other bacteria (including CNS) are identified. In order to reduce the economical and mental workload, the farm started to use a polyvalent mastitis vaccine based on *E.coli* J5 and *S.aureus* (STARTVAC<sup>®</sup>) from September 2016. The cows were vaccinated with program protocol, i.e. the first dose with 45 days before the expected parturition date (EPD), the second dose with 10 days before EPD and 52 days after EPD. The data from the farm was evaluated on number of monthly mastitis cases, number of mastitis cases by bacteria, the length of stay in mastitis treatment-receiving herd, the transition of number of monthly emergency cases (cases with severe systemic symptoms that needed urgent medical treatment by veterinarian) and cases require intravenous infusion treatment, and the transition of number of deaths/culls cases.

**Results:** The number of mastitis cases from May to September 2016 was 220 cows in average, and it statistically decreased to 165 cows in average during May 2017 to September 2017. This decrease was due to decrease of number of mastitis cases caused by other bacteria (including CNS) other than *S.aureus*, Coliforms and Streptococci. In August 2017, the number of Coliform mastitis cases increased due to bedding; however the length of stay in treatment-receiving herd tended to be shortened from 9.3 days in average before vaccination to 8.3 days in average after the vaccination. The number of emergency cases before the vaccination was 15 cows in average, and it decreased to 2.6 cows in average after the vaccination. The number of treatment (intravenous infusion) received cases before the vaccination was 20.8 cows in average, and it also decreased to 9 cows in average after the vaccination. The number of death and cull cases decreased from 3.8 cows before the vaccination to 2.6 cows after the vaccination.

**Conclusion:** Efficacy of the vaccination with a polyvalent mastitis vaccine based on *E. coli* J5 and *S. aureus* (STARTVAC<sup>®</sup>) was evaluated in a large scale farm. It resulted in reducing the number of mastitis cases, shortening of treatment period, reducing severe mastitis cases and deaths/culls. Number of large scale farms with more than 10,000ton milk production is rapidly increasing in Japan. A polyvalent mastitis vaccine was considered to be one of the possible mastitis management tools in such a large scale farm as a result of this study.

HH-13

## Efficacy of a polyvalent mastitis vaccine at a large scale dairy farm

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HH-14

### Use of antimicrobial intramammary treatments in dry cow management in Finnish Dairy Herds

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**Objectives:** Importance of dry period and dry cow management for health and milk production of dairy cows is widely recognized. Drying-off practices differ greatly between countries and dairy farms, but antibiotic dry cow therapy (DCT) is an important part of most mastitis control programs. The DCT is either administered to all quarters of all cows (blanket DCT, BDCT) or given selectively to only treat cows or quarters with indications of intramammary infections (selective DCT, SDCT). Use of antimicrobial DCT is believed to be low, but has not been previously studied in Finland. The objectives of this study were to survey Finnish dairy herds regarding the use of antimicrobial DCT and to assess its association with herd characteristics.

**Material and Methods:** A questionnaire about farm characteristics and dry cow management was designed using close-ended questions. An online survey, accessible to all dairy farmers of the Finnish dairy herd recording system (appr. 5,400), was launched in collaboration with the major Finnish dairy company and a company responsible for the official milk recording system. The survey was open between January and May 2017, and the Dillman method was followed. Data analyses were performed with SAS v9.4 (SAS Inst. Inc, Cary, NC, USA).

**Results:** In total, 715 dairy producers (13% of farms) across the country participated in the survey. The reported herd sizes were small, with only 28% of farms with more than 60 cows. The proportion of farms with tie-stall milking, parlour, and automatic milking system (AMS) was 55%, 23%, and 22%, respectively. Based on their responses, 93% of farms had a milk production over 8,000 kg/year and 65% had bulk tank somatic cell count (SCC) under 150,000 cells/ml and 98% below 250,000 cells/ml. Thus, the responding farms were representative of the Finnish dairy industry.

Dry cow therapy is most often administered selectively in Finland: 78% of the responding herds applied SDCT, 9% of farms did not use DCT at all, and 13% of farms used BDCT. In 96% of the farms cows were dried off gradually, by reducing milking frequency and/or restricting feeding and in 86% of farms, cows produced 15 kg/day or less at dry-off.

A significant trend (Jonckheere-Terpstra test for k samples,  $p < 0.005$ ) was observed with increasing herd size and proportion of farms using BDCT, e.g., 9% of small farms (< 30 cows) vs. 23% of the bigger farms (> 60 cows) reported using BDCT. Additionally, the percentage of farms using BDCT was higher in farms with AMS (23%) than in farms with parlour or tie-stall milking (12% and 10%, respectively).

Farmers' own experience was the most commonly reported reason (64% of farms) for choosing a particular approach to DCT, followed by veterinary advice (35% of farms) and other farmers' experience/advice (1%). Of the farms using BDCT, in 46% of the farms veterinary recommendation was the reason for the selected approach. An indication for the use of BDCT recommended by veterinarians is likely high prevalence of mastitis caused by contagious pathogens in the herd.

Bacteriological examination of milk samples at dry-off was carried out in 82% and 64% of farms that used SDCT and BDCT, respectively. The importance of microbiological diagnosis before antimicrobial treatments is emphasized in Finland, seen in these numbers. In farms using SDCT, bacteriological diagnosis of milk samples was the preferred method for identifying cows for the treatment, followed by using information on clinical mastitis history and high SCC. Probably based on good udder health, a high number of farms using SDCT (71%) reported treating only up to one-fourth of their cows at dry-off. Further analyses on milk quality in farms using different DCT approaches will be conducted.

**Conclusions:** Use of antibiotic DCT in Finland is limited, with a majority of farms (78 %) using selective DCT or no DCT (9%). Blanket DCT was most commonly used in larger farms with AMS. A high percentage of farms analysed milk for mastitis pathogens at dry-off before treatment, both in farms using selective and blanket DCT.

HH-15

### Impact of postpartum subclinical hypocalcemia on uterus recovery and reproductive performance of dairy cattle

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The main cause of perinatal diseases of dairy cattle is postpartum hypocalcemia, and it has a big impact on productivity. There is a report that subclinical hypocalcemia even with no obvious symptom (low calcium under 8.6mg/dl) causes a perinatal disease and decreases reproductive performance. This time, in S Town in Tokachi, Hokkaido, the investigation is conducted to study the incidence status of subclinical hypocalcemia and the impact on reproductive performance.

At 9 dairy farms in S Town in Tokachi area, the investigation was conducted on 757 Holstein breed multiparous cows, which gave birth during the period from June 2016 to September 2017. From the result of the blood sampling and the biochemical test during 0 to 3 postpartum days (within 96 hours), blood calcium level under 8.6mg/dl was considered as subclinical hypocalcemia. Cows with no obvious symptom after calcium injection at clinical hypocalcemia or and multipara cows received calcium injection that included in this study. The average blood calcium level and the incidence rate of hypocalcemia were compared by farm and by postpartum day. Ultrasonography was used to check metritis during 7 to 13 postpartum days and intrauterine retention during 21 to 27 postpartum days. The result of investigation on 120-day fertility rate and open period was compared to the incidence of subclinical hypocalcemia by postpartum day, and the impact was analyzed and studied statistically.

The average calcium level and the hypocalcemia under 8.6mg/dl incidence rate were 8.43 mg/dl and 59% on 0 postpartum day, 8.38 mg/dl and 55% on the 1<sup>st</sup> postpartum day, 8.72 mg/dl and 49% on the 2<sup>nd</sup> postpartum day, and 9.02 mg/dl and 28% on the 3<sup>rd</sup> postpartum day, respectively. 47% had hypocalcemia under 8.6mg/dl as a whole. Considering the reproductive per-



formance, incidence status of hypocalcemia during 0 to 1 postpartum day had no significant impact (120-day fertility rate: 48% with hypocalcemia, 51% with normocalcemia, Open period: 130 days with hypocalcemia, 130 days with normocalcemia). However, hypocalcemia during 2 to 3 postpartum days significantly reduced 120-day fertility rate (120-day fertility rate: 37% with hypocalcemia, 52% with normocalcemia, Open period: 143 days with hypocalcemia, 130 days with normocalcemia). The incidence rate of hypocalcemia under 8.6mg/dl by farm is 15% to 67% as a whole, and the rate during 2 to 3 postpartum days showed a significant difference from 7% to 50%. The metritis incidence rate is notably higher with cows, which had hypocalcemia during 1 to 3 postpartum days (26% with hypocalcemia under 8.6mg/dl, 13% with normocalcemia), and the rate of intrauterine retention is also remarkably higher (25% with hypocalcemia, 18% with normocalcemia).

The incidence rate of postpartum subclinical hypocalcemia is high until the 3<sup>rd</sup> postpartum day, and a significant difference was identified among farms. The investigation showed that subclinical hypocalcemia occurred during 2 to 3 postpartum days reduce the reproductive performance of cows. Considering the incidence rate of metritis and the rate of intrauterine retention are higher among cows with subclinical hypocalcemia during 1 to 3 postpartum days, one of the factors of lower reproductive performance is considered to be metritis and slow uterus recovery caused by subclinical hypocalcemia.

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HH-16

### Integrating prediction of parturition and timing supplementation of calcium for prevention of subclinical hypocalcemia

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In spite of all the advancements in strategies to prevent hypocalcemia, the subclinical presentation of the disease remains highly prevalent among dairy cows with detrimental effects on health and reproduction. A recent study by our group demonstrated that plasma calcium concentrations in multiparous cows developing hypocalcemia began to fall at ~9 hours before calving. This declines in calcium coincide with a pivotal time when adequate plasma levels are required to maintain proper uterine and immune function to prevent dystocia, uterine prolapse, and retained placenta. The data suggest that the shortcoming of the strategies to prevent subclinical hypocalcemia may be the inadequate level of calcium during the time of utmost importance for disease prevention. Thus, an approach to improve calcium metabolism based on a correct prediction of parturition might be the holy-grail for effective prevention of subclinical hypocalcemia that translates into improvement for health and reproduction in dairy cows. Moocall<sup>®</sup> is a tail mounted sensor that uses an algorithm to predict when cows are likely to calve by measuring tail movement patterns triggered by labor contractions. The device sends an SMS text alert when tail movements reach a certain level of intensity over a period that suggests parturition will occur within 1-2 hours. We hypothesize that calcium supplementation at time of prediction parturition (~2 hours before calving) improves calcium concentration at the first 24

hours after calving.

**Objectives:** To evaluate device prediction of parturition and its association with calcium profile and clinical signs associated with impending parturition. To determine if a prepartum oral bolus supplementation at the device prediction of parturition can improve calcium metabolism during first 24 postpartum.

**Materials and methods:** A total of 14 multiparous cows had the device mounted on their tail approximately three before the calving expected date. Blood samples were collected twice daily to evaluate whole blood and plasma calcium levels. Clinical signs of impending parturition (relaxation of the pelvic ligaments, udder hyperplasia, udder edema, teat filling, relaxation of the tail, vulva edema, vaginal mucus discharge) were evaluated every 12 hours. Cows were randomly assigned to 1 of 2 treatment groups. In the first group, CALC, (n =7) cows received an oral bolus containing 43 g of calcium following the second message predicting parturition followed by one bolus at 0 and 24 hours after calving. The second group, CONT (n =7) served as control and cows did not receive calcium supplementation at Moocall prediction, but cows were supplemented with the same oral bolus of calcium at 0 and 24 after parturition.

**Results:** Moocall predicted parturition correctly in over 92.8% (13 of 14) of the cows with a mean time of 270.3 minutes (SEM = ± 35.3) between the first message and calving. Over 80% of cows calved between 3 and 6 hours after the first message false positive alarms occurred in 6 cows. Clinical signs associated with impending parturition (relaxation of the pelvic ligaments, udder hyperplasia, udder edema, teat filling, relaxation of the tail, vulva edema, vaginal mucus discharge) at approximately hour -12 before calving were poorly correlated with the prediction of parturition having an area under the curve < 0.65. As expected, we found no differences ( $p>0.05$ ) in plasma calcium concentration between cows in CALC and CONT before calving. However, CALC cows supplemented at the prediction of parturition had increased ( $p<0.05$ ) plasma calcium at calving (CALC = 2.26 mmol/L ± 0.05 vs. CONT = 2.09 mmol/L ± 0.03) and 24 hours after calving (CALC = 2.28 mmol/L ± 0.05 vs. CONT = 2.16 mmol/L ± 0.03) than CONT herdmates.

**Conclusions:** Moocall prediction of parturition was accurate for most cows, but with a broader (1.5 to 6 hours) range than expected (2 hours). However, Moocall prediction of parturition allowed timing intervention with calcium supplementation. Prepartum oral calcium supplementation improved calcium metabolism in the first 24 hours post-calving suggesting that a reduction in the incidence of subclinical hypocalcemia might be possible with the strategy investigated in the current study.

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HH-17

### Assessment of the association of *Mycobacterium avium* subs. *paratuberculosis* serostatus with birth to calving intervals from first to fifth calving, in Portuguese dairy cows

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**Objectives:** To assess the association of *Mycobacterium avi-*



um subs. *Paratuberculosis* (MAP) seropositivity on Birth to Calving Intervals (BTCI), from first to fifth calving, in the Portuguese Dairy Cows, taking into account the infection at herd level, the parity, 305 corrected milk production and average Somatic Cell Counts.

**Materials and Methods:** All cows aged over 30 months, from 329 farms (in total 15,300 animals) were MAP tested by indirect biphasic ELISA (IDEXX MAP Ab, IDEXX Laboratories, Inc. Westbrook, Maine USA). These cows represent 6.45% of the Portuguese dairy cow's population and 18.5% of cows (and 20% of farms) registered in the National Dairy Improvement Association (NDIA). 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 for the farm, in four levels: Strongly Negative (SNEG - all cows tested in the farm, minimum 60 test results, all results negative), Negative (NEG - less than 60 tests, all cows tested, all results negative), Non-Negative (NNEG; all cows tested, at least one dubious result and no more than one positive result) and Positive (POS; all cows tested and at least two POS cows). Records from 40,065 30,611 calving dates were available from 12,749 cows. Intercalving intervals and Birth to Calving intervals one to fifth (BTC-1 to -5) and Intercalving intervals from one to fourth (ICI-1 to -4) were calculated. Outcome variables BTC were investigated using PTB status and farm PTB Level as explanatory variables, calving season, farm, farm status, parity, 305 corrected milk production and average somatic cell counts and BTC-1 as variables. Multilevel mixed models will be used for the analysis, with IBM SPSS Statistics, version 23 and SuperMixe 2.1.

**Results:** Of the total cows, 90.9% were NEG, 0.58% were DUB and 4.5% were POS. Correspondingly 4.67% of calvings/lactations were also positive. As to farms, 14.6% were S\_NEG, 39.0% NEG, 18.2% N\_NEG and 29.2% were POS. 58.3% of the cows were NEG animals in positive farms. Average 305 corrected milk production was 9,444 kg; POS cows' average was 9,528. Average SCC was 260,000 cells per ml and 272,000 for the POS cows. The total 30,611 BTC had the typical National distribution from first to fifth parities. The proportion of BTC-5 was similar for POS and NEG cows (10.8% and 11.5% respectively). We observed 1,418 POS BTC and 29,024 NEG BTC. Average BTC-1 to BTC-5 and standard deviations were 843.2 (116.3); 1,258.3 (153.8); 1,676.6 (184.1); 2,099.6 (217.2); 2,532.3 (252.0) for NEG cows and 820.1 (110.3); 1,250.2 (150.1); 1,672.9 (191.9); 2,057.4 (220.7); 2,427.9 (210.6) for POS cows respectively. There is a tendency for increasing differences in BTC between POS and NEG cows. POS cows reach the first and fifth lactation 14 and 104 earlier than NEG cows. As to ICI, ICI-1 an ICI-2 were similar for POS and NEG cows (424 and 418 in NEG; 430 and 423 in POS); but ICI-3 and ICI-4 were 39 and 62 days shorter in POS than in NEG cows (423 and 433 in NEG; 385 and 370 in POS). The average time from first to fifth calving in POS cows was 1608 days, which is 90 less than the corresponding average of NEG cows.

**Conclusions:** The POS cows reach first calving earlier than NEG cows. There is an increasing difference in BTC-2 to BTC-5 between POS and NEG cows. POS cows reached fifth lactation about 104 days earlier than the NEG ones and they have shorter ICI in the two last calving's. The authors had previously observed that in POS cows, cumulative corrected milk production over 5 lactations was lower: first and second lactations' av-

erage 305 corrected milk productions were higher and then experienced major a drop in lactations 4 and 5. The variation in reproductive performance is similar of the one observed for milk production. It is important to further investigate if more efficient cows (based on milk production and reproduction performances) are more prone to MAP infection or PTB evolution. Additional insight on the influence of MAP and farm MAP status in reproductive performance in Portuguese dairies will be provided upon completion of our work.

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HH-18

### Monitoring heifer growth: use of thoracic tape to estimate heifer's body weight and body mass index as a practical tool to monitor dairy heifers growth

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**Introduction:** Healthy, productive herd replacements are the result of a good management that starts before conception and continues until they enter the milking herd. The only way to know how heifers are growing is by measuring them several times a year, to compare with established goals. On the other hand, an adequate heifer development allows a reduction in the age at first calving (AFC) to optimize dairy rearing and profit. The most general recommended AFC are 22-24 months.

To monitor the development of heifers, it is recommended to evaluate at least three parameters (weight, height and BCS) and four phases (birth, weaning, insemination and parturition). In the different phases achieving a percentage of the farm mature cow weight and height are considered as the main indicators of heifer's development. Moreover, a regular measurement of heifer growth allows adjusting rations and sanitary management to meet goals. To monitor the development of heifers, it is recommended to evaluate at least three parameters (weight, height and BCS), which are considered to be the main indicators of heifer development and at least in four phases (birth, weaning, insemination and parturition).

Body weight can be measured accurately using a calibrated scale or estimated with different techniques the heart girth with a tape being the most commonly used; while hip or wither height can be measured with a measuring stick.

Moreover, in the human side, a very useful combined parameter frequently used to determine a person's weight in regard to their height is the body mass index (BMI).

We hypothesize if a parameter like this could be useful in order to evaluate both parameters at the same time, weight and height, as a measure of heifer growth and development.

**Objective:** The objective of the present study was on the one hand to check the usefulness of using the thoracic perimeter tape (either using a thoracic tape or measuring in cm and applying different formulas) to estimate the BW of calves at different ages; and on the other hand to evaluate the usefulness of the body mass index in order to monitor heifer development at





different phases during rearing; and assess if this index could be used as a practical tool for implementing a monitoring program for growing of rearing heifers at flock level.

**Material and methods:** The study involved two veterinarians who measure a total of 1,225 heifers using different calibrated electronic scales to determine body weight (Braxtan BR80, Iconix FX21 and M.O.L.Y., Tecnozoo), a girth tape to indirectly estimate the weight and a measuring stick at the withers to measure the height. For the concordance analysis, we classified the heifers according to their age and body weight. Additionally, BMI was calculated in 673 heifers and for the established goals dividing the body weight by the height.

For the analysis, different scatter plots were built to represent the results and randomness of errors distribution. Additionally, to assess the concordance between tape and electronic scale, the intraclass correlation coefficient (ICC) was determined using SPSS 15.0.

**Results:** Dispersion diagrams comparing the scale weight with the thoracic tape estimation after applying different formulas showed best correlation with Heinrich's formula (2007) and randomized distribution of the residuals. The concordance between both techniques was very good from 0 to 18 months old heifers; without notable differences between both veterinarians. However, in heifers older than 18 months the ICC was reduced (ICC = 0.882 and 0.701, 18-24 and > 24 months). Related to the BMI, its distribution was useful and practical to evaluate at the same time weight and height as it moves from 0.5 (41kg and 81 cm) to 4.6 (649kg and 141 cm).

**Conclusion:** BMI can be an interesting index to evaluate heifer's development. To determine the heifers body weight a calibrated electronic scale is the most accurate method in all ages. However, in those cases a scale is not available; the estimation based on the thoracic perimeter has been shown to achieve an excellent concordance until 18 months of age, which makes it a practical tool for monitoring until conception.

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HH-19

## Dry off methodology and effect on daily milk production curves in Dutch dairy cows

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**Objectives:** The objectives of this study were:

To describe the distribution of dryoff (DO) methodology applied in Dutch dairy herds and To investigate the effect of an apparent gradual dryoff methodology on milk production levels in the last 60 days of lactation.

**Materials and methods:** Data provided by Dairy Data Warehouse from lactating cows (n=42,633 cows from 656 Dutch commercial dairy herds) were analyzed to investigate the impact of dry-off management on milk yield before dry-off.

By definition a gradual DO is applied in the event where any intervention to reduce milk production through reducing milking frequency and/or feeding regime is applied prior to the last milking, and an abrupt DO is applied when no changes in ei-

ther, milking frequency or feeding regime, is applied.

As no information on the type of dry-off procedure performed at herd or animal level were available, two proxies for the type of DO management ("abrupt" and "gradual") were developed based on daily milk meter data for the last 60 days before the day of dry-off.

Classification of milk production curves were done using expert-based labelling and a Multi-Layer Perceptron classification algorithm. Accuracy in the prediction of the type of DO management was internally validated to be 85%. This method based on parameterization of lactation curves is advantageous as it focusses directly on the outcome of a presumed gradual DO technique being the reduced amount of milk (besides physiological declines) obtained from an animal in the period prior to DO.

To identify possible sub clusters in the gradually DO group, a combined k-means + Dynamic Time Warping (DTW) clustering approach was used.

Having empirically observed that in almost all lactation curves from cows dried-off "gradually" a reduction of milk yield is within the last 30 days of lactation, the focus was limited to 30 days for the clustering analysis, with daily milk data expressed as fraction of the average yield between day -30 and -25 to DO.

**Results:** Distribution of gradual vs abrupt DO management in the Netherlands was 31,87% (13,587 cows) and 68,13% (29,046 cows) respectively according to the classification algorithm.

Running the described clustering routine on the 13,587 animals dried of "gradually", two further clusters were identified. A "slower decline" group (6,329/13,587 animals) where daily milk yield declines gradually from about day -25 to day -1 by more than half. On the contrary, a "faster decline" group (6,895/13,587 animals) levels off the relative daily milk yield between 1 and 0.9 from day -30 until day -5 and then a 30% decrease in milk yield is seen in the last 4 days.

**Conclusions:** Cows dried off in included Dutch herds showed characteristic patterns in late-lactation milking curves, allowing for discrimination into "gradual" and "abrupt" DO groups, with approximately 2/3 of cows being dried off "abruptly" and the remaining third manifesting a non-physiological reduction of milk yield in the last 30 days prior to dry off.

Among the cows dried off "gradually" the average evolution of the milk production curve differed with one cluster of cows having a slow decline in milk per day from approximately 25 days before the day of last milking, and another group with a steeper decline from approximately 5 days before the dry off. The latter may be seen as a more aggressive strategy with less milk losses overall, while the former might represent a gentler approach. Regardless of the observed steepness, it is however interesting to notice that, in both groups, an intervention is visible to some extent from day -5 onwards, suggesting that, as a general trend, actions are commonly taken in the last week of lactation. Further studies are necessary to determine the extent of the milk production losses observed among cows dried off "gradually".

The Study was supported by CEVA Santé Animale (France) and done with the collaboration of Dairy Data Warehouse (The Netherlands).



HL-01

### An unusual presentation of toe ulcers in a pasture-based dairy herd

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**Objectives:** Toe ulcers, as defined by the ICAR claw health atlas, are a well-known disease of the claw horn capsule and cause of lameness in cattle. Prevalence of these lesions is usually low compared to other claw horn lesions like sole ulcers and white line disease. Toe ulcers are usually located in claw zone 1 or 5 with most lesions being in the lateral claw of a hind limb. Toe ulcers are usually attributed to overzealous claw trimming, housing on abrasive concrete floors, or thin soles. This paper describes the seasonally increased incidence of an unusual form of toe ulcers in a pasture-based dairy herd.

**Materials and Methods:** Fortnightly lameness visits were carried out on a 600-cow spring-calving pasture-based dairy farm in the Manawatu region of New Zealand. All cows with abnormal locomotion scores were presented at these visits for veterinary lameness examination in a hydraulic WOPA foot-trimming chute. Regular lameness visits were carried out from October 2016 to March 2017. Claw lesions and affected claws were recorded and the cows were treated according to the lesions that were present. There was no preventative functional claw trimming performed in this herd.

**Results:** Preliminary data analysis showed that white line disease was the most prevalent lesion found in lame cows in the months of October 2016 (90%), November 2016 (73%), December 2016 (100%) and January 2017 (75%). However, in February 2017 there was an unexplained increase in toe ulcers with an uncommon location and presentation. These toe ulcers made up 65% of all lameness cases in February while white line disease accounted for only 30%. All toe ulcers were located in the medial claw of either the left or right hind limb. The lesions were painful; cows were scored with a lameness score of 3-4 on the Sprecher scale. In all cases there was a lentil-sized, punctate lesion in zone 5 of the sole horn towards zone 2/3. While close to the white line in many instances lesions were independent of it and sited in the sole horn, and were often surrounded by an area of horn haemorrhage. The amount of under-run horn when paring the lesion was usually moderate, and no tracking to the tip of the toe was observed. Signs of toe necrosis were not present.

The lesion responded well to debridement and topical antibiotic spray. The lateral claw of the affected limb received a hoof block to elevate the lesion and relieve the pain associated with it. Although, NSAID treatment was considered on a case-by-case basis, elevation provided good relief for pain and only cows with other disease (e.g. foot rot) required additional treatment.

**Conclusions:** Sole bruising and white line disease are the most common causes for lameness in dairy cattle in New Zealand. While toe ulcers can be diagnosed sporadically in New Zealand, they are more commonly found in the front feet in heifers rather than in the hind feet of mature cows. The seasonal increase in the incidence of toe ulcers with an uncommon presentation was not attributable to any of the known risk factors for development of toe ulcers, and did not coincide with seasonal management factors such as mating which have

been described to contribute to an increased prevalence of clinical lameness in seasonally-calving, pasture-based dairy cattle. More research is required to determine the mechanisms behind the development of these claw horn lesions in order to implement effective preventative measures on farm.

HL-02

### Bovine Digital Dermatitis in Victoria, Australia

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**Objectives:** To estimate the prevalence of DD in Victoria, Australia, and to investigate which pathogens are consistent with typical DD lesions.

Bovine digital dermatitis (DD) is a contagious disease and an important cause of lameness in cattle worldwide. *Treponema* species are likely to be the primary pathogens of DD; however, it is considered that DD is a polymicrobial disease for which the causative pathogens are not fully understood. It is likely that there is a seasonal pattern in the occurrence of DD. In Australia, limited published reports on DD are available, although there have been anecdotal reports of increasing prevalence in Victoria. Prior to this study, a pilot study among 13 herds in Victoria, Australia was conducted. All of these herds were positive for DD, with a mean herd-level prevalence of 19.11% (Coombe et al., unpublished).

The prevalence and causative pathogens of DD are not clear yet in Australia and this information is important to aid in the development of education, treatment methods and preventative measures.

**Materials and methods:** Sampling and examination were conducted at three knackeries in Victoria, Australia. A knacker is a processing facility for deceased cattle that are not fit for human consumption or transport. Every visit consisted of five days of examination; two knackeries were visited twice and one was visited once. All limbs of mature cows processed through the knackeries were examined for lesions consistent with DD. On examination, limbs were classified as N (normal), A (active DD-lesion), D (dried or chronic DD-lesion) or S (suspected case of DD). N was defined as no visible lesion or palpable thickening of the epithelium, A was defined as a red, erosive, acute or ulcerative lesion, D indicated a chronic, hyperkeratotic, wart-like proliferated lesion with thickening of the epithelium and S was classified as a palpable thickening of the epithelium but no visible lesions. Additionally, a total of 18 skin biopsies were taken (6 from each each knacker), which consisted of two biopsies from normal skin, two biopsies from active lesions and two biopsies from dried lesions. DNA extraction and amplification from the biopsies was performed and diversity profiling was completed.

**Results:** Of all cows examined (n = 823), 88.6% were dairy cattle and 11.4% were beef cattle. The total prevalence of DD was 29.8%. The prevalence of DD was significantly higher in dairy cows (32.2%) than in beef cows (10.8%). Of all the cows with DD (n = 245), 19.6% had active lesions and 84.9% had dried (chronic) lesions, and 67.2% of the examined cows with



DD had more than one foot affected.

The diversity profiling showed no clear clustering between active, dried and normal skin biopsies. The presence of *Mycoplasma*, *Fusobacterium*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and *Firmicutes* was compared between the dried, active and normal skin biopsies using a one-way ANOVA, but no significant difference was found. Two biopsies of normal, healthy skin tissues were positive for *Treponema*-species, and of dried and active lesions 5 out of 12 biopsies were positive for *Treponema*. There was no significant difference in the presence of *Treponema* between the three sample groups.

**Conclusions:** The calculated prevalence of lesions demonstrates that DD is endemic in Victoria, Australia. Additionally, *Treponema* was isolated from some of the samples from affected cows. Since the presence of *Treponema* was not limited to samples from animals with DD-like lesions, and considering the similarity of microbial compositions in samples (as compared with other studies), the results of this study might suggest that the aetiology of DD in Australia is different from the aetiology of DD in other countries. More research is necessary, to investigate the true prevalence, risk factors and causative pathogens, to enable treatment and preventative strategies to be developed.

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HL-03

### Systematic review of prevalence of lameness and leg lesions on dairy farms: 2006 to 2016

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Lameness is widely accepted as one of the most important animal welfare concerns facing the dairy industry today. Previous work has shown that leg lesions and lameness vary across countries, regions and even farms. Our aim was to summarize the peer-reviewed literature published over the last decade on the prevalence of lameness (n=34 papers) and leg lesions (n=25) in dairy cows across the globe. We undertook a systematic search of databases (Web of Science<sup>TM</sup>, PubAg, PubMed, and Social Sciences Citation Index) using the combined terms for lameness "dairy AND (survey OR cross-sectional OR on-farm) AND (lameness OR gait score OR locomotion) and hock lesions "dairy AND (survey OR cross-sectional OR on-farm) AND (hock OR skin OR knee OR lesion OR leg). For lameness, only papers describing the gait or locomotion scoring system and described cut points for clinical and severe lameness were included. Articles were included if the primary focus was lameness or hock lesions and published between January 1, 2006 and December 31, 2016. In the case of lameness and hock lesions our search process identified 177 and 191 papers, respectively. Selection and assessment of the retrieved papers were performed first based upon paper title using the eligibility criteria above. The second stage involved screening the full manuscripts using the eligibility criteria above, plus a search for the presence of a calculated farm prevalence of lameness and hock lesions. From the papers assessed 34 matched the crite-

ria and were used for the prevalence of lameness and 25 for hock lesions on-farm. The articles undertaken over the last 10 years has shown that the global prevalence of clinical lameness in lactating dairy cattle exceeds 25%. Independent of country or housing system, the 34 on-farm studies averaged more than 20% clinically lame cows and 5% severely lame cows. Although there is considerably less work on the prevalence of hock lesions on dairy farms, the work undertaken by others over the last decade suggests that hock and knee lesions are also a concern. Leg lesions follow the same pattern with great variability between housing systems and countries, but on average 39.1% of cows have a leg lesion and on average 8.0% with a severe hock lesion in the papers found. These results indicate that lameness and leg lesions remain prevalent around the world, especially in free stall facilities, and highlights the need to place importance on efforts to reduce their occurrence.

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HL-04

### The Prevalence of Lameness in UK Finishing Cattle

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**Objectives:** The prevalence of lameness in UK dairy cattle is reported to be 36.8% (Barker et al. 2010). The prevalence of lameness in UK finishing cattle, however, is largely unknown. Furthermore, there is limited information regarding lameness in finishing cattle internationally. A number of observational studies in various countries have monitored lameness prevalence on arrival for slaughter, which will only represent those that are suitable for transport, and have reached slaughter weight. Lameness prevalence or incidence based on treatment records is at a similar risk of underestimation, as previous studies have indicated that dairy farmers frequently under diagnose lameness. These type of studies report an incidence per 100 animal years of 1.04 cases in Nebraska and Kansas (Terrell et al. 2017) and 1.19 – 3.28 in Italy (Compiani et al. 2014), with lameness accounting for 16% of health problems and 5% of deaths in five US feedlots (Griffin et al. 1993).

Lameness causes significant production losses in dairy cattle (Hassall et al. 1993) and sheep (Nieuwhof & Bishop 2005) and is likely to have a similar impact in beef finishing units. The absence of information regarding the effects on production in UK finishing cattle makes effective prevention planning difficult, and may even lead to lameness not being appreciated as an important condition within the industry.

This study identified the prevalence of lameness across 18 finishing units that finish cattle to slaughter weight in housed systems. Foot lesions were also identified.

**Materials and Methods:** A cross sectional observation study was carried out on farms located in England and Wales between June and October 2017. Animals of interest were housed cattle, in the finishing period and having being reared solely for beef production. 18 farms were recruited, and 1685 animals in total underwent locomotion scoring once by the same researcher, on a hardstanding, using a 5 point locomotion scoring sys-



tem. High scoring (lame) animals and a control sample of low scoring (sound) animals underwent examinations of all eight digits (and the distal limb) to identify the presence of any lesions.

**Results:** Initial results suggest that 33% of cattle scored were Limousin Cross breed, 14% British Blue Cross, and 9.4% Holstein Fresian. The mean age was 20.7 months (Standard Deviation 5.6 months). The mean prevalence of lameness was 7.8%, ranging from 2% to 21.2%. In total, 305 animals received a foot inspection, 127 lame and 178 sound. Claw overgrowth was found on 24.1% of claws belonging to lame animals, compared to 11.5% of claws in sound animals, showing a significant difference ( $P < 0.01$ ). Details of other lesions identified on both lame and sound animals are discussed, along with the severity of lesions, and are compared to dairy cattle.

**Conclusions:** The lameness prevalence found in this study is lower than that in dairy cattle. This may be a result of cattle age, breed, husbandry and management differences, and the typical time spent in the production system (short duration for finishing animals compared to longer retention for dairy cattle). In some cases, lame finishing cattle may be prematurely sent to slaughter, lowering the on farm prevalence. The variation in prevalence between farms indicates that management related factors are influencing lameness prevalence on farm. Further knowledge of lameness risk factors could improve performance, efficiency and welfare. Furthermore, an overgrown claw is significantly more likely to be present on a lame animal. This association suggests that prevention of overgrown claws may be important in the control of lameness in beef cattle.

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HL-05

### Distribution of weight and pressure on the claw soles in young calves

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**Objectives:** The objective of this study was to measure load and pressure distribution on the claw soles, and their respective heel and toe areas, in healthy young calves. The intention was to gain insight into the forces and pressures acting on the claws of calves and the development of claw diseases resulting from abnormal weight bearing.

**Materials and Methods:** The variables were measured in 6 Brown Swiss and 6 Holstein calves using a force plate, a pressure plate and appropriate software. The calves were healthy, 3 to 5 months old, and had a normal posture with normally shaped claws that were free of lesions. Measurements were made in either both forelimbs or both hind limbs simultaneously. The calves were encouraged to stand squarely and quietly during measurements. A total of 500 data sets were recorded during a 2-sec measurement period. Each calf underwent 3 measurements in the forelimbs and 3 measurements in the hind limbs. One data set was selected from each of the 3 measurements by means of a random generator and the calculated

means of the 3 data sets were used for further calculations. A digital template was used to define the contact surface of the lateral and medial claws with the ground and to divide the sole into a "heel" and a "toe area". Differences between limbs, claws and sole areas were analysed using a paired t-test. Differences were considered significant at  $p < 0.05$ .

**Results:** The mean load exerted by the calves was  $1,623 \pm 190$  N (means, standard deviation). The load placed on the claws of the forelimbs ( $879 \pm 106$  N, 54.2%) was significantly greater than the load placed on the claws of the hind limbs ( $744 \pm 93$  N, 45.8%). The medial claws carried more weight than the lateral claws; this difference was significant in the front feet (65.9%, medial claws) but not in the hind feet (55.1%, medial claws). The load acting on the "heel area" of the claws was significantly greater (72.5%, front feet; 62.4%, hind feet) than those acting on the "toe area". In the front feet, the contact surface of the medial claws was greater than that of the lateral claws; this difference was significant in Brown Swiss calves but not in Holstein calves. The lateral claw contact surfaces of the hind feet were significantly larger than the medial claw contact surfaces in Brown Swiss calves but were the same size in Holstein calves. The mean pressure acting on the front feet was significantly greater than the pressure acting on the hind feet ( $123 \pm 25$  kPa, front feet;  $111 \pm 16$  kPa, hind feet). Mean pressures were always greater in the medial claws ( $147 \pm 31$  kPa, front feet;  $127 \pm 33$  kPa, hind feet) compared to the lateral claws ( $88 \pm 39$  kPa, front feet;  $93 \pm 25$  kPa, hind feet). Maximum pressures were measured most often at the heel area of the medial claws (in 72% of the forelimb claws and in 50% of the hind limb claws).

**Conclusions:** Similar to adult cows, there was a greater weight bearing by the front feet compared with the hind feet and there were greater pressures acting on the heel area compared with the toe area of the sole in young calves. The main difference between calves and adult cows was that in calves, the medial claws of the hind feet tended to carry more weight, had higher mean pressures and received more often maximum pressures than the lateral claws. These findings confirmed earlier observations. Thus, it appears that the medial claws of cattle are intended to carry more weight than the lateral claws. Which factors cause this relationship to be reversed in the hind feet of dairy cows, in which the lateral claws carry more weight, requires further studies.

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HL-06

### Effect of different ground conditions on pressure distribution under the bovine claw measured with foil-based sensors

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**Objectives:** Dairy cows kept on hard flooring systems are subjected to a significantly higher risk of developing claw diseases and lameness (1). Therefore, the effects of different floorings on pressure load of bovine claws have been investigated in the past (2, 3). As most surveys used stationary pressure measur-



ing plates no direct influences of flooring on the claw could be captured (4). In this study, thin pressure sensitive sensors were attached under the claws of walking dairy cows to analyse the direct kinetic effects of different flooring conditions on the claw sole surface.

**Materials and methods:** Ten adult dairy cows (age 3-5 years) without signs of lameness were equipped with sensor foils (M3200E, Hoof™System, Tekscan Inc, Boston, MA, USA) cut to the size of the claws. The Sensors were attached under both claws of their left hind limb by use of a leather claw shoe. The system was calibrated based on manufacturers instructions before. After attaching the sensors and data loggers in a treatment crush, cows walked a straight line of 15 m distance on concrete flooring or rubber mats (KURA, Gummiwerk KRAIBURG GmbH&Co. KG, Waldkraiburg, Germany). Three walking sequences with at least five regular steps were recorded for each flooring. In the subsequent data analysis overall contact area, mean pressure and maximum pressure were determined and the courses of vertical Ground Reaction Forces (GRF) of medial and lateral claws were examined.

**Results:** For both flooring types, the lateral hind claw was subjected to higher loads than the medial claw. The average pressure was significantly higher on concrete ( $54.6 \pm 13.5$  N/cm<sup>2</sup>) than on rubber ( $42.9 \pm 8.8$  N/cm<sup>2</sup>). Similarly, the maximum pressure showed significantly higher values on concrete ( $147.3 \pm 31.7$  N/cm<sup>2</sup>) than on rubber ( $100.6 \pm 24.9$  N/cm<sup>2</sup>). The overall contact area tends to be larger on rubber flooring than on concrete. The vertical force-time curves show differences between the examined cows but generally describe biphasic courses with local peaks at  $29 \pm 5$  % and  $79 \pm 3$  % of stance phase time. Moreover, the force-time curves differ noticeably between lateral and medial claws.

**Conclusions:** With the present study, a new method for kinetic analysis of claw floor interaction in live dairy cows could be established. The Tekscan Hoof™System was applied successfully under walking dairy cattles' claws. For the first time kinetic measurements were performed separately for the lateral and medial claw while walking more than one step. This provides new insights in detailed biomechanics of the bovine claw. On rubber flooring mechanical strain to the claw was significantly lower than on concrete. Average pressure values are comparable to the findings of van der Tol (5), although the present study detected higher vertical GRFs, which could be due to an unrestricted gait of the cows. Force-time curves show a biphasic course, which was already found by Nuss et al. (6).

Prospective studies should include a larger number of animals as well as further floorings like pasture or slatted concrete.

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HL-07

### Detection of hind limb pathologies in cattle with the aid of the cow pedogram

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**Objectives:** Foot and limb disorders are a major health concern in cattle. The aim of this study was to evaluate if the cow pedogram could differentiate between cows with no signs of orthopaedic pathologies and such with pathologies of various locations (hind foot versus hind limb proximal to the fetlock joint).

**Materials and Methods:** Twelve dairy cattle with no signs of orthopaedic pathologies (no pathology group; group C) and 67 cows with unilateral hind foot and limb pathologies (pathology group, group L) were included in the study. Group L consisted of cows with pathologies at one of two different localisations: foot pathologies up to and including the fetlock joint, LOC1 (n=43); limb pathologies located proximal to the fetlock joint up to the hip joint, LOC2 (n=24). The result of a thorough orthopaedic examination of all 4 limbs (presence or absence of pathological findings) was used as the gold standard for group allocation. The cows were equipped with 2 standalone 3D accelerometers (400 Hz)<sup>1</sup>, which were fitted at the level of the metatarsus to both hind limbs. The pedogram variables (temporal events, kinematic outcome = gait cycle duration and relative stance-phase and swing-phase durations; peaks, kinetic outcome = foot load, toe-off) were extracted using the validated semi-automated software Cow-Gait-Analyzer<sup>2</sup>. The variables were calculated as the absolute differences across limbs within cows ( $\Delta_{MT}$ ). For comparison between different groups, one-way ANOVA and receiver operator characteristic analysis (ROC) were performed to determine the performance of the pedogram at the cow level and to calculate the optimal cutoff values for the different gait cycle variables.

**Results:** A significant difference between group C and group L was found for the following variables: relative stance-phase and swing-phase durations and peaks of foot load and toe-off. The variable relative stance phase duration allowed the correct allocation of each cow with a sensitivity and specificity of 100% (threshold of  $\Delta_{MT} = 2.09\%$ ). Peak foot load was significantly different between LOC1 and LOC2. However, the performance of



the cow pedogram was poor to differentiate between LOC1 and LOC2.

**Conclusions:** With the variables of the cow pedogram, differentiation between proximally and distally located orthopedic pathologies is not possible with a sufficient sensitivity and specificity. However, comparison of various pedogram variables between affected and healthy contralateral limb represents a highly sensitive tool for detection of cows with orthopedic pathologies, even if no sign of lameness is visible.

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HL-08

### Sense of sensors: behavioral aspects of dairy cattle in the transition period related to their locomotion scores

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A problem that is often underexposed in the dairy industry is lameness. Due to claw disorders, for example (inter)digital dermatitis, heel horn erosion, toe tip necrosis, laminitis, white line disease, toe and sole ulcerations, many cows at dairy farms all over the world are lame to some degree. Claw disorders are painful and associated with diseases. Besides the impact on animal welfare, lameness is associated with an increased culling rate, a reduced milk yield and, therefore, economic losses. Although a claw problem sometimes is not associated with clear lameness, because pain experience is often masked unless it is severe, it can affect a cow's behavior.

Because lameness has different degrees, this study investigated the relationship between locomotion score and behavioral aspects of dairy cattle as measured by sensors in the transition period. During one year, 370 cows from 8 dairy farms in the Netherlands were involved in this investigation. These cows were equipped with two sensors, one leg-sensor and one neck-sensor, which measured the following: number of steps, lying time per day, number of lying bouts per day, duration per lying bout, eating time per day, number of eating bouts per day, duration per eating bout and ruminating time per day.

The locomotion score was measured visually on a scale of 1-5. The locomotion score of each cow was determined at 4 moments: at the beginning and end of the dry period, and at 4 and 8 weeks in the postpartum period. The cows were divided into 5 groups (1-5), depending on the degree of lameness. Locomotion groups 1 and 2 were taken together for analysis as the non-lame group.

Cows in locomotion group 3, 4 and 5 had reduced daily eating times compared to non-lame cows (locomotion groups 1 and 2), respectively 39, 63 and 135 minutes/24 h. Cows in locomotion group 4 and 5 had longer daily lying times than non-lame cows, respectively 19 and 93 minutes/24 h and longer lying bouts, respectively 7 and 32 minutes/lying bout. In comparison with non-lame cows, the number of lying bouts per day only decreased in locomotion group 5, with an average of 1.7 lying

bouts less per day. As for ruminating time, cows in locomotion group 4 differed from the non-lame cows, with an average of 22 minutes less per day. Cows in locomotion groups 3, 4 and 5 took fewer steps than non-lame cows, respectively 532, 986 and 2156 steps per day. Furthermore, a certain pattern of locomotion scores of the cows is present during the transition period. There is a decrease of locomotion scores 1 and 2, and an increase of the scores 3, 4 and 5. At the beginning of the dry period, most cows are classified in locomotion group 1 or 2, while 8 weeks after calving is the moment with the least non-lame cows.

In conclusion, lame cows show reduced daily eating times compared to non-lame cows, increasing with the degree of lameness. Cows in locomotion group 4 and 5 have longer daily lying times and longer lying bouts than non-lame cows, the number of lying bouts only decreased in locomotion group 5. Lame cows take fewer steps than non-lame cows, decreasing with the degree of lameness. Only cows in locomotion group 4 have shorter ruminating times than non-lame cows. The information regarding behavior of the cows in different locomotion groups is potentially useful for the identification of cows with a claw problem, as an early warning system. With this data, a diagnostic tool could be made for early detection of cows with claw disorders.

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HL-09

### Evaluation of lameness in cattle under traditional pastoralists' management in Semi-arid zone of Bauchi and Yobe States, North Eastern Nigeria

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**Objectives:** To give an overview of incidences of lameness, identify predisposing factors and type of lesions predominantly associated with lameness in traditionally managed cattle in North Eastern Nigeria.

**Materials and Methods:** A total of 2234 cattle were randomly selected and examined for lameness in 8 Local Government Areas (LGA's) of Bauchi and Yobe states of NE Nigeria. A total of 1187 cattle from 46 farms were examined in Potiskum, Fika, Fune and Nangere LGAs in Yobe state, and 1047 cattle from 37 farms in Jama'are, Katagum and Shira LGA's of Bauchi State. The climate is hot dry tropical savanna with an average annual temperature of 35 °C and average annual rainfall of less than 1000 mm. Farmers were sensitized and agreed to participate in the study. The herdsmen responded to verbal interview on major causes of lameness in their herds. Digital and hoof lesions, traumatic injuries and other causes of lameness were recorded. Examination of lameness was performed visually at farm premises in motion and under physical restraint to ascertain the location and types of lesions. All examinations were performed by registered veterinarians or experienced veterinary assistants. Animals were considered lame using the



## DairyNZ Lameness Scoring Guide.

**Results:** The overall average prevalence of lameness using visual locomotion score (VLS) in Yobe and Bauchi farms were  $4.2 \pm 0.37$  and  $3.9 \pm 0.62$  % ( $P=0.86$ ). Distribution of the incidence by farms ranged between 2.9 and 5.2%. Most of the affected animals were adult bulls. There was no planned hoof examinations and claw trimmings in all the farms. Treatment of lameness was performed with the help of local veterinary clinic where available. All the animals were reared either under semi intensive (31.9%) or extensive (68.1%) management systems. Free grazing with crop residues were used as the major feeds. High dietary protein balanced diet were not used in any of the farms. Average incidence of gross hoof lesions in the period of study were hoof cracks 17.2%, sole ulcer (pododermatitis) 28%, interdigital ulcer (interdigital cleft) 29.3%, and fracture 5.2%. Major predisposing factors include infection (FMD) 27.8%, trauma 41.1%, nutrition 19.3%, snake bite 9.1% and others 2.7%.

**Conclusion:** Traumatic injuries were found to be the most common cause of lameness in the study area, while FMD remained a major infectious cause of foot abscesses in cattle in the Semi - arid zone of Yobe and Bauchi states of Nigeria.

## HL-10

### Accuracy of Corium-parallel trimming technique using of Magnetic Resonance Imaging (MRI) system in the bovine claws

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**Introduction:** The beginning of the bovine claw trimming in Japan was learned by the equine farriers many years ago. According to the traditions, there was an important theory to understand sole horn anatomy. The sole horn is consisted of the SEI-KAKU and KO-KAKU based on hydration status. SEI-KAKU is called hydrated horn looked like wetly, dense, delicate and not pare off any farther. KO-KAKU is also called dehydrated horn that are more whitely and rough compared with SEI-KAKU.

It is easily to define KO-KAKU layer, it contains a pith and chalky cells obviously. One of the Japanese traditional hoof trimming method, called on "Corium-parallel trimming", is a technique that are making of a weight bearing surface paralleled to the sole of the corium. It is needed to understand of this technique, border of layer between SEI-KAKU and KO-KAKU are parallel to the sole of corium. The aim of this study is an accurate of Corium-parallel trimming to using a Magnetic Resonance Imaging (MRI) system to detect of the border layer between the SEI-KAKU and KO-KAKU.

**Materials and methods:** Thirty cadaver feet that were brought from a slaughterhouse within three days after slaughtered were examined by MRI.

A cadaver foot trimmed by corium-parallel trimming was also examined by MRI.

**Results:** Quite clear images were taken. (Osirix MD, Pixmeo Sari) It was obvious that the border layer between SEI-KAKU

and KO-KAKU is parallel to the sole of corium. A image of a cadaver foot trimmed by corium-parallel trimming showed that the weight bearing surface was parallel to the sole of corium.

## HL-11

### Comparison of orthopaedic hoof blocks for the treatment of lame cattle under New Zealand pasture conditions in regard to wear and block duration

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**Objectives:** Lameness in cattle has significant negative implications on cow welfare as well as milk production and fertility. Claw horn lesions are the most important reason for lameness and can be treated effectively by therapeutic trim and elevation of the diseased claw using an orthopaedic block. There is a large range of commercial hoof blocks available but data is limited on their suitability for cows kept in a pasture-based system where walking distances are significantly longer than in indoors systems.

The objectives of this study were therefore to investigate wear and loss rate of three commercial hoof blocks in New Zealand pasture-based dairy cows.

**Materials and Methods:** 68 Friesian and Friesian x Jersey cross dairy cows with unilateral hind limb lameness attributable to a claw horn lesion were recruited for the study. The contralateral healthy claw was treated with a hoof block and blocks were allocated from a randomised table blocked for the three different products, a plastic shoe (Cow Slip), a wooden block (BoviBond) and a foam block (Walkease). Blocks were checked during milking by the farmer (present/ not present) and date of loss was recorded. Measurements for block wear and fit were obtained on day 14 and day 28 after block placement. To establish block wear a reference block corresponding to the treatment block was used to identify six fixed landmarks and depth measures were taken with electronic callipers at the landmarks on the block used for treatment. Blocks were removed on day 28 unless further elevation of the diseased claw was indicated.

**Results:** Random allocation meant that there was no difference in distribution of blocks between right and left hind feet as well as medial and lateral claws ( $X^2=1.3$ ;  $p=0.25$ ). Block duration was significantly different between blocks with mean survival time >26 days for the plastic shoe, 20 days for the wooden block and 14.8 days for the foam block. Compared with the wooden block, the hazard ratio for being lost was 0.25 for the foam block and 1.9 for the plastic shoe. The plastic shoe endured for an average walking distance of 8.52 km compared to 6.20 km (wooden block) and 4.58 km (foam block). Height reduction (wear) of the blocks was greatest for the wooden block (mean 10.1 mm) followed by the foam block while the plastic shoe showed least wear. Wear was greater in the first 14 days compared to days 15-28 in all products for all landmarks.

**Conclusions:** The plastic shoe stayed on for the longest period of time, and thus endured for the furthest walking distance. Additionally, it showed less wear than the other two products indicating that it might be the product best suited to the New Zealand pastoral dairy system, where herds commonly walk



long distances every day.

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HL-12

### Clinical evaluations and histology to test the effect of amino acid complexed trace mineral supplementation on digital dermatitis in growing-finishing feedlot cattle

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**Objectives:** Digital dermatitis (DD) or hairy heel warts is an infectious claw disease associated with major outbreaks of painful lameness in beef and dairy cattle. Currently, DD in beef cattle fed in confinement is underreported or often confused with footrot. Quantitative measures for economic losses in cattle affected by DD are lagging behind the extremely high prevalence of DD (Evans et al 2016, Muelling et al 2014).

To evaluate the effect of amino acid complexed trace minerals (AAC) supplementation on the prevalence of digital dermatitis in beef feedlot cattle, we used clinical evaluations of cattle and histological examination of skin biopsies during the finishing period of two field cohorts of growing-finishing beef cattle in two Midwestern commercial feedlot settings in the USA.

**Materials and Methods:** Two randomized longitudinal field trials, I and II, were conducted for 4 and 11 months, respectively to quantify the effect of AAC on the prevalence of DD in a cohort of 1077 steers and 1120 beef heifers (4 AAC and 4 Control pens each). Trial I has been reported by Kulow et al (2017). Four clinical evaluations of M-stages on all hind feet were conducted either in a stand-up restraint chute or by means of so-called alley checks and records were made using the DD Check App (Tremblay et al 2015, Berry et al 2012, Dopfer et al 1997). The DD acute stages, M2, are associated with pain, while the chronic M4 and M4.1 stages are more associated with the reservoir formation of DD (Kulow et al 2017, Muelling et al 2014). Evaluations were completed either before 60 days on AAC compared to after 60 days on AAC (trial I) or before 60 days on AAC compared to after 100 days on AAC (trial II).

A total of 144 skin samples from Trial I were submitted for histopathological evaluation of DD-associated invasion of treponemes into different layers of the skin (epidermis and dermis).

All steers were followed to slaughter to obtain individual carcass information. Animal use was approved by the University of Wisconsin Animal Care and Use Committee protocol V01525.

**Results:** For both field trials, the relative risk for observing an increased prevalence of DD lesions in steers after being fed the respective diets for 60 days or over 90 days compared to before was significantly higher in the Control group compared to the AAC group.

The final logistic regression models resulted in a statistically significant interaction between time points before and after supplementation when comparing AAC groups that had less rapid spread of DD lesions to Control groups with more rapid spread

of DD. This difference in the spread of DD was interpreted as an effect of the AAC supplementation. Histology showed that DD lesions harbored treponemes more deeply and in larger numbers in DD affected skin and the skin invasion was significantly reduced when cattle were supplemented with AAC.

The production parameters of average daily gain, hot carcass weight (trial data I and II), and final live weight (data for trial I only) were all negatively impacted when steers were observed to have M2 lesions compared to steers with no M2 lesions over the study period. In trial II, cattle fed AAC were observed to have heavier finishing and carcass weights than Controls.

**Conclusions:** The long-term consequences of DD in feedlot cattle are unclear; therefore prevention is key towards controlling DD, particularly during the last 100 days prior to harvest. It has become clear that DD associated treponemes reside deep in the skin for long periods of time and therefore reservoirs of infection are common among cattle.

We have systematically gathered convincing data and evidence that DD is a long-term problem in finishing beef cattle in areas where it has been become endemic. The velocity at which DD can spread is significantly decreased when cattle are supplemented AAC. Carcass yield is significantly reduced when DD is present in the feedlot. Supplementation with AAC may help to manage the state of the disease allowing for implementation and noticeable benefits from preventive measures such as foot baths and improved hygiene on feedlots. This effect of AAC is not expected to completely eliminate DD from beef cattle and concentrations of AAC supplemented should be closely aligned with body weight and dry matter intake of the cattle.

The major result of these trials is part of an awareness campaign for prevention and control of DD in beef cattle to raise welfare in these animals that are currently suffering from pain associated with severe lameness.

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HL-13

### New ways to preserve claw health – comparative study on the efficacy of antibiotics and antibiotic-free treatments for digital dermatitis (Mortellaro disease)

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**Objectives:** Digital dermatitis (DD) is a globally occurring infectious problem on cattle farms which is predominantly caused by *Treponema* spp. (Cornelisse, van Asten et al. 1997, Wilson-Welder, Alt et al. 2015). This important infectious claw dis-





ease leads to severely disrupted skin with erosive and painful lesions (Read and Walker 1998), typically located on the hind legs at the coronary band. Due to the high number of infected animals large quantities of antibiotics are used worldwide (Cutler, Cramer et al. 2013). In addition to an antibiotic spray, non-antibiotic topical treatments are on the market (Fiedler, Sauter-Louis et al. 2015). Even after topical treatment is judged as effective by positive clinical evaluation, many relapses occur throughout the herds, supposedly caused by encysted *Treponema* in the epidermal and dermal layers of the skin. To reduce the amount of dispensed drugs it is necessary to find an effective, pain reducing and sustainable treatment without a negative impact on the environment.

**Materials and methods:** A total of 100 cows on five dairy farms with free stall barns (Southern Germany) were included in the study. Every cow was scored at least once 6 months prior to the start of the study to group the cows into type 1-3 cows. A modified DD lesion score was used to evaluate the feet. Every cow with a M2-lesion was trimmed on day 1 and randomly assigned to one of the five different treatment groups. The topical treatments include a bandage and the single usage of salicylic acid, zinc- and copper chelates, chlortetracycline and a polyurethane wound dressing or the combination of the last two. Punch biopsies and photographs were taken on day 1, 14 and 28. The biopsies ( $\varnothing$  6 mm) were taken under local anesthesia and the cows were treated with an NSAID to avoid any additional pain. By scoring the lesions on farm, examine the skin samples histologically and detect the presence or absence of treponemes in the tissue in the laboratory, a healing process was evaluated.

The experimental set up was evaluated and accepted by the District Government of Upper Bavaria and the number of approval is NTP-ID: 00007101-1-3.

**Results:** All five different treatments show the potential of a positive clinical outcome of the locally /topic treated lesions but the success of the local treatments deviate obviously between the groups. The histological evaluations present different cell reactions dependent on the treatment. The wound closure differs from 3-6 layers of corneocytes to a profound hyperkeratosis. By comparing the treatment groups, it is obvious, that also antibiotic-free treatments have the possibility to improve the stage of disease and lead to an improvement or healing of acute DD-lesions. Also the starting point of being an acute or chronic diseased cow has an impact on the outcome and the effectiveness of a treatment.

**Conclusions:** Antibiotics seem not to be mandatory for the success of a topical treatment for digital dermatitis. The positive clinical outcome was achieved without any environmental changes or footbaths on one of the farms. Early detection of DD-lesions has to be improved to enhance a positive effect of local treatment. By looking at the different resources necessary for treatment regarding costs for products and labor, the environmental impact with long term efficacy, a cost effective treatment without the risk of antibiotic resistance is possible.

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**Key words:** cattle, digital dermatitis, histology, topical treatment, treponemes



IV-01

## The application of bovine hyperimmune serum against selected outer membrane antigens of Gram-negative bacteria in prevention and treatment of respiratory tract infections in dairy calves

### Alternative for antibiotics

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**Objective:** Bovine respiratory disease (BRD) in one of the most expensive problems in dairy calves. Facultative pathogenic, Gram-negative bacteria play important role. Passive immunization of calves with hyperimmune sera is today rarely used to support the health of growing calves. Aim of the study was to evaluate the efficiency of prophylactic and treatment-supporting application of hyperimmune serum against Gram-negative bacteria conservative antigens that induce broad interspecies cross-reactivity.

**Materials and Methods:** Production of hyperimmune (HI) and normal (N) bovine serum

The five liters of blood was taken from jugular vein from ten healthy cows in experimental herds. Five cows selected from this group were hyperimmunized subcutaneously with recombinant *Histophilus somni* Hsp60, *H.somni* OMP40 and their conjugates with core oligosaccharide from *Actinobacillus pleuropneumoniae* LPS, with Emulsigen adjuvant. The immunization took place on day 0, than 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 11<sup>th</sup> week. The five liters of blood were taken from each cow on week 8<sup>th</sup>, 11<sup>th</sup> and 14<sup>th</sup>. Serum harvested from normal (N, before immunization) and hyperimmunized cows (HI) was separately pooled and protected with 0,5% phenol. Antibody against examined antigens was evaluated by ELISA in IgG<sub>1</sub>, IgG<sub>2</sub>, IgM and IgA classes.

Experiment.1. Passive immunization of calves with HI and N bovine serum

Twenty calves received 100 ml HI or N serum subcutaneously on first day of life and on the day of transfer to calf barn (HI and N experimental groups). The control group (C) -twenty calves did not receive the serum. The clinical examination of calves and collection of blood samples were between 24-72 hour of life, on 25 day (+/- 1 d.) , on the day of transfer to calf barn (6-8 weeks of life) and every month until the 6 month of life. In the blood concentration of IgG<sub>1</sub>, IgG<sub>2</sub>, IgM, haptoglobin (Hp), fibrinogen (Fb), serum amyloid A (SAA) was estimated.

In cases of respiratory tract infections nasal swabs were taken for bacterial culture and seroconversion against most common respiratory viral pathogens (BVDV, BRSV, BHV-1, PI-3V) and *Mycoplasma bovis* was examined.

Experiment 2. Application of bovine HI serum in supporting the treatment of BRD in calves.

The study was performed in calves with respiratory tract infections diagnosed between 2<sup>nd</sup> week and 3<sup>rd</sup> month of life. On the first day of standard treatment, 20 calves were injected subcutaneously with 100 ml of HI serum. Control group were calves treated the same way, but without serum application. Clinical

score of illness was performed according to [https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf\\_respiratory\\_scoring\\_chart.pdf](https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_respiratory_scoring_chart.pdf)) at the beginning of treatment and on 7<sup>th</sup> and 14<sup>th</sup> day after, and every month until 6 months of life. At the same time points blood was taken and the same parameters as in Exp.1 were estimated, including seroconversion. At the start of treatment bacterial culture was made from nasal swabs.

**Results:** In Exp.1. The calves in the experimental group with HI serum showed the BRD until 6 month of life less frequent than calves in other experimental group with N serum or without (14.3 vs. 39.3 vs 37.85% respectively). The moderate changes in IgG<sub>1</sub> and IgG<sub>2</sub> concentrations in HI serum protected calves were observed during whole observation period , whereas in N and C groups they fluctuated and increased periodically. Hp and SAA concentrations were the lowest in HI calves at winter season, but not differed from N and C groups in summer-autumn. No differences occurred in Fb concentration.

In Exp.2. within 14 days of treatment 90% and 40% calves which received HI serum at the beginning of BRD treatment, showed decrease of clinical signs in cases of moderate and severe course of illness, respectively. In control group within 14 days clinical signs disappeared in 30% and 10% in moderate and severe cases respectively. Immunoglobulin and acute phase protein concentrations not differed between groups in the later period of observation.

**Conclusions:** Analysis of clinical, immune and inflammatory parameters confirm the protective role of developed HI serum at prophylactic and treatment-supporting application. The developed HI serum may be efficient aid in health protection of dairy calves.

**Comments:** Supported by NCBR project PBS3/A8/33/2015 The elaboration of subunit vaccines for cattle and pigs based on recombinant antigens Hsp60 and OMP40 and core lipopolysaccharide from Gram-negative bacteria.

IV-02

## Innate Immunity and Rumen Resiliency Using Fermentation Bioactives

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Cargill Inc

This presentation highlights epidemiologic surveys in both feedlot and dairy cattle demonstrating a health impact from feeding an all natural product that uses bioactives from a proprietary fermentation process. The fermentation bioactives have demonstrated impacts on the innate immune system. Notably macrophage activation, neutrophil recruitment and natural killer cell activity. Additionally, the bio actives have multiple peer reviewed studies showing production impacts related to rumen microbial resiliency.

The feedyard surveys were conducted on a yard in the western US. Three of the surveys were conducted in cattle destined for the all natural market. They reveal a significant reduction in the incidence of bovine respiratory disease and the concomitant reduction to treat cattle with antibiotics. Two of the surveys were in "conventional cattle". The cattle were surveyed feeding the fermentation bioactives vs metaphylaxis. The cattle fed the



fermentation bioactives out-performed the metaphylaxis cattle in health and production parameters.

The dairy survey involved the analysis of 25 large dairy herds. Incidence of mastitis was analyzed in the previous 4 years. On year 3 the intervention of the fermentation bioactive was implemented. In that survey 16/25 herds saw a significant reduction in the incidence of mastitis.

These studies indicate an overall impact on mastitis in dairy cows and BRD in feedlot cows by impacting the innate immune system, as well as providing rumen microbial resiliency

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IV-03

### Local immune responses in stressed and unstressed beef cattle

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Seventy-five beef calves were randomly assigned into one of three groups of 25 calves. Group one were administered an intranasal viral vaccine containing TS BHV-1, TS PI3 and MLV BRSV while undergoing no stress. Group 2 were administered the same vaccine approximately 12 hours after a 9 hour transportation and group 3 were kept as unvaccinated controls that were also transported. IgA against BRSV and BHV1 and interferon alpha, beta and gamma were measured in nasal secretions. Results of this trial will be discussed.

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IV-04

### Efficacy and safety of pegbovigrastim in EU clinical field trials

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**Objectives:** Many diseases that dairy cows experience in the early post-partum period are a consequence of both management and physiological factors in the dry period (Kelton 1998). Diseases attributable to immune function in this period include mastitis, retained placenta (RP) and metritis (Nonnecke 2003) (Hammon 2006). Innate immune suppression is manifested as a decrease in neutrophil function: myeloperoxidase (MPO) activity, Reactive Oxygen Species (ROS) activity and effective migration (Detilleux 1995) (Kehrli 1999). Immune modulation is possible by administration of the cytokine, bovine granulocyte colony-stimulating factor (bG-CSF) (Kehrli 1990). Pegylated bG-CSF, or pegbovigrastim (Imrestor®, Elanco Animal Health) has been shown to increase the number, MPO function, activity and migration of neutrophils (Kehrli 1991) (Kimura 1999). Imrestor has consistently shown to aid in the reduction of clinical mastitis in early lactation in both Mexico and the USA (Ruiz 2016) (Canning 2017). In this study, we investigated pegbovigrastim effects on mastitis and animal safety at 35 sites in the European Union.

**Materials and Methods:** This study was executed from August

2013 through July 2014, at 35 commercial dairy farms located in the United Kingdom, Germany, Hungary and The Netherlands, representative of typical farm husbandry styles in Europe. Cows were milked either twice or three times daily and were evaluated for signs of clinical mastitis and general health at least twice per day to assess clinical safety. Cows in the treated group were administered 15 mg of pegbovigrastim subcutaneously, approximately 7 days prior to their anticipated calving date. The second syringe was given within 24 hours after calving. The control group received an equivalent amount of sterile saline. Treatments were applied blinded by a designated treatment administrator. Animals were physically examined by a licensed veterinarian or trained designee on the day of enrollment (Day -7). The clinical status of each quarter was recorded twice daily from first milking to day 30 post-calving. Clinical mastitis scoring was executed according to published EU guidance documents (see below). If a cow exhibited one or more abnormal quarters or milk at any of the milking times, duplicate quarter milk samples were obtained for microbiological analysis.

**Results:** A total of 2561 animals (1509 cows, 58.92 % of the population /1052 heifers, 41.08 % of the population) were enrolled and 96 removed due to protocol deviations. Of the 2465 animals that were evaluated for mastitis, 1235 were treated with pegbovigrastim and 1230 with an equivalent amount of saline. From 1230 cows in the control group, 156 showed clinical signs of mastitis (12.68 %) and of the 1235 cows in the pegbovigrastim group, only 116 developed mastitis (9.39%). This represents a relative reduction in the incidence of clinical mastitis in the first 30 days after calving of 25.95% ( $p=0.0095$ ). Approximately 400 milk samples were submitted for microbiological evaluation. Isolates included Gram-positive pathogens such as *Staph. aureus*, *Strep. dysgalactia*, and *Strep. uberis*. Gram-negative pathogens included *E. coli*, *Serratia marcescens* and *Klebsiella pneumoniae*. Abnormal observations were typical of those seen at commercial dairy farms during the periparturient period, including issues relating to lameness, reproductive health and metabolic disorders. These however, were not directly correlated to the use of Imrestor. The average number of days between the first and second treatment were 9.0 days for the control group and 9.3 days for the treated group, indicating that gestation length was not affected by treatment

**Conclusion:** In this 35 site, EU study, it was demonstrated that pegbovigrastim provided a statistically significant, 25.95% ( $p=0.0095$ ) relative reduction in the incidence of naturally occurring clinical mastitis in the first 30 days post partum compared to the control group. The administration of Imrestor to periparturient dairy cows and heifers was shown to be both safe and efficacious when administered approximately 7 days prior to predicted calving and within 24 hours after calving. Abnormal health observations were typical of those to be expected in commercial dairy farming and no impact on gestation length was observed.

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IV-05

### Relationship between hemorrhagic bowel syndrome and *Clostridium perfringens* type A toxin

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**Objective:** Hemorrhagic bowel syndrome (HBS) medical treatment has had limited success. We investigated whether the a toxin produced by type A *Clostridium perfringens* (CPaT), which causes enterotoxemia, influences the onset of HBS. To investigate the status of natural antibody titer to CPaT (CPaTAT) and factors affecting CPaTAT in dairy cows, we examined CPaTAT in healthy cows and cows affected with HBS or other gastrointestinal diseases. We also investigated the duration of CPaTAT persistence after vaccination, with a vaccine containing CPa-Toxoid in dairy cows.

**Materials and Methods:** CPaTAT was examined in 115 Holstein dairy cows (age 2–89 months) fed at five dairy farms in the Nemuro area of Hokkaido, Japan. CPaTAT was also examined in 36 cows that were treated by surgical operation for gastrointestinal diseases including 17 with HBS, and in 8 cows that died prior to surgery and underwent pathological autopsy (a total of 44 cows). CPaTAT was also observed over a 231-day period in 5 dairy cows administered a vaccine containing CPaToxoid and in 6 dairy cows administered a vaccine not containing CPaToxoid (a total of 11 cows fed in different farms).

**Results:** In the healthy cows from 5 dairy farms, which had not been vaccinated with CPaToxoid, the CPaTAT varied between 1:50 to 1:1600. The titers were higher in older aged cows than younger cows. The titers increased more below 12 months of age and first calving. The geometric means of CPaTAT with HBS, abomasal diseases (displacement, ulcer, torsion), and intestinal diseases except for HBS such as enteritis and torsion were 1:1200, 1:1900 and 1:1200, respectively. These titers were extremely high compared with those of healthy cows. (1:340). After the vaccination containing CPaToxoid, CPaTAT values were higher than the non-CPaToxoid ones. Significantly higher titers were recognized between 31 and 154 days after CPaToxoid vaccination.

**Conclusions:** Our results suggested that CPaT may influence the onset of gastrointestinal diseases, abomasal diseases, and

HBS in dairy cows. Vaccination prior to calving with a vaccine including CPaToxoid may help to prevent the occurrence of serious gastrointestinal diseases.

IV-06

### *In vitro* and clinical study on antiviral activity of an anti-PD-L1 rat-bovine chimeric antibody against bovine leukemia virus infection

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Programmed death-1 (PD-1), an immunoinhibitory receptor on T cells, is known to be involved in immune evasion of pathogens through its binding to PD-ligand 1 (PD-L1) in many chronic diseases. We previously reported that PD-L1 expression was upregulated in cattle infected with bovine leukemia virus (BLV) and that an anti-PD-L1 antibody that blocks the PD-1/PD-L1 interaction reactivated T cell function *in vitro*. Therefore, this study aimed to assess the antiviral activities of anti-PD-L1 antibody *in vivo*. First, we inoculated the anti-bovine PD-L1 rat monoclonal antibody 4G12 into a BLV-infected cow. However, this did not induce T-cell proliferation or reduction of BLV provirus loads during the test period, and only bound to circulating IgM+ B cells until one week post-inoculation. We hypothesized that the lack of *in vivo* effects was due to its lower stability in cattle. So, we established an anti-PD-L1 rat-bovine chimeric antibody (Boch4G12). Boch4G12 was able to bind to bovine PD-L1 specifically, to interrupt the PD-1/PD-L1 interaction, and to activate the immune response in both healthy and BLV-infected cattle *in vitro*. Therefore, we experimentally infected a healthy calf with BLV and inoculated it intravenously with 1 mg/kg of Boch4G12 once it reached the aleukemic (AL) stage. Cultivation of peripheral blood mononuclear cells (PBMCs) isolated from the tested calf indicated that the proliferation of CD4+ T cells was increased by Boch4G12 inoculation, while BLV provirus loads were significantly reduced, clearly demonstrating that this treatment induced antiviral activities. Therefore, further studies using a large number of animals are required to support its efficacy for clinical application.

IV-07

### Overcoming T-cell exhaustion in chronic infections of cattle: a pilot clinical study of anti-PD-1 immunotherapy in bovine leukemia virus infection

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**Objectives:** Progressive exhaustion of T-cell functions is considered to facilitate the immunopathogenesis of several chronic infections, where pathogens evade immune elimination and establish persistent infection. We have reported that an immunoinhibitory signaling via programmed death-1 (PD-1)/PD-ligand 1 (PD-L1) downregulates T-cell functions in cattle with bovine leukemia virus (BLV) infection, Johne's disease, bovine anaplasmosis, and mycoplasmosis. We therefore hypothesized that blockade of the PD-1/PD-L1 interaction restores the effector function of T cells and prevents disease progression in these diseases. To test this hypothesis, this study aimed to establish the blocking antibody targeting bovine PD-1 molecule, to characterize its biological function, and to confirm its clinical efficacy in a pilot clinical trial of BLV-infected cattle.

**Materials and methods:** The nucleotide sequences of the variable regions of the heavy and light chains of anti-bovine PD-1 monoclonal antibody (mAb) were combined with the constant regions of modified bovine IgG1 and bovine Ig lambda, respectively. Stable high-producer cell lines expressing anti-bovine PD-1 chimeric antibody (chAb) were established with the use of the mammalian expression system in Chinese hamster ovary (CHO)-DG44 cells transfected with plasmid vectors encoding the constructed chAb sequence. The chAb was produced by shaking cultivation of the established cell clones and purified from the culture supernatant by affinity chromatography with Protein G/A.

The binding ability of anti-PD-1 chAb was confirmed by flow cytometric analysis with membrane-bound bovine PD-1-expressing cells. Surface plasmon resonance analysis was performed to determine the binding affinity of anti-PD-1 chAb with the use of a Biacore instrument and polyhistidine-tagged bovine PD-1 protein. To evaluate the ability of the chAb to block PD-1/PD-L1 interaction, a flow cytometry-based binding assay was conducted using bovine PD-1-Ig protein and bovine PD-L1-EGFP-expressing cells in the presence of the chAb.

To confirm the clinical efficacy of anti-bovine PD-1 chAb in cattle, BLV-infected young and adult cattle (Holstein breed) were administered the purified chAb intravenously. Peripheral blood was collected before and more than once a week after inoculation. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples, labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE), and cultured with heat-inactivated whole BLV proteins or BLV gp51 peptide mix for 6 days. After the cultivation, proliferations of CD4<sup>+</sup> and CD8<sup>+</sup> T cells were analyzed by flow cytometry. In addition, BLV proviral loads were measured by quantitative real-time PCR targeting BLV *tax* gene with extracted genomic DNA. The concentration of DNA was measured by UV absorbance at 260 nm with a spectrophotometer.

**Results:** Anti-bovine PD-1 bovinized chAb was stably expressed and produced with the use of the CHO-DG44 cell expression system and successfully purified from supernatants using Protein G/A resins. As expected, the heavy and light chains of the chAb were detected at approximately 50 and 25 kDa in SDS-PAGE, respectively.

Flow cytometric and Biacore analyses determined that the binding ability and affinity of the chAb were similar to that of the original anti-bovine PD-1 mAb. The chAb was also capable of

blocking PD-1/PD-L1 binding at the same level as the original mAb.

The immunomodulatory and therapeutic effects of the chAb were evaluated by *in vivo* administration of the antibody to BLV-infected animals. The chAb inoculation resulted in activation of the proliferation of BLV-specific CD4<sup>+</sup> T cells and decrease in the proviral load of BLV in the peripheral blood.

**Conclusions:** This study demonstrates that the established anti-bovine PD-1 chAb retains equivalent biochemical functions to that of the original mAb and anti-PD-1 immunotherapy is a significant strategy for the regulation of anti-viral T-cell response in cattle and prevention of the disease progression of BLV infection.

This trial is the first attempt of antibody-based immunotherapy in cattle. Therefore, the result requires confirmation in additional clinical trials with a large number of BLV-infected animals from different herds or farms. Additionally, a clinical efficacy of this immunotherapy in cattle with other chronic diseases awaits further experimentation.

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IV-08

### A study to compare serological response to vaccination with an inactivated Bovine Respiratory Disease vaccine given at various inter-vaccination intervals

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**Objectives:** To evaluate the effectiveness of an inactivated Bovine Respiratory Disease vaccine (Bovilis MH+IBR) at various inter-vaccination intervals (primary to booster intervals).

The trial aimed to determine the serological response 14 days after booster vaccination with a prime to boost interval of 10 days, 14 days, 3 months, 4 months, 6 months and 9 months compared to the serological response 14 days after booster vaccination with a prime to boost interval of 3 weeks (previous label recommendation).

**Method:** A randomised controlled study of nine month old mixed sex Angus calves was conducted under field conditions in Southern Australia:

Eight groups of cattle with 10 to 12 animals each Group 1: non-vaccinated controls Group 2: booster 10 days after first vaccination Group 3: booster 14 days after first vaccination Group 4: booster 3 weeks after first vaccination Group 5: booster 3 months after first vaccination Group 6: booster 4 months after first vaccination Group 7: booster 6 months after first vaccination Group 8: booster 9 months after first vaccination

Blood samples were collected: Pre vaccination After primary vaccination (just prior to boost vaccination) 14 days after boost vaccination At additional time points in Groups 2 to 7 to demonstrate residual circulating antibodies up to 9 months after primary vaccination

**Results:** Infectious Bovine Rhinotracheitis (IBR) results



The proportion of positive cases in all vaccinated groups (Groups 2 to 8) was significantly different, both after primary vaccination and 14 days after booster vaccination, from those in the unvaccinated Control Group (Group 1).

The results demonstrate that the proportion of calves defined as positives did not differ between Groups 2 to 8 vs. Group 4 for both after primary vaccination and 14 days after booster.

#### *Mannheimia haemolytica* (MH) results

1. Nearly all calves enrolled in this trial were positive to MH infection. This may have been due to MH being a commensal organism in the bovine upper respiratory tract and the trial was conducted in a field situation.

Positive cattle were not excluded from the trial since the objective of the trial in comparing serological response between the groups could still be met if non-parametric results were analysed.

2. The MH antibody titres 14 days after booster vaccination in all vaccinated groups (Groups 2 to 8) were significantly different from those in the unvaccinated Control Group (Group 1).

3. All vaccinated groups (except Group 3) had similar serum MH antibody concentrations to Group 4 (current label). Group 3 had a higher serological response than group 4 (rank sum 156.5 vs Group 4 rank sum 96.5), which demonstrates a superior serological response compared to the current vaccination schedule.

**Conclusion:** The serological response to IBR vaccination supported the hypothesis that the proportion of positive cases will not be different when boosters are administered at the different intervals studied. Similarly, the serological response to MH vaccination supported the hypothesis that, when compared to the serological response when boosters are given at 3 weeks, the serological response will be equal or greater when boosters are administered at different intervals.

IV-09

### **Mucosal Immune Response in Young Calves Following Intranasal Administration of Bovine Coronavirus, Modified Live Vaccine**

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**Objectives:** A novel intranasal Bovine Coronavirus (BCV) vaccine (BOVILIS® Coronavirus, MSD Animal Health, USA) has recently been licensed in the United States. The purpose of this study is to evaluate the mucosal immune response following intranasal (IN) administration of the BCV vaccine to young calves in the presence or absence of maternal antibodies.

**Materials and Methods:** Sixty Holstein male and female calves were collected at birth prior to suckling. Calves in groups one through four received 160 grams of IgG via a commercial colostrum replacer (Calf Choice Gold, Saskatoon Colostrum

Company) within two hours of birth and were designated maternal derived antibody positive (MDA+). Calves in groups five through seven were given electrolyte in water but no colostrum. These groups were designated maternal derived antibody negative (MDA-). Calves were 2-4 days of age on study day zero.

All calves were tested prior to day 0 for serum total protein, total IgG and BCV specific antibodies to verify (Zero or >0) maternal antibody status. MDA+ calves were randomly assigned to four groups and MDA- calves were randomly assigned to 3 groups. MDA+ calves received 1 dose (2ml) of BCV vaccine IN on day 0 (group 1) or 2 doses of vaccine on day 0 and 21 (group 2). Group 4 MDA+ calves received 1 dose of a commercial IN IBR vaccine (Nasalgen, MSD Animal Health, USA) to serve as positive controls. MDA- calves received 1 dose of BCV vaccine on day 0 (group 5) or two doses of the same vaccine on days 0 and 21 (group 6). Group 3 (MDA+) and group 7 (MDA-) calves were unvaccinated controls.

Blood and nasal secretion samples were collected at designated time points from day 0 to 42.

**Results:** BCV IgA titers in nasal secretions collected from MDA- calves were significantly ( $p < 0.05$ ) higher by day 14 following vaccination than in all other groups. MDA- calves revaccinated at day 21 showed a significant ( $P < 0.01$ ) secondary vaccination response. BCV IgA titers in MDA+ calves did not differ statistically from unvaccinated controls. There was a significant ( $p < 0.01$ ) negative correlation between serum BCV titers on day zero and mucosal IgA response by day 21 in vaccinated calves. Calves with higher levels of maternal antibodies on day 0 had lower IgA titers in nasal secretions 21 days after vaccination. Fewer calves in the MDA+ groups responded to vaccination (2/14) compared to MDA- calves (10/14).

In calves that received a second vaccination at day 21 the response was also greater ( $p < .001$ ) in the MDA- groups. A larger proportion of calves in the MDA+ groups responded to secondary vaccination (4/8) than to primary vaccination, but still fewer and of lower magnitude compared to MDA- calves (7/8) vaccinated twice.

The average response of MDA+ calves in group 4 was positive for Bovine Herpes Virus (BHV-1) IgA demonstrating that MDA+ calves in the study were capable of producing an antibody response in the presence of maternal antibodies to a previously studied immune antigen.

A serum BCV neutralization antibody increase was evident after vaccination in MDA- calves but not in MDA+ calves when compared to controls.

Detectable levels of interferon gamma were present in nasal secretions collected from Group 4 (IBR IN vaccinates) on days 2 and 4 post-vaccination but not in any of the BCV vaccinated or control groups ( $P < 0.001$ ).

**Conclusions:** The results of this study demonstrate that

1/ An IgA response to intranasal vaccination with a BCV vaccine is reduced but not eliminated by the presence of maternal antibodies.

2/ A secondary vaccination at a 3 week interval is an effective strategy for -exploiting immune memory in calves with low transfer of maternal antibody and to a lesser extent in calves with high maternal antibody levels.

3/ The production of Interferon-gamma is not stimulated by the BCV vaccine regardless of colostrum status.



4/ The demonstrated ability of this novel vaccine to stimulate both a mucosal and systemic antibody response in colostrum deprived calves is valuable given the high incidence of failure of passive transfer on many commercial dairies (Can Vet J. 2011 May; 52(5): 524–526).

5/ Further study is needed to elucidate the full protective mechanism of this BCV vaccine.

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IV-10

### Stimulation of passive immunity by vaccination of dry cows with a multivalent inactivated respiratory vaccine.

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**Objectives:** During the first months of life new-born calves are more susceptible to infections, among which bovine respiratory disease (BRD). BRD is a complex and multifactorial disease caused by viruses and bacteria, and is one of the most important cattle diseases. The viral pathogens associated with BRD include: bovine herpesvirus type 1 (BHV-1), parainfluenza-3 virus (PI3), bovine viral diarrhoea virus (BVDV), and bovine respiratory syncytial virus (BRSV). Bacterial pathogens associated with BRD include: *Mannheimia haemolytica* (Mh), *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis* and *Mycoplasma dispar*. Calves may be vaccinated early in life. However, calf immunization may be adversely affected by interference from maternal antibodies or unfavorable environmental conditions. Therefore, the effective vaccination of pregnant cows and the subsequent colostrum intake by their offspring can enhance the immune response of new-born calves. The aim of this study was to investigate the level of passive immunity acquired by calves that have been fed colostrum derived from their own dam depending on the moment of pre-partum vaccination against Mh, PI3 and BRSV.

**Materials and methods:** Forty-eight pregnant Holstein Friesian cows from a commercial 550 head dairy farm vaccinated for BRD were divided in four groups (6 heifers and 6 cows each). The animals in three groups were vaccinated once against Mh, PI3 and BRSV (Bovilis® Bovipast RSP, MSD Animal Health) (with a single dose) at respectively 30 (group 30), 60 (group 60) and 90 (group 90) days before parturition, and one group of unvaccinated dams served as control. Directly after parturition the new-born calves were separated from their dams. The calves were kept according to the standard breeding regulations, and orally fed 4L colostrum derived from their own dams within 6 hours after birth. Rectal temperatures, general condition and the presence of respiratory signs were recorded daily. Blood samples were collected from cows and heifers at the time of vaccination and at parturition, and weekly from the 48 newborn calves during 12 weeks. The total level of colostrum immunoglobulins (Ig) was determined in by the Clinical pathology service of Istituto Zooprofilattico Sperimentale delle Venezie by electrophoresis. Mh specific antibody levels were determined on the blood samples using an in-house ELI-

SA from the Centre for Diagnostic Services of MSD Animal Health (Boxmeer, The Netherlands). A commercial ELISA kit was used to obtain the antibody levels on blood of BRSV (Svanovir BRSV Ab, Sweden) was determined in serological service of Istituto Zooprofilattico Sperimentale delle Venezie. The results are presented as arithmetic means. The differences between the arithmetic mean values recorded in the experimental and control groups at the same time point were analysed using test ANOVA.

**Results:** No respiratory signs were observed in any of the calves throughout the study. The colostrum of unvaccinated control cows had an average Ig concentration of 91.1 mg/ml while group 30, group 60 and group 90 had an average Ig concentration of respectively 94.43 mg/ml, 70.70 mg/ml, 80.92 mg/ml. The colostrum quality was very high for all groups including the control animals and not significantly different.

The Mh specific antibody levels, valued as average of all the samples in a group, were significantly higher for group 30 and 60 compared to the control group ( $p < 0.001$ ). The titers declined slightly over time, but remained higher in the vaccinated groups than in the control group throughout the experiment.

The BRSV antibody titers were significantly higher in the calves from the vaccinated dams compared to calves from the control group throughout the study ( $p < 0.01$ ). Although from the 11th week there was a rise in BRSV's titers in the control group.

**Conclusions:** Makoschey et al. (2012) and Dudek et al. (2014) already showed the positive effect of vaccinating pregnant cows with a multivalent inactivated vaccine (Bovilis® Bovipast RSP) on the level of passive immunity in their offspring. Our results confirmed that the vaccination of dry cows at 60 and 30 days before parturition gives higher serum titers of Mh and BRSV specific antibodies in calves receiving colostrum from their proper dams compared to calves that received colostrum from unvaccinated dams. There are no differences in the vaccine titres of calves from vaccinated cows 90 days before calving. Pre-partum immunization of the cows with the specific multivalent inactivated vaccine against BRD (Bovilis® Bovipast RSP) may effectively stimulate the level of passive immunity in their offspring.

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IV-11

### FMD-LL3B3D Vaccine Platform: Safe, Highly Potent, Fully DIVA Compatible, Inactivated Foot-and-Mouth Disease Virus Vaccines

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Traditional foot-and-mouth disease virus (FMDV) vaccines are used to control FMD around the world in spite of drawbacks - (1) large quantities of virulent FMDV are used, with the risk of



virus escaping from manufacturing facilities or incomplete inactivation during the vaccine formulation process; (2) traditional vaccines produced from wild type FMDV are not fully compatible with a DIVA approach, since small amounts of nonstructural proteins (NSPs) may still be present; and (3) they do not fully protect animals from persistent infection. A novel, marked FMD-LL3B3D vaccine platform under development by Zoetis, Inc. and The United States Department of Agriculture - Agricultural Research Service, consists of an attenuated virus platform containing negative markers in the NSPs 3B and 3D<sup>pol</sup>. This vaccine platform allows for the easy exchange of capsid coding sequences. In contrast to wild-type FMD vaccine viruses, the FMD-LL3B3D vaccine viruses induce no clinical signs of FMD and no shedding of virus in cattle or pigs when inoculated as a live virus. This vaccine platform may use existing FMD vaccine manufacturing technology and significantly lowers biosafety risks associated with FMD vaccine production. Upon exclusion from the Select Agent Program, the vaccine platform may be used to produce high potency, fully DIVA compatible FMD vaccines in the United States. Cattle immunized with a variety of chemically inactivated FMD-LL3B3D vaccine constructs were protected from challenge with parental virus. Two negative markers allow the FMD-LL3B3D vaccines to be fully DIVA compatible. This vaccine platform, currently undergoing development in the United States, provides opportunities for safer and higher potency FMD vaccines in support of global control and eradication programs.

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IV-12

### **A non-inferiority study of low dose intradermal rabies vaccine and standard dose intramuscular vaccination**

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Massey University

Intradermal vaccination delivers antigen directly to an area that has a significant population of antigen-presenting cells so can produce equivalent or higher protective antibody titres than conventional intramuscular or subcutaneous vaccination even when given at a lower dose rate.

In developing countries the cost of vaccination limits the use of prophylactic rabies vaccination, especially in livestock. Low dose intradermal vaccination has been shown to be effective in humans and dogs; if intradermal vaccination was effective in cattle the cost of vaccination would be markedly decreased thereby increasing the proportion of cattle vaccinated in endemically-infected areas.

However there have been very few studies of intradermal rabies vaccination in cattle, all of which have been small-scale. As of January 2018 there are no rabies vaccinations which have been licensed for intradermal use in cattle. This study was undertaken to compare the antibody response in cattle vaccinated using a standard intramuscular (im) dose (1 ml) of an inactivated rabies vaccine (Raksharab, Indian Immunologicals) with that of cattle vaccinated with 0.2 mL of the same vaccine intradermally (id). The study was undertaken in the Haa province of Bhutan, an area of Bhutan where rabies is not endemic so that the change in antibodies response would only be

due to a vaccine response.

100 cattle on 27 farms were selected for the study. Up to 4 cattle were randomly assigned on each farm to either id or im vaccination; on 10 farms where more than 4 cattle were kept an extra cow was included in the study as an untreated control to confirm the absence of rabies infection in the study area. Virus neutralising antibody response (VNA) was measured using a fluorescent antibody virus neutralisation test on the day of vaccination (day 0) and 14, 30, 60 and 90 days later. The effect of vaccine route on the proportion of animals with a protective titre (> or= 0.5 IU/mL) at each timepoint was assessed using a repeat measures general linear model with a binomial response and a logit link, while the effect of vaccination route on log VNA was assessed using a linear mixed model.

Overall 89% of im vaccinated animals produced a protective response (> or= to 0.5IU/mL) but only 71% of id vaccinated cattle. This difference was significant ( $P < 0.02$ ) on days 14 and 30 post vaccination – with 36 and 56%, respectively, of id vaccinated animals having a protective response on those days compared to the equivalent figures of 78 and 76% for im vaccinated cattle. VNA titres were lower for id vaccinated animals than im vaccinated cattle ( $p < 0.001$ ); on both days 14 and 30 mean back-transformed VNA titres of id vaccinated cattle were >0.6 IU/mL lower than the mean titres of the im vaccinated cattle. Thus although low dose id vaccination did produce a detectable antibody response it was inferior to im vaccination. Further research is required to establish whether increasing the dose or using multiple vaccinations would improve the response to id vaccination using this vaccine in cattle.





BC-01

## Dairy UK Johne's survey – winning farmer engagement

### A survey of 394 farmers opinions of the UK approach to Johnes control

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**Introduction:** Successful Johne's control requires a complex mixture of science, policy, practical interventions but most of all an ability to engage and enthuse the farmer to apply the necessary changes at farm level

Dairy UK hosted a conference in February 2017 to launch Phase 2 of the UK National Johne's Management Plan (NJMP). The successful adoption of the NJMP by UK dairy farmers will allow BCVA trained vets to assess risks of Johne's Disease (JD) transmission and develop, in conjunction with their clients, a veterinary led robust JD management plan. For this to be a success the blocks to progress and opportunities must be clearly identified.

**Method:** A convenience or non random survey of 394 farmers was conducted in January 2017 to assess dairy farmers' attitudes to Johne's control. The results of this survey were shared at the conference and 4 key questions were further discussed on approaches to testing, segregation of cows, creating a robust control plan and effective communication with farmers.

**Results:** 394 farmers non-randomly selected completed a survey. Of those surveyed, 71% had developed a robust JD control plan in conjunction with their vet and a further 22% has created a plan based on their own research and talks. A further 4% planned to start JD control soon. No farmers surveyed failed to believe in the need to control the risks of JD.

47% of farmers were happy with their control plan and had no concerns. The key problems with JD control related to segregation of high risk animals (53%), TB testing interfering with results (40%) and uncertainty on when to cull test positive cows (38%).

The major benefit of effective JD control appeared to be an improved overall health of the herd (83%), reduced forced culling (63%) and improved fertility (50%). Other economic gains were cited such as improved farm margins (40%) and better market opportunities for my processor (42%). Farmers also cited that they had less worry and anxiety now they were in control of JD (41%) and 35% believed they had improved mastitis and udder health through more effective JD control.

When asked how they felt about JD control 78% classified themselves as firm believers and would recommend it to other farmers. A further 13% were controlling JD for the benefit of their processor. 7% knew they should control JD but it remained low on their priority right now. The latter two groups were further analysed as an Unconcerned group and compared to the Proactivists in the detailed analysis

Farmers were asked to score themselves on a scale of 1-10 as to on how likely that they would recommend Johne's control and Protection to another farmer.

51% were classified as Promoters (score 9-10), 28% Passives (score 7-8) and 21% Detractors (score 0-6). The NP score was

29 indicating that the scheme was broadly positively accepted. The level of satisfaction amongst respondents was high and satisfactory as measured by belief and Net Promoter Score respectively

The key driver for future engagement was financial incentives from their milk processor (50%), more on farm training on JD and practical control (27%) and more evidence that JD control works (28%). Further help on understanding risks (24%) and opportunities to visit farms who have controlled disease (20%) would be valued.

Both Proactivists and Unconcerned farmers supported financial incentives (58% and 47% respectively) and on farm training. However, the Unconcerned group required more convincing the rewards outweigh the benefits (31% vs 17% for Proactivists) and more evidence that Johne's control works (49% vs 21% for Proactivists).

**Summary:** The UK JD control program has been broadly accepted by the farmers with a positive Net Promoter Score. A social norm for control has been created through a nationwide JD engagement program. Extension of the program to the non-Proactivists farmer will require different approaches with a combination of education and incentives focusing on strengthening the belief that JD control works rather than pursuing other classical approaches such as herd certification.

BC-02

## Effective herd level control of paratuberculosis in beef cow herds: a 20 year history of UK national control programme

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Paratuberculosis control programmes have been running in many countries for decades and the focus of control has largely been the dairy herd. In 1998 a voluntary programme for the control and accreditation of freedom from paratuberculosis was launched in the United Kingdom. This programme developed to accommodate a risk-based approach to provide an assessment of risk that was based on the presence or absence of test positive animals in herds and adopted a progressive management programme to reduce the risk of introduction of infection and the spread of infection within the herd. After two decades there are 2690 herds that participate in the programme delivered by one provider. From the beginning the participants were largely the beef cow herds with few from the dairy sector. The programme has used a test and cull strategy, following standard testing methodology. In 2017 of the participating herds 1070 (40%) were accredited at the lowest risk level (risk level 1) and all major beef breed societies in the United Kingdom require herds to declare their herd's paratuberculosis risk level at the point of sale. This programme has been successful in raising awareness of paratuberculosis in the beef cow sector; reduced the financial loss experienced in infected herds through early culling of test positive animals with favourable carcass sales; and it has provided a pool of animals of high genetic merit with a measure of the risk of paratuberculosis infection. This paper will describe the programme and the lessons learned from



twenty years of active paratuberculosis control in the beef cow sector.

BC-03

### Evaluation of the proportion of fetal infection with *Mycobacterium avium* subspecies *paratuberculosis* based on histopathological typing of cows diagnosed with paratuberculosis

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**Objectives:** The elimination of calves from cows infected with paratuberculosis (PTB) is one of the recommended control measures since there is abundant evidence of in utero fetal infections. There are many reports on fetal infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cows with clinical signs of PTB or high fecal shedding. However, it is difficult to eliminate all calves born to cows diagnosed with PTB because of the economic losses incurred by the farm. Moreover, there have been a few case studies on fetal infections, based on histopathological examination, which would evaluate more precisely the transmission of MAP and its effect on the body. In this study, we have reported on the proportion of fetal MAP infections based on histopathological examination of the ilea of pregnant cows diagnosed with PTB, to determine the elimination and for those calves.

**Materials and methods:** In Tokachi District, Hokkaido, between July 2016 and March 2017, the ileoceca of twenty-two pregnant cows diagnosed with PTB were histopathologically examined. The lesions were classified into four types based on severity: "N type", showing no specific PTB pathology; "Tuberculoïd type" (T), when tuberculoïd lesions (multinucleated giant cells) were observed (mild form); "T and L type" (T/L), when both T and L were observed (moderate form); and "Lepromatous type" (L), when lepromatous pathology (epithelioid cell cluster) was observed (severe type). The amount of MAP in the tissue was also dependent on the histopathological typing (N<T<L). The fetal tissues (including heart, lung, kidney, liver, spleen, ileum, jejunum, colon and meconium), umbilical cord, caruncle, umbilical blood and amniotic fluid were cultured and subjected to histopathological examination.

**Results:** The histopathological types of the ilea from the 22 pregnant cows were classified as follows: 7, 8, 2, 5 (N, T, T/L, L, respectively). Isolation rates of MAP from fetuses increased depending on the level of severity of the types (0%, 25%, 50%, and 60% for N, T, T/L, and L, respectively). MAP was most often isolated from the liver (4 fetuses), and less frequently from the heart, spleen, kidney and ileum (1 fetus each). MAP was also isolated from the umbilical cord of 3 fetuses and the caruncle of 6 fetuses. In all the fetal tissues examined in this study, there were no pathological findings of PTB and no acid-fast bacilli.

**Conclusions:** We found that the most severe lesion type (L) was associated with the highest rate of fetal MAP infection. The results also suggest that fetal infection in cows with lesions classified as type N, are rare. Additionally, we found the highest rate of MAP isolation from the liver than from other organs, in agreement with the results of earlier research. This higher pro-

portion of isolation from the liver than from other organs, along with our findings from the umbilical cords and caruncles, suggests that MAP disseminates hematogenously via the umbilical cord, depending on the disease condition. The absence of histopathological observations specific for PTB in any of the fetuses suggests that if lesions did form, in case of a severe MAP infection, embryonic death or abortion might have resulted. This may explain why the fetuses in this study did not have any lesions. Our results, especially the high rate of fetal infection in cows with lesions classified as type L, suggest that an effective control strategy for PTB would be to eliminate the last calf born to a cow diagnosed with PTB.

BC-04

### Prostaglandin E<sub>2</sub> suppresses immune responses in cattle with Johne's disease

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**Objectives:** Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), is a chronic bovine infection and widespread in many countries including Japan. In Map-infected cattle, the expression of immunoinhibitory molecules, programmed death-1 (PD-1), PD-ligand 1 (PD-L1), and lymphocyte activation gene 3 (LAG-3), is upregulated, but the detailed mechanism of immunosuppression has not been fully elucidated. In humans, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is known to have a suppressive role in immune cells including T cells, natural killer cells, dendritic cells, and macrophages via its receptors, EP2 and EP4. However, in the field of veterinary medicine, little information is available on the immunosuppressive function of PGE<sub>2</sub>, and its association with bovine chronic infections is largely unknown. In this study, the functional and kinetic analysis of PGE<sub>2</sub> was conducted on Johne's disease in which immunosuppression is frequently observed.

**Materials and Methods:** Serum PGE<sub>2</sub> concentration in Map-infected and uninfected cattle was determined by ELISA. Peripheral blood mononuclear cells (PBMCs) from uninfected cattle were cultured with PGE<sub>2</sub>, the proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> cells and PD-L1 expression were assayed by flow cytometry. In addition, IFN- $\gamma$  and TNF- $\alpha$  production in the culture supernatant was determined by ELISA. Subsequently, PBMCs from Map-infected cattle were cultured with a COX-2 inhibitor and/or anti-PD-L1 antibody (Ab) in the presence of the Map antigen. The proliferation of CD8<sup>+</sup> cells was assayed by flow cytometry, and IFN- $\gamma$  production was determined by ELISA.



**Results:** PGE<sub>2</sub> was upregulated in cattle with Johne's disease. PGE<sub>2</sub> inhibited the production of Th1 cytokines and cell proliferation, and upregulated PD-L1 expression in PBMCs. A COX-2 inhibitor promoted CD8<sup>+</sup> cell proliferation and production of Th1 cytokines. Similarly, blockade of the PD-1/PD-L1 pathway induced CD8<sup>+</sup> cell proliferation and IFN- $\gamma$  production *in vitro*. Interestingly, combined treatment with the COX-2 inhibitor and anti-PD-L1 Ab enhanced CD8<sup>+</sup> cell proliferation more effectively.

**Conclusion:** Our results show that PGE<sub>2</sub> may contribute to the progression of Johne's disease by the suppression of Th1 responses, and a COX-2 inhibitor has the immunostimulatory effect on bovine immune cells. Moreover, co-targeting of PGE<sub>2</sub> and PD-L1 could be a novel immunotherapy against Johne's disease.

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BC-05

### The investigation of a persistent outbreak of bovine tuberculosis using a novel enhanced cattle testing programme and evaluation of environmental contamination.

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**Objectives and Introduction:** An intensive dairy herd suffered a chronic endemic infection of bovine tuberculosis for more than five years. The whole herd, comprising over 1100, animals were tested using the Single Comparative Intradermal Tuberculin Test (SCITT) every 60 days or so in accordance with the UK statutory testing and control programme. Between October 2012 and December 2017 there were 101 reactors disclosed by routine testing, with none showing visible lesions at post mortem examination.

A comprehensive disease investigation in 2015 suggested that the low prevalence infection within the herd was being maintained by undisclosed infected and infectious animals being retained, with the possible reintroduction of infection from environmental contamination of grazing pastures from wildlife.

An enhanced testing programme was introduced in 2015 using novel organism based tests (Actiphage (Swift 2016) and rd4 qPCR (Taylor 2007)) to detect infected cattle that were not being detected by the routine SCITT, and to determine the environmental risks by testing badger latrines around the farm for the presence of *Mycobacterium bovis* (King 2015).

Once infected cattle and environmental risks were identified, a robust bTB management programme was introduced to manage the disease and enhance the statutory bTB controls.

**Methods:** The routine statutory SCITT testing programme detected several bTB reactors at each routine test. However, it was noticed that several animals gave detectable reactions to the bovine tuberculin but were not classified as reactors because of an accompanying reaction to the avian tuberculin, and so were retained in the herd under the UK interpretation guidelines. These animals (any animal that has shown any form of bovine reaction over 2mm increase in skin thickness) were classified as "high risk" animals and submitted for enhanced testing using a bacteriophage based method combined with

PCR (phagePCR, Actiphage) on whole bovine blood samples, and rd4 qPCR on bovine blood and faeces samples.

Concurrently, in 2015, a complete survey of badgers around the farm showed a significant population (although there was no evidence of badgers entering the farm buildings or feed stores). 273 badger faecal samples were taken from 26 latrines and tested using the RD4 qPCR method to detect the presence of *Mycobacterium bovis*.

**Results:** During the period of the study, 142 high risk cattle (those that had a positive skin reaction to bovine tuberculin but had not been classified as official reactors) have been tested using the enhanced testing programme. Of those 142 high risk animals, 92 animals had at least one positive Actiphage test (65%), and 18 were found to have detectable *Mycobacterium bovis* in their faeces as determined by qPCR (13%).

Of the 273 badger faecal samples tested for the presence of *Mycobacterium bovis*, 32% had detectable *M. bovis* and 69% of the latrines had at least one positive sample.

**Discussion:** The enhanced testing programme demonstrated a significant number of infected animals were retained in the herd after the routine SCITT surveillance programme. Many of these infected animals were shedding organisms in their faeces, providing a source of infection to all animals in contact, and so acting as a reservoir of infection.

A significant source of infection in the grazing environment was demonstrated in badger latrines, creating a biosecurity risk to grazing young stock that would later join the adult herd.

A programme of targeted culling and risk management to control the disease in the herd, above and beyond the statutory controls, has been devised and implemented on the farm, including vaccination of the badger population and minimising the risk of spread from cattle potentially shedding *M. bovis* in their faeces.

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BC-06

### Occurrence of and countermeasures against bovine salmonellosis in Hokkaido

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#### ◁Occurrence of bovine salmonellosis in Hokkaido▷

In Japan, *Salmonella enterica* serovar Dublin (SD), Enteritidis (SE) and Typhimurium (ST) cause notifiable bovine salmonellosis. In Hokkaido, bovine salmonellosis has been reported since 1970 and mostly occurred in beef cattle. However, bovine salmonellosis in dairy cattle has been seen since 1987. The occurrences of bovine salmonellosis in dairy and beef cattle between 2000 and 2015 was 737 farms (3,519 heads), and 117 farms (374 heads), respectively.

Regarding the serotype distribution of isolates from bovine salmonellosis, ST was the most prevalent, followed by SD in the latter half of the 1980s. However, the distribution has diversified since the 1990s. Forty-six serotypes were reported from 1998 to 2015. A monophasic variant of ST, serotyped as 4:i:-(4:i:-), has been the most prevalent since 2012. SD has not been isolated in recent years, but it was isolated in 2015.

#### ◁Survey on bovine salmonellosis in Hokkaido▷

We examined the occurrence of salmonellosis caused by SD, ST and 4:i:- from 2011 to June 2016. SD had a tendency to require a longer period for countermeasures than the other two serotypes. Comparing the onset age and symptoms, there was no significant difference in the incidence in calves and adult cattle in ST and 4:i:-. Diarrhea and fever were observed in almost all cases. On the other hand, 88% of SD occurred in calves and yearlings. Respiratory symptoms were observed in 63% of cases, in addition to diarrhea and fever. Regarding antibiotic susceptibility, many isolates of the three serotypes were multidrug resistant. Fluoroquinolone resistance was not observed in the three serotypes except for one strain of 4:i:-, which showed resistant to marbofloxacin.

#### ◁Countermeasures against bovine salmonellosis in Hokkaido▷

Upon occurrence of salmonellosis, we try to eliminate *Salmonella enterica* from the farms by cleaning and disinfecting the environment, performing a periodic examination of the whole herd and environment, and isolating and treating diseased and carrier cattle. It is important that these countermeasures should be taken under the cooperation of clinical veterinarians, municipalities, agricultural cooperatives, and livestock hygiene service centers in addition to farmers.

BC-07

### Whole-genome-based study of *Salmonella* Dublin in cattle and humans in Denmark

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**Background:** *Salmonella* Dublin is a cattle-adapted salmonella serovar causing intestinal as well as systemic infections in its bovine host. Infection with this serovar is costly to the farmer, mainly due to production losses. *S. Dublin* may be transmitted to humans via contact to infected animals or consumption of contaminated meat or unpasteurized milk. In humans, *S. Dublin* infections are sometimes lethal. Despite the efforts to eradi-

cate *S. Dublin* from the Danish cattle population since 2007, it remains present in around 7% of the dairy cattle herds, and clinical cases of human infection continue to be reported in Denmark every year. Therefore, it is of interest to understand, whether Danish cattle are the source of infections in human patients. Furthermore, it is important for the success of the eradication program to increase the understanding of mechanisms of transmission of bacteria between herds and persistence of infection within cattle herds.

**Aims:** The aim of this study was to investigate whether Danish dairy cattle herds that remained test-positive for *S. Dublin* despite the national control measures were continuously infected with the same strain of the bacterium, or whether the persistent infections were more likely due to breakdown of external biosecurity. In addition, we investigated whether Danish human cases were infected with domestic *S. Dublin* cattle strains.

**Material and Methods:** A total of 196 isolates of *S. Dublin* from 58 cattle herds, collected in high prevalence regions of Denmark from 1996 to 2016, were subjected to whole genome sequencing (WGS) with MiSeq (Illumina). The isolates were classified into whether they originated from persistently or non-persistently infected herds according to a set of surveillance criteria. In addition, whole genome sequences of 46 isolates from clinically infected humans were compared with the cattle strains, and further genome sequences of strains sequenced in other countries were included.

**Results and Discussion:** The WGS comparison based on the single nucleotide differences in the core genome grouped Danish *S. Dublin* strains originating from cattle into three major clusters with some geographical associations. The majority of the isolates collected from the same cattle herd were closely related, even when the isolates were collected from the herd over an extended period of time (up to 16 years apart). This indicates existence of local herd-specific source(s) of infection in the farm environment or persistence in the animals in most herds. None of the genes in the accessory genome of *S. Dublin* were specifically associated to strains originating from persistently infected herds. This suggested that strains in persistently infected herds were not likely to have particular traits of importance for persistence. The comparison of Danish human and cattle isolates resulted in human isolates clustering closely with cattle isolates, indicating that Danish human cases were most likely acquired from Danish cattle. Comparison to genomes of non-Danish isolates supported this observation.

**Conclusion and Perspectives:** Previously, typing of *S. Dublin* has not allowed separation between strains with sufficient typing power to perform detailed analysis on herd level. WGS is a novel typing approach that offers this level of details. The study provided better insight into circulation of clones in cattle herds in Denmark over the years, in particular it revealed that the lack of success in controlling *S. Dublin* in some herds was not due to break down of external biosecurity, but due to persistent infection with the same strain over time. Thus, internal biosecurity measures need to be improved in such herds. The study also underpinned the importance of the eradication program to prevent future *S. Dublin* cases in humans in Denmark.



BC-08

## The maternal microbiome plays a key role in determining the offspring's early-life microbial community of *Bos taurus*

### Maternal microbiome effect on the offspring

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Natural transference of maternal microbes to the neonate, especially at birth via the vaginal canal, has recently been recognized in humans; however, the same process has not yet been documented in bovines. Therefore, our study was designed to elucidate the potential vertical transfer of bacterial organisms from cows to their neonatal calves and investigate its microbial influence on calf health during the pre-weaning period. Here, by applying deep sequencing, we compared the bacterial communities in vaginal and fecal samples from 81 pregnant Holstein dairy cows (273 days carried calf) from a single farm versus those in nasopharyngeal and fecal samples collected at 3, 14 and 35 days of life from their respective progeny. Multivariate analysis with Unifrac revealed unique profiles of bacterial communities by sample type, wherein the dissimilarities between sample types were mainly accounted for by the presence or absence of operational taxonomic units (OTUs) rather than by their relative abundances. However, the microbiota of the calf upper respiratory tract (URT), regardless of calf age, was found to be highly similar to the maternal vaginal microbiota. Moreover, the calf fecal microbiota clustered closely to the maternal fecal microbiota, progressing toward an adult-like state over the first 35 days of life when only relative abundances of taxa were considered. Ninety OTUs were shared between cow and calf fecal microbiota, whereas 253 OTUs were shared between the maternal vaginal microbiome and the calf URT microbiota. *Bacteroidetes*, *Ruminococcus*, *Clostridium*, and *Blautia* were the top four genera identified in dam and calf fecal samples, regardless of calf age. *Mannheimia*, *Moraxella*, *Bacteroides*, *Streptococcus* and *Pseudomonas* were the top five genera identified within the most abundant bacterial genera in dam vaginal and calf URT samples across all days of calf life examined. Furthermore, the genus *Mannheimia* was relatively more abundant in the vaginal microbiota of dams whose progeny were diagnosed with respiratory and middle ear disease. Our results provide evidence that the dam vaginal microbiome influences the initial bacterial colonization of the calf URT, and that it may have a beneficial effect on the health of the calf respiratory tract and middle ear.

BC-09

## Risk factors for *Mycoplasma mastitis* outbreak in Hokkaido, Japan

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**Objective:** *Mycoplasma mastitis* is an important contagious mastitis because of its high infectivity and agalactia. There was

an outbreak of the disease from 2014 to 2015 in Nemuro, Hokkaido, Japan. The objective of the study is to reveal the risk factors associated with the *Mycoplasma mastitis* outbreak.

**Materials and Methods:** Descriptive epidemiology was performed on 40 farms in Nemuro where *Mycoplasma mastitis* occurred from April 2014 to July 2015, and 882 farms in the same area where the disease didn't occur.

A case-control study involving two questionnaire surveys were conducted. In the first survey, 1:2 matching was performed for 39 infected farms by agricultural cooperatives and size category, and 73 non-infected farms were matched; in total 112 farms participated in the study. The questions asked knowledge about *Mycoplasma mastitis*, milking and barn hygiene management. An additional survey was conducted in 107 farms which participated in the first survey, to overcome the limitations of possible behavior change after the outbreak and unspecified questions. For farmers who answered that they changed their hygiene management after the outbreak, their answers were updated.

Movement records of cows in the participating farmers in the first survey were collected. The frequencies of movement, use of common ranch and purchase of cows were compared between infected and non-infected farms using Wilcoxon test.

For individual level risk factors, clinical records of cattle in the infected farms were collected.

**Results and Discussion:** Descriptive epidemiology revealed that many cases of this outbreak occurred in the period from January to March 2015. The infected farms kept significantly more cattle than the non-infected. There was heavy snow in winter 2015, and the result suggested that worsened rearing environment because of the heavy snow could be a risk factor in the outbreak.

In the farm level univariable analyses, the number of milking cows, the number of calves, type of a barn for milking cows, conscious wipe of teats and machinery ventilation at a calf barn are factors significantly associated with the outbreak of the mastitis. On the other hand, there were several variables suggesting higher hygiene management among infected farms.

In the individual cattle univariable analyses on movement, the age of cattle moved in and out excluding selling off was significantly younger in the infected farms than non-infected ( $p < 0.05$ ).

The analyses are still going on. For the farm-level analysis, multivariable analysis using generalized linear models will be performed. For the individual level analysis, the relationships with *Mycoplasma mastitis* and histories of respiratory diseases, tympanitis, and arthritis will be analyzed using the mixed-effects models. The variables on cattle movement and histories of said diseases with p-values less than 0.2 were selected and multivariable analysis will be conducted using mixed-effects model.

BC-10

## Rapid screening method for identification of bovine milk mycoplasmas and *Acholeplasma laidlawii*

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**Objectives:** *Mycoplasma* mastitis is increasingly generating considerable interest in bovine dairy industry. It is common knowledge that mycoplasmas can cause clinical or subclinical mastitis in cattle either as an individual or co-invader pathogen. *Mycoplasma* mastitis is usually be a missing component from routinely mastitis detection due to the specific culture and lab requirements. The study objectives were to develop a rapid and accurate screening method for identification of *Mycoplasma* mastitis, investigate relative merits of conventional culture-based method versus DNA amplification for detecting *Mycoplasma* mastitis in bovine milk, and discriminate between field isolates of different species of mycoplasmas and *Acholeplasma laidlawii* using high resolution melting assay (HRM).

**Methods:** A total of 368 individual cow milk samples were collected from two commercial dairy farms in South Australia. At the time of collection, Milk samples were aseptically collected from each functional quarter in sterile 50 mL tubes. Samples were kept on ice and sent immediately to the PC2 laboratory at the School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, South Australia. Milk samples were subjected to conventional *Mycoplasma* culture and remaining sample contents were frozen at -20°C and retained for molecular analysis. Samples were cultured for detection of mycoplasmas using Mycoplasma enrichment broth and Mycoplasma agar according to the manufacturer's instructions. Samples were incubated under 10% CO<sub>2</sub>-enriched conditions at 37±1°C for 14 days under 10% CO<sub>2</sub>-enriched conditions. Cultures were considered positive when growth of at least one *Mycoplasma*-like colony was detected. DNA extraction was performed directly from milk using QIAmp DNA extraction kit according to manufacturer's instructions. Six separate PCR reactions with six different primers pairs were used in this study. The first universal primer was designed to target 16S rRNA gene at genus-level, while the other five primers were species-specific and were previously published elsewhere. In-vitro amplification of DNA to detect *Mycoplasma* and *Acholeplasma* spp. was conducted for each primer pair separately. Amplifications were performed for 35 PCR cycles conditions using T100™ Thermal Cycler. The resultant PCR products were analysed by 1.5% agarose gel electrophoresis and visualised by staining with Gel Red. Agreement between sets of data for the aforementioned detection methods was identified using Cohen's Kappa coefficient test. A real-time PCR-HRM assay was performed using the Eco real-time PCR system from (illumina) with AccuMelt™ HRM SuperMix. A subset of 40 samples were sequenced targeting the 16S rRNA gene for confirmation.

**Results:** Of 368 milk samples collected at individual cow level from a single dairy farm in South Australia, 192 (52%) tested positive for *Mycoplasma* using a conventional culture-based method, compared to 269 (73%) using our specifically designed universal *Mycoplasma* PCR-based method for testing DNA extracted directly from milk. PCR results using previously published species-specific primers yielded 256 (70%) positive *Mycoplasma* samples. Detected species included *Acholeplas-*

*ma laidlawii*, *M. arginini*, *M. bovirhinis* *M. bovis* and *M. canadense*. Infection by two or more of the abovementioned mycoplasmas showed highest prevalence. Our universal PCR demonstrated best concordance with species-specific PCR (Cohen's Kappa= 0.747 ± 0.031). The real time PCR-HRM assay was able to detect and discriminates between different field isolates of mollicutes. This assay showed clearly distinct melting curve profiles of *Acholeplasma laidlawii*, *M. arginini*, *M. bovirhinis* *M. bovis* and *M. canadense*.

**Conclusion:** In conclusion, due to rapidity and higher sensitivity compared to the conventional culture method for surveying *Mycoplasma* mastitis in dairy herds, we recommend screening of milk samples using our universal PCR method. The real-time PCR-HRM assay was able to identify and discriminate between mastitis causing mycoplasmas and other mollicutes which are often milk contaminants.

BC-11

### Evaluation of a new qPCR-assay for diagnosis of bacterial infections in calves

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**Objectives:** The Danish Green Development and Demonstration Project - Diagnostic tests for veterinary practice (VetDiagnostics) aims to promote prudent use of antibiotics in cattle and pig production. An important step is to develop improved diagnostic methods that can guide the veterinarian in the choice of prevention, and choice of drug. Bovine Respiratory Syndrome is the most important reason for use of antimicrobials in calf production in Denmark, and this study aimed to develop and validate a rapid quantitative PCR test to be used in private veterinary practice for detection of pathogens associate with this disease in calves.

**Material and methods:** We designed primers and probes for detection of *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. To validate PCR methods, we first selected 15, 16, 10, and 3 isolates of the four target-species. In addition, 135 different non-target isolates were selected. We then compared different extraction procedures. Finally, we performed a validation trial. We collected 65 tracheal fluid samples from clinically sick and apparently healthy control calves in 10 farms. Veterinarians sedated the calves and performed a tracheal lavage with 50 ml isotonic saline and collected 15 -30 ml of tracheal fluid. This was transported on the same day to DNA Diagnostic (Risskov, Denmark) where the qPCR test (Pneumo 4) was performed at the day of arrival. At the same day, 3 ml of sample was shipped to Copenhagen University (Copenhagen, Denmark) for culture and 2 ml to DTU-vet (Copenhagen, Denmark) for *Mycoplasma bovis* and virus detection.

**Results:** DNA obtained from pure cultures of *M. haemolytica* (n=15), *P. multocida* (n=16), *H. somni* (n=10), and *M. bovis* (n=3) all tested positive in the qPCR test (Pneumo 4). In addition, all 135 different non-target isolates tested correct nega-



tive. These isolates included *Mannheimia* and *Pasteurella* species that are closely related to the target bacteria. Comparison of DNA extraction procedure revealed that the protocol developed by DNA-Diagnostic (Risskov Denmark) for mastitis PCR gave the best yield of high quality DNA from the tracheal fluid samples.

Of the 65 samples, 20 were found to be positive for *M. haemolytica* by the qPCR test; only 4 of these were positive by culture. Similarly, 34 samples were positive for *P. multocida* by the qPCR test; 19 of these were positive by culture. The corresponding results for *H. somni* were 4 positive by the qPCR test and 0 positive in culture, and for *M. bovis* 30 positive by the qPCR test and 16 positive in the PCR test performed at DTU-vet (Copenhagen, Denmark).

The efficiency of the qPCR test was evaluated on a quantified genomic DNA ten-fold dilution to estimate the copy number of nucleic acids. The qPCR test was found to be accurate in detecting target nucleic acids down to 10 copy number per PCR reaction. The correlation between Ct values of the qPCR test and bacterial colony forming units (CFU) of target bacteria was tested in triplicates on three different isolates for each target. The CFU/0.5 ml in the diluted samples was calculated from the plates containing between 10 and 300 colonies. The correlation curves for all four targets shows an acceptable correlation between CFU/0.5 ml and Ct value.

**Conclusion:** The newly developed qPCR test (Pneumo 4) was found to be useful in detecting the major bacterial causes to Bovine Respiratory Disease. The test can be performed in 3 hours and can be done in the private veterinary practice laboratory. Work is in progress to include the major virus associated with this disease.

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BC-12

### Oral feeding with a probiotics *Bacillus subtilis* C-3102 modulates the immune and metabolic functions in dairy cattle, resulting in prevention of mastitis

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**Objectives:** Mastitis, which is an inflammatory disease occurring in the udder of dairy cattle, causes a huge economic loss in dairy industry. There are many reports that probiotic additives normalize intestinal microbiota and control gut inflammation. However, the effect of probiotics on dairy mastitis remains unclear. It has been reported that oral feeding with a probiotics *Bacillus subtilis* C-3102 (BS) conduces to good health and growth for chickens and pigs. In this study, we investigated the effect of BS on the incidence of mastitis in dairy cattle.

**Materials and methods:** Dairy cattle were bred in Miyagi Prefecture Livestock Experimental Station and fed a diet with or without BS ( $3 \times 10^9$  cells/head) twice a day for 11 months from 1 month before parturition. The numbers of onset of

mastitis, days of medical care and somatic cell count (SCC) in milk were monitored within 3 months after parturition. The metabolic analyses were carried out once a month throughout the experimental period, to measure the concentrations of glucose, urea nitrogen, non-esterified fatty acid (NEFA), total cholesterol, cortisol, and thiobarbituric acid reactive substances (TBARS) in plasma. The immunological studies were also conducted using a flow cytometry to address the frequencies of peripheral granulocytes, monocytes, B cells and T cell subsets (CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>,  $\gamma\delta$ , WC1<sup>+</sup> $\gamma\delta$  and CD8<sup>+</sup> $\gamma\delta$ ). The sensitivity of peripheral granulocytes to cyclophilin A (CypA), which is known to be highly expressed in the udder of dairy cattle with mastitis, was also determined by an *in vitro* chemotactic assay.

**Results:** The numbers of onset of mastitis and days of medical care in BS-feeding group were statistically lower than those in control group. The average of milk SCC in BS-feeding group was constantly below than 100,000 cells/ml throughout the experimental period and was significantly lower than that in control group. After parturition, the concentration of total cholesterol was rapidly recovered to the original level in BS-feeding, but not in control group. In the middle and late stages of lactation, the concentrations of urea nitrogen, cortisol and TBARS were lower in BS-feeding group than those in control group. There is not a difference in the frequencies of granulocytes, monocytes, T cells, B cells and subsets of CD3<sup>+</sup> T cells in peripheral blood between two groups. However, the sensitivity of peripheral granulocytes to CypA was increased in control group, but reduced in BS-feeding group after parturition.

**Conclusions:** In dairy cattle, oral feeding with a probiotics *Bacillus subtilis* C-3102 modulates inflammatory immune responses and the metabolic functions after parturition, resulting in prevention of the mastitis recurrence. These results indicate that the use of BS as a feed additive may contribute to increase not only the feed requirement but also the milk production in dairy industry.

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BC-13

### Genetic Characteristics of CTX-M-type ESBL-Producing *Enterobacteriaceae* Involved in Mastitis Cases on Japanese Dairy Farms, 2007 to 2011

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**Objective:** The aims of this study were to determine the genetic characteristics, antimicrobial susceptibility, and genetic relatedness of CTX-M-type extended-spectrum  $\beta$ -lactamases (ESBLs) (CTX-M)- and plasmid-mediated AmpC  $\beta$ -lactamase-producing *Enterobacteriaceae* isolated from bovine mastitis cases.

**Materials and Methods:** We isolated 28,900 Gram-negative bacilli from a total of 258,888 quarter milk samples obtained from 176,808 cows affected by mastitis, between 2007 and 2011. Of these, 419 strains were cefazolin-resistant and oxidase-negative. Then, the CLSI ESBLs confirmatory test-positive, and metallo- $\beta$ -lactamases-negative by SMA-test, *Enterobacteriaceae* isolates (n=65) were identified using the ID 32 E



API system. The MICs of 23 antimicrobials were determined by the CLSI broth microdilution. These 65 isolates were analyzed by multiplex PCR for the presence of *bla*<sub>CTX-M</sub> genes, and plasmid-mediated AmpC  $\beta$ -lactamase genes. The CTX-M types of the CTX-M-positive isolates were identified by bidirectional sequencing using group-specific PCR primers for *bla*<sub>CTX-M-1 group</sub>, *bla*<sub>CTX-M-2 group</sub> and *bla*<sub>CTX-M-9 group</sub>. AmpC-positive isolates were analyzed using type-specific PCR primers (e.g., *bla*<sub>CMY-1</sub> and *bla*<sub>CMY-2</sub> genes), and *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes were analyzed and bidirectionally sequenced. Random amplified polymorphic DNA (RAPD)-PCR analysis for *Klebsiella pneumoniae*, PFGE, multilocus sequence typing (MLST), and O-, H-serotyping for *Escherichia coli* were performed.

**Results:** Sixty-five isolates were identified as CTX-M-producing strains. Fifty-one isolates (78.5%), which included 41 *K. pneumoniae*, 6 *Klebsiella oxytoca*, 2 *Citrobacter koseri*, 1 *E. coli*, and 1 *Enterobacter aerogenes*, harbored *bla*<sub>CTX-M-2</sub>; 10 *E. coli* isolates (15.4%) harbored *bla*<sub>CTX-M-15</sub>; and 4 isolates (6.2%, 2 *K. pneumoniae*, and 2 *E. coli*) harbored *bla*<sub>CTX-M-14</sub>. No isolates contained the plasmidic AmpC gene. Four (40.0%) of 10 CTX-M-15-producing *E. coli* isolates also harbored *bla*<sub>TEM-1</sub>. The isolation rate of strains producing CTX-M-2/15/14 in bovine mastitis was 0.22% of the 28,900 Gram-negative bacilli isolates.

Isolates producing CTX-M exhibited high resistance to oxyimino-cephalosporins, however, they exhibited high susceptibility rates to imipenem, gentamicin, and amikacin. The CTX-M-15-producing *E. coli* showed higher rates of resistance to ceftazidime, aztreonam, SXT, and kanamycin than CTX-M-2/14-producers did.

The 41 CTX-M-2-producing *K. pneumoniae* isolates from 15 farms revealed 32 RAPD types. The 18 isolates from farm F revealed 16 RAPD types. There was not a predominant RAPD type. The 13 *E. coli* isolates from 7 farms belonged to 10 STs, and showed 12 PFGE types. Two isolates each of *E. coli*, which were isolated from two different cows each on three farms, had the same ST (ST23, ST58, and ST10) and closely related PFGE types.

**Conclusions:** The CTX-M producers were dominated by CTX-M-2-producing *K. pneumoniae* and CTX-M-15-producing *E. coli*. There was no predominant clonal type and clonal diversity was found even in *K. pneumoniae* strains isolated from a single farm, suggesting that these were opportunistic infections from a wide variety of environmental sources. The two isolates each of *E. coli* showing the closely related genotype, which were isolated from the different cows on the same farm, suggest a contagious infection or an infection from an environmental point source.

**Reference:** Mamoru Ohnishi, et al. 2013. J. Clin. Microbiol. 51:3117-3122.

BC-14

## Associations of respiratory pathogens and the upper and lower respiratory tract microbiotas in calves with bovine respiratory disease

### Respiratory pathogens and microbiota associations

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**Objectives:** Bovine respiratory disease (BRD) complex is a major economic and public health burden that affects young bovines worldwide, with a multifactorial etiology involving the presence of one or several viruses and/or bacteria favored by altered host immunity and disturbed environmental factors. These respiratory pathogens act in the presence of a very complex network of resident bacterial species called the "microbiota". Recent evidence suggests that the upper respiratory tract (URT) microbiota plays an important role in respiratory health and disease susceptibility in cattle. Whereas only few data are available for the LRT. The objectives were (i) to describe the mosaic structures of the URT and LRT microbiota and their interactions in healthy calves and calves with BRD and (ii) to determine if well-known respiratory viruses or bacteria have an impact on the URT and LRT microbiota.

**Materials and methods:** Deep nasal swabs (NS) and bronchoalveolar lavages (BAL) were performed in 3 to 5 calves of 23 herds suffering from acute BRD and not vaccinated nor treated with antibiotics. Similar samples were taken in healthy calves of 6 similar herds. DNA of calves from the same herd were extracted and pooled for sequencing. The V1-V3 hyper-variable region of the bacterial 16S ribosomal RNA gene was amplified from the pools and sequenced using the Illumina Miseq platform. Comparisons and diversity analysis of the bacterial communities based on measures of taxonomic richness (Alpha diversity metrics) and distances between samples (Beta diversity metrics) were done. Different types of transformation of the count tables were investigated and the log and log-CSS that showed a good separation between the NS and BAL samples on the first axis of a PCA. Then, sparse PLS-DA was performed after the individual contribution has been removed with, a multi-level approach as implemented in the R package mixOmics. An integrated analysis was also performed to describe the complex relationships between microbiota of URT and LRT and the presence of respiratory pathogens (BRSV, PI3, BCov, *M. Haemolytica*, *P. multocida*; *H.somni*, *M. bovis*).

**Results:** Preliminary results indicate significant differences of bacterial communities in terms of taxonomy, richness and diversity between the URT and the LRT of calves with BRD. At the phylum level, the LRT is associated with high relative richness of *Proteobacteria* while the URT is mostly associated with *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. At the genus level, *Mycosplasma* were found to be the most abundant in both URT and LRT. For the other top ten taxons found, 8 were significantly different between the URT (*Corynebacterium*, *Streptococcus*, *Moraxella*, *Staphylococcus*, *Pasteurella*, *Mannheimia* and *Ureaplasma*) and LRT. (*Stenotrophomonas*, *Alcaligenes*, *Pseudochrobactrum*, *Pseudomonas*, *Delftia*, *Achromobacter*, *Fusobacterium*, and the species *Cilia-associated respiratory bacterium*). Differences of microbiota between healthy or diseased calves are under investigation. The impact of respiratory pathogens on the microbiota was also investigated. In the URT the presence of *P. multocida* and *H.somni* is associated with an important number of resident bacteria. A negative association was found between the presence of BRSV and bPI3 (association with a small number of bacteria) and the presence of *H. somni*. In the LRT *M.haemolytica* is associated with a large number of bacteria, while its absence is associated with the





presence of *P.multocida* and *H.somni*. Finally the presence of a respiratory virus (BRSV, bPI3 or BCoV) is associated with specific bacterial taxa in the URT while results are less reliable for the LRT (lower discrimination quality).

**Conclusion:** As a preliminary conclusion, the microbial ecology of the calves during BRD seems to be diverse with a clear distinct structure of the microbiome observed between the upper and lower respiratory tract of the calves. Some patterned relationships were observed, notably between the presence of a known respiratory pathogen (bacteria or virus) and the respiratory structure of either the URT or the LRT microbiomes, raising the question of how these pathogens interact between themselves and with the resident microbiota.

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BC-15

### The PD-1/PD-L1 pathway contributes to the immunosuppression in bovine mycoplasmosis

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**Objectives:** Bovine mycoplasma is the pathogen causing pneumonia, mastitis, arthritis and otitis in cattle. Once clinical symptoms develop, it is difficult to completely recover from this disease. It is well known that this pathogen displays immunosuppressive characteristics. These characteristics could be associated with the disease progression of bovine mycoplasmosis. However, the mechanisms involved in bovine mycoplasmosis have not been fully elucidated.

Previous studies revealed that the expression of the immunoinhibitory receptors, such as programmed death-1 (PD-1), was upregulated on exhausted T cells in various chronic infections. Exhausted T cells have low effector functions and allow pathogens to establish persistent infection. Thus, immunosuppression observed in exhausted T cells is regarded as the common mechanism of immune evasion in chronic infections. In this study, we investigated the roles of the PD-1/ PD-ligand 1 (PD-L1) pathway on the immunosuppression in cattle with bovine mycoplasma.

**Materials and methods:** To examine the decrease in interferon- $\gamma$  (IFN- $\gamma$ ) production in bovine mycoplasmosis, we performed enzyme-linked immunosorbent assay (ELISA) to measure IFN- $\gamma$  production from peripheral blood mononuclear cells (PBMCs) isolated from cattle with bovine mycoplasmosis. Then, the expression levels of PD-1 and PD-L1 in PBMCs from cattle with mycoplasmosis were analyzed by flow cytometric

analysis. Finally, in order to examine the potential of anti-PD-1 and anti-PD-L1 antibodies as a therapeutic method for bovine mycoplasmosis, the effects of the blockade of the pathway by antibodies on IFN- $\gamma$  production from antigen-specific T cells were evaluated using PBMCs culture.

**Results:** IFN- $\gamma$  production was significantly decreased in cattle with bovine mycoplasmosis compared to that in uninfected cattle. Concomitantly, flow cytometric analysis revealed that the proportion of PD-1<sup>+</sup> T cells as well as PD-L1<sup>+</sup> monocytes was increased in cattle with bovine mycoplasmosis. Interestingly, the increases in the proportion of PD-1<sup>+</sup> T cells were negatively correlated with IFN- $\gamma$  production from PBMCs in bovine mycoplasmosis. In addition, the blockade of the PD-1/PD-L1 pathway *in vitro* by anti-bovine PD-1 and anti-bovine PD-L1 antibodies significantly upregulated the IFN- $\gamma$  production from PBMCs stimulated with Mycoplasma-specific antigen.

**Conclusions:** These results suggest that the immunosuppression in bovine mycoplasmosis could be mediated by the PD-1/PD-L1 pathway. In conclusion, the blockade of the PD-1/PD-L1 pathway may be an effective method to control bovine mycoplasmosis.

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BC-16

### Identification of multiresistant *Gallibacterium anatis* strains in a clinical outbreak of infectious bronchopneumonia in beef cattle

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**Background and Objectives:** *Gallibacterium anatis* (*G. anatis*) is historically considered an opportunistic pathogen of intensively reared poultry and domestic birds. In recent years it emerged as a multiresistant pathogen causing high mortality in these species and occasionally sepsis in humans. This report describes an outbreak of bronchopneumonia in neonatal calves associated with multiresistant *G. anatis* strains.

**Methods:** In an 900 head Belgian Blue beef herd, an outbreak of bronchopneumonia occurred in the neonatal calves. Animals showed fever, increased respiratory rate, nasal discharge, positive tracheal reflexes and depression. Broncho-alveolar lavage (BAL) samples were taken from animals displaying lung consolidation on lung ultrasonography to identify the causative pathogen(s). BAL samples were cultured on Columbia blood and pleuropneumonia-like organism agar. Species confirmation was done by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Susceptibility testing for 12 commonly used antimicrobials (trimethoprim, sulfonamides, ampicillin, tetracycline, florfenicol, doxycycline, neomycin, penicillin, tylosin, tulathromycin, enrofloxacin and ceftiofur) was performed with disc diffusion.

**Results:** Four animals were sampled and *G. anatis* was isolated from all of them. In three cases the bacteria were abundantly present in pure and dominant culture, whereas in the fourth



case contaminants were present next to *G. anatis*. All isolates were resistant to tetracycline, sulfonamides, tylosin, tulathromycin and enrofloxacin. For trimethoprim, ampicillin, florfenicol, doxycycline, neomycin and penicillin, susceptibility patterns varied. All isolates were resistant to a minimum of 8 antimicrobials tested, confirming multiresistance in every isolate. All isolates were susceptible for ceftiofur. No other pathogens were identified.

**Conclusions:** To the authors knowledge, this is the first description of a clinical outbreak of bronchopneumonia in cattle associated with *G. anatis*. Multiresistance was frequent in this outbreak, potentially causing treatment failure.

BC-17

### The prevalence of *Leptospira borgpetersenii* sv Hardjo and *Leptospira interrogans* sv Pomona in Victorian dairy herds, 2017

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**Objectives:** While previous studies of *Leptospira* Hardjo in Victoria demonstrated a relatively high prevalence of exposure in both cattle (40%; Milner et al. 1980) and humans (22%; Sutherland 1988) these estimates need to be revised since leptospirosis vaccination programmes are now commonplace on Victorian dairy farms.

**Materials and methods:** This was a cross-sectional study to estimate the prevalence of *Leptospira borgpetersenii* sv Hardjo and *Leptospira interrogans* sv Pomona in dairy herds in South-Western Victoria. Fifty-three herds were enrolled into the study. Herd managers were asked to present 15 late-lactation cows that had fertility issues (cows that had not conceived or had delayed calving to conception intervals). Furosemide 500 mg was injected into the tail vein of eligible cows and a mid-stream urine sample of the second voiding collected to increase the likelihood of sampling leptospira (Nervig RM, Garrett LA 1979). At the time of each herd visit a questionnaire was administered to herd managers asking them to provide details of methods used for controlling leptospirosis, including vaccination. Urine samples were pooled at the herd level and tested for leptospira spp. using qPCR. Pooled samples were then tested individually and samples that were positive, were tested for *Leptospira* Hardjo and *Leptospira* Pomona using qPCR.

**Results:** Laboratory analyses are ongoing. Three of the 53 pooled urine samples have returned a positive result. The leptospira positive pools returned a minimum of three positive individual cow urine samples. Testing of individual cow urine samples identified an additional positive herd (with one weak positive and one inconclusive result), giving an apparent prevalence of 8 (95% CI 1 to 13) leptospira-positive herds per 100 herds at risk.

Based on the 53 completed questionnaires, leptospirosis vaccination programs were non-compliant with label directions in 36 herds: 69 (95% CI 51 to 76) out of 100 herds that routinely vaccinate for leptospirosis were doing so incorrectly. Of the 53

herds that took part in this study, only 1 herd was completely unvaccinated.

**Conclusion:** Based on the findings from this study we estimate that close to one out of 10 dairy farms in South-Western Victoria are leptospirosis positive. While most herds are vaccinating for leptospirosis, most are doing so incorrectly. We conclude that herd managers need to be better educated regarding leptospirosis vaccination programs.

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Nervig RM, Garrett LA, 1979. Use of furosemide to obtain bovine urine samples for leptospiral isolation. Am J Vet Res Aug 1979; 40 (8); 1197-1200

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PA-01

### Experimental infection of Friesian bulls with *Theileria orientalis* (Ikeda) and effects on the haematocrit, live weight, rectal temperature, activity and fertility

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**Objectives:** To infect a group of mature Friesian bulls with *Theileria orientalis* (Ikeda) and measure the effects of acute infection on the haematocrit, rectal temperature, weight, activity and fertility over a follow-up period of 20 weeks.

**Materials and methods:** Ten naive 2-year-old Friesian bulls were infected with *T. orientalis* (Ikeda) by intravenous injection of 30ml of infected blood, with a further 7 naive bulls left as uninfected controls. The infected blood was sourced from 2 acute cases of *Theileria* associated bovine anaemia on a local dairy herd experiencing a severe outbreak of infectious anaemia. After infection the bulls were blood sampled, had rectal temperatures taken and weighed 3 times weekly for 13 weeks and then once weekly for a further 7 weeks. Activity pedometers were attached to the bulls and the bulls were semen and libido fertility tested every 2 weeks throughout the experiment. Semen was collected by artificial vagina using a heifer in oestrus restrained in a service crate. The bulls were kept as one group on a property where ticks had not been previously reported and were treated with flumethrin 1% pour-on tickicide every 4 weeks.

**Results:** All 10 of the treatment bulls were successfully infected with *T. orientalis* (Ikeda). The first bull was confirmed *T. orientalis* (Ikeda) PCR positive on Day 19 after infection, 8/10 bulls were positive by Day 26 and by Day 33 all 10 were positive. On a group basis the maximum *Theileria* count for the infected group peaked on Day 52 at 155750 organisms/ $\mu$ l, and on an individual basis the average *Theileria* count peaked at 298050 organisms/ $\mu$ l. DNA sequencing confirmed that all the infected bulls and none of the control bulls were uniquely infected with *T. orientalis* Ikeda type.

Overall 6/10 (60%) infected bulls became clinically anaemic (HCT < 0.25) with the lowest HCT recorded by an individual bull = 0.19 on Day 54. On a group basis, the lowest average HCT was on Day 59 (average HCT = 25.0) for the infected group and on an individual basis was at an average of 68.7 days (average HCT = 22.8). The HCT of the infected bulls was significantly lower than the control bulls for the period 47 to 80 days post infection ( $P < 0.05$ ). On average there was 18 days between the peak in *Theileria* DNA and the HCT nadir for individual animals.

There was no pyrexia recorded in the infected bulls. The infected bulls had on average a lower rectal temperature from Day 5 to Day 42 with the maximum difference occurring on days 19 and 21 post-infection. Over the entire study period there was no significant difference in the weight gain of the two bull groups, however the control bulls were on average heavier from Day 70 to Day 138, equivalent to the convalescent period for the infected bulls. The infected bulls took fewer steps than the control bulls on 101/111 monitored days (91%) and on average took 189 steps per day less than the control bulls.

There was no observed change in wave motion score of semen

between infected and control groups ( $P = 0.29$ ) and no change in forward motion between infected and control groups ( $P = 0.56$ ). The percentage of normal sperm ( $P = 0.003$ ) was slightly lower in the infected bulls (91.9%) compared to the control group (94.3%), although the density of sperm in an ejaculate ( $P < 0.005$ ) was higher in infected bulls ( $1.45 \times 10^{10}$  sperm per mL) compared to control bulls ( $1.14 \times 10^{10}$  sperm per mL). Time to first mount ( $P = 0.74$ ) and time from first mount to second mount ( $P = 0.2$ ) was not significantly different between infected and control groups. The number of mounts was similar between infected (2.33) and control (2.36) groups ( $P = 0.93$ ) and there was no interaction between mount frequency and time from infection ( $P = 0.22$ ).

**Conclusions:** The study showed that it is relatively easy to infect cattle with *T. orientalis* (Ikeda) by intravenous inoculation and that in mature bulls the effect of infection is moderate, with no drastic adverse effects on fertility, weight gain and activity observed. However, slightly over half of the animals became clinically anaemic and HCT was significantly lower for just over 30 days indicating there was a clear impact for this period of time on the animals' health. The study also replicated the findings from other studies which found that the infection intensity of *T. orientalis* (Ikeda) peaks before the lowest HCT is reached.

PA-02

### Efficacy of imidocarb and diminazene aceturate in the treatment of natural bovine babesiosis

#### Comparative effects of imidocarb and diminazene aceturate in bovine babesiosis

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Bovine babesiosis is widespread in Africa including Nigeria. The search for easily accessible chemotherapeutic agents for its control is continual. The aim of this study was to evaluate the response of some cattle to diminazene aceturate and imidocarb for the treatment of this disease in a commercial herd of cattle with semi-intensive system of management in Ibadan, Nigeria.

The study was carried out using 124 cattle of various breeds and both sexes. The animals were those found to be free from helminthosis and other haemoparasites using standard laboratory techniques. Blood glucose and haematological parameters (Packed Cell Volume, Haemoglobin concentration and Red Blood Cell counts) were determined using glucometer One Touch Basic<sup>R</sup> (LifeScan) *in situ* and standard laboratory methods respectively. Serum potassium levels were determined using high-performance liquid chromatography. Clinical signs, signalments, PCV values and Giemsa staining technique were used to confirm the infection. The animals were randomly divided into two equal treatment groups A and B imidocarb dipropionate (Imizol<sup>R</sup> containing 120 mg/mL of imidocarb dipropionate) and diminazene aceturate (Dophaniil<sup>R</sup> in a sachet containing 1.05g diminazene aceturate) respectively. All cattle were treated fifth week after the onset of clinical signs and when the arithmetic PCV value was 24% or less. All the signalments, haematological parameters and serum potassium levels of the animals were monitored weekly.



*Babesia bovis* (60%) and *B. bigemina* (40%) were detected in all the cattle. A significant ( $P < 0.05$ ) reduction in PCV, Hb concentration, RBC counts, with hypoglycemia and hypokalaemia was observed in all the cattle. Cattle in group A had significantly ( $p < 0.05$ ) faster recovery of all the parameters determined. Improvement in rectal temperature ( $38.5 \pm 0.1^\circ\text{C}$ ), capillary refill time ( $1.6 \pm 0.2$ secs), heart rate ( $76.1 \pm 1.2$ beats/min) and respiratory rate ( $15.3 \pm 1.0$  breaths/min) for group A animals was much better than those in group B with  $39.1 \pm 1.1^\circ\text{C}$ ,  $2.4 \pm 1.0$  secs,  $79.5 \pm 1.0$  beats/min and  $17.2 \pm 0.1$  breaths/min respectively. Recovery from hypoglycemia and hypokalaemia followed the same trend.

Imidocarb dipropionate was therefore found to be more potent than diminazene aceturate in the treatment of natural bovine babesiosis. The effects of the two drugs are discussed.

PA-03

### Occurrence and genotyping of *Cryptosporidium* spp. and *Giardia* in calves in Algeria

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**Background:** *Cryptosporidium* and *Giardia* are an important worldwide zoonotic parasites of a public and veterinary importance. Only a few studies restricted to some regions have reported the molecular epidemiology of these two pathogens in farm animals in Algeria. Thus, this study was performed in order to investigate the occurrence and molecular characterization of *Cryptosporidium* spp. and *Giardia* in calves in different regions in Algeria.

**Materials and methods:** To study the occurrence of *Cryptosporidium* and *Giardia* infection in young cattle, a total of 62 faecal samples were obtained from calves less than three months old. The primary diagnosis was established by the detection of *Cryptosporidium* oocysts or *Giardia* cysts using immunofluorescence (IF) assay (Merifluor® *Cryptosporidium/Giardia* Kit). Fecal specimens were screened for presence of *Cryptosporidium* by Nested-PCR at the small subunit ribosomal RNA (rRNA) gene, followed by restriction fragment length polymorphism (PCR-RFLP) and sequence analyses to determine parasite species in the resulting amplicons. *Cryptosporidium* was subtyped by PCR-sequence of analysis of the polymorphic 60 kDa glycoprotein gene.

For *Giardia*, samples were screened by nested-PCR analysis (followed by RFLP) using the glutamate dehydrogenase and triose phosphate isomerase to examine occurrence and genotype distribution of *G. duodenalis*.

**Results:** The presence of *Cryptosporidium* oocysts and *Giardia* cysts was reported respectively in 40/ 62 (65%) and 16/62

(25.8%) samples. The *Cryptosporidium* species identified from 23/40 (58%) microscopy positive samples were *C. parvum* 19/23 (82.6%) and *C. bovis* 4/23 (17.4%). From 14 *C. parvum* isolates, two subtypes were recognized within the subtype family IIa including IIaA16G2R1 (8/13) and IIaA15G2R1 (1/13) while IIaA16G1 (4/13) was the only subtype within IIa subtype family. *Giardia duodenalis* genotypes identified from 15/16 (93.75%) microscopy positive samples were ruminant-specific assemblage E (10/15), zoonotic assemblage A (3/15) and mixed assemblage (2/15).

**Conclusion:** These findings showed that calves are a potential reservoir for both *Cryptosporidium* and *Giardia*. The presence of zoonotic *C. parvum* subtype families (IIa, IIb), as well as the zoonotic *Giardia duodenalis* assemblage A, highlights the importance of zoonotic transmission of these two gastro-intestinal parasites.

**Keywords:** *Cryptosporidium*, *Giardia*, molecular typing, nested PCR, calves, zoonosis, Algeria.

PA-04

### Cryptosporidiosis and biosecurity

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More than 100 years after the first description of *Cryptosporidium* parasites by Edward Tyzzer, the treatment and prevention of cryptosporidiosis remain a challenge for scientists.

*Cryptosporidium* spp. has a worldwide distribution and this protozoan is considered the agent of an emerging zoonosis.

Because of its substantial economic impact in farms and in preventing the spread of a zoonotic agent, the control of cryptosporidiosis remains a major challenge for a successful One Health response.

WHO estimates that nearly two-thirds of all human pathogens originate from zoonoses, making it important to adopt a global 'one-health approach' involving veterinary and human sectors to control and prevent zoonotic pathogens.

*Cryptosporidium* may occur in 30-50% of diarrheic calves on a worldwide basis and, in some countries, is the most important cause of neonatal diarrhea in young ruminants.

Although cattle should no longer be blamed as the single source of human crypto, more than 90% of human infections are caused by *C. hominis* and *C. parvum* and the transmission from cattle to people is nevertheless an important public health concern.

*Cryptosporidium* spp. is transmitted via the fecal-oral route. The low infectious dose of directly infective oocysts, prolonged survival in moist environments and resistance to most of disinfectants explain the difficulty of eradication when the parasite is established on a farm.

Veterinarians' recognition of the zoonotic potential of cryptosporidium is paramount for a successful One Health response.

The vet practitioners play a key role in communicating and implementing biosecurity measures on farm: they should inform



the livestock owner of the hazards that result from contact with visibly and invisibly infected animal and provide him the basic knowledge of sanitary measures required to protect people and environment.

The aim of this presentation is to suggest a framework for on-farm biosecurity that should be implemented when cryptosporidiosis occurs.

tance and allow prudent worm control methods to be employed.

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PA-05

### **Using pooled samples and Mini-FLOTAC to assess efficacy of cattle anthelmintics on a 5,000-head heifer-rearing unit in Tasmania, Australia.**

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**Objectives:** Anthelmintic resistance is an established problem in Australian cattle herds. However, testing for efficacy is not routinely conducted due to perceived expense and inconvenience. This study trialled a simple and inexpensive anthelmintic efficacy testing method using the Mini-FLOTAC device at 5 eggs per gram sensitivity, to replace the current standard protocol for faecal egg count reduction test (FECRT) using McMaster egg count chambers.

**Materials and Methods:** The study was conducted on a on a 5,000-head heifer-rearing unit in Tasmania, Australia. Faecal samples were taken post-rectum prior to treatment, and again 14 days post-treatment, from mobs of 40-100 head. Fifteen randomly selected cattle were sampled per mob. Samples were transported to Dawbutts lab by post. One gram from each of 5 samples were pooled to form a 5g composite sample, resulting in 3 composite samples per mob for worm egg counts. Copro-culture was performed on mob samples and larval differentiation conducted microscopically after 7 days incubation at 26°C to identify parasites to genus level. Analysis of before and after faecal egg counts was done using RESOLOOT NEW (Microsoft Excel).

**Results:** A total of 46 FECRTs were conducted in the period May 2017 to January 2018. Five anthelmintic treatments were trialled: moxidectin injectable doramectin injectable doramectin injectable concurrently with levamisole oral abamectin + levamisole pour-on oral combination of abamectin, levamisole and oxfendazole.

Pre-treatment worm egg counts (n=138) had a mean of 81 eggs per gram and ranged from 0 to 620 epg for a pooled sample of 5 head. *Cooperia* and *Ostertagia* worms were identified in almost all mobs, while *Trichostrongylus* and *Oesophagostomum* were seen occasionally. Efficacy of the 5 treatments against *Cooperia* and *Ostertagia* ranged from 0-100%.

**Conclusions:** 'Before and after' testing using Mini-FLOTAC at 5 eggs per gram sensitivity is an inexpensive and simple method to assess the efficacy of cattle anthelmintics. The collection and submission of faecal samples was easily incorporated into the work schedule of the co-operator farm. This study revealed advanced levels of resistance to commonly-used anthelmintics in Tasmanian dairy heifers. Mini-FLOTAC and pooled testing should be used more widely to diagnose anthelmintic resis-



VR-01

### Computer simulation of spread of Bovine Leukemia Virus in a dairy farm

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**Objective:** Enzootic Bovine Leukosis (EBL) is caused by Bovine Leukemia Virus (BLV). Most infected cattle are asymptomatic, 20-30% of them develop persistent lymphocytosis, and 2-5% of them develop EBL. The most important route of infection is bloodsucking by tabanid flies. BLV seropositive rate in Japanese cattle is high; about 40% of dairy cattle and 30% of beef cattle are seropositive.

The objective of the study is to assess effectiveness of BLV countermeasures by simulating how BLV spreads among cattle in a dairy farm.

**Materials and methods:** The simulation of spread of BLV based on a dairy farm in Hokkaido, Japan. Hokkaido is the biggest dairy area in Japan, and about half of cow's milk in Japan is produced there. The monthly simulation for 5 years was done and change of BLV prevalence was calculated. The following points are considered in the simulation: age, sex, infection status (Non-infected/Asymptomatic/Persistent lymphocytosis/EBL), pregnancy stage, lactation number, lactation stage. The simulation was done in the statistical software R (version 3.4.3).

**Results and future study plan:** Change of BLV prevalence was simulated.

Simulations based on other real dairy farms will be done. In the additional simulation, farm profile will be considered: type of barn (freestall/tiestall), use of common ranch, introduction of cows from other farms, whether the farm graze cattle or not, etc.

The final objective of the study is to assess effectiveness of BLV countermeasures, by simulating change of BLV prevalence by applying a countermeasure.

VR-02

### A cohort study on the economic loss associated with bovine leukemia virus infection in dairy production in Hokkaido, Japan

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**Objectives:** Bovine leukemia virus (BLV) infection is a global health problem in cattle but was overlooked in Japan due to unapparent economic damage. This study was conducted to quantify the economic loss from the disease in dairy farms to understand the actual significance for the dairy industry if a disease is not controlled.

**Materials and methods:** A cohort study was conducted with

10 commercial dairy farms in which infection of BLV was confirmed within the Nemuro and Kushiro region of Hokkaido, Japan in 2015 and 2016. Of the ten farms, nine have participated Hokkaido Dairy Milk Recording & Testing Association. On the farmer's consent, we collected milk production data from this Association. Similarly, we gathered beef production data of which the dairy cow was removed by the farm at meat hygiene inspection centers and a meat processor. For the analysis of the impact of BLV infection on meat weight, Generalized Linear Models (GLM) with gamma errors were used for the univariable and multivariable analyses. For the analysis of the effect of BLV infection on the frequencies of mastitis, Generalized Linear Models (GLM) with zero-inflated Poisson errors were used.

**Results:** The mean meat weight for an asymptomatic or aleukemic cow and a non-infected cow was 333kg. In contrast, the mean of carcass weight for persistent lymphocytosis cow was 303kg and there was a significant difference ( $p=0.05$ ). The frequency of mastitis during a secretion of milk period in persistent lymphocytosis cows was significantly higher than non-infected cows ( $p=0.03$ , risk ratio = 1.37).

**Conclusions:** The persistent lymphocytosis cow was confirmed to cause a significant economic loss in dairy farms. The expected values of economic losses are still under analysis and will be shown in the talk in the conference.

VR-03

### Risk factors associated with the introduction of BoHV1 into a herd

#### An European literature review

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Bovine herpesvirus 1 (BoHV1), the causative agent of infectious bovine rhinotracheitis (IBR), is endemic in the Netherlands and voluntary programs for the control of BoHV1 are conducted. Certified IBR-free herds are subject to a risk of (re) introduction of the virus. BoHV1 introduction into a free herd leads to animal health issues and economic losses for the farmer. In the Netherlands preparations are ongoing to start a mandatory national program for the eradication of BoHV1. The aim of our review was to determine the relevance of risk factors for introduction of BoHV1 from scientific literature. During the BoHV1 eradication campaign, communication with farmers, veterinarians and other professionals in the cattle industry is vital. Explaining biosecurity measures for BoHV1 in an applied manner to farmers and their advisors should reduce the risk of introduction of BoHV1 into a herd. The evidence from scientific literature can help in discussions about the risk perception and ranking of risk factors.

A literature search was carried out in the search engines Web of Science and PubMed in January 2016. As search criteria, 'risk factor' in combination with different synonyms for BoHV1 were entered in the search engines and resulted in 456 hits. After checking for duplicates and excluding articles irrelevant to



the topic, 76 unique publications remained. The abstracts of these studies were read by two authors and categorized as 'approved', 'rejected' or 'doubtful' on the basis of the requirements for the literature study. Only studies that were performed in Europe, were written in English, Dutch, French or German with an English summary and that determined risk factors for introduction of BoHV1 into cattle herds were included. Solely European studies were incorporated because they were considered most representative for the Dutch cattle industry. The separate allocations by two persons were then compared and discussed together for definite approval. Finally, the full text of eighteen publications were read by the first author and included in the final review. The review included studies from eight different European countries: Belgium (2), Estonia, Ireland (3), Italy (2), the Netherlands (5), Spain, Switzerland and the United Kingdom (3).

Risk factors were classified in the following categories: herd factors, management factors, animal factors, purchase related factors, direct animal contact factors, neighborhood factors, visitor factors and other risk factors. Most studies quantified the risk factors with crude odds ratios (OR), the odds of a particular event occurring in an exposed group compared to the odds of occurrence of the event in a non-exposed group.

Risk factors with consistently high odds ratios in several studies were considered most relevant for introduction of BoHV1 into cattle herds. Those factors were herd size, purchase of cattle, participation in cattle shows, communal grazing, type of housing, distance to neighboring farms and professional visitors. These risk factors seem most important when eradication is considered. The literature review indicated that other ruminants (e.g. sheep, wild ruminants) were a negligible risk.

Farm management and demographic factors can have a large impact on the control of BoHV1. The consistently high risk of adding purchased cattle to a herd highlights the importance to consider the antibody BoHV1 status of cattle prior to transportation, as to prevent concomitant introduction. This is not common practice in the Netherlands. In conclusion, the review showed that a closed farming system and the use of protective clothing for all professional visitors can to a large extent prevent introduction of the virus.

large variation in prevalence of IBR between EU member states. Up to now voluntary programs for the control of BoHV1 are conducted in the Netherlands and a national plan for mandatory eradication is in preparation. The aim of such a program would be to achieve freedom from BoHV1 circulation in the Dutch cattle population. Nevertheless, approximately 900,000 cattle, the majority veal calves 2-3 weeks old, from 21 different European countries are imported on an annual basis, which poses a risk of reintroduction of disease when a low prevalence of freedom from disease is achieved. It is therefore important to gain insight on the magnitude of the import risk and assess the effectiveness of different risk mitigating measures.

Quantification of the import risk of IBR was achieved in five steps:

**1. Selection of the risk factors.** Several risk factors such as import of animals, semen, embryos, use of contaminated trucks, etc. were assessed. Import of live cattle was assumed to be the main import risk.

**2. Quantification of import movements.** This information included 1) the origin (country disease status, herd level prevalence) and amount of imports, 2) risk of transport and 3) type of receiving herds (either dairy, suckler, veal, beef, trade, young stock raising or small scale herds). This information was obtained using the national identification and registration database together with existing import regulations.

**3. Quantification of disease specific information.** Concerning IBR, it is important to distinguish acutely from latently infected cattle at the moment of import. Other infection statuses in the risk release pathway were susceptible and maternally protected.

**4. Development of the stochastic simulation model.** The simulation model was constructed in MS Excel (Microsoft Corp., 2013) with @Risk 6.2.0 (Palisade, 2014<sup>®</sup>). The outputs were stratified to cattle herd type and different age categories. The output was subdivided into the risk of importing acutely or latently infected animals. Results were based on 5,000 iterations after which the model outputs were stable.

**5. Adding risk mitigating scenarios.** These scenarios included prohibition of import from high risk countries, channelling of veal calves, vaccination with marker vaccines or blood testing (gE or gB antibody ELISA) prior to export.

The model output predicted that IBR infected animals are imported regularly and lead to a substantial total of 571 (5<sup>th</sup> and 95<sup>th</sup> percentile: 431-781) cattle herds per year (from in total approximately 36,000 cattle herds) that will be newly infected because of import in a free situation. Most infections are related to import of latently infected cattle (77%) and occur in beef and suckler herds. Veal herds most often import acutely infected cattle. In the model we assumed that import of a single IBR infected animal (either acutely or latently infected) would always result in an infected receiving herd. If in practice however the virus does not reactivate in the imported latently infected animals, subsequent impact of such an introduction remains limited. To mitigate the risk of infections, a scenario in which vaccination was applied in cattle younger than 4 months old in combination with older cattle being tested for gB antibodies in blood prior to export was feasible for the industry to implement and would reduce the number of newly infected herds to 82 per year (86% reduction).

The approach of our study was able to effectively quantify the risk of importing BoHV1 in a free situation. The impact on re-

VR-04

## Probability of reintroduction of BoHV1 in the Netherlands through cattle imports

### A quantitative risk analysis

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This study presents a stepwise approach to quantify import risks of infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus 1 (BoHV1), in the Dutch cattle industry using stochastic simulation modelling. In Europe, several countries have a program to control and eradicate IBR, resulting in a



ceiving herds was not assessed, neither was an economic evaluation conducted. The model and subsequent output represent the situation in 2016, the results may be overestimated by the time the Netherlands becomes a BoHV1 free country because many European countries are currently conducting control and eradication programs for IBR. A similar modelling procedure can be applied to quantify the risk of import of other infectious cattle diseases.

VR-05

### Bovine respiratory syncytial virus infection enhances *Pasteurella multocida* adherence on respiratory epithelial cell lines

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**Objectives:** Bovine respiratory syncytial virus (BRSV) can be considered as a primary bovine respiratory disease complex (BRDC) pathogen and has an ability to predispose the respiratory tract to secondary bacterial infection. However, the detail synergy effect of interaction between BRSV and bacterial infection is still unknown. To elucidate it, we investigated co-infection status of BRSV and bacterial interaction.

**Materials and methods:** Respiratory swab samples ( $n=101$ ) were collected from cattle with pneumonia symptom ( $n=29$ ), pneumonia diseases history and recovered ( $n=38$ ), non-pneumonia diseases symptom ( $n=24$ ) and asymptomatic disease ( $n=10$ ) in Miyazaki, Japan during the period of 2015-2017. BRSV, bovine herpesvirus 1, bovine viral diarrhoea virus, bovine parainfluenza virus 3, *Pasteurella multocida* (PM), *Mannheimia haemolytica*, *Mycoplasma bovis*, and *Histophilus somni*, were detected by RT-PCR/PCR. The adherence of bacterial affected by preceding BRSV infection in respiratory epithelial cells was assessed by colony forming unit assay (CFA), flow-cytometry analysis (FACS) and indirect immunofluorescence assay (IFA) using epithelial cell lines (A549, HEp-2, MDBK). Adherence of bacteria to the cells was studied using BRSV-infected and uninfected monolayers as a control.

**Results:** A total of 81 cattle (81%) were found to have BRDC-related genes, including 56% has multi-agent cases. From multi-agent pattern, PM was shown 87% as a common bacteria detected paired with viruses. *In vitro* co-infection model of PM on BRSV-infected epithelial cells, PM adherent was increased 2 to 8 fold than the control cases. By FACS and IFA, we observed increasing fluorescence intensity and distribution of the FITC-labeled PM adherence over the epithelial surface due to time and MOI fashioned of BRSV infection.

**Conclusions:** We found the BRSV and PM were the most common multi-pathogen infection cases. BRSV enhanced the cell-adherent ability of PM on respiratory epithelial cells. Therefore, these interactions might have related to serious symptoms of BRDC.

VR-06

### Towards a better understanding of the pathogenesis and transmission of Influenza D virus in cattle

#### New influenza D virus in cattle

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**Objectives:** Since its discovery in 2013 in the United-States (Hause *et al.*, 2013), the novel influenza D virus (IDV) of the *Orthomyxoviridae* family was found spread among swine and ruminants on four continents so far, confirming a worldwide distribution. Cattle are suggested to be the main host of IDV and its detection during bovine respiratory disease (BRD) outbreaks (and their significant correlation) suggests that this virus could be a respiratory pathogen of young cattle (Mitra *et al.*, 2016). To date very little is known about the biological features of this virus. To understand the physiopathological, virological, immunological and clinical aspects in addition to the aerosol transmission potential of IDV, an experimental infection was carried out.

**Materials and methods:** Experimentation was performed under EEC guidelines (86/609/CEE) and official French ethical agreement. Sixteen calves, free of IDV and respiratory pathogens (BRSV, BCoV, BPI3, BVDV, BoHV-1, *M. haemolytica*, *P. multocida*, *H. somni*, *M. bovis*), were distributed into two separate pens. In the first pen 8 calves (Infected group) were intranasally nebulized at day 0 (D0) through a mask with  $10^7$  TCID<sub>50</sub> per calf of a French IDV strain D/bovine/France/5920/2014. Three calves (indirect contact group) were placed 4 meters apart from the infected group in a setup disallowing any direct contact between the animals. The second pen contained 5 non-infected calves (control group). Calves were examined from 3 days before challenge (D-3) to the end of experimentation (D23) for clinical signs, gross and microscopic lesions (D7 for 3 infected calves and D23), and IDV replication in both upper (nasal swabs (NS) from D1 to D16) and lower (bronchoalveolar lavages (BAL) at D0, D3, D7, D10 and D16 for infected and control calves) respiratory tracts. The immune response was assessed for antibody (hemagglutination inhibition assay) and T cell responses. Transmission of IDV via aerosols was evaluated by viral detection in NS in the contact group and by air samples collected using the wet cyclone sampler Coriolis (15 min corresponding to 4.5m<sup>3</sup> of ambient air at D0, D1, D2, and every two days until D18).





**Results:** Infected calves showed mild to moderate respiratory clinical signs between D5 to D10 characterized by coughing, tachypnea, dyspnea and, for few calves, labored breathing with abnormal lung sounds (wheezing). The mean clinical scores indicated significant differences between infected and control calves at D7 and D8. All infected animals recovered. IDV genome was detected by RT-qPCR in nasal swabs from D1 to D12 with a peak mean titer of  $1.4 \times 10^9$  IDV RNA copies/mL at D4. IDV was also detected in BALs at D2, D7 and D14 with mean titres of 1.4, 0.7 and  $14 \times 10^4$  IDV genome copies/mL in 3, 2 and 1 calves, respectively. One calf in the contact group acquired IDV infection and started shedding virus at D11. Of the 30 pooled air samples collected over the course of the experiment, IDV genome was detected at D3, D5, D7, D9 and D13 in a range of  $8 \times 10^4$  to  $1.1 \times 10^6$  IDV RNA copies/mL. Gross lesions of interstitial pneumonia with mild intensity were observed in infected calves euthanized at D8 and confirmed by histology. Immunochemistry clearly indicated the presence of IDV in the lungs. Finally calves started an antibody response at D10 for the infected group and D22 for one calf of the contact group. T-cell assay (on lymphocytes collected at D0 and D23) showed a specific proliferation at D23 in 2 out of 5 infected calves. Respiratory host response by transcriptomic analyses of BAL is under way.

**Conclusions:** We showed that IDV is a respiratory pathogen of calves with moderate virulence in both the upper and lower respiratory tracts. Moreover, in addition to direct contact, IDV also showed its ability to transmit on short distances via aerosol droplets. Altogether, these results, the frequent IDV detection in calves affected by BRD (Mitra *et al.*, 2016) and the high IDV seroprevalence in France (personal data) suggest that this virus could be a new initiator pathogen of this major syndrome.

VR-07

### Pseudocowpox virus on dairy farms in Slovenia

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Eroded papules are sporadically observed in calves raised in separate calf rearing facility of large commercial dairy farms, with more than 800 Holstein cows. Calves from 3 dairy farms belonging to the same firm are gathered in the calf rearing facility and stay there from 3-7 days old until weaning. The skin lesions on the muzzle, nostrils and oral mucosa usually appear in calves in the first month of life, especially in those suffering from severe and long-lasting diarrhea. Skin scrapings of papules and swabs from erosions were taken from 2 calves with most severe and obvious lesions.

The sampled material was inoculated on bovine turbinate cell culture where it caused cytopathic effect. Parapoxvirus has been identified by electron microscopy in the cell culture supernatant. Polymerase chain reaction (PCR) with primers targeting the ORF045 gene was performed from sample material. The PCR amplicons were subjected to direct sequencing. To determine the genetic characteristics, the sequences were analyzed and compared with sequences deposited in GenBank. Nucleo-

side sequences of the amplicons indicated from 97% to 99% similarity with pseudocowpox virus and lower homology with other parapoxviruses. Medical records of cows on farms were studied to see if pseudocowpox was clinically appearing also in milking cows. Just few records indicated skin lesions that could be connected to pseudocowpox that resolved without any complications and treatment as was the case in calves. No disease was observed in workers of the farms.

**Keywords:** nostrils, mouth, papules, erosion, calf, parapoxvirus

VR-08

### Development of Recombinant Nucleocapsid Protein-based Enzyme-linked Immunosorbent Assay for Serological Detection of Winter Dysentery Disease

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**Objectives:** Development of an indirect enzyme-linked immunosorbent assay (ELISA) based on the recombinant nucleocapsid (rN) protein was developed for antibodies detection against Winter Dysentery Disease and produced by *Escherichia coli* protein expression system.

**Materials and methods:** 231 dairy cow sera from 33 dairy herds in 6 farms in western and 27 farms in central regions, Thailand were collected. RNA was extracted from diarrheic cattle feces. The cDNA samples were synthesized from the extracted RNA. The N PCR products were performed. T&A and pQE80L vector were constructed. The rN protein was expressed in *E. coli* strain M15 and purified using 2 M urea. The rN ELSA was established. Statistical analysis had many values such as mean  $\pm$  SD, Cohen's kappa value, correlation coefficient (r).

**Results:** It was identified to be 48 kDa as a 6x His-tagged protein. The conditions of the ELISA method were optimized. The rN protein was standardized with a coating antigen concentration of 5  $\mu$ g/well. The dilution of the primary antibodies was identified as 1:50 by checkerboard titration. The cut-off OD<sub>corr450</sub> value from mean  $\pm$  2SD was established at 0.049. The specificity and sensitivity between the rN-ELISA and commercial ELISA kit were 96.3 and 84.8%, respectively. The Cohen's kappa value of 0.71 revealed substantial agreement. The correlation coefficient (r) of absorbance values was 0.68 and indicated that the strength of the two tests was good.

**Conclusions:** The recombinant Nucleocapsid protein-based Enzyme-linked Immunosorbent Assay was successfully developed. It might be helpful for Bovine coronavirus diagnosis and surveillance.



VR-09

### Studies on the Prevention of Bovine Papilloma Virus Infection in Dairy Cattle

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Bovine papilloma virus (BPV) induces diseases of considerable veterinary importance in cattle and other ruminants. BPV infects the skin from wounds such as puncture scratches by bloodsucking insects. Papilloma grows as tumor at the site of infection, papillomas of the teats and udder are often observed and it is associated with mastitis in dairy cows. Since an efficient treatment method for papilloma has not been established, prevention of transmission is most important. Reducing blood-sucking insects was shown to be effective in reducing the incidence of papilloma in dairy herds. The present study aimed to examine the effects of piretrin, piretroid and mint oil on blood-sucking insects and which compound is more effective for reducing the biting of bloodsucking insects, to reduce the transmission of BPV and to evaluate the impact of increasing the frequency of treatment from thrice a week to daily.

Experiments were conducted at the Field Center of Animal Science and Agriculture of Obihiro University, Japan. In experiment 1, a total of 58 grassing Holstein heifers were treated with chrysanthemum powder (pyrethrin 10%), bayticol (pyrethroid), ETB (pyrethrin), peppermint oil diluted in salad oil at 10 % and 2%, then applied to the teats thrice a week. In experiment 2, a total of sixty heifers were divided into groups and treated with chrysanthemum powder, ETB, peppermint oil (10 %, 3% and 2%), and then applied to the teat once a day. Teats were daily observed and the number of insect bites (appearance of small hemorrhagic red dots) were counted on the whole teats, only fresh wounds were regarded as bite.

In experiment 1, a significant decrease in the number of insect bites were observed in all treatment groups except for the Bayticol-treated group compared with untreated group. However, no significant difference was observed among the different drugs. As the number of insect bites in untreated and treated groups increased, it was difficult to differentiate which treatment was more effective. In experiment 2, a significant effect was confirmed in the following order for the chrysanthemum powder, peppermint oil 3%, then ETB, then peppermint oil 10 % and 2% group. In addition, heifers treated with chrysanthemum powder, peppermint oil 30 and ETB clearly decreased the number of insect bites compared with the first day just before application to teats. Based on the results of the present study including effectiveness and cost of treatment, we suggest that daily application of chrysanthemum powder is effective in reducing the number of insect bites and in reducing the risk of infection and transmission of bovine papilloma virus in a dairy herd.

IM-01

### Investigations on electrocardiographic abnormalities associated with hyperkalemia in 130 hospitalized neonatal diarrheic calves

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**Objectives:** Hyperkalemia in neonatal diarrheic calves can potentially result in serious cardiac conduction abnormalities. The present study aimed to document the sequence of electrocardiographic (ECG) changes that are associated with increasing plasma potassium concentrations [K<sup>+</sup>] in a large population of affected animals.

**Materials and methods:** Data from 130 hospitalized neonatal diarrheic calves (age up to 21 days) were used for analysis. The relationship between ECG variables and increasing [K<sup>+</sup>] was characterized by means of segmented linear regression analysis.

**Results:** Hyperkalemic calves had significantly ( $P < 0.05$ ) longer QRS durations as well as deeper S wave, and higher T wave and ST segment amplitudes in lead II than calves with venous blood pH and [K<sup>+</sup>] within the reference range. The first ECG changes in response to an increase in [K<sup>+</sup>] were found to be an increase in voltages of P, Ta, S, and T wave amplitudes. Segmented linear regression indicated that P wave amplitude decreased when [K<sup>+</sup>] >6.5 mmol/L, S wave amplitude voltage decreased when [K<sup>+</sup>] >7.4 mmol/L, QRS duration increased when [K<sup>+</sup>] >7.8 mmol/L, J point amplitude increased when [K<sup>+</sup>] >7.9 mmol/L, and ST segment angle increased when [K<sup>+</sup>] >9.1 mmol/L. P wave amplitude was characterized by a second common break point at [K<sup>+</sup>] = 8.2 mmol/L, above which value the amplitude was 0.

**Conclusions:** In addition to increased S and T wave amplitude voltages, alterations of P and Ta wave amplitudes are early signs of hyperkalemia in diarrheic calves which is consistent with the known sensitivity of atrial myocytes to increased [K<sup>+</sup>].

IM-02

### An observational study to assess the value of a computer-aided lung auscultation system to predict the risk of bovine respiratory disease (BRD) relapse and mortality when collected at the time of first BRD treatment

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**Objectives:** The objective of this study was to assess the efficacy of a computer-aided lung auscultation (CALA) system (Whisper<sup>®</sup> Veterinary Stethoscope) in predicting relapse and



mortality risk among cattle treated for bovine respiratory disease (BRD).

**Materials and Methods:** The CALA system records sounds from the thorax of a calf and processes them with a proprietary machine-learning algorithm. A score from 1 to 5 is generated which is designed to reflect the severity of BRD (1=minimal lung pathology; 5=severely compromised lung tissue) for the calf.

A CALA score was generated for calf-fed Holstein (N=2,023) and mixed-breed, auction market derived, beef steer and heifer (N=703) feedlot calves at the time of first BRD treatment. Data were collected at one U.S. feedlot from 29 July 2015 to 15 April 2017. Animals were deemed to be "sick" with BRD based on subjective criteria such as general appearance, attitude, gauntness, reluctance to move, etc., were individually sorted from pen mates, and moved to the hospital facility. The population was further differentiated based on a predefined rectal temperature cutoff of > 40.5 °C (Holstein, N=823; beef, N=261) or < 40.5 °C (Holstein, N=1,200; beef, N=442). The CALA score was not used to determine treatment options.

Calves were followed for 120 days after enrollment in the study. Data analyses were performed by generalized linear mixed models evaluating the calf as the experimental unit. All necessary clustering effects were accounted for in all models. The outcome risks of BRD relapse and mortality were evaluated across the sample population. Where the number of degrees of freedom permitted, all biologically plausible two and three-way interactions were assessed following determination of main effects. A backwards step-wise model building procedure was implemented to determine a final multivariable model. Confounding and collinearity were assessed by standard methods. An alpha level of 0.05 was used for inclusion and adjusted for multiple comparisons.

**Results:** The CALA score collected at the time of first BRD diagnosis was observed to be significantly associated with the risk of BRD relapse ( $p<0.05$ ). The model-adjusted BRD relapse risk estimates and 95% confidence intervals (95% CI) for each of the CALA scores are as follows: CALA=1 (0.49, 95% CI; 0.34, 0.71), CALA=2 (0.54, 95% CI; 0.47, 0.63), CALA=3 (0.63, 95% CI; 0.56, 0.71), CALA=4 (0.87, 95% CI; 0.70, 1.00), CALA=5 (0.97, 95% CI; 0.70, 1.00).

Likewise, the CALA score collected at the time of first BRD diagnosis was observed to be significantly associated with the risk of BRD mortality ( $p<0.05$ ). The model-adjusted BRD mortality risk estimates and 95% CI for each of the CALA scores are as follows: CALA=1 (0.12, 95% CI; 0.6, 0.21); CALA=2 (0.17, 95% CI; 0.14, 0.21); CALA=3 (0.22, 95% CI; 0.18, 0.25); CALA=4 (0.30, 95% CI; 0.23, 0.40); CALA=5 (0.61, 95% CI; 0.42, 0.87)

**Conclusions:** In this study, the CALA score, collected at the time of first BRD treatment, was significantly associated with the risk of BRD relapse and BRD mortality. The risk of BRD relapse and mortality were observed to increase as the CALA score increased from 1 to 5.

IM-03

### Evaluation of administration of tildipirosin and a multivalent inactivated respiratory vaccine to control bovine respiratory disease in pre-weaned dairy calves.

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**Objectives:** Bovine respiratory disease (BRD) is a major health condition that affects efficiency of calf-rearing programs due to mortality, morbidity, treatment cost, and reduced performance (growth and milk production). The control of BRD involves management practices to reduce pathogen load and vaccination programs to improve the immune status of the herd. Alternatively, dairy calves at high risk of developing BRD typically receive antibiotics in a metaphylactic manner to reduce disease morbidity. The objective of this study was to evaluate the effect of revaccination alone or in combination with the antibiotic tildipirosin to control BRD in high risk dairy calves.

**Materials and methods:** The study was conducted on a commercial dairy farm located in Chile. The BRD incidence on this farm previous to applying vaccination or metaphylactic treatment was above 30%. A total of 300 Holstein dairy calves (148 males and 152 females) at high risk for developing BRD were enrolled in the study. At two weeks of age, calves were randomly assigned to four treatment groups. The treatments in the different groups were:

BP+TIP: Tildipirosin (Zuprevo, MSD Animal Health) according to label and an inactivated respiratory vaccine (Bovilis® Bovipast RSP, MSD Animal Health) at 2 weeks of age and re-vaccinated at 6 weeks of age (N=80).

TP: Tildipirosin according to label (at 2 weeks of age) and saline solution (at 6 weeks of age) (n=80).

BP: An inactivated respiratory vaccine (Bovilis® Bovipast RSP) at 2 weeks of age and re-vaccinated at 6 weeks of age (n=80).

CON: Saline solution at 2 weeks and at 6 weeks of age (n=60).

After enrollment, calves were allocated in collective pens containing 10 animals per pen that received the same treatment. During the experimental period (80 days post-enrollment), calves were fed with the same diet and were observed twice daily for BRD clinical signs by the farm veterinarian who was blinded to treatment. BRD incidence was determined using a respiratory disease score (RDS) and rectal temperature (BRD incidence = RDS>2 plus rectal temperature >39.5°C, or RDS=3). The experimental unit for all analyses was the individual calf. BRD incidence was tested for statistical significance by GLIMMIX procedure. In all cases statistical significance was assessed at  $P<0.05$ .

**Results:** No mortalities due to BRD were observed in the study. The incidence of BRD was statistically lower ( $P<0.05$ ) in animals receiving TIP (8.9%), BP (16.5%), or BP+TIP (11.3%) compared to those receiving CON (32.5%). There was no statistical difference ( $P>0.05$ ) in BRD incidence in animals receiving combination of metaphylaxis and vaccination (BP+TIP) compared to those receiving TIP or BP alone. There were no



statistical differences ( $P>0.05$ ) among treatment groups in body weight at birth, weaning weight or average daily gain (ADG).

**Conclusion:** In the present study consisting in an 80 day experimental period, the efficacy of respiratory vaccination was confirmed by the reduction of the BRD incidence compared to the control group. Also, the administration of tildipirosin to control BRD was effective to reduce the incidence of the disease. The combination of the metaphylaxis and respiratory vaccination did not differ compared to these two practices alone; however, further research including long term evaluation of health and performance may provide additional information to determine the best practices for healthcare in replacement dairy heifers.

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IM-04

### Measuring veterinary medicine storage and use on United Kingdom dairy farms

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**Objectives:** The use of prescription veterinary medicines (PVM) on dairy farms in the United Kingdom (UK) is currently not well understood, despite potential implications for the development and transmission of antimicrobial resistance and animal health. Farmers in the UK are in the privileged position of being able to store PVM on farm for use following veterinary protocols and guidance, meaning most treatment decisions and implementations occur without the direct supervision of a veterinarian. Currently in the UK, veterinary medicine use is measured at a national level, with measurement at an individual veterinary practice level likely to be instituted in the near future. Despite detailed on-farm medicine records being a requirement for dairy farmers, there is currently limited data available for measurement of veterinary medicine storage and use at the individual farm level. This longitudinal prospective study aims to compare PVM use from veterinary prescription data, on-farm medicine records and on-farm medicine waste bins.

**Materials and Methods:** Twenty-six dairy farms with a broad range of management systems, herd sizes and production goals were enrolled in September 2016. A full PVM inventory was taken along with a structured management survey. Medicine waste bins were placed on farms and participants were asked to dispose of all used medicine packaging into these bins. Participants were asked to continue using and recording veterinary medicines in the usual way. All farms were followed for a 12-month period, with bins emptied and audited on a quarterly basis. At the end of the study, farm medicine records and veterinary sales data were obtained, and medicine waste bins were removed.

**Results:** Medicines were recorded and stored in a variety of different ways. A wide range of different medicines were stored, in varying quantities. Antimicrobials classified by the World Health Organisation to be of highest critical importance for human health (fluoroquinolones, 3rd & 4th generation cephalosporins) were stored on 89% of farms; these accounted for between 0% and 30% of the total weight of antimicrobials. Expired antimicrobials were present on 74% of farms, and were used on 69%. Total quantity of antimicrobials stored did not correlate well with the

number of lactating cows, youngstock or volumes of milk produced. Median herd size was 161 lactating cows and median annual production was 1.6 million litres, both slightly higher than UK averages of 142 and 1.2 million respectively.

Compliance with use of medicine waste bins was good, with farmers reporting ease-of-use as a contributing factor. On-farm medicine record quality varied widely between farms; some farmers kept very accurate and up-to-date records, while others kept no records at all. Veterinary sales data were accurate and easy to obtain, and correlated well with actual on-farm use, particularly when combined with a pre- and post-audit medicines inventory.

Data analysis is ongoing with full results available from March 2018.

**Conclusions:** PVM use on UK dairy farms is difficult to measure accurately. Veterinary sales data provide more granular data than current UK national estimates, however sales data are prone to overestimating the amount of medicine being administered on farms and are subject to time lag (medicines that are bought and stored but not administered within a given time period). On-farm medicine records provide a more temporally accurate measure of use and generally provide information on dose rate, course length and the identity of the individual animal being treated. However, the quality of on-farm records varies widely, and the current format of these records is such that collating data is inefficient and time-consuming. Medicine waste bins provided the most accurate record of PVM use on many of the participating farms, capturing data on expired medicine use, off-license use and wasted medicines. Waste bins, however, were at risk of underestimating use where participants forgot to use them. In the short term, moving away from national PVM use estimates and towards veterinary prescription data will improve the quality of data. Ultimately an integrated, user-friendly computerised medicine book may provide the best quality data.

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IM-05

### Performance of multiple diagnostic tools in assessing the progression of respiratory disease in calves infected with IBR followed by *Mannheimia haemolytica*

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**Objectives:** This study's objective was to evaluate the performance and applicability of multiple diagnostic tools measuring physiological, clinical and pathological changes as indicators of early bovine respiratory disease (BRD) in calves experimentally inoculated with Infectious Bovine Rhinotracheitis virus (IBR) and *Mannheimia haemolytica* (Mh).



**Materials and Methods:** Holstein steers, negative for IBR and Mh antibodies, were acclimated and determined to be clinically normal for a period of 8 days prior to study initiation. Thirty animals were randomized to necropsy days and held in one feedlot pen. Steers were inoculated intranasally on study day 0 with IBR, and intrabronchially on study day 6 with Mh. The study was conducted from May 11 to 24, 2017. Several clinical, physiological and pathological parameters were measured, including: rectal temperature, computer-aided lung auscultation (CALA [Whisper<sup>®</sup> Veterinary Stethoscope, MSD Animal Health; DeSoto, KS, USA]), complete blood cell counts, chute-side blood differential (Q Scout BLD, Advanced Animal Diagnostics; Morrisville, NC, USA), pulse oximetry, bilateral thoracic ultrasonography, clinical illness scores, body weight, and gross pathology (including percent lung consolidation). Animals were euthanized on study days 6, 7, 9, 10, 11 and 13.

The calf was considered the experimental unit. Data were analyzed with generalized linear mixed models comparing changes from baseline (pre-challenge) data to repeated measures of data collected following challenge. An alpha of 0.05 was utilized in this study.

**Results:** The challenge model effectively reproduced naturally occurring BRD and was consistent with reported IBR and Mh disease processes. Clinical scores were normal (0) pre-challenge, varied following IBR challenge (study days 0 to 6) from 0 to 3 (severe depression), and following Mh challenge ranged from 1 (mild depression) to 4 (moribund) on days 6.5 to 9, and from 2 (moderate depression) to 4 (moribund) on days 10 to 13. Oxygen saturation ranged from 93 to 100% (mean = 97.6%) on study days 0 to 6, 73 to 100% (mean = 94.5%) on days 6.5 to 10, and 82 to 100% (mean = 92.0%) on days 11 to 13. No lung consolidation was detected with thoracic ultrasound at the beginning of the study. On study day 6, a maximum of 5% lung consolidation was observed by ultrasound, followed by a maximum of 40% on days 6.5 to 10, and 50% on days 11 to 13. Computer-aided lung auscultation scores (1 to 5) ranged from 1 (normal) to 3 (moderately acute) on study days 0 to 6, from 1 to 4 (acute) on days 6.5 to 10, and from 1 to 3 on days 11 to 13. Blood leukocyte differential ranged from 7,000 cells/ $\mu$ l to 4,000 and 6,000 cells/ $\mu$ l for neutrophils and lymphocytes, respectively, from days 0 to 6. A large increase was observed for both cell types on days 6 to 7 (post Mh challenge) then subsequently declined to approximately 3,000 cell/ $\mu$ l by day 13. Rectal temperatures ranged from 38.3 °C to 41.5 °C (mean = 39.9 °C) on study days 0 to 6, 36.8 °C to 42.5 °C (mean = 40.9 °C) on days 6.5 to 10, and 38.0 °C to 40.9 °C (mean = 39.9 °C) on days 11 to 13. Average body weights were 210.9 kg pre-challenge, 206.4 kg on study days 0 to 6, 196.4 kg on days 6.5 to 10, and 184.6 kg on days 11 to 13. Fibrinous to muco-purulent tracheitis, consistent with IBR disease, was observed in calves necropsied on study day 6 (pre-Mh challenge). For calves necropsied on days 7 to 13 (post-Mh challenge), the lungs progressively showed severe consolidation of the cranio-ventral portions. Fibrinous pleural adhesions and fibrinous to purulent pleuritis were also found. Necrotic laryngitis and normal to necrotic tracheas also were observed at necropsy on days 7 to 13.

Diagnostic test results (for each individual test) were significantly different across study days ( $p < 0.05$ ).

**Conclusions:** All of the diagnostic tools were able to detect statistically significant changes over time as disease progressed throughout the study. The application of these diagnostic methods, either individually or in some combinations, may be of value

for improving BRD diagnostic accuracy in cattle.

IM-06

### Factors associated with pulmonary inflammation in calves as determined by cytology on non-endoscopic broncho-alveolar lavage samples

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**Objective:** Pulmonary inflammation in calves can be due to infectious and non-infectious causes, with possible interactions. Non-endoscopic broncho-alveolar lavage (BAL) is a practical and cheap method currently used for pathogen identification in cattle. Cytological analysis of these BAL samples can provide insights in the level of pulmonary inflammation in calves. The objective of the present study was to determine which clinical signs, lung ultrasound findings and BAL characteristics are associated with total nuclear cell count and the differential count of pulmonary leukocytes.

**Materials and methods:** A cross-sectional study was conducted on 332 calves (Holstein Friesian ( $n=177$ ) and Belgian Blue ( $n=155$ )) from 59 conveniently selected herds between January and April. Animal selection criteria were judgment as clinically healthy by the farmer, no previous antimicrobial use, age between 1 and 6 months and indoor group housing. Animals were clinically examined and lung ultrasound performed. Broncho-alveolar lavage fluid (BALF) was collected using a non-endoscopic method and instillation of 40 mL of saline. Bacterial culture was performed and species confirmation was done by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). The total nucleated cell count (TNC) of the recovered lavage fluid was determined manually using a haemocytometer. Cyto-centrifuge preparations of BALF (1200 rpm for 10 minutes) were evaluated to determine the 400 cell differential cell count. A mixed model with herd as a random factor was used to identify factors influencing BALF total and differential cell counts.

**Results:** Of the calves, 49.1% (163/332) demonstrated lung consolidation on ultrasound. Pathogen isolation rates were 33.7% (112/332) for *Pasteurella multocida*, 15.4% (51/332) for *Mannheimia haemolytica*, 3.6% (12/332) for *Histophilus somni* and 3.3% (11/332) for *Mycoplasma bovis*. Isolation rates were not influenced by clinical signs or ultrasonographic findings. Mean TNC was  $1.9 \times 10^9$  cells/L (standard deviation 1.8; range 0.0-13.7). Of the calves, 63.0% (209/332) had an increased TNC ( $> 1.0 \times 10^9$  cells/L), but only from 61.2% (128/209) of these a pathogen could be isolated. In contrast, from 37.4% (46/123) of the low TNC's a pathogen could be isolated. In the final multivariable model TNC's were associated with isolation of *P. multocida* ( $P < 0.01$ ), volume of recovered lavage fluid ( $P < 0.001$ ) and presence of erythrocytes ( $P < 0.01$ ).

Mean differential cell count (%) of leukocytes in BALF were 42.8% macrophages (standard deviation 19.1; range 2.4-92.3), 36.6% neutrophils (standard deviation 23.9; range 0.0-97.4) and 5.5% lymphocytes (standard deviation 5.3; range 0.0-45.8). In the final multivariable model isolation of *P. multocida*



( $P < 0.01$ ), increased respiratory rate ( $P < 0.05$ ) and a positive trachea reflex ( $P < 0.05$ ) were positively associated with BALF neutrophil percentage. Macrophage percentage in BALF was inversely associated with the recovered volume of BALF ( $P < 0.05$ ) and *P. multocida* ( $P < 0.05$ ) isolation and was significantly higher in calves maintaining sternal recumbency ( $P < 0.01$ ). The lymphocyte percentage in BALF increased with an increasing age ( $P < 0.05$ ) and the presence of erythrocytes in BALF ( $P < 0.05$ ).

**Conclusions:** Subclinical bronchitis and pneumonia are widespread in group-housed calves. Observed neutrophil percentages are markedly higher than reported in experimental studies in healthy calves using endoscopic lavage. Clinical signs, ultrasonography and bacterial culture explained very little of the variation in total and differential leukocyte counts in BALF from calves, suggesting that other factors like respiratory viruses or non-infectious environmental factors affect pulmonary inflammation.

IM-07

### Non Invasive Mechanical Ventilation (CPAP Mode) on premature calves for neonatal hypoxia treatment

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**Objective:** The objective of this research was to evaluate the influence of non invasive mechanical ventilation (CPAP Mode) on arterial blood gas analyser parameters, pulmonary artery pressure and cardiac output of premature calves

**Materials and Methods:** Twelve neonatal calves were used in this research, all born by caesarean section between 270 and 285 days of gestation. The EVITA 2® ventilator was used in CPAP mode for non invasive mechanical ventilation with a facial mask. Animals were submitted to two ventilation procedures: FiO<sub>2</sub> (oxygen fraction) variation (21,28,50 and 100%) with PEEP (Positive End Expiratory Pressure) equal as zero, and PEEP variation (0,5,10 and 15) with FiO<sub>2</sub> 28%. The parameters evaluated were: arterial blood hemogasometry (pH, PaO<sub>2</sub>, PaCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, BE, SO<sub>2</sub>), pulmonary artery pressure and cardiac output .

**Results:** In comparison to the initial values (FiO<sub>2</sub> 21%), with the FiO<sub>2</sub> variation (21,28,50 and 100%) the PaO<sub>2</sub> and SO<sub>2</sub> arterial blood parameters increased showing lung response to the oxygen offer. A reduction in pulmonary artery pressure was also observed in animals receiving 50% and 100% of FiO<sub>2</sub>. Cardiac output did not change. Increasing PEEP from 0 to 15, PaO<sub>2</sub> and SO<sub>2</sub> increased gradually, with PaO<sub>2</sub> ranging from 72.31 ± 7.37 to 106.55 ± 7.12, and SO<sub>2</sub> ranged from 88.75 ± 2 , 65 to 96.64 ± 0.88, showing that this experimental procedure may have beneficial effects on the oxygenation of neonatal calves. Pulmonary artery pressure and cardiac output did not change.

**Conclusions:** It was concluded that the use of non-invasive mechanical ventilation with CPAP mode with increased FiO<sub>2</sub> or increased PEEP helped prematurely ventilated calves to revert to their neonatal hypoxia. Aside from this, the use of 50% or 100% FiO<sub>2</sub> has an effect on pulmonary artery pressure reduc-

tion. The increase of PEEP up to 15 showed to be safe and can help to improved oxygenation.

IM-08

### Comparison of Causative Agents of Bovine Diarrhea and Acute Phase Proteins between Healthy and Diarrheic Korean Indigenous Calves

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**Objectives:** This report is to identify the causative agents of calf diarrhea from Korean indigenous calf and their relationship between causative agents and calf diarrhea and their effects on acute phase proteins (APPs).

**Materials and Methods:** Ten local Korean indigenous cattle farms were selected for the investigation of causative agents of calf diarrhea and APP. Serum and feces were collected from calves up to 60 days of age and transferred to a laboratory for further researches. The evaluation of fecal scores were performed based on the calf health scoring guide developed at the University of Wisconsin School of Veterinary Medicine and feces level 2 and 3 was determined as diarrhea. Real-time PCR were performed for the detection of *Cryptosporidium*, *E. coli* K99, bovine coronavirus (BCV), bovine rotavirus (BRV), and bovine viral diarrhea virus (BVDV) and Ct value below 40 was considered as positive. Flootation method was performed *Eimeria* spp. For analyzing APPs, serum Amyloid A (SAA) and haptoglobin (Hp) were analyzed by commercial ELISA kits and fibrinogen (Fb) was analyzed by the difference of the concentration of plasma protein to serum protein. Statistical analysis was performed using the SPSS 23.0 software package (SPSS, Chicago, Illinois, USA).

**Results:** Totally, 544 calf feces [302 normal feces (level 0: 153, level 1: 187) and 236 diarrheic feces (level 2: 116, level 3: 88)] were collected from 10 local farms in 2016-2017. The infection rate (%) of *Cryptosporidium*, *E. coli* K99, BCV, BVDV, BRV, *Eimeria* spp., in normal feces and diarrheic feces was 2.1/8.3, 0/0.5, 7.4/10.3, 1.2/0, 5.0/15.2, 25.0/31.4, respectively. The averages of each APPs (SAA, Hp, Fb, mg/dL) in normal feces and diarrheic feces were 21.6/23.7 ( $p = 0.10$ ), 7.1/14.1 ( $p < 0.01$ ), 630.2/753.7 ( $p < 0.01$ ), respectively.

**Conclusion:** In total, the feces and serum from 302 non-diarrheic Korean indigenous calves and 236 diarrheic calves were collected for the detection of causative agents of calf diarrhea (*Cryptosporidium*, *E. coli* K99, BCV, BVDV, BRV, *Eimeria* spp.) and the evaluation of APPs (SAA, Hp, Fb). All diarrhea-causative agents except *E. coli* K99 (no from normal feces) and BVDV (no from diarrheic feces) were detected from normal feces and diarrheic feces. Except BVDV, the infection rates were higher in diarrheic calves than healthy calves. Also, Hp and Fb



showed statistical significance between normal feces and diarrheic feces and the average of SAA in diarrheic calves was higher when compared to healthy calves. However, statistical significance was not found from the detection number of causative agents and APPs. APP seems to be related to clinical signs, not to specific causative agents.

IM-09

### Does the Physicochemical Approach Improve Detection of Acid-base Disorders in Diarrheic Calves?

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**Objectives:** To determine the level of agreement of the traditional (Henderson-Hasselbalch [HH], base excess; [BE]) and the physicochemical (simplified strong ion difference; sSID) approaches to detect metabolic acid-base (AB) imbalances in diarrheic calves with pH and pCO<sub>2</sub> values within the normal ranges.

**Materials and Methods:** Retrospectively, medical records were reviewed for all calves presented to a teaching hospital between 2010 and 2016. All calves included in the study met the following inclusion criteria: < 21 days of age, duration of diarrhea (increased frequency and water content in the feces) < 48 hours, measurement of blood gases and basic serum metabolic profile within the 1st hour of hospitalization before to parenteral administration of fluids. Clinicopathologic data of pVCO<sub>2</sub> (mmHg), pH, HCO<sub>3</sub> (mmol/L), BE (mmol/L) and plasma [Na<sup>+</sup>] (mmol/L), [K<sup>+</sup>] (mmol/L), [Cl<sup>-</sup>] (mmol/L), [L-lactate] (mmol/L), and [TP] (g/L) were recorded. The sSID variables for calves were calculated as strong ion difference (SID) = (Na<sup>+</sup>+K<sup>+</sup>)-(Cl<sup>-</sup>+L-Lactate<sup>-</sup>); total plasma concentration of non-volatile weak acids [A<sub>tot</sub>]= 0.343 x TP(g/L) and unmeasured strong ions [USI] = A<sub>tot</sub>/(1+10<sup>(7.08-pH)</sup>) - SID - HCO<sub>3</sub>. AB disorders were defined when any of the respective variables were outside of the normal ranges (HCO<sub>3</sub> < 20 or >30 mmol/L), (BE < -2 or > 6 mmol/L), (sSID < 38 or > 48 mmol/L), (Atot < 15 or > 22 mmol/L), (USI < -2 or > 3 mmol/L). McNemar's test evaluated if pairs of approaches were equally likely identifying AB disorders and Kappa coefficient test assessed the level of agreement between pairs of approaches in detecting AB disorders.

**Results:** A total of 37 calves hospitalized for diarrhea with normal venous pH (mean ± SD; 7.39±0.03 and 7.41±0.03, respectively) and pCO<sub>2</sub> (41±4.5 mmHg and 42±4.5 mmHg, respectively) were included. In these groups, HH approach detected AB disorders in 11/37 (30%) calves, BE in 9/37 (24%) calves, and sSID in 25/37 (68%) calves. McNemar's test demonstrated that HH/BE were equally likely to detect AB disorders (P=0.50), but HH/SID and BE/SID were not (P<0.05). There was fair agreement between HH/BE (κ=0.4, 95%CI:0.1 - 0.72), and a poor agreement between HH/SID (κ=0.18, 95%CI:0.05 - 0.41) and BE/sSID (κ=0.17, 95%CI:0.01-0.37), for detection of AB disorders.

**Conclusions:** The physicochemical approach improved detection of underlying mixed metabolic disorders in diarrheic calves

with normal venous pH and pCO<sub>2</sub>. In addition to pH, HCO<sub>3</sub><sup>-</sup> and BE, clinicians should calculate the quantitative effect of the strong electrolytes, unmeasured anions and protein on the AB balance of diarrheic calves.

IM-10

### Predicting the level of metabolic acidosis in neonatal calf with diarrhea: diagnostic value of urinary pH and construction of decision trees based on clinical signs.

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**Background:** Metabolic acidosis is common in calves with neonatal diarrhea. Specific treatment is based on venous and oral rehydration. Amount of bicarbonate or metabolic bases necessary for acidosis correction is usually estimated from clinical signs assessment, when laboratory equipment is not available. But clinical assessment of acidosis gives different results between studies, depending on the clinical signs that are considered.

**Objectives:** The first aim was to assess the additional diagnostic value of urinary pH for predicting metabolic acidosis in neonatal calf diarrhea. The second objective was to build decision trees including different individual criteria and clinical signs to predict the base excess level (or bicarbonate concentration) in diarrheic calves.

**Material and Methods:** A total of 152 calves with complete data over 193 Charolais beef calves with diagnosis of neonatal diarrhea admitted to a veterinary practice during a 2-year study period were included in the study. Physical examinations were carried out following a standardized protocol and included, among others, the assessment of suckling and palpebral reflexes, posture/ability to stand, degree of depression and degree of dehydration (enopthalmos). Urine PH was assessed by a pH-meter, while blood pH was measured on an electrolyte and blood gas analyzer (Vetstat, IDEXX) together with Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, PCO<sub>2</sub>, tCO<sub>2</sub>, and HCO<sub>3</sub><sup>-</sup>. Base excess was calculated based on biochemical results.

Multiple Correspondence Analysis was carried out to explore relationship between clinical signs and base excess level. Classification trees with cross validation-based model selection and Random Forest Analysis were applied to predict base excess, either on the continuous scale or after discretization in 4 groups, based on the most relevant clinical signs.

**Results:** Calves were between 1 and 21 days (median 8) days old. Base excess ranged between -25.3 and 6.3 mmol/L and 142 calves (93.4%) had blood pH<7.35. Base excess was significantly less pronounced in calves younger than 5 days (-6.83 +/- 9.48) than in older ones (-15.43 +/- 6.62). No relation between urinary pH and either blood pH, base excess or bicarbonate concentration could be evidenced, indicating that urine pH is of no help when assessing the degree of metabolic acidosis. Posture, age, degree of depression, hypothermia and palpebral reflex were the most significantly signs associated



with base excess level, with clear segregation of the lowest and uppermost base excess values.

Several classification trees with different combinations of clinical signs and levels of complexity were fitted. Whatever the modeling approach, the accurate prediction of base excess ( $\pm 5$  mmol/L) was associated with correct prediction rates of only 44% to 49%. However when loosening the prediction accuracy ( $\pm 10$  mmol/L), prediction rates as high as 84% were found. Finally for almost a third of the calves, the decision trees underestimated the base excess.

**Conclusions:** Our results indicate that urinary pH is useless to predict the base excess in calves with neonatal diarrhea. Classification trees allowed an accurate prediction of base excess level in only about half of the calves but adequately segregated the lowest and uppermost base excess values.

IM-11

### Evaluation of a portable ion-selective electrode meter for measuring potassium concentrations in whole blood and plasma of dairy calves

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**Objectives:** An ion-selective electrode (ISE) handheld meter (LAQUAtwin B-731; Horiba Ltd.) has recently become available for the measurement of potassium concentrations [ $K^+$ ] in biological fluids. The ISE meter has the potential to facilitate the diagnosis and treatment of potassium balance disorders of critically ill cattle. The objective of this study was to characterize the analytical performance of the ISE meter in a study population of dairy calves with a broad range of plasma [ $K^+$ ].

**Materials and methods:** Whole blood and plasma samples from 125 hospitalized calves (age up to 3 months) were used for analysis. The accuracy of the meter against the reference method (indirect ISE, Cobas c311, Roche) was assessed using Passing-Bablok regression and Bland-Altman plots.

**Results:** The [ $K^+$ ] in whole blood as measured by the ISE meter in direct mode ranged from 2.4 to 9.9 mmol/L. The meter measured whole blood [ $K^+$ ] as 3.8% higher than plasma [ $K^+$ ]. Passing-Bablok regression for whole blood [ $K^+$ ] measured by the meter against plasma [ $K^+$ ] determined by indirect potentiometry revealed a linear relationship that was almost identical to the line of identity. However, the Bland-Altman plot indicated that the meter measured plasma [ $K^+$ ] 5.1% lower than the reference method. This result was consistent with analytical differences of direct and indirect ISE methods in respect to variation in the plasma protein concentration.

**Conclusions:** The LAQUAtwin B-731 meter provides an accurate, rapid and low-cost tool for the diagnosis of potassium derangements in critically ill calves, particularly when whole blood samples are analyzed

IM-12

### Effect of dehydration and acidemia on the potassium content of muscle tissue and erythrocytes in calves with neonatal diarrhea

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**Objectives:** The aim of this study was to determine the effect of dehydration and acidemia on intracellular potassium (K) content in diarrheic calves.

**Material and Methods:** Severely dehydrated and acidemic calves with naturally occurring diarrhea were included in this prospective case control study. Blood samples and muscle biopsies were obtained immediately before initiation of treatment (T0) and subsequently after complete rehydration and correction of acidemia (T2). Blood samples were used to perform blood gas- and blood biochemical analysis and to determine the K content in erythrocytes. Muscle biopsies were used to determine muscle tissue K and dry matter.

**Results:** Twentyseven animals were included in the study. At admission calves showed pronounced acidemia and dehydration. Plasma potassium concentration [K] decreased from 5.44[4.76-6.17] to 4.16[3.99-4.31] mmol/L between T0 and T2. The erythrocyte K content increased from 73.63 $\pm$ 13.73 to 77.64 $\pm$ 15.97 mmol/L but was associated with a concomitant decline of the erythrocyte volume. Muscle K with 79.84 $\pm$ 11.51 and 78.62 $\pm$ 11.93  $\mu$ mol/g wet weight at T0 and T2 and muscle dry matter with 20.89 $\pm$ 1.5 at T0 and 20.13 $\pm$ 1.0 % at T2 remained unchanged and in the range determined in healthy control calves. While changes in plasma [K] were associated with the degree of dehydration, neither dehydration nor acidemia notably altered the K content of muscle tissue or erythrocytes.

**Conclusions:** Severe dehydration and acidemia in diarrheic calves was not associated with notable changes of K content of muscle tissue or erythrocytes. These results do not support the concept of intracellular K depletion in calves with neonatal diarrhea.

IM-13

### Bone resorption status in parturient dairy cows: a perspective of bone markers related to osteoclast activity

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**Objectives:** Most dairy cows develop varying degrees of hypocalcemia at calving due to a sudden loss of calcium (Ca) into colostrum. Osteoclastic bone resorption is important for the control of circulating Ca concentrations. We previously found that the number of osteoclasts increased prior to parturition in





dairy cows. However, the precise mechanism of bone resorption in Ca homeostasis around parturition is not well understood. Osteoprotegerin (OPG) protects against loss of skeletal mass by regulating the differentiation of osteoclast cells (osteoclastogenesis). The aim of this study was to demonstrate osteoclastic bone resorption in parturient dairy cows by measuring the sequential changes in peripartum serum OPG levels. Circulating levels of tartrate-resistant acid phosphatase 5b (TRAP5b), a bone resorption marker exocytosed by osteoclasts, were also evaluated in those cows.

**Materials and Methods:** This study used 18 primiparous ( $n = 9$ ) and multiparous ( $n = 9$ ) parturient Holstein Friesian cows. They calved normally at the farm of Obihiro University of Agriculture and Veterinary Medicine. All were declared clinically healthy during the experimental period. The blood samples were collected 21 days before the expected calving date (means; 20.3 days prior to calving) and over 5 days after calving (immediately after calving, and 12 hours, 2 and 5 days after parturition). Serum OPG levels were measured using a commercial ELISA kit. Plasma TRAP5b levels were measured by a fluorometric method using naphthol-ASBI-phosphate. Serum Ca concentrations were measured by a biochemical autoanalyzer. The data were analyzed in the mixed model for repeated measures.

**Results:** At 21 days prepartum, multiparous cows had a significantly higher serum OPG level than primiparous cows ( $7.5 \pm 0.4$  vs.  $5.0 \pm 0.6$  ng/mL;  $P < 0.01$ ). Immediately after calving, multiparous cows exhibited a significant decline in both serum OPG ( $3.9 \pm 0.3$  ng/mL;  $P < 0.01$ ) and Ca levels ( $2.1 \pm 0.08$  mmol/L;  $P < 0.01$ ). Primiparous cows did not exhibit these changes. The activity of TRAP5b peaked ( $P < 0.01$ ) at calving in both primiparous and multiparous cows ( $2.1 \pm 0.2$  and  $3.1 \pm 0.3$  U/L, respectively).

**Conclusions:** OPG inhibits osteoclastogenesis by acting as a decoy receptor for the receptor activator of NF $\kappa$ B ligand, known as RANKL. The circulating levels of TRAP5b are known to correlate well with the number of osteoclasts. In the present study, the peripartum fluctuations in circulating OPG and TRAP5b revealed the different bone resorption patterns between primiparous and multiparous cows. Multiparous cows seemed to preserve their bone mineral content during late pregnancy and the serum OPG levels at calving likely to be associated with Ca requirement. Therefore, we suggested circulating OPG as well as TRAP5b was an informative indicator of the osteoclastic bone resorption in dairy cattle. Further studies are needed to articulate the biological significance of circulating OPG in periparturient dairy cows with various health status.

IM-14

### Prognostic value of preoperative plasma L-Lactate concentrations in calves with acute abdominal emergencies

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**Objectives:** L-lactate is associated with disease severity and

mortality in critically ill humans and animals. The aim of the present study was to assess the prognostic accuracy of preoperative plasma L-lactate concentrations (cL-lactate) in a large study population of hospitalized calves suffering from acute abdominal emergencies.

**Materials and Methods:** The medical records of 594 calves up to an age of 6 months that were admitted to our hospital for reasons of an acute abdominal emergency were retrospectively analyzed. L-lactate was determined as part of a routinely performed clinical biochemistry panel on admission and before surgical intervention was carried out.

**Results:** Increased cL-lactate ( $>2.2$  mmol/L) were evident in 75% of calves. The highest median cL-Lactate (8.9 mmol/L) was observed in calves with a diagnosis of a torsio mesenterialis intestini. The overall survival rate until hospital discharge was 31%. Non-survivors had significantly ( $P < 0.001$ ) higher median cL-lactate (4.9 mmol/L) than survivors (3.1 mmol/L). Survival rates in the 9<sup>th</sup> (9.1-13.6 mmol/L) and 10<sup>th</sup> decile of cL-lactate ( $>13.6$  mmol/L) were 12% and 10%, respectively. A binary logistic regression analysis indicated, that the odds for non-survival increased by a factor of 1.14 (95% CI: 1.08-1.19) for every mmol/L increase of cL-lactate ( $P < 0.001$ ). Based on the Youden-Index, a receiver-operating characteristics (ROC) analysis identified a cutpoint for cL-lactate of 4.56 mmol/L, which had a sensitivity and specificity of 53.5% and 69.2%, respectively, for predicting non-survival in this study population. The area under the ROC curve for cL-lactate was 0.653 (95% CI: 0.607-0.699)

**Conclusions:** Increased cL-lactate are a prognostic factor in calves with acute abdominal emergencies. Although the survival rate of affected calves with markedly elevated cL-lactate is poor, the results of the ROC analysis indicate that it is not possible to reliably predict the outcome only based on a single measurement of cL-lactate.

IM-15

### Prognostic relevance of sequential plasma L-Lactate measurements in calves with acute abdominal emergencies

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**Objectives:** L-lactate is a well-established prognostic biomarker and has also attracted increasing attention in bovine medicine due to the availability and validation of cheap and portable L-lactate analyzers. In human and equine patients serial and postoperative measurements of plasma L-lactate concentration (cL-lactate) are known to provide more accurate prognostic information than single measurements before initiation of therapy. The aim of the present study was therefore to evaluate the prognostic value of sequential cL-lactate measurements in calves with acute abdominal emergencies.

**Materials and methods:** A prospective study was carried out involving 84 hospitalized calves up to an age of 7 months requiring surgical intervention for reasons of an acute abdominal



emergency such as gastrointestinal ileus or peritonitis. Values for cL-lactate were determined on admission and 6, 12, 24, 48, 72 hours post-surgery.

**Results:** The overall rate of non-survival until hospital discharge and after an additional 3-month period was 71% and 76%, respectively. At all sampling times during the first 48 hours after surgical intervention, non-survivors had significantly ( $P < 0.05$ ) higher cL-lactate and by tendency a lower percentage clearance of initial cL-lactate than survivors. A binary logistic regression analysis indicated, that the odds for non-survival during the 3-month observation period increased by a factor of 1.34 (95% CI: 1.09-1.65) for every mmol/L increase of cL-lactate on admission, but by a factor of 5.29 (95% CI: 1.69-16.56) and 5.92 (95% CI: 1.29-27.27) at 12h and 24h, respectively. The area under the receiver operating characteristics curve for cL-lactate at 12h was 0.91, and a cutpoint of  $>2.7$  mmol/L was identified which had a sensitivity and specificity for predicting non-survival of 68% and 100%, respectively.

**Conclusions:** A delayed normalization of cL-lactate in response to treatment is associated with an increased risk of mortality in calves with acute abdominal emergencies. Serial measurements of cL-lactate are therefore a clinically useful tool to identify patients with a high mortality risk at an early stage after surgical intervention was carried out.

was analyzed using a two way ANOVA based upon the Latin square design. The two independent variables were both the experimental infusions and time of solution infusion.

**Results:** Calves showed a significant alkalinising effect (increase pH) after infusion of sodium acetate, sodium bicarbonate and sodium benzoate at all sampled times (T1 to T5;  $P < 0.05$  for all comparisons). The alkalinising effect of sodium bicarbonate was significantly greater than sodium acetate ( $P < 0.05$ ). Calves showed a significant acidifying effect (decreases arterial plasma  $\text{HCO}_3^-$  and BE) after infusion of hydrogen acetate ( $P < 0.05$ ). Using the physicochemical approach it was determined that increased SID (increased), but not  $A_{\text{tot}}$  and USI resulted in a significant alkalinising effect after infusion of sodium acetate ( $P < 0.05$ ), sodium bicarbonate ( $P < 0.05$ ) at all times.

**Conclusions:** Sodium bicarbonate and sodium acetate alkalinising effect (increase blood pH) is subjected to different explanations. The traditional approach explains the alkalinizing effect of these solutions by providing bicarbonate or a bicarbonate precursor, while the physicochemical approach takes into account the increase in SID associated with an greater plasma sodium concentration relative to plasma chloride concentration.

IM-16

### Mechanistic effect of intravenous fluids solutions on blood pH of healthy calves.

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**Objectives:** To determine the mechanism underlying the changes in blood pH after infusion of different acidifying and alkalinizing electrolyte solutions to healthy calves using the traditional and physicochemical approach.

**Materials and Methods:** Prospective study. Using a Latin square design, xx (number of calves) calves were infused with five different solutions over a five-week period, each animal received one infusion per week. One litre of each solution was given intravenously at a constant rate of infusion over a one-hour period. Electrolyte solutions infused were: 1) sodium acetate; 2) hydrogen acetate (acetic acid); 3) sodium benzoate; 4) sodium bicarbonate. Arterial and venous blood samples were collected for acid-base analysis before treatment (T0) and then at 60 (T1), 90 (T2), 120 (T3), 180 (T4) and 300 (T5) minutes after treatment. Clinical data including heart rate, respiratory rate, temperature, central venous pressure and electrocardiogram were recorded at 10 minutes intervals. Clinicopathologic data of arterial  $\text{paCO}_2$  (mmHg), pH,  $\text{HCO}_3^-$  (mmol/L), BE (mmol/L) and venous plasma  $[\text{Na}^+]$  (mmol/L),  $[\text{K}^+]$  (mmol/L),  $[\text{Cl}^-]$  (mmol/L),  $[\text{L-lactate}]$  (mmol/L), and  $[\text{TP}]$  (g/L) were measured. Other calculated variables included strong ion difference (SID) =  $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{L-Lactate}^-)$ ; total plasma concentration of non-volatile weak acids  $[A_{\text{tot}}] = 0.343 \times \text{TP}(\text{g/L})$  and unmeasured strong ions  $[\text{USI}] = A_{\text{tot}} / (1 + 10^{(7.08 - \text{pH})}) - \text{SID} - \text{HCO}_3^-$ . The data



JB-01

## Evaluation of fecal pH for metabolic acidosis caused by diarrhea in Japanese Black Calves

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### Abstract

**Objective:** Diarrhea in calves causes dehydration symptoms and metabolic acidosis, which in the worst case can lead to death, so need appropriate evaluations of clinical conditions and treatments. In the present study, we examined that fecal pH can be used for evaluation of metabolic acidosis.

**Material and Methods:** Fecal samples were obtained 140 diarrhea from 89 calves and 18 normal from healthy calves. Collecting samples were used for measured pH using pH meter B712 (Twin pH; Horiba, Ltd.). Blood samples were obtained from all calves and measured venous blood pH, HCO<sub>3</sub><sup>-</sup>, BE<sub>ecf</sub> using i-STAT analyzer (Fuso Pharmaceutical Industry, Japan) immediately. Statistical analysis was performed using Pearson's correlation coefficient, Spearman rank correlation coefficient, ROC curve and odds ratio (EZR version 1.35).

**Results:** 1) The Pearson's correlation coefficient between clinical score and blood pH, HCO<sub>3</sub><sup>-</sup>, BE<sub>ecf</sub> were  $r = -0.474$ ,  $-0.413$ , and  $-0.432$ , respectively, and negative correlations were observed.

2) The Pearson's correlation coefficient between fecal pH and blood pH, HCO<sub>3</sub><sup>-</sup>, BE<sub>ecf</sub> were  $r = 0.682$ ,  $0.712$ , and  $-0.716$ , respectively, and positive correlations were observed.

3) We calculated the difference between fecal pH, blood pH, HCO<sub>3</sub><sup>-</sup> and BE<sub>ecf</sub> on the treatment day and the following day. Spearman rank correlation coefficients between fecal pH and blood pH, HCO<sub>3</sub><sup>-</sup> and BE<sub>ecf</sub> were  $r = 0.642$ ,  $0.707$ ,  $0.709$ , respectively, and positive correlations were observed.

4) From the ROC curve, the cutoff value of metabolic acidosis was calculated as fecal pH 5.75, clinical score 2. Sensitivity and specificity were fecal pH : 0.720, 0.938, clinical score : 0.633, 0.578, both criteria : 0.893, 0.709, respectively.

5) As a result of PCR, pathogens were detected in 33 specimens out of all 73 specimens. Infectious diarrhea tended to be more sensitive and specific than Noninfectious diarrhea.

**Conclusion:** This results suggest that the fecal pH strongly correlated with blood pH, HCO<sub>3</sub><sup>-</sup>, BE<sub>ecf</sub>, and could also be used to determine the effect of treatment for metabolic acidosis. Furthermore, it was found that metabolic acidosis can be detected with considerable accuracy by combination of fecal pH and clinical score. We hope that fecal pH contributes to accurate treatment for metabolic acidosis.

Nakatsu City Large Animal Center

**Background:** Cranial meningocele is a congenital defect that results from focal failure of neural tube closure during embryogenesis. It has been assumed to be influenced by hereditary, environmental factors, lack of folic in mother and so on. The accurate frequency of occurrence in Japan is unknown, but it may be rare.

**Case Presentation:** A one-day-old male Japanese Black calf weighing 20kg had a large swelling in the frontal region from birth, which was human baby's head size. By deep palpating, its cranial bone seemed to have a hole and a protrusion from a part of it. In ultrasonography, we could observe fluid and fibrin in the hernia sac. Aseptic puncture by a needle permitted drainage of about 1 liter of murky red-tinged fluid. The hernia sac had an abrasion, so it was likely to be already infectious. In addition, the calf showed some clinical symptoms including very severe neurological signs of astasia, opisthotonus, nystagmus and head rotation. Any findings supported extensive brain abnormalities or brain hypoplasia, so we carried out an appraisal killing under anesthesia. When we held an autopsy, we found the frontal bone had a hole which was a diameter of 5cm, and a bony protrusion was 3cm high. Sections of brain were unclear and the volume was very small. These matched with ante-mortem physical findings. Furthermore, both viral and parasitic tests relating to abnormal labor using whole blood of parent and child and spinal fluid of child were negative. We diagnosed this calf as meningocele with brain hypoplasia.

**Discussion:** A calf with severe neurological symptoms must be euthanized along the idea of animal welfare, but some reports show successful surgical repairs for relatively mild meningocele before unexpected infection. Proper evaluation of clinical signs and application of diagnostic imaging tools can reveal calves which have possibilities to survive. Due to very few information about meningocele on Japanese textbooks, they tend to judge that every case has a poor prognosis. We would like to spread to all veterinarians that mild cases are adaptable enough for surgical treatment.

JB-02

## A case of cranium bifidum with meningocele in Japanese Black calf

\*Eri Kaneda



NU-01

### Hind limb weakness associated with hypophosphataemia and inadequate dietary macro mineral content

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**Objectives:** To determine the aetiology of an unusual syndrome affecting a Holstein dairy herd in Australia and provide appropriate treatment.

**Materials and Methods:** The investigation was carried out at a 175 cow, year-round calving, Holstein dairy herd in the Illawarra region of Australia that experienced multiple cases of unilateral and bilateral mild flexion of the metatarsophalangeal joints over a two year period. The syndrome usually affected cows 80-120 days-in-milk with duration of clinical signs lasting several weeks, followed either by spontaneous recovery or progression to sternal recumbency. At the time of the investigation one cow was recumbent and 6 cows were ambulating with unilateral or bilateral metatarsophalangeal flexion. The herd grazed kikuyu in summer and autumn and rye grass in winter and spring. Daily concentrates fed consisted of 5kg wheat, 4kg dried distillers grain, 114g acidbuf, 11g salt (NaCl), 11g limestone, 2.8g magnesium oxide, 23g vegetable oil and 30g of a micro mineral supplement. Average milk production was 22L/cow/day.

A blood sample was collected from the jugular vein of an untreated down cow, 120 days-in-milk, with a three week history of bilateral flexion of the metatarsophalangeal joints prior to recumbency and a 36 hour history of recumbency. Whole blood was submitted to the University of Sydney laboratory for full blood count and serum biochemical analysis.

The lactating cow ration was analysed using CPM-Dairy version 3.0.8 (University of Pennsylvania, 2006). Two models were developed. Model 1 was based on the average cow producing 22L of milk, eating 9.7kgDM of kikuyu pasture and a total dry matter intake (DMI) of 18.3kg. Model 2 was based on a high-producing, peak lactation cow producing 40L of milk, eating 14.5kg kikuyu pasture and a total DMI of 22.7kg. Both models were used to assess the macro mineral content of the ration relative to National Research Council (NRC) requirements.

Model 2 was used to determine the changes required to correct macro mineral deficits. The ration was modified by increasing salt (NaCl) from 11g/cow/day to 100g/cow/day, increasing limestone from 11g/cow/day to 70g/cow/day and adding dimagnesium phosphate ( $MgHPO_4 \cdot 3H_2O$ ) at a rate of 60g/cow/day. Following ration changes the herd was monitored by daily observation for 8 months for signs of metatarsophalangeal joint flexion or recumbency.

**Results:** Full blood count revealed a neutrophilia ( $7.52 \times 10^9$  cells/L) and lymphocytopenia ( $1.1 \times 10^9$  cells/L) consistent with a stress leukogram. Biochemical analysis revealed a moderate hypophosphataemia (1.06 mmol/L) and moderate elevation in AST (1004 U/L). Low creatinine (53 $\mu$ mol/L) and low urea (4.5 mmol/L) were consistent with declining body mass and reduced protein intake while recumbent, and hyperproteinaemia (serum protein 88g/L) was consistent with dehydration.

Model 1 revealed that dietary sodium met only 60% of NRC requirements, whereas calcium, phosphorus, magnesium, potas-

sium and chloride met 131%, 128%, 149%, 193% and 184% of requirements respectively for cows producing 22L/day of milk.

Model 2 estimated that dietary calcium, phosphorus and sodium met only 95%, 97% and 50% of NRC requirements, whereas magnesium, potassium and chloride met 114%, 203% and 163% of requirements respectively for cows producing 40L/day of milk. When Model 2 was modified to represent a cow producing 45L/day of milk, dietary calcium met only 88% and dietary phosphorus met only 91% of requirements.

Increasing supplemented salt, limestone and dimagnesium phosphate to 100g, 70g and 60g respectively increased calcium, phosphorus and sodium to 123%, 112% and 114% of requirements respectively for cows producing 40L/day of milk.

All 6 ambulating cows that had mild metatarsophalangeal joint flexion showed resolution of clinical signs within 4 days of the ration change. No further cows were identified with clinical signs consistent with this syndrome in the 8 months following the ration change.

**Conclusion:** This syndrome appears to be associated with hypophosphataemia and dietary macro mineral deficits in high-producing cows, mostly 80-120 days in milk. Correction of dietary calcium, phosphorus and sodium to levels above NRC requirements resulted in resolution of clinical signs and prevention of new cases.

NU-02

### Phosphorus balance in muscle tissue and muscle function in dairy cows undergoing dietary phosphorus deprivation during the transition period.

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Phosphorus (P) deficiency is believed to be associated with periparturient recumbency in dairy cows. The objective of this study was to determine effects of dietary P deprivation on muscle P homeostasis and muscle function in transition dairy cows.

Thirty-six dairy cows in late gestation were randomly assigned to either dietary P deprivation from 4 weeks before to 4 weeks after parturition or to a control treatment with adequate dietary P supply. Blood and muscle biopsies were obtained and electromyographies were conducted on biceps femoris and intercostal muscles in regular intervals. Muscle tissue was analyzed for the total P, ATP, ADP and creatine phosphate content.

P deprivation resulted in pronounced hypophosphatemia without causing any signs of clinically apparent muscle weakness or recumbency. Content of different P-containing compounds in muscle tissue showed minor changes over time, a treatment effect however could not be identified for any of biochemical parameters studied in muscle tissue. Electromyographies of bi-



ceps-femoris muscles did not reveal significant treatment effects. Subtle treatment effects suggestive of mild neuromyopathy were however observed in intercostal muscles. Pathologic spontaneous activity of intercostal muscles also became more frequent over time in P deprived animals.

These results suggest that prolonged and pronounced dietary P deprivation in transition cows results in marked hypophosphatemia but has only minor effects on the intracellular P balance of muscle tissue. While clinical signs of muscle weakness could not be induced, subclinical disturbances of muscle tissue function particularly of the intercostal muscles are likely to be attributable to dietary P deprivation.

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NU-03

### Effect of dietary phosphorus deprivation ante partum on the calcium homeostasis of periparturient dairy cows

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Hypocalcemia is an important metabolic disorder in periparturient dairy cows. The objective of the present study was to determine the effect of feeding phosphorus (P) deficient diets ante partum (a.p.) on plasma calcium (Ca) homeostasis in dairy cows around parturition.

Eighteen multiparous dairy cows were fed either a P-deficient ration or a control ration with adequate P content during the last four weeks of gestation until day ten post partum. Blood was obtained before initiation of P-deprivation and -10, -2, 0, +2 and +10 days relative to parturition. Plasma concentrations of Pi [Pi], Ca [Ca], the bone resorption marker CrossLaps [CTX] and parathyroid hormone [PTH] were determined. Cows were also monitored for signs of clinical hypocalcemia around parturition.

Clinical hypocalcemia occurred in 3/9 control and 0/9 P-deprived cows. [Ca], [CTX] and [PTH] did not differ between groups until parturition. P-deprived cows had significantly higher [Ca] than control cows on days 0 (2.46±0.11 vs. 2.27±0.41 mmol/L) and +2 (2.61±0.13 vs. 2.35±0.25 mmol/L). At parturition [PTH] was significantly increased in control cows compared to P-depleted cows. [CTX] was significantly higher in P-deprived than in control cows on day +2.

Feeding a P-deficient diet for four weeks a.p. positively affected [Ca] of periparturient dairy cows. Bone resorption in periparturient P-deprived cows seemed to occur with only marginal increases of [PTH] suggesting either greatly increased sensitivity of bone to PTH or bone mobilization independent of PTH. Future studies must explore potentially negative effects of P-deprivation a.p. on health and metabolism of the fresh cow.

NU-04

### Intravenous calcium supplementation at calving induces fluctuations in circulating calcium and hypocalcemia when compared to voluntary oral calcium supplementation.

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**Objectives:** The discontinuity in serum calcium (Ca) clearance at calving and the delay in adaptation of absorption lead to hypocalcaemia risk in dairy cows. To assess the efficacy of prophylactic treatments to mitigate hypocalcemia, serum total Ca (tCa) and ionized Ca (iCa) were monitored in 24 multiparous Holstein cows after parturition.

**Materials and methods:** Cows were blocked by calving sequence and by calcaemic status at calving in two categories: normocalcaemic (iCa > 1.10 mmol/L; n=8) or hypocalcaemic (iCa < 1.10 mmol/L; n=16). Cows in each block were randomly assigned to 2 treatments: Ca-Oral (n=12) or Ca-IV (n=12). Ca-Oral was given a 20 L commercial Ca suspension (total of 47.7 g Ca), that consisted of Ca carbonate, Ca formate, Ca propionate, other minerals and vitamins, and was voluntarily consumed (Farm-O-San Reviva<sup>®</sup>, Trouw Nutrition, Amersfoort, the Netherlands). Ca-IV received a 450 mL intravenous Ca solution (total of 12.5 g Ca); that contained 298.4 mg/mL of Ca gluconate, 33.3 mg/mL of magnesium chloride and 82 mg/mL of boric acid (AmosCAL<sup>®</sup>, Kommer-Biopharm B.V, Heiloo, the Netherlands). Pre-calving diets were formulated with a positive DCAD (dietary cation-anion difference) of 182 meq/kg DM and contained 4.1 g Ca/kg dry matter (DM). Treatments were administered once immediately after parturition. Blood samples for Ca analyses were collected at 0 h (before treatment administration), 1, 3, 10 and 18 h relative to treatment, and at 0700 and 1900 for the next 2 consecutive days to represent the 24, 36, 48 and 60 h sampling time points. Data were standardized by T0 (0 h relative to treatment administration) for evaluation of treatment effects.

**Results:** Incidence of calving-related disorders during the first 30 days of lactation did not differ between treatment groups ( $P > 0.20$ ). In Ca-IV cows, both iCa and tCa concentrations peaked at 1 h (1.52 mmol/L for iCa and 2.88 mmol/L for tCa) and declined to a nadir at 24 h post treatment administration (0.94 mmol/L for iCa and 1.74 mmol/L for tCa). While iCa and tCa were higher at 1 and 3 h in Ca-IV cows ( $P < 0.0001$ ), concentration of iCa was greater for Ca-Oral cows at 24, 36 and 48 h ( $P < 0.05$ ) and for tCa at 36 h ( $P < 0.05$ ).

**Conclusions:** Our data indicate that intravenous Ca supplementation immediately induced a state of hypercalcaemia followed by hypocalcaemia 24 h later, possibly by downregulation of Ca metabolism. In contrast, oral Ca supplementation did not present a marked Ca signal immediately after the intervention and was better able to sustain calcaemia. This suggests that oral administrations, as demonstrated in this study, represent a better standard Ca prophylaxis protocol compared to intravenous Ca administration.



NU-05

### Usefulness of supplementation of selenium and Vitamin E during transition period in dairy cows

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**Objectives:** The transition period between 3 weeks prepartum to 3 weeks postpartum is the most sensitive period during the reproductive cycle of dairy cows. The most commonly noted perinatal disorders in dairy cows include clinical mastitis, hoof infections, placenta retention, recumbency or clinical and sub-clinical ketosis.

The aim of the study was to evaluate the influence of vitamin E and selenium supplementation on selected blood biochemical parameters in dairy cows during the transition period.

**Material and methods:** The study was conducted on 20 Holstein Friesian cows divided into two groups, Group I (experimental, 10 cows) was intramuscularly administered a Vitamin E and selenium supplement (30ml) (tocopherol acetate 50mg, sodium selenite 0.5mg, solvent 1ml) 5 days prepartum. Group II was the control with no supplementation. The BCS of all cows was determined at level 4 five days prepartum. Blood samples were collected from all cows on sampling dates (5 days prepartum, on the day of parturition, and 5, 10 and 15 days postpartum). Serum total protein, glucose, cholesterol, triglyceride, NEFA (non-esterified fatty acid), BHB (betahydroxybutyrate), Vitamin E and selenium levels were determined in the collected samples. AST (aspartate aminotransferase), GGTP (gamma-glutamyl transpeptidase) and GSH Px (glutathione peroxidase) activity were measured as well. The results were analyzed statistically in onefactor orthogonal design. The significance of differences between mean values in groups and examinations were verified by the Students t test.

**Results:** Total protein concentration in serum were similar in experimental and control group animals. The lowest glucose levels were reported 10 days postpartum (3.06 mmol/l in experimental group, 3.09 mmol/l in control). No significant differences were observed between sampling dates or animal groups. Cholesterol concentration peaked (no significantly) on the last sampling date in both experimental (4.83 mmol/l) and control group (4.51 mmol/l) animals. Triglyceride levels were similar in both groups.

NEFA concentrations in both groups increased on successive sampling dates, but remained within the reference range. BHB concentrations were a little bit higher in control group in all sampling dates. Selenium and Vitamin E concentrations were significantly higher in experimental group animals. The highest level of selenium was observed in the experimental group in the last sampling (91 micrograms/l) The level of GSH Px activity was observed in the same group in the end of the experiment (77.28 U/gHb). AST and GGTP activities were similar in all groups during entire experiment.

**Conclusions:** Selenium and Vitamin E supplements had no significant effect on biochemical parameters. A single dose of selenium and Vitamin E administered 5 days prepartum fulfilled the demand for selenium in high yielding cows during transition period and may have positive impact on the health of the cows

due to the reduction of the risk of oxidative stress and its associated consequences.

NU-06

### Effects of anti-lipopolysaccharide antibody administration on ruminal fermentation and hepatic transcriptomic adaptation during subacute ruminal acidosis in Holstein bull cattle

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**Objectives:** Subacute ruminal acidosis (SARA) is responsible for lipopolysaccharide (LPS) release in the rumen, which triggers systemic or hepatic inflammatory responses. Until recently, various types of probiotics or yeast have been used to improve rumen fermentation and prevent SARA incidence in cattle. However, there have been no studies on anti-LPS antibody, despite its potential to control rumen-produced LPS. Therefore, we investigated the effects of anti-LPS antibody administration on ruminal fermentation and LPS activity, as well as hepatic transcriptomic adaptation under SARA challenge.

**Materials and methods:** Four fistulated Holstein bull cattle (164 ± 14 kg; 5–6 months of age) were used in a replicated 3x3 Latin square design. The cattle were treated with 0 (control group), 2, or 4 g immunoglobulin yolk containing anti-LPS antibody (EW Nutrition Japan., Gifu, Japan) per head (administration groups) once daily for 14 consecutive days (days 1–14). Cattle were fed a high-forage diet during the first 11 days (days 1–11; pre-challenge), a high-grain diet for 2 days (days 12 and 13; SARA-challenge), and then a high-forage diet for 1 day (day 14; post-challenge). Ruminal pH was measured continuously every 10 min. Rumen fluid samples were collected at 08:00 and 14:00 on days 11, 12, and 13, and at 08:00 on day 14 to analyze total volatile fatty acid (VFA), VFA components, NH<sub>3</sub>-N and lactic acid concentrations, and LPS activity. Liver tissue was biopsied at 08:00 on day 14 in the control and 4 g administration groups. Biopsy was performed in the 10<sup>th</sup> to 12<sup>th</sup> intercostal space using an 18-gauge automated biopsy gun under ultrasound guidance. Total RNA was extracted from the liver tissue, and mRNA expression profiles were examined by one-color microarray analysis. Differentially expressed genes were analyzed using ingenuity pathway analysis (IPA).

**Results:** The 1 h mean ruminal pH during the SARA- and post-challenge periods was higher in the 2 g and 4 g administration groups, and the ruminal LPS activity during the post-challenge period was significantly lower in the same groups compared to the control group. The concentrations of acetic and lactic acid, and the ratio of ruminal acetic acid to propionic acid, were significantly lower in the administration groups. The concentration of propionic acid was significantly higher in the same groups compared to the control group. *In silico* analysis revealed that the most significant canonical pathway involved xenobiotic metabolism signaling, and the highest-scored network involved factors involved in immune cell trafficking, which indicated the inhibition of immune modulatory



genes.

**Conclusions:** During the post-challenge period, the 2 g and 4 g anti-LPS antibody administration groups showed significantly lower ruminal LPS activity. This suggests that the antibody bound to endogenous LPS in the rumen, suppressing its activity. This treatment may also help to suppress the hepatic inflammatory responses caused by translocated ruminal LPS.

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NU-07

### The Possible Role of High Oxalate pasture in producing Subclinical Hypocalcaemia in a beef breeding herd in Central Queensland

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**Objective:** A case of widespread clinical ketosis in a beef breeding herd is reviewed with the investigation of the role of high oxalate containing pasture species in producing subclinical hypocalcaemia in beef breeders under certain grazing conditions. The effect of subclinical hypocalcaemia/ketosis under similar conditions on calf wastage and conception rates is also investigated.

**Methods:** Biochemistry records pertinent to the case were re-examined. Current literature and conference proceedings were reviewed with reference to the effects of subclinical hypocalcaemia in cattle and to oxalate toxicity in cattle from plant ingestion. Anecdotal evidence was collected from veterinarians and beef breeders.

**Results:** The laboratory results showed a blood calcium level of 1.7mmol/litre indicating a marked hypocalcaemia as well as ketosis. A wilted lush growth of Buffel Grass (*Cenchrus ciliaris*) containing high levels of oxalic acid is strongly suspected of producing the hypocalcaemia. Literature shows that Buffel Grass can contain up to 6% by dry matter weight of oxalates under certain conditions reducing the amount of available calcium to the grazing animal by up to 20%. Evidence from veterinarians and breeders showed an increase in cases of lengthened parturition time when breeders were grazed on lush Buffel Grass. Reduced conception rate is also indicated on one property.

**Conclusion:** The grazing of monoculture pastures containing high oxalate levels under particular conditions can put beef cattle at significant risk of subclinical hypocalcaemia. The effects included lengthened parturition time and clinical and subclinical ketosis impacting on general herd health and conception rates.

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NU-08

### Oral supplementation with organo-modified clinoptilolite influence the relative content of serum $\gamma$ globulin fractions without influencing metabolic profile parameters of primiparous dairy cow during periparturition period

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**Objectives:** Our previous results showed positive effect of oral supplementation with organo-modified clinoptilolite on the colostrum quality in primiparous dairy cows [1]. We have further investigated possible effects of oral supplementation with organo-modified clinoptilolite in primiparous dairy cows during periparturition period on the blood serum proteins homeostasis, energy status, lipid and mineral metabolism.

**Methods:** Total number of 36 primiparous Holstein dairy cows, were divided in two groups: 1) 20 cows were daily orally supplemented with 150g of organo-modified clinoptilolite (Minazel plus<sup>®</sup>, Patent Co, Serbia) during the late pregnancy (21 days before until 2 days after parturition), and 2) control group of 16 unsupplemented dairy cows. All experimental animals were clinically healthy during the study. Blood serum samples were obtained five times during the study: at 21 days and 5 days before parturition (Day -21 and -5), and at Day 1, 2, and 7, after parturition (Day +1, +2 and +7). Blood serum protein, albumin, urea, glucose, beta-hydroxybutyrate, total cholesterol, triglyceride, total calcium, phosphorus, and magnesium, concentrations were determined using standard methods on automated spectrophotometric chemistry analyser (Biosystem A15, Barcelona, Spain). Serum globulin fractions were analysed by agarose gel electrophoresis method. The relative content of protein fractions ( $\alpha$ ,  $\beta$  and  $\gamma$  globulins) was quantified by densitometry analysis and their absolute concentrations were determined based on the total serum protein concentration.

**Results:** Oral supplementation with the organo-modified clinoptilolite had no effect on total serum protein, albumin, urea, glucose, beta-hydroxybutyrate, total cholesterol, triglyceride, total calcium, phosphorus, and magnesium concentrations, which were within the reference range in both experimental groups. Oral supplementation also did not influence absolute concentration of  $\alpha$ ,  $\beta$  and  $\gamma$  globulins. In the agarose gel electrophoresis two protein fractions of  $\gamma$  globulins were present: slow or cationic  $\gamma$  globulin (predominantly contains IgG<sub>2</sub>; [2]) and fast, anionic  $\gamma$  globulins (predominantly contains IgG<sub>1</sub>; [2]). In both experimental groups on Day -21 relative content of  $\gamma$  globulin fractions was similar (58 $\pm$ 5:54 $\pm$ 6%,  $p>0.05$ , slow  $\gamma$  globulin fraction; 42 $\pm$ 5:46 $\pm$ 6%,  $p>0.05$ , fast  $\gamma$  globulin fraction, treated vs. control). From Day -5 to +7 there was an increase in the concentration of slow  $\gamma$  globulin and decrease in the concentration of fast  $\gamma$  globulin in the both groups. However, oral supplementation with the organo-modified clinoptilolite resulted in the significant changes of  $\gamma$  globulin fractions relative content at Day -5 and +1, compared the control group. At the Day -5 there was a slightly but significant decrease in slow  $\gamma$  globulin fraction relative content (65 $\pm$ 3:70 $\pm$ 5%,  $p<0.01$ , treated vs. control) and an increase of fast  $\gamma$  globulin fraction relative content (35 $\pm$ 3:32 $\pm$ 5%,  $p<0.01$ , treated vs. control) in the supplemented group. The opposite effect was observed at Day +1, with significantly increased slow  $\gamma$  globulins fraction relative content (65 $\pm$ 3:61 $\pm$ 6%,  $p<0.05$ , treated vs. control) and decreased fast  $\gamma$  globulin fraction relative content (35 $\pm$ 3:39 $\pm$ 6%,  $p<0.05$ , treated vs. control) in the supplemented group compared to the control



group of animals.

**Conclusion:** The oral supplementation with organo-modified clinoptilolite did not have any apparent adverse effects on the protein metabolism, energy status, lipid and mineral metabolism in primiparous dairy cows during periparturient period. However, our results indicate that oral supplementation with organo-modified clinoptilolite could have beneficial effect on the relative content of fast  $\gamma$  globulin fractions (mostly IgG<sub>1</sub>), with increased relative content on Day -5 and decreased relative content on Day +1, reflecting their increased synthesis and transport to the colostrum.

**Reference:** 1. Fratrić N, Stojić M, Ilić V, et al. Proceeding of the World Buiatrics Congress 2016, p. 664, Dublin, Ireland

2. Kickofen B, Hammer DK, Scheel D. Hoppe-seyler's Z Physiol Chem. 1968, 349-351.

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NU-09

### Effects of pH and the plasma concentration of chloride and L-lactate on the ionized calcium concentration in plasma from healthy and critically ill calves

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**Objectives:** The ionized calcium concentration ( $cCa^{2+}$ ) is the biologically active form of calcium and the plasma  $cCa^{2+}$  is the preferred method for evaluating calcium status in animals. A pH-corrective equation has been developed for human plasma, whereby measured  $cCa^{2+}$  ( $cCa^{2+}_m$ ) at measured pH ( $pH_m$ ) is corrected to  $pH=7.40$  ( $cCa^{2+}_{7.40}$ ), such that:  $\log_{10} cCa^{2+}_{7.40} = \log_{10} cCa^{2+}_m - 0.23 \times (pH_m - 7.40)$ . The validity of this corrective equation for bovine plasma is unknown. It is well known the high chloride (acidogenic) rations mitigate the incidence of periparturient hypocalcemia in dairy cows; however, the underlying mechanisms have not been fully elucidated. We hypothesize that the pH-corrective equation for bovine plasma differs from that of human plasma, and that  $cCa^{2+}$  is also dependent on the plasma chloride ( $cCl^-$ ), L-lactate ( $cLactate$ ), and albumin ( $cAlbumin$ ) concentrations.

**Materials and methods:** The *in vitro* plasma  $\log(cCa^{2+})$ -pH relationship was determined by  $CO_2$  tonometry of 465 plasma samples from 10 healthy calves. Plasma  $cCl^-$  was altered by equivolume dilution of plasma with electrolyte solutions of varying  $cCl^-$ . The *in vivo* plasma  $\log(cCa^{2+})$ -pH relationship was investigated using clinicopathological findings extracted from the medical records of 689 critically ill calves with neonatal diarrhea.

**Results:** The pH-corrective equation for bovine plasma was similar to that for human plasma when plasma  $cAlbumin$  and  $cCl^-$  were within the reference range. Plasma  $cCa^{2+}$  in critically ill calves was associated with blood pH, total calcium concentration,  $cCl^-$ , and  $cLactate$ . Interestingly, the plasma  $cCa^{2+}$  in-

creased by 0.0032 (*in vitro*) and 0.0023 (*in vivo*) mmol/L for every 1 mmol/L increase in  $cCl^-$ .

**Conclusions:** Our finding that plasma  $cCl^-$  influences  $cCa^{2+}$  provides an additional and novel mechanism by which ingestion of high chloride (acidogenic) rations mitigates the incidence of periparturient hypocalcemia in cattle and reemphasizes the importance of strong electrolytes in acid-base disturbances.

NU-10

### Effect of soluble carbohydrate diets on reticuloruminal pH and motility, and on hematological and biochemical health indicators in cattle

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**Objectives:** Reticuloruminal health has been monitored using many parameters including production performance, hematology and blood biochemistry, ruminal pH and microflora, fecal consistency and composition. Continuously monitored reticuloruminal pH enables the early detection of ruminal acidosis, but pH monitoring boluses are expensive and have a maximal life span of less than 200 days due to electrode-drift. We hypothesized that a) motion-sensing boluses in the reticulorumen would provide an effective indicator of reticuloruminal health, and b) that sugar- and starch-based challenges would have differential effects. Our objectives were to use reticuloruminal pH boluses in parallel with prototype motion-sensitive boluses and relate data from both to health parameters, hematological and biochemical changes in cows challenged with a high starch (HSt) or a high sugar (HSu) diet compared with a control diet (CO).

**Materials and methods:** 6 adult, non-lactating, rumen-fistulated Jersey cows were fitted with a smaXtec rumen bolus (Moletech, Devon, UK) and a prototype motion-sensitive bolus in a 3 X 3 Latin square design for a total duration of 9 wk. During the first 2 wk of each 3-wk period, cows were fed a maintenance diet of 500 g rolled barley and 32 kg grass silage (as fed). In wk-3 of each 3 wk period, the animals were fed one of 3 diets: maintenance diet (CO); a starch based diet (HSt) of 6.32 kg of barley and 17 kg of silage (as fed); a sugar-based diet (HSu) of 3.77 kg of barley, 3.33 kg of cane molasses, 20 g of urea and 17 kg of silage (as fed). Animals were monitored daily using 4 health parameters: diarrhea, inappetence, depression and ruminal tympany, each scored from 0 (not present) to 2 (severe). Blood samples were taken before feeding on the first (T1 - before the delivery of the challenge diet), the second (T2) and the last day (T7) of each challenge week for a total number of 9 sampling times. Hematology and biochemistry included total protein, albumin, globulin, liver enzymes, glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), packed cell volume, red blood cells, neutrophils, monocytes and lymphocytes. The data were analyzed using R (V3.0.2 R Development Core Team 2010). Differences between T2 and T1 and





between T7 and T2 were calculated for each of the biochemical and hematological variables. The effects of treatment and time-point were determined by Kruskal-Wallis rank sum test for univariate relationships and then by generalized linear model (GLM) including the effects of treatment, cow ID, and time. Models with two or three terms (treatment always forced in the model) were compared using ANOVA and the best model with the lowest Akaike information criterion (AIC) was selected.

**Results:** HSt and HSu both significantly reduced the hourly median pH, hourly minimum pH and increased standard deviation (all  $p < 0.0001$ ). Neither HSt nor HSu had a significant direct effect on the median value of the inter-contraction period ( $p > 0.1$ ). HSt significantly increased the amplitude of reticuloruminal contractions ( $p = 0.0018$ ). The HSu diet was more likely to not be completely eaten than the HSt diet ( $p = 0.001$ ). HSu and HSt diets were more likely to cause diarrhea than the maintenance diet ( $p = 0.0003$ ). Kruskal test showed a significant reduction ( $p = 0.0035$ ) in the lymphocyte count in HSt cows between T1 and T2, and a tendency for BHB ( $p = 0.06$ ) to decrease on the HSu diet between T1 and T2, while NEFA tended to decrease over the same time in animals on the HSt diet ( $p = 0.08$ ). GLM showed a significant increase in neutrophils at T2 in HSt cows ( $p = 0.02$ ), while monocytes showed a significant reduction on T7 independent of the diet ( $p = 0.02$ ).

**Conclusions:** The high starch and high sugar diets both induced ruminal pH changes consistent with soluble carbohydrate-induced acidosis. Measurements of reticuloruminal motility require improvement to be as useful as pH measurement. The increased occurrence of diarrhea in animals on HSt and HSu diets, the reduction in lymphocytes and increased neutrophils on the HSt diet, are consistent with a rapid inflammatory effect of highly soluble carbohydrate, but suggest that barley starch-based diets might have more pronounced inflammatory effects than the sugar-based diets.

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NU-11

### Effect of bypass methionine hydroxy analogue supplementation in commercial concentrate diet on milk productive performance and post partal health of dairy cow

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**Objectives:** The objective of this study was to determine the effect of bypass methionine hydroxy analogue (HBMi) supplementation in a commercial concentrate diet on milk production, their compositions and general health during post-parturition period in dairy cattle.

**Materials and methods:** Thirty-five crossbred Holstein-Friesian cows in small holder dairy farms were separated into two groups (Control;  $n = 15$  and HBMi;  $n = 20$ ). Both groups were fed by the same basal diet from 2<sup>nd</sup> weeks pre-parturition until 6<sup>th</sup> weeks post-parturition within the same farm. The basal roughage was a corn husk from agricultural by-product. HBMi (isopropyl ester of 2-hydroxy-4-methylthio-butanoic acid) was on-top fed at 20 grams/cow/day over a commercial concentrate diet in morning meal. Milk yield and milk sample were collected

every week. Blood sample, body weight and body condition score (BCS) were collected at -2, 1, 3 and 5 weeks post-parturition. Data were analyzed by using the MIXED model and repeated measurement analysis packages in R studio program.

**Results:** No significant difference ( $P > 0.05$ ) was observed on milk production and their compositions, serum metabolic profiles, body weight and BCS. Whereas, milk protein yield tends to increased ( $P = 0.06$ ) in HBMi when compared with control group. Moreover, HBMi have no negative effects on serum metabolic profiles and were kept in a normal range.

**Conclusions:** HBMi may be use as an alternative way to improve economic impaction by enhancing milk protein yield in small holder dairy farms under feeding with low quality roughage in field trail.

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NU-12

### Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows

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**Objective:** The objective of this study was to assess the agreement between the concentration of beta-hydroxybutyrate (BHB) in blood and milk of cows and to determine the cut-off value of BHB in milk serum for identifying subclinical ketosis.

**Materials and Methods:** Ninety-four samples of blood and milk of Holstein cows in the first third of lactation were analysed. Blood was taken from the tail vein (*vena caudalis mediana*), and milk samples were collected at the same time. The blood serum was separated by centrifugation at room temperature (1,800 g, 15 min). The milk samples were centrifuged at 4500 rotations for 15 minutes to get skimmed milk. Skimmed milk samples were centrifuged in Eppendorf tubes for 30 minutes on 13000 rotations to get a milk serum. BHB concentrations were measured in blood serum and milk serum using biochemical analyser RX Daytona (RANDOX Laboratories Ltd., UK) and Rayto 1904cv (Rayto Electronics Inc. Shenzhen, China). A kinetic enzyme test with BHB dehydrogenase (reagent RANBUT, Art RB 1007) and software setting of manufacturer RANDOX was used. Measurements in the milk serum were carried out in parallel and as a result the mean value of the both measurements was used.

Statistic analysis was performed with SPSS (ver. 22) software package (IBM Analytics, USA) (Arbuckle 2013). Descriptive statistic for BHB in milk and blood was calculated as frequency distribution with parameters of central tendency and variation. Correlation between BHB in blood and milk was determined by Pearson correlation coefficient and linear regression. A difference between correlation coefficient in group of cows with blood BHB  $< 2.0$  mmol/l and  $> 2.0$  mmol/l was determined. Receiver operating characteristic (ROC) curve was used to illustrate performance of milk BHB for diagnostics of subclinical ketosis (analysis area under curve-AUC of ROC curve, sensitivity and specificity for optimal cut-off value of BHB in milk). For



ROC analysis cows were divided in accordance with BHB concentration in blood, to positive and negative to subclinical ketosis based on the cut-off at  $>1.00$  mmol/l. The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

**Results:** The concentration of the BHB in milk was approximately ten times lower than in the blood. The average concentration of BHB in the blood serum samples was  $1.14 \pm 0.68$  mmol/l (median: 0.90 mmol/l) while in the milk it was at  $0.116 \pm 0.086$  mmol/l (median: 0.089 mmol/l).

Comparing the concentration of BHB in blood and milk, a statistically significant positive correlation ( $r = 0.705$ ,  $p < 0.001$ ) was found. Regression analysis showed that increase of blood BHB concentration for 1 mmol/l means increase of milk BHB concentration for 0.11 mmol/l. Much stronger correlation between the value of BHB of blood and milk was found when the value of BHB in blood was below 2.0 mmol/l ( $r = 0.658$ ,  $p < 0.001$ ) in comparison to samples with BHB levels above 2.0 mmol/l ( $r = -0.292$ ,  $p = 0.206$ ). For this reason, there is a much greater dispersion of results with increasing values of BHB. Statistically significant cut-off value of BHB in milk for subclinical ketosis was determined. The results of ROC analysis showed the best sensitivity and specificity at the border line value 0.080-0.100 mmol/l. Concentration of BHB in milk at  $>0.080$  mmol/l ( $AUC = 0.91 \pm 0.03$ ,  $p < 0.001$ ) was significant indicator for subclinical ketosis in dairy cows with 94% sensitivity and 74% specificity. At the cut-off value of 0.100 mmol/l, the sensitivity was 86%, and specificity 82%.

**Conclusions:** Statistically significant correlation between the concentration of BHB in blood and milk serum of cows was confirmed. The best sensitivity and specificity for measurements of BHB in milk were observed at the cut-off value of 0.080 mmol/l. This cut-off is suitable for practical use because it allows detection of the most cows with subclinical ketosis. BHB in milk serum is a good indicator of subclinical ketosis in dairy cows.

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NU-13

### An Innovative Management Approach to Ketosis Treatment in Dairy Cattle

#### Reducing energy demand instead of increasing energy intake to more effectively treat ketosis in lactating dairy cows

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Subclinical ketosis affects approximately 40% of dairy cows in North America. Current recommended treatments for ketosis focus on providing cows with more energy. However, the most effective treatments, such as oral propylene glycol, only resolve ketosis approximately 50% of the time. The objective of this research was to evaluate the effect of decreasing milking frequency from two milkings/day, to one milking /day for two weeks; in conjunction with a standard treatment of five days of oral propylene glycol in cows diagnosed with ketosis. Ketosis

was defined as a blood beta-hydroxybutyrate concentration of greater than or equal to 1.2mmol/L. From November 2016 to September 2017, 104 ketotic cows from the University of Guelph Livestock Research Innovation Centre were studied. 55 cows were randomly allocated to the once/day milking group, and 49 were enrolled in the twice/day milking group. All cows inhabited the same pen during their time on trial, and all cows were milked in a DeLaval VMS robot equipped with Herd Navigator. Blood, milk, and urine samples were collected in a 21-day period to analyse ketones over time in the cows. Disease occurrence was recorded up to 60 DIM and reproductive performance, examining days to first breeding, and pregnancy on the first breeding were explored. CanWest DHI herd recording data was collected on a weekly basis, for a period of 15 weeks, examining milk production, milk component data, and SCC. Results indicate that both heifers and cows milked once/day for a two-week period are significantly more likely to recover from ketosis than heifers and cows milked twice/day during treatment ( Fisher's exact test;  $P < 0.005$ ).

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NU-14

### The Difference Analysis of Rectum Microbiome Community in Chinese Holstein cows with or without Ketosis

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The objective of this study was to evaluate changes in microbiome in the rectum content of dairy cattle with and without ketosis. We first measured plasma  $\beta$ -hydroxybutyric acid (BHBA) level in 350 postparturient dairy cows in a farm near Nanning China and identified 22 cows as having ketosis with BHBA  $>1.2$  mmol/L (KET group). We then selected 22 cows with BHBA  $<0.60$  mmol/L from the same herd as pair controls based on calving dates and parity (CON group). Rectum fecal samples were collected by rectal examination and were analyze for microbiome profile using high-throughput DNA sequencing at the 16S hyper-variable V4 region. Total genome DNA was extracted from each fecal sample and 16S V4 regions were amplified used specific primer with barcodes. The average effective sequences of per sample was 84983 (range: 64090 – 94470). PCoA test showed distinctive microbiome clusters between KET and CON cows. Beta diversity analysis was then used to evaluate differences in species complexity. At the Phylum level, the percentage of *Euryarchaeota* was significantly lower ( $p < 0.05$ ) in the KET group than in the CON group. At the Genus level, the percentages of *Ruminococcaceae-UGG-014*, *Methanobrevibacter*, *Erysipelotrichaceae-UGG-009*, and *Atopobium* were all significantly lower ( $p < 0.05$ ), while the percentage of *Lachnospiraceae* was significantly higher ( $p < 0.05$ ), in KET cows compared with CON cows. The high percentage of *Lachnospiraceae* microorganisms might be a causative factor for ketosis because they are associated with butyrate production. Findings from our study provide a comprehensive picture of rectum microbiome ecosystem between KET and CON cows which could be helpful for the development of interventions to prevent ketosis.



NU-15

### Flagging cows with poor metabolic adaptation during early lactation using cluster analysis

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**Objectives:** Hyperketonemia and 'ketosis' are used interchangeably to refer to the clinical signs associated with poor metabolic adaptation to negative energy balance during early lactation. Historically, the hyperketonemia was observed in the first described clinical cases. Today, there is increasing evidence that elevated blood ketones are not consistently associated with any abnormal clinical signs. Although, clinical signs of poor metabolic adaptation during early lactation are often referred to as "clinical ketosis", to differentiate it from hyperketonemia Poor Metabolic Adaptation Syndrome (**PMAS**) is a better descriptive term. Blood ketone levels are good predictors for negative health outcomes later during lactation, but they cannot effectively flag cows with PMAS. It was our aim to re-evaluate the commonly-used measures of metabolic health (input variables) that were available (i.e., blood beta-hydroxybutyrate acid, milk fat to protein ratio, blood **non-esterified fatty acids (NEFA)**) to characterize risk classes for PMAS.

**Materials and Methods:** Twenty-six Bavarian farms equipped with automatic milking systems were enrolled for weekly visits for an average of 7 weeks when veterinarians performed physical examinations of the cows (between 5 and 50 days in milk) and blood and milk samples were collected. Resulting data included 790 observations from 312 cows (309 Simmental). Principal component analysis (PCA) was conducted on the three input variables, followed by K-means cluster analysis of the first two orthogonal components. The five resulting clusters were ascribed to PMAS risk classes (low risk, intermediate risk, high risk) based on the presence of concurrent PMAS risk factors, clinical signs, milk testing results, and blood values associated with PMAS.

**Results:** The three resulting risk classes of PMAS were most significantly associated with blood NEFA levels. NEFA cut-off values were evaluated for their ability to separate risk classes for PMAS. Our resulting NEFA cut-off values (less than 0.39 [0.360 - 0.410 95% CI] mmol/L to identify Low Risk PMAS cows, and at least 0.7 [0.650 - 0.775 95% CI] mmol/L to identify High Risk PMAS cows) were very similar to those determined for Holsteins diagnosed with ketosis in conventional milking settings as reported in literature.

**Conclusions:** The fact that NEFA flags cows with PMAS better than hyperketonemia should be validated with data from additional locations, breeds, and milking systems. A future NEFA prediction model using milk Fourier-transform infrared spectroscopy data as predictors will help diagnose PMAS cows in need of diagnostic and therapeutic attention during the early

post-partum period.

**Key words:** ketosis, cluster analysis, negative energy balance, non-esterified fatty acid

NU-16

### Metabolomics and performance data indicate a higher adaptive flexibility in the transition period in Fleckvieh than in Holstein dairy cows

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**Objectives:** Dairy cows experience tremendous metabolic changes in the transition period. Over the last decades, genetic selection for high milk yield has increased negative energy balance as nutritional requirements for milk production cannot be met by feed intake alone. Therefore, mobilisation of body reserves is required to bridge the energy deficit. This situation results in metabolic stress, which can be associated with several production diseases. Most studies have been focused on investigating metabolic changes in Holstein cows, the dominant dairy breed in Europe. However, there is a lack of studies analysing metabolic pathways in the transition period in different dairy breeds. In this context, metabolomics is a powerful tool to analyse a plethora of metabolites at a certain time. This displays a fingerprint of the biochemical and functional activity of cells and allows insights into the metabolic condition of an animal. Thus, the aim of the present study was to investigate adaptation processes related to transition in two modern dairy breeds, German Holstein (HF) and the dual-purpose breed German Fleckvieh (FV) using a metabolomics approach. We hypothesized that the metabolic adaptation to lactation is more balanced in Fleckvieh, resulting in less metabolic stress and a higher adaptive flexibility.

**Materials and Methods:** The study comprised 18 Holstein and 10 Fleckvieh cows that were kept under the same management, environmental and feeding conditions. The selection was based on milk recording data from the current and previous lactation with the aim to create two homogenous groups of animals. Blood samples were taken at two different time points, 42 days before calving [day -42] and 21 days after parturition [day +21]. At the day of blood sampling, all animals were clinically examined and various health and production variables were assessed using a standardised protocol. Blood samples were subjected to biochemical analysis (Cobas c 311, Roche Diagnostics, Mannheim, Germany) and plasma insulin concentration was measured using a commercially available bovine ELISA kit (Mercodia, Uppsala, Sweden). Furthermore, a metabolomics approach was performed using the AbsoluteIDQ p180 panel (Biocrates Life Science AG, Innsbruck, Austria) to identify serum metabolites by liquid chromatography-mass spectrometry. Processed data was evaluated by univariate data analysis, and metabolomics data was also subjected to multivariate data analysis using the MetaboAnalyst 3.5 software.

**Results:** The transition from gestation to early lactation result-



ed in body weight loss ( $p < 0.05$ ), but group differences after calving were initially not apparent. However, body weight decreased to a greater extent in HF than in FV during the next weeks. The concentration of  $\beta$ -hydroxybutyrate (BHBA) was significantly increased in both breeds at day +21 ( $p < 0.05$ ), but HF displayed higher BHBA levels than FV ( $p < 0.01$ ). As expected, the insulin concentration was lower at day +21 in both breeds, however, paired t-test only revealed a significant reduction in HF ( $p < 0.001$ ). Metabolomics revealed 170 metabolites which could be assigned to acylcarnitines, amino acids, biogenic amines, glycerophospholipids and sphingolipids, respectively. Protein metabolism was identified as different between the two breeds. The level of free amino acids was already higher at day -42 in HF cows ( $p < 0.05$ ). After calving, the concentration increased in both groups but was still higher in HF than in FV at day +21 ( $p < 0.001$ ). Further analysis revealed that branched-chain amino acids were significantly lower in FV at day +21.

**Conclusions:** The results of the present study demonstrate that genetic breeds may transition differently from gestation to early lactation. Despite similar milk yields, body reserves were mobilised to a greater extent in HF than FV cows post calving, which was reflected by continued weight loss, increased BHBA concentrations as well as higher levels of circulating amino acids. The latter indicates that body protein degradation is particularly increased in HF to meet energetical requirements. Increased BCAA levels have been associated with poor metabolic health and insulin resistance in humans. Intriguingly, these metabolites were displayed on lower levels in the blood of FV cows at day +21 than in HF cows. The authors conclude that adaptive flexibility to metabolic changes might be more pronounced in FV than in HF.

was performed between November 2015 and 2016. The trial involved exact documentation of clinical and production state throughout the trial, as well as liver biopsies, blood and urine sampling at day -14 prepartum, and day 7 and 28 postpartum. MS-based metabolomics analysis of blood and liver samples were performed using the AbsoluteIDQ p180 kit (Biocrates Lifes Sciences) and an Agilent 1290 Infinity UPLC-System attached to a ABSciex5500 Qtrap LC-MS/MS System for mass spectroscopy. Urine samples were analyzed using a Bruker AVANCE III spectrometer equipped with a TCI-Cryo-Probe and a sample jet system (Bruker BioSpin, Germany). Statistical analysis was performed using SIMCA 14.0 (Umetrics, Sweden) software package for multivariate data analysis.

**Results and Conclusions:** Clinical and production data are currently being processed and analyzed. First results of the metabolome analysis (creating principal component analysis (PCA) plots) show a clear clustering of the samples according the sampling time in urine and blood, indicating a strong partum effect and a postpartum adaptation to milk production. In the liver samples, also a clear differentiation between prepartum and postpartum samples is observed, however, the postpartum samples of day 7 and 28 are mingled and separated into two distinct clusters. The respective contribution plots show that the two clusters differentiate due to an increase of mainly acylcarnitines, certain glycerophospholipids (lysophosphatidylcholines) and sphingolipids in one of the two groups, possibly indicating differences in the liver lipid metabolism. Ongoing analyses are currently focusing on the metabolic characterization of the partum effect and the integration of clinical data.

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NU-17

### Alterations in the blood, urine and liver metabolome of dairy cows throughout the transition period.

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**Objectives:** Dairy cows in modern production systems are at a high risk to encounter metabolic disorders during the transition period. Reasons for individual differences in susceptibility as well as the underlying pathomechanisms are still widely unknown. Furthermore, the identification of prognostic markers that allow the development of practical cow-site tests are needed. The aim of the current study is to analyze the metabolome of transition dairy cows to characterize the metabolic alterations in the liver, blood and urine in relation with clinical and production data to identify possible prognostic markers.

**Materials and Methods:** An on-farm trial involving 80 German Holstein dairy cows (lactation number:  $3.9 \pm 1.8$ , 305 d milk production in previous lactation:  $10.944 \pm 2013$  kg, mean  $\pm$  SD)



SP-01

## Characteristics of liquid organic fertilizer from Bali cattle's urine using decomposer and different fermentation time

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Environmental pollution is now increasingly alarming. This happens because of the use of chemical fertilizers on uncontrolled farmland. The organic farming system is one of the government programs implemented in Indonesia. Implementation of organic farming system one of them is the use of organic fertilizer on agricultural crops. The use of chemical fertilizers and commercial decomposers is still difficult to apply by farmers in the countryside because it is not environmentally friendly and the price is quite expensive. The development of liquid fertilizer from Bali cattle's urine and the use of decomposers from natural ingredients needs to be studied further. The objective of this study was to evaluate the characteristics of liquid organic fertilizer from Bali cattle's urine processed using local decomposers with different fermentation processes. Raw material from cow urine was obtained from cattle ranchers. In this research, there are 2 types of local decomposer applied in fermentation process, namely (1) extract of Bali cattle's feces and (2) extract of local banana root. Time of fermentation process applied for 1 week, 2 weeks and 3 weeks. The research was conducted in 2 steps, namely (1) making of decomposer extract and (2) implementation of fermentation process. The experimental research was conducted by experimentally laboratory applying Completely Randomized Design (CRD) with factorial pattern. The data obtained were statistically tested using ANOVA to determine the effect of treatment. The results showed that application of local decomposer and fermentation time had significant effect ( $P < 0.05$ ) on C-Organic, N-Organic and C/N ration of liquid fertilizer, but no significant effect ( $P > 0.05$ ) on pH value. The average value of C-Organic content obtained ranged from 9.59-12.02%; N-Organic levels of 1.03-1.35% with a C/N ratio of 8.33 to 11.03 and pH of 8.76 to 8.95. The final result of this study concluded that the application of banana root extract as local decomposer with fermentation time of 2 weeks shows the best characteristic accordance with the terms and conditions by the government.

SP-02

## Large scale dairy conversion to organic farming: the veterinarian's role

### The role of the veterinarian in transitioning into sustainable agricultural production

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**Objectives:** Organic farming is growing across the globe as consumers demand what they perceive to be improved approaches to farming and food production. Organic farmers typically can gain a premium on their product, because consumers are willing to pay more for what they perceive as a higher quality

product.

Dairy veterinarians are uniquely placed to assist and advise their clients on this transition, but it often places them in an uncomfortable role outside of their typical comfort zone. However, working alongside dairy farmer clients on this journey can strengthen the relationships and provide new opportunities for veterinarians in areas of sustainability and alternative farm management.

This paper will report on our learnings as veterinarians as we transitioned into the organic space, and the opportunities available for veterinarians to engage in this area.

**Materials and Methods:** A large, corporate group of farms in the Southland area of New Zealand identified an opportunity to move further up the value chain by producing organic product.

The group was made up of 6 dairy farms, a total of 2978 hectares and 6235 milking cows. All farms had been conventionally farmed for many years, using seasonally calving, pastorally based New Zealand management systems. The individual farms varied in size from 572ha to 1400ha.

The process of conversion involved a significant amount of preliminary work. Initially, processor contracts had to be negotiated and set up, because local processors did not distinguish between conventionally farmed and organically farmed milk. A decision on which organic standard was used had to be made, and that in turn determined the nature of the conversions.

The group had achieved some significant animal health and welfare goals under conventional farming, with significant reductions in bulk milk somatic cell count and improvements in reproductive performance. Early on, the group agreed to partner with us as veterinarians and stewards of animal health and welfare to aim to ensure that these standards were maintained.

**Results:** Initially, the land was converted to organic in 2016. This was followed by the stock in 2017, so that the farms were fully organic by the end of the 2017 season.

Stocking rates were reduced over all farms, from a mean of 2.1 (conventional) to a mean of 1.8 (organic). This also gave the opportunity to remove a number of cows, eg those with high SCC or low reproduction, so that the final milking cow number was 5400.

Farmers were trained in organic and sustainable methods, in particular with regards to animal health management and treatments. A major consideration was the change to treatment options. All veterinarians who serviced the farms had to be aware of organic requirements and regulations. A key veterinarian was assigned to coordinate all animal health activity across all the farms.

Significant farm staff training was implemented around disease detection, management and treatment. In addition, alternative treatment methodologies were investigated. These particularly focused on novel detection and treatment regimes for managing clinical mastitis to reduce the need for antimicrobial use without compromising animal welfare.

**Conclusion:** As organic dairy farming becomes more popular, enabling and advising farming clients as they firstly decide, then proceed with this conversion will become an increasingly important area of expertise for dairy veterinarians. Abandoning traditional therapeutic options is uncomfortable, and the role of exploring novel therapies without compromising animal welfare can be challenging.



The dairy veterinarian must call on many other aligned disciplines to help clients successfully transition from conventional to organic farming. In turn, they will often need to turn away from treatment and management practices that they may have embraced for a long period. This can be quite challenging to veterinarians, but should be seen as an opportunity to expand their breadth of knowledge and develop more multi-disciplinary skills.

Consumers, and hence farmers, are demanding ever greater levels of sustainability, and challenging orthodoxies around farming practices, and animal health management and treatment. Developing a greater understanding of these areas is of value to both veterinarians and their clients.

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SP-03

### Farmer Action Groups- A participatory approach to instigating change on farms

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#### Objectives:

- Establish and follow a series of Farmer Action Groups (FAGs) across South West England
- Assess whether farmers engage with participatory methodology
- Evaluate effectiveness of participatory methodology in enacting change on farms, particularly regarding antimicrobial use

**Materials and Methods:** This research seeks to adapt and test the 'Stable Schools' model used widely in Denmark with organic dairy farmers. The methodology harnesses the potential of peer-to-peer learning to achieve practical, farmer-led change by prioritising local-level experience and expertise, which is critical for sustainable dairy farming. Farmer Action Groups (FAGs) work to support farmers to reduce antimicrobial usage and improve herd health and welfare.

Five FAGs were established in South West England, each made up of 5-8 dairy farmers meeting approximately every 6 weeks on each other's farms to discuss medicine usage and share successes and challenges. Meetings lasted 3 hours and involved a farm walk and facilitated discussion, all of which were audio recorded. Medicine audits were carried out on each farm at the start and end of the project to quantify changes in antimicrobial use. The outcome of each meeting requires the farmers to co-create an Action Plan for the host farm of practical measures to reduce antimicrobial usage without adverse impacts on herd health and welfare. Each farm is re-visited by the FAG several months later and the Action Plan is scored, discussed and reflected upon.

**Results:** Thirty farmers participated and many more were approached, by veterinary practices, via agricultural shows and through advertisements in the farming press. The majority of farmers exposed to the project have been contacted through specific recruitment meetings in collaboration with the levy board. Over 900 farms were invited to recruitment meetings,

and 40 farms (4%) attended at least one meeting. Of these, 23 farms (58%) signed up to the project. Thirteen farms (21%) withdrew from the project after attending at least one meeting. Collaboration with the levy board and a well respected member in the community was pivotal in recruitment of participants to the project.

Participating farmers have been very positive in feedback, with enthusiasm for the approach and 30 Action Plans completed. From transcribed group discussions and interviews with participants, the role of the facilitator has been pivotal for the running of the groups. Some participants credit the facilitator's reputation as a motivational factor for joining the project and also necessary to assist the farmers with gaps in knowledge and to audit any mis-information in discussions.

After just 12 months farmers have already implemented measures such as: re-designing sheds to reduce the incidence of bovine respiratory disease, using paraprofessionals such as foot trimmers more frequently to address disease processes early, increasing discussions with veterinarians about the medicines used on farm and improvements to calf feeding and colostrum management. Preliminary results from discussions and medicine reviews indicate an overall reduction in antimicrobial use for some participants and a switch from CIA use.

The Action Plans consist of around 10 practical steps. Average completion rates for the Action Plans to date are 50% (n=8). All Action Plans that have been re-visited so far contain at least one practical step that has been attempted. Participants have found the process enlightening and helpful, and regard the co-creation of the Action Plan with a group of like-minded farmers relevant and important. Thematic analysis of interviews continues and more in-depth results will be available by July 2018.

**Conclusions:** FAGs help foster autonomy to create practical on-farm change. The sharing of on-farm successes and challenges with a cohesive group of farmers has given participants the confidence to reduce their reliance on antimicrobials. Professional, credible facilitation is essential for the running of the FAGs and for the recruitment of farmers to the project. Farmer engagement and time-consuming recruitment has implications for policy making in this area.



PH-01

### Comparative study on the efficacy of aqueous ozone, sodium hypochlorite, and peracetic acid in the disinfection of various *Salmonella* contaminated surfaces in dairy operations.

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**Objectives:** To characterize and compare the microbial killing capacity of aqueous ozone (aqueous-O<sub>3</sub>), sodium hypochlorite (SHC), and peracetic acid (PAA) on various surfaces (plastic, nylon, rubber, and wood) contaminated with *Salmonella Typhimurium* and *S. choleraesuis* (aSTC).

**Materials and Methods:** In a crossover design, 14 strips (7.5 X 2.5 cm) (substrates) of each material were randomly assigned to one of 3 groups, treatment (n=6), positive control (n=6), and negative control (n=2). The substrates were soaked in sterile dairy cattle feces inoculated with 10<sup>8</sup> – 10<sup>10</sup> microbes of aSTC for 60 minutes. The substrates in the treatment groups were exposed to 50 mL of 9 ppm of aqueous-O<sub>3</sub>, 200 ppm SHC, or 400 ppm PAA for 4 minutes. Following exposure, each strip was swabbed and quantitative bacterial cultures were performed using 3M™ Petrifilm™ rapid aerobic count plate (RAC) and plate reader. Data were analyzed by using Kruskal-Wallis test, repeated measures analysis of variance, and Linear regression. P<0.05 was considered significant.

**Results:** Plastic (smooth) surfaces were free of aSTC with 4 minutes exposure to aqueous-O<sub>3</sub>, SHC, or PAA. On nylon surfaces, aqueous-O<sub>3</sub> reduced aSTC population by 4.8-log<sub>10</sub> (P<=0.001), compared to SHC and PAA that reduced aSTC cell counts below the detectable limit. On rubber surface, SHC was the least effective against aSTC, achieving only 2.5-log<sub>10</sub> reduction (P=0.002), compared to aqueous-O<sub>3</sub> (4.2-log<sub>10</sub>, P<=0.001), and PAA that completely decontaminated the rubber surface from aSTC. On complex surfaces (wood), PAA showed the highest reduction rate (4.1-log<sub>10</sub>, P<=0.001) in aSTC cell counts, compared to aqueous-O<sub>3</sub> and SHC that achieved 1.9-log<sub>10</sub> (P<=0.001) and 2.1-log<sub>10</sub> (P<=0.001) reduction in aSTC population, respectively. The results of univariable linear regression identified a significant impact (P<=0.001) of type of surface on the reduction rate of aSTC for three disinfectants.

**Conclusions:** Smooth surfaces were most effectively decontaminated. Peracetic acid of 400 ppm can provide an effective means to reduce the *Salmonella* load on various surfaces in dairy operations to a safe level. However, the low production of harmful residues make ozone an attractive alternative disinfectant for improving farm hygiene and biosecurity.

PH-02

### The effect of vaccination practices on injection site lesions in UK beef cattle

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**Objectives:** The aim of this study was to investigate the prevalence of injection site lesions (ISLs) in UK beef cattle, and to investigate if, and how, injectable vaccines may contribute to ISLs.

**Materials and methods:** Four abattoirs were visited between April 2009 and April 2010. The abattoirs selected for the study were a convenience sample, based on throughput of beef cattle and location. All carcasses on two days were examined for lesions. Carcasses were inspected visually by Food Standards Agency and slaughterhouse staff at two control points. Any lesions or abnormal tissue was recorded and removed from the carcass.

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:

##### Abattoir results

2853 beef carcasses were examined at both the slaughter line and cutting/deboning areas and 117 lesions were recorded (4.1%). Forty seven percent of these were found in the rump and 42% were found in the neck. The remaining 11% were located in other sites such as ribs and flank.

The diameter of lesions ranged from 1 to 23 cm. Twenty three percent were described as small (diameter of 1-7.9cm), 67% as medium (8-15.9cm) and 10% large (16-23cm). There appeared to be larger lesions in the rump compared to the neck, however this difference was not significant.

##### Questionnaire results

For vaccines to be administered subcutaneously, the correct route of administration was chosen by 86% (n=26/30) of respondents. For intramuscular vaccines, the correct route was chosen by 79% (n=19/24) of respondents.

When asked to indicate the site on the animal where respondents administered the vaccine, 60% of respondents injected in the neck (n=40/66) and 33% respondents injected in the rump (n=22/66). Seventy two percent (n=21/29) of respondents administered vaccines in the correct site according to the data-sheet recommendations.

There was a trend (p=0.22) towards a significant difference in distribution between site of vaccine administration (60% in neck, 33% in rump) and site of lesions (42% in neck, 47% in rump).

With regards to vaccination compliance, respondents were asked 'When administering this vaccine by injection (using e.g. a syringe, vaccinator gun), which of the following apply on your farm?'. New needles were used to start the vaccination session by 43% of respondents. The needle was changed when broken or blunt by another 43% of respondents.

When asked 'What instructions did you follow when administering this vaccine?', 56% of respondents followed the instructions on the box/bottle, and 40% referred to the datasheet. 20% said that they did what they have done previously and did not need instructions.

**Conclusions:** This study demonstrates areas of improvement for veterinarians to focus on when discussing vaccination and



injection techniques. ISLs have the potential to cause economic loss due to trimming and reduced carcass value and to compromise animal welfare and meat quality. In the current climate where consumer trust is challenging the food animal industry, it is crucial to ensure the best quality produce and animal welfare.

Deficits in compliance with recommended injection protocols may be contributing to the occurrence of ISLs. Efforts could be focused on reducing risk factors, such as using the correct route and site of administration, or following datasheet recommendations with regard to needle usage

The role of vaccination in the occurrence of ISLs appears limited and is unlikely to be the predominant cause of ISLs. The veterinarian has a key role in reducing ISLs by providing advice when prescribing vaccines, particularly with regard to compliance with current datasheet recommendations on site of administration and appropriate needle usage.

PH-03

### Genotypic comparison of *Streptococcus agalactiae* isolated from cattle, fish and human in northern Thailand

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*Streptococcus agalactiae* has been well recognized as a contagious pathogen causing bovine mastitis worldwide and become a major mastitis causing pathogen in northern Thailand. *S. agalactiae* is also known to cause a variety of infections in many species such as fish and human. Genotypic comparison among *S. agalactiae* strains isolated from multiple species has never been reported in Thailand. We aimed to investigate genetic variations of *S. agalactiae* strains isolated from bovine mastitis cases, and compare with isolates from fish and human. The distribution of *S. agalactiae* strains was investigated using capsular typing and multilocus sequence typing (MLST) techniques. A preliminary result revealed that 3 capsular types and 3 sequence types (ST) were detected from 14 *S. agalactiae* isolates. All *S. agalactiae* strains isolated from bovine mastitis cases were capsular type Ia and ST103 (6/6). A strain of *S. agalactiae* with capsular type II and ST652 was identified from a human case. Moreover, *S. agalactiae* strains with capsular type III and ST283 were detected among isolates from fish (5/5) and from human (2/3). In conclusion, the present study provides the first insight in genetic diversity of *S. agalactiae* isolated from animal and human in Thailand. Genetic variations of *S. agalactiae* isolated from cattle and fish are more conserved within species compared with human strains. Even though a small sample size was used in this study, a diversity of genetic materials can be detected using capsular typing and MLST. A further study with more sample size is necessary to conclude any genetic relationship among *S. agalactiae* strains. In northern Thailand, where various kinds of livestock farming are presented, cross-species transmission can potentially occur. Genetic investigation of a pathogen using molecular tools

will help identify mode of transmission and thereby improve control and prevention of such infectious diseases.

PH-04

### Q fever in milk cattle herds as a potential threat for humans

#### Q fever in milk cattle threat for humans

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**Introduction:** Q fever is zoonosis caused by *C. burnetii*. Prevalence of this zoonotic factor increased in recent years. Infected animals very often are asymptomatic but shed *C. burnetii* in milk. Therefore consumption of raw milk could be a threat for humans.

**Objective:** The aim of the study was to estimate the prevalence and genotyping of *Coxiella burnetii* strains occurring in dairy cattle herds in Poland. Altogether 515 milk samples from 173 herds were collected from different regions of Poland at the turn of 2014 and 2016. The qPCR (IS1111 transposon-like repetitive region) was performed using Adiavet COX RealTime PCR kit. MLVA was performed using 6 variable loci. Amplification products were run on ABI 3500 Genetic Analyser and electropherograms were evaluated with GeneMapper software. MST was performed for 9 spacers.

**Results:** Out of 173 tested herds, 61 (35.26%) were positive in real-time PCR detecting the IS1111 element. The 33 milk samples with low Ct values were included in genotyping by MLVA-6 and MST methods. Four complete MLVA genotypes, including one new, were obtained from 16 tested samples. In four of the samples, genotypes were almost complete, i.e., one of the six markers did not amplify. All complete genotypes differ in one of six loci and can be the microvariants of one genotype. According to available databases three of them were found before in samples collected from cattle in Spain, France, Saudi Arabia, Netherlands, Switzerland, Hungary and also in human samples from France. Multispacer sequence typing (MST) revealed that most of the strains belong to ST20 genotype which frequently occurs in European and North-American cattle. In some samples a new allele for Cox37 was observed, but it needs further investigations including whole genome sequencing.

**Conclusion:** Molecular characterization of *Coxiella burnetii* strains circulating in milk cattle herds was crucial due to lack of current data. Moreover, determination of the prevalence of this bacteria and characterization of its genotypes was essential to assess the potential risk for humans. The research confirmed that the level of prevalence of *C. burnetii* in milk cattle herds is significant and similar to other European countries. This study revealed that the majority of the genotypes identified in Poland are observed in many countries but, fortunately, they rarely caused the disease in humans or data about prevalence of *C. burnetii* in humans are underestimated. Therefore, the threat for humans cannot be ruled out. Currently it is not known which factors can induce the virulence in *C. burnetii* strains which was observed during huge outbreaks in the Netherlands.





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PH-05

### An integrated platform for rapid testing of pathogens in the meat/poultry supply chain

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**Objectives:** To develop and validate an integrated system for rapid in-field detection of hazardous food pathogens (*Campylobacter*, *E.coli* O157 and *Salmonella*). This will help to improve meat quality assurance and reduce foodborne infections in humans. The FSA Foodborne Disease Strategy 2015 estimates that each 1% reduction in food poisoning case numbers would mean around 10,000 fewer cases and save the UK economy around £15 million per year, this would be greater worldwide.

The system uses Loop-mediated AMPlification (LAMP) assays which provides bacterial concentration without the need for culture enrichment. Although regulatory testing requirements still have to be satisfied, this system offers the opportunity for rapid proactive management of food pathogen threats and is now being used commercially in the UK.

**Materials and methods:** The testing platform combines sample preparation, immunomagnetic separation of the bacteria and LAMP assay. Once the target bacteria are specifically selected and concentrated, they are added to purpose-built tubes containing the LAMP reagents that will fit into a small instrument where DNA release and amplification take place, and fluorescence interrogation of the relevant piece of the device will read the results.

LAMP assays for detection of *Campylobacter*, *Salmonella* spp and *E.coli* O157 based on the DNA amplification technology LAMP were developed by Fera Science Ltd.

Carcass samples were collected from abattoirs as per the FSA standard protocol by RAFT Solutions Ltd. Each carcass was swabbed in duplicate using a sterilised sponge swab soaked in 10ml of 0.9% unbuffered sodium chloride solution (inside a resealable plastic bag to maintain sterility). Samples were taken after final inspection by Meat Hygiene Service officials and before chilling had occurred. A side of the carcass to sample was chosen at random and swabbed by starting at the back leg of the animal and using a firm pressure rub the sponge across the carcass in a sweeping motion, this was repeated with a second sponge. The sponge was carefully enclosed in the bag and resealed to avoid cross contamination & the bag was labelled using a permanent marker pen.

Carcass swabs were transported in a chilled polystyrene box to Fera Science for use to optimise the LAMP assays, the duplicate samples were transported in the same way to an accredited laboratory for culture assessment as a validation.

On arrival at Fera Science Ltd samples were stored in a refrigerator until testing. The swab was spiked with a serial dilution of *Salmonella* Typhimurium/Enteritidis then squeezed repeat-

edly for two minutes and an aliquot of the saline solution removed and placed into an eppendorf tube. The eppendorf underwent 85°C cells lysis for five minutes before a 5ul aliquot was transferred to a tube containing LAMP reagents and placed in the developed small instrument where DNA release and amplification took place. Fluorescence interrogation of the relevant piece of the sample read the results in 20-50 minutes. Natural carcass swabs from turkey and chicken were tested for *Campylobacter* using LAMP and microbiology methods in parallel and a wider panel of pathogens was tested on both pigs and beef cattle in abattoirs.

**Results:** For *Campylobacter*, there was a very good correlation of results, with LAMP being able to detect the bacteria in 100% of swabs contaminated with more than 800 cfu/swab (determined by traditional microbiology methods), 40% of swabs with counts between 800 and 200 cfu/swab and 12% of swabs with less than 200 cfu.

**Conclusions:** Direct detection of pathogens in carcass swabs has been successfully achieved using LAMP technology with no enrichment culture, no DNA purification & results in less than 1 hour. Further work is ongoing to refine the process with additional pathogens.

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PH-06

### Bile in gallbladder as an important contamination source of *Campylobacter* species in slaughter cattle

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**Introduction:** *Campylobacter* is the leading cause of zoonotic enteric infections in Japan and many other developed countries, and the public health burden of campylobacteriosis is increasing. Many risk factors for campylobacters have been identified and healthy cattle is considered as one of the infectious sources for a variety of *Campylobacter* species, such as *C. jejuni*, *C. coli*, *C. hyointestinalis*, and *C. fetus*. *Campylobacter* species have been isolated from not only intestinal contents but also from bile and liver of cattle. To control the bacterial contamination in meat products, quantitative assessment of campylobacters in liver and gallbladder was carried out at an abattoir.

**Materials and Methods:** Liver and bile samples were collected from 108 healthy beef cattle after evisceration and viable counts of campylobacters were determined by a direct-plating technique using modified Cefoperazone Charcoal Deoxycholate agar (mCCDA). The suspected colonies on the highest dilution plates were subjected to biochemical tests and PCR for identification. *C. jejuni* strain 99-a, *C. coli* strain 98-a, and *C. fetus* strain 98-b isolated in this study and *C. hyointestinalis* ATCC35217 were used to assess the bacterial growth in bile after incubation at 37°C under microaerobic conditions. Each bacterial suspension was made in 10 mM phosphate buffered saline (PBS) pH 7.2, and inoculated with *Campylobacter*-free cattle bile which had been heated at 60°C for 30 min. The number of each *Campylobacter* species spiked in the bile was approximately 10 cfu/ml. After determined incubation periods, the viable counts in the bile were measured by the direct-plating method.



**Results and Discussion:** *Campylobacter* species were isolated from 49 (45%) bile and 6 (5%) liver specimens examined. Numbers of campylobacters in bile and liver ranged from  $10^3$  to  $10^7$  (median  $10^5$ ) and  $10$  to  $10^2$  (median  $10$ ) cfu per ml and per g, respectively. These *Campylobacter* species were identified as *C. fetus*, *C. jejuni*, and *C. coli*. Multiple infections involving two species were observed in 16 cattle. *C. fetus* and *C. jejuni* were the predominant species in bile. Growth of *C. fetus*, *C. jejuni*, and *C. coli* in spiked bile samples revealed an initial exponential growth phase followed by a period with no apparent increase in colony count for 28 days. It appeared that these campylobacters can survive in bile for a long period. However, *C. hyointestinalis* did not grow in the bile. From these results, bile in gallbladder as well as feces in cattle was considered as an important contamination source of *Campylobacter* species in processing plants.

PH-07

### A one-year investigation of the dynamics and antimicrobial susceptibility of bacteria isolated from cows with mastitis on a large-scale dairy farm in Japan

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Bovine mastitis is an inflammatory condition caused by a variety of microorganisms affecting the mammary gland or udder of dairy cows, being responsible for high economic loss in the dairy industry worldwide. Bacteriological examinations including antimicrobial susceptibility testing of milk samples from affected cows are vital for proper management, as cows with mastitis require treatment with optimally effective antimicrobial agents. On large-scale dairy farms, so-called "mega" farms housing thousands of cows, mastitis in individual animals is difficult to control and can have a potential impact on animal welfare and hygiene. Therefore, it is important to collect basic data on the dynamics of antimicrobial-resistant bacteria isolated from cows with mastitis and conduct epidemiological analysis for effective prevention and control of mastitis. In the present study, we conducted bacteriological examinations to isolate major causative bacteria from cows with mastitis. We further tested antimicrobial-resistant isolates to confirm existence of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum  $\beta$ -lactamases (ESBLs) during the course of one year at a mega dairy farm in Japan, together with molecular epidemiological analysis. Moreover, we conducted molecular typing of ESBLs-producing *E. coli* and *K. pneumoniae* strains from individual cows.

**Materials and Methods:** The present study was conducted from August 2016 to July 2017 on a mega dairy farm in Kyushu, Japan, housing approximately 1890 milking cows. In this farm, routinely all suspected mastitis samples were checked for microbiologically so far. Aliquots of milk (2-5 ml) from cows with suspected mastitis were collected in sterilized 15-ml tubes and kept on ice. The samples were transferred to our laboratory at

-20°C within 48 h after sampling. A total of 1549 samples were obtained from 952 cows including individuals that had suffered repeated infections. Bacteriological testing of the milk samples was carried out using standard microbiological techniques, as described elsewhere. Suspected colonies on various selective media at each dilution were subjected to identification by MALDI-TOF MS and the numbers of bacteria were counted. Antimicrobial sensitivity testing (for ampicillin, oxytetracycline, ceftazolin, cefuroxime, and enrofloxacin) was carried out by the disk-diffusion method. To detect MRSA, the *mecA* gene was amplified by PCR. ESBL production by *E. coli* and *K. pneumoniae* was assessed by the Double Disk Synergy Test (DDST). The ESBLs were further confirmed by their genotypes using 5 primer pairs to amplify the specific ESBLs gene blaCTX-M, blaTEM, and blaSHV. Molecular subtyping profiles of *E. coli*, *K. pneumoniae*, and *Serratia marcescens* were analyzed using randomly amplified polymorphic DNA (RAPD)-PCR.

**Results and Discussion:** The bacteria most frequently isolated from milk samples from cows with mastitis were *E. coli* (18.9%), followed by *K. pneumoniae* (12.3%), *Streptococcus dysgalactiae* (7.8%), *S. aureus* (7.7%), and *S. marcescens* (6.3%). *E. coli* was isolated most frequently during summer to autumn, and the isolation rates of *K. pneumoniae* and *S. dysgalactiae* were 2 to 3 times higher in winter than in other seasons. Some cases of mastitis caused by *Klebsiella* recurred in the same cow for a relatively long period.

All isolates of *S. aureus* were susceptible to oxytetracycline, ceftazolin, and cefuroxime. No strains carrying the *mecA* gene were found. Among *E. coli* and *K. pneumoniae* isolates, 10.2% and 62.3% were resistant to ampicillin and 7.2% and 11.5% were ESBL-producing strains, respectively. The results of genotyping and RAPD-PCR analysis suggested that there was no plasmid transmission between *E. coli* and *K. pneumoniae*, and that some clonal populations among ESBL-producing strains may be prevalent at this dairy farm. A high proportion of *S. marcescens* isolates (88.7%) showed ceftazolin resistance, probably due to the fact that the ceftazolin was the main antimicrobial employed on the farm. However, RAPD-PCR analysis showed that many of *S. marcescens* isolates were polyclonal.

PH-08

### In vivo characterization of the ileal microbiome of healthy cattle using Ion Torrent PGM analysis

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**Objectives:** There is limited data that fully describes the *in vivo* commensal intestinal microbiome of ruminants. The importance of a healthy microbiota associated with the gastrointestinal tract of many species is well described in the literature. Dysbiosis of the microbiome can adversely affect productivity and overall homeostasis. This study focused on describing the commensal microbiome of the bovine ileum, *in vivo*, with the goal to further characterize similarities or changes in bacterial diversity between the lumen and the epimural surface of the ileum utilizing a cannulated calf model.

**Materials and Methods:** Six crossbred dairy yearling steers were surgically fitted with indwelling ileal t-shaped cannulas.



Following a post-op recovery period, luminal contents (LS) and epimural surface (ES) biopsies were collected from each steer over a four-week period. Each animal was collected three times, randomly, in order to provide consistency and assess potential variation due to individual, environmental, and bacterial factors. Epimural biopsies were collected using a flexible video-endoscopy via the indwelling t-shaped cannula. The bacterial populations and their relative abundance, were analyzed using 16S rDNA Ion Torrent PGM metagenomics.

**Results:** The top five phyla present in the LS consisted of Firmicutes (60%), Bacteroidetes (26%), Spirochetes, Proteobacteria, and Fusobacteria (8.9% combined). In contrast, in the ES, Firmicutes (49.5%) were less common and Bacteroidetes (31%) more so, followed by Spirochetes, Proteobacteria, and Fusobacteria comprising 12.5% of the total population. The primary phyla Firmicutes and Bacteroidetes composed over 80% of the microbiome present in both sample locations. Furthermore, differences were observed in the relative bacterial populations between the luminal contents and epimural surface biopsies when analyzed at other taxonomic levels.

**Conclusions:** Understanding the changes and similarities of the ileal microbiota in cattle in vivo is imperative, as many intestinal infectious diseases that affect cattle, and consequently food safety, occur in the small intestines. Dysbiosis of the commensal microbiome can adversely affect the ability of the gut to absorb or secrete metabolic byproducts. This study demonstrated differences in the natural microbiota of the LS and ES of the ileum. Further study is warranted to explore the impact of medical therapy and or environmental effects on the metabolically-active gut microbiome of ruminants.

RE-01

## New aseptic technique of semen collection for microbiological culture in bulls

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The objective of present research was to develop a new aseptic technique of semen collection using clinical healthy beef bulls. The investigational hypothesis was that semen from clinically healthy bulls collected in accordance to this new protocol would produce semen samples free of microorganisms. Twenty-five mature beef bulls declared satisfactory potential breeders according to the Society for Theriogenology with an additional negative semen smear stained to detect the presence of white blood cells and normal blood cell counts were utilized. At the time of semen collection the bulls were without sexual contact with any female for at least 3 days and fasted for at least 12 hours. Bulls were placed in a clean chute, the ventral part of the abdomen was brushed and cleaned, the preputial hairs were clipped, urination stimulated, the preputial orifice was initially closed, then opened to allow urine to flow out, and antiseptics of the preputial area was performed with povidone scrub for 5 minutes. Finally the preputial cavity was infused twice with povidone solution (10%; from 250 to 500 ml at each flush) using a sterile catheter maintained 5 minutes for every time by closing the prepuce opening using sterile clumps. Next, a slight massage of the accessory sexual gland especially the seminal glands by per rectum palpation was performed. Lastly, the electroejaculator probe was introduced in the rectum and switch on, after protrusion and erection, the first 2 to 4 jets of pre-seminal fluid emitted were discarded and the new aseptic system of semen collection was attached to the free portion of the penis. A minimum of 1 ml of the semen was collected. Immediately, a second semen sample using a non-sterile collection device for evaluation of motility and spermatozoa morphology was collected. The new system of aseptic semen collection consisted of: 1- semen collection handle attached a 2- disposable director cone (open end) that was 3- connected to Falcon tubes (17 X 120 mm; 15 ml polypropylene conical tube), and 4- a transparent plastic bag with seal 10 cm X 15 cm containing the blue cap of the Falcon tube. All these items were wrapped in a rectangular plastic bag (15 cm X 50 cm) and sterilized. The first semen sample was submitted to the microbiology laboratory that was blinded about this investigation. The semen sample was cultured on blood agar incubated aerobically and anaerobically, as well as PEA (Phenylethyl Alcohol Blood Agar; for Gram positives) and Tergitol (for Gram negatives) media. In addition, selective media for the isolation of *Brucella* sp. (Tryptose and modified Farrell's agar), *Campylobacter* sp. (modified Skirrow's agar), and *Mycoplasma* (modified Hayflick's; agar and broth) were used. The second semen sample was evaluated for motility, spermatozoa morphology, and presence of white blood cells. The first drop of the second sample was evaluated for mass motility (raw semen) and individual motility (diluted semen sample) using a photonic microscope. The spermatozoa morphology was evaluated by mixing one drop of raw semen with one drop of eosin-nigrosine stain on a warm slide that was placed on warm table (37° C) and keep for 30 seconds, then a smear was performed. The minimum criteria for spermatozoa morphology was 70% normal spermatozoa. Finally, a third drop of raw semen was used to perform a smear that was further



stained with Diff-Quick to detect the presence of white blood cells. From these 25 clinically healthy bulls semen collected by this new aseptic procedure in 22 samples no bacteria or mycoplasma were cultured. Therefore, this protocol produced a significant increase in the proportion of samples with negative microorganisms cultured (88%; Confidence Interval=70 to 96;  $P < 0.0001$ ). It can be concluded that this new aseptic collection technique of seminal fluid was satisfactory in collection samples free of microorganisms in clinically healthy bulls.

RE-02

### Scrotal circumference and plasma concentrations of testicular and metabolic hormones from pre-puberty to post-puberty in Japanese Black beef bulls with normal, abnormal fresh and low-fertility post-thaw semen

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(Weerakoon and Sakase contributed equally in this study.)

**Objective:** The present study was designed to compare scrotal circumference and plasma concentrations of insulin-like peptide 3 (INSL3), testosterone, inhibin and insulin-like growth factor-I (IGF-I) at pre-puberty, puberty and post-puberty in Japanese Black beef bulls with normal, abnormal fresh and low-fertility post-thaw semen.

**Materials and methods:** Scrotal circumferences of Japanese Black beef bulls ( $n=57$ ) were recorded monthly from 8 to 21 months of age (pre-puberty 8-11 months, puberty 12-17 months, post-puberty 18-21 months). Blood samples were obtained monthly from the bulls ( $n=66$ ) from 8 to 21 months of age. Semen was collected weekly from 12 months of age until at least 18 months. Fresh semen was evaluated for volume, the rate of sperm with highly progressive motility, sperm concentrations, and rate of sperm morphological defects. The normal fresh semen was frozen and examined for post-thaw sperm motility and fertility. Bulls were judged as having normal post-thaw semen ( $n=45$ ) if all the above examinations met criteria, and judged as having abnormal semen ( $n=21$ ) if at least one of the examinations did not meet the criteria for 6 months. The abnormal semen were divided into two groups based on their semen parameters; abnormal fresh ( $n=16$ ) and low-fertility post-thaw semen ( $n=5$ ). The abnormal fresh semen was further divided into morphological defects only ( $n=9$ ) and morphological defects plus low motility ( $n=7$ ). The plasma concentrations of testosterone and IGF-I were measured by EIA, and plasma INSL3 and inhibin concentrations were assayed by TRFIA.

**Results:** Scrotal circumferences did not differ among the normal, abnormal fresh and low-fertility post-thaw semen groups at pre-puberty and puberty. However, at post-puberty, the scrotal circumferences in the abnormal fresh semen group were smaller than those in the normal semen group ( $P < 0.05$ ). Plasma INSL3 concentrations were lower in the abnormal fresh and low-fertility post-thaw semen groups than in the normal semen group at all the periods ( $P < 0.005$ ). When the abnormal fresh semen group was further classified into two categories, the

plasma INSL3 concentrations were clearly reduced in the morphological defects plus low motility semen group compared to the normal semen group at all the periods ( $P < 0.001$ ). However, plasma testosterone concentrations did not differ significantly among the normal, abnormal fresh and low-fertility post-thaw semen groups at all the periods. Plasma inhibin concentrations were lower in the abnormal fresh and low-fertility post-thaw semen groups than in the normal semen group at all the periods ( $P < 0.001$ ). Plasma IGF-I concentrations were lower in the abnormal fresh semen group than in the normal semen group at puberty and post-puberty ( $P < 0.05$ ). The plasma IGF-I concentrations were reduced in the low-fertility post-thaw semen group compared to the normal semen group at all the periods ( $P < 0.001$ ).

**Conclusions:** Declines of scrotal circumferences were observed only at the post-puberty in bulls with the abnormal fresh semen. Blood plasma INSL3 and inhibin concentrations showed reductions in bulls with the abnormal fresh and low-fertility post-thaw semen in all three periods, *i.e.*, pre-puberty, puberty, and post-puberty. Furthermore, plasma concentrations were clearly declined at all the periods for both of INSL3 in bulls with the morphological defects plus low motility and IGF-I in bulls with the low-fertility post-thaw semen. Thus, lowered blood concentrations of INSL3, inhibin and IGF-I at pre-puberty and puberty might be associated with abnormal semen in the beef bulls. Also, those three hormonal concentrations, but not the scrotal circumferences, could be used as indicators to predict the seminal abnormalities before puberty in beef bulls.

RE-03

### Effects of early weaning on postpartum endometrial environment in beef cows

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**Objective:** Although uterine involution is a critical step during the postpartum period in order to get ready for the next breeding, effect of early weaning on the endometrial environment from the cytological point of view remains unclear. The objective of the present study was to clarify the effect of early weaning on postpartum endometrial environment in beef cows with a particular emphasis on the endometrial cytology.

**Materials and Methods:** A total of 86 Japanese Black cows reared in four farms were used. Postpartum endometrial cytology, ovarian condition, and metabolism were compared between early weaning group (WG;  $n = 37$ ) and suckling group (SG;  $n = 49$ ) at three weeks (w3) and five weeks (w5) after parturition. Within the same farm, endometrial cytology and metabolism were observed in WG ( $n = 22$ ) and SG ( $n = 19$ ) from two (w2) to six weeks (w6) after parturition.

**Results:** The percentages of polymorphonuclear neutrophils (PMN%) of WG was lower than that of SG at W3 ( $P < 0.1$ ) and W5 ( $P < 0.05$ ). The ratio of numbers of cows having CL at W3 or W5 in WG (86.5%) was higher than that in SG (57.1%) ( $P < 0.01$ ). Average concentration of non-esterified fatty acid (NEFA) in WG was lower than that in SG at W5 ( $P < 0.1$ ). Average concentration of  $\beta$ -hydroxybutyrate (BHB) in WG was lower than



that in SG at W3 and W5 ( $P < 0.01$ ). Within the same farm, average PMN% in WG was lower than that in SG at W2 ( $P < 0.1$ ), W4 and W5 ( $P < 0.05$ ). Average concentrations of NEFA and BHB in WG were lower than those in SG at W2 to W6 ( $P < 0.05$ ). PMN% in WG was correlated with concentration of NEFA ( $P < 0.05$ ) and BHB ( $P < 0.05$ ) at W3 ( $P < 0.05$ ).

**Conclusion:** Early weaned cows had less endometrial PMN% than that in suckled cows during early postpartum period. Poor nutritious status that resulted from energy consumption and fat mobilization by suckling may be involved in the delay not only in the resumption of ovarian cyclicity but also restoration of the endometrium in the process of uterine involution.

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RE-04

### Prognostic indicators of survival time in Norwegian Cattle suffering from Uterine prolapse

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Bovine uterine prolapse is an uncommon but life threatening postpartum condition. This study aimed to identify prognostic indicators which could be used by veterinary surgeons to optimise treatment decisions of cows suffering from the condition. Practicing veterinary surgeons in Norway were contacted and asked to fill out a questionnaire on cases of bovine uterine prolapse they attended in a spring calving season. The questionnaires gathered data on signalment, clinical presentation, treatment and outcome. Questionnaire data were supplemented with culling data from the Norwegian Dairy and Beef Herd Recording Systems. Survival data were analysed using Cox Proportional Hazard modelling techniques. Start Day was determined to be the day of prolapse. Two analyses were performed. In both analyses stop day was determined to be the day of death or the point of right censoring which was set to Day 30 or Day 180 in the different analyses.

Data from 126 cases of bovine uterine prolapse were collected, survival data from 2 of these were missing and they were removed from the study leaving 48 dairy cattle cases (26 first parity, 6 second parity and 16 third parity and older) and 76 cases from beef cattle (43 first parity, 18 second parity and 15 third parity and older). Dairy cows were more likely to be assessed by the attending veterinary surgeon to be in a poor clinical state than beef cows. Replacement of the uterus was the primary course of treatment in 95 of the cases, in one case the uterus was amputated and in the others the animal was emergency slaughtered (23 cases) or euthanased (5 cases). Beef cows were more likely to be treated than dairy cows ( $p < 0.000$ ). Cows in a worse general clinical state were less likely to be treated than those in a better clinical state ( $p = 0.003$ ). Cows in a normal or slightly reduced clinical state were more likely to be alive at 30 ( $P = 0.027$ ) and 180 ( $p = 0.052$ ) days post-prolapse than those in a poor clinical state. Cows below body condition score 2.5 of a five point scale tended to have lower chance of survival at Day 30 after treatment than those in higher body condition scores ( $p = 0.11$ ). The occurrence of dystocia ( $n = 50$ ), vaginal prolapse ( $n = 34$ ), milk fever ( $n = 10$ ) all had no effect on the survival of treated cattle at 30 or 180 days. Occurrence of these

conditions did not increase or alter likelihood of treatment. Whether the cow was standing or recumbent did not affect the decision to treat or the survival times. The chances of survival at Day 30 were higher in those animals in which replacement of the uterus took less than 20 minutes compared to those in which replacement took more than 20 minutes. ( $p = 0.009$ ).

This study found that the opinion of the veterinary surgeon on the clinical state of the animal and the time to replace the uterus were the two factors which could be used as prognostic indicators of survival after uterine prolapse. In addition there was a tendency for thin cows ( $BCS < 2.5$ ) to have a lower chance of survival than cows in better condition.

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RE-05

### The effect of clinical endometritis on the calving to conception interval of dairy cows

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**Objectives:** To determine the proportion of clinical endometritis in dairy herds in the Riverina, New South Wales (NSW), Australia, and to investigate its effect on the calving to conception interval (CCI).

**Materials and methods:** The source population was lactating cows in the period between calving and conception on three pasture-based, year-round calving, dairy herds in the Riverina, NSW. Each farm was visited by veterinarians fortnightly between January 2015 and August 2017 to assess cows' reproductive status and health.

At each visit, the reproductive tract was examined by *per rectum* palpation and ultrasonography for pregnancy diagnosis (all cows were bred by artificial insemination) and assessment of cyclicity (presence of a CL), and by *per vaginum* examination to assess reproductive tract discharge. Cows received treatment to enhance reproductive management and health as required. Clinical endometritis was diagnosed by the presence of abnormal reproductive tract discharge according to the following classification: *per vaginum* purulent discharge  $\geq 21$  days post-calving or mucopurulent discharge  $> 26$  days post-calving.

The proportion of clinical endometritis of the total number of cows and the total number of calvings was calculated. The calving-to-conception interval (CCI) was estimated from the days between calving and the insemination date associated with the first positive pregnancy diagnosis. A Kaplan-Meier survivor function was used to visually assess and estimate median time to conception post-calving dependent on the presence or absence of clinical endometritis. Mixed effects Cox-proportional hazard models were used to estimate the total and direct effects of endometritis, as well as other potential risk factors on



CCI such as body condition score [BCS], ambient temperature and milk yield.

**Results:** Six hundred and fifty-nine cows were included in this study. The proportion of cows that experienced clinical endometritis during the study period was 22% (95% CI 18.9-25.4%) and the proportion of post-calving monitored periods (PCMPs) in which clinical endometritis was observed was 16% (95% CI 13.8-18.5%). The proportions of clinical endometritis per cow and per PCMP did not differ significantly between the three farms ( $X^2 = 3.37$ ,  $df = 2$ ,  $P = 0.19$  and  $X^2 = 5.41$ ,  $df = 2$ ,  $P = 0.07$ , respectively). A Kaplan-Meier survivor function, demonstrated that the median CCI was 176 days and 118 days for PCMPs with and without clinical endometritis, respectively (difference between groups = 58 days,  $P = 0.0001$ ). Cox proportional hazards regression showed that the total and direct effects of clinical endometritis significantly decreased the rate of pregnancy (CCI was increased) by approximately 0.6 times relative to the rate of pregnancy in cows without endometritis throughout the post-calving period (total effect hazard ratio = 0.62,  $se = 0.18$ ,  $P = 0.01$ ). High peak milk yield (>32 L) and a parity >2 also significantly extended the CCI (a reduction in pregnancy rate of 0.75 and 0.6 times respectively). In this study, we did not observe an effect of BCS or ambient temperature on CCI.

**Conclusions:** The proportions of cows and PCMPs with clinical endometritis that were observed in this study are consistent with those reported in other studies (approximately 5-35% in dairy herds, dependent on location, diagnostic method, and definition of clinical endometritis). The current study shows that despite regular veterinary assessment and treatment of clinical endometritis on the farms in this study, clinical endometritis significantly extended CCI. Although the incidence of clinical endometritis will differ between farms according to herd and management conditions, the association between clinical endometritis and extended CCI observed in this study is likely to be generalisable to a wider population of dairy cows. Therefore, we suggest that in the absence of more effective treatments for clinical endometritis, increased efforts to identify and minimise the risk factors associated with clinical endometritis - for example, poor transition management - could be cost-beneficial given the findings of this study.

RE-06

### Reproducibility of endometrial cytology and association between mRNA expressions of pro-inflammatory factors and uterine health in cows

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**Objectives:** of this study were to assess i) the reproducibility of polymorphonuclear cells (PMN)-counts obtained by cytobrush (CB) at 5 endometrial sites and ii) the association between uterine health status and mRNA expression of pro-inflammatory factors.

**Material and Methods:** Cows with vaginal discharge were defined as clinical endometritis (CE). A cut-off of  $\geq 5\%$  PMN at

CB distinguished subclinical endometritis (SE) from healthy cows (H). From cows between 28 to 34 days in milk, 5 endometrial samples were collected by CB (Kasimanickam et al., 2004) at i) *corpus uteri* (CU), ii) left horn base (LHB), iii) left horn tip (LHT), iv) right horn base (RHB), and v) right horn tip (RHT). Endometrial expressions of normalized mRNA of Cyclooxygenase (COX) 2, Cluster of Differentiation (CD) 45, CD 66, Interleukin (IL) 1 $\alpha$ , IL 1 $\beta$ , IL 8, Mucin (MUC) 4, and MUC 16 were analyzed.

**Results:** In total, 195 CB-samples from 19 healthy, 9 SE, and 12 CE were harvested. Endometrial samples from 7 healthy, 7 SE, and 4 CE cows were analyzed for mRNA expression of pro-inflammatory factors. Proportion of PMN was higher in groups CE and SE than in group H ( $P < 0.05$ ). Reproducibility between different sites was calculated by Interclass correlation coefficient (ICC) and was 0.61. Samples from the RHB demonstrated the greatest ICCs (0.66 to 0.85). Interleukin 1 $\alpha$ , IL 1 $\beta$ , and IL 8 were higher expressed in SE and CE at site CU. The site CU can be regarded as indicative for endometritis by analyzing IL 1 $\alpha$  and IL 1 $\beta$  with cut-off 4.27 and 0.46, respectively. The highest Youden-index for the diagnosis of endometritis by IL 8 was found in sites LHT and LHB (cut-off 1.25 and 1.96, resp.).

**Conclusions:** The RHB represented the best sampling site for CB-technique in this study. It has to be noticed that this study design did not allow the comparison with a gold standard ("truth"). Cut-off levels of 1 $\alpha$ , IL 1 $\beta$ , and IL 8 expression might be used as indicators for subclinical and clinical endometritis.

RE-07

### Comparison of cow-side diagnostic techniques to assess subclinical endometritis in dairy cows

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**Objective:** Endometrial cytology is the gold standard to diagnose subclinical endometritis (SCE) in dairy cows. However, its use in practice is limited because samples need to be stained and evaluated microscopically, so it is not a cow-side (point-of-care) test. The objective was to compare cytobrush (CB) and low volume lavage (LVL) cytologic outcomes and to assess Brix refractometry and leukocyte esterase (LE) test strips as surrogate tests for SCE.

**Materials and methods:** Holstein cows ( $n = 178$ ) between 29 and 35 days postpartum were sampled with both CB and LVL techniques. CB samples were rolled onto microscope slides. CB tips were then placed in a tube containing 1 mL of saline solution and gently mixed for 30 seconds. A drop of this suspension was used to measure the Brix index using a portable refractometer. The LE strip was inserted in the same tube for 5 seconds, and the color recorded after 2 minutes. The liquid (0.9% NaCl) collected after the LVL was similarly evaluated (Brix and LE), then centrifuged and smeared onto a microscope slide for cytologic evaluation. The polymorphonuclear cells and LE cut-points were set at 5% and >1 (small amount of leukocytes), respectively.

**Results:** The concordance correlation coefficient (CCC,  $\rho_c$ ) for



PMN% between CB and LVL was  $\rho_c = 0.6$  [95% confidence interval (CI) 0.49-0.69] and the Kappa for agreement was  $\kappa = 0.4$  [sensitivity (Se) = 0.75, specificity (Sp) = 0.72]. The CCC between cytology and Brix for the CB was  $\rho_c = 0.29$  (CI 0.15-0.42) but  $\rho_c = -0.08$  (CI -0.23-0.06) between cytology and Brix from the LVL. For Brix values from CB and LVL  $\rho_c = 0.13$  (CI -0.01-0.27). For LE agreement between CB and LVL samples  $\kappa = 0.55$  (Se 0.84, Sp 0.76). Agreement between LE and cytology from CB ( $\kappa = 0.48$ ; Se 0.92, Sp 0.52) and LVL ( $\kappa = 0.42$ ; Se 0.77, Sp 0.65) were similar.

**Conclusion:** There was moderate agreement of cytology from CB and LVL samples. However, the Brix index had poor correlation with cytology. Either CB or LVL are viable techniques to diagnose SCE cow-side via LE with good agreement between diagnosis by cytology and LE.

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RE-08

### Associations of periparturient plasma biochemical parameters, endometrial leukocyte esterase and myeloperoxidase, and bacterial detection with clinical and subclinical endometritis in postpartum dairy cows

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**Objective:** This study was aimed at demonstrating associations between peripheral biochemical parameters, endometrial leukocyte esterase (LE) and myeloperoxidase (MPO), and bacterial detection with the degree of endometrial inflammation, and determining the best time postpartum for diagnosing endometritis to predict subsequent fertility in dairy cows.

**Materials and Methods:** Plasma albumin, blood urea nitrogen (BUN), total cholesterol (T-cho), NEFA, and BHBA concentrations were analyzed in 43 Holstein cows at 3, 5, and 7 weeks postpartum (W3, W5, and W7). Endometrial samples were collected at W3, W5, and W7 to examine LE and MPO activities, bacterial detection rates, and PMN% profiles. The 43 cows were divided into healthy (HE), subclinical endometritis (SE), and clinical endometritis (CE) groups, classified differently at W3, W5, and W7 based on the definitions of SE and CE for each of the three weeks pp.

**Results:** LE level had an association with PMN% in all weeks pp ( $P < 0.05$ ). Albumin and BUN levels had weak negative associations with endometrial PMN% at W3. Pathogenic bacterial detection rates were higher in the cows with endometritis at W3 and W5. Conception rate at first artificial insemination tended to be lower ( $P = 0.057$ ) in the cows diagnosed with endometritis at W3 than in the healthy cows.

**Conclusion:** Associations were found between endometrial LE and endometritis, but not for MPO and endometritis. Diagnosing endometritis in W3 may be the best moment to predict subsequent fertility.

RE-09

### The reproductive performance of 80 problem breeder cows after receiving a therapeutic uterine lavage.

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**Objectives:** A retrospective study analysing the fertility performance of 80 problem breeder cows after receiving a therapeutic uterine lavage.

**Materials and Methods:** This study was undertaken on 190 intensively managed Holstein Friesian cows with poor reproductive history on 7 properties in North West England and Southern Scotland.

Animals were selected and defined as a 'problem breeder cow' if the individual had received four or more services. 80 treatment animals and 110 controls were obtained. Each treatment animal received a therapeutic uterine lavage and was followed for two services post intervention, whilst the controls received no treatment.

As per conventional Bovine Embryo Transfer, the main instrument used for uterine lavage is a 20 FR gauge 2-way foley catheter with a continuous flow system. Under caudal epidural anaesthesia and after disinfection of the perineal region using 5% chlorhexidine solution, a sterile, 20 FR gauge 2 way foley catheter was passed through the cervix to the palpable bifurcation of the uterine horns. From here it was advanced 2cm along the right uterine horn and the catheter balloon inflated with 5ml of air. The catheter stylette was then removed and the Y-tubing continuous fluid flow system attached to the foley catheter. Approximately 50mls of sterile sodium lactate solution was introduced into the uterine lumen in aliquot fashion, for up to six occasions and recovered via the continuous flow system. Fluid flow was controlled by the operator using halkey Roberts clamps on the inflow and outflow tubes. This procedure was repeated for the left uterine horn.

Post uterine lavage, cyclical cows with a corpus luteum were administered prostaglandin per the intramuscular route, and were inseminated after heat detection. Those with follicles in the absence of a corpus luteum where enrolled on an ov-synch program followed by fixed timed artificial insemination as per data sheet recommendations.

No antibiotics were administered.

Ultrasound pregnancy diagnosis was performed at 30 days post service.

**Results:** Studied cows ranged from 1 to 8 lactations (mean 2.5) and the number of services prior to uterine lavage intervention ranged for 4 to 11 (mean 6.5).

Statistical analysis was performed using Epi-Info version 3.5.4 (CDC) statistical software and results were deemed statistically significant when  $p < 0.05$ .

51 out of 80 (64%) studied cows fell pregnant on average 31 (95% C.I. 26-43) days after flush wash intervention. However, no statistical difference was seen when compared to control animals OR 1.58 (95% C.I. 0.87-2.84)  $P > 0.05$ .

**Conclusion:** A hostile uterine environment is detrimental to embryo viability and implantation and may account for a signifi-



cant proportion of embryonic wastage within the industry. Many chronic cases of endometritis can go undiagnosed as no overt clinical signs are present, yet either through the direct action of bacterial species present, or through secondary uterine inflammation, may cause infertility in individual animals. Although not found to be statistically significant within this study, uterine lavage should be considered as a therapeutic intervention in individual problem breeder cows.

## RE-10

### The effect at herd level of more frequent testing for endometritis and subsequent treatment of positive cows with a single intrauterine infusion of 500 mg cephalixin compared to a more traditional single test and treatment.

\*Mick Clews

Vetora Reporoa NZ

**Objectives:** This study follows on from one reported at World Buiatrics Conference 2016 "Assessment of the daily post partum change in proportion of cows testing positive with a commercially available instrument designed to indicate endometritis".

The first study showed the odds of finding a metrichex positive (MC+) cow with the Metrichex (MC) device decreased significantly as days post partum (pp) increased.

This follow on study attempts to quantify any change in reproductive performance from treating these MC+ cows early with an intrauterine infusion of 500mg cephalixin (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".

The specific objective was to measure any change in reproductive performance at the herd level that was associated with more frequent herd MC (and subsequent treating of cows found to be MC+) than the traditional NZ system of a single herd MC (and treatment of MC+ cows) just prior to mating.

**Materials and Methods:** The 15,547 cows from the first study were randomly divided into a control group (CG) (7762) and a treatment group (TG) (7785). Of these 14,776 cows (7388 CG and 7388 TG) were still present at the planned start of mating (PSM) and included in this second study.

All herds were visited on three separate occasions being about 69, 48 and 27 days before the herd's next PSM. All cows greater than 7 days pp at the day of the visit were examined for signs of endometritis using the Metrichex device. Presence of purulent or muco purulent material was considered to be MC+. Any cow in the TG found to be MC+ was treated with Metriclean at that visit. MC+ cows in the CG were recorded but only treated if MC+ at the third visit. This simulated the single MC/Metriclean herd visit that is the standard protocol in most NZ herds.

All cows were examined by ultrasound for pregnancy at about 84 and 130 days after the PSM.

**Results:** The incidence any cow being MC+ at one or more of the visits was similar in both groups (95%CI=-0.81 to 1.87%,

p=0.4387) (22.3% CG and 21.8% TG).

MC+ cows found at one of the first two visits that were subsequently Metrichex negative (MC-) 21 days later were classified as "apparently cured".

There was no difference (95%CI= -0.6367 to 4.4532%, p=0.1329) in the proportion of cows with an "apparent self cure" (CG 87.17%) and the proportion of cows with an "apparent treatment cure" (TG 90.09%).

MC+ cows in the TG (cows that were treated at whichever visit they were found to be MC+) had a better reproductive outcome than MC+ cows that were in the CG (were only treated if still MC+ at the third visit). They had a 9.6% (95%CI=6.1397 to 13.0374, p=0.0000) higher 6 week pregnancy proportion (59.05% vs 49.45%), a 3.25% (95%CI=0.2930 to 6.1977, p=0.0288) higher 84 day pregnancy proportion (78.16% vs 74.90%) and conceived 8 days earlier (Kaplan Meier median days to conception 36 days (34-37) vs 44 days (42-47), log rank test p=0.0001).

The TG as a whole (both MC+ and MC-cows) had a better reproductive outcome though than the CG as a whole. The TG as a whole had a 2.40% (95%CI=0.7788 to 4.0114, p=0.0035) higher 6 week pregnancy proportion (55.12% vs 52.72%), a 1.65% (95%CI= 0.3052 to 2.9963, p=0.0154 ) higher 84 day pregnancy proportion (78.8% vs 77.15%) and conceived 2 days earlier (Kaplan Meier median days to conception 39 days (38-40) vs 41 days (40-42), log rank test p=0.0081).

**Discussion:** More frequent MC and treating of MC+ cows gave a superior reproductive outcome than the traditional single MC and treatment of MC+ cows that occurs in the NZ system.

Because the reproductive outcome of cows that "apparently self cure" is significantly inferior to the reproductive outcome of cows that "apparently cure with treatment" it is presumed that at least a proportion of these "apparent cures" are due to a decreased sensitivity of the test as days pp increases. ( i.e "apparent cures" are not necessarily "cured" but rather become increasing undetectable to the MC as days pp increases.)

## RE-11

### Post-insemination intrauterine treatment with cephalixin in dairy cows with mild endometritis

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Dairy cows in estrus but with signs of clinical endometritis (CE) are often not inseminated or undergo an intrauterine treatment after artificial insemination (AI). Decades ago, the so-called Aström method was described as an intrauterine infusion of an iodine-potassium solution 2 to 4 days after AI. Nowadays, it is common to use antibiotics instead of iodine solution and the treatment is performed only a few hours after AI. The objective of this study was to evaluate the effect of this modified Aström treatment on pregnancy rates (proportion of cows pregnant after AI).

A total of 341 dairy cows were included in the study at the day of AI. Before enrollment, vaginal discharge was evaluated with





the Metricheck device. Animals with clear discharge were assigned to a healthy comparison group (HE; n = 113) and animals with cloudy discharge or fleck of pus in the mucus were divided into a treatment and a control group. The treatment group (MET; n = 108) received  $6 \pm 1$  hours after AI an intrauterine treatment with cephapirin (Metricure<sup>®</sup>, Intervet Deutschland GmbH). The control group (CO; n = 103) remained untreated. Pregnancy diagnosis was performed 39 days after AI by ultrasound. Non-pregnant animals were inseminated at the next estrus. Thus, treatment effects were evaluated for the first or second AI after treatment.

The results showed that the pregnancy rate after the first AI was similar in all groups (HE: 37.7%; MET: 31.5%; CO: 31.1%). After the second AI, the pregnancy rate was numerical higher in MET (73.2%) compared with CO (62.5%). Interestingly, the lowest pregnancy rate was observed in HE with 58.3% pregnant cows. However, these differences were not significant.

In summary, the post-insemination treatment with cephapirin had no significant positive effect on pregnancy rates after AI in cows with mild endometritis. One reason could be that the time for recovery of the inflamed endometrium is too short before the embryo enters the uterus. Furthermore, bacteriological analysis of uterine samples that were collected in this study will show which bacterial species were involved in post-insemination endometritis and their susceptibility to cephapirin.

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## RE-12

### **Efficacy of Mycobacterium Cell Wall Fraction in the treatment of clinical and subclinical endometritis in dairy cows**

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**Objectives:** The main objective of the present on-going study is to evaluate the efficacy of Mycobacterium Cell Wall Fraction (MCWF; Amplimune<sup>®</sup>) on the reproductive performance of dairy cows with clinical or subclinical endometritis. We hypothesized that treatment with MCWF will induce higher influx of polymorphonuclear (PMN) cells in the uterus and improve the reproductive performance of dairy cows with clinical or subclinical endometritis by clearing infection at higher rate. **Material and methods:** Experimental study protocol was reviewed and approved by institutional Animal Care Committee prior to the study start. Five hundred lactating Holstein cows between 21 and 35 days postpartum were included in the study. On Day 0, cows were examined clinically, by rectal palpation to detect presence or absence of a CL, using the Metricheck instrument for evaluation of cervical discharge, and a cytobrush sample was taken for endometrial cytology (infiltration of PMN, cut-off point 10% per 300 cells). Following vaginal and cytology examination, cows were allocated to disease groups as follows: cows with no evidence of clinical endometritis (further subdivided into normal and subclinical); cows without purulent discharge (score 0) and cows with clinical endometritis [purulent discharge; score of 1 (only strands), 2 (<50% pus) and 3 (>50%

pus), and were assigned at random to treatment groups. Cows with discharge score of 0 and 1 received no treatment (control) or MCWF (1.25mg of MCWF) via intrauterine application (IU) while cows with discharges score of 2 and 3 received either MCWF IU or Exceede<sup>®</sup> (Ceftiofur free crystalline acid; Zoetis Animal Health, 6.6 mg/kg subcutaneously). Although the impact of MCWF on treatment of endometritis and reproductive performance will be assessed at 200 days in milk (DIM), for this report purposes pregnancy rate was evaluated at 100 DIM. Pregnancy rates to 100 DIM was analyzed using general mixed models for binomial data with a logit link. Proportion of pregnant animals across DIM were compared using survival curves calculated according to the Kaplan and Meier algorithm and compared with the log rank test. **Results:** The incidence of endometritis (clinical and subclinical) in the farm studied was 29.2%. Only numerical differences were found in the 100 DIM pregnancy rate according to the definitive diagnosis and the treatment received. The most notable differences were observed between normal cows receiving MCWF and untreated controls in which pregnancy rate was 44.4% compared to 29.2%. Furthermore, cows diagnosed with subclinical endometritis and treated with MCWF had 57.1% pregnancy rate compared to 46.7% in control group at 100 DIM. There were no differences between cows with clinical endometritis with score 1 in both MCWF treated and control groups. There was a numerical difference observed in cows with clinical endometritis with score 2 and 3 receiving Exceede<sup>®</sup> having 30.8% pregnancy rate compared to 18.2% in the MCWF treated group. Analysis of the survival curves of the normal cows treated or not treated with MCWF indicated that although there were no statistically significant differences between the groups, there is a on going trend suggesting that cows treated with MCWF become pregnant earlier. The median days open for cows treated with MCWF was 89 days and 100 days for those in the untreated control group. **Conclusion:** Preliminary results of this study indicate that MCWF treatment in lactating cows without purulent discharge (normal or with subclinical endometritis) between 21 to 35 DIM may have a positive effect of fertility, suggesting that MCWF may have a beneficial effect on uterine recovery after parturition.

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## RE-13

### **Efficacy of Mycobacterium Cell Wall Fraction on intrauterine influx of polymorphonuclear cells in dairy cows-dose comparison study**

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**Study Objective:** The main objective of present study was to evaluate the efficacy of three different doses of Mycobacterium Cell Wall Fraction (MCWF; Amplimune<sup>®</sup>) following intrauterine (IU) infusion as chemoattractant in dairy cows without signs of clinical endometritis. In addition, the functional activity of polymorphonuclear leukocytes (PMNLs) attracted by MCWF was measured by Reactive Oxidative Species (ROS) tests. We hypothesized that an influx of functional PMNLs in the uterus



could be achieved by IU administration of different MCWF doses in a dose-dependent manner. It is anticipated that the increased presence of PMNLs will have a beneficial effect on reproductive performance in dairy cows by reducing the incidence of clinical and subclinical endometritis and improving the conception rate. Our hypothesis is based on the ability of MCWF to increase IL-1 concentration, accelerate ovulation and enhance angiogenesis, leading to increased embryo survival. **Materials and methods:** The study was conducted on a commercial dairy operation with 1200 milking Holstein cows. The experimental study protocol was reviewed and approved by an institutional Animal Care Committee prior to study start. Forty lactating cows averaging 45 days postpartum were selected. Inclusion criteria included history of easy calving, no retained placenta and absence of clinical endometritis. Prior to MCWF treatment, the baseline status of the uterine lining was determined by cytology examination using a cytobrush. Stained slides were examined to determine the percentage of PMNLs per 300 cells. Following cytology assessment, cows were randomly assigned to four experimental groups with 10 cows per group. On day 0, cows in the control group were treated IU with 0.33g of oyster-glycogen dissolved in 60 ml Saline, while animals in the MCWF groups received an IU dose of 2.5ml, 5ml or 10ml MCWF diluted in saline to a total volume of 60ml. Twenty-four hours later, a uterine cytology sample was taken from the same spot (*corpus uteri*) and was subjected to new cytomorphology analysis and PMNL counts. In addition, uterine lavage samples were obtained immediately after the second cytology sampling. PMNL activity was analyzed by Flow-Automated-Cell Sorting (FACS) using a Phagoburst™ kit within 4 hours post-collection. The Oxidative burst index (OBI) of individual cells was calculated by multiplying the percentage of oxidative burst-positive PMNLs and their mean fluorescence intensity (MFIs) using the following formula ( $OBI = [(\% \text{ phagocytic cells}) \times (MFI)]/100$ ). All data were subjected to statistical analysis using a one-way ANOVA followed by the Kruskal-Wallis multiple comparison test. **Results:** None of the cows had elevated PMNL counts prior to the treatment as determined by cytology. At 24h post-treatment, PMNL counts in all groups were elevated with statistically significant differences only in the groups receiving 5ml and 10 ml of MCWF ( $p < 0.0012$  and  $p < 0.0048$  respectively). Even though there were no statistically significant differences observed in the control and 2.5ml MCWF groups, there was a trend in PMNL increase with 54% and 14% increases respectively ( $p < 0.052$  and  $p < 0.16$ ). FACS analysis on ROS activity revealed a similar ROS percentage for all experimental groups (39.7, 37.6, 40.6 and 38.9% for control, 2.5ml, 5ml and 10ml MCWF groups respectively). Calculated OBI demonstrated that cows receiving 5ml and 10ml of MCWF had the highest reaction (OBI=779.7 and 812.02 respectively) compared to control (OBI=578.8) and 2.5ml MCWF (OBI=221.9). Data on reproductive performance showed that 9/10 cows in the 2.5ml and 5ml MCWF groups and 7/10 cows in the 10ml MCWF and control group were pregnant at 200 days post-insemination. In total, 3 cases of subclinical endometritis and 1 case of clinical endometritis were observed in all MCWF-treated cows (4/40), while cows in the control group were diagnosed with 2 subclinical and 2 clinical cases (4/10) within 60 days post-treatment. **Conclusions:** MCWF has the potential to attract significant numbers of PMNLs in the uterus following IU administration. MCWF-attracted PMNLs have a stronger oxidative burst activity and could lead to accelerated clearance in the case of postpartum infection. A dose of 5ml MCWF appears sufficient for IU administration. Larger studies are underway to demonstrate efficacy of a 5ml MCWF dose in prevention and treatment of subclinical

and clinical endometritis in dairy cows.

RE-14

### Vascularization to preovulatory follicle and corpus luteum- A valuable predictor of fertility in dairy cows.

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The aim of the present study was to predict pregnancy rate based on vascularization to follicle and corpus luteum (CL) in dairy cows. 26 Holstein Friesian cows were synchronized using Ovsynch protocol. On day 10 of the protocol, vascularization and morphological characteristics of follicle was assessed and cows underwent artificial insemination (AI). Vascularization was assessed using Doppler ultrasonography and quantified as pixel<sup>2</sup>. Morphological evaluation and vascularization to CL was assessed on day 12 and 21 following AI and blood samples were obtained for estimation of plasma progesterone (P4). Pregnancy diagnosis was performed on day 60 and was grouped into normal, complicated and non-pregnant. The overall conception rate was 76.92% (20/26). Complications in pregnancy were intrauterine growth retardation, late embryonic death and infection. Cows with a highly vascularized follicle (>550pixel<sup>2</sup>) underwent normal pregnancy, whereas those that had moderately (250-550pixel<sup>2</sup>) and poorly (< 250pixel<sup>2</sup>) vascularized follicle experienced complications or remained non-pregnant, respectively. There was no significant difference between luteal blood flow (LBF), P4 concentration or morphological characteristics of CL between the three groups ( $P > 0.05$ ) on day 12. LBF to day 21 CL alone was not beneficial in differentiating among groups ( $P > 0.05$ ), but LBF with turbulence suggested an increased turbulence in CL of complicated pregnant cows (CPCL) (66.67%) compared to pregnant cows (PCL) (16.67%). On comparing LBF of day 12, 21 PCL vs CPCL, there was an increase in LBF ( $P < 0.05$ ) from day 12 to 21 in PCL alone. Non-pregnant cows experienced a decrease in CL volume, increased LBF with turbulence (100%) and low P4 from day 12 to 21 suggestive of luteolysis. Therefore, assessment of ovarian structures using Doppler ultrasonography was valuable in predicting functional capacity of follicle and CL and fertility in dairy cows.

RE-15

### Effects of the function of corpus luteum at the beginning of Shortsynch program on conception rate of lactating dairy cows

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**Objectives:** Several fixed-time artificial insemination (TAI) programs were developed to increase insemination rate for cattle. Shortsynch program is one of simple methods for TAI. In the Shortsynch program, prostaglandin (PG) PGF<sub>2α</sub> was administered to a cow having functional corpus luteum and follicle, gonadotropin releasing hormone (GnRH) was administered two days later, and on the following day TAI was applied. In this program, since the treatment for follicle recruitment is omitted, the cost and effort of hormonal treatment can be reduced. However, there is only a few information for the factors having effects on conception rate after Shortsynch program. The objective of this study was to clarify the effects of the function of corpus luteum at the beginning of Shortsynch program on conception rate of lactating dairy cows.

**Materials and Methods:** After examination by ultrasonography, lactating dairy cows who has large corpus luteum (>20mm in diameter) and large follicle (>10mm) were chosen for treatment. Shortsynch program was performed as follows; 1) cows were received PGF<sub>2α</sub>, (cloprostenol, 500mg), 2) GnRH (fertile-rin, 100mg) were administrated on 56 hours after PGF<sub>2α</sub> treatment, 3) cows were inseminated at 16-20 hours after GnRH treatment. In Experiment 1, thirty-six lactating cows (123.8±9.4 days in milk) were received Shortsynch program. Estrus cycle of these cows were unknown. In Experiment 2, fifteen lactating cows (114.0±10.0 days in milk), who has dominant follicle of second follicular wave (14-16 days after estrus), were treated with Shortsynch program. In both experiments, blood sample for analysis of CL function were collected at the start of Shortsynch program. Plasma progesterone (P4) concentration were examined by EIA.

**Results:** In Experiment 1, conception rate was 47.2% (17/36). Cows having plasma P4 more than 2ng/ml tend to be higher conception rate (57.9%, 11/19) compared with cows having plasma P4 less than 2ng/ml (35.3%, 6/17). In Experiment 2, conception rate was 60.0% (9/15). Plasma P4 levels were more than 2ng/ml at the beginning of Shortsynch program in all cows.

**Conclusions:** Cows having plasma P4 less than 2ng/ml at the beginning of treatment might be in the regression stage of CL. Therefore, insemination with inappropriate timing in these cows may have caused low conception rate. Present study indicated that Shortsynch program should be applied to the cow who has a functional CL.

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RE-16

### Earlier emergence of large follicles rather than early ovulation as an indicator of subsequent fertility in lactating dairy cows

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**Objectives:** Postpartum resumption of ovarian activity plays an important role in subsequent fertility of lactating dairy cows. It has been accepted that too late ovarian resumption indicated by postpartum first ovulation extends calving interval and has

negative impact on dairy management. It is still controversial whether early resumption of ovarian cycle has positive effect on subsequent fertility. Our previous study demonstrated that lactating dairy cows experienced postpartum first ovulation at 31 days on the average after parturition, and before that, one or more large follicles (LF) could develop but some of the follicles became atretic. Only few studies have focused on the influence of early ovarian activities on the subsequent fertility, concerning with not only early ovulation but also earlier emergence of LF. The main objectives of our study were to evaluate whether early ovulation and earlier emergence of LF have beneficial effects on subsequent reproductive performance.

**Materials and methods:** Data were collected from 499 lactations (188 primiparous and 311 multiparous) of 241 Holstein dairy cows from an experimental herd. Based on our previous study, ovulations which occurred before postpartum 27 days was defined as an early postpartum first ovulation. The occurrence of the first ovulation was determined by the presence of corpus luteum (CL) at 26 days postpartum (range from 25 to 27 days) by ultrasonography. Before the CL confirmation, emergence of LFs with a diameter of > 10 mm at 10 days postpartum (range from 9 to 11 days) was defined as an earlier emergence of LF. Early postpartum first ovulation was observed in 260 lactations out of the 499 (Group-C1; 52.1 %), and the others were 239 lactations (Group-C2; 47.9 %). Emergence of one or more LFs was confirmed in 446 lactations (Group-F1; 89.3 %), and the others were 53 lactations (Group-F2; 10.6%). Submission rates for artificial insemination and pregnancy rates in each pair were compared using chi-square test or Fisher's exact test, and calving to first service interval (FS), days open (DO) and services per pregnancy (SP) were compared using Student's *t*-test. Based on a 5-point scale, body condition scores (BCS) were assigned at the each ultrasonographical scanning and were compared in each pair using Student's *t*-test.

**Results:** No significant differences were detected in submission and pregnancy rates between Group-C1 and C2 (83.9 % versus 82.4 % and 77.7 % versus 78.2 %, respectively), and in FS, DO and SP (86.4 + 26.2 versus 87.7 + 22.8, 107.9 + 41.7 versus 107.6 + 42.2 and 1.62 + 0.94 versus 1.53 + 0.88, respectively). There were no significant differences in BCS between the two groups at both 10 and 26 days postpartum (2.88 + 0.21 versus 2.87 + 0.23 and 2.73 + 0.20 versus 2.70 + 0.21, respectively).

For earlier emergence of LF, submission and pregnancy rates were significantly higher in Group-F1 than in Group-F2 (84.5 % versus 71.7 % and 79.3 % versus 66.0 %, respectively *P* < 0.05), but no significant differences were confirmed in FS, DO and SP (86.6 + 24.7 versus 90.4 + 23.3, 107.3 + 42.6 versus 112.2 + 34.2 and 1.58 + 0.92 versus 1.60 + 0.77, respectively). Higher BCS at both 10 and 26 days postpartum were recorded in Group-F1 than in Group-F2 (2.89 + 0.21 versus 2.76 + 0.27 and 2.73 + 0.20 versus 2.61 + 0.25, respectively *P* < 0.01).

**Conclusions:** Our results from the large number of lactation data set clearly indicated that the occurrence of early postpartum ovulation has no beneficial effect on subsequent fertility. Earlier emergence of follicles with > 10 mm diameter could be a good indicator of subsequent submission and pregnancy rates in lactating dairy cows, rather than early postpartum ovulation. Body condition of cows might be involved with this earlier emergence.



RE-17

### Does the side (left or right) of preovulatory follicle location at artificial insemination affect the conception rate in lactating dairy cows?

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**Objectives:** Although some factors, such as season, parity, semen type (conventional vs. sexed) and milk production, affect the conception rate following artificial insemination (AI) in lactating dairy cows, there is little information about the influence of ovarian characteristics, such as preovulatory follicle (PF) location at estrus, on fertility in lactating dairy heifers. We previously reported that PF development in the left ovary was associated with increased conception rates in dairy heifers, suggesting the side of the PF was important factor on fertility in dairy heifers. However, the locational effect of the PF on fertility is still unknown in lactating dairy cows. Our study aimed to compare conception rates in lactating dairy cows between PFs that developed in the left ovary and those that developed in the right ovary at estrus.

**Materials and methods:** Postpartum lactating Holstein cows at nineteen commercial dairy farms in Hokkaido, northeast Japan, were used. In total, 2,327 AI were analyzed (postpartum day of AI, 125.5 ± 62.4; parity: 2.1 ± 1.3; means ± SD). Estrus was detected by visual observation, and rectal palpation was performed to confirm the PF and regressed corpus luteum. PF locations were examined using rectal palpation, and cows were divided into two groups on their PF locations: 1) the PF located in the left ovary (L-PF); and 2) the PF located in the right ovary (R-PF). Semen was deposited in a uterine body in this trial. Pregnancy was diagnosed by rectal palpation 50-60 days after AI.

**Results:** The incidence of PF development in the left ovary was lower than that in the right ovary in dairy heifers (L-PF vs. R-PF : 920 vs. 1407). The conception rate was 48.4% in all cows. Conception rate was significantly higher in the L-PF (57.4%) than in the R-PF (42.5%). The conception rate was significantly higher in less than 125 days postpartum (48.6%) than more than 125 days postpartum (59.1%). In addition, season, parity and semen type (conventional or sexed semen) did not affect conception rates.

**Conclusions:** PF development in the left ovary was associated with increased conception rates in lactating dairy cows.

RE-18

### A New Hypothesis on the Development and Healing of Follicular Cysts in Dairy Cows

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**Objective:** It is known that 82% of cases of fatty liver, a peripartum disorder in dairy cows, occur within 4 months after parturition. Additionally, 70% of cases of follicular cysts occur with-

in 3 months of calving. Thus, the objective of this study was to understand the association between liver disorders and follicular cysts, and to study the therapeutic effect of a liver activator on follicular cysts.

**Methods & Results 1):** Steroid hormones produced in the body are conjugated with glucuronic acid in the liver; therefore, the activity of the conjugating enzyme liver UDP-glucuronosyl-transferase (UGT) was measured, and its association with liver disorders was studied. The liver UGT activity in 62 cows with follicular cysts and 8 normal cows with a normal estrous cycle was 2.19±0.15 and 4.28±0.22 pM/min/mg protein, respectively. The liver UGT activity in the follicular cyst group was significantly lower than that in the normal estrous cycle group ( $p < 0.01$ ). The results of histopathological examination by liver biopsy showed liver disorders (fatty liver and/or hepatitis) in 30 of 62 cows with follicular cysts (48.4%). The liver UGT activity was 1.57±0.12 pM/min/mg protein in the liver disorder group, and 2.18±0.23 pM/min/mg protein in the liver disorder-free group. In both cases, the value was significantly lower than that observed in the normal estrous cycle group ( $p < 0.01$ ). The liver UGT activity measured at the same stage as that used for the normal estrous cycle group, 60-90 days postpartum, was 1.27±0.20 pM/min/mg protein in the liver disorder group and 3.10±0.51 pM/min/mg protein in the liver disorder-free group, with a significant difference between the normal estrous cycle group and the liver disorder group, but no significant difference between the normal estrous cycle group and the liver disorder-free group. Liver UGT activity was classified into three levels -low, medium, and high- and its association with liver disorders was investigated. Of the cows in the low activity group ( $< 2.0$  pM/min/mg protein), 71.8% had liver disorders, compared to 22.2% in the medium activity group (2.0-3.0 pM/min/mg protein), and 8.3% in the high activity group ( $> 3.0$  pM/min/mg protein). This suggests that liver UGT activity is strongly associated with liver disorders, and closely associated with follicular cyst development in dairy cows.

**Methods and Results 2):** The therapeutic effect of the liver activator MPG (*N*-(2-mercaptopropionyl)glycine; tiopronin), on follicular cysts was compared in cows that had follicular cysts and were administered only MPG, and in cows that had follicular cysts and were administered MPG after human chorionic gonadotropin (hCG) had been ineffective. A total of 53 dairy cows diagnosed with follicular cysts after two rectal examinations at a 7-day interval were used in the study. Luteinization was noted in 13 of 15 cows (86.7%) that were administered MPG after initial diagnosis. Similarly, luteinization was noted in 9 of 16 cows (56.3%) that were administered MPG after the initial treatment with hCG after diagnosis was ineffective. Moreover, luteinization was observed in 7 of 12 cows (58.3%) that were simultaneously administered hCG and MPG after initial diagnosis. Luteinization was noted in 7 of 10 (70%) cows that were simultaneously administered hCG and MPG after initial hCG treatment was ineffective. Thus, we verified that MPG administration to cows with follicular cysts has a therapeutic effect similar to that of hCG, which is conventionally employed, and that MPG is also effective in cases in which hCG is ineffective.

**Conclusions:** The present study offers a new hypothesis regarding the development and mechanism of healing of follicular cysts in dairy cows, based on the observation that a high percentage of cows that develop follicular cysts have low UGT activity and demonstrate complications in liver disorders. Liver disorders are closely associated with the development of follicular cysts, and administration of a liver activator to cows with



follicular cysts has a therapeutic effect similar to that of an hCG preparation.

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RE-19

### Application of hormonal intervention in treatment of ovarian disorders in dairy cattle herd in Vinhphuc province, Vietnam

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**Objectives:** Ovarian disorders is one of the important disease that significantly reduce reproductive performance in dairy cows, while it have not been given enough attention in Vietnam. The study was performed to investigate the incidence of ovarian disorders and to apply treatment of prostaglandin F2alpha (PGF2a), Gonadotropin releasing hormone (GnRH) and intravaginal progesterone releasing device (CIDR®) in dairy cattle herd in Vinhphuc province, Vietnam from 02/2017 - 12/2017.

**Methods:** Of 152 cows which did not express estrus until > 100 days post-partum were examined by trans-rectal palpation to diagnose the cause of anestrus, and were allocated to one of three groups:

Group 1. Cows with persistent corpus luteum were injected with 2ml PGF2a analogue (Ovuprost, Bayer, Vietnam)

Group 2. 2ml GnRH (Ovurelin, Bayer, Vietnam) were administered to cows with cystic ovarian

Group 3. Cows with no follicle and CL were diagnosed as inactive ovary and were given Ovsynch plus CIDR based protocol (GnRH + CIDR - 7 days - PGF2a + CIDR removal - 1 day - GnRH).

All treatments were conducted at the initial examination day (referred as day 0), and AI was carried out in cows displayed estrus. Sixty days after AI, pregnancy were confirmed by palpation per rectum.

The cows were housed in tie-stall barn, and fed a total mixed ration consisting primarily of corn or grass silage and concentrate.

**Results:** Out of a total of 152 cases, 70 (46.06%) had prolonged luteal phase, 65 (42.76%) had inactive ovaries and 17 had cystic ovarian (11.18%).

After treatment by hormonal intervention, 130/152 (85.53%) cows had normal ovarian cycle regardless of type of ovarian disorders. When compared with others, inactive ovaries cows had higher estrus resumption rate (90.77% versus 81.43%; 82.35% for group 1 and group 3, respectively,  $P > 0.05$ ).

In addition, 75/120 (62.50%) cows were conceived after two services, and 10 cows which are waiting for pregnancy diagnosis were not calculated.

RE-20

### The Incidence of reproductive disorders in dairy cows under smallholder farms

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The objective of this study was to know the incidence of reproductive disorders in smallholder dairy farms. The study was conducted in 12 small dairy farms in Enrekang Regency, Indonesia. A total of 80 dairy Holstein Friesian cattle consisted of 51 dairy cows and 29 dairy heifers were used in the present study. All dairy cattle at each farm were housed in tie-stall barns. Reproductive examination was conducted to determine the incidence of reproductive disorders both vaginoscopy and palpation per rectum. The incidence of reproductive disorders was 30.0%; 31.0% in dairy heifers and 29.4% in dairy cows. Uterine infection was the most reproductive disorder suffered to the dairy cattle (12.5%), followed by inactive ovaries and cyst (10% and 5%, respectively). The dairy cattle suffered from reproductive disorders increased the likelihood to mate (artificial insemination; AI) greater than three times as well as to become pregnant. In the population of dairy cattle, 48% AI was conducted greater than three times. The pregnancy rate for the dairy cattle suffered from reproductive disorders was only 20%, with interval from calving to conception was 550 days in average. It can be concluded that high incidence of reproductive disorders in smallholder dairy farms. The occurrence of reproductive disorders decreased the reproductive performance of the dairy cattle in smallholder farms.

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RE-21

### Characterization of pregnancy losses between 30 and 210 days of gestation in grazing dairy cows

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**Objectives:** The objective of this study was to characterize pregnancy losses in grazing dairy cows between 30 and 210 days of gestation in a large commercial dairy farm in Argentina.

**Materials and methods:** A retrospective study including a total of 14,153 records of first, second and third plus pregnancies (PREG) within the same lactation from cows of 1, 2 or 3 plus lactations (LACT) calving from January 1st, 2010 to December 31st, 2016 was used. Pregnancy diagnosis (PD) was performed every two-weeks between 30-44 days post AI. Preg-



nancies were recorded as single or double during each diagnosis. Pregnancy losses (PL) after confirmation of pregnancy (n=3,504) were classified as: 1) cows that had a dead embryo at PD with ultrasonography 30-44 days after, 2) cows that were diagnosed not pregnant at the PD reconfirmation between 60 and 90 days post AI, 3) cows that returned to estrus and were diagnosed not pregnant at the next examination after detected in heat, and 4) cows that were diagnosed pregnant and returned to estrus 30 days after PD and were inseminated. Percent (PERC) of PL, days of gestation (DG) to PL, and days in milk (DIM) to PL were analyzed by the Glimmix procedure of SAS using a model that included the effect of LACT number, PREG number and the interaction of LACT by PREG and the PDIFF option to detect differences between LACT and PREG groups. Statistical significance was set at  $P < 0.05$ . Results are presented as LSM $\pm$ SE.

**Results:** The PL were similar between LACT number (23.64%,  $P > 0.95$ ), and PREG number (23.68%,  $P > 0.26$ ). The interaction of LACT by PREG was not significant ( $P > 0.10$ ). The DG to PL were different between LACT number ( $P < 0.03$ ). While LACT1 and LACT2, and LACT2 and LACT3 had similar DG to PL (95.53 $\pm$ 1.04 vs. 89.97 $\pm$ 1.04,  $P > 0.28$ ; 89.97 $\pm$ 1.04 vs. 81.76 $\pm$ 1.05,  $P > 0.11$ ). Conversely, LAC1 had longer DG to PL than LAC3 (95.53 $\pm$ 1.04 vs. 81.76 $\pm$ 1.05,  $P < 0.006$ ). The DG to PL were similar between PREG (88.93 $\pm$ 1.03,  $P > 0.42$ ). The interaction of LACT by PREG was not significant ( $P > 0.99$ ). The DIM to PL were different between PREG number ( $P < 0.0001$ ). PREG 1 had lower DIM to PL compared to PREG2, and PREG2 compared to PREG3 (222.05 $\pm$ 1.01 vs. 369.92 $\pm$ 1.02 vs. 496.81 $\pm$ 1.05,  $P < 0.0001$ ). The DG to PL were similar between LACT number (344.33 $\pm$ 1.03,  $P > 0.42$ ). The interaction of LACT by PREG was not significant ( $P > 0.82$ ).

**Conclusions:** In conclusion, prevalence of PL was similar for all LACT and for all PREG numbers. The DG to PL were 13.8 days longer in LACT1 compared to LACT3; and DIM to PL was 148 days later in lactation in PREG2 compared to PREG1, and 127 days later in lactation in PREG3 compared to PREG2.

**Key Words:** pregnancy losses, dairy cows.

## RE-22

### Pregnancy losses in dairy cows with singleton and twin pregnancies with cavitory and non-cavitory corpora lutea

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**Objectives:** Embryonic/fetal mortality in dairy cattle is a well-known and increasing problem all over the world. In this study, the effect of twin pregnancy, fetal laterality, and the number of corpora lutea (CL) and cavitory CL on late embryonic/fetal mortality was evaluated in Holstein-Friesian cows.

**Materials and methods:** A total of 1253 dairy cows were enrolled in this study with a positive pregnancy diagnosis by means of transrectal ultrasonography between Day 29–42 after AI. Pregnancy was confirmed by transrectal palpation between

Day 57–70 after AI and at the time of drying-off as well.

**Results:** Out of 1253 pregnancies, 105 (8.4%) were diagnosed as twin gestations at the first pregnancy diagnosis. At the time of calving, the number of fetuses and stillbirths were also recorded. Embryonic/fetal loss did not differ between singleton- and twin-carrying cows at the confirmation of pregnancy (4.6% vs. 4.8%,  $p > 0.05$ ) between Day 57–70 of gestation, moreover, at drying-off also a non-significant difference was detected between singleton and twin carrying groups (10.7% vs. 10.5%,  $p > 0.05$ ).

In cases of singleton pregnancies more losses occurred between Day 29-42 and 57-70 of gestation in cows with cavitory than in animals with non-cavitory CL (12.1% vs. 3.6%,  $p < 0.05$ ) and, surprisingly, in cows with double CL than in cows carrying single CL (7.3% vs. 3.6% %,  $p < 0.05$ ). Between Day 57–70 of gestation and drying-off this difference was still significant (20.7% vs. 3.7%,  $p < 0.001$ ) between cavitory vs. non-cavitory CL, while it was non-significant between cows with one CL (5.7%) vs. double CL (3.7%). The occurrence of cavities in cases where a single CL was present was not affected by hormone therapy prior to AI (either PGF2 $\alpha$  or OvSynch; 4.4% vs. 5.4%, respectively,  $p > 0.05$ ), however, the number of CL was reduced by pharmacological treatments (11.6 vs. 19.6%,  $p < 0.0005$ ). In singleton pregnancies, laterality had a borderline effect on pregnancy losses at the time of drying off ( $p=0.048$ ). In cases of twin pregnancies there was no difference between bilateral and unilateral pregnancies at the different time points ( $p > 0.05$ ). The length of singleton and twin gestation was 278.2  $\pm$  10.5 and 267.4  $\pm$  31.2 days, respectively ( $p < 0.01$ ). The stillbirth ratio was also higher in twin carriers than in singleton carriers (5.3% vs. 19.5%,  $p < 0.001$ ).

**Conclusions:** Although the role of the number of CL and cavitory CL in maintaining pregnancies requires further evaluation, our study highlights the importance of follow-up twin pregnancies to decrease stillbirth rate.

## RE-23

### Effect of local progesterone treatment in conditions associated with higher abortion rates in dairy cows

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**Objectives:** The increased use of ultrasonography has allowed more detailed intrauterine examinations in pregnant dairy cattle. Transuterine migration (TUM) of embryos to the uterine horn contralateral to the corpus luteum as well as ultrasonographically confirmed fetal membrane detachment (MD) is associated with higher abortion rates, although normal parturition can still occur in some of these cases. In the present study, progesterone was intravaginally placed in cows diagnosed with TUM or MD to examine its effectiveness for abortion prevention.

**Materials and methods:** The study included 117 dairy cows that were diagnosed with TUM or MD during ultrasonographic pregnancy diagnosis between January 2010 and October 2017 at 16 dairy farms around the Osaka area, Japan. 45 cows received intravaginal progesterone (CIDR 1900, Zoetis, Tokyo,



Japan) for a duration of approximately a month, and their abortion rates were compared to those of the remaining untreated cows by Fisher's Exact test.

**Results:** Of 56 cows with TUM, 15 cows were treated with progesterone and showed a significantly lower mean abortion rate of 40.0% vs. 73.2% in TUM cows that were untreated ( $P = 0.03$ ). Progesterone treatment in 30/61 cows with MD resulted in a lower abortion rate of 30.0% compared to 54.8% in untreated MD cows ( $P = 0.07$ ).

The mean gestation period before progesterone treatment was 36.6 days, and the mean treatment duration was 34.1 days. Abortion occurred between 32 and 157 days of gestation (mean, 63.2 days; median, 56 days; mode, 49 days). Cows that did not experience abortion maintained pregnancy to term (around 280 days) and calved normally.

**Conclusions:** Progesterone treatment resulted in lower abortion rates in cows with ultrasonographically confirmed TUM or MD. While implantation in the uterine horn contralateral to the corpus luteum has been empirically associated with higher abortion rates, the role of local progesterone concentration in the maintenance of pregnancy has been increasingly recognized. The results of the present study confirmed that using exogenous progesterone to increase the local progesterone concentration in the non-corpora luteum side of the uterus is an effective strategy to prevent abortion. Decreased progesterone levels can also cause fetal membrane detachment by compromising lymphocyte-regulated immune tolerance. Exogenous progesterone likely helped maintain pregnancy by supplementing the decreased endogenous progesterone level.

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RE-24

### Factors affecting bovine pregnancy-associated glycoprotein (bPAG) distribution in dairy cows with confirmed Late Embryonic Death (LED).

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**Objectives:** Bovine pregnancy-associated glycoproteins (bPAG) are mainly secreted by trophoblastic binucleate cells and are stated as an indicator of trophoblast secretory function. bPAG level in body fluids have been used as a marker of pregnancy and fetoplacental well-being in cattle they are suspected to be an effective indicator of physiological mechanisms associated with mortality. In the case of embryonic mortality circulating concentrations of bPAG have been shown to be measurably lower compared to animals that maintained a pregnancy. Because the embryonic and fetal mortality is a major cause of reproductive failure in cattle, a circulating marker that can be used to predict pregnancy loss would be very useful. Secretory function of the trophoblast is affected by many factors, which are changing circulation of PAG, therefore the aim of this study was to determine the factors affecting the PAG concentration in the blood, milk and urine of cows, which experienced pregnancy failure. This finding will possibly refine diagnostic application of PAG.

**Materials and methods:** The study was performed on group of 76 Holstein Friesian cows (between first and fifth lactation). All cows underwent pregnancy examination (ultrasonographic examination per rectum) and the blood, milk and urine samples were collected directly after artificial insemination (day 0) and afterwards on 14, 21, 28, 35, 49, 63, 77, 91 and 105 days of gestation. Combined measurements of PAG concentrations in plasma, milk and urine were performed. The double-antibody radioimmunoassay III (RIAIII) was used for estimation PAG, estrogen, progesterone concentration in blood, milk and urine. RIAIII is characterized by the highest sensitivity for the PAG molecules secreted by the placenta during the first weeks of pregnancy. After 105 days all cows were divided into 2 groups: cows with diagnosed late embryonic death (LED) and pregnant cows (P). The correlation coefficient was used to assess possible linear association between variables such as: cows body weight, number of given births and milk yield.

**Results:** Circulating concentrations of PAG on days 28 to 31 were significantly lower in plasma and milk in cows that experienced late embryonic mortality, than animals which maintained a pregnancy. However, such observations have not been confirmed in every other study. Therefore, based on the estrogen plasma concentration LED were divided into 2 separate groups: with low estrogen concentration (LE) ( $E2 < 2\text{pg/m}$ ) and high estrogen concentration (HE) ( $E2 > 2\text{pg/ml}$ ). We demonstrated significantly lower ( $p < 0.0001$ ) PAG concentration in LED group with LE compared to HE (mean $\pm$ SEM) in plasma (LE:  $2.03 \pm 0.91$  [ng/ml]; HE:  $2.99 \pm 0.52$  [ng/ml]) and milk (LE:  $0.47 \pm 0.05$  [ng/ml]; HE:  $0.49 \pm 0.04$  [ng/ml]) but not in urine (LE:  $1.45 \pm 0.17$  [ng/ml]; HE:  $1.35 \pm 0.12$  [ng/ml]). concentration of PAG was elevated in urine of LED after abortion, and reduced during pregnancy, correlated its duration. Pregnant cows demonstrated moderate uphill correlation ( $r = +0.51$ ) between the concentration of the PAG in the blood and P4 concentration in this period and no correlation of P4 concentration to the concentration of the PAG in urine. A strong positive linear relationship was demonstrated between concentrations of E2 and plasma PAG ( $r = +0.73$ ), and no relationship was observed between E2 and PAG concentration in urine. Additionally, in cows over two lactations statistically significant decrease in plasma PAG concentrations and increase in urine PAG concentrations were observed. Body weight, milk yield remain without relationship. Results demonstrated lower influence of studied factors on urine PAG than plasma PAG concentration.

**Conclusions:** This work demonstrated possibility to measure biologically important correlations of PAG concentration, especially in urine. On the other hand, high concentrations of PAG in the urine of cows with LED after abortion suggest the existence of nontrophoblastic source of synthesis and secretion of PAG molecules or similar proteins. In the context of late embryonic mortality diagnosis, PAG urine test proved to be equally sensitive method as ultrasound.

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RE-25

### Effect of induced expulsion of dead fetuses on the fertility of dairy cows

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**Objectives:** Fetal death is not an uncommon finding during ultrasonographic pregnancy diagnosis in dairy cows. The purpose of the present study was to investigate whether PGF2alpha-induced expulsion of the uterine content would improve the future fertility of the dam.

**Materials and methods:** The study included 535 Holstein cows diagnosed with fetal death between November 2012 and April 2016 at 32 dairy farms around Osaka, Japan. Fetal death was diagnosed by transrectal ultrasonography (50S TRINGA, Esaote-Pie Medical, Maastricht, The Netherlands) between 28 and 90 days of gestation based on the absence of fetal pulse and mobility, detached fetal membranes, fetal autolysis and/or echoic amniotic fluid. Of the 535 cows, 342 were intramuscularly treated with a single 25-mg dose of PGF2alpha (5 mL of PRONALGON F®, Zoetis, Tokyo, Japan) immediately after diagnosis to expel uterine contents, while the remaining 193 cows were untreated. Time to resume cycling was compared between these two groups of cows by log-rank test using statistical analysis software R (Toukei Kagaku Kenkyujo, Japan).

**Results:** Of the 342 cows treated with PGF2alpha, 273 (79.8%) became pregnant; 71 cows (20.7%) became pregnant after the first insemination performed within around 3 days of PGF2alpha treatment, while 75 cows (21.9%) became pregnant by inseminations performed within around 24 days of PGF2alpha treatment. In total, 154 cows (45.0%) were pregnant within a month after PGF2alpha treatment. The mean time to the first insemination was 12.4 days, and the mean time to successful insemination was 49.1 days. On average, 2.2 inseminations were required for pregnancy. Of the 193 untreated cows, 119 (65.3%) became pregnant. The mean time to the first insemination was 39.3 days after diagnosis, and the mean time to successful insemination was 76.3 days. In 47 (24.4%) of the untreated cows, fetal tissues and annex membranes were still present at 2-week to 1-month follow-up examination. Comparison of Kaplan-Meier curves for these reproductive performances showed that the treated cows became pregnant significantly earlier than untreated cows ( $P < 0.01$ ).

**Conclusions:** PGF2alpha-induced expulsion of the uterine content at the time of diagnosis resulted in earlier pregnancies in treated dams compared to those untreated. Retained fetal tissues and annexes may delay the return to normal cyclicity due to the functioning fetal membrane. Ultrasonographic pregnancy diagnosis, therefore, plays a significant role in dairy management by allowing early detection and treatment of fetal death, resulting in shorter days open.

RE-26

### Effect of clinical mastitis around the time of insemination on the fertility of grazing dairy cows

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**Introduction:** The fertility of high-producing dairy cows has decreased during the last decades, concomitantly with an increase in milk yield. This lower fertility has partly been associated with pregnancy losses. Late embryonic losses (LEL), diagnosed by ultrasonography 30–44 days post-AI, represent a big component of these losses. Therefore, defining the risk factors for LEL would be very important to later develop strategies to control this problem.

**Objective:** The objective of the present study was to assess the risk factors for LEL in grazing dairy cows.

**Material and methods:** A data set of a commercial dairy farm having productive, reproductive and health records of dairy cows calving between Jan 1, 2011 and Dec 31, 2015 (n=13,551) was used in this study. Pregnancy was diagnosed by ultrasonography at 30–44 days post-AI. At this time, cows showing lack of a heart beats, membrane detachment, disorganization and echoic floating structures including embryo remnants were defined as having LEL (CASE). Standard disease definitions were used for diagnosis. Cows having retained fetal membranes, metritis, clinical endometritis and/or pyometra were classified as uterine disease (UD). Cows having clinical mastitis and/or clinical lameness were classified as non-uterine disease (NUD).

A case-control study was carried out with a temporal matching design to assess the risk factors for LEL. Four cows were randomly selected from non-case records (CONTROL, positive pregnancy diagnosis on the same date of each case) per every CASE of LEL included. The logistic model (Proc GLIMMIX, SAS) included the fixed effect of year of LEL (2011 through 2015), season of LEL (summer, fall, winter, spring), parity (1, 2, 3 plus), UD, NUD, anestrus, daily milk yield (MILK) and days in milk (DIM) to the event. Statistical significance was set at  $P < 0.05$  and a tendency at  $P < 0.10$ .

**Results:** A total of 642 cases of LEL were reported during the 5-year period. From 13,551 lactations started in this study period, 10,149 had a positive pregnancy diagnosis and were defined as non-cases, among which 2,568 were finally included in the study as controls. From all the included records, 6.3, 20.5, 27.2 and 14.7 % of cows suffered from LEL, UD, NUD and anestrus, respectively. Year and season had no effect on the odds for LEL ( $P=0.509$  and  $0.870$ , respectively). Parity had an effect on the odds for LEL given that cows from parity 2 (Odds Ratio [OR]=1.165, 95% Confidence Interval [95%CI]=0.897–1.512,  $P < 0.001$ ) and parity 3+ cows (OR=1.581, 95%CI=1.236–2.023,  $P < 0.001$ ) had higher odds of LEL than primiparous cows. The UD had no effect on the odds for LEL (OR=1.090, 95%CI=0.886–1.341,  $P=0.415$ ); whereas, NUD increased the odds for LEL (OR=1.298, 95%CI=1.044–1.616,  $P=0.019$ ). Anestrus had no effect on the odds for LEL (OR=0.931, 95%CI=0.704–1.231,  $P=0.615$ ). An increase of 1 SD in milk was associated with a 28% higher odd for LEL (OR=1.281, 95%CI=1.144–1.435,  $P < 0.001$ ). Finally, DIM also increased the odds for LEL (OR=1.008, 95%CI=1.007–1.010,  $P < 0.001$ ) given that the chance increased 0.8% per day.

**Conclusions:** The odds for LEL increased with parity, with the presence of NUD, with daily milk yield, and with DIM in grazing dairy cows. Conversely, the odds for LEL were not associated with year, season, and presence of UD or anestrus.

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**Key words:** late embryo loss, risk factors, milk yield, fertility, grazing dairy cow.

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RE-27

### Milk production, cell count, reproductive performance, and survival of crossbred cows in Australia

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**Objectives:** To compare milk production, cell count, reproductive performance, and survival between a) F1 Jersey (J) x Holstein-Friesian (HF) crossbred dairy cows ('J-HF') with their purebred parent breeds and b) F2 3-breed cross animals (Australian Red (AR) x J-HF) with F2 backcross animals (HF x J-HF).

**Materials and methods:** A retrospective cohort study was conducted using data from milk-recording herds extracted from the Australian Herd Improvement Scheme database. Lactations commencing from 1990 to 2013 inclusive were used. Milk production and individual cow cell counts were analysed using herds with at least one year with both valid breed data and high quality reproductive data. Milk, protein and fat yields for the first 300 or 305d of lactation were received from ADHIS (for simplicity, referred to as '305'-d yields). Protein and fat concentrations were calculated for each lactation as 305-d protein or fat yield, respectively, divided by 305-d milk yield; these were expressed as g/100mL milk. Individual cow cell counts greater than 10d and less than or equal to 305d after calving were used. The numbers of these counts that were greater than 250,000 cells per mL milk were counted for each lactation; each lactation was also classified as having none or at least one count that was greater than 250,000 cells per mL milk. Each cow's survival interval commenced with her first calving. Each cow's survival interval ended with culling or was right censored in the last recorded calving date for the herd. Confounding by herd was minimised by using only herds with all breeds being compared and fitting herd as a random effect. For the J-HF versus their purebred parent breed comparisons (depending on the breed and trait), 2591 to 1625582 lactations were used for production, reproduction, cell count and between 4012 to 35963 cows were used for survival comparisons. For the comparisons of the F2 cows (depending on the breed and trait), 543 to 23465 lactations were used for production, reproduction, cell count and 669 to 5499 cows were used for survival.

**Results:** For all reported differences, P was less than or equal to 0.001.

J-HF versus their purebred parent breeds: J-HF cows produced 604 litres less milk and 6.2kg less protein than purebred HF cows but 5.3kg more fat and had higher protein and higher fat concentrations (0.25 and 0.51g per 100 mL milk higher, respectively) than HF cows. The J-HF cows produced 1,093 litres, 29.5kg and 26.3kg more milk, protein and fat respectively than J cows but had 0.19 and 0.49g per 100ml milk lower protein and fat concentrations, respectively. There were no significant

differences between J-HF cows and their purebred parent breeds for the cell count variables. J-HF cows had higher 3-w submission rates (SR) (crude 77%) than either of the parent breeds (55% for HF and 70% for J), and higher first service conception rates (CR) (49%) than HF (39%). J-HF cows were more likely to survive than either of the parent breeds (hazard ratio for being culled 0.8 compared with HF or J).

F2 3-breed cross animals (AR x J-HF) versus F2 backcross animals (HF x J-HF): The 3-breed cross cows performed significantly better than the backcross for all production traits except milk volume, where there was no significant difference. There were no significant differences between 3-breed and backcross animals for the cell count variables. SR and CR did not differ significantly. However, a higher percentage of 3-breed cross animals became pregnant by week 6 of the breeding period (crude 6 week in calf rate 71% for 3-breed cross animals compared with 63% for backcross cows). The 3-breed cows were more likely to survive (hazard ratio for being culled 0.73 when compared with the backcross cows).

**Conclusions:** In Australian dairy herds, J-HF cows produce less milk and protein than purebred HF, but more than purebred J cows. The J-HF cows produce more fat than the pure breeds and have higher protein and fat concentrations than HF, but less than J cows. J-HF animals perform better in some measures of reproduction, and survive longer than the pure breeds. Farmers and advisors need to know the best breeds to use over J-HF cows, namely whether to use a third breed in a 3-way rotational breeding strategy or a backcross system (where one of the parent breeds is used over J-HF cows). On balance, the results from this study indicate that if the Australian Red were used as that third breed, this is preferable to a backcross strategy.

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RE-28

### Relationships between Body Condition Score, milk production and reproduction in seasonally calving, pasture fed New Zealand dairy cows

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Body condition score, milk protein, 6-week in calf rate

**Objectives:** The study was carried out to assess the relationship between body condition score (BCS), milk production and reproduction for 8,500 seasonally calving dairy cows on 10 commercial, New Zealand pastoral dairy farms. BCS was measured at routine events (milking or other scheduled management tasks) and involved no extra animal handling. Therefore, under Section 5(2) of the Animal Welfare Act 1999, the approval of an animal ethics committee was not required.

**Materials and methods:** BCS was visually assessed once a month from July to December by a trained veterinarian using the DairyNZ 10-point range for every cow in the herd. All farms were scored in the same order over a period of 10 working days so that the interval between scoring was a consistent 30 days for each cow. The planned start of calving for all herds was at the end of July and terminated 10 weeks later. Mating for all cows started 85 days after the planned start of calving



and typically consisted of 6 weeks of artificial insemination followed by 4 weeks of bull mating.

Cow and lactation level data was extracted from the national herd database and combined with the BCS data using mixed multivariable logistic regression and general estimating equations to model the logit of the probability of pregnancy after 6 weeks of mating. Models were adjusted for herd, year, cow age, breed, days in milk, BCS at calving, BCS change between calving and mating, volume adjusted milk protein concentration pre-mating and where data was available, pasture neutral detergent fibre (NDF) and dry matter (DM) content pre-mating.

**Results:** After adjusting for the other variables 6 week in calf risk was positively associated with BCS greater than or equal to 5.0 at calving (DairyNZ 10-point range) and negatively associated with BCS less than 5.0. Cows that lost more than 0.5 BCS units between calving and mating were significantly less likely to become pregnant within 6 weeks of mating as were cows failing to gain BCS over the mating period. Milk protein percent below the median and low pasture DM was also associated with a lower 6-week in calf risk.

Cows above BCS of 5.0 at calving were more likely to lose more BCS after calving and more likely to continue to lose weight during mating.

**Conclusions:** These results highlight the importance of achieving BCS targets at calving for successful reproductive outcomes and indicate that BCS loss, milk protein content and pasture characteristics may all be useful indicators identifying cows at risk of poor reproductive outcomes.

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RE-29

### The behaviour of Nelore cows of different ages as an indicator of efficiency in a pasturing system in the Bolivian tropic.

#### The behaviour of Nelore cows of different ages

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Bovine production is moving to the warm tropics due to the fact that this ecosystem -because of its higher luminosity and temperature- has a richer production of biomass, when compared with the mild zone. However, the characteristics associated with the biological efficiency such as reproduction and longevity have been worsening despite their importance for the viability of the agricultural business. The objective of this work has been the evaluation of the productive and reproductive behaviour of adult and growing Nelore cows in pasturing condition in the Bolivian tropic. This investigation work was carried out between October 2016 and November 2017 in the Cattle Ranch E7 in Colonia Japonesa San Juan, Santa Cruz, Bolivia (17°17" south latitude and 63°50" west longitude). The climate is typical of Savannah to subtropical wet Forrest. With an annual average temperature of 24.1°C (maximum average: 29.1°C and minimum average 19.4°C); it is 300 above sea level with an annual relative humidity of 68%. The average annual rainfall is 1,881 mm;

occurring only in six months, having a wet season with heavy rains and a dry season. Feeding was based on pastures of *Brachiaria decumbens* and *Brachiaria brizantha*, with a smaller contribution of other species such as *Brachiaria humidicola* and *Panicum maximum*. 100 Nelore cows were used (28 ≥ 5 years old (Adult group: GA) and 72 ≤ 5 years old (Growing group: GC), in which the following variables were registered: delivery-delivery interval (ipp) in days, body condition (ec) from 1 to 5, live weight (pv) in kilograms and weight of calf at birth (pt) in kilograms. Ec and pv were checked every 30 days throughout the study, the calf weight was obtained with a manual electronic scale (POCKET BALANCE; made in Germany) on the day of birth. Data as regards live weight was obtained at the same time and with a calibrated electronic scale ICONIX, New Zealand Ltd). The interval delivery-delivery was obtained between the date of delivery and the previous one for each cow. The existence of significant differences between the groups GA and GC were proved through the variance analysis to a classification criterion and multiple comparison tests HSD of Turkey-Kramer HSD ( $p \leq 0.05$ ). Results showed that adult cows had shorter ipp (GA: 379.9±28; GC: 505.6±18 days,  $p \leq 0.003$ ), better body condition (GA: 2.4±0.06; GC: 2.2±0.02,  $p \leq 0.002$ ), higher live weight (GA: 461.3±7; GC: 418.3±5 kg,  $p \leq 0.0001$ ) and higher calf weight with no significant differences  $p \geq 0.05$  (GA: 34.1±0.8; GC: 32.3±0.5 kg). Due to their better body condition and their live weight, the adult cows were able to give birth to almost a calf per year, having the calf a weight which gives them higher survival chances. Keeping a bigger number of adult cows in the rodeo - close to 80%, would mean - from a zootechnical concept- that the characteristics associated to biological efficiency such as reproduction and longevity of animals would increase the sustainability of the productive system. It is concluded that adult cows have a better productive and reproductive behavior, and therefore, it would be important to keep a higher percentage of them in the rodeo for the systems under study.

Key words: production; reproduction; longevity, Nelore cows, pasturing system, tropic.

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RE-30

### Fluctuation of eating time in the dry period affects fertility parameters in subsequent lactation

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During the last years, the development and the use of sensors on dairy farms, has given the opportunity to monitor behavior of cows in a continuous way. Therefore, it is possible to implement this data in daily management processes. To investigate the relationship between eating behavior during the transition period and fertility in dairy cattle, we studied the 1) interval between calving and first insemination; 2) number of inseminations; and 3) expected calving interval of dairy cows, in 1141 cows on 17 farms. These cows were equipped with Nedap



Smarttag Neck sensors from 42 days (d) before until 28 d after parturition, that continuously measured the time cows spent eating during this period. A Cox proportional hazard model was used to analyze eating time in relation to fertility variables. A larger standard deviation of the observed eating time from 30 days to 2 days *pre partum* resulted in a higher number of inseminations and a longer expected calving interval. A longer interval between calving and first insemination, higher number of inseminations and longer expected calving interval were observed due to a larger difference in eating time between day 2 before parturition and day 2 after parturition. The individual farm effect was of substantial influence on the fertility parameters as well, which was taken into account in statistical analysis. Furthermore, a difference between primiparous and multiparous cows was found concerning the fertility parameters too. In heifers, a shorter interval between calving and first insemination; a lower number of inseminations; and a shorter expected calving interval were found. The results of this study underline the important relationship between eating time during the transition period and fertility parameters after parturition. This study demonstrates, for the first time, that more fluctuation in eating time during the period from thirty days before parturition until two days before parturition correlates with a greater risk of reduced fertility in dairy cows.

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RE-31

### **The effect of Bio-active peptides from FPP (Fermented potato protein) on reproductive and production performance in dairy cows: a field trial in on Belgian Dairy Herd**

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**Introduction:** Milk yield and reproductive performance are both considered fundamentally important to the economic viability of dairy operations. The reproductive efficiency of dairy cattle has decreased for several decades as evidenced by decreased conception rates, more services per conception, and longer calving intervals in modern dairy cattle. Milk production has steadily increased during the period of reproductive decline. The greater nutritional and metabolic demands of high milk production, therefore, may be responsible for the decline in reproductive performance. Transition cow management and nutrition have been recognized as the most important variables in reproductive performance, as well as in milk yield.

The growth hormone/insulin-like growth factor (IGF) system plays a critical endocrine role controlling nutrient metabolism in dairy cattle. In liver, growth hormone receptor (GHR) and IGF-1 are dynamically regulated by lactation and energy balance. Lianol® Dairy contains natural messenger molecules that support the secretion of the hormone Insulin like Growth Factor (IGF-1) in the liver.

The aim of our study was to determine to effect of feeding Lianol® Dairy postpartum on reproductive parameters and milk yield on a Belgian dairy farm.

**Materials and Methods:** A randomized clinical trial was conducted on a well managed dairy herd, not suffering with transi-

tion problems (e.g. ketosis, hypocalcemia, acidosis,...) in Flanders, Belgium. Cows and heifers received either 30gram/cow/day Lianol® Dairy during the first 100 days of lactation (n=72) or received no additive (n=72) via an automatic feeder in the automated milking system (AMS). Data was collected by the herd management system and the AMS software. Prior to statistical analysis, observations were explored and checked for unlikely values. No data were excluded for this reason. At the moment of writing this abstract, the datasets were not yet fully completed. Nevertheless, some preliminary descriptive results can already be mentioned. The association between supplementation (yes or no) and milk yield during the first 120 days in milk will be done by using mixed models with test-day MY (kg) as outcome variable. The association between supplementation (yes or no) and conception rate at first service will be examined using contingency tables and  $\chi^2$  analysis. To compare the expected (confirmed) calving interval from supplemented and control animals, survival curves of both groups will be plotted using a Kaplan-Meier survival analysis. The dataset will be fully complete by April 2018.

**Results:** In primiparous animals the average conception rate at first service was 29.4% (n=17) and 36.8% (n=19) in the control group and the supplemented group, respectively. In multiparous cows the conception rate was 21.7% (n = 46) in the control group and 33.3% (n=42) in the supplemented group. The confirmed expected average calving interval was 360 days (SD=38) in the control group and 354 days (SD=38) in the supplemented group for the primiparous animals. Also, in the multiparous animals the average calving interval was 377 days (SD=58) and 359 days (SD=51) in the control group and the supplemented group, respectively.

In the heifers, average daily milk production in the first 60 days in lactation was 26.2kg (SD=6.9) and 27.2kg (SD=7) in the control and the supplemented group respectively. Average daily milk production in the multiparous cows was 37.3kg (SD=11.5) in the control group and 35.5kg (SD=10.7) in the supplemented group.

**Conclusions:** Preliminary results shows that supplementing a natural IGF-1 stimulating feed additive to dairy cattle during the beginning of lactation can affect both reproductive performance and milk yield. The effects seems to depend on parity.

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RE-32

### **Use of sexed semen in first and second lactation cows**

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Using sexed semen in dairy cattle has been traditionally restricted to heifers, due to the low fertility rates obtained with this practice; however, since the sexed semen quality seems to have improved in later years, showing improvement in efficiency, it seems feasible its use in lactating cows.

Results of sexed semen used in first and second calf cows are presented in this paper. Positive results obtained not only in adequate fertility but in faster grow of replacement calf invento-



ries, allow consider using this practice in a more extensive way.

Fertility obtained among groups of first and second calf cows bred with or without sexed semen will be compared, using general fertility rate as reference value.

147 dairy females using sexed semen and 131 dairy females using conventional semen were used in this work, which were classified in 3 groups: Virgin heifers(VH), first lactation cows(1LC) and second lactation cows(2LC), during 2 consecutive years. During first year, all females were bred with common frozen semen with the purpose of establishing base reproductive parameters; during the second year, sexed semen was used for all the females.

The following parameters were evaluated: Days to first service(D1S), and calving interval(CI), for which Variance Analysis test(ANOVA) and Tuckey test were used to determine difference among groups; additionally pregnancy rates at first(%1S) second(%2S) and third service(%3S) as well as females born(%FB) were evaluated using the Squared Chi test to determine the difference among groups. In every case, a level of significancy of 95% was used.

No significative differences were found among D1S( 75.34 days), CI(445.4 days) between first and second lactation. Regarding pregnancy rates for 1LC values of %1S 25.08 and 29.37, %2S 25.08 and 29.37, %3S 43.51 and 48.79 were found using or not sexed semen respectively, for 2LC, values of %1s 31.83 y 25.42, %2S 24.91 y 24.38,%3S 43.26 y 50.2 using or not sexed semen respectively. As for VH values of %1S 46.25 y 42.67, % 2S 32.62 y 28.98, %3S 21.15 y 28.35.

Lower values were found in cow groups using sexed semen however there are no significative differences.

Regarding %FB values of 77.26 and 49.05 with or without sexed semen respectively, founding significative difference between groups.

Comparing fertility rates between heifers and milking cows using or not sexed semen show no significative difference in this work but do show besides significative difference in the number of females born in percentage, a great potential to increase much faster the size of herds by increasing the number of replacements and milking cows using sexed semen not only in heifers but also in 1<sup>st</sup> and 2<sup>nd</sup> lactation cows.

This herd was formed in 2013 when 150 pregnant heifers were bought. Up to the date, the total inventory is 446 females: 232 milking cows and 214 heifers meaning an spectacular increase of 297.33% in only 4 years. Of course no heifers have been bought since.

RE-33

### Effect of a novel synchrony program including a second treatment of Prostaglandin F<sub>2α</sub> on fixed time artificial insemination results in pasture-based dairy herds

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**Objective:** To evaluate the effect of an additional prostaglandin F<sub>2A</sub> injection, within a standard Ovsynch program, on first-service conception rate in pasture-fed dairy cows.

**Methods:** We conducted a randomized clinical trial involving 1703 dairy cows selected from five split calving, pasture-fed dairy herds in Southwest Victoria, Australia. At the appropriate time in the mating cycle in each herd, lactating cows were allocated to two groups. The control group (n = 851), received the original Ovsynch program: gonadotropin-releasing hormone at day 0, prostaglandin F<sub>2α</sub> at day 7, Gonadotropin-releasing hormone at day 9 and fixed time artificial insemination on day 10. The treatment group (n = 852), received a modified Ovsynch program, which incorporated an additional injection of prostaglandin F<sub>2α</sub>: gonadotropin-releasing hormone at day 0, prostaglandin F<sub>2A</sub> at day 7, prostaglandin F<sub>2A</sub> at day 8, gonadotropin-releasing hormone at day 9 and fixed time artificial insemination on day 10. A subset of animals from each group in each herd (n=95 treatment cows and n=99 control cows) had blood samples taken at the time of the first prostaglandin injection and at the time of artificial insemination. Serum progesterone concentrations were measured in these cows to assess the proportion with complete corpus luteum regression and the likelihood of ovulation at the time of artificial insemination.

**Results:** After adjusting for the effect of herd, conception rates were 7.0% (95% CI 2.3% to 11%; Chi-square test statistic 8.26; P<0.01) greater in cows in the treatment group compared with cows in the control group. The proportion of cows with plasma progesterone concentrations greater than or equal to 1ng/mL in the treatment group was 13.5% (95% CI 2% to 22%; Chi-square test statistic 4.77; P=0.03) lower than that of cows in the control group.

**Conclusion:** Compared with the original Ovsynch program, a modified Ovsynch program involving an additional injection of prostaglandin on day 9 increased first conception rates in pasture-fed, split calving dairy cows in Southwest Victoria by 7.0%. Compared with the original Ovsynch program, we estimate that a total of 14 (95% CI 8 to 43) cows need to be treated using the modified Ovsynch program to return one additional pregnancy.

RE-34

### A new intravaginal progesterone-releasing device from Vietnam: Plasma progesterone response after insertion and efficacy of estrus induction in cattle.

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**Objectives:** Vietnam have studied to create intravaginal progesterone releasing device (referred to as ProB) in order to reduce the dependence on exotic products in bovine reproductive management. ProB consists of a T-shaped spine coated with silicon rubber, loaded with 1.3gr progesterone evenly dispersed therein.

The study was performed so as to investigate progesterone plasma concentration after insertion of ProB into vaginal of ovariectomized cows. In addition, the effect of Ovsynch plus ProB protocol on resumption of normal ovarian cycle was eval-



uated in anestrus dairy herd.

**Methods:** Experiment 1. Four ovariectomized cows consisting of 03 HF cows and 01 Red Sindhi cow were randomly inserted 03 ProB and 01 CIDR; after 7 days of insertion, all intravaginal devices were removed from cows.

Blood samples were collected daily at 7:00-9:00 hours into tubes with micronized silica particles from cows via the tail vein puncture at 30 minutes before insertion, 7 days during insertion and 1 day after removal. Immediately after collection, blood samples were stored at 4°C and centrifuged. The plasma obtained after separation were decanted into Eppendorf tubes and stored at -20°C until analysed by ELISA.

Experiment 2. Two hundred and three HF cows (anestrus until >100 days post-partum) which were fed in both of commercial farms and smallholder farms were assigned to one of four groups. Cows in smallholder farms were received Ovsynch plus ProB (group 1, n=50) or CIDR (group 2, n=50) protocols; cows in commercial farms were administered Ovsynch plus ProB (group 3, n=51) or CIDR (group 4, n=52) protocols.

Treatment as follows: D0, an intravaginal P4 device and GnRH; D6, PGF<sub>2α</sub> and P4 were removed; D7, GnRH.

Estrus expression was detected by visual observation in the next 7 days after treatments.

**Results:** Experiment 1. Thirty minutes after insertion P4 circulating levels increased dramatically (mean ± SE; 6.09 ± 1.82 ng/ml) and reached a peak of 8.56 ± 2.16 ng/ml. Then, the levels dropped gradually to 2.71 ± 0.79 ng/ml at the day 7. In cows given CIDR, plasma progesterone concentration raised rapidly to 17.75 ng/ml after a half hour of insertion and declined slightly to 5.79 ng/ml at the day 7. To sum up, both devices induced a high and quick elevation of pP4 circulating levels in the experimental cows and remained these concentration above 2 ng/ml until removal.

Experiment 2. The results of resumption normal cycle in cows fed in small-scale farms after given ProB-based protocol or CIDR-based protocol were both higher than in cows kept in intensive farms (82.0% vs 74.51%; 78.0% vs 69.23%) (P>0.05). In total of two experimental locations, the rate of estrus in cows given ProB-based protocol or CIDR-based protocol were 78.22% and 73.53%, respectively (P>0.05).

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RE-35

## The influence of insemination timing, post enrolment in an oestrus synchronisation programme, on twin pregnancy rates in dairy cattle.

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**Objectives:** To determine if the timing of artificial insemination (AI) following an Ov-synch or prostaglandin (PGF<sub>2α</sub>) oestrus synchronisation programme was a risk factor for twin pregnancies in dairy cattle.

**Materials and methods:** Reproductive data was collected from a high producing, freestall, year-round calving dairy herd

over a 12 year period in NSW, Australia. During the latter part of the study period the herd expanded from 2000 to 3500 milking Holstein cows.

Weekly veterinary reproductive visits included pregnancy diagnosis by trans-rectal ultrasonography at 32 - 39 days post-insemination. The farm utilised a combination of natural heat, PGF<sub>2α</sub> and Ov-synch synchronisation programmes, with all breedings by AI. Over the 12 year period 12,267 pregnancies were recorded in lactating cows.

Insemination data, days in milk, parity, previous twin history, synchronisation details, pregnancy results and number of calves born were recorded using Dairy Comp 305. A Multivariable Generalised Linear Mixed Model (GLMM) with binomial distribution was conducted using Genstat (Schall 1991 Biometrika method, VSNi), with twin births as the response variate and cow ID as the random factor. Variables entered into the model included; days in milk, parity, year, history of twins, Ov-synch breeding, PGF<sub>2α</sub> breeding, Ov-synch intervals, PGF<sub>2α</sub> intervals and season of insemination (Spring, Summer, Autumn, Winter).

**Results:** Twin births accounted for 1,065 (8.6%) calving events. Twinning following natural heat was 8.1%, following Ov-synch was 8.64%, and following PGF<sub>2α</sub> breeds was 10.2%. There was no significant difference in twinning following Ov-synch breeds overall. Twinning was significantly increased following PGF<sub>2α</sub> synchronisation. Closer examination of the relationship between synchrony program and twinning outcomes revealed that insemination on days 1-4 post PGF<sub>2α</sub> administration resulted in a lower incidence of twins (8.5%) compared to breedings that occurred 5-9 days following PGF<sub>2α</sub> administration (14.47%). When cows were inseminated on day 10 according to a standard Ov-synch program the incidence of twins was 7.16%. Cows that were observed to cycle and bred during the Ov-synch program had a higher incidence of twins with breeding on days 8-9 resulting in 14.3% twins and breeding on days 1 - 7 resulted in 12% twins. The difference in the incidence of twins according to breeding interval was statistically significant (P < 0.001)

Other variables found to significantly influence the risk of twinning included season, parity and year. A higher percentage of twins were conceived in Autumn (9.51%) and winter (9.51%). Spring (7.25%) and summer (8.23%) had a lower incidence of twins.

Breedings of first lactation cows produced the lowest incidence of twins (7%), the highest incidence was observed with second lactation cow breedings (10.15%) with a stepwise reduction in incidence in lactations 3 (9.5%), 4 (8.1%), and 5 (7.1%). A significant increase in twins was observed in the last 2 years of the study period which corresponded to the implementation of a double PGF<sub>2α</sub> in the Ov-synch.

**Conclusion:** On initial glance, synchronisation programmes appear to have no effect on twinning however on closer examination the timing of AI following synchronisation enrolment is significant. The increased incidence of twin births following the introduction of a double PGF<sub>2α</sub> within an Ov-synch raises the question as to its impact on the mechanism of twinning however further research is required to confirm this.



RE-36

### Evaluation of Reproductive Performance of Dairy Heifers Subjected to two Estrus Detection Systems and treated with two Prostaglandin Formulations

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**Objectives:** Efficient and accurate identification of estrus is critical for the success of estrous detection based reproductive management. We hypothesized that management of dairy heifers with the aid of an automated estrous detection system (AES) would improve service rate and consequently the reproductive performance of dairy heifers. In addition we hypothesized that treatment of heifers with cloprostenol (CLO) would improve pregnancy per service compared with treatment with dinoprost (DIN). The objectives were to evaluate the reproductive performance of dairy heifers managed with the aid of an AES vs. a mounting detector device (MD) and synchronized with CLO vs. DIN.

**Material and Methods:** Holstein heifers (n = 1,019) from a dairy herd in the Southeast of the USA were enrolled in the experiment at approximately 11 months of age when they were fitted with a Heat Rumination Long Distance collar (SCR Ltd., Netanya, Israel). At approximately 12 months of age heifers were randomly assigned to an estrous detection treatment (EDT: AES vs. MD) and PGF<sub>2α</sub> treatment (CLO vs. DIN) according to estrous cycle day at PGF<sub>2α</sub> treatment (d 4 to 6 vs. d 7 to 26). Heifers in the AES treatment were serviced according to estrus detected by changes in activity and rumination, whereas heifers in the MD treatment were serviced when detected in estrus by farm personnel according to the activation of the KAMAR device (Steamboat Springs, CO). Heifers were treated with PGF<sub>2α</sub> (same formulation throughout the experiment) every 14 d or until serviced. The experiment had a 2x2 design according to estrous detection treatment (AES vs. MD) and PGF<sub>2α</sub> treatment formulation (CLO vs. DIN) and was analyzed as such, controlling for estrous cycle day at PGF<sub>2α</sub> treatment. Binary variables were analyzed by logistic regression using the LOGISTIC procedure. Hazard of service and pregnancy were analyzed by the Cox proportional hazard ratio using the PHREG procedure. Statistical significance was considered at  $P < 0.05$  and tendency at  $0.05 < P < 0.10$ .

**Results:** Estrous detection treatment did not ( $P=0.17$ ) affect the hazard of service. There was a tendency ( $P=0.06$ ) for CLO heifers to have increased hazard of service (AHR=1.14, 95%CI=0.99-1.30) than DIN heifers. The interaction between EDT and PGF<sub>2α</sub> treatment did not ( $P=0.65$ ) affect the hazard of service. Among heifers receiving artificial insemination for first service, EDT ( $P=0.78$ ), PGF<sub>2α</sub> treatment ( $P=0.19$ ), and the interaction between EDT and PGF<sub>2α</sub> treatment ( $P=0.81$ ) did not affect pregnancy at 75 d after first service. Among heifers receiving embryo transfer for first service, there was a tendency ( $P=0.06$ ) for AES treatment to increase pregnancy at 75 d after estrus compared with MD treatment (AOR=1.52, 95%CI=0.98-2.36), but PGF<sub>2α</sub> treatment ( $P=0.71$ ) and the interaction between EDT and PGF<sub>2α</sub> treatment ( $P=0.75$ ) did not affect pregnancy at 75 d after estrus. Hazard of pregnancy was not affected by EDT ( $P=0.25$ ), PGF<sub>2α</sub> treatment ( $P=0.51$ ), and by

the interaction between EDT and PGF<sub>2α</sub> treatment ( $P=0.54$ ).

**Conclusions:** In a commercial dairy farm in the Southeast of the USA, reproductive management with an AES did not improve hazard of pregnancy, despite slightly increased pregnancy to embryo transfer. Despite a tendency for increased hazard of service, treatment with cloprostenol did not improve hazard of pregnancy of dairy heifers.

RE-37

### Evaluation of an ear-attached accelerometer for detecting heat events in indoor housed dairy cows

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**Objective:** For a successful and efficient dairy farming a good reproductive performance is essential. In particular, a reliable heat detection and the determination of the optimal insemination time is important. Visual heat detection is often challenging for farmers, because of a decreased duration of visual signs of heat, a less expressive estrus behavior and a high proportion of heat events occurring during night hours. Hence, various automated estrus detection technologies were developed to assist the farmers.

The company Smartbow (Weibern, Austria) developed the ear-attached sensor system SMARTBOW for a wireless, continuous and real-time monitoring of physiological and pathophysiological conditions in cows. The electronic ear tag consists of an accelerometer for detecting head and ear movements in three dimensions (x-, y- and z-axis). By processing the data, information on an animal's activity, rumination and localization inside the barn is provided. Estrus detection by the SMARTBOW system is primarily based on an increased activity and behavioral changes of an animal by considering its physiological state.

The objective of this study is to evaluate the reliability and efficiency of different algorithms developed for heat detection under field conditions.

**Materials and Methods:** 500 multiparous Holstein Friesian dairy cows from a commercial farm housing 2,700 cows in total were enrolled in this study and equipped with ear-attached accelerometer. The cows were kept in groups of approximately 200 animals in free-stall barns on concrete floors. All animal related events (e.g. heats, inseminations, disease) were entered into the herd management software by the farm personnel.

In this study, a heat followed by an artificial insemination which led to pregnancy was defined as 'true heat event' and is used as golden standard. Inseminations resulting from an ovsynch protocol were considered in the statistical analyses.

For the evaluation of the heat detection algorithms, heat alerts provided by the SMARTBOW system were retrospectively compared with insemination and reproduction data entered into the herd management software. A heat alert provided by the



SMARTBOW system is classified as 'true positive' (TP) if the heat alarm matches with the golden standard. Alerts which occurred outside of a 'true heat' event are defined as 'false positive' (FP). Based on these classifications, performance measures of the system, e.g. the sensitivity and the positive predictive value will be calculated.

**Results:** Currently, statistical analyses are performed using different algorithms for heat detection. The final results will be presented at the conference.

**Conclusions:** In recent years, several heat detection systems were brought to the market by various companies. As for many systems reliable heat detection rates are not reported, systems should be independently tested under field conditions in order to evaluate their potential effectiveness and economic benefit.

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RE-38

### **The use of genetic selection to improve herd reproductive performance of dairy cattle in Northern Victoria, Australia**

*A retrospective cohort study to examine the efficacy of the daughter fertility Australian Breeding Value, and to explore the attitudes and intentions of dairy farmers toward selection of high daughter fertility ABV sires.*

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**Introduction:** In 2013, in order to halt the decline of cow fertility within the Australian dairy industry, geneticists developed the Daughter Fertility Australian Breeding Value (ABV). This genetic estimate allows farmers to select AI sires who produce higher fertility daughters, improving herd reproductive performance over successive generations.

As with any new management tool, farmers can be slow to adopt changes until they are aware that the practice exists, have the skills to implement it, and are confident that it is worthwhile. Accordingly, there is a need to validate the use of the Daughter Fertility ABV in order to better demonstrate its value. There is also a need to describe farmer usage and attitudes towards the Daughter Fertility ABV in order to identify barriers and improve uptake.

**Objectives:** With this in mind, the first objective of this study is to determine whether the Daughter Fertility ABV is an effective tool for improving cow fertility, using field data collected from clients at Rochester Veterinary Practice. The second objective is to explore farmer attitudes and intentions towards genetic selection for daughter fertility, with the end goal of identifying key attitudes, information sources and perceived barriers that encourage or inhibit farmers from using the ABV.

**Materials and methods:** The study is split into two parts. The first part is a retrospective cohort study, using historic herd records and pregnancy testing data for over 82,000 cows collected from 35 dairy farms in Northern Victoria, Australia. Using

veterinary epidemiological principles, a multivariate linear regression analysis was performed in order to quantify the association between the Daughter Fertility ABV and phenotypic expressions of cow fertility. These include Australian industry standards such as the 6-week in-calf rate, 3-week submission rate and conception rate.

The second part of this study explored farmer attitudes and intentions towards genetic selection for daughter fertility, using the Theory of Planned Behavior as a social research framework. 33 farmers were interviewed about their salient beliefs, social norms and perceived barriers regarding the selection of high daughter fertility ABV sires. These results were then used to test the strength and prevalence of these beliefs amongst the wider population of Rochester Veterinary Practice clients (n = 160) via a postal survey. Principal component analysis identified clusters of demographic and attitudinal beliefs that had an impact on farmer intentions to select high daughter fertility ABV sires.

**Results:** Results are preliminary at the time of writing this abstract. However, all analyses will be fully completed by the time of the conference in 2018.

**Conclusions:** It is anticipated that the Daughter Fertility ABV will have a positive correlation with 6-week in-calf rate, 3-week submission rate and conception rate. However, the strength of this association is currently unknown.



RT-01

### Establishing a robust ovum pick up (OPU) and *in vitro* embryo production (IVP) system for use in UK cattle breeding.

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**Objectives:** Since 2005 there has been a steady decline in the number of *in vivo* produced embryos from around 800,000, to 660,000 in 2015, while the number of transfers of *in vitro* produced embryos has steadily increased to around 613,000 in 2015 (Perry, 2016). However, this has been largely driven by a steady increase in the number of transfers in South America (especially with *bos indicus* cattle) since 2001. Although there has been an annual increase of around 30% in the numbers of OPU/IVP embryos transferred in North America since 2011, there has been little change in the numbers transferred in Europe (Perry, 2016), and there are very few embryos transferred in the UK. Here we report the first large scale retrospective analysis of factors influencing the establishment of a commercially robust ovum pick up (OPU) and *in vitro* embryo production (IVP) platform in the UK.

**Materials and methods:** Over a 5-year period, a robust OPU/IVP system was gradually developed and validated for use in the UK. Various datasets including two IVP laboratories and 6 OPU teams were analysed that spanned this period. Here we investigate some of the key factors that affect successful and consistent OPU/IVP embryo production in the UK; whether these be donor effects, cow-side techniques and protocols, laboratory techniques and protocols or recipient traits. The benefits of utilising follicle stimulating hormone (FSH) and 'coasting' as part of a standard protocol were investigated. Coasting refers to short-term (typically 48h) hormonal withdrawal after FSH stimulation, and prior to OPU, with or without prior dominant follicle removal (DFR). A composite score is proposed that combines oocyte numbers and oocyte quality (sCOC) and this was tested against OPU/IVP outcomes.

**Results:** There were differences ( $P < 0.001$ ) in the mean number of follicles aspirated by the various OPU teams ranging from 6.5 to 14.9, as well as the number of oocytes collected ( $P < 0.001$ ). There was an indication ( $P = 0.055$ ) that blastocysts per oocyte rate varied across the teams. These findings emphasise the need for a meticulous standardised set of protocols, and that the skill and experience of the operators are crucial.

There was an interaction ( $P < 0.001$ ) between ovarian stimulation/coasting and semen type, with stimulation/coasting improving embryo development when sexed, but not unsorted semen, was used for IVF. There was an increase ( $P < 0.001$ ) in blastocyst yield per OPU cycle in stimulated vs non-stimulated cycles ( $-1.2$  vs  $0.4$ ). There were differences between two commercial products containing different levels of FSH and luteinising hormone (LH); Pluset® resulted in a greater ( $P < 0.001$ ) mean number of follicles aspirated, a higher ( $P < 0.001$ ) proportion of oocytes that were suitable for insemination and more ( $P = 0.003$ ) blastocysts per oocyte; while Folltropin® numerically was associated with a higher pregnancy rate per transfer (this was not significant due to the relatively small number of pregnancies recorded at the time of reporting).

Analysis of stimulated, (either with Folltropin® or Pluset®) and

non-stimulated cycles from the largest dataset, from a single OPU team using one IVP laboratory (to reduce confounding variables), demonstrated that several measures of oocyte quality and competence differed. Grade 1 cumulus oocyte complexes (COCs) as a proportion of total COCs recovered ( $P < 0.05$ ), oocytes that cleaved as a proportion of total COCs ( $P < 0.001$ ), and blastocysts as a proportion of COCs ( $P < 0.001$ ), were all greater for stimulated than non-stimulated cycles irrespective of stimulation product used. It was shown that the Log Mean sCOC was highly correlated ( $P < 0.001$ ) with both the proportion of blastocysts produced per oocyte collected, and to the total number of embryos produced per cycle.

**Conclusions:** This study demonstrated that there is a relationship between our composite score of COC quality and number of COCs aspirated and blastocyst yields. Data collection and analyses are ongoing, to identify other key performance indicators within the OPU/IVP embryo transfer (ET) system, with a view to refining the model.

RT-02

### Efficacy of a single measurement of plasma anti-Müllerian hormone concentration for ovum pick-up donor selection of Japanese Black heifers in herd breeding programs

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**Objectives:** Anti-Müllerian hormone (AMH), secreted from the granulosa cells of growing ovarian follicles, is a good predictor of not only follicle and oocyte number at ovum pick-up (OPU) but also their quality (Gamarra *et al.*, 2015). Additionally, a recent study of Japanese Black (JB) heifers revealed that circulating AMH concentrations in heifers aged 10 or 11 months correlate with the number of embryos collected after superovulation at 13 to 18 months (Hirayama *et al.*, 2017). Based on the above-mentioned findings, we speculated that a single measurement of AMH concentration in heifers aged approximately 10 months may be useful in predicting their breeding potential of OPU-donor heifers undergoing routine OPU treatment 3 to 4 months later. The objective of the present study was to evaluate the relationship between a single plasma AMH concentration in heifers aged approximately 10 months and the number of oocytes recovered after OPU treatment at 13 months of age. Additionally, the AMH concentrations were compared among sister heifers from the same parents using the embryo transfer (ET) technique in recipient cows.

**Materials and Methods:** Female JB heifers from a single farm with the same feeding and environment were used in this study. All heifers were produced via embryo transfers from recipient cows. Blood samples were obtained from 50 heifers aged 7.3 to 10.2 months (average, 8.7 months). Plasma AMH concentrations were measured using a commercially available ELISA kit (Beckman Coulter, Inc., Brea, CA, USA). Based on the 25th, 50th (median), and 75th percentiles of mean AMH concentrations, the OPU-donor heifers were divided into 3 groups: group





H - high AMH concentration; group MH – intermediate concentration; and group L – low concentration. Among them, 25 heifers were selected as OPU-donors (group H: n = 10; group MH: n = 6; and group L: n = 9). Heifers without measured plasma AMH concentrations were used as controls (group C: n=84). OPU was conducted in 13-month-old heifers 3 times every 2 weeks by three skilled technicians. Both the number of follicles (2–9 mm), determined via ultrasound examinations, and the number of collected oocytes after OPU were counted and compared among the groups.

**Results:** Plasma AMH concentrations in the JB heifers ranged from 28 to 1998 pg/mL, and the mean  $\pm$  standard deviation (SD) was 363.5  $\pm$  353.4 pg/mL. The 25th, 50th (median), and 75th percentiles of AMH concentrations were 139, 232, and 469 pg/mL, respectively. The mean numbers of follicles at the first and third OPU trials were not significantly different for each group of heifers (29.8  $\pm$  4.4 and 29.4  $\pm$  14.0 in group H, 22.7  $\pm$  7.9 and 22.0  $\pm$  5.7 in group MH, 21.3  $\pm$  7.6 and 19.1  $\pm$  6.2 in group L, and 20.1  $\pm$  8.7 and 20.2  $\pm$  6.2 in group C, respectively). Finally, the mean numbers of follicles from the 3 OPU trials were 29.6  $\pm$  10.2 in group H, 22.5  $\pm$  5.8 in group MH, 20.2  $\pm$  6.4 in group L, and 20.1  $\pm$  7.2 in group C. Group H had a significantly higher ( $p < 0.05$ ) mean number of follicles compared to other groups. The mean numbers of collected oocytes at the first and third OPU trials were not significantly different for each group of heifers (26.0  $\pm$  9.9 and 26.7  $\pm$  16.2 in group H, 21.2  $\pm$  13.2 and 19.2  $\pm$  7.4 in group MH, 18.1  $\pm$  5.4 and 17.4  $\pm$  6.7 in group L, and 15.6  $\pm$  7.7 and 16.1  $\pm$  7.5 in group C, respectively). Finally, the mean numbers of collected oocytes from the 3 OPU trials were 26.2  $\pm$  12.5 in group H, 20.7  $\pm$  8.7 in group MH, 17.4  $\pm$  6.2 in group L, and 16.1  $\pm$  7.3 in group C. Group H had a significantly higher ( $p < 0.01$ ) mean number of collected oocytes compared to groups L and C. In the present study, 2 to 5 ET derived sister heifers were born from 13 donor cows. Although some sister heifers from the same donor parents showed similar AMH concentrations (such as 3 heifers from donor A; 226, 271, and 373 pg/mL or 2 heifers from donor B; 436 and 516 pg/mL), extremely different AMH concentration were observed among other sister heifers (such as 5 heifers from donor C; 35, 161, 246, 271, and 720 pg/mL or 3 heifers from donor D; 157, 921, and 1998 pg/mL).

**Conclusion:** These results clearly indicated that the heifers with the highest AMH concentrations (higher than 469 pg/mL) measured at 7.3 to 10.2 months of age had the highest numbers of follicles to be punctured in their ovaries and gave the highest numbers of collected oocytes at OPU conducted at 13 months of age. The results of this study showed that a single measurement of plasma AMH concentration in heifers is practically useful in selecting excellent OPU-donor heifers in herd breeding programs. Additionally, it was suggested that the differences observed between heifers within each family could be due to genetic differences between sisters or due to environmental differences related to the nutritional conditions of the recipient cows. Overall, a single measurement of plasma AMH concentration may contribute to the acceleration of intensive breeding of JB herds.

RT-03

## A Novel Approach to Monitoring Working Dairy Breed Natural Service Sires

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**Objectives:** While natural service sires can contribute substantially to the reproductive performance of seasonally calving, pasture-based dairy herds, relatively little is known about the how bulls partition their time, how far they walk, how often they mount and serve or how much time is spent grazing, ruminating and resting.

This information is important, since it can be used to plan periods of rest, or to rotate bull teams into and out of the herd. Provision of an optimal bull to cow ratio and ensuring that those bulls that are run with the herd are adequately rested and sound are important first steps to achieving herd reproductive targets.

The aim of this study was to quantify working bull activity (the amount of time mating, the amount of time grazing, and the amount of time walking per day) in an effort to better define daily metabolizable energy requirements.

**Materials and Methods:** This was a prospective observational study on an 800-cow dairy farm (run as two separate herds) near Smithton in north-western Tasmania, Australia. At the start of the study on the 22<sup>nd</sup> of December 2016 triaxial accelerometer collars fitted with global positioning system (GPS) units (obtained via the Tasmanian Institute of Agriculture and the Commonwealth Science and Industrial Research Organisation (CSIRO)) were deployed on ten working bulls. The collars were kept on the bulls for 14 days, unless broken or deemed unstable, in which case they were removed. Collared bulls were part of a 30-bull team used on the farm and the herd manager ran each of the collared bulls with the milking herd as he saw fit.

Throughout the 14-day follow-up period direct observations of bull behaviour in the paddock were made by the first author and assistant. Monitored behaviours included grazing, ruminating, resting, walking, mounting or serving. Each bull was continuously observed for periods of four hours each day and a record of the start time, end time and details of the type of behaviour expressed recorded using a microcomputer tablet application.

Details of bull location and movement were recorded on a secure digital card housed within the accelerometer collar applied to each bull. At the conclusion of the follow-up period, records of bull activities (from the tablet application) and the accelerometer readings were downloaded for analysis and submitted to CSIRO, Data61 Computational Intelligence Group, Hobart. Learning algorithms were then applied to the data to associate accelerometer readings from the sensor data with the observed movement categories.

**Results:** The learning algorithm was better able to predict periods of grazing ( $F = 0.86$ ) and walking ( $F = 0.91$ ) compared with ruminating ( $F = 0.19$ ) and resting ( $F = 0.38$ ). Grazing and walking behaviours were mapped for each bull over the study peri-



od and analysis is continuing.

Insufficient data were available to derive an algorithm that predicted mounting and serving behaviour with confidence.

GPS logging data for each bull will be analysed to provide an estimate of the number of metres walked per day and to compare those estimates with bulls from the same team that were at rest.

**Conclusions:** This study represents the first analysis of the daily activity of a commercial pasture based natural service sire and provides insight into the distance walked per day, the relative frequency of behaviours exhibited, and energy used. A better knowledge of how natural service sires behave in the paddock can provide herd managers and veterinarians with a better understanding of how bulls might be used, rotated and rested to optimise reproductive performance.

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RT-04

## Objective analysis of fresh and frozen bovine semen using CASA and flow cytometry

### Significant differences between populations of semen in practice

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RAFT Solutions Ltd

**OBJECTIVES:** Quality of frozen semen for artificial insemination (AI) is routinely assessed by semen production centres (SPCs) prior to release. Such assessments are not always independently verified. Once semen has been released there is generally no further routine quality assessment prior to use. Incorrect transport and storage of frozen AI straws may result in deterioration of semen quality. Semen may be handled a number of times after the initial quality control procedures at stud and this could result in significant reduction in fertility. Significant differences have already been found between insemination technicians with respect to motility and acrosome integrity of semen from the same ejaculates, indicating that storage and transport protocols can have an impact on semen quality. Opportunities exist to assess semen used on pedigree dairy/beef as well as commercial dairy/beef farms. Currently this is the only independent service offering computerised semen analysis to cattle farms in the UK (SemenRate).

**MATERIALS/METHODS:** Frozen/thawed, extended bull semen from 50 Yorkshire cattle farms were analysed using computer assisted semen analysis (CASA) and flow cytometer. Data was cross-checked by veterinary surgeons with semen evaluation experience. Semen analysis parameters were compared to subsequent fertility performance on farm to build a multi-parametric database of results (with eight European partners) to enable analysis of the parameters of most significance in terms of subsequent fertility outcomes on farm. The commercial service established (SemenRate) has been extended across mainland UK. The means of different populations of semen used in practice were compared using the t-test.

**RESULTS:** For mitochondrial activity conventional and sexed semen were significantly different ( $p < 0.0001$ ). Viability was significantly different for dairy vs beef sires ( $p = 0.0432$ ). Viability

and acrosome integrity was significantly different on dairy vs beef farms ( $p = 0.0043$ ). CASA motility was significant for conventional vs sexed semen ( $p = 0.0182$ ) and on beef vs dairy farms ( $p = 0.0129$ ), as was progressive motility ( $p = 0.0024$  and  $p = 0.0243$  respectively). Morphology was significantly different for conventional vs sexed semen, beef vs dairy sires, and beef vs dairy farms ( $p = 0.0257$ ,  $p < 0.0001$ ,  $p = 0.0271$ ).

**CONCLUSIONS:** The use of flow cytometry in genuinely multi-parametric analysis is generally not routinely occurring in SPCs in the UK or in Europe. There are significant differences between the different types of semen in commercial use. The correlation between semen quality parameters and field fertility was not being assessed currently in the UK and so now an EU collaboration will aim to establish figures relevant to the UK and across the EU. CASA technologies are currently principally used by breeding/semen companies to assess semen to ensure minimum standards prior to commercial release. SemenRate offers an independent service to vets and farmers. The results of semen analysis are clearly only part of an overall fertility solution on participating farms. As well as the technical laboratory based skills required to run the analysis and scientific knowledge required to interpret results, the veterinary herd health input required to identify and manage risks associated with poor semen quality on farm are needed in partnership with the referring veterinary surgeon. Solutions to the issues causing poor quality semen may be identified and corrected, resulting in use of better quality in the future. Alternatively managing the risk of suboptimal semen usage can help minimise the impact on fertility on that farm. For example, a farmer may be advised to use extra straws of semen per insemination of batches found to have 'compensable' defects. Likewise appropriate advice on semen identified as having non compensable defects would be given.



SR-01

## Crossed fetal protection against Border Disease Virus (BDV) infection: efficacy assessment of three vaccines directed against Bovine viral diarrhoea virus (BVDV)

### Border disease virus vaccination

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**Objectives:** Border Disease (BD) is a worldwide viral disease of sheep which is responsible for significant economic losses. BD control is based on the detection and elimination of persistently infected (PI) lambs, biosecurity measures and vaccination of ewes. Vaccination is done to protect fetus against Border Disease Virus (BDV) infection and to avoid the birth of PI animals. As no BDV vaccine exist, cross vaccination is carried out in the field using half doses of vaccines directed against Bovine Viral Diarrhoea virus (BVDV), a closely related pestivirus. Since there is no data in literature, we assessed the efficacy of three commercialized BVD vaccines for fetal cross protection of sheep against BDV infection.

**Materials and methods:** Experimentation was performed under EEC guidelines (86/609/CEE) and official French ethical agreement. Four groups of BDV free sheep (negatives for antibodies and virus) were used. In three groups 11 ewes were vaccinated with BVD vaccines (half dose used for cattle) by one shot of live Mucosiffa BVDV-1 (Ceva, group 1) or Bovela BVDV-1/BVDV-2 (Boehringer, group 2) vaccines or two injections of killed Bovilis BVD-1 vaccine (Merck SA, group 3), respectively. The fourth control group (18 sheep) was not vaccinated. Ninety-one days after vaccination, all ewes were challenged at 52 days of gestation (Day 0 or D0) by intramuscular injection of  $2.10^5$  TCID<sub>50</sub>/animal of BDV genotype 6 strain 6390. Clinical examinations were recorded daily from 3 days before inoculation (D-3) to D18 and for abortion until the end of the experimentation. Haematological examination was performed daily 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, IdVet Montpellier, France), and antibody response kinetics was measured by NS2-3 competitive ELISA (IdVet, Montpellier France) and seroneutralisation. The ewes were euthanized two months after challenge (D64 to D66) at 117-119 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 and thymus by RT-qPCR.

**Results:** No clinical signs were observed in ewes after infection and no abortion was observed. A transient leucopenia, mainly characterized by lymphopenia, was observed in all groups starting at D3. The duration of leucopenia was significantly higher (D3 to D10) in non-vaccinated ewes compared to groups 1 and 2 (D3 to D6). BDV could be detected in all infected groups with significant higher duration in non-vaccinated one. Seroconversions for NS2-3 antibodies were observed in

sheep immunized by live vaccines between 30 and 79 days post vaccination (before challenge) for 8 and 7 out of 11 ewes of groups 1 and 2 respectively. All ewes of groups 1 and 2 were ELISA positive 14 days after challenge. In groups 3 and 4, ewes were ELISA negative at D0. They seroconverted between D7 and D35 for group 3 (killed Bovilis vaccine) and D14 to D49 for control group 4. Using seroneutralisation assay against BVDV-1, the mean neutralizing titres at D0 were  $7.3 \pm 3.8$ ,  $7.8 \pm 1.5$ ,  $6.1 \pm 3.3$  and 0 Log<sub>2</sub> ED<sub>50</sub>/mL for groups 1 to 4 respectively. At the end of experimentation (D69) titres were  $13.0 \pm 2.1$ ,  $13.5 \pm 0.9$ ,  $13.3 \pm 0.9$  and  $9.4 \pm 1.4$  Log<sub>2</sub> ED<sub>50</sub>/mL. Significant differences ( $p < 0.001$ ) were observed between vaccinated groups and the control one. Seroneutralisation assays against BDV-6 are under way. Twenty-one, 22, 21 and 41 fetuses were collected in groups 1 to 4. All fetuses except one (group 2) were viable, with correct measures and no malformations. Groups do not show statistical differences. Finally all fetuses were seronegative and BDV was found with mean loads of 4.9, 4.1, 4.4, 4.1 and 3.8, 3.5, 3.1, 3.3 log<sub>10</sub> RNA copies/100 mg of tissue in brain and thymus of all ewes of Mucosiffa, Bovela, Bovilis and control groups respectively (no statistical differences between groups).

**Conclusions:** Despite extensive using in the field and the development of an antibody response, this study indicates, in the proposed model, an absence of fetal protection by BVDV vaccines against BDV genotype 6 infection of sheep. Hypotheses will be discussed in relation to existing scientific data (cross protection and genetic diversity of pestiviruses) and to the method used (route and dose of challenge, vaccine dose and time-span between vaccination and challenge).

SR-02

## Resilience and susceptibility to paratuberculosis in sheep

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The success of pathogenic mycobacteria is underpinned by factors such as their ability to persist within host cells, remain dormant and subsequently reactivate to cause clinical disease, and their propensity to disperse surreptitiously within populations. The latter is made possible by the chronicity of disease and the often healthy appearance of infectious individuals. While there is considerable understanding of host-pathogen interactions at the cellular level and disease pathogenesis at the whole animal level there is a lack of knowledge as to why some animals are resistant and others susceptible to disease. This impacts our ability to identify animals that will remain healthy despite exposure to the pathogen, and those that should be treated, quarantined or culled to prevent spread of the infection. To assess resilience, sheep were exposed to the pathogen, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and disease progression was monitored over 2.5 years. Variation in susceptibility of different breeds of sheep (Merino, Poll Dorset, Border Leicester, Suffolk-Merino) to paratuberculosis was also assessed in a controlled experiment. An early strong IFN $\gamma$  response differentiated resilient sheep from susceptible individuals prior to the onset of clinical disease. Commonly



used diagnostic tests, faecal shedding and antibody ELISA, were more ambiguous. Faecal shedding of MAP was transient in the majority of resilient animals and therefore should not be used for diagnosis of MAP infection in younger sheep. Some resilient animals could be diagnosed as having disease using a commercial antibody ELISA. While there were differences in the severity of disease (pathology and the number of animals with clinical disease) between breeds during the trial period, the quantity of faecal shedding was comparable in sheep with clinical disease in all breeds studied. Poll Dorset sheep had the strongest early IFNg response and the lowest proportion of infected animals and clinical disease. Host responses in animals that are resilient to MAP infection are as complex as in susceptible sheep. While there is a spectrum of disease severity amongst the breeds studied, all were equally capable of spreading disease.

SR-03

### Vitamin B12 is bioavailable in sheep when blended in combination injectable clostridial vaccine

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**Objectives:** As vitamin B<sub>12</sub> binds readily to proteins, including tetanus toxoid (Soldner, 2011), the aim of this study was to demonstrate that vitamin B<sub>12</sub> (in the form of hydroxocobalamin acetate [HA]) is bioavailable following injection into lambs when formulated in combination with a multivalent clostridial vaccine.

**Materials and methods:** HA equivalent to 1mg/ml of B<sub>12</sub> was blended into the test vaccine containing antigens derived from five clostridial species (*Clostridium tetani*, *C. perfringens* type D, *C. septicum*, *C. novyi* type B, and *C. chauvoei*) and *Corynebacterium pseudotuberculosis*. In a parallel-group, controlled, randomised, longitudinal intervention study this new vaccine was compared to a reference vaccine. This reference vaccine (Guardian 6 in 1, MSD Animal Health Australia), is identical to the test vaccine but does not contain B<sub>12</sub>.

The study commenced in December 2014 in the central North Island of New Zealand on a commercial sheep and beef farm with a history of B12 deficiency. The study population of male and female Romney/Suffolk/Texel lambs (n=105) born in the spring of 2014, were sourced from a mob deemed as marginally B<sub>12</sub> deficient (liver B12 mean [range] 318 [190-440] nmol/kg, n= 5). Ranked on live weight, the lambs were allocated into three equally sized groups; A, B and C. All lambs were vaccinated twice (2ml subcutaneously), on day (D) 0 and again on D28. Group A was injected with the test then the reference product (2mg B<sub>12</sub>). Group B was injected both times with the test product (2mg B<sub>12</sub> x2). Group (control) C was given the reference vaccine on both occasions. All three groups were grazed as a single mob on ryegrass/clover pastures for the duration (97 days) of the study.

Serum B<sub>12</sub> concentration was repeatedly quantified from a random selection of 20 lambs per group, prior to treatment (D0) and 6 times after treatment (D7, 28, 42, 56, 70 and 97). B<sub>12</sub> concentration from liver biopsy samples was quantified from

different groups of randomly selected lambs on D0 (n = 4 per group), D42 (Group A and B n = 5 per group, Group C n = 10), D56 (n = 10 per group), and in duplicate on D70 (Group A and C n = 20x2 per group, Group B n = 12x2).

Serum data were analysed using mixed models to account for within-animal dependence associated with repeated measurements. Liver samples were analysed using linear regression. Animal ethics approval was granted for this study.

**Results:** Mean [95%CI] serum B<sub>12</sub> concentrations were markedly (>3x) elevated 7 days after the first injection in treated lambs (A and B, n=40, 2072 [1840-2305] pmol/ml) compared with non-supplemented lambs (C, n=20, 668 [577-769] pmol/ml; p<0.001). Compared to the control group mean serum concentrations [95%CI] remained elevated at all time points post treatment in group A (P<0.035, D97: 642 [540 to 745], and 496 [418 to 574] pmol/ml for groups (n=20) A, and C, respectively). Compared to the control group the mean serum B<sub>12</sub> in Group B were significantly greater on D42, D56 and D70 (P<0.017).

Mean liver B<sub>12</sub> concentration at D0 of 293 [260 to 325] nmol/kg was borderline marginally deficient (<280nmol/kg). Mean liver concentrations [95%CI] on D42 were 429 [354 to 503], 603 [528 to 677], and 307 [254 to 359] nmol/kg for groups A (n=5), B (n=5) and C (n=10), respectively. All group means were significantly different from each other (P<0.012). Mean liver concentrations [95%CI] on D56 were 380 [330 to 431], 598 [547 to 649], and 319 [268 to 370] nmol/kg for groups (n=10) A, B and C, respectively. Concordance between the within-animal livers samples was, using the criteria of McBride (2005), poor (Concordance correlation coefficient 0.68 95%CI 0.53 to 0.83). Mean liver concentrations [95%CI] on D70 (averaged within-animal) were 423 [380 to 465], 511 [457 to 565], and 351 [309 to 393] nmol/kg for groups A (n=20), B (n=20) and C (n=12), respectively. All group means were significantly different from each other (P<0.019).

**Conclusions:** The study demonstrates that the vitamin B<sub>12</sub> is bioavailable when formulated into a vaccine containing numerous proteinaceous antigens. This supplementation causes an initial significant increase in serum B<sub>12</sub> and a prolonged increase in serum and liver B<sub>12</sub> concentration. These levels are comparable to those seen when lambs were injected with 2mg of B<sub>12</sub> in an aqueous solution (Grace et al, 1998).

SR-04

### Field efficacy of Meloxicam when used in sheep to alleviate pain and inflammation from routine husbandry procedures

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**OBJECTIVES:** In the Australian sheep industry, a surgical operation to remove excess skin wrinkle from around the breech area in young lambs is used to provide ongoing benefits of reduced blowfly strike and dag accumulation. At the same time, lambs are tail docked and castrated. However, these procedures are often undertaken without any form of pain relief, and represent a risk to productivity and acceptable welfare standards.



Metacam 20® (meloxicam 20 mg/ml, Boehringer Ingelheim) has been recently approved for use in sheep in Australia, New Zealand and Canada. Meloxicam is a non-steroidal anti-inflammatory drug of the oxicam class and in sheep is given as a single subcutaneous injection of 1.0mg/kg bodyweight from 2 weeks of age.

An experiment was designed and undertaken to assess the effect of Metacam 20 on reducing pain associated with routine husbandry procedures (castration, tail docking and mulesing) (RHP), measured by behavioural signs of pain in lambs after the procedure. It was hypothesized that lambs treated with Metacam 20 would return to normal behaviour sooner and show less pain associated behaviours than those not treated.

**MATERIALS AND METHODS:** The study was a randomised block design with three treatments. Lambs were blocked according to their live weight into 4 replicates with each replicate assigned a separate paddock. Within each block (n=21), lambs were randomly allocated to one of three treatment groups. Treatments were either Metacam 20 given subcutaneously 20 minutes prior to undergoing RHP (MET), saline solution given subcutaneously 20 minutes prior to undergoing RHP (NOMET) or placed in cradle and removed as per normal practice without any RHP (SHAM). Lambs were identified with a unique number on both sides and placed in allocated paddocks with their dams. Observation commenced 30 minutes after animals entered their paddocks, and repeated hourly for eight hours on day 1. The observer scanned sequentially for each lamb within the block over 25 minutes and recorded the behaviour. On the days following treatment (Day 2 and Day 3) each group was monitored once. Lambs were weighed on days -2, 1, 2, 3, 4, 5 and 7.

Each behavioural observation was assessed by fitting General Linear Models (GLM). The approach used a logit transformation and binomial distribution with treatment and observation period as fixed effects and block, lamb, date and observer as random effects. Each behaviour category was treated as a binary function (e.g. yes or no). For live weight and wound score, data was analysed using appropriate GLMs. All statistical analyses were performed using GENSTAT 17th edition (VSN International Ltd, Hemel, Hempstead, UK).

**RESULTS:** There was a significant effect of treatment for three of the observation categories over the three days of observation; hunched standing ( $P<0.001$ ), normal walking ( $P<0.001$ ) and stiff walking ( $P<0.001$ ). Normal standing trended to significance at  $P = 0.051$ . Back transforming values to percentages, application of Metacam increased the incidence of normal walking by 39% and reduced the incidence of stiff walking by 42% compared to NOMET lambs. The number of lambs observed with normal standing was 12% greater in lambs treated with Metacam and for the inverse measurement of hunched standing, observed behaviour decreased by 15% compared with NOMET lambs. There were no significant differences between treatments for lamb live weight throughout the observation period.

**CONCLUSIONS:** Metacam 20 significantly increased the incidence of normal behaviours (such as normal walking) and significantly decreased the incidence of pain associated behaviours (Stiff walking and hunched standing) after routine husbandry procedures over the 3 day observation period when compared with lambs that did not receive pain relief. Metacam 20 should be considered an important tool in managing welfare of lambs undergoing routine husbandry procedures.

SR-05

### Effect of copulation on estrus duration, LH response, and ovulation in Boer goats

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The objectives of this investigation were to determine the effect of copulation on estrus duration, LH response and ovulation in Boer goats. A controlled randomized study, with two replicates, in which does were divided at each replicate in treatment (COP) and control (CON) groups was performed. All the does were pluriparous and estrous synchronized with CIDR (progesterone 300 mg) maintained in the vagina for seven days, and received 50 µg of GnRH at device insertion and 5 mg of natural prostaglandin F-2<sub>α</sub> im at CIDR removal. The COP group received two copulas within the first 4 hours of estrus onset, and the CON group was only permitted to be mounted. Estrus was detected twice a day during the first 24 hours after pessary removal and then every 4 hours by using bucks with canvas apron, as teasers, led by leash for 96 hours. Blood was collected throughout all the estrus period after each estrus detection and analyzed for LH by radioimmunoassay (RIA). In addition, at the second replicate ovulation time and number of ovulations were also monitored by transrectal ultrasonography using a linear 7.5 MHz probe beginning 24 hours after estrus onset and repeated every four hours until all the preovulatory follicles disappeared. Estrus onset was  $36.7 \pm 10.5$  hours and  $35.5 \pm 13.6$  hours for CON and TRE groups, respectively ( $P=0.82$ ). Estrus duration for the same groups was  $40.3 \pm 9.9$  hours and  $28.3 \pm 4.7$  hours, respectively ( $P=0.001$ ). The LH peak time for the CON group was  $17.7 \pm 6.3$  hours, and for the COP group, it was  $10.9 \pm 2.6$  hours ( $P=0.004$ ). The LH magnitude for the same groups was  $31.5 \pm 16.2$  ng/mL and  $34.9 \pm 20.7$  ng/mL, respectively ( $P=0.34$ ). The LH peak duration was not different between groups (CON:  $7.3 \pm 1.6$  hours versus COP:  $7.2 \pm 2.4$  hours;  $P=0.94$ ). The first ovulation time for CON and COP groups was  $33.7 \pm 3.9$  hours and  $29.1 \pm 3.2$  hours ( $P= 0.05$ ), and the last ovulation time for the same groups was  $37.7 \pm 3.9$  hours and  $32.6 \pm 2.5$  hours, respectively ( $P=0.02$ ). The overall time from LH peak to ovulation was  $18.6 \pm 4.8$  hours without differences between groups (CON:  $16.3 \pm 5.6$  hours versus COP:  $20.6 \pm 3.3$  hours;  $P=0.15$ ). The number of ovulations for the CON group was  $2.2 \pm 0.4$ , and for COP group, it was  $2.1 \pm 0.4$  ( $P=0.96$ ). It was concluded that copulation reduced estrus duration, hastened LH peak and ovulation in Boer goats.

SR-06

### Inhibitory effects of long-term repeated treatments of sustainable GnRH antagonist, degarelix acetate, on caprine testicular functions

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**Objective:** In order to examine whether a sustainable GnRH antagonist, degarelix acetate, can be used for chemical castration in goats, we investigated the effects of long-term repeated treatments of the antagonist on testicular hormonal secretion, size, ultrasound image, histology and spermatogenesis.

**Materials and methods:** Male Shiba goats (3-6 months of age, n=4) were treated subcutaneously with degarelix acetate (4 mg/kg body weight) every 4 weeks for 24 weeks (7 times in total). Scrotal circumferences and surface temperatures were measured and ultrasound examinations of testes were performed weekly for the 24 weeks. Blood samples were taken from jugular vein on Days -7, 0 (just before the first treatment), 1, 2, 3, 4, 5, 7 and then weekly until 29 weeks after the first treatment. Testes and epididymides were collected by surgical castration one week after the last (7<sup>th</sup>) treatment (25 weeks after the first treatment). Testes and epididymides were also collected from goats without GnRH antagonist treatments (9-12 months of age, n=4) and used as control samples. Plasma testosterone and insulin-like peptide 3 (INSL3) concentrations were measured by EIA and TRFIA, respectively. Ultrasound image of testicular tissue was analyzed by an image analysis software and expressed as pixel intensity. The highest and lowest temperatures of scrotal surface were calculated by a software for a thermography camera. Testicular tissue was fixed in Bouin's solution and stained by hematoxylin and eosin, followed by observations of seminiferous tubules and interstitial tissue. Sperm numbers were counted in homogenates of epididymides.

**Results:** Plasma testosterone concentrations decreased ( $P<0.05$ ) 1 day after the first treatment (Day 1) and maintained lower levels until 29 weeks ( $P<0.05$ ). Plasma INSL3 concentrations declined at Day 2 ( $P<0.05$ ) and remained lower levels until 29 weeks after the first treatment ( $P<0.05$ ). A significant reduction in scrotal circumferences was observed at 8 weeks after the first treatment ( $P<0.05$ ) and decreased from 8 weeks to 24 weeks ( $P<0.05$ ). The scrotal circumference at 24 weeks was approximately 70% of that at Day 0. Pixel intensity decreased at 4 weeks after the first treatment ( $P<0.05$ ) and further reduced to about 35% of that at Day 0 (day of the first treatment). There was no effects of repeated treatments of GnRH antagonist on scrotal surface temperature. The weights of testes and epididymides were reduced at 24 weeks after the first treatment compared to those in untreated goats ( $P<0.05$ ). Areas of seminiferous tubules were smaller at 24 weeks after the first treatment than those in untreated goats ( $P<0.05$ ), and no spermatozoa in the seminiferous tubules and atrophic Leydig cells in the interstitial tissues were observed in the GnRH antagonist treated goats. Sperms were absent in homogenates of epididymis at 24 weeks after the first treatment while there were  $16.5 \times 10^9$  sperms per epididymis in untreated goats.

**Conclusions:** The secretion of plasma testosterone and INSL3 concentrations decreased within a few days after the first treatment of degarelix acetate and these secretions remained inhibited for 6 months due to the effects of monthly repeated treatments. Pixel intensity of ultrasound testicular images reduced at 1 month and testicular volumes declined at 2 months after the first treatment. The suppression of spermatogenesis in seminiferous tubules and absence of sperm in epididymides were confirmed at 6 months after the first treatment. Thus, repeated treatments of the long-acting GnRH antagonist are an

effective chemical castration method for goats.

SR-07

### Comparison of fresh sexed sorted and conventional semen in a commercial embryo transfer program involving East Friesian dairy ewes in New Zealand

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**Objectives:** NZ sheep dairying has the aspirational target of building to a 2 billion NZ dollar industry by the year 2030. Currently the NZ milking flock consists of mostly cross bred animals with very few purebred milking sheep. In 2016 protocols for the importation of new dairy genetics from parts of Europe were agreed, allowing the first importation of dairy sheep genetics into NZ for over 25 years.

Embryo transfer has been suggested as one of the ways to increase the speed of dissemination of this imported genetic material. This study sought to establish if the use of sexed sorted semen was a potentially viable way of increasing the numbers of females produced from these programs.

**Materials and methods:** 22 multiparous purebred East Friesian ewes were selected as embryo donors for the study. An intra vaginal progesterone releasing device (CIDR), (Zoetis NZ) was inserted 15 days before the proposed breeding date. 8 days later a second CIDR was inserted with concurrent injection of prostaglandin (0.5ml Cyclyase, Agrihelth NZ). 3 days after this, being 4 days before the proposed breeding date, a series of 7 single injections of follicle stimulating hormone (FSH) (Foltraphin, Bioniche) was started at approximately 12 hour intervals (i.e. over 3 and one half days) in reducing dose rates being 2.5, 2.0, 1.5, 1.5, 1.0, 1.0 and 0.5ml respectively. Concurrent with the first FSH injection a dose of 300IU equine chorionic gonadotropin (Novormon eCG, AgriHealth NZ) was given. 36 hours before the time of breeding both CIDRs were removed.

Semen was collected from a single ram 16 hours before the insemination time and fresh semen sex sorted by Sexing Technologies NZ. The same ram was collected a second time 4 hours before the time of insemination to be used in a conventional non sorted way.

Ewes were divided randomly into two groups. 15 ewes were inseminated once to fresh sex sorted semen (SS) (approximately 4 million sperm dose) and 7 ewes with conventional fresh semen (CS) (approximately 100 million sperm dose).

A CIDR was re inserted to donor ewes 4 days after inseminations with embryos collected using a conventional surgical technique 6 days after insemination. Embryos were graded for quality with all grade 1 and 2 embryos implanted immediately into synchronized Romney recipient ewes. Each recipient received 2 embryos.

Lambing was undertaken in small paddocks with three hourly monitoring and identification of offspring.

**Results:** A total of 318 embryos and unfertilised ova (UFO) were recovered (14.45 per ewe). There was not a statistical difference between the two groups in this regard. SS (15.53) compared to CS (12.14,  $p=0.6038$ ). The percentage of implant-



able embryos (Grade 1 and 2) was similar in both groups. SS (70.39%) and CS (76.47%,  $p=0.2859$ ) leaving a similar number of embryos to be implanted SS (10.9) compared to CS (9.3,  $p=0.7296$ ).

Lambs born as a percentage of those embryos implanted for SS embryos (65.24%) was not different to that of CS embryos (69.23%,  $p=0.5653$ ). It follows then that number of live lambs born as a percentage of embryos and UFO recovered for SS embryos (45.92%) was not different to that of CS embryos (52.94%,  $p=0.2681$ ).

The number of lambs born per donor for SS (7.1) was not statistically different to that of CS (6.4,  $p=0.8151$ ).

**Conclusions:** Although care must be taken with interpretation of the results, as this study did involve the use of a single sire, it has show that SS semen can be used in commercial embryo transfer programs using EF ewes in NZ without significantly affecting lambing rates. It is then suggested that this technology could be adapted to more quickly speed up the dissemination of dairy specific genetics as they enter NZ from overseas.

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SR-08

### The characteristic of cyclic reticulo-ruminal motility recorded by telemetry electromyography method in sheep.

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**Objectives:** Reticulum and rumen are functionally considered as a single unit, the reticulorumen, in which motility is responsible for proper rumination cycles. The specialized contractions of reticulorumen provides constant mixing of partially digested material (cycle A) and its flow into the abomasum as well as a cyclic eructation (cycle B) and rumination (cycle C). The reticulorumen motility may be investigated during long-term electromyography (EMG) combined with the telemetry recording system. Wireless measurements of EMG signals from freely moving conscious animals reduced stress of animals and the number of artifacts, which allows to obtain and analyze reliable myoelectrical data.

**Materials and methods:** The study was carried out on 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 conducted under general anesthesia, when three silver bipolar electrodes were inserted into muscular layers of the reticulum, the rumen and the abomasum. The combination of electrodes was connected to the three-channel transmitter (TL10M3-D70-EEE, DSI, USA), which was placed under abdominal skin. The EMG signal sent by the transmitter 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.

**Results:** In reticulo-ruminal motility three types of cycles were determined: cycle A, cycle B and cycle C using burst and bundle parameters. The burst represented single action potentials, while

the bundle- multiple action potentials, for which mean amplitude [mV], mean root mean square (RMS) [mV] and duration were analyzed. Cycle A was defined as a single or double bursts forming bundle in reticulum, before occurrence of single bundle in ventral rumen sac. Cycle B was defined as a single or double bursts forming bundle in reticulum, then double bursts forming bundle in ventral rumen sac. Cycle C was determined as triple bursts forming bundle in reticulum, followed by single or double bursts forming bundle in ventral rumen sac. During the rest period with no food intake, the number of ruminal cycles remained constant  $12\pm 0.2$  to  $16\pm 0.1$  in 15 minutes intervals, which means that appeared with frequency 0.013-0.018 Hz. We found that percentage of each type of cycle changed in different intervals. The cycles A and B occurred equally with percentage 43.75%-50.00% and 50.00%-56.25% respectively, when cycle C did not occur. Cycles C appeared every 30 minutes and lasted for average 15-30 minutes with significant advantage (57.14%-73.33%). At this time only intermittent cycles A (6.67%-14.29%) and B (20.00%-28.57%) occurred. Repeated occurrence of rumination phase during 30 minutes intervals was repeatable in all animals from start point of synchronization, as a result of head reaction.

**Conclusions:** The obtained results suggests that EMG signal registered in the wall of non-stimulated gastrointestinal tract in sheep is not random, but it occurs in an orderly array and percentage. Those EMG signals of each rumination cycles formed specific pattern of reticulo-ruminal motility during rest period. It may be concluded, that the telemetry EMG registration is an accurate and useful technique to determinate the reticulo-ruminal myoactivity in sheep.

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SR-09

### The evidence of the tyrosine kinase III receptor CD117 and vimentin immunopositive cells in the gastrointestinal tract of the sheep

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**Objectives:** The interstitial cells of Cajal (ICC) have been reported as the pacemaker cells of gastrointestinal motility essential for discharging slow triggering waves. They have been found in the wall of the gastrointestinal tract (GI) of several species, including humans and bovines but were not considered in ovine forestomachs and abomasum. The ICC could easily be distinguished from smooth-muscle cells, mast cells fibroblasts and neurons, the other main cell types present in the tunica muscularis of GI, based on immunoreactivity for Kit/CD117- receptor of tyrosine kinase and vimentine. We demonstrated the evidence and the morphological characteristics of the immunofluorescent reaction both against c-kit and vimentine in the forestomachs and abomasum of adult sheep.

**Materials and methods:** The fresh whole thickness samples of rumen, omasum and abomasum were collected from 5 mature sheep. Afterwards, collected material was stained with hematoxylin-eosin and immunofluorescently (IF) labeled. Double



IF labeling was performed using primary polyclonal Chicken IgY anti-Vimentin antibody and polyclonal Goat IgG anti human CD117/c-kit antibody in combination with secondary antibody conjugated with AF 568 (goat anti-chicken IgG) and AF 660 (donkey anti-goat IgG), respectively. The ICC were identified based on cell nuclei stained by Hoechst and fluorescence detected simultaneously in 580 - 610 and 680 - 730 emission band using confocal microscope (Leica TCS SP8) and scanning cytometer (TissueFAXS PLUS).

**Results:** The cells with characteristic morphology and immunophenotype were identified inside the wall of rumen, omasum and abomasum of ovine tissues. The cells were classified as ICC basing on fusiform, triangular or star-like shape of cell body with dendritic processes forming cellular network and double anti-CD117 and anti-vimentine positivity. The fusiform ICC were the predominant cell type in the rumen, while triangular or star-like cells predominated in omasum and abomasum. Vimentin reactivity was mainly localized within the cell processes, while CD117 has a patchy pattern in cell body. The ICC were localized in the tunica muscularis, predominantly near the small blood vessels. ICC were abundant in the inner layer of the tunica muscularis of rumen and seemed to be consistently associated with this layer. In all samples, ICC were found in the outer layer of the tunica muscularis. Furthermore, ICC were found between the two muscle layers in the rumen.

**Conclusions:** The ICC are present in the forestomach and abomasum of ovines and may be identified by immunofluorescence methods. The ICC seemed to create a functional network in gastrointestinal tract of small ruminants, which requires detailed research in future.

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SR-10

### Diagnostic advances in ovine virulent footrot

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**Objectives:** Virulent ovine footrot is a severe multifactorial disease caused by *Dichelobacter nodosus*, an obligate parasite of the feet of ruminants. It affects sheep and goats worldwide. While it is not possible to reproduce the clinical signs of this disease without *D. nodosus*, an interaction with the complex microflora in the skin is needed for pathogenesis. Footrot occurs only after *Fusobacterium necrophorum*, a faecal organism, and other bacteria colonise macerated interdigital skin, which is infected with *D. nodosus*. Elaboration of proteolytic enzymes by *D. nodosus* leads to underrunning of the horn of the hoof and severe pain. Lameness can affect a high proportion of sheep in a flock. Transmission and expression of disease depend on relatively warm, moist conditions and the sheep being genetically susceptible. Merino sheep are most susceptible.

Early researchers described a mild form of footrot that preceded or followed outbreaks of severe footrot. It is now termed "benign" footrot and is due to strains of *D. nodosus* with low virulence. The classification of outbreaks as benign or virulent is made at flock-level and in most states of Australia, it is approached systematically through flock history and clinical examination. This assessment can be complex and is usually conducted by an experienced veterinarian. A diagnostic dilem-

ma arises when severe lesions are observed in a small proportion of sheep or when mild lesions are seen in many sheep. This can be due to relative resistance of the host (eg in cross-bred sheep), unfavourable environmental conditions for transmission and disease expression (cold, dry), use of chemical footbaths or antibiotics to mask lesions, or absence of virulent strains of *D. nodosus*. Consequently, laboratory tests may be needed for confirmation. Accurate diagnosis is important because benign footrot causes insignificant economic or welfare impacts and is difficult or impossible to eradicate. In some jurisdictions in Australia, a diagnosis of virulent footrot leads to quarantine and compulsory eradication, the costs of which have a major economic impact on the farm.

The objectives of this study were to investigate the utility of laboratory diagnostic tests for virulent footrot under Australian conditions.

**Materials and methods:** There is a correlation between the proteolytic activity of a *D. nodosus* isolate measured by elastin or gelatin digestion and its tendency to cause underrunning of the hard horn of the hoof in susceptible sheep under appropriate environmental conditions. Assays for protease activity require anaerobic culture and are time consuming. Recent developments in DNA extraction methods have enabled foot swabs to be directly tested for the presence *D. nodosus*. Competitive qPCR assays for the alleles of the acidic protease gene (*aprV2/aprB2*), which encode the protease responsible for elastase activity, were reported in Europe. In the first studies published these assays discriminated virulent and benign strains of *D. nodosus*. In the present study which involved 40 flocks of sheep, the elastase test, the gelatin gel test and an *aprV2/aprB2* qPCR test were evaluated against clinical examination using diagnostic test evaluation principles of the OIE.

**Results:** Relative to clinical diagnosis, a high rate of false positive test outcomes was obtained with both the gelatin gel test and the *aprV2/aprB2* qPCR. The agreement between the elastase and gelatin gel test was slight: 84% of isolates classified as benign by the elastase test were classified as virulent by the gelatin gel test. The lowest rate of false positives was obtained with the elastase test.

**Conclusion:** It is technically possible to reliably detect *D. nodosus* DNA directly from foot swabs, but it seems the virulence of *D. nodosus* is determined by more than the acidic protease gene. Consequently, the results of laboratory tests must be interpreted with caution in the light of clinical examination. Reliance solely on tests for protease or a single gene would cause unnecessary quarantine and control measures to be imposed on farmers. Further research is required to understand the virulence factors of *D. nodosus*. If supportive evidence is required from the laboratory for confirmation of virulence and final diagnosis, the elastase test is recommended as it has the lowest rate of false positives of the available tests.

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SR-11

### Diagnosis and molecular characterization of *Cryptosporidium* and *Giardia* in sheep in Algeria

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**Background:** *Cryptosporidium* parasites are common enteric pathogen of humans and wide range of animals worldwide. The parasite is involved in numerous outbreaks of diarrheal illness. Yet, *Cryptosporidium* related studies in small ruminants have received little attention compared to cattle. Cryptosporidiosis in small ruminants can lead to severe diarrhea, anorexia, depression and weight loss, causing high morbidity and mortality rate in the flocks, especially in kids and lambs, resulting in considerable economic impact, in addition to the public health significance. Little is known on the occurrence and identity of *Cryptosporidium* and *Giardia* species in sheep and goats in Algeria. This study aimed to investigate occurrence and genotype distribution of *Cryptosporidium* spp. and *Giardia* in sheep.

**Materials and methods:** A total of 84 faecal samples were collected from lambs less than three months old. They were screened for presence of *Cryptosporidium* by nested-PCR analysis of the small subunit ribosomal RNA (rRNA) gene, followed by restriction fragment length polymorphism (PCR-RFLP) and sequence analyses to determine *Cryptosporidium* species present. *C. ubiquitum* was further subtyped by sequence analysis of the polymorphic 60 kDa glycoprotein gene. For *Giardia*, samples were screened by nested-PCR analysis (followed by RFLP) using the glutamate dehydrogenase and triose phosphate isomerase to examine occurrence and genotype distribution of *G. duodenalis*.

**Results:** The presence of *Cryptosporidium* oocysts and *Giardia* cysts was reported respectively in 36/ 84 (43%) and 15/84 (17.9%) samples. The *Cryptosporidium* species identified from 21/36 (58%) microscopy positive samples were *C. parvum* 16/21 (76.19%) and *C. ubiquitum* 5/21 (23.8%). From 15 *C. parvum* isolates, two subtypes were recognized within the subtype family IIa including IIaA21G2R1 (3/15) and IIaA13G2R1 (1/15) while IIaA16G1 (11/15) was the only subtype within IIb subtype family. *Giardia duodenalis* genotypes identified from 12/15 (80%) microscopy positive samples were the ruminant-specific assemblage E (9/12), the zoonotic assemblage A (1/12), and mixed assemblages (2/12).

**Conclusion:** This study reported for the first time the identification of both *Cryptosporidium* and *Giardia* from lambs in Algeria. The presence of zoonotic *C. parvum* subtype families (IIa, IIb) and *C. ubiquitum*, as well as the zoonotic *Giardia duodenalis* assemblage A, indicates that lambs can play an important role as a potential reservoir for zoonotic transmission.

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**Objectives:** The use of feed additives, including medicinal herbs, to mitigate methane (CH<sub>4</sub>) production in ruminants has recently drawn a great attention of researchers. Garlic can be added in diets of small ruminants in the form of bulbs, leaves, essential oils, or active compounds (allicin, diallyl disulfide, propyl propane thiosulfinate, propyl propane thiosulfonate) to decrease CH<sub>4</sub> production. Studies conducted *in vitro* indicated methanogenic properties of garlic. Although small in number, the results of different *in vivo* studies on goat and sheep are contradictory (indicating positive and negative effects). Therefore, the present study takes a meta-analytical approach to investigate the effects of garlic (bulbs, leaves, essential oils, or active compounds) on CH<sub>4</sub> production in sheep and goats. Meta-analysis uses statistical approaches to combine and summarize the findings of many studies with a greater statistical power.

**Materials and methods:** Studies in the present meta-analysis included those published on peer-reviewed journals from January 2007 to November 2017 in English or indexed on ISI Web of Knowledge. The keywords to search the relevant studies used sheep OR goat AND methane AND garlic OR *Allium sativum*. The references of collected papers were also reviewed to search the relevant papers. The experiments that consisted of a control group together with group(s) receiving garlic (bulbs, leaves, essential oils, or active compounds) were included in this study. In addition, studies that measured CH<sub>4</sub> production *in vivo* were included while those that examined this parameter *in vitro* were excluded from the database. Comprehensive Meta-Analysis version 2.2 was used for statistical analysis and effect size for CH<sub>4</sub> production (grams per day) was calculated based on mean difference (MD) with the confidence interval (CI) 95%. Random effects model was used in this meta-analysis and calculation of heterogeneity on *I*<sup>2</sup> statistic was performed.

**Results:** After filtering the studies through inclusion and exclusion criteria, the remaining 7 experiments comprising of 10 comparisons between a control group and treatment groups with garlic (bulbs, leaves, essential oils, or active compounds) were finally used for meta-analysis. The findings of the meta-analysis suggested a trend in decrease of CH<sub>4</sub> production (MD= -2.151 g/d, CI= -4.632 to 0.330, P= 0.08) as due to garlic (bulbs, leaves, essential oils, or active compounds) added in the diets. Heterogeneity results indicated almost 55%.

**Conclusions:** The findings showed that garlic (bulbs, leaves, essential oils, or active compounds) as a feed additive may tend to lower CH<sub>4</sub> production in small ruminants. In addition, the heterogeneity results suggest that a variety of factors, including type of garlic product (bulbs, leaves, essential oils, or active compounds), dose, properties of basal diet, and type of the animals receiving treatment, can affect CH<sub>4</sub> production to a great extent.

SR-12

## A meta-analysis of *in vivo* studies on the effects of garlic in reducing methane production in small ruminants

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SR-13

### Preliminary data on the occurrence of the main bacterial agents that cause respiratory diseases in sheep in the State of São Paulo and Rio de Janeiro - Brazil

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Ovine respiratory disease is important due to its high morbidity and mortality rates. *Pasteurella multocida*, *Mannheimia haemolytica* and some species of *Mycoplasma* are the major cause of this disease. **Objective:** The aim of this study was to determine the main bacterium present in the lower respiratory tract of healthy sheep and with respiratory disease. **Materials and methods:** Seventy-seven sheep (male and female, two months to three years old) from 4 farms of São Paulo State and from 6 farms of Rio de Janeiro State, Brazil were enrolled in this study. The properties were semi-intensive system. Evaluation of vital parameters and a specific examination of the respiratory tract were performed. Animals were classified as healthy (G1 = 61.04%, 47/77) and unhealthy (G2 = 38.96%, 30/77). Tracheo-bronchial lavage samples were collected after antiseptics of the trachea region. An Intracath® was introduced and twenty milliliters of sterile saline 0.9% was instilled, with subsequent recovery of up to 5mL. Samples were kept in Stuart's medium at 4 ° C and specific transport medium for *Mycoplasma* spp in nitrogen until analysis. The detection of aerobic bacteria, with special attention to *P. multocida* and *M. haemolytica* was performed by plating samples on blood agar. Colonies were classified according to their morphological characteristics and biochemical tests were performed to identify the species. Cultivation and isolation of *Mycoplasma* spp were performed in SP-4 agar and broth medium. The PCR methodology was also used to detect *Mollicutes*. Initially, with generic primers to detect *Mollicutes* and then the positive were submitted to PCR again to detect *M. mycoides* subsp. *capri* and *M. agalactiae*. **Results:** In G1, growth of *Bacillus* sp. (6.38%, 3/47), *Staphylococcus* spp (12.67%, 6/47) and *Streptococcus* sp. (6.66%, 2/47) was observed. Non-fermenting Gram-negative bacteria were found in 6.38% (3/47) of the samples. The concomitant presence of two species was also observed. *Staphylococcus* sp. and *Bacillus* sp. with 6.38% (3/47), *Bacillus* sp. and *E. coli* with 2.17% (1/47). Positive cultures with "fried-egg" colonies were observed in 8.51% (4/47) of samples. *Mollicutes* microorganisms were detected in 17.02% (8/47) of samples by PCR. *M. mycoides* subsp. *capri* was detected in 2.12% (1/47). Undetermined species were observed in 10.63% (5/47) samples. In G2, 16.66% (5/30) were of *Bacillus* sp., being possible to find this agent together with others like *Staphylococcus* spp (3.33%, 1/30) and *E.coli* (3.33, 1/30). Other bacteria observed in this group were *Staphylococcus* sp. (6.66%, 2/30), non-fermenting Gram-negative bacteria with 16.66% (5/30) and two *Klebsiella* species: *K. oxytoca* (3.33%, 1/30) and *K. pneumoniae* (3.33%, 1/30). Moreover, *Mollicutes* microorganisms were detected in 46.66% (14/30) of samples by PCR. Colonies of *Mycoplasma* spp were observed in 33.33% (10/30). Colonies of *P. multocida* and *M. haemolyti-*

*ca*, commonly associated with pneumonia in ruminants, were not observed in this study. **Conclusions:** Respiratory disease is common in Brazilian sheep farming, however, studies on the identification of infectious agents responsible for this condition are still scarce in the country. This work was the first to detect *M. mycoides* subsp. *capri* in the respiratory tract of sheep in the State of São Paulo and Rio de Janeiro. The studies should continue. There is a lack of information about sheep respiratory disease in Brazil and its importance to the local economy.

SR-14

### Relationship of lipid factors in Seminal Plasma and Blood Serum of Afshari Rams

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**Objective:** The aim of this study was to investigate relationship of seminal plasma cholesterol and blood serum cholesterol in Afshari rams.

**Material & Methods:** In this study, four Afshari rams was selected with a mean weight of 50 ± 5 kg and mean age of 2.5 years old. At the same time, blood and semen samples were collected for evaluation of total cholesterol, triglycerides, HDL, LDL, while BHBA was measured only in blood samples. After adding semen to the extender, sperm motility, morphology and viability were evaluated by the CASA software, Papanicolaou, and Eosin-Nigrosin staining, respectively. The sperm parameters were assessed at pre-freezing and post-thawing in seasonal and non-seasonal breeding.

**Results:** The results of this study show that cholesterol and HDL levels of young ram were significantly higher in seasonal breeding than non-seasonal breeding in both blood and semen samples (p<0.05). However, semen cholesterol levels of old ram was not significantly different in non-seasonal and seasonal breeding, but it was significantly in triglycerides and HDL. Blood cholesterol, HLD and LDL were significantly in seasonal breeding compared to non-seasonal breeding (p<0.05). Motility, morphology, and viability of young rams were significantly higher in seasonal breeding than non-seasonal breeding at pre-freezing (p<0.05).

**Conclusion:** Semen and blood Lipid factors were positively correlated which could improve the sperm quality parameters of Afshari can ram in the season and non-seasonal breeding.

**Keywords:** Lipid, Sperm, Afshari Ram, Blood, Seminal Plasma

SR-15

### Impact of different dietary protein levels on goats minerals, and blood parameters

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The analysis of haematological parameters allows to monitor and evaluate the health and nutritional status of animals. In this study, the experiment was conducted to determine impact of different dietary protein levels on goat's blood minerals, and other blood parameters of Tswana goats reared in extensive production systems. Twenty-five female Tswana weaner goats' with similar body weight and age were used for this experiment. Animals were grouped into three treatment of eight goats each in a randomized block design according to live weight. Animals were fed as follow: protein 23.51g and energy 8.55g per kg DM and then they were given Lucerne ad libitum. Blood samples were collected on the first day of the experiment and then weekly until the end of the experiment. Collected samples were analysed for blood biochemistry and haematological parameters using the IDEXX haematology Analyser.

The results obtained revealed that different levels of protein supplementation in Tswana goats significantly affected blood glucose, albumin, albuglobulin and urea ( $P < 0.05$ ) respectively. However, no statistical significant difference were observed on serum total protein, globulin, lipase, triglycerides and cholesterol and they fell within the reference value of the goats. In addition, it was also noted that haematological parameters were influenced significantly ( $P < 0.05$ ) by physiological stages of animals.

Haematology analysis remain a tool to evaluate the well-being of animals

**Keywords:** Supplementation, Haematology, protein, Albumin, protein, energy, growth performance, production

Cerromonte", Spain). Within individuals treated for GT (clinically diagnosed based on neurological symptoms), sheep with the highest  $\beta$ -hydroxybutyrate (BHB) blood concentrations were selected ( $n=9$ ; TOX). Matching healthy controls ( $n=9$ ; CON) were chosen based on lambing date, lactation number ( $4 \pm 3$  lactations), and number of carried lambs ( $2 \pm 1$  born lambs). Body condition parameters were recorded and a fasting blood sample (prior to morning feeding) was collected in late gestation ( $6 \pm 2$  days before parturition).

**Results:** There were no differences in body weight or body condition score between groups. As expected, TOX sheep had decreased glucose (58.8 vs. 69.4 mg/dl;  $P < 0.02$ ), and increased non-esterified fatty acids (1.57 vs. 0.72 mM;  $P < 0.0004$ ; NEFA) and BHB (2.00 vs. 0.84 mM;  $P < 0.0004$ ) blood concentrations, compared to CON sheep. Circulating cholesterol was decreased in TOX sheep (83.8 vs. 98.4 mg/dl;  $P < 0.03$ ); but fructosamine, lactate, triglycerides and urea concentrations did not differ between groups. Gestational toxemia increased circulating tumor necrosis factor  $\alpha$  (8.4 vs. 5.9 pg/ml;  $P < 0.002$ ) and decreased haptoglobin (2.4 vs. 7.1 mg/dl;  $P < 0.03$ ), but did not change interleukin-6 concentrations. Haptoglobin concentrations were negatively correlated with both BHB ( $r = -0.62$ ;  $P < 0.006$ ) and NEFA ( $r = -0.60$ ;  $P < 0.009$ ) levels. Tumor necrosis factor  $\alpha$  concentrations tended to be and were positively correlated with BHB ( $r = 0.42$ ;  $P < 0.09$ ) and NEFA ( $r = 0.82$ ;  $P < 0.0001$ ) levels.

**Conclusions:** In summary, GT in sheep appears to be associated with alterations in biomarkers of inflammation.

SR-16

## Gestational toxemia in lactating sheep is associated with alterations in circulating inflammatory biomarkers

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**Objectives:** Gestational toxemia (GT) is a late pregnancy metabolic disease characterized by the disruption of glucose and lipid homeostasis. Metabolic dysregulation leads to hepatic failure and neurological disorders, which frequently result in the death of both the ewe and its lamb/s. The etiopathology of GT is poorly understood. Several risk factors have been identified (e.g. age, number of fetuses, nutritional status, etc.); however, the large individual variability in GT susceptibility suggests that other factors are likely involved. Recently, inflammation has been associated with metabolic diseases both in cows and sheep. Thus, the study objective was to evaluate changes in inflammatory biomarkers between toxemic and healthy sheep.

**Materials and methods:** The current dataset was retrospectively obtained as a subset from a larger experiment ( $n=334$ ) conducted at a Lacaune sheep high-yield dairy farm ("Granja



SU-01

### Transrectal guidance of the ovaries reduces operative time during bovine laparoscopic ovariectomy

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**Objectives:** Bovine ovariectomy is considered a necessary farm animal management technique, not only for research on reproductive endocrinology, but also for improving feeding efficiency in feeder cattle and for treating ovarian diseases, such as ovarian tumors and granulosa cell tumors. Ovariectomy used to be performed by colpotomy or laparotomy, laparoscopic ovariectomy. Laparoscopic ovariectomy offers various advantages. Minimal invasion of the abdominal cavity is not only esthetically advantageous, but also therapeutically beneficial. However, in laparoscopic ovariectomy, the uterus and ovaries are difficult to visualize when over fattening results in pronounced fat deposits in organs or when insufficient withholding of food makes it impossible to secure sufficient intra-abdominal space; as a result, the surgery is prolonged, thereby placing significant burden on the cow. Rectal palpation is a physical examination commonly used for the diagnosis of reproductive disturbance and pregnancy. Using this technique, the ovary or uterus can be palpated within a few seconds. Thus, we investigated the effect of transrectal guidance of the ovaries by an assistant on operative time during laparoscopic ovariectomy.

**Materials and methods:** In total, 24 Holstein dairy cows (28.2–98.9 months, 1–5 parities, weight 406–698 kg, BCS 2.75–4.00) were from a commercial dairy farm. These cows were randomly divided into two groups: with (guidance group) and without (control group) transrectal guidance of the ovaries by an assistant (12 cows each). All cows were submitted to general status examinations and blood tests prior to surgery and on days 1 and day 14 after surgery. All surgeries were performed by a veterinary surgeon who performs laparoscopic examinations and surgeries on a daily basis, a camera assistant, and a surgical technician. In the control group, two pairs of grasping forceps were introduced through ports 2 and 3 to identify the uterus, which was then grasped with forceps; we then followed along the right and left uterus horn until we confirmed the ovaries. In the guidance group, the designated assistant grasped the ovaries via a transrectal approach and pulled them to a position where they could be visualized with the laparoscopic camera. The ovary was pulled with the grasping forceps, and the extended mesovarium was cauterized close to the ovary with a vessel-sealing device inserted through port 3. Blood samples were obtained from the jugular vein prior to surgery and 1 day and 14 days after surgery. Collecting samples were used for counting of red blood cells and white blood cells, Platelet and hematocrit.

**Results:** The time required from skin incision to the completion of bilateral ovariectomy in the control group (63 ± 25.2 min) was significantly longer than that in the guidance group (24 ± 6.6 min) ( $P < 0.01$ ). None of the 24 cows demonstrated any abnormalities at general status examinations or blood tests prior to surgery or at day 1 or day 14 after surgery; furthermore, no differences were observed in blood tests between the groups. In general, it was necessary to use large traumatic forceps with relatively large teeth to avoid losing hold of the uterine horn;

this procedure can cause bleeding and lacerations in the uterine serosa. However, in the present study, an assistant grasped the ovaries via a transrectal approach, which enabled us to avoid injuring the uterine serosa. We can therefore confirm that the transrectal assistance for ovariectomy by laparoscopy in cows helps to protect the cow from physical damage. As long as specialized instruments are on hand, laparoscopic ovariectomy can be performed with minimal invasiveness and with little risk of complications, making it safe and ideal for cows.

**Conclusions:** In a previous study, the duration of ovariectomy in cows was reported to be 120–150 minutes. In the present study, however, transrectal examination enabled us to complete ovariectomy in a much shorter period of time. This results indicate that the combined technique of using laparoscopy and a transrectal assistant is effective for ovariectomy in cows because it reduces operative time, physical damage, and the burden on the operator.

SU-02

### Comparison of one-step laparoscopy-guided abomasopexy versus omentopexy via right flank laparotomy for the treatment of dairy cows with left displacement of the abomasum

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This work carried out in the region of Entre Douro and Minho between October 2011 and March 2013.

Surgical techniques are the most commonly applied and developed for resolution of displaced abomasum (LDA). The objective of this study was to compare two surgical techniques for the resolution of the LDA in dairy cows, taking into account the time to return to milk production. The techniques are: Abomasopexy by laparoscopy-Guided versus Omentopexy via right flank Laparotomy. This work involves 127 cows Holstein-Friesian lactating with LDA, 64 cows were operated by Laparoscopic technique and 63 cows were operated by laparotomy via right flank. Cows with retained fetal membranes, metritis, dystocia were considered in this study, cows with clinical mastitis were excluded from the study. Collect a blood sample (5 ml) in medium coccygeal vein and analyzed total protein, total bilirubin, calcium, glucose, B-hydroxybutyrate, sodium, potassium and chlorine. Data are summarized the Mean ± SD, and value of  $P > 0.05$  was not significant considered statistically for all analyzes, except Chlorine variable with a value of  $P < 0.05$ . Variables that were not normally distributed logarithmically were transformed before statistical analysis was performed. Milk production was recorded at different time intervals: before pathology, surgery day, 8 th, 15 th, 30 th days after surgery. Differences by treatment group of milk production over time was evaluated with a Repeated Measures Analyses of Variance "ANOVA".

There is no difference between the groups for the biochemical parameters analyzed ( $P > 0.05$ ) with the exception of Chlorine that has lower average values in Omentopexy group ( $P < 0.05$ ).

Milk production average during the study period was not significantly different between Laparoscopy and Laparotomy groups (



P = 0, 42 ), although significant change trough post- surgery team was observed, but no interaction between time and groups was observed.

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SU-03

### Efficacy of laparoscopic abomasopexy for left displaced abomasum in late-pregnant cows

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ISO VETERINARY SERVICE

**Objectives:** Recent increased occurrences of left displaced abomasum (LDA) lead to great economical loss. Majority of LDAs commonly occur postpartum, but some of LDAs are also found during late pregnancy. In Japan, right flank omentopexy is selected as the surgical technique for the postpartum LDAs. However, applications of this surgical technique for LDAs during late pregnancy are extremely hard, because of frequent interruptions of the supermassive pregnant uterus. This study introduces good surgical outcomes due to applications of laparoscopic abomasopexy for LDAs in late-pregnant cows.

**Materials and Methods:** Fifteen heads of female late-pregnant cows aged average 6.6 years (ranging 4 to 11 years), and calved average 3.3 times (ranging 1 to 6 times). The animals have presented anorexia, and diagnosed from ping sound of abomasum within the left chest walls by auscultation. Laparoscopic abomasopexy was performed at average 266.9 gestation day (ranging 263 to 274 gestation day) with the following surgical procedure; 1) a first 10-mm-diameter trocar is bluntly introduced into the left flank wall caudal than the last rib. Endoscope is inserted through the trocar into the abdominal cavity for observation of the big and going-up abomasum within the spaces between the left abdominal wall and the rumen; 2) Under endoscopic observation of abdominal cavity, a second 13-mm-diameter trocar is introduced in the eleventh and twelfth intercostal space; 3) the sharp cannula (the sharp needle is set into the cannula) is inserted through a second trocar into the abomasal lumen under endoscopic observation; 4) after removal of the sharp needle, a toggle bar is inserted into the abomasal lumen, and abomasal gas is escape through the cannula; 5) Soon after endoscopic observation of downward moving of the reduced abomasums, using one-hundred-thirty-cm-long needle and one-hundred-fifteen-cm-long applicator, the suture of toggle pin, which is tied to apex of the needle, goes outside through the ventral abdominal wall; and 6) the suture is tied over a gauze stent without tension. Tied suture is cut at 7 days after surgery. The cure rates due to laparoscopic abomasopexy for late-pregnant cows were statistically investigated compared with those of right flank omentopexy (n = 314), and laparoscopic abomasopexy during postnatal periods (n = 256) with chi-square test.

**Results:** All animals appeared quick recovery of appetite, and experienced normal deliveries without the recurrences. Perinatal diseases were also not found during 5 months after surgery. The cure rate due to laparoscopic abomasopexy for late-pregnant cows was 100% (15/15), and identical with the cure rate of 92.0% (289/314) due to right flank omentopexy, and comparatively high than 80.9% (207/256) due to laparoscopic abomasopexy during postnatal periods, with no significantly differ-

ences.

**Conclusions:** The late-pregnant cows with LDA commonly receive right flank omentopexy after delivery due to parturition induction. This procedure frequently relates to low milk productions and occurrence of perinatal diseases. During late pregnant periods, left flank abomasopexy is recommended, but is not easy for repair of LDA, because of relationship between body size of the animals and arm length of operators and interruptions of the supermassive pregnant uterus. Laparoscopic abomasopexy allows high cure rate, because the positional repairs of the abomasum are possible based on the endoscopic recognitions of another abnormality of the abdominal organs as well as the dislocated abomasum without interruptions of the pregnant uterus. Moreover, laparoscopic abomasopexy is minimally invasive surgical technique, which is very important from a point of view of animal welfare.

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SU-04

### Can Interlocking Nail Stabilization be an alternative technique for long bone fractures in Calves?

#### Interlocking Nail Stabilization in Calves

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The aim of the study to report the clinical and radiographic outcome after use of an interlocking nail for stabilization of long bone fractures in calves.

A total of 21 different breed, age, weight and sex calves with the front and hind limb fractures were obtained to the Selcuk University, Faculty of Veterinary Medicine, Department of Surgery. Proximal or distal diaphyseal transverse, oblique fractures of the *humerus*, *radius*, *femur* and *tibia* were evaluated. 3rd generation of interlocking pin was used in this study. Equipment was produced by Orthovet in Izmir. 6, 8, 9 and 10 mm in diameter was used for interlocking pin. Interlocking pin was placed for 15 calves. The distribution of cases are 7 *femur* (46.6%), 4 *tibia* (26.6), 2 *humerus* (13.3%) and 2 *radius* (13.3%), respectively.

Six out of the 15 patients (40%) were good condition without any problems in the post-operative period. In 8 patients (60%) were ex in the post-operative period. They have shown various causes (infection, opening of suturing area, etc.). The success of 40% in results is particularly linked to the post-operative care conditions. Despite the short period of the operation and good fixation, low success rate was attributed not to provide adequate environment in the post-operative period.

This study is showing that calves with the fractures are particularly occur during birth and incongrute helping. It is causes severe economic losses in the calves in our country. Interlocking pin is intended to use widespread application for calf orthopedics.



SU-05

### Randomised prospective trials to study effects of reduced antibiotic usage in abdominal surgery in cows

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Societal concerns on antibiotic resistance urged us to evaluate the use of prophylactic and postoperative antibiotic treatments for caesarean section (CS) and exploratory laparotomy (LA) in the teaching hospital. To this end, we conducted a randomized prospective trial for both abdominal surgeries. Cows submitted for CS received either a prophylaxis with 5 gram of ampicillin-sodium IV only (CSL) or in combination with post-operative IM injections of ampicillin-trihydrate 20% for three days (10 mg/kg, 2 dd)(CSH). Cows that underwent LA were given no antibiotic prophylaxis (LAN) or 5 gram of ampicillin-sodium IV (LAL). Cows in all groups were found healthy after a follow-up period of 10 days given their normal temperature, feed intake and clinical appearance. More antibiotic treatments were needed for the treatment of complications for CS compared to LA groups, but did not differ within surgery type between protocols. We observed that CSL cows required more treatments for diseases that were not related to the surgery than CSH cows (OR 2.8 (CI 1.2-7.2)). The percentage of infected sutures was higher for LAN cows compared to LAL cows (OR 2.6 (CI 1.5-4.9)). We estimated that 29 CSH treatments are needed to prevent 1 CS cow with serious complications related to the surgery in the CSL group. Likewise, 53 LAL treatments would prevent 1 LA cow with complications related to the surgery in the LAN group. We therefore concluded that it is possible to reduce antibiotic prophylaxis in CS and LA cows. The low number of clinically detected complications could effectively be treated by postoperative antibiotics.

SU-06

### Neurosurgical treatment, clinical findings and imagistic diagnosis of coenurosis cerebri in sheep.

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**Objectives:** Coenurosis is a disorder of the central nervous system in sheep, caused by *Coenurus cerebralis*, the larval stage of *Taenia multiceps*, a tapeworm, which parasite in the small intestine of carnivores, surgical removal of cysts is the only effective therapy to date.

Objectives of the study were to evaluate the imagistic investigations for accuracy of diagnosis, to describe and to evaluate the neurosurgical procedure for the cyst removal.

**Materials and methods:** This retrospective study, during the period between October 2014 and October 2017, describes the clinical evolution, imagistic diagnosis and surgical treatment of cerebral coenurosis from acute to chronic infestation in 18

sheep who were naturally infected, from 7 different flocks with an average of 150–250 animals each. Two Sarda sheep, four Merinos, five Tigaia(Tzigai) seven Turcana(Tsurcana) breed, ten males and eight females, between 5-18 months of age, weighting 11–44 kg, were examined because of neurological signs. All 18 sheep showing clinical signs of infestation with *Coenurus cerebralis* were subjected imagistic examinations (radiographical examination(Rx) and computed tomography (CT)), and subsequently treated surgically for removal of the cyst by craniotomy.

Xylazine was given intramuscularly at a dose of 0.05 mg/kg bodyweight, induction of anaesthesia was then achieved by intravenous injection of ketamine at a dose of 2.2 mg/kg bodyweight, followed by intubation and general anaesthesia maintenance with Isoflurane. The sheep were secured in ventral recumbency and area between horns were aseptically prepared with Betadine and draped. A linear sagittal skin incision on occipital region were performed with careful haemostasis using electrocautery, and after H periosteal incision the resulted flap was undermined and elevated. Craniotomy was performed using an oscillating saw blade(osteotome) a squared bone flap(approximately 2 by 2cm) was removed. The duramater was incised once the bone flap had been removed. After craniotomy and durotomy, commonly because of intracystical pressure and superficial localization, the cyst protrude and appeared as water filled balloon. The cyst was gently grasped by using one or two a mosquito artery forcep and gently pulled out by twisting the forceps. When the cyst was profound localized within the nervous substance gently exploration was made with an 18G needle and fluid aspiration for cyst deflating and intracranial decompression. Then the bone flap was placed on site, periosteal flap suture by simple interrupted with Vicryl 3-0, and skin incision were closed by simple interrupted with non absorbable Prolene 2-0.

**Results:** Symptoms were related to cyst localization, and the most prominent clinical symptoms were: weight loss(n=15), unstable gait or incoordination(n=11), ataxia(n=11), circling behavior(n=9)loss of herd instinct(10), head tremors(n=10), obtunded mental status(n=7), drowsiness and bilateral reduction of the menace reaction(n=7), nistagmus(n=5).

Radiographical examination(Rx) and computed tomography (CT) allow the diagnosis and accurate localization of and the cyst. Cysts were most commonly localized in the parietal and frontal lobes of the brain. In 3 sheep(n=3) cysts were very large and fluid volume within the cyst ranged between 100 to the largest one 170ml. The cysts were localized in left lobe in seven sheep(n=7), in right lobe in eight sheep(n=8), and in three sheep the cyst invaded left and right lobes. In one sheep(n=1) was a severe haemorrhage because a severe bleeding from central artery and intraoperative death. One sheep(n=1) that was in recumbency in last 5 days was unable to stand without support in next day, and it was euthanased. Sheep recovery started from the first hours after the surgery in cases of subdural cyst removal, 50% of the sheep(n=9) standing within 3-4 postop. In this study the successful and recovery rates was 88,88%, similar with the literature reported recovery rates(75%–90%).

**Conclusions:** The only effective therapy is based on neurosurgical removal of the coenurus cyst under general anaesthesia of the animal; the approach has a very good success rate, dependent on accurate localisation of the lesion(by radiographical examination and computed tomography).



The observation allow to consider the CT the golden standard for accurate cyst localisation.

There was a successful clinical response to the removal of the cyst.

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SU-07

### Unilateral rostral mandibulectomy for gingival vascular hamartoma in two calves

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**Objectives:** A vascular hamartoma is a neonatal malformation with a neoplasm-like structure composed of large numbers of disorganized capillaries. The rostral mandibular gingiva is one of the most common locations for such lesions in newborn calves. For most gingival vascular hamartomas in calves, surgical resection results in a good prognosis without recurrence. However, recurrence has been reported in some cases, and was associated with osteolysis due to deep invasion of the mass. The present report introduces therapeutic efficacy of unilateral rostral mandibulectomy for the invasive gingival vascular hamartomas.

**Materials and Methods:** A 2-month-old female Holstein calf (Case 1) and a 5-month-old female Japanese black calf (Case 2) presented with acute heavy hemorrhage from the mouth, and significant pain-related behavior. Case 2 experienced 2-times recurrences at 1 month after surgical resection, and soon after thermocautery applied to the base part of the mass, since the mass was first found at 2 month old. Osteolytic changes appeared within the mandibular bones adjacent to the masses on radiographic or computed tomographic images.

**Results:** Case 1 and Case 2 was placed in the right and left lateral recumbent position under anesthesia, respectively. An incision with cautery was made 3 mm from the border of the mass in the gingival mucosa on the buccal and lingual aspects of the canine tooth and three incisors of the affected mandible. After blunt dissection of the gingival mucosa from the mandibular bone, the first cut was applied to the exposed mandibular bone on the rostral aspect of the affected region using an oscillating saw, placed as far from the mass as possible. The second cut was made on the sublingual aspect of the mandibular bone, directed obliquely from the caudal region of the canine tooth to the apex of the mandibular symphysis. The bony segment including the mass and the teeth was removed from the mandibular bone using a bone chisel. The rostral and sublingual gingival mucosae were apposed over the cut surface of the bone with absorbable suture material. Two animals had a normal appetite and were not restricted from eating. One week after surgery, intact healings without suppuration were observed in the surgical wound. The animal's cosmetic appearances had not changed. At 2 years and 1 year after surgery, no recurrence was found in Case 1 and Case 2, respectively.

**Conclusions:** Unilateral rostral mandibulectomy is the useful

surgical technique for intact removal of pathological region of mandibular bones and prevention of postoperative recurrence in invasive gingival vascular hamartomas in calves. Moreover, because such bony involvements may predict recurrence, radiography and CT can be also used for surgical planning for gingival vascular hamartomas in calves.

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SU-08

### Subluxation of the Elbow in a Cow: Clinical Signs, Diagnostic Imaging, Treatment and Long-term Follow-up

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**Objectives:** The aim of this report was to describe the clinical signs, results of diagnostic imaging and treatment of radioulnar subluxation in a dairy cow and to present the short- and long-term prognosis.

**Material and Methods:** A 3-year-old Red Holstein dairy cow, 7 months pregnant, was presented with acute 4/5 mixed lameness of the right forelimb. The lameness started when the cow was on pasture but the cause was not identified. On clinical examination, the carpus was mildly flexed and there was occasional knuckling of the fetlock. There was a painful effusion of the elbow joint and radiographs showed radio-ulnar subluxation with lateral displacement of the radius and ulna.

**Results:** With the cow under general anesthesia and in left lateral recumbency, the elbow joint was positioned in maximum flexion by one surgeon and the limb was rotated medially. A second surgeon reduced the radio-ulnar joint subluxation by applying lateral pressure to the radius. No additional devices were necessary. After reduction, no internal or external fixation was provided. Recovery from anesthesia and the clinical course were uneventful. The cow was confined to a box stall bedded with wood shavings for 6 weeks during which time the lameness grade gradually decreased. Clinical and radiographic examination 6 weeks after reduction confirmed that the radius and ulna were in a normal position. The cow was discharged from the clinic with the recommendation of another 6 weeks of box rest and 4 weeks of hand walking exercise. Four months after reduction, the cow had calved and returned to her intended use in the herd. One year later, clinical and radiographic examination of the cow at the farm showed no lameness and only mild arthrosis.

**Discussion and Conclusion:** Subluxation of the elbow joint is rare in cows. We describe successful closed reduction of a radio-ulnar subluxation in a dairy cow. Cattle are generally able to protect injured limbs when lying down or rising, therefore and for economic reason, conservative treatment was preferred over surgical fixation. Complete clinical and radiographic healing was achieved after 4 months of box stall confinement and the cow was re-introduced into the tie-stall herd with pasture access.



SU-09

### Femoral head ostectomy for treatment of chronic coxofemoral luxation in a Shorthorn cow

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**Objectives:** To describe the feasibility and outcome of femoral head ostectomy in a clinical case of chronic coxofemoral luxation in a Shorthorn cow.

**Materials and methods:** A 2-year-old shorthorn cow was diagnosed with a dorsal coxo-femoral luxation of 2 weeks duration by the referring veterinarian and admitted to the Veterinary Teaching Hospital. Upon arrival she was able to ambulate with a grade 3 out of 5 right hind lameness. While standing, a marked protrusion was present just cranial and dorsal to the ischium, presumed to be the greater trochanter. Closed reduction was not attempted because of the chronic nature of the injury. A femoral head ostectomy was performed due to the poor prognosis for open reduction and chronicity of the condition. Based on clinical experience, a guarded prognosis for breeding soundness was communicated. Under general anesthesia, a 25-cm long curvilinear incision was made starting caudal and dorsal to the acetabulum and extending distal and lateral along the long axis of the femur. Blunt dissection between the superficial and middle gluteal muscles allowed exposure of the luxated femoral head. External rotation of the limb aided exposure of the femoral head and neck. An oscillating bone saw was used to transect the femoral neck and remove the femoral head. The wound was copiously lavaged and the muscles apposed using #2 polydioxanone suture in cruciate pattern; the skin was sutured closed using #2 polypropylene suture in a cruciate pattern. Para-operative pain management was initiated prior to the surgery and continued for 30 days using meloxicam (0.5 mg/kg bdwt, every 48 hours, orally). Locomotor comfort was evaluated twice daily during the entire course of hospitalization. Electrical stimulation therapy (E-stim) was initiated the day after surgery. The hair was clipped and the areas of electrode contact were cleaned with betadine and isopropyl alcohol before each session. Transdermal functional electrical stimulation pads were placed at the proximal and distal ends of the biceps femoris and gluteal muscle bellies. Electrical intensity was slowly increased until a visible muscle contraction was seen. Treatment length was initiated at 5 minutes twice daily, and increased 1-2 minutes per day for a maximum treatment time of 20 minutes twice daily. The cow improved while hospitalized and was discharged 17 days after surgery. Instructions were given to the owner to continue the meloxicam for 7 days after discharge, to continue E-stim therapy for no more than 5 consecutive days per week until the mobility of the limb returned to near normal. Long term follow-up by video recording 4 months after the surgery showed that she was fully weight bearing, active, and does not hesitate to utilize the limb, but the cow had a mild persistent gait deficit (Grade 2 out of 5) with a minimal degree of lameness.

**Results/Conclusions:** This case report is the first report of a femoral head ostectomy being performed on an adult beef cow for the treatment of chronic coxofemoral luxation. FHO may represent an acceptable alternative to euthanasia or culling in

valuable breeding cows. A significant factor in the rehabilitation of this cow was the use of E-stim therapy to rapidly rebuild lost muscle mass associated with chronic lameness and pain.

SU-10

### Clinical findings and treatment outcomes of non-infectious joint disease in Japanese Black calves

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**Objectives:** Ten Japanese Black (JB) calves diagnosed with non-infectious joint disease (NJD) were analyzed with respect to their clinical findings and treatment outcomes. The purpose of this study was to examine the pathophysiology and clinical manifestations in JB calves with the disease.

**Materials and methods:** Medical records of ten JB calves diagnosed with NJD which were presented to the Veterinary Teaching Hospital, University of Miyazaki from 2013 to 2017 were submitted to this study. Their history, physical, radiological (n=7) and synovial fluid analysis (SNF, n=6) findings were evaluated, retrospectively. SNF analysis comprised of specific gravity (SG), total protein concentration (TP), cell count, differential leukocyte count (DLC) and microbiological examination included detection of *Mycoplasma bovis* by real-time PCR assay. Arthrotomy was performed in nine calves and arthrocentesis in one calf. The outcomes were evaluated based on improvement of clinical signs.

**Results:** This study comprised of seven females and three male calves. The median of age was 90 days (range: 30 ~ 240 days) and the median of body weight was 77.5 kg (range: 46 ~ 256 kg). In this study, carpal joint (n=4) was the most affected site, followed by hock (n=3), shoulder (n=1), stifle (n=1) and fetlock (n=1) joints respectively. Radiological findings (n=7) were chiefly characterized by subchondral bone cysts (n=2) and osteolysis (n=2) in four calves, while lesions could not be clearly defined in three calves. SNF analysis (n=6) resulted in a high TP concentration (n=4) and low TP concentration (n=2) respectively. The results of *Mycoplasma bovis* by real-time PCR was negative in all cases. Among nine calves receiving the surgery, six calves had good prognosis while three calves showed guarded to poor outcomes.

**Conclusions:** In this study, clinical signs and radiological lesions of JB calves with NJD had resemblance to previous reports of osteochondrosis in cattle and equines. Further research including pathology will be required to determine the diagnosis as osteochondrosis and to discuss the etiology and pathogenesis in JB calves with the disease.

SU-11

### Substance P monitoring to quantify nociceptive responses during and after umbilical surgery in calves

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**Objectives:** Plasma cortisol is a well-known indicator for stress, pain and fear, but substance P is believed to be more sensitive and accurate concerning the objective assessment of pain in ruminants. Several studies have described substance P in calves and cattle undergoing different surgical and painful procedures, such as castration, dehorning and electroejaculation. The aim of this study was to describe and evaluate plasma concentrations of substance P in calves undergoing umbilical surgery with two different analgesic protocols.

**Material and methods:** Umbilical surgery was performed in 21 calves (aged  $38 \pm 9$  days) with umbilical hernia but otherwise sound general condition, according to a strictly standardized surgical protocol. Calves were randomly assigned to either CON (control group,  $n = 10$ ) or MET (metamizole group,  $n = 11$ ) and treated with meloxicam (0.5 mg/kg body weight (BW) intravenously) and additionally either with a placebo (0.9% sodium chloride solution, CON) or metamizole (40 mg/kg BW intravenously, MET). Anesthesia was initiated with xylazine (0.2 mg/kg BW intramuscularly) and ketamine (2 mg/kg BW intravenously). Umbilical surgery was performed under general anesthesia with isoflurane (1-5% inspiratory isoflurane concentration, depending on the effect on the calf) with ventilation by intermittent positive pressure. All animals were treated with benzyl penicillin procaine (20.000 IU/kg BW subcutaneously) from the day before surgery until day 4 after the surgery. Blood samples for determination of plasma substance P (PSPC) and plasma cortisol (PCC) concentrations were taken 60 minutes before surgery (baseline concentration) and 5, 15, 30, 45, 60, 90, 150 and 510 minutes after the start of surgery.

**Results:** PSPC increased in both groups 5 minutes after start of surgery. In CON, PSPC was significantly ( $p < 0.05$ ) higher 5, 30 and 90 minutes after start of the surgery compared to the baseline concentration. PSPC was lower in MET than in CON at all times, but not significantly. Calves of MET reached baseline PSPC 60 minutes after surgery, whereas calves of CON did not reach baseline concentrations during the trial. A linear mixed model showed that mean PSPC was 214.5 pg/mL lower in MET than in CON (95% confidence interval: -509.7 to 80.8 pg/mL). There was no statistically significant difference ( $p = 0.25$ ) in AUC between CON and MET. Correlation between PSPC and PCC was weak but significantly positive ( $\rho = 0.255$ ,  $p < 0.0004$ ).

**Conclusion:** The results of this study show that PSPC increases during umbilical surgery and is significantly positively correlated with PCC. Metamizole, administered as an additional non-opioid drug to meloxicam, results in lower PSPC during surgery, representing lower nociception of pain in calves. Thus, in the opinion of the authors, substance P is a suitable and objective biomarker that can be used for the assessment of nociception in ruminants.

SU-12

## Influence of metamizole on nociception in calves undergoing inhalation anesthesia for umbilical surgery

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**Objectives:** In preventing nociception in calves undergoing surgery, the commonly used anesthesia regimes (xylazine, ketamine, isoflurane, meloxicam) may be insufficient. For this reason, a double-blind study in calves was conducted, in order to examine if additional application of metamizole (dipyrone) prior to umbilical surgery reduces the surgery-induced cortisol release, as a possible sign of a reduction in nociception.

**Animals and methods:** 26 calves with uncomplicated umbilical hernia but otherwise clinically normal were randomly allocated to a metamizole group (MG) and a control group (CG). The groups differed only in the presurgical application of either 40 mg/kg metamizole intravenously (i.v.) (MG) or an equal volume of sterile 0.9 % sodium chloride solution (CG), 60 minutes before skin incision (SI). All calves received meloxicam (0.5 mg/kg) i.v. 60 minutes before SI. General anesthesia was induced by injection of xylazine (0.2 mg/kg, intramuscularly) and ketamine (2 mg/kg, i.v.), maintenance after intubation by isoflurane (ET<sub>iso</sub> 1.4%) in medical oxygen. Animals were mechanically ventilated by intermittent positive-pressure ventilation (IPPV). The surgical procedure was strictly scheduled, and all manipulations standardized, and time-slots for each step were defined (before beginning of the surgical intervention, positioning of cloth clamps, positioning of the navel clamp, incision of the skin, preparation of subcutaneous tissue, incision of the peritoneum, ligation of the umbilical vein, manipulation of the guts, suture of the peritoneum and fascia, suture of the subcutaneous tissue, suture of the skin, end of surgery, end of anaesthesia, after end of anesthesia). Heart rate, mean arterial blood pressure and reactions to noxious stimuli were recorded from beginning until the end of anaesthesia 60 minutes after SI. Plasma concentration of cortisol (PCC) was measured 60 minutes before SI, and at 5, 30, 150, and 510 minutes after SI. For statistical analysis, multiple linear models, linear mixed models, and the Fisher's exact test were used.

**Results:** None of the calves showed any movements of the legs or other signs of defensive reactions during surgery. In both groups, heart rate dropped continuously whereas mean blood pressure increased but, (apart for HR in time-slot 'positioning of the navel clamp': MG: 86 beats/min versus CG: 78 beats/min), there were no significant differences between groups. PCC was significantly lower 150 minutes after SI in the MG, compared with the CG (median: 11.6 versus 39.1 nmol/L,  $p < 0.01$ ) and, considering the whole course, was estimated to be 11.9 nmol/L lower on average in the MG than in the CG. The median of the maximal PCCs was higher in the CG (59.3 nmol/L) than in the MG (37.9 nmol/L) ( $p = 0.08$ ). While 92.3% of the animals in the MG reached the maximal cortisol concentration intraoperatively, 53.8% of those in the CG recorded it postsurgically ( $p = 0.03$ ).

**Conclusions:** The additional presurgical use of dipyrone (met-



amizole) results in a significantly lower increase of plasma cortisol concentration in the course of an umbilical surgery, and leads to a quicker decrease of the cortisol level after the end of the surgical procedure, indicating a possible reduction in nociception and an improvement of the analgetic management.

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SU-13

### Post-operative complications after elective caesarean sections in Belgian Blue cattle, performed in a clinical setting.

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**Objectives:** A caesarean section (CS) is a routinely applied surgical procedure in the double-muscled Belgian Blue breed. Due to foeto-maternal disproportion, 90% - 95% of the Belgian Blue calves are born via elective CS. For Belgian veterinary students, the CS is an essential day-one-skill they should master before graduation. A CS, being an invasive surgical procedure, requires excellent operational skills to minimize post-operative complications. The latter may result in reduced fertility or can cause problems during subsequent CS. The aim of the present study was to investigate the occurrence of post-operative complications after an elective CS in a clinical educational setting.

**Materials and Methods:** Data of 1,083 surgery reports of CS performed on Belgian Blue cattle in the teaching hospital of the Faculty of Veterinary Medicine (Ghent University) were used as input for the study. All CS were performed according to a standard protocol, by graduated veterinarians assisted by two undergraduate students. The surgery reports contain clinical information about the cow, findings during prepartal vaginal examination and the CS itself. In addition, retention of foetal membranes, fever within the first three days post-partum and post-operative complications during the first ten days after calving (skin emphysema, internal haemorrhage, wound infection, wound dehiscence, peritonitis and death) were recorded. The prevalence of post-operative adhesions was calculated by comparing the prevalence of adhesions in cows that underwent two consecutive CS (N=354).

**Results:** Only operations in which a life calf was born (N=1,053) were included in the study. The results of the descriptive analysis show that 250 (23.7%) animals hadn't expelled their foetal membranes within 12 hours post-partum. Fever (>39.5°C) was present in 13.7% of the animals. In total 30.4% of the animals had suffered from at least one post-operative complication, wound emphysema being the most prevalent one (21.3%). Only a mere 9.1% of the animals had suffered from at least one severe post-operative complication such as wound infection (1.4%), wound dehiscence (0.7%), internal haemorrhage (0.3%) and peritonitis (0.2%). Two animals (0.2%) died during the CS due to diffuse bleeding of the uterus during the operation. In 35.4% of all cows that had experienced at least one previous CS (N=562), adhesions were noticed during surgery, while only 8.9% of these adhesions were classified as severe, involving the uterus and seriously impeding the surgical procedure.

**Conclusions:** This study reveals that severe post-operative complications are relatively seldom present in elective CS. The high occurrence of retained foetal membranes (23.7%), could be a cause of concern since this might lead to metritis and even peritonitis. Post-operative adhesions, with a potentially negative impact on future fertility and hence feasibility of a subsequent SC, were a rare finding in the present setting (8.9%). However, this amount might be an underestimation since not all cows become pregnant again (infertility due to adhesions, (endo)metritis,...), not all cows return to the clinic for their next CS and not all data on the most recent CS are known yet. We furthermore conclude that an elective CS can be taught to undergraduates with only minimal post-operative complications using the current standard operation procedure.

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SU-14

### Use of Blood L-Lactate as a Prognosis Factor for the management of surgical Digestive Disorders in cattle.

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Digestive diseases are common in cattle and a surgery is often necessary to cure. In cattle medicine, an economical approach is actually essential and we must improve prognosis accuracy using tools to decide whether to operate, cull or euthanize the animal. Blood L-lactate (BL) as prognosis factor was previously used for abomasal displacement in dairy cattle. In a preliminary study performed at the Clinique for Ruminant of the Veterinary Teaching Hospital of the veterinary of Liège based on 17 calves, BL before surgery appears to be a reliable prognosis factor with a threshold above which digestive surgery is not indicated due to the poor prognosis. We herein determine if BL variations could be used as a prognosis predictor before and after surgery in calves and adult cattle for digestive diseases.

All data were collected at the Clinic for Ruminant of the Veterinary Teaching Hospital of the University of Liège. Two studies were performed between September 2013 and June 2017. The first study concern 156 animals referred for digestive problems between January 2014 and June 2017, 66 adult animals and 90 calves, divided in two groups depending on the outcome, positive (P) or negative (N) outcome. BL was measured at the arrival after clinical examination using L-lactate Epoc® handler (LAC1). Post-operative L-lactate was measured 12h (LAC2) and 48h post-surgery (LAC3). Analysis of ROC curves indicated an AUC > 0,5 for LAC1 in calves, with a p-value of 0,039, but no reliable cut-off value could be determine. In adult cattle, no significant difference was found in LAC1 between the two groups. However, in both calves and adults cattle, LAC2 and especially LAC3 could predict outcome after surgery. The second survey deepens this aspect, studying the data of 52 adults cattle referred to the University Veterinary Clinic (CVU) for colic with a surgical intestinal disease diagnosed by celiotomy between September 2013 and May 2017. BL was measured before (LAC1) using Epoc® device, 12h (LAC2) and 48h (LAC3) after the surgery, using Accutrend®plus device. The evolution of BL over time was investigated and differences between two groups (P and N) were analyzed. The level of BL significantly



decreases over time within each group and postoperative levels are significantly lower in the group with the P ( $p < 0.5$ ). ROC curves show that four parameters based on the level of BL are useful to predict the outcome (i.e.  $AUC > 0.5$ ): LAC2, LAC3, the decrease (%) between LAC1-LAC2 and LAC2-LAC3. A P is predicted when LAC2 is  $< 1.73$  mmol/L or when the decrease of BL is  $> 50\%$  12h after surgery, or when LAC3 is  $< 1.94$  mmol/L or when the decrease of BL is  $> 70.5\%$  48h after surgery.

We herein demonstrate that in calves younger than 6 months, BL could be a good prognostic tool for digestive surgery, but no threshold could be highlighted yet, so other parameters should be taken into consideration. BL before surgery cannot be used to determine outcome after surgery in adult cattle, but results shown that the BL measured after the surgery and it changes over time allow reliable predictions of the outcome of digestive surgeries in adult cattle, with a best prediction using BL 48h after surgery.

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SU-15

### Case report: Correction of carpal angular deformities with closing wedge osteotomy in goat

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**Objectives:** Congenital angular limb deformities is very rare in ruminants. These deformities can originate from many sources: incomplete carpal/tarsal cuboidal bone ossification, growth plate abnormality, diaphyseal malformation and malunion, fracture, and ligament rupture. In our case patient was affected carpus valgus angular deformities.

**Materials and methods:** The goat was brought to the clinic in age of 24 weeks with angular deformities of forelimbs. Orthopedical and X – ray examination diagnosed valgus carpal deformities of both forelimbs. The cause of angular deformities were the curvature of distal metaphyses of the antebrachia. The pivot angle was measured to mark the degree of deformities. On the left limb  $28^\circ$  and on the right limb  $30^\circ$ .

A month before the patient was demonstrated a bridging of distal physis of radius was performed by cerclage and cortical screws. This procedure was without effect. After agreement with the owner, a closing wedge osteotomy was performed on both forelegs. The incision by oscillatory saw was performed by wedge resection at the transition of distal metaphyses and diaphysis at radius and ulna. After repositioning, the fragments were fixed with a plate and 4.5 mm cortical screws. The procedure was performed on both sides. Post operative medical therapy included systemic antibiotics and NSAID. The limbs were immobilised with a bandage splint. Directly after standing after surgery, the patient was bearing weight on both limbs. After 10 days the bandage was removed and stitches were removed. The limbs were immobilised with a cast for 5 weeks.

After 5 weeks, an X-ray was performed to show the forming callus. In the next 4 weeks, the X-ray examination was again performed showing ossified callus on both forelimbs and it was decided to remove the plates.

**Results:** Approximately at a half a year old goat both side clos-

ing wedge osteotomy was performed. The reason was the valgus carpal deformities of both carpal joints. The procedure was successful. The healing time was 3 months.

**Conclusions:** Based on this case, we are convinced that both side closure wedge osteotomy is suitable for dealing with angular deformities in sheep and goats. Although this treatment is time consuming and financially more demanding, it is particularly interested in breeders who keep sheep and goats as pets. We will continue to address this issue in the future.



TP-01

## Milk residues in lactating dairy cattle following concurrent use of veterinary medicines

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**Objectives:** Veterinarians in New Zealand commonly prescribe the use of more than one restricted veterinary medicine (RVM) at a time for the treatment of clinical cases of disease in dairy cattle, and are responsible for providing the appropriate milk withholding period (WHP) instructions for any combination of products that they authorise. Individual veterinary medicine WHPs are generated via residue studies in healthy cattle treated with a single medicine alone, and there is scarce data available for when 2 or more medicines are administered concurrently. It is not known if there is any effect on milk residues and the subsequent WHP when 2 products are administered during the same treatment period. This study was to determine milk residues in lactating dairy cows following concurrent administration of injectable or intramammary RVM product pairs.

**Materials and methods:** A randomised field study was undertaken on two commercial New Zealand dairy farms. Lactating, healthy dairy cows that had not been treated with an antibiotic or anti-inflammatory product during the 30 days prior and being milked twice daily were enrolled and randomly assigned to 19 groups of 5 cows. Each study group was treated with 2 RVM products following the standard label course of treatment (dose, frequency and number of treatments). Treatments included parenteral and intramammary antibiotics and non-steroidal anti-inflammatory (NSAID) medicines. All intramammary products were administered to all four quarters of each cow at each treatment time, and some of these contained multiple antibiotic actives. For each group after the treatment course was complete, the longest milk WHP was observed with milk discarded, then at the next four subsequent milkings a composite milk sample was collected using proportionate herd test meters. Each milk meter was used only once per milking to collect one sample, and was hot-washed after each milking before next use.

Milk samples were analysed for the presence and concentration of multiple antibiotics using Liquid Chromatography-Mass spectrometry. The concentration of antibiotic in each milk sample was compared to the maximum residue limit (MRL) permitted in milk according to the NZ Ministry of Primary Industries 'Food Notice: Maximum Residue Levels for agricultural compounds'.

**Results:** Milk samples obtained from the first milking after the observed WHP were slightly above an antibiotic MRL in 2 out of 95 cows, which were from different study groups. These study groups were treated with an intramammary antibiotic and a non-steroidal anti-inflammatory product. In both cases the milk samples from the other 4 cows in the group at the same time point returned a result which was far below the MRL. Milk samples that tested below the MRL were returned by one of the outlier cows at the 2<sup>nd</sup> milking after the observed WHP, and the other cow at the 3<sup>rd</sup> milking after the WHP.

The incidence of antibiotic concentration above the MRL threshold in this study was rare. As the regular excretory route for intramammary antibiotics is via milk removal, the two cows with results above MRL could be attributed to low milk produc-

tion (reducing excretion of the antibiotic from the mammary gland), or due to tissue damage or fibrosis inside one or more quarters as a result of prior mastitis or other trauma to the mammary tissue. It is unusual in practice to treat all four quarters simultaneously with intramammary antibiotic product, as occurred in this study. Thus a higher quantity of antibiotic was administered to each cow than would be customary in practice.

It should be noted that the milk samples were not analysed for anti-inflammatory medicine actives, and the study was undertaken with the objective to assess individual cow data with no interpretation attempted at a herd bulk milk tank level, for which milk residue limits may be different due to milk processor or regulatory residue limits.

**Conclusion:** This study provides the first data for New Zealand registered veterinary medicines used concurrently in lactating dairy cattle, for antibiotic residues detected in milk following the longest milk WHP. Concurrent administration of two veterinary medicines to dairy cattle following label directions, for the majority of product pairs and cows, did not incur residue concentrations above the MRL for each antibiotic active at the first milking beyond the longest label milk WHP for the medicine pair.

TP-02

## Antimicrobial peptides produced by mammary gland bacteria with activity against major mastitis pathogens

### Funded by Sistema General de Regalías de Risaralda

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Bovine mastitis is an inflammation of the mammary gland, usually caused by bacteria. Treatment of mastitis is performed with antibiotics, which have the disadvantage to generate residues in milk, becoming a risk factor in the emergence of antibiotic resistant strains. **Objective:** The aim of this study was to search for molecules with antimicrobial activity and originated from other intramammary bacteria. **Methodology:** Several bacteria strains (CNS, n=269; *Streptococcus* spp., n=75; *Enterococcus* spp., n=11; and LAB, n=548) were isolated from 275 milk composite samples collected in 11 Colombian dairy herds. Those isolates were tested for antimicrobial activity against *Staphylococcus aureus* and *Streptococcus agalactiae*. Antimicrobial activity was assessed by the agar well diffusion method. Descriptive statistics was used to present preliminary results. **Results:** Several isolates (3.3%) exhibited antimicrobial activity against both indicator strains. Some of them (5.9%) showed activity only against *Staphylococcus aureus*, and 6.1% only against *Streptococcus agalactiae*. These antimicrobial substances were characterized as bacteriocin-like inhibitory substances by their sensitivity to proteolytic enzymes. The sensitivity tests *in vitro* are still under way, and further clinical trials *in vivo* are needed. **Conclusions:** These preliminary results suggest that antimicrobial peptides produced by bacteria species from the udder are molecules with a high potential as an alternative



treatment for bovine mastitis. Antimicrobial peptides might be used as mastitis treatment with no withdrawal time in comparison to antibiotics, and will be safer for human health.

TP-03

### Pharmacokinetics of enrofloxacin in the peripheral blood and bronchoalveolar region after single subcutaneous administration in calves

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**Introduction:** The calf pneumonia is mainly recognized as alveolar pneumonia. Therefore, it is important for systemically administered antimicrobial agent to distribute in bronchoalveolar region effectively to act against the targeted bacteria. In the present study, we investigated the distribution of enrofloxacin (ERFX) in the peripheral blood and bronchoalveolar region after subcutaneous administration to healthy calves.

**Material and Methods:** Clinically healthy 4 Holstein bull calves were used twice each in this study. A single dose (5mg/kg) of commercial ERFX (Baytril® 5% injectable solution, Bayer, Tokyo Japan) was administered to all calves by subcutaneous injection. The blood and bronchoalveolar lavage fluid (BALF) samples were taken before and administration. Peripheral blood samples were collected at 0 (before administration), 1, 2, 3, 6, 10 and 24 hr after administration via the jugular vein. The ERFX concentration, ciprofloxacin (CPFX) concentration and concentration of urea nitrogen (UN) were measured each. BALF was collected at 0 (before administration), 2, 6, 10 and 24 hr after administration. And the ERFX concentration, ciprofloxacin (CPFX) concentration and concentration of urea nitrogen (UN) were measured each after the supernatant and cell pellets were separated. The ERFX concentration in pulmonary epithelial lining fluid (ELF) was determined by UN in plasma and alveolar cells in BALF. The ERFX concentration determined by a volume of  $1.28 \mu\text{l}/10^6$  BALF cells.

**Results:** The ERFX concentration in plasma rose to an average of 1,272 ng/ml at 1 hr after administration, and reached the peak value (average 1,450 ng/ml) at 2 hr after administration.

ERFX concentrations in ELF reached the peak value (average 9,372 ng / mL) at 2 hrs after ERFX administration, and it decreased gradually. But relatively high concentrations were maintained after administration at even 24 hrs. ERFX concentration in BALF cells rose to an average of 4,409 ng / mL at 2 hrs after ERFX administration, and reached the peak value (average 5,241 ng / mL) after ERFX administration at 6 hrs. On the other hand, ERFX concentration in ELF increased 8.9 times on average compared to ERFX concentration in plasma at 6 hrs after ERFX administration. ERFX concentration in BALF cells reached 8.2 times on average compared to ERFX concentration in plasma at 10 hrs after ERFX administration.

**Discussion:** The subcutaneously administered ERFX to healthy calves could distribute in the bronchoalveolar region, the outside the body, with high concentration for a long time. ERFX was thought to be the effective tool for treatment of calf

bacterial pneumonia. At the present time, high-dose administration of ERFX (7.5 mg / kg BW ; Baytril® one shot injection solution) is also measured.

TP-04

### The Predictive Value of Using Antemortem Bacterial Culture and Susceptibility Testing to Determine Clinical Outcome of Bovine Respiratory Disease in Feedlot Cattle

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**Abstract:** Bacterial culture and antimicrobial susceptibility testing are commonly used diagnostic tests to help determine the bacterial pathogens involved in causing BRD and to select antimicrobial agents for the treatment or control of BRD.

The objective of this study was to determine how closely the results of antemortem bacterial culture and susceptibility testing correlated with clinical outcome in feedlot cattle that were determined to be ill due to BRD.

Over one thousand heifers were procured from sources with a perceived history of tulathromycin resistance and transported to a research facility in Texas, USA. Within 36 hours of arrival at the research center, all cattle enrolled in the study were processed using standard feedlot arrival protocols. Each animal was weighed individually and two ear notch samples were taken and sent to two separate laboratories for diagnosis of BVDV persistent infection. As soon as possible after test results were available, animals identified as persistently infected with BVDV were removed from study pens and excluded from the study. A Deep Nasopharyngeal Swab (DNS) was used to collect a sample for bacterial culture. After wiping the external nares with a clean paper towel to remove external debris, a sterile double guarded culture swab was inserted into the ventral nasal meatus until the pharyngeal mucosa was contacted or a distance from the nares to the medial canthus of the eye was reached. Animal identifications were recorded and swabs were individually labeled and stored in AMIES transport media without charcoal, kept under refrigeration, and then shipped overnight on ice packs to the laboratory. All animals then were given an injection of tulathromycin per label (2.5 mg tulathromycin/kg BW) to control the further clinical signs of BRD. After a 7 day treatment moratorium, animals were eligible for first pull treatment of BRD if they were identified with clinical signs of BRD based on rectal temperature and a Clinical Attitude Score (CAS) of 1 and rectal temperature = or > 39.7°C or a CAS = or > 2 regardless of rectal temperature. Prior to treatment, all animals were again sampled for bacterial culture and susceptibility testing using the DNS procedure. As well, a nasal swab (NS) was taken for viral PCR testing. Animals were not eligible for additional treatment of BRD for another period of 7 days after administration of tulathromycin for first pull treatment unless animals were scored a CAS > 3 which resulted in those animals receiving emergency treatment or euthanasia and removal from the study pens. All animals that died or were euthanized during the study were necropsied by the investigating veterinarian and sufficient testing was performed to determine a definitive diag-



nosis for death.

All DNS samples were submitted to a university microbiological laboratory and were cultured for identification of *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. Tulathromycin susceptibility was determined using one representative colony from each sample for all *Mannheimia haemolytica* or *Pasteurella multocida* isolates. All *M. haemolytica* and *P. multocida* isolates were determined to be either susceptible (S), intermediately susceptible (I), or resistant (R) to tulathromycin using standard microdilution techniques and CLSI definitions.

Data were recorded, stored, and analyzed in a centralized data management system, SAS version 9.3. Data were summarized in contingency tables and analyzed for Sensitivity (Sen), Specificity (Spec), Positive Predictive Value (PPV), and Negative Predictive Value (NPV). Clinical outcome was defined as Treatment Success if the animal continued to score a CAS=0 or CAS=1 with a rectal temperature <39.7°C until the end of the study. Treatment Failure was defined as an animal scoring a CAS of 1 and rectal temperature = or > 39.7°C or a CAS = or > 2 regardless of rectal temperature, or death due to BRD. In determining the Predictive Value of the Susceptibility Test, True Positive tests were analyzed and defined as either susceptible isolates (MIC <16 ug/ml) that were cultured from animals that were Treatment Successes or non-resistant isolates (MIC <64 ug/ml) that were cultured from animals classified as Treatment Successes. True Negative tulathromycin susceptibility tests were defined as resistant isolates (MIC >64 ug/ml) from animals classified as Treatment Failures or non-susceptible isolates (MIC >16 ug/ml) from animals that were classified as Treatment Failures. False Positive tulathromycin susceptibility tests were defined as susceptible or non-resistant (susceptible and intermediate) isolates from animals classified as Treatment Failures and False Negative tulathromycin susceptibility tests were defined as resistant or non-susceptible (resistant and intermediate) isolates from animals that were classified as Treatment Successes.

**Results:** To be presented at WBC.

TP-05

### The influence of irrigation on transdermal absorption of flunixin meglumine in calves

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**Objectives:** Flunixin meglumine is a non-steroidal anti-inflammatory agent which is typically administered either orally or intravenously. Recently Finadyne Transdermal<sup>®</sup> has been introduced and enables the administration of flunixin as pour on solution for cattle. According to the manufacturer's instructions, Finadyne Transdermal<sup>®</sup> should be applied on dry skin on the back of the animal. Unfortunately, due to variable weather and

housing conditions, this cannot always be guaranteed.. The main objective of the study was to evaluate the potential effect of light or heavy irrigation (mimicking rain) might have on absorption.

**Materials and Methods:** Nine male calves (Holstein Frisian and cross breed) were included in the study. The calves were administered flunixin meglumine at the approved dosage of 3.33 mg/kg bodyweight as pour on down the midline of the back. The application was done under three different and general conditions:

Dry condition (control). Light irrigation, mimicking light rain (maximum of 2.5 mm/h, with a maximal rate of 0.25 mm/6 min) Heavy irrigation, mimicking heavy rain (more than 7.6 mm/h or more than 0.76 mm/6min)

All calves underwent nine different experimental arrangements in different order (cross over study allocating the treatments following a 9x9 Latin square design):

Control dry conditions Light irrigation starting immediately after treatment for 30 minutes Light irrigation starting 30 minutes after treatment for 30 minutes Light irrigation starting 60 minutes after treatment for 30 minutes Light irrigation starting 240 minutes after treatment for 30 minutes Heavy irrigation starting immediately after treatment for 30 minutes Heavy irrigation starting 30 minutes after treatment for 30 minutes Heavy irrigation starting 60 minutes after treatment for 30 minutes Heavy irrigation starting 240 minutes after treatment for 30 minutes

A wash-out period of 4 days was abided between subsequent treatments.

Blood samples (5 ml) were taken before each treatment at 0 min, 15 min, 30 min, 60 min, 120 min, 240 min, 8 h, 12 h and 24 h after application. An isocratic HPLC method was developed and performed to quantify flunixin concentrations by UV-detection in bovine serum. Pharmacokinetic parameters such as maximal concentration (C<sub>max</sub>), time to maximal concentration (T<sub>max</sub>) and area under the curve (AUC) were calculated, and concentration-time curves were created.

**Results:** Light irrigation resulted in a significant decreased absorption (decreased C<sub>max</sub>, T<sub>max</sub> and AUC) only when irrigation started immediately after application of flunixin. The effect of heavy irrigation reduced the absorption and bioavailability of flunixin significantly if irrigation starts within an hour after application of the drug. The subsequent time point when heavy irrigation started after flunixin administration was at 4 h, at that time no effect was found on C<sub>max</sub>, T<sub>max</sub> or AUC. From our study, it is not possible to conclude on absorption kinetic in a situation when irrigation would have started between 1 and 4 hours after flunixin application.

**Conclusions:** Irrigation may have an effect on the absorption and bioavailability of flunixin meglumine after transdermal application under certain conditions. It seems reasonable to believe that the results might be transferable to rain conditions. Therefore, weather conditions should be considered within 4 h after application, especially heavy rains requires shelter to allow sufficient absorption.



TX-01

**Bracken Toxicosis.****An acute outbreak in a group of fattening cattle.**

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**Objectives:** To determine the aetiology of an acute onset illness in a group of recently purchased beef heifers in northern England.

**Materials and Methods:** The clinical investigation was undertaken on an extensive beef farm in northern England, where 1300 fattening animals were kept. All were purchased as stores and taken to finishing.

Affected animals were amongst a group of Aberdeen Angus cross Charolais calves which had been purchased from northern Scotland ten weeks prior and had been grazed on extensive, reclaimed hill pasture since their arrival.

The day prior to presentation the owner treated one heifer for bovine respiratory disease but the following day that animal had deteriorated and another heifer was exhibiting similar clinical signs.

On clinical examination the previously treated heifer was pyrexia (temperature 40.7°C), dull and depressed. She had a facial skin tent >3 seconds, was tachycardic (130 beats per minute), had a CRT of >3 seconds and her mucus membranes and vaginal mucosa were pale. Scleral haemorrhages were present in both eyes and a patch of dried blood was present on the animals left thorax at an injection site at where the client had administered antibiotics the previous day. The respiratory rate was elevated (70 breaths per minute). Thoracic auscultation revealed significant wheezes and crackles diffusely spread across all lung fields. A bilateral muco-purulent nasal discharge was noted. Rumen fill was poor and no rumen contractions were audible. Gaseous distension of the large intestine was audible on percussion of the right sub lumbar fossa. On rectal examination fresh blood was present within a small quantity of dry, firm faeces.

The second heifer was dull and depressed. She was pyrexia (temperature 40.4°C), tachycardic (110 beats per minute). Mucus membranes were pale and she had a CRT >3 seconds. No scleral haemorrhages were noted. The respiratory rate was elevated (60 breaths per minute). Thoracic auscultation revealed significant wheezes and crackles particularly in a cranio-ventral orientation. A bilateral muco-purulent nasal discharge was noted. Rumen fill was poor and the animal had rumen contractions present but at a reduced rate of one per three minutes. She exhibited signs of tenesmus and passed a quantity of melaena.

On the basis of clinical findings consistent with a haematological crisis, an EDTA blood sample was taken from the most severely affected animal and submitted to the local Veterinary laboratory (AHVLA) for haematological examination. A blood transfusion was performed in both affected animals.

Both animals appeared brighter twelve hours post transfusion but were euthanased over the following two days when they became recumbent and clinically septicemic. They were then submitted to AHVLA for necropsy examination.

**Results:** Haematological findings demonstrated an acute, haematological crisis with pancytopenia. Necropsy findings in-

cluded widespread necrotic foci on the liver, in the wall of the small intestine and on the diaphragm. Intestinal, cardiac and respiratory haemorrhages, multi-focal thrombo-emboli in all lung lobes, extensive blood splashing and a profuse pure growth of *E.coli* from internal organs indicating a terminal colisepticaemia. Histopathology revealed a preferential depression of myeloid series cells and megakaryocytes.

Clinical, haematological, necropsy and histopathological findings were consistent with a diagnosis of acute ptaquiloside toxicity.

**Conclusion:** The animals had been grazed for ten weeks on extensive, reclaimed hill pasture. The area had received little rainfall over the previous two months and grass had been grazed to a very low level and showed areas of scorching. Further investigation revealed a large area of Bracken fern (*Pteridium aquilinum*), on the upper slopes of the pasture that had been heavily grazed by the herd. The shortage of grass caused extensive grazing of the bracken fern during the period of drought and led to the occurrence of acute ptaquiloside toxicosis.

TX-02

**Determination of micotoxins in dairy feedstuffs typically used in Chile**\*Pedro Melendez<sup>1</sup> Nicole Orellana<sup>2</sup> Maria Marin<sup>2</sup> Pablo Pinedo<sup>3</sup> Macarena Perez<sup>4</sup><sup>1</sup>University of Missouri, <sup>2</sup>University Santo Tomas, <sup>3</sup>Colorado State University, <sup>4</sup>Alltech

**Objective:** Mycotoxins are toxic secondary metabolites produced by fungi (molds) that cause a detrimental effect when animals are exposed. Exposure is usually by consumption of contaminated feeds but may also be by contact or inhalation. There are more than 400 mycotoxins described in the literature, consequently their biological effects is extremely variable. Mycotoxins can affect liver, kidney, central nervous system, reproductive tract (estrogenic effects), digestive tract, and skin to name a few. Therefore, the fungal toxins are chemically diverse, representing a variety of chemical families. Only few mycotoxins have been extensively researched, and even fewer have good methods of analysis available. The objective of the present study was to determine the presence of mycotoxins in feedstuffs (concentrates, forages and total mixed rations) in Chile.

**Material and Methods:** The study was conducted in dairy farms from different agro ecological regions of Chile (Central region [33.3 S, 71.4 W to 35.4 S, 71.3 W]: 14 farms, South-Central region: [35.5 S, 72.6 W to 37.8 S, 72.7 W] 17 farms and Southern region: [35.6 S, 71.4 W to 41.5 S, 72.9 W] 8 farms). According to the production system (grazing, confinement, mixed) a sample of forages, total mixed rations and concentrates were obtained at random during a visit of the farm in the fall season of 2014. Samples were dried off, stored in vacuum plastic bags and submitted to a toxicological lab (Kentucky, USA). The analysis was conducted by Mass Spectrometry (Acquity UPLC / ESI-TQD MS, Waters Corp., Milford, MA, USA) A descriptive analysis by feedstuff and agro ecological region was conducted using the Fisher exact test.



**Results:** Most of mycotoxins were below the Chilean and International accepted minimum levels, except for Trichothecens type B (> 470 ppb) in total mixed rations and forages and in all the studied regions. No differences were found among mycotoxins within feedstuffs except for Trichothecens type B (higher in total mixed rations [477 ppm] and forages [866 ppb] than concentrates [183 ppb], Fusaric acid (higher in forages [976 ppb] than total mixed rations [394 ppb] and concentrates [279 ppb] and Fumonisin (higher in concentrates [3933 ppb] and total mixed rations [2497 ppb] than forages [1060 ppb]). No differences were found among agro ecological regions except for Ochratoxins (higher in South area [5.56 ppb] than Central-South area [1.45 ppb] and Central area [0.14 ppb]), Fusaric acid (higher in Central-South area [1026 ppb] than South area [353 ppb] and Central area [174 ppb] and Fumonisin (higher in Central area [3336 ppb] and South-Central area [2253 ppb] than South area [290 ppb]). There were farms and feedstuffs without the presence of mycotoxins.

**Conclusions:** It is concluded that mycotoxins are present in forages, concentrates and total mixed rations and they vary according to feedstuffs and agro-ecological regions in Chile.

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TX-03

### Supplementation with difructose anhydride III (DFA III) reduces mycotoxin absorption in cattle by modifying the tight-junction network

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**Objectives:** Mycotoxins are a major factor that adversely affects cattle production. Several strategies to mitigate the effects of mycotoxins have been evaluated, but their application and efficacy in cattle practice is still under investigation. We previously developed a urinary mycotoxin monitoring system in cattle that initially focused on the detection of zearalenone (ZEN), a nonsteroidal estrogenic mycotoxin, and proposed it as a useful mycotoxin exposure monitoring method at the farm level (Takagi et al., 2014, 2016 in *WBC*). The objective of the present study was to evaluate the potential protective effects of difructose anhydride III (DFA III) supplementation in cattle feed using this urinary ZEN monitoring system. DFA III belongs to the group of small cyclic disaccharides used as prebiotics. It is also known to affect calcium homeostasis.

**Materials and Methods:** Female Japanese Black cattle from two fattening herds (Herd 1: n = 10, Herd 2: n = 20; age: 9-10

months; mass: ~300 kg) were used in this study. DFA III (40 g/day; 20 g top-dressed mornings and evenings) was administered as a supplement to half of each herd for 2 weeks (Herd 1: n = 5; Herd 2: n = 10; DFA III group); the other cattle in each herd served as controls (Herd 1: n = 5; Herd 2: n = 10). Urine and blood samples were collected on the day before DFA III supplementation (Day 0), on Days 9 and 14 after supplementation, and again on Day 23 (9 days after supplementation ceased). Zen concentrations in urine and its metabolites, a-zearalenol (a-ZOL) and b-ZOL, were measured using LC/MS/MS. Additionally, serum Ca, inorganic phosphorus (iP) and Mg concentrations were measured, as DFA III is known to affect the paracellular transport of calcium and also likely affects other bivalent ions through the intestinal epithelial cells barrier.

**Results:** The concentrations of ZEN in the mixture of roughage and concentrates fed to the heifers was 0.27 mg/kg in Herd 1 and 0.22 mg/kg in Herd 2. These findings confirmed that the ZEN contamination levels of the dietary feeds were at approximately the same level in both herds and were below the threshold levels applied in Japan (< 1 mg/kg). The urinary ZEN concentrations on Day 0 also revealed that each herd had approximately the same level of ZEN contamination. When comparing the DFA III and control groups, significant differences (P < 0.05) in ZEN were confirmed on Day 14 (11.0 vs. 22.2 pg/mg of Creatinine), in a-ZOL on Day 23 (11.3 vs. 25.2 pg/mg of Crea), in b-ZOL on Day 14 (8.6 vs. 25.4 pg/mg of Crea) and Day 23 (12.7 vs. 34.3 pg/mg of Crea), and in the ΣZEN (ZEN+a-ZOL+b-ZOL) on Day 9 (11.6 vs. 38.3 pg/mg of Crea) and Day 14 (31.0 vs. 63.9 pg/mg of Crea), respectively. Moreover, although no differences were observed in either serum Ca or Mg, the iP concentration in DFA III-supplemented animals was significantly higher than the control on Day 23 (8.4 vs. 7.7 mg/dL), suggesting a possible role of DFA III in tight-junction functions of intestine epithelial cells.

**Conclusions:** These results suggest that the DFA III supplementation of dietary feed alters ZEN and ion adsorption levels in cattle. Our results indicate for the first time that DFA III supplementation may reduce the levels of mycotoxins that reach systemic circulation and are excreted in the urine. This preventive effect may be associated with an improved tight junction-dependent intestinal barrier function. At the same time, this practical approach reconfirmed that monitoring urinary mycotoxin is useful for evaluation of the effects of dietary supplements that may prevent mycotoxin adsorption. Further field studies are necessary to create a database for the assessment of DFA III as a dietary supplement to reduce mycotoxin adsorption in cattle and other animal species.

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TX-04

### Outbreak of *Lantana camara* poisoning in tropically adapted cattle with long term follow up

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**Background:** *Lantana camara* is a poisonous plant of ruminants present worldwide. It contains lantadenes, which cause primary liver disease with associated photosensitisation. Mini-





mal research has been published in regards to treatment efficacy, case progression or prognosis in cases of natural intoxication of tropically adapted cattle. This report follows a single case series of a natural lantana intoxication outbreak in a group of young tropically adapted cattle.

**Case series:** A group of 50 Droughtmaster steers and heifers aged 4 to 7 months were placed in a paddock containing orange flowering *Lantana camara*. Seven days later, a portion of the calves developed lesions of photosensitization consistent with lantadene intoxication. Veterinary care was provided 3 days after signs were first noted. The calves were moved to a lantana free paddock on the day the signs were first noted. Fourteen calves had evidence of photosensitization. Photosensitization was defined as a progressing dermatologic lesion with vesicle and bulla formation, serum exudation, ulceration, exfoliation, scab formation, skin necrosis, and skin sloughing. For the purposes of the study, 14 unaffected calves of the same sex were identified and used as controls.

All 28 calves received a clinical examination. Ten of the 14 affected calves were mildly obtunded, two were moderately obtunded and two were severely obtunded with one severely obtunded calf having difficulty rising. All 14 control calves were bright, alert and responsive. Twelve of the 14 affected calves were icteric, while 0 of the 14 control calves were icteric. Urine was obtained from the control calves and 12 of the 14 affected calves. Three animals were isosthenuric (urine specific gravity (USG) 1.008-1.012) with 2 being controls and one diseased. Two calves were hyposthenuric (USG <1.008) with one control and one diseased. Twenty one calves were *hypersthenuric* (USG >1.012) with 11 controls and 10 diseased. *Signs of photosensitization were observed on the nares (14), ears (13), periorbitally (12), vulva commissures (6), perineum (6), back (4) and tongue (1). Primary treatment of the affected individuals was activated charcoal at 5 mg per kg by oral rumen tube with 12 litres of water, and oxytetracycline (200mg/ml) at 20 mg per kg intramuscular was administered for secondary bacterial infections. A follow up evaluation was performed at 2 ½ months. No new cases were identified. All cattle were eating and appeared to be bright. The distribution of the dermatological changes due to photosensitization was unchanged from the initial visit. Blood samples were collected from the 4 calves with the most severe photosensitization lesions. Biochemical analysis was focused on hepatic and renal parameters (GGT, GLDH, total bilirubin, BUN, creatinine). All laboratory values of GGT, GLDH, total bilirubin, BUN, and creatinine were within the normal reference range limits of the Laboratory Services, School of Veterinary Science, Faculty of Science University of Queensland. All calves survived and were successfully marketed 9 months after the initial examination with no apparent differences in body weight and body condition. Five of the affected calves had hair loss on the ears at time of marketing. The remaining dermatologic abnormalities had resolved.*

**Conclusions:** Treatment with activated charcoal and oxytetracycline appeared to be effective in this natural outbreak of lantana intoxication. Urine specific gravity was not a useful prognostic indicator as both control and diseased animals were isosthenuric. Further investigation would be required to establish the validity of USG as a prognostic indicator. Commonly reported sites of lantana induced photosensitization in Droughtmaster cattle include the ears, nares, and periorbital area. Renal and hepatic laboratory parameters were not increased at the 2 ½ months follow up evaluation, suggesting that the provision of appropriate care facilitated the resolution of any initial

liver or renal damage and supporting the notion that cattle are able to recover metabolically from acute lantana toxicosis. Long-term aesthetic effects, characterized by hair lost up for up to 9 months, do occur with lantana intoxication. This altered appearance of the animal has the potential to affect marketability and market value of recovered cattle. Long-term hair loss has been a previously unreported consequence of lantana intoxication.



TD-01

## Improving biosecurity for Foot-and-Mouth Disease (FMD) control in countries from Asia, the Far East and Oceania

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**Objective:** Within the South East Asian region and beyond, transboundary animal diseases (TADs) and FMD in particular, cause negative impacts on national economies and smallholder farmer livelihoods. To inform the improvement of biosecurity and reporting practices in livestock production systems, a survey was conducted of OIE (World Organisation for Animal Health) Delegates representing Asia, the Far East and Oceania, to assist efforts aimed at progressing global FMD control and eradication.

**Materials and methods:** Responses to an online questionnaire were received from 26 (of 36) OIE (World Organisation for Animal Health) Delegates representing Asia, the Far East and Oceania, to inform progression of biosecurity on smallholder farms, at the village-level, in commercial enterprises and at international borders. The questionnaire consisted of six sections. Section 1 contained 5 questions on the role of respondents, their involvement in policy and extension work and their considerations on the role of OIE in improving biosecurity. Sections 2-6 requested responses to 22 questions covering the 5 principles of biosecurity. Responding countries were categorised according to FMD and Income status, with: FMD1 being FMD Free without vaccination and FMD-free zones WITHOUT vaccination (n = 11) and FMD2 being FMD free zones WITH vaccination and FMD present (n = 15) countries. Income status 1 countries had high and upper middle incomes (n = 12) and Income 2 countries were low income and lower middle income (n = 14). Data was analysed using Genstat 18<sup>th</sup> edition to determine the differences between the categories in identifying biosecurity and reporting practices associated with FMD. Univariable binomial logistic regression was conducted to identify variables associated with FMD freedom (FMD1/FMD2 1/0) (P<0.1), these were then tested for collinearity and correlated values were removed. A backwards stepwise procedure was used in the multivariable model building to identify significant practices.

**Results:** Countries categorised as FMD2 status were less likely to discourage the sale and movement of sick or infected animals at the smallholder level (P = 0.015), ensure wild birds did not have access to water used for commercial poultry, undertake negative disease reporting (P = 0.062) and more likely to recommend the use of systemic antibiotics in the treatment of FMD (P = 0.008).

From the final four significant variables used in the multivariable modelling only 'the promotion of vaccination for FMD and other TADS of incoming animals at the smallholder (or village) level' (P = 0.001) was retained in the final model.

**Conclusions:** The univariable analysis indicated that there were numerous activities that were associated (P<0.2) with an FMD1 status. These biosecurity messages can be further promoted to strengthen biosecurity in FMD2 countries. In developing countries this can prove challenging due to the nature of livestock ownerships and trade by livestock keepers and traders. While the multivariable modelling only retained one vari-

able of significance, the act of vaccinating all incoming animals at either the village or smallholder level against FMD and other TADs is known to be able to play an integral role in regional disease control as many of these animals can be involved in extensive trade routes. Further, as the sale of sick and infected animals is common in many developing countries, extension efforts to limit or prevent the sale and movement of infected and sick animals, plus vaccination and promoting sale of vaccines, are important strategies in minimising the spread of TADs such as FMD.

Improved reporting systems and inclusion of 'negative disease reporting' is considered important in improving surveillance for FMD, providing evidence of absence of disease as countries move towards eradication. In the global FMD control and eradication challenge, improving biosecurity and reporting practices is vital to reduction and elimination of disease. Further research to investigate the actual biosecurity practices and reporting behaviours at the different levels of FMD2 countries is warranted to ensure any disparity between reported and actual practices can be identified and remediated.

TD-02

## An evidence-based herd health program for tropical smallholder beef production in Southeast Asia

### Livelihood benefits of beef vaccination

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**Objective:** Develop an evidence-based herd health program for tropical smallholder beef production in Southeast Asia from recent research on the epidemiology, financial impacts and estimated benefits of routine vaccination of episodes of Foot-and-Mouth Disease (FMD), Haemorrhagic Septicaemia (HS), and Blackleg (BL), on smallholder farmer livelihoods.

**Materials and methods:** Following continuing high demand for cattle for meat in rapidly developing Southeast and Eastern Asian communities, research attention on supporting these markets has included investigations of infectious disease risk management. Clinically diagnosed outbreaks of FMD, HS, and BL, were investigated in Laos and Cambodia, with epidemiological studies including Financial Impact Survey Questionnaires (FISQs), conducted with smallholder owners of infected large ruminants. Financial losses were determined, including direct losses due to mortality using estimation of 100% of pre-loss sale value, and morbidity using estimated difference between the expected sale price pre-outbreak and 4-6wks following onset of the outbreak, with costs of treatments included. This information enabled partial budget analysis to estimate benefits of vaccination programs at the village, and where appropriate, at the national levels (FMD).

**Results:** For endemic FMD, estimated losses due to FMD per household varied between provinces in Laos, being 60, 40 and 16% of annual household income (p < 0.001), contributing to losses in annual household income of 213, 181 and 60% of the



income from sales of large ruminants. Partial budget analysis indicated an average net benefit of USD22 and USD33 for cattle and buffalo, respectively based on an outbreak every 20 years.

For endemic HS, at the village level, 24% of all households were affected with an estimated mean village herd morbidity of 10.1% and mortality of 28.8%, and affected farmers reporting HS disease morbidity and mortality at 42.7% and 63.6%, respectively. The estimated mean cost per affected household was USD 952.50 based on ownership of 5 large ruminants and the impact per affected animal was USD 375.00, reducing the pre-disease value by 66.1%. Partial budget analysis revealed a net benefit of USD32.42 for cattle based on an outbreak every 20 years.

For BL, although occurring only sporadically, at the village level, 27% of all households were affected with a mean village herd morbidity of 4.19% and mortality rate of 2.03%. The estimated financial losses of BL at the village level was USD12,878, with losses per affected household of USD822±692 (CI: 518-1,125), being 37% and 122% of annual household income and income from the sale of large ruminants, respectively. Partial budget analysis revealed a net benefit of USD3.09 for cattle based on an outbreak every 20 years.

**Conclusions:** Although there is limited information on the financial impacts on smallholder farmer livelihoods of livestock diseases, these studies indicate that FMD, HS, BL are important tropical diseases causing severe clinical and financial impacts in smallholder beef production in Mekong countries and that routine vaccination offers significant financial benefits. Current investigations are also determining the financial benefits of control of *Toxocara vitulorum* (Tv) and *Fasciola gigantica* (Fg) in smallholder herds, with routine endoparasite control required in many beef herds where habitat favours parasite transmission. Improved farmer knowledge, attitudes and practice in use of biosecurity interventions plus routine vaccination and parasite control programs, particularly in moderate to high risk areas for each of these diseases is required, with this information potentially offering profound improvements in disease control policy and practice.

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TD-03

### Can medicated molasses blocks deliver superior health and productivity from smallholder large ruminants in developing countries?

#### Improved large ruminant production and health from medicated molasses blocks

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**Objective:** Progress of serial field studies aimed at addressing production and health inefficiencies through use of urea, triclabendazole or fenbendazole medicated molasses nutrient blocks for improved production and control of endemic *Fasciola gigantica* or *Toxocara vitulorum* and other endoparasite infestations.

**Materials and methods:** A series of applied field studies were designed and implemented in Laos to examine the potential for urea medicated (UMMB), and triclabendazole (TMB) or fenbendazole (FMB) medicated molasses blocks offered to large ruminants, to improve smallholder health and production outcomes in a developing country where productivity is compromised by low growth rates and widespread endoparasitism, with limited farmer knowledge of parasites and facilities for administration of therapeutics.

**Results:** The studies compared production and health outcomes between cohorts enrolled into one of three groups: (i) access to urea, triclabendazole or fenbendazole medicated molasses blocks; (ii) access to unmedicated molasses blocks (UMB); and (iii) control groups without access to blocks. Data and faecal samples were obtained at weeks 1, 4, 8 and 12 for assessment of growth rates and faecal egg counts (FEC). In the initial FBZ trial (n=24), complete elimination of the FEC was observed in the FMB group and not in the UMB and control groups, although a subsequent larger trial displayed inconsistent results, presumably due to differences in animal numbers accessing the blocks between cohorts. In the initial TBZ trial (n=241), the reduction in FEC following exposure to blocks for 12 weeks was ~90%, with live weight increases from ~175kg to ~192kg in weeks 1 and 12 respectively (P=0.01) and an average daily weight gain (ADG) of 201g/day. Reduction in FEC in the UMB group was also observed, by ~19%, with live weight increases from ~180kg to ~190kg in weeks 1 and 12 respectively (P=0.3), with an ADG of 124g/day. This study suggests that productivity was enhanced with use of UMB to Lao cattle and this response was enhanced when an anthelmintic was added to the blocks (as Fasinex®, Novartis Animal Health Australia, Pty Ltd, at 0.1kg per 20kg block for provision to up to 10 animals), delivering parasite suppression or potentially therapeutic doses on ad-libitum feeding of the TMB. Results from current trials with urea and FMB will also be presented.

**Conclusions:** Improving the efficiency of large ruminant production in developing countries is a major challenge for sustainable livestock agriculture in meeting the increasing demands of food insecurity in a climate changing world. Although further work is required to determine the most efficacious dose rates of medications and the duration of exposure of animals to achieve optimum therapeutic potential of medicated blocks, this approach offers a practical and potentially efficacious parasite management and nutritional supplementation strategy for tropical smallholder farmers. This is particularly important in Laos and other developing countries where administration of medication to large ruminants is difficult due to low animal husbandry skills and lack of animal restraint facilities. Provision of urea, triclabendazole or fenbendazole medicated molasses blocks may improve utilisation of poor quality forage and assist control of endemic *Fasciola gigantica* or *Toxocara vitulorum* and other endoparasitic infestations, offering potentially transformational improvements in large ruminant production efficiency and contributing to improved rural livelihoods.

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TD-04

### Investigation of infectious reproductive pathogens of large ruminants: are neosporosis, brucellosis, leptospirosis and BVDV of relevance in Lao PDR?

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*N. caninum*, bovine viral diarrhoea virus, *Brucella abortus* and *Leptospira interrogans* serovar Hardjo are globally significant reproductive pathogens that cause abortion and reproductive loss in large ruminants. Prevalence information is lacking in Lao People's Democratic Republic (Laos) despite the poor reproductive performance of cattle and buffalo. Serological examination of frozen cattle ( $n = 90$ ) and buffalo ( $n = 61$ ) sera by commercially available enzyme-linked immunosorbent assays provided the first reported screening of some of these pathogens in the Laos. Seroprevalence differed amongst these large ruminant species, with *N. caninum*, BVDV and *L. interrogans* serovar Hardjo antibodies found in 68.9% (95% CI  $\pm$  11.6), 4.9% (95% CI  $\pm$  5.4) and 3.3% (95% CI  $\pm$  4.5) of buffalo sera, respectively, and in 7.8% (95% CI  $\pm$  5.5), 10.0% (95% CI  $\pm$  6.2) and 22.2% (95% CI  $\pm$  8.6) of cattle sera, respectively. Buffalo sera had a significantly higher seroprevalence of *N. caninum* compared to cattle ( $p < 0.001$ ) and cattle sera had a significantly higher seroprevalence of *L. interrogans* serovar Hardjo compared to buffalo ( $p = 0.003$ ). Variability was also observed across provinces for *N. caninum* in buffalo ( $p = 0.007$ ) and for *L. interrogans* serovar Hardjo in cattle ( $p = 0.071$ ), suggesting provincial risk factors conducive to pathogen transmission. BVDV and *N. caninum* seropositivity were negatively associated in buffalo ( $p = 0.018$ ) and cattle ( $p = 0.003$ ). In buffalo, *L. interrogans* serovar Hardjo and BVDV seropositivity were associated ( $p = 0.035$ ,  $p = 0.039$ ). The identification of antibodies against three major abortifacient pathogens in Laos prompts further research to determine if infection is associated with low reproductive efficiency and the risk factors for infection. This is needed for the development of evidence based prevention strategies for improved large ruminant reproductive management among smallholders in Laos.

TD-05

### Participatory evidence-based planning for community-based control of bovine brucellosis in Tanzania

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**Objectives:** Brucellosis is one of the most prevalent zoonoses in the world. Since 2015, a series of socio-epidemiological studies have been conducted in Tanzania by the authors. This study was conducted to understand the view of communities on the preferred control option of brucellosis in the study areas.

**Materials and methods:** Four participatory rural appraisals were conducted with the agro-pastoralists who participated in the cattle surveillance in 2016, in the four villages in Mvomero

District, Tanzania. Prior to the appraisals, results of risk factor analyses for bovine brucellosis, and willingness-to-pay study for vaccine were returned, and characteristics of the disease in animals and humans were explained. The survey team answered to the questions related with brucellosis by the participants, and qualitative data on the culture and belief on the risky behaviours associated with human infection were collected. Finally, participants were encouraged to discuss about sustainable and community-based voluntary control of brucellosis.

**Results:** Risky behaviours such as drinking raw cattle blood and milk were common particularly among Maasai tribe, and they believed that raw cattle blood provides strong energy. One village preferred to talk without research team about disease control. All the villages came to the same conclusion of continuous conduct of calf vaccination paid by themselves. A Maasai leader mentioned that adults cannot stop risky behaviours, but education can change behaviour of young generation. Technical supports from both medical and veterinary authorities were perceived to be important.

**Conclusions:** Provision of evidence-based information on brucellosis was confirmed to raise motivation of community-based control of brucellosis among cattle keepers. One Health brucellosis control among medicine, veterinary medicine, and education may be a key to success.

TD-06

### Perception and behaviours associated with community-based bovine brucellosis control among agro-pastoralists in Tanzania

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**Objectives:** Brucellosis is a worldwide zoonosis. The disease causes abortion in livestock and reduces milk production in livestock thereby inflicts economical damage on farmers. It also causes undulant fever, arthritis and general malaise in humans through the consumption of infected unheated milk. In many developing countries, governments do not keep enough budget for the disease control. In such situation, community-based control with vaccination is considered to be a possible solution. This study investigated the farm-level prevalence and risk factors for bovine brucellosis and perception and behaviours associated with community-based brucellosis control among agro-pastoralists in Morogoro Region, Tanzania.

**Materials and methods:** A cross-sectional study involving herd milk diagnosis by indirect enzyme-linked immunosorbent assay and questionnaire survey was conducted in 124 farms in 2016. Questions included potential risk factors, knowledge of brucellosis, willingness-to-pay for cattle vaccination, and item count technique (ICT) for selling behaviour of cows that experienced abortion. For the willingness-to-pay for vaccination, *Brucella* vaccine price was set as 3,000 Tanzania shilling (approximately 1.3 USD) per shot. Risk factor analysis for bovine brucellosis and analysis of factors associated with willingness-to-pay were conducted using classical tests and generalised linear models.

**Results:** Most farmers had little knowledge about brucellosis (disease name: 13.7%, symptoms: 3.2%, transmission from



cattle to human: 2.4%, and Brucella vaccine: 2.4%). The farm-level bovine brucellosis prevalence was 44.4% (55/124, 95%CI: 35.5–53.5). No risk factors for bovine brucellosis were identified; however, using a veterinary service was identified as a preventive factor (OR = 0.39, 95%CI: 0.18–0.84,  $p = 0.02$ ). For scenarios of vaccinating all cattle and only calves, 59.7% and 89.5% of farmers were willing to pay for vaccination, respectively. Being a Maasai tribe member was a hesitating factor for vaccinating all cattle (OR = 0.39, 95%CI: 0.19–0.83,  $p = 0.01$ ) and using a veterinary service was an encouraging factor for vaccinating calves (OR = 4.0, 95%CI: 1.2–13.0,  $p = 0.02$ ). The ICT found that 45.1% of farmers sold cows that experienced abortion. This estimate was not statistically different from that obtained by direct questioning (34.1%, SE = 7.5%, binomial  $p$  value = 0.27, factor score = 1.32), suggesting that farmers did not hesitate to sell such cows. The Maasai conducted more risky behaviours for human infection such as drinking raw milk ( $p = 0.06$ ) or blood ( $p < 0.01$ ) and helping delivery with bare hands ( $p = 0.03$ ) than other tribes.

**Conclusions:** Community-based brucellosis control programmes with calf vaccination may be feasible in the study areas. A One Health approach including the promotion of health education and expansion of veterinary services is crucial for disease control.

TD-07

### ***Theileria parva* survey by simple FTA card-based polymerase chain reaction in dairy cattle in Mbarara, Uganda**

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**Background:** East coast fever (ECF) is one of the most severe protozoan diseases caused by *Theileria parva* mainly in East and Central Africa. The parasite is transmitted by ticks, *Rhipicephalus appendiculatus* and causes anemia and high mortality of cattle especially exotic dairy breeds. In 2016, JICA Partnership Project, Safe Milk Promotion in Mbarara Project started, and we set tick-borne disease control as one of the objectives of this project. In this study we evaluated the infection status of *T. parva* in calves aged 1 to 6 months in 30 dairy farms by polymerase chain reaction (PCR) assay combined with simple FTA card-based DNA extraction method.

**Materials and methods:** In total, 418 cattle blood samples were collected from 30 dairy farms in Mbarara, Uganda. Around 125  $\mu$ l of the blood was kept on FTA Classic Card, and remaining blood was kept at -30°C until further use. Three pieces of 4 mm<sup>2</sup>-cut blood contained FTA card were washed with TENT buffer or 20 mM NaOH solution, and used for PCR template. *T. parva* p104 gene was amplified by nested PCR, and the PCR products were analyzed by agarose gel electrophoresis.

**Results:** In preliminary experiment, among 10 randomly selected samples, the positive rate by using FTA card DNA extraction method with TENT buffer and NaOH were 10% and 50%, respectively. Interestingly the positive rate by using common DNA extraction kit of whole blood remained low at 20%. Based on the method of FTA card with NaOH DNA extraction, the average of infection rate was 45% (N=418). The infection rates at farm levels differed from 0 to 100%.

**Conclusions:** In this study, high infection rate of *T. parva* (45%) was observed in dairy cattle in Mbarara, although there were big differences at farm level. FTA card-based DNA extraction is convenient and low-cost method because it does not require refrigerator, incubator, and centrifuge machine for the storage and extraction of DNA. Moreover the cost can be restrained by using simple NaOH solution. Our data shows that the simplified FTA card-based PCR method is reliable and low-cost technic for diagnosis of ECF.

TD-08

### **Prevalence of sub-clinical mastitis and its association with milking practice in Mbarara, Uganda**

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**Objectives:** A three-year project, Safe Milk Promotion in Mbarara Project has been being conducted sponsored by Japan International Cooperation Agency since September 2016 to improve dairy farming productivity in Mbarara, Uganda. Prevalence of sub-clinical mastitis was surveyed and its risk factor analysis was conducted by checking milking practice and hygiene management to control mastitis.

**Materials and methods:** Thirty farms participated in the project and the farm information of herd size, milk yield and herd management practices were collected. California mastitis tests (CMTs) were conducted for 607 milking cows which included 2405 quarters to generate information on quarter-level and cow-level mastitis prevalence in 30 farms in Mbarara district from February to May 2017. Teat tip scoring was also performed for 2062 quarters. Milk samples whose CMT score greater than or equal to 2 ( $n = 576$ ) were collected for bacterial culture tests. Milking practices were surveyed at the sampled farms using check-list at the milking time. Descriptive epidemiology was performed for the information collected. For risk factor analysis, univariable analyses were performed to analyze relations between the prevalence of mastitis and milking practice, a relation between teat tip score and mastitis and relations between milking practice and mastitis caused by contagious pathogens, using generalized mixed effect models with binomial errors.



**Results:** The mean, median and range of herd size (adult cows) of 30 farms were 35.9, 28.5 and 4 - 250, respectively. The parity were 2.45, 2 and 1 - 11, the individual milk yield (l/day) were 9.5, 8 and 1 - 32 and the body condition score were 3.0, 3.0 and 1.75 - 4.0, respectively. The cow-level prevalence of CMT positive was 69.0% (419/607) and the quarter-level was 39.3% (946/2405). Teat tip sore results were the following: normal, 81.4% (1979/2062); smooth, 16.6% (342/2062); rough, 2.0% (41/2062); very rough, 0%. The bacterial culture results: no growth, 48%; *Corynebacterium bovis*, 15%; *Staphylococcus aureus*, 11%; Coagulase negative staphylococci, 10%; Other Streptococci, 5%; *Truperella pyogenes*, 4%; *Streptococcus agalactiae* 1%; Coliforms, 1%; Others, 5%. From milking practices survey, all the farms conducted hand milking without wearing gloves; 13.8% of the farms (4/29) conducted hands disinfection before milking; drying hands, 7.1% (2/28); pre-milking, 13.8% (4/29); pre-dipping, 0% (0/29); drying teats, 24.1% (7/29); squeezing teats properly, 20.7% (6/29); post-dipping, 8.3% (2/24). Grazing was common in the farms (93.1%, 27/29) and most of legs and udders of cows at the farms were clean (89.7%, 26/29). From univariable analyses, cows in farms that they were relaxed at the end of milking had lower prevalence of CMT positive (63.8% (308/483), OR = 0.18, 95% CI: 0.04-0.67,  $p < 0.01$ ) than cows in those that they were not (90.9%, 80/88). Teats which had smooth (44.7% (151/338), OR = 1.89, 95% CI: 1.25-2.89) and rough (61.0% (25/41), OR = 4.26, 95% CI: 1.70-11.07) score had a higher prevalence of CMT positive than those which had normal score (32.5%, 540/1663). Cows in farms that they use one towel for one cow for teats wiping had a lower prevalence of mastitis caused by the contagious pathogen (62% (44/71), OR = 0.25, 95% CI: 0.05-0.88) than cows in those that they do not (83.7%, 159/190).

**Conclusions:** From this study, the high prevalence of sub-clinical mastitis, especially, caused by contagious pathogen was observed in Mbarara, Uganda. From this fact, production loss for dairy farming from mastitis is concerned. Also, the low quality of milk could be a problem for consumers especially in Uganda where a cold chain system is not well developed. Although the teat tip score was not too bad when it is compared to that of machine milking, the damaged teats were observed even in farms where only hand milking is conducted. A milking practice of not to damage teats is important and proper teat squeeze is required to prevent mastitis. Since using the same towel which was used for a mastitis cow to wipe teats to the other cows can spread contagious bacteria, using one towel for one cow is required to control mastitis caused by contagious pathogens. From the evidence of this study, an intervention study will be held for the future study to control mastitis in the 30 farms.

UH-01

### Recurrent mastitis – persistent or new infections?

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Clinical bovine mastitis represents the economically most important infectious disease in dairy cows. Up to more than 50% of clinical mastitis cases on farm level can be recurrent cases. The reason for recurrent mastitis can be either a persistent infection of the bovine mammary gland or a reinfection of a quarter or udder after bacteriological cure with a pathogen of the same or another species. Different reasons require different management actions, therefore the objective of the present work was to identify the range of persistent cases and cases of reinfection within the recurrent cases of mastitis using cultural methods and molecular biological analysis (RAPD and PFGE). Milk samples of clinical mastitis cases were collected in three Northern German dairy farms from 2011 to 2015. In total 2,380 mastitis cases were examined on quarter level (1,160 1<sup>st</sup> cases in lactation, 1,220 recurrent cases in lactation). In 220 recurrent cases the same pathogen species compared to 1<sup>st</sup> cases were identified. With molecular methods in 130 cases the same bacterial strain as in the 1<sup>st</sup> cases could be confirmed. These preliminary data show that most (app. 90 %) of the recurrent cases are new infections with other bacterial species or other bacterial strains. Therefore, not only treatment and culling rules but also preventing of new intramammary infections are important to prevent recurrent mastitis cases in dairy farms.

UH-02

### Mastitis pathogens on dairy farms in Southern Germany

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The Bavarian Animal Health Services (TGD) are a non-governmental not for profit organization that provides diagnostic and veterinary consulting services to Bavarian livestock farmers. Traditionally dairy farmers call upon the services of the TGD either if they have high bulk tank somatic cell or bacteria counts or if they want to monitor the mastitis pathogens on their farm to adjust management practices proactively.

The **objective** of this study was to describe the distribution of mastitis pathogens on Bavarian dairy farms visited by the TGD.

**Materials & Methods:** In 2017, technicians of the udder health division of the TGD visited 1,948 of the roughly 30,000 dairy farms in Bavaria and collected aseptic quarter milk samples from lactating cows of those farms. At the time of sample collection, the technician also performed a California mastitis test (CMT). The CMT scores were 0 for normal milk, 1-3 for increasing agglutination and 'S' for visibly abnormal milk. All milk samples were preserved with boric acid and were submitted to the TGD Bayern's udder health laboratory for standard microbiological testing. Descriptive summary statistics were conducted



in SAS 9.4 (SAS Institute, Cary, NC, USA).

**Results:** Quarter milk samples were scored 0 (n=248,686), 1 (n=444,411), 2 (n=18,215), 3 (n=11,245) and S (n=4,060). Most samples (84.2%) showed no growth. However, when the samples were separated by CMT score then this changed to 93%, 66%, 52%, 39%, and 25% for CMT scores 0, 1, 2, 3 and S, respectively. Among the isolates CNS (33%), *S. aureus* (22%), *S. uberis* (16%), *S. dysgalactiae* (8%), Enterococci (5%), *Lactococcus* ssp. (5%), *S. agalactiae* (4%), *S. canis* (1%), Streptococci (esculin-positive, 1%), *T. pyogenes* (1%) and *E. coli* (1%) were the most common isolates. Overall the prevalence of gram-negative bacteria was negligible as they contributed combined less than 6% of all mastitis pathogens, including for S-classified samples. Depending on CMT score, the primary pathogen shifted from CNS for CMT scores 0, 1 and 2 to *S. aureus* and *S. uberis* for scores 3 and S, respectively.

In **conclusion**, the likelihood of pathogen detection in quarter milk samples increased with increasing CMT. Subclinical mastitis was predominately associated with CNS and *S. aureus* while clinical mastitis was most commonly associated with *S. uberis*. Infections with gram-negative pathogens were rare in Bavaria.

UH-03

### Different concentration of antimicrobial components in bovine milk infected by different pathogens

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**Introduction:** Mastitis is a frequent infectious disease in dairy cows. To prevent this disease, immune functions such as innate and acquired immunities in the mammary gland are important. Innate immunity includes antimicrobial components such as lactoferrin, defensin, S100, and cathelicidin. These antimicrobial components contribute to the innate immune function in the mammary gland. Mastitis is caused by the various environmental and contagious pathogens, although it is unknown whether production of antimicrobial components depend on these different pathogens. Therefore, the present study was undertaken to investigate the antimicrobial components concentration in milk infected with some pathogens in order to clarify their relationships.

**Materials and Methods:** Milk samples were collected from udders infected with *Corynebacterium bovis* (Cb), Yeast-like fungi (Yf), *Staphylococcus aureus* (SA) and *Escherichia coli* (*E. coli*) and healthy udders. Milk was centrifuged and skim milk was used for measurement of antimicrobial components including lactoferrin, lingual antimicrobial peptide (LAP, a beta-defensin) and S100A8.

**Results:** LAP concentration in milk infected with Yf was significantly higher than that infected with Cb and healthy one. Milk infected with SA had a significantly higher LAP concentration than healthy one. Lactoferrin in healthy milk had significantly lower concentration compared with those infected by Cb, Yf

and SA. Yf-infected milk showed the highest lactoferrin concentration that was significantly higher than that of *E. coli*. Conversely, S100A8 concentration was highest in healthy milk that was a significantly higher than those of other infected milks.

**Conclusion:** These results suggest that secretion of innate component may be stimulated by different pathogens. These differential antimicrobial component secretion may be considered as a strategy of mammary gland for defense against pathogen infection.

UH-04

### Alternative management options for a herd outbreak of prototheca mastitis

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**Background and Objectives:** Prototheca are microscopic algae that are found all over the world with *Prototheca zopfii* being the most commonly reported cause of bovine protothecal mastitis<sup>1,2,3</sup>. Both environmental and contagious transmission of prototheca can occur<sup>4</sup>. Once cows are infected the algae can spread from cow to cow through the milking process. Poor teat disinfection and / or a high level of teat end damage may contribute to the spread of infection<sup>2</sup>. All commercially available intra-mammary antibiotics are ineffective against these algal organisms. Once a cow is infected the only available course of action is culling from the herd.

Two seasonal calving dairy herds in New Zealand with high infection rate of Prototheca mastitis presented an opportunity to determine if culture identification and isolation of infected cows rather than immediate culling was a viable management option.

**Materials and Methods:** Between June 2016 and June 2017 two dairy herds in the Waikato region of New Zealand were diagnosed with high rates of prototheca mastitis. The farmer of Herd 1 was prepared to cull cows immediately but due to the financial implications the farmer of Herd 2 favoured identifying cows and milking them until they were culled at the end of the seasonal lactation. The decision to isolate prototheca infected cows and milk them last requires consideration of (1) the intermittent shedding of prototheca (2) the limitations of milk culture (3) the sensitivity of ancillary tests such as RMT (rapid mastitis test) (4) the potential risk of milk and faecal shedding allowing further spread of infection (5) farmer stress and economics

#### Herd 1; 463 out of 1500 cows with prototheca mastitis (31%)

At the end of July 2016, 30 samples were taken from cows that had a high reading on the RMT with 22 positive for Prototheca. The 900 cows that were already calved were rapid mastitis tested and any cow with a RMT score >0 was milk sampled for culture. Freshly calved cows were sampled in batches 4-7 days post calving. Multiple quarter composite samples were taken with any positive samples being culled from the herd immediately. A total of 463 cows were culture positive and culled from the herd.



### Herd 2 259 out of 700 cows with prototheca mastitis (37%)

In early August 2016 *Prototheca* was isolated in 9 out of 16 samples. The decision was made to;

RMT test all cows in milk and take milk samples from any RMT positive cows and To bring the first herd test forward and take milk samples from all cows over 150,000 ISCC. Take milk samples from all cows with strong RMT quarters at their 8<sup>th</sup> milking after calving.

117 out of 186 cows with a ISCC > 150,000 at first herd test were prototheca positive (62.9%). Cows infected with prototheca were milked in a separate herd that was milked last. With a hot wash was put through the milking machines morning and night and iodine based teat spray (maintained at 0.5%) applied manually<sup>5</sup>

Through the process of milk culturing after RMT tests and individual Somatic cell count testing, a total of 253 cows were identified as milk culture positive for prototheca. The ISCC of prototheca positive cows ranged from 62,000 to 4,600,000. A final whole herd milk culture was done at the end of the season (excluding cows already in the prototheca positive herd). 412 cows had composite milk samples taken from all 4 quarters with 6 cows were identified as infected with prototheca.

A total of 259 cows were culled at the end of the 2016/17 season. The total milk solids production was only 3% down on the previous season and the income from the infected cows assisted financially as replacement cows were organised.

**Conclusions:** These cases highlight the importance of investigating the prototheca infection status of the herd if individual cases are identified. The low level of infection in cows retained in the prototheca positive herd of farm 2 indicates that isolation and milking infected cows in a separate herd is a potential management option if immediate culling is not possible.

UH-05

### Comparison of milk L-lactate concentration and the California Mastitis Test in identifying quarters with a high somatic cell count at dry-off in lactating dairy cattle

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**Objective:** To compare the clinical performance of measuring milk L-lactate concentration ([lact]) and the California Mastitis Test (CMT) in identifying quarters with a high somatic cell count (HSCC) at dry off. The accurate identification of HSCC quarters might be helpful in implementing a selective dry cow treatment program.

**Materials and Methods:** Quarter foremilk samples (419) were collected at dry-off from 105 dairy cows. A Delaval<sup>®</sup> cell counter was used as the reference method to measure quarter somatic cell count (SCC), with HSCC defined as SCC ≥ 1,000,000 cells/mL. Milk [lact] was measured at 37°C using the Lactate plus<sup>®</sup> hand-held meter. The area under the receiver operating curve (AUC), sensitivity (Se) and specificity (Sp) at the optimal cut point, as well as the positive likelihood ratio (+LR) and kap-

pa coefficient (κ) for diagnosing HSCC, were calculated from the results of logistic regression analysis. P<0.05 was considered significant.

**Results:** Milk [lact] ranged from <0.3 to 6.6 mmol/L and 19% (67/419) of the quarters were identified as having a HSCC. Milk [lact] ≥ 0.4 mmol/L (optimal cut-point) had an AUC=0.92, Se=0.93, Sp=0.80, +LR=4.7, and κ=0.52 for diagnosing quarters with HSCC. For comparison, the CMT ≥ 1 (optimal cut-point) had an AUC=0.96, Se=0.91, Sp=0.94, +LR=15.2, and κ=0.37 for diagnosing quarters with HSCC.

**Conclusions:** Measuring milk [lact] using the Lactate plus<sup>®</sup> hand-held meter is a clinically useful method for identifying quarters with HSCC at dry-off. However, a CMT ≥ 1 provided a more accurate and less expensive test for identifying HSCC quarters.

UH-06

### Does boric acid preservation of milk samples affect differential somatic cell count analysis?

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In Germany, quarter milk samples for microbiologic analysis are often preserved in boric acid for transport. Recently, automated milk leukocyte differentiation has been introduced in various commercial systems to provide additional information about the health and immune status of the udder beyond the classic somatic cell count.

**Objectives:** The objectives of this study were 1) to compare the differential somatic cell count (DSCC) results of bronopol and boric acid conserved milk samples, 2) to evaluate whether storage time (days 2-5) would affect the DSCC results of boric acid conserved milk samples and 3) to assess, if the ratio of boric acid to milk would alter the DSCC results.

**Materials & Methods:** At one dairy and at one milking the udder health of all cows entering the milking parlor was assessed by one assessor utilizing a California mastitis test (CMT). Quarters of cows with a CMT of at least "Trace" were enrolled in the study and 250 ml was manually stripped per quarter into a clean plastic cup prior to milking cluster attachment. Thirty ml aliquots (32 ± 4.8 ml) of each sample were then immediately filled into eight prepared milk sample containers. The containers were prefilled either with bronopol (**Control**), 3.6 ml boric acid (**d1, d2, d3, d4, d5**), 7.2ml (double concentration, **DC**) or 10.8 ml boric acid (triple concentration, **TC**). Then the samples were cooled and transported to the laboratory of the Milchprüfung Bayern e.V., where the samples Control, d1, DC and TC were analyzed according to manufacturer specifications using a Fossomatic 7DC (FOSS, Denmark). The other samples (d2-d5) were stored at 5°C until they were analyzed on day 2, 3, 4 and 5, respectively. The statistical analysis was completed in SAS 9.4 (SAS Institute, Ithaca, NY, USA) and alpha was set at 0.05. The DSCC and SCC of the different samples were compared using a Spearman correlation. In addition, the observations were visually compared through scatterplots (e.g., differences (Delta) of DSCC of different samples plotted over SCC).





**Results:** In the end samples of 109 quarters were taken. However, only 98 bronopol conserved and > 100 of each the boric acid conserved milk samples met the quality criteria of the Fossomatic 7DC (good separation=1). The Spearman correlation coefficients of the SCC of the different samples was highly correlated (>0.92,  $P < 0.01$ ). Likewise the correlation coefficient of the Control to DSCC of samples d1 – d5 remained fairly stable (range: 0.79 to 0.84) over time ( $P < 0.01$ ), but it markedly decreased with increasing boric acid to milk ratio (Control to DC: 0.72 and TC: 0.64,  $P < 0.01$ ). Exclusion of samples with SCC < 200.000 cells/ml improved the correlation coefficients between DSCC of Control and all other samples (range: 0.77 to 0.87). However, the scatter plots (Delta of DSCC of bronopol and DSCC of boric acid preserved samples) revealed increasing fanning of the Delta (range: >48.1) with decreasing cell count of the sample. This did not improve, even when a cutoff of 1.2 Mio. cells/ml or a DSCC > 50 of control samples was chosen.

In conclusion, boric acid affects the differentiation of white blood cells in milk samples using the Fossomatic 7DC system and thus prevents reliable DSCC analysis.

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UH-07

### Analysis of factors affecting milking claw vacuum levels using a simulated milking device

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**Objectives:** Bovine mastitis is typically caused by microbial infection of the udder, but the factors responsible for this condition are varied. One potential cause is the milking system, and although previous studies have investigated various methods for inspecting these devices, most have not assessed methods for evaluating the milking units. The objective of this study is to evaluate milking unit performance.

**Materials and Methods:** We analyzed the factors that affect the vacuum inside the milking claw by using a simulated milking device and by measuring milking claw vacuum when adjusting the water flow rate with pulsation in five stages (1.9kg, 3.8kg, 5.7kg, 7.6kg, and 8.7kg/min). The factors analyzed in each milking system were the vacuum pressure settings (high and low line operating system), milk tube length (200-328 cm), aperture diameter (14-22.2mm), constricted aperture diameter (12mm), tubing configurations, lift formation (0-80cm), claw type (bottom and top flow) and use or non-use of a milk sampler.

**Results:** Claw vacuum in the highline system dropped dramatically as the flow rate increased, meanwhile in the low line system dropped gradually. Longer milk tube, thinner aperture diameter, horizontal configuration, and higher lift height induced the lower claw vacuum as well as using top flow claw and milk sampler.

**Conclusions:** This study suggest that a diagnostic method using a simulated milking device should be considered when inspecting modern milking systems.

UH-08

### Farm level risk factors for bovine mastitis in Dutch automatically milking herds

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**Objectives:** Automatic milking systems (AMS) are installed on a rapidly growing number of dairy farms worldwide. Management to support good udder health on these farms is hampered because it is largely unknown to what extent risk factors for mastitis on AMS farm differ from farms using a conventional milking system (CMS). The aim of this study was to identify farm level factors associated with somatic cell count (SCC) and farmer-reported clinical mastitis incidence in Dutch dairy herds that use an AMS.

**Materials and Methods:** We collected data through an extensive questionnaire combined with on farm recordings of cow, stall and milking machine hygiene on 135 farms. In this way, we obtained data on 115 risk factors and linked these to 4 dependent variables related to the udder health status of the herd: the  $\log_{10}$  transformed annual average of the herd SCC ( $\log_{10}$  (HSCCav)), the  $\log_{10}$  transformed annual variance of the herd SCC on each test day ( $\log_{10}$  (HSCCvar)), the annual average percentage of new high SCC cases (NHSCC) and the farmer reported annual incidence rate of clinical mastitis (IRCM).

To identify associations between risk factors and dependent variables, we used two approaches. First, we employed linear regression models for  $\log_{10}$  (HSCCav),  $\log_{10}$  (HSCCvar) and NHSCC, and a negative binomial regression model for IRCM. We used multiple imputation to deal with missing values in our data. Secondly, we extracted principal components from the explanatory variables using non-linear principal components analysis (NLPCA) and regressed these against the same 4 dependent variables. Stepwise backward method based on the model AIC was used in model selection, a difference of AIC > 2 was considered as threshold for significance.

**Results:** In the regression models, we found feeding dry cows with hay, a higher proportion of cows laying outside cubicles and higher hygiene score of teats before cleaning and not participating in a *Salmonella* control program to be positively associated to  $\log_{10}$  (HSCCav). The  $\log_{10}$  (HSCCvar) was positively associated to a smaller milk quota (i.e. smaller farms), not participating in a *Salmonella* control program and not using a feed mixer. A large number of variables was related to NHSCC, suggesting that this variable captures more between-herd variation, which is supported by the fact that the coefficient of variation of this variable was substantially higher than that of  $\log_{10}$  (HSCCav) and  $\log_{10}$  (HSCCvar). NHSCC gives information about the dynamics of intra-mammary infections on a farm and we found higher NHSCC to be associated with a lower 305-d milk yield, a higher proportion of teats not covered by spray, not feeding dry cows with leftovers from milking cows, not participating in a *Salmonella* control program, and variables related to manure removal. The farmer-reported IRCM was positively associated to performing checks of the AMS while milking.

The NLPCA with 3 dimensions explained 16% of the variance in the risk factors. Based on variable loadings and quantifications, we interpreted being high on the first principal component



as the older and larger farms with poor cow hygiene and poor AMS hygiene, whereas the second principal component was related to smaller farms with poor cow hygiene.  $\log_{10}$  (HSC-Cav) was positively related to the first principal component,  $\log_{10}$  (HSCCvar) was positively associated with the second principal component, NHSCC was positively associated with both the first and the second principal component; IRCM was negatively related to the second principal component.

**Conclusions:** Altogether, our study shows that cleanliness of cows and of the AMS is related to better udder health, and that larger herds tend to have higher but less variable herd SCC. This work therefore confirms the importance of hygiene in relation to udder health and suggests that, specifically on larger AMS herds, udder health control needs more attention.

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UH-09

### Comparison of two commercial presentations of recombinant bovine somatotropin on milk yield in grazing dairy cows in a non-inferiority trial.

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**Background:** Recombinant bovine somatotropin (rbST) has been widely researched in the dairy industry. Numerous studies have investigated the effect of rbST on milk production, indicating an increase in milk yield in treated cows (St-Pierre et al., 2014). However, there are not enough studies to compare the effects of two commercial presentations of rbST available in the Colombian market, where the dairy systems are mainly based on grazing. Therefore, studies are needed to generate more information to help producers decide which formulation would be best suited for their own husbandry conditions.

**Objective:** The objective of this study was to demonstrate a non-inferiority of one commercial presentation (Bomate<sup>®</sup>, Merck & Co. Inc., Kenilworth, NJ, USA) over the other (Lactotropin<sup>®</sup>, Elanco Animal Health, Greenfield, IN, USA) by assessing their effect on milk yield in dairy cows maintained under a commercial grazing dairy system.

**Materials and methods:** The effect of two commercial presentations of rbST on milk yield was evaluated in a non-inferiority trial. The non-inferiority margin was set at  $\delta = 5.0$  (95% CI: 2.5; 7.5) kg of milk/cow/d. Using an average milk yield of 23 kg/cow/d, a one-sided significance level of 5%, and an estimated power of 80%, the trial sample size was calculated to be a minimum of 6 cows per group; however, we used a larger than calculated sample size in this study. Twenty-four multiparous Holstein cows were selected in a commercial dairy herd in Sabana de Bogota, Colombia, and were randomly allocated to one of two groups: Group A (n=12, 500 mg of rbST in a vitamin E lecithin base formulation, Bomate<sup>®</sup>, Merck & Co. Inc.), and Group B (n=12, 500 mg of rbST in a zinc sesame oil base formulation, Lactotropin<sup>®</sup>, Elanco Animal Health). Both formulations were administered according to manufacturers' instructions every 14 d over 20 cycles starting between 15 and 80

DEL. There were no differences in parity ( $3.8 \pm 2.1$ ) and DEL ( $57.8 \pm 14.3$ ) between groups at the beginning of the injection cycles. Cows were milked twice a day, and milk yield was recorded once a week. Five cows (3 cows from Group A, and 2 cows from Group B) were removed from the study as well as from the statistical analysis due to health issues not related to the treatments. Mixed regression models in Stata 15.1 (StataCorp analyzed data. College Station, TX, USA) were used for data analysis.

**Results:** Average milk yield in Group A was  $27.5 \pm 6.7$  kg/cow/d, and  $25.1 \pm 9.5$  kg/cow/d in Group B. The average difference in milk yield between groups was 2.7 kg/cow/d, indicating that milk yield was numerically higher in Group A. However, the lower bound of the 95% confidence interval (CI) for the difference was lower than 0, and the upper bound for the difference was higher than the critical value,  $\delta$ . There was a significant interaction of time x treatment on the average milk yield ( $P < 0.01$ ). There were no effects of parity ( $P > 0.05$ ) and DEL at the beginning of the trial on milk yield ( $P > 0.05$ ).

**Conclusions:** Our results indicate that milk yield in grazing dairy cows treated with Bomate<sup>®</sup> was numerically higher when compared to Lactotropin<sup>®</sup> over the whole lactation. However, this effect may not be considered as a single effect of the rbST treatment but depending upon time.

**References:** St-Pierre, N.R., G.A. Milliken, D.E. Bauman, et al. 2014. Meta-analysis of the effects of Sometribove zinc suspension on the production and health of lactating dairy cows. J. Am. Vet. Med. Assoc. 245:550–564.

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UH-10

### A Bayesian microsimulation to evaluate the cost-effectiveness of specific interventions for mastitis control during lactation and the associated reduction in antimicrobial use

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**Objectives:** The control of mastitis in dairy cows is poorly understood especially when caused by pathogens behaving in an 'environmental' manner, that is, being transmitted via the cow's environment, rather than directly from other cows. There are very few intervention studies that have measured the clinical efficacy of specific mastitis interventions within a cost-effectiveness framework so there remains a large degree of uncertainty about the cost-effectiveness of specific interventions. As pressure increases on producers to reduce the amount of antimicrobials used in livestock production, it is important to understand what impact specific mastitis control interventions are likely to have on farm-level antimicrobial use in addition to farm economics. The objectives of this study were to use a Bayesian framework to investigate the cost-effectiveness of mastitis control interventions to reduce intramammary infections acquired from the environment during lactation, and to predict the associated reduction in antimicrobial use as a result of the interventions.

**Materials and methods:** Data were assimilated from 75 UK dairy farms that participated in a British national mastitis control



programme during 2009-2012 in which the majority of intramammary infections were acquired during the lactation. The data consisted of clinical mastitis and somatic cell count (SCC) records, herd management practices and details of interventions that were implemented by the farmer as part of the control plan.

The outcomes used to measure the effectiveness of the interventions were i) changes in the incidence rate of clinical mastitis from 30 days after calving and ii) the rate at which cows gained new infections during lactation (measured by SCC changes between subsequent monthly milk recordings from <200,000 cells/ml to >200,000 cells/ml). A Bayesian one-step micro-simulation model was constructed such that posterior predictions from the model incorporated uncertainty in all parameters. The incremental net benefit of specified interventions was calculated across 10,000 Markov chain Monte Carlo iterations, to estimate the cost-benefit of each mastitis intervention. The impact of specific interventions on antimicrobial use was calculated simultaneously within the same Bayesian framework.

**Results:** Interventions identified as being cost-effective in most circumstances included effective fly control, the use of drying agents in cubicle beds, good ventilation of the lactating cow accommodation and effective scraping of housing, feeding and loafing areas. These were all associated with reductions in antibiotic use, the degree to which, depended on the treatment protocols used.

**Conclusions:** The results of this study highlighted the efficacy of specific mastitis interventions in UK conditions which, when incorporated into a cost-effectiveness framework, can be used to optimize decision making in mastitis control. This intervention study provides an example of how an intuitive and clinically useful Bayesian approach can be used to form the basis of an on-farm decision support tool and how it can be used to predict likely reductions in antimicrobial use, as well as informing economic decisions.

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UH-11

### Gene expression of the teat canal epithelium in dry and lactating cows

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**Objectives:** A delay in the closure of the teat canal by the keratin plug early in the dry period can increase the risk of mastitis in dairy cows. This study investigated the changes in gene expression of the teat canal epithelium before and after dry off.

**Materials and methods:** Teat canal biopsy samples from dairy cows enrolled in a larger study were extracted with a curette on the last day of lactation (Day 0) and on Day 11 after dry off (n=3 per time point). Total RNA was extracted and the samples were sent for RNA-seq analysis using Illumina HiSeq 2500. The resulting reads were mapped to the reference cattle genome (UMD3.1) and gene based read counts were analysed using DESeq2 in order to identify significantly differentially expressed

genes.

**Results:** Comparison of samples between Day 0 and Day 11 of the dry period identified 14 up-regulated and 22 down-regulated genes. Most of the downregulated genes were related to mitosis, inflammatory response pathways, protein trafficking, and membrane trafficking (e.g. Heat Shock Protein A6 (HSPA6), Kinesin Family Member 23 (KIF23), Marker of proliferation KI67 (MKI67), Synaptotagmin 4 (SYT4), Heat shock protein B8 (HSPB8), Cysteine and Glycine Rich Protein 2 (CSRP2)). Other downregulated genes were either involved in pathways related to apoptotic signalling or in the maintenance of tissue structure (e.g. Glutamate-cysteine ligase (GCLC) and Activating Transcription Factor 3 (ATF3)). Upregulated genes encoded mainly proteins related to protection of the epithelium such as Peptidyl arginine deiminase type I (PADI1) and Metallothionein 1E (MT1E). Keratin expression showed no significant differences between Day 0 and Day 11. However, although this was not significant at a false discovery rate (FDR) correct p-value of <0.1, the expression levels of some keratins might suggest that during the dry period the tissue in the teat canal becomes less active in terms of multiplication but may be more differentiated e.g. Keratin 75 (KRT75) and Keratin 17 (KRT17) were downregulated whereas Keratinocyte differentiation-associated protein (KRTDAP) was upregulated.

**Conclusions:** Cell proliferation and immune response in the teat canal appear to be diminished during the first stage of the dry period. Keratin synthesis was not significantly different between the times analysed. This study provides an unprecedented description of the complexity of the bovine teat canal epithelium cell transcriptome. It showed 36 differentially expressed genes in the teat canal before and after dry off that are candidate genes to be investigated in future studies. Further detailed studies on the physiology of the teat canal are necessary in order to develop strategies to improve cow's protection against mastitis in the early dry period.

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UH-12

### Recovery of subclinical intramammary infections during the dry period

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**Objectives:** This study aimed to evaluate the elimination of four different mastitis pathogens, *Streptococcus (Strep) agalactiae*, *Mycoplasma (M.) bovis*, *Staphylococcus (Staph.) aureus* and *Strep. uberis*, from infected udder quarters during the dry period by using quantitative polymerase chain reaction (qPCR) method. The second purpose of this study was to evaluate the inflammatory response to subclinical IMI. Elimination rates of *Staph. aureus*, *Strep. agalactiae* and *Strep. uberis* during the dry period and inflammatory response to IMI caused by these udder pathogens have been described in previous studies. To our knowledge, there are no published studies about the elimination of *M. bovis* IMI during the dry period or about the associations between *M. bovis* IMI and local inflammatory response.

**Materials and methods:** Cow udder quarter aseptic milk samples were collected at the dry-off and at first milking after calv-



ing between November 2014 and May 2015 from one Estonian dairy herd. All dairy cows were treated with cloxacillin based antibiotic product at dry-off (Noroclox DC, 600 mg, Norbrook Laboratories Limited, Newry, Ireland). Bacterial DNA of *M. bovis*, *S. aureus*, *Str. agalactiae* and *Str. uberis* was detected by using commercial qPCR test kit Mastit 4B (DNA Diagnostic A/S, Denmark). Milk Hp concentrations (mg/l) were determined by a method based on the ability of Hp to bind to haemoglobin adapted to be used for milk.

**Results:** In total, 1001 udder quarter milk samples were collected at dry-off and during first milking after calving from 133 dairy cows. Among detected udder pathogens, *Strep. uberis* had the highest elimination rate (100%). Elimination rates for *M. bovis*, *Staph. aureus* and *Strep. agalactiae* were 87%, 94% and 96%, respectively.

The milk Hp concentration was ( $p = 0.067$ ) higher in milk samples positive for *Strep. agalactiae* at the dry-off. After calving, the milk Hp concentration was significantly higher in milk samples positive for *Staph. aureus* ( $p = 0.003$ ) compared to milk samples negative for *Staph. aureus*. There was no significant association between milk Hp concentration and *M. bovis* IMI either at the dry-off or after calving.

**Conclusions:** In conclusion, the recovery from subclinical IMI during the dry period was high. Results of this study indicate that spontaneous recovery from subclinical *M. bovis* IMI occurs during the dry period. However, this should be evaluated in further studies with larger sample size. We can conclude that the milk Hp concentration can be used as an indicator of the presence of IMI at the dry-off and after calving together with bacteriological results.

UH-13

### Segmented linear regression confirms that a quarter somatic cell count greater than 100,000 cells/mL at dry-off indicates an abnormal secretion in dairy cattle

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**Objectives:** Descriptive studies have suggested that a quarter somatic cell count (SCC) >100,000 cells/mL indicates inflammation in bovine milk. To the author's knowledge, this SCC cutpoint has not been formally evaluated using quantitative statistical techniques. The objective of this study was to apply segmented regression to determine the SCC cutpoint for identifying the presence of inflammation (altered secretion concentration) in bovine milk.

**Materials and Methods:** Quarter foremilk samples ( $n=454$ ) were collected from 115 dairy cows at dry-off. Quarter SCC was measured using a Delaval<sup>®</sup> cell counter. Milk pH, electrical conductivity (EC),  $[Na^+]$ ,  $[K^+]$ , and  $[Ca^{2+}]$  were measured using ion-selective hand-held meters (LAQUAtwin). Milk [lactose] was measured using a hand-held glucose meter. Segmented linear regression was used to characterize the relationship between each continuous predictor (y axis) and  $\log_{10}SCC$  (x axis) by applying nonlinear regression to two sequential linear regression equations with a common break point ( $X_c$ ) such that: if

$x < X_c$  then  $y = b_0$ ; if  $x > X_c$  then  $y = b_0 + b_1(x - X_c)$ . The value of  $b_1/b_0$  provides an index of the sensitivity of a change in the predictor variable relative to the mean value for a healthy quarter.

**Results:** SCC breakpoints identified were:  $[Na^+]$ , 97,724;  $[K^+]$ , 100,000; [lactose] and pH, 120,226; EC, 125,893; and  $[Ca^{2+}]$ , 141,254 cells/mL. Values for  $b_1/b_0$  were:  $[Na^+]$ , +1.53; EC, +0.46;  $[K^+]$ , -0.42; [lactose], -0.38;  $[Ca^{2+}]$ , -0.21; and pH, +0.05.

**Conclusions:** A quarter SCC >100,000 cells/mL at dry-off indicates the presence of inflammation in bovine milk. Milk  $[Na^+]$  provides the best indicator of healthy milk of the six studied variables.

UH-14

### Identifying effective herd and cow selection criteria for selective dry cow therapy in Australian pasture based dairy herds

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**Objectives:** Antimicrobial resistance is of increasing importance in the dairy industry world wide and with it the imperative to reduce antibiotic usage. One area of dairy production in focus is the blanket use of long acting intramammary antibiotics at the end of the lactation when cows are dried off. The aim of the study is to determine if similar milk quality and udder health can be obtained in Australian pasture based dairy systems without the blanket use of antibiotics at dry off. Whilst similar studies have been conducted overseas no such investigation comparing the efficacy of internal teat sealants alone to internal teat sealants and intramammary antibiotics has occurred in pasture based Australian conditions. With dry cow antibiotics making up more than 85% of antibiotic usage on some dairy farms it is imperative that we provide on ground practical data to allow veterinarians and producers to make informed decisions around the use of antibiotics when drying off. This in turn will lead to a reduction in antibiotic usage in the Australian dairy industry and aid in reducing the level of antimicrobials used in food producing animals globally.

**Materials and Methods:** Twelve herds in the Macailster irrigation district supplied 1,774 cows for enrollment into this investigation. These herds were negative for *Mycoplasma bovis* and *Streptococcus agalactiae* on a bulk tank PCR, had an average bulk milk cell count of <250,000cell/ml over the past 12 months and herd tested (tested the individual somatic cell count of each cow) at least three times during the lactation. Eligible cows had an individual somatic cell count (ICC) of <150,000cells/ml at all herd tests and no case of clinical mastitis for the current lactation. The cows were split into two groups based on odd and even ear tags. The control group ( $n=853$ ) received long acting intra-mammary antibiotics (600mg Cloxacillin) and internal teat sealant (65% bismuth subnitrate in a paraffin base) at dry off while the treatment group ( $n=921$ ) received only internal teat sealant. Drying off was performed by farmers after undergoing a training program demonstrating the correct technique for drying off and highlighting the importance of hygiene. In the following lactation the ICC from each group was monitored at regular herd tests. All cases of clinical masti-



tis within the first 30 days after calving will also be submitted for microbiological culture. The rate of clinical mastitis in the dry period and in the subsequent lactation, and the ICC distribution of each group will then be compared. The economics of the new proposed selective dry cow therapy will also be investigated.

As part of the project, data including age, breed, frequency of herd testing and days in milk will be collected and analysed to refine potential cow selection criteria and allow for the development of more tailored protocols specific to a farm or group of cows.

**Results:** Preliminary results are positive, showing a similar rate of clinical mastitis in both groups of calved animals. Clinical mastitis cases are currently being cultured and herd test data is being collected and analysed.

**Conclusions:** It is imperative that we find practical alternatives to current dry off practices that reduce antibiotic usage without compromising milk quality or udder health. In collating more information on selective dry cow therapy in pasture based systems we will enable producers, veterinarians and the industry to make informed choices allowing for a reduction in antibiotics whilst maintaining the current quality of production.

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UH-15

### Performance of milk leukocyte differential test in selective dry cow therapy approach

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**Objectives:** Bacteriological culture is the gold standard to identify cows for treatment in selective DCT, but it is a lengthy and expensive method. Milk leukocyte differential (MLD) is a tool available for on-farm use that has the potential to be used for selective DCT. The objectives of this study were to determine the ability of QScout MLD test (AAD, Inc., Morrisville, NC, US) to detect intramammary infection (IMI) in late-lactation dairy cows and to compare udder health and milk production between cows treated with complete and selective DCT using QScout MLD test.

**Materials and Methods:** To validate the ability of the MLD test, a cross-sectional study was conducted on a dairy herd in North Carolina (USA). Cows that were over 280 DIM and had no visible changes to the composition of their milk were enrolled. Milk samples from each quarter were collected for the MLD test, somatic cell count (SCC), and bacteriological culture. Classification for infection with MLD test was obtained using 12 different thresholds. Bacteriological culture was used as the gold standard to determine sensitivity, specificity and predictive values (PV) of the MLD test at each threshold.

To validate the use of MLD test for selective DCT, a randomized controlled trial was conducted on a dairy herd in Idaho (USA). Cows were enrolled the day prior to dry off and were randomly assigned to blanket or selective DCT. At enrollment and after parturition, milk samples were taken for MLD test and bacteriological culture. Quarters were tested individually and

classified as positive or negative using the QScout MLD test. Cows in the blanket DCT were all treated with intramammary cephalosporin benzathine and teat sealant. Cows in selective DCT with at least one MLD positive quarter were treated with intramammary cephalosporin benzathine and teat sealant in all quarters; cows with all MLD negative quarters were treated with only teat sealant. After parturition, quarters were classified for intramammary infection (IMI) using the postpartum bacteriological culture results. Through the lactation, clinical mastitis cases were recorded, and milk production and SCC were obtained monthly from DHI reports. At the quarter-level, the odds of postpartum IMI were compared between complete and selective DCT using logistic regression models. At the cow-level, the number of clinical mastitis events per cow-day at risk were compared using a negative binomial regression model; and the milk production and SCC (log transformed) were compared between complete and selective DCT treatment using linear regression models with repeated measurements structure. Statistical analyses were conducted using SAS Studio 3.6 (SAS Institute, Cary, USA).

**Results:** In the cross-sectional study, 94 (373 quarters) cows were enrolled. The median SCC at the quarter-level was 67,500 cells/mL. The true prevalence of IMI was 12.1% in quarters, and 31.9% in cows. At the quarter-level, the MLD test sensitivity and specificity ranged between 44.4% to 73.3% and 75.5% to 91.5%, respectively. Positive PV ranged from 29.7% to 42.6%, and negative PV from 92.1% to 95.4%. At the cow-level, sensitivity and specificity ranged between 50.0% to 76.7% and 54.0% to 81.0%, respectively. Positive PV ranged from 44.2% to 55.6%, and negative PV from 77.3% to 82.9%.

In the randomized controlled trial, 300 cows (1,193 quarters) were enrolled. At dry-off, cows had a median SCC of 43,000 cells/mL, and a median milk production of 34.5 kg. At the quarter-level, the proportion of postpartum IMI did not differ between complete and selective DCT (odds ratio = 0.93;  $P = 0.56$ ). At the cow level, the number of clinical mastitis per cow-day at risk did not differ between complete and selective DCT (relative risk = 1.05;  $P = 0.90$ ). Milk production varied between monthly tests ( $P < 0.01$ ), but not between complete and selective DCT groups ( $P = 0.90$ ; group by test interaction:  $P = 0.58$ ). Similarly, SCC (log transformed) varied between monthly tests ( $P < 0.01$ ), but not between complete and selective DCT groups ( $P = 0.50$ ; group by test interaction:  $P = 0.40$ ).

**Conclusion:** The results of these studies showed the value of QScout MLD test to identify IMI in dairy cows in late-lactation to guide selective DCT. They also demonstrate that udder health and production parameters did not differ between cows treated with blanket and those determined to receive selective DCT by QScout MLD.

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UH-16

### Economic optimization of selective dry cow treatment

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**Objectives:** In the animal industry, prudent and restricted use of antimicrobials is of major importance in order to reduce the risk of development of antimicrobial resistance. On dairy farms, blanket dry cow treatment is widely used, not only to eliminate existing intramammary infections (IMI) but also to prevent new IMI to occur. With an otherwise unchanged management, selective dry cow treatment, aiming not to use antimicrobials at drying off in non-infected cows, increases the incidence rate of clinical mastitis and the somatic cell count post-partum.

The economic impact of selective dry cow treatment may vary for different herd situations and for different levels of antimicrobial use at drying off, likely influence decisions of dairy farmers, and therefore need further attention. Studies describing and evaluating economic consequences of selective dry cow treatment on herd-level can be used by dairy farmers and their advisors to help to optimize economic decisions on dry cow treatment. The objective of this study was therefore to develop a mathematical model in which economic costs are minimized, while the percentage of cows to be dried off with antimicrobials is restricted, accounting for effects of clinical and subclinical mastitis and antimicrobial use.

**Materials and Methods:** Based on data from a large field trial, a linear programming model was built with the goal to minimize the costs associated with antimicrobial use at drying off. To enable calculations at the herd-level we created an example 'average' herd. Cows were projected on three types of herds with different bulk tank somatic cell count (BTSCC), and were categorized in groups based on parity and somatic cell count from the last test recording before drying-off.

Economically optimal use of antimicrobials was determined while restricting the maximum percentage of cows dried off with antimicrobials from 100% to 0%, revealing the economic consequences of different approaches. A sensitivity analysis was performed to evaluate the effect of variation in the most important input variables, being the effect of dry cow antimicrobials on the incidence of clinical and subclinical mastitis, and the milk price.

**Results:** Total costs of mastitis per cow to be dried off per year varied from € 45 in a herd with a low-BTSCC where 100% of the cows were allowed to be dried off with antimicrobials to € 56 on a herd with a high-BTSCC where the maximum percentage of cows to be dried off with antimicrobials was 0. Clinical and subclinical mastitis incidence in the different situations varied from 9.9% to 16.1% and from 8.2 to 19.6% respectively. Antimicrobial use varied from 0.6 animal defined daily dosages when no dry cow antimicrobials were allowed (0%) in herds with a low-, an average-, and a high-BTSCC to 3.9 animal defined daily dosages when 100% of cows were allowed to be dried off with antimicrobials in a herd with a high-BTSCC.

The effect of clinical mastitis on the total costs of mastitis is greater than the effect of BTSCC and influences the economic consequences of selective dry cow treatment. In herds with a low incidence of clinical mastitis selective dry cow treatment was more beneficial than a blanket approach and even using no dry cow antimicrobials at all (0%) was found to be cheaper than blanket dry cow treatment. In all types of herds, blanket dry cow treatment was always more expensive than selective dry cow treatment.

**Conclusions:** From an economic perspective, blanket dry cow treatment was found not to be the optimal approach, although

differences between approaches were small. Selective dry cow treatment was economically more beneficial than blanket dry cow treatment with greater economic profits in herds with a low incidence of clinical mastitis and a low BTSCC. In all types of herds, the use of dry cow antimicrobials can be reduced without economic consequences, although not always to 0%. In herds with a low incidence of clinical mastitis the use of no dry cow antimicrobials at all is cheaper than blanket treatment. The udder health situation as a whole, including the incidence of clinical mastitis and the BTSCC, has a much bigger economic effect than the dry cow treatment approach. Altogether economics is not an argument against reduction of the use of dry cow antimicrobials by applying selective dry cow treatment.

UH-17

### Quarter-based selective dry cow therapy using on-farm diagnostics: preliminary results of a randomized controlled trial

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**Introduction:** The general recommendation in many countries is to use blanket dry cow therapy (BDCT). Selective dry cow therapy (SDCT) combined with an internal teat sealant (ITS) could be a potential alternative to reduce quantity of antimicrobials used in dairy production. When using SCC records and on-farm culture to differentiate infected and healthy cows, a SDCT+ITS protocol allowed a reduction of dry-off antimicrobials use by 22% (Cameron et al., 2013). However, since many cows may have only one infected quarter, a selection at quarter-level could further improve the antimicrobial usage reduction associated with a SDCT approach.

**Objectives:** The study goal was to assess the efficacy of an on-farm culture system using Petrifilm® for targeted treatment decisions at quarter-level at dry-off; with three hypotheses: (1) an on-farm quarter-milk culture system could be used to identify infected quarters at dry-off; (2) prevention of new intramammary infections (IMI) in uninfected quarters could be achieved by using an ITS only; (3) a quarter-based SDCT approach could result in a substantial reduction (> 50%) of antimicrobial usage at dry-off, without any harmful effect on udder health or milk production in the subsequent lactation.

**Materials and Methods:** A total of 569 cows (2,251 quarters) from 9 dairy herds (range: 26-169 cows/herd) with bulk tank SCC < 250,000 cells/ml in Quebec, Canada were enrolled and randomly allocated to:

- i. Two positive control groups:
  - intramammary (IMM) infusion of antimicrobial alone to all cows (BDCT; group 1);
  - IMM infusion of antimicrobial and ITS to all cows (BDCT+ITS; group 2);
- ii. Two SDCT groups with on-farm culture using Petrifilm®:
  - IMM infusion of antimicrobial to infected quarters and ITS to



healthy quarters (SDCT/ITS; group 3);

→ IMM infusion of antimicrobial and ITS to infected quarters and ITS to healthy quarters (SDCT+ITS/ITS; group 4).

Thus, an average of 142 cows were enrolled per group (range: 132-153 cows). For SDCT groups, on a day prior to dry-off, single quarter milk samples were collected and cultured on-farm on Petrifilm® Aerobic count plate. At drying off, each quarter in SDCT groups was treated according to culture results on Petrifilm®: quarters with greater-than or equal to 5 cfu/0.01ml were considered infected and quarters with < 5 cfu/0.01ml were considered healthy. For all enrolled cows, single quarter milk samples were also collected for bacterial identification using laboratory-based bacteriological culture followed by MALDI-ToF identification on: 1) a day prior to dry-off (S1); 2) day 3 to 4 after calving (S2); 3) day 5 to 18 (S3) after calving; and 4) for all clinical mastitis (CM) cases (S4) up to 120 DIM. IMI and new IMI (NIMI) were defined using laboratory-based milk bacteriological culture. Descriptive statistics on prevalence of IMI at dry-off, NIMI during dry period and CM up to 120 DIM were computed. As preliminary analyses, a chi-square test was used to compare results between treatment groups.

**Results:** In SDCT groups, 57.8% (range: 32.1 – 74.5%) of quarters were classified as uninfected based on Petrifilm® and, therefore, did not receive antimicrobials. Based on laboratory bacteriology (i.e. culture and MALDI-ToF), 71.5% of quarters were classified as uninfected at dry-off. The prevalence of IMI at dry-off was 31.2, 30.4, 26.9 and 25.5% for 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups, respectively. The incidence of NIMI over the dry period was 15.4% in general and 16.0, 13.3, 17.9 and 14.6% for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups, respectively, when considering all relevant pathogens for bovine mastitis. The incidence of NIMI caused by environmental pathogens only (NIMI<sub>env</sub>) was 12.2% with a proportion of 13.0, 11.3, 14.1 and 10.3% for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups, respectively. Incidence of clinical mastitis during the first 120 DIM was 5.4% in general and 5.1, 7.1, 5.3 and 3.7% for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups, respectively. Preliminary analyses did not show any significant difference among the different treatment groups, for prevalence of IMI at dry-off, NIMI or NIMI<sub>env</sub> incidence during the dry period, and for CM incidence during the first 120 DIM.

**Conclusion:** Analyses are still ongoing to evaluate the impact of quarter-based SDCT on SCC and milk production in the subsequent lactation. But for now, we can conclude that an important reduction in use of antimicrobials can be achieved using quarter-based SDCT, without negative impacts on main udder health parameters.

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UH-18

### Effect of dry cow treatment of a teat sealant alone or in combination with antibiotic when applied at the cow or quarter level.

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**Introduction:** In the EU, the concern around the prophylactic use of antibiotics has led to further scrutiny of dry cow therapy use and the classes of antibiotics used. Ultimately, a quarter level selective dry cow treatment approach may potentially further reduce antibiotics compared to cow level treatment and could result in more prudent use and better overall dry period outcomes.

**Objective:** The objective of the study was to compare cow level to quarter level dry cow therapy.

**Material and Methods:** Six commercial dairy farms in the UK, with a bulk milk somatic cell count that reflects the average currently in the UK, and an ability to demonstrate compliance with the study protocol.

Cows, within herds, are stratified 'infected' when the last 3 monthly cow somatic cell counts (CSCC) are all > 200k cells/ml and any clinical mastitis (CM) occurrence within the same time frame, or 'uninfected', when all last 3 monthly CSCC are < 200k cells/ml, and no CM occurrence in the same time frame, before being randomly allocated to one of three treatment groups; Cow Level Treatment (CLT), Quarter Level Treatment (QLT) 0 and QLT1, based on a CMT test result threshold of > 0 or > 1 respectively.

At dry off and within a week post-calving all quarters are sampled for bacteriology and SCC analysis and also tested by CMT post-calving. In the CLT group, all quarters of all cows are treated with a teat sealant (TS) and also received antibiotic treatment in all quarters (AB), if being considered 'infected'.

Within the QLT0 and QLT1 groups quarters within cows were allocated to treatments, based on a California Mastitis Test (CMT) score >0; (score 0 = TS and score 1, 2 or 3, TS+AB) versus CMT score >1 (Score 0 or 1 = TS and score 2 or 3, TS+AB) respectively.

Successful dry period outcome were/will be determined using bacteriology, SCC (<200k), and by CM incidence in the 1<sup>st</sup> 100 days in milk. For statistical analysis, the quarter will be the experimental unit.

Preliminary analysis was undertaken using SCC outcomes, with quarters with an SCC <100K being defined as 'uninfected'. Groups were compared using the chi2 test with allowances made for multiple comparisons using a Layered Bonferroni Correction.

**Results:** This is the first study comparing cow and quarter level dry cow treatment using different CMT thresholds at dry off.

At the time of abstract submission, 43.7% of animals (328 cows, 1312 quarters) had been enrolled and post calving CMT and SCC data were available for 166 animals. The authors performed a preliminary statistical analysis on these data. Results are very interesting but preliminary, only based on SCC data, and should therefore be interpreted with care. That is why the authors decided to refrain from showing results in this abstract at this early stage, but we will be able to show results including all cows combined with a full statistical analysis at the time of WBC Congress.



UH-19

### Study on Udder Health and Milk Quality, and its Relation to Dry Cow Therapy in Sahiwal Cows

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Sahiwal, a breed of Zebu cattle produces the most milk of all zebu breeds and has many advantages over the exotic breeds, for example; high level of tick and parasite resistance along with tolerance to high environmental temperature. Due to its unique characteristics, this breed has been exploited for up liftment of local breeds in parts of Asia, Africa and Australia. However, very little research has been done till date on this breed despite all its characteristics, The present study was undertaken to evaluate the udder health and milk quality, and role of dry therapy in the control of mastitis this breed of cattle. In the first part of the study, 102 Sahiwal dairy cows maintained under a semi-intensive system at an organized dairy farm were assessed for udder health status and milk quality. The animals were bucket machine milked twice daily, morning 3:30 am and evening 3:00 pm. The milk sampling were collected during the evening milking and processed for bacteriology (microbial procedures of National Mastitis Council, USA) and somatic cell count (SCC, using Somatic cell counter, Delta Instruments, The Netherlands). The health status of udder quarters was defined as healthy, specific, non-specific and latent mastitis on the basis of bacteriology and SCC of quarter foremilk using International Dairy Federation criteria. A cow with at least one specific or nonspecific mastitis quarter was categorized into mastitis group. The results showed 43.13% of cows affected with sub-clinical mastitis. The quarter wise, 18.14% of quarters comprising 9.56% specific and 8.58% nonspecific mastitis were found to affect with subclinical mastitis. The coagulase negative staphylococci (59%), *Staphylococcus aureus* (25.64%) and *Corynebacteria* (10%) were found the chief isolates in specific mastitis. The cow composite milk SCC ranged from 20 to 3393  $\times 10^3$  cells/ml with about 1/3<sup>rd</sup> of cows (32%) possessing milk SCC above 500  $\times 10^3$  cells/ml. The 38% Cows were having milk SCC less than 200  $\times 10^3$  cells/ml. The 23% were in the range of 200-400  $\times 10^3$  cells/ml. The second part of study evaluated the effect of dry cow therapy on quarter infections and milk quality. The 40 cows approaching dry period were randomly assigned to groups: Treatment group and Control group. In the treatment group, immediately before drying off, each functional quarter of the cows were dry treated with an intramammary suspension of Cepravin dry cow (cephalonium dihydrate 250 mg, MSD Animal Health). The cows in the control group received no treatment. The quarter foremilk/ cow composite milk samples were collected at dry off and 5-7 days post calving, and subjected to bacteriology, California mastitis test (CMT), SCC, electrical conductivity (EC, Digital conductivity meter, Eutech Instruments, CON 700) and milk biochemical composition (fat, total Protein, lactose and solids not fat) analysis applying infrared spectrophotometer method (Lactoscan LA from Milkotronic Ltd., Bulgaria). The dry cow therapy could eliminate 10/11 (90.91%) quarters infections as compared to 7/15 (46.67%) infections in control group present at dry off; a significant difference ( $\text{Chi}^2 = 5.49$ ;  $p = 0.019$ ). Also, the establishment of new infections was significantly lesser in the treatment group (7.25%) than the control (19.67%); ( $\text{Chi}^2 = 4.40$ ;  $p = 0.03$ ). A significant effect was seen,

of dry cow therapy, on the biochemical composition of milk with respect to lactose, solids not fat and total protein contents of milk. Further, the therapy resulted in an appreciable fall in the mean milk electrical conductivity (9.55 to 5.98 mS/ cm), SCC (4399 to 292 $\times 10^3$  cells/ml) and CMT score (2.03 to 0.20) at cow level. The proportion of quarters with CMT score <1 (i.e. with low SCC) at calving was found significantly higher in dry treated cows (94.94%) than that in control group (22.37%,  $\text{Chi}^2 = 13.37$   $p = 0.000$ ). For the present study and available data on the udder health in crossbred cows and buffaloes, it may be concluded that with regard to milk composition and SCC, Sahiwal cows behave like buffaloes. The dry cow therapy may be employed for the prevention of mastitis and improvement of milk quality in this milch breed of cattle.

UH-20

### Incomplete Milking in Early Lactation Increases the Odds of Eliminating Intramammary Infections: Results from a Randomized Controlled Trial

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**Objectives:** This study aims to evaluate the impact of an incomplete milking during the first 5 days in milk (**DIM**) on early lactation (1) incidence of intramammary infection (**IMI**), (2) IMI elimination rate, and (3) clinical mastitis (**CM**) risk. Our hypothesis was that, by reducing hyperketonemia in incompletely milked cows, immunosuppression would be prevented, leading to an improved udder health.

**Material and Methods:** This study was a randomized controlled trial conducted on multiparous cows from 13 commercial dairy farms. Multiparous cows were randomly allocated, using a random number generator, to be: incompletely milked during the first 5 DIM (a maximum of 10, 12, and 14 kg/d on days 1-3, 4, and 5 respectively; **INC** group); or milked conventionally (**CON** group). Dairy producers could not be blinded to group allocation (INC or CON).

To evaluate IMI incidence and elimination, quarter-milk samples were collected twice (2<sup>nd</sup> and 3<sup>rd</sup> weeks in milk). Somatic cell counts (**SCC**) was measured on quarter-milk samples using a Fossomatic cell counter. To calculate IMI incidence, quarters without IMI (< 100,000 cells/mL) at 1<sup>st</sup> sampling were monitored again one week later to detect those that developed IMI ( $\geq 200,000$  cells/mL). To calculate elimination rate, only quarters with IMI ( $\geq 200,000$  cells/mL) at 1<sup>st</sup> sampling were considered, and we observed whether they eliminated that IMI (< 100,000 cells/mL) on 2<sup>nd</sup> measurement. Cases of CM were recorded by participating producers in their electronic health records. The effects of the incomplete milking on odds of acquiring an IMI, on odds of eliminating an existing IMI, and on odds of CM between 0-30 DIM were determined using generalized mixed models using herd and, for quarter analyses, cow as random intercepts.

**Results:** Cows from control group produced a mean of 5.3, 19.6, 25.2, 28.7 and 29.9 L of milk/d on DIM 1, 2, 3, 4 and 5, respectively. In total, 3,173 quarter-milk samples from 807





cows (412 CON and 395 INC) were collected throughout this study. The first and second quarter milk samples were collected at 11.5 DIM (range: 5 to 18) and 18.6 DIM (range: 14 to 25), respectively. On first and second sampling, samples were missing for 121 and 163 quarters, respectively. Therefore, 2,889 pairs of quarter-milk samples were available for the analyses. At the first sampling, 2,662 quarters were free from IMI, and, therefore, at risk of acquiring a new IMI. The odds of new IMI for INC were 0.73 (95% CI: 0.37, 1.4;  $P=0.30$ ) times those of CON. Regarding IMI elimination, 137 quarters were considered infected on first sampling. Odds of cure of an IMI for INC were 2.8 (95% CI: 1.3, 6.3;  $P<0.05$ ) times those of CON.

Data on CM are still being compiled; using preliminary data from 583 cows, odds of CM 0-30 DIM for CON were 1.7 (95% CI: 0.64, 4.4;  $P=0.30$ ) times those of INC.

**Conclusion:** Incomplete milking during the early lactation increases the odds of IMI elimination, but does not affect development of new IMI, nor CM incidence in early lactation.

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UH-21

### The effect of celery extract (*Apium graveolens*) on the lactation edema, mastitis and milk composition in Holstein dairy cattle

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**Objectives:** Celery plants have antibacterial and diuretic properties because for the composition such as phthalide and essential oil. The purpose of this study was to evaluate the impact of alcoholic extract of celery on lactation edema, mastitis and milk composition in Holstein dairy cattle.

**Materials and methods:** In this study number of 40 nulliparous dairy cow was randomly at 4 treatment groups: placebo as oil-based, celery extract 0.5%, celery extract 1% and control group. Alcoholic extract of celery was produced in oil-based as ointment and applied on the udder tissue during 7 days postpartum. The elimination of lactation edema was assessed during an administration ointment. After 7 days, milk samples were collected and tested milk composition [fat, protein, lactose, solid non-fat (SNF), total dry matter, and the somatic cell count (SCC)].

**Results:** The result of this study shows that the elimination of lactation edema was shorter in treatment groups and placebo compared to the control group. Also, the elimination of lactation edema was been shorter in treatment groups than the placebo group. However, milk composition wasn't significant differences in between the groups expect of SCC. The SCC was significantly differences in treatment groups compare to the control group.

**Conclusion:** In conclusion, the consumption of alcoholic extract of celery (at oil-base) was improved lactation edema at udder tissue, decrease of SCC and mastitis in dairy cattle subsequently.

**Keywords:** Celery, Lactation edema, Mastitis, Milk Composi-

tion, Dairy Cattle

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UH-22

### The severity of periparturient udder edema in dairy cattle is inversely associated with the cross-sectional area of the cranial epigastric veins draining the mammary gland

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**Objectives:** Periparturient udder edema is common in primiparous dairy cattle but the mechanism of edema development remains unknown. The objectives of this study were to compare udder edema severity, cross-sectional area of the cranial epigastric vein, and blood flow within the cranial epigastric vein between primiparous and multiparous cattle.

**Materials and Methods:** The cross-sectional area of the right or left cranial epigastric vein of 50 Holstein cattle (14 primiparous, 12 second lactation, 24 third or more lactations) was determined ultrasonographically using a 5 MHz linear probe (EXAGO 90-1119 ECM) daily from 3 days antepartum to 2 days postpartum. The depth and width of the vein were measured and the cross-sectional area calculated using the formula for an ellipse. Mean venous blood flow velocity was measured by placing the ultrasound probe along the longitudinal axis of the vein and mean blood flow calculated from the area and velocity measurements. Udder edema severity was scored daily using a validated 10-point visual scale. Data was analyzed using Spearman's correlation coefficient ( $r_s$ ) and mixed models analysis.

**Results:** Venous cross-sectional area and blood flow increased from day 3 antepartum to day 2 postpartum but cross-sectional area was much lower ( $P<0.001$ ) at parturition in primiparous cattle ( $1.4\pm 0.5\text{cm}^2$ ) than second lactation ( $4.2\pm 0.9\text{cm}^2$ ) and third or more lactation cattle ( $7.2\pm 2.5\text{cm}^2$ ). Udder edema score was inversely correlated with venous cross-sectional area ( $r_s=-0.49$ ).

**Conclusions:** Periparturient udder edema develops as a result of inadequate venous drainage of the mammary gland relative to the marked increase in mammary blood flow associated with lactogenesis.

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UH-23

### Treatment of Staphylococcus aureus mastitis with recombinant bovine GM-CSF

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**Objectives:** Bovine mastitis is the most prevalent and costly production disease in dairy herds worldwide, and it most commonly develops in response to intramammary bacterial infection. Currently, the main therapy for mastitis is the administration of antimicrobial drugs; however, this approach is associated with a risk of the development of antimicrobial resistant bacteria. In particular, *Staphylococcus aureus* (SA) mastitis is known to be highly resistant to antibiotic therapy because the organism forms biofilm in the mammary gland tissues, survives in the host cells, and easily leads to chronic disease. Antimicrobial resistance of zoonotic organisms in domestic animals has become a potential threat to global public health; therefore, the development of alternative therapies to antibiotics is urgently required from a food safety standpoint. One such alternative strategy is the modulation of immune responses using recombinant cytokines. The present study was designed to investigate the effect of the intramammary infusion of recombinant bovine granulocyte-macrophage colony-stimulating factor (rbGM-CSF) on quarter milk levels of immune cell states and shedding pattern of somatic cell count, and SA count in quarters affected with SA subclinical mastitis.

**Materials and methods:** For this experiment, eighteen Holstein dairy cows with naturally SA infected-subclinical mastitis were used. The cows whose milk SCC had been exceeding the level of 1,000,000 cells/mL were selected as members of the GM-CSF group (n=9) and the control group (n=9). The rbGM-CSF was produced in transgenic silkworm. As the GM-CSF group, the infusion of rbGM-CSF (400 µg/5 mL/quarter) into the cistern through the catheter was conducted immediately after the morning milking on day 0. As the control group, 5 mL of phosphate buffered saline (PBS) was also infused into the cistern. Blood and milk samples were collected prior to the treatment (day 0) and 0.25, 1, 2, 3, 7 and 14 days post infusion of rbGM-CSF or PBS. Chemiluminescence (CL) activity, mononuclear cell populations, SCC and bacterial count in milk were measured in order to evaluate the therapeutic effect of rbGM-CSF. Criteria of mastitis in the cattle was determined using the measurement of SCC.

**Results:** None of the cows showed any abnormal clinical signs or any visible local reactions in the areas infused with rbGM-CSF or PBS. The SCC had a transient rise on day 0.25 after the infusion of rbGM-CSF and were followed by a smooth and significant decline on days 3, 7 and 14. At the end of experiment, SCC of cows infused with rbGM-CSF was lower compared with day 0. Seven of nine cows (77.8 %) in the GM-CSF group showed a decreased SCC on day 14 compared to day 0. The SA count in the GM-CSF group were lower than the control group at day 7. In the GM-CSF group, the CL activity in milk tended to be higher on day 0.25 after the infusion of solution and was significantly lower on day 14. Furthermore, the percentages of CD4+ and CD4+CD45RO+ cells increased on days 1, 2, 3, 7 and 14 post infusion of rbGM-CSF.

**Conclusions:** The results suggest that the intramammary infusion of rbGM-CSF produced in transgenic silkworm has a high potential as a therapeutic agent for mastitis of dairy cows. Our study indicates that the infusion of rbGM-CSF accelerates the bactericidal activity of polymorphonuclear leukocytes and the memory function of helper T cells in the mammary gland infected with mastitis, and these cells induce the enhancement of innate immunity in the mammary gland.

UH-24

### Automatic analysis of infrared thermograms using computerized active shape modeling in experimentally induced *E. coli* - mastitis

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**Objectives:** Infrared thermography is a noninvasive method to measure changes in bovine udder surface temperature and thus may be a helpful tool for early mastitis detection. However, the manual analysis of thermograms requires a high amount of time. The aim of this study was to ascertain whether a computerized automatic segmentation and evaluation of thermal images gains comparable results to manual analysis as a 'gold standard'.

**Material and methods:** Acute mastitis was induced by intramammary *E. coli* inoculation into the hind right quarter (HR) of five healthy Holstein-Friesian cows (approved by the Animal Ethics Committee). As a placebo treatment, 2mL of sterile physiological saline were instilled into the hind left quarter (HL) of the same animal. Thermograms were taken every two hours in a period of 24 hours before and after infection (pi).

The images were interpreted using two methods. A manual method (Man), using a polygon-tool (ThermaCAM Researcher Pro 2.8, FLIR Systems) and, a computerized automated image analysis method (Aut), using active shape modeling. The latter detects the silhouette of the udder and creates two regions of interest (ROIs) in each thermogram: the hind surface of the udder including the teats, divided by left and right hindquarter and, in a second step, 5% of the pixels inside each ROI, originating from the outer borders, were automatically excluded. For each image, values for average (Avg) and maximum (Max) udder surface temperature were calculated. For statistical analysis: paired t-test, Pearsons correlation analysis, one-way ANOVA, Dunnett's post-test and Receiver-Operating-Characteristic (ROC)-curves were used.

**Results:** Manually ascertained maximal and average temperatures were consistently higher than computerized automatic analysis by active shape models throughout the whole trial and in both hind quarters (means of differences: HR Max: 1.06, HR Avg: 0.72°C, and HL Max: 0.78°C, HL Avg: 0.73°C, all p-values < 0.0001). The results of both methods are highly correlated (Max HR: r=0.90, MaxHL: r=0.92, Avg HR: r=0.99, Avg HL: r=0.98, all p-values < 0.0001). In both methods surface temperature peaked between 13 and 15 hours pi. Compared to temperatures measured 24 hours before, significant elevations (all  $\geq 1.32^\circ\text{C}$ ,  $p < 0.05$ ) could only be detected in both methods using Max. In ROC-analysis, both methods provide good results for sensitivity and specificity at different threshold values, depending on the method used: Aut Max HR: threshold  $\geq 37.42^\circ\text{C}$ , sensitivity=93.75%, specificity=94.96%; respectively: Man Max HR: threshold  $\geq 38.65^\circ\text{C}$ , sensitivity=93.75%, specificity=96.40%.

**Conclusions:** It is presumed that warmer temperatures in manual analysis occur due to involvement of hot regions, e.g. udder-thigh cleft, whereas automatic segmentation leaves



these regions out. Still, temperature curves for each method showed similar progresses. Therefore, automatic segmentation of infrared images is a quick and promising approach to enhance early mastitis diagnosis.

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UH-25

### Use of infrared thermography in pre-partum dairy heifers to predict elevated somatic cell count and intramammary infection at calving

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**Objectives:** Bacterial intramammary infection (IMI) is the main cause of mastitis. In early lactating heifers it can affect negatively welfare, milk production, longevity in the herd and economics of the farm. Early detection of higher risk animals for subclinical and clinical mastitis at calving, with a non-invasive and contactless tool, would allow gathering useful information to establish when IMI occurs. And, better prevent its potential negative impact in udder health, without the risks of the traditional methods to assess nulliparae IMI. This study aimed to evaluate the usefulness of infrared thermography (IRT) to detect changes in udder surface temperature of primigravid dairy heifers that could be indicative of an elevated somatic cell count (SCC) and/or intramammary infection at calving.

**Material and methods:** The study was conducted on a commercial dairy farm with approximately 700 lactating cows. Replacement heifers were reared on an off-site rearing unit returning to the main unit approximately 6 weeks prior to the expected calving date. One hundred twenty Holstein-Friesian heifers in the last trimester of gestation were enrolled in the study and submitted twice to IRT previously to calving. The first set of IRT images were collected approximately two months prior to calving and the second set two weeks before calving, using a FLIR E300 infrared camera. Each IRT image set included one caudocranial and one ventrodorsal projection of the udder. Ambient temperature, shed humidity and rectal temperature were measured. IRT images were analysed using ThermoCAM Research 2.10 Pro. Descriptive parameters were obtained through the application of the software geometric analysis tools. Milk samples were collected aseptically from individual quarters for standard aerobic bacteriological culture and SCC measurements by flow cytometry at calving. IMI was defined as the presence of  $\geq 1000$  colony-forming unit (cfu) per millilitre, at single colostrum quarter sample. Udder surface temperatures and temperature gradients were used for the statistical analysis, which was performed using SPSS software version 22.0.

**Results:** The synchronization of IRT image collection with farm routines allowed an easy, fast and non-contactless collection of udder surface temperatures non-invasively. However the current software for IRT image analysis can be laborious. Ventrodorsal projection of the udder was shown to be a valid option to assess udder surface temperature when compared to commonly used caudocranial projection ( $dT_{\max; r} = 0.9$  and  $r = 0.97$  two months pre-calving and two weeks pre-calving, respectively) and permitted to detect a suggestive lower temperature pattern in quarters with likely worst outcome (IMI, major pathogens and higher SCC). Even though IRT was unreliable to predict IMI and elevated SCC ( $p > 0.05$ , binomial logistic regression and ordinal logistic regression, respectively), presenting a lack of accuracy to discriminate between infected (mainly with CNS bacteria; 89.7%) and not infected quarters (AUROC very close to 0.5). From the total of quarter-milk samples cultured ( $n = 440$ ), 36% of the quarters were culture-negative ( $n = 160$ ), 39% culture-positive with  $< 1000$  cfu/ml ( $n = 171$ ), 20% IMI quarters ( $n = 86$ ) and 5% were classified as contaminated ( $n = 23$ ;  $\geq 3$  bacterial morphotypes). SCC geometric means at calving were 336, 365 and 867 ( $\times 1000$  cells/ml) for culture-negative, culture-positive with  $< 1000$  cfu/ml and IMI quarters, respectively.

**Conclusion:** IRT presented limited potential as early detection tool for screen of IMI with minor pathogens. However, it showed an unusual thermal pattern in quarters with high SCC, major pathogens and higher CFU that may deserve additional study. Further studies in herds with higher rate of IMI with major pathogens and clinical mastitis at calving are recommended to better understand udder skin temperature as indicator of udder problems in dairy heifers.

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UH-26

### Application of microRNA biomarkers in Bovine Mastitis Milk

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MicroRNA in liquid samples can serve as biomarkers of many diseases. The primary aim of this study was to identify the miRNA biomarkers of mastitis milk. The first study was designed to decide the suitable reference miRNA in milk for qPCR assay. Based on previous report of next generation sequencing data, we chose miR-92a, miR-375, and let-7g as candidate reference genes. The Normfinder software was identified miR-92a as the most stable reference gene. The candidates were then validated by normalizing the expression levels of miR-146a, which is well known as an inflammation associated miRNA. The significance levels were most remarkable and reproducible when miR-92a used as the reference. Based on the results, miR-92a would be the best reference gene for relative quantification of miRNA expression in bovine mastitis milk. The second study was designed to identify the miRNA biomarkers of bovine mastitis. The expression of inflammation-related miRNA in milk from mastitis-affected and normal cows was analyzed using qPCR. We found that the expression of miR-21, miR-146a, miR-155,



miR-222, and miR-383 was significantly upregulated in California mastitis test positive (CMT+) milk. We further analyzed these miRNA using a chip-based QuantStudio Digital PCR System. The digital PCR results correlated with those of qPCR, demonstrating upregulation of miR-21, miR-146a, miR-155, miR-222, and miR-383 in CMT+ milk. In conclusion, we identified upregulated miRNA in CMT+ milk. These miRNA exhibited sensitivity and specificity greater than 80% for differentiating between CMT+ milk and normal milk. Our findings suggest that inflammation-related miRNA expression in the bovine milk was affected by mastitis, and miRNA in milk have a potential for use as biomarkers of bovine mastitis. The third study used next generation sequencing (NGS) technic to investigate miRNA in bovine mastitis milk. 25 miRNA were differentially expressed including miR-146a and miR-222, the bovine mastitis biomarkers which we identified in the second study. In addition to miRNA, we found that snRNA played a role in bovine mastitis. The genome-wide views of miRNA profiles in the third study provide insights into bovine mastitis and inflammatory diseases.

UH-27

### ***Streptococcus uberis* genotypes involved in intramammary infections: Comparison of small-scale farms to a large scale dairy herd**

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**Objectives:** *Streptococcus (Sc.) uberis* is a major mastitis pathogen related to intramammary infection (IMI). *Sc. uberis* can be found ubiquitously throughout the cow's environment (e.g. straw bedding, manure, skin, feces). Acute clinical mastitis cases, recurrent infections and persistent chronic subclinical IMI have been described. Clinical *Sc. uberis* IMI become more frequent after drying-off and in the early calving period. Consistent with its environmental origin, many authors reported a high level of *Sc. uberis* strain diversity within investigated herds. However, persistent infections with dominant *Sc. uberis* clones could also be observed. Potential cow-to-cow spread is controversially discussed. Thus, the different clinical and epidemiological manifestations of *Sc. uberis* are not finally clarified yet. Genetic fingerprint related prediction could be included in an early warning system to enhance the control and prophylaxis of *Sc. uberis* mastitis.

The aim of this study was to determine the *Sc. uberis* genotype variety found among small-scale dairy herds in comparison to a large herd based on pulsed-field gel electrophoresis (PFGE). The objective was to show that among a heterogeneous group of *Sc. uberis* strains some predominantly host-adapted clones could be recurrently isolated from IMIs and are geographically widespread.

**Materials and methods:** In this study 127 randomly selected small-scale Austrian farms from four Austrian federal areas [Lower Austria (n=67), Upper Austria (n=8), Salzburg (n=51) and Styria (n=1)], and one Italian farm were included in comparison to a large-scale Slovakian dairy herd.

Quarter milk samples were collected on all farms from August 2014 to September 2015. The dairy herd sizes included an average number of 30 dairy cows in Austria and South Tyrol (mainly Simmental breed) and 2500 dairy cows in Slovakia (Holstein Friesian breed). A total of 311 *Sc. uberis* isolates were collected during the study phase originating from 26% and 74% subclinical and clinical cases, respectively. The isolate confirmation included phenotypical and genotypical characterization. In detail, growth characteristics on modified Rambach agar medium, biochemical profiling, Lancefield grouping and 16S rRNA sequencing were performed for the species level confirmation. A *Sma*I based PFGE protocol was developed and applied to identify strain similarities on the genetic level.

**Results:** Preliminary results indicated a high variability of *Sc. uberis* profiles in all investigated farms. The PFGE analysis with *Sma*I of the 311 isolates resulted in 204 different profiles. Identical PFGE profiles were detected in Austria in different quarters of the same cows at the same sampling date (n=7/185 individual cows), co-infection among cows within the same dairy herd (n=9/128) and during a case of recurrent mastitis. Similar PFGE profiles were detected in different farms (n=8/128) at the same geographical location. Five PFGE types of *Sc. uberis* strains were widely distributed amongst farms and could be isolated in quarter milk samples from multiple dairy cows in Lower Austria, Upper Austria and Salzburg.

In the large Slovakian dairy herd several PFGE profiles (n=17) were shared between cows suffering from mastitis, or persisted in one quarter for several weeks even after treatment (n=6/49 cows).

**Conclusions:** *Sc. uberis* IMI is caused by strains with a wide heterogeneity of PFGE types. In the large herd, the molecular epidemiological results indicate that specific strains of *Sc. uberis* might be involved in contagious transmission events and led to persistence in several cases. Further research needs to be undertaken to elucidate the molecular mechanisms that contribute to the capability of these strains to produce a recurrent infection. The global spread of contagious strains should be clarified applying multi-locus sequence typing.

UH-28

### **Efficient herd surveillance for mastitis using the modular *bactotype* Mastitis qPCR**

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**Objectives:** Mastitis is the single most common and costly disease of dairy cattle worldwide. For routine mastitis diagnostics, it is important to rapidly and correctly identify the causative agents. Currently, bacterial culture remains widely used but it is time- and labour-intensive.

For a fast turnaround and reliable detection of mastitis causing bacteria, a combination of optimized milk extraction and an efficient modular qPCR test system was developed.

DNeasy Mastitis Mini is designed for manual DNA extraction from milk based on silica membranes. MagAttract Mastitis Kit is designed for automated extraction based on magnetic beads.



The bactotype Mastitis Screening PCR kit is intended to screen for *S. aureus*, *S. uberis* and *S. dysgalactiae*, *S. agalactiae* and *Mycoplasma bovis*.

The other qPCR kit modules can identify the following contagious and environmental bacteria:

bactotype Mastitis HP3: *S. aureus*, *S. agalactiae* and *M. bovis*

bactotype Mastitis HP2+: *Mycoplasma*, *S. agalactiae* and *M. bovis*

bactotype Mastitis Env: *Klebsiella* and *E. coli* (one channel), *S. dysgalactiae*, *S.uberis* and *T. pyogenes*.

An endogenous internal control will help to monitor DNA extraction from milk.

**Material and methods:** For this study, 970 bovine milk samples sourced from mastitis testing laboratories in Germany and Switzerland were analysed for the presence of *S. aureus*, *S. agalactiae*, and *M. bovis* DNA, using the bactotype Mastitis HP3 PCR Kit. Milk sample quality varied from normal through to sour, coagulated milk.

To test the two new DNA extraction kits for milk, DNA from milk samples was isolated in parallel using the DNeasy Mastitis Mini Kit and MagAttract Mastitis Kit. DNA was isolated from 400 µl milk samples according to the kit instructions. Results from the new DNA extractions were then compared to another commercially available method using the bactotype Mastitis HP3 PCR Kit detecting *S. aureus*, *S. agalactiae* and *M. bovis*.

Additionally, bacterial cultures were serially diluted and results from bacterial culture were compared to those of the new modular mastitis qPCR detection system.

**Results:** 44, 34 and 370 out of 970 samples tested DNA positive for *S. agalactiae*, *M. bovis* and *S. aureus*, respectively. Results correlated well between the new modular mastitis system and another commercially available qPCR test system.

Diagnostic sensitivity of the bactotype Mastitis HP3 PCR Kit was 99.4–100.0%, diagnostic specificity 99.9–100.0% and diagnostic efficiency 99.8–100.0%, based on the respective three pathogens.

Using in vitro DNA, between 10 -100 copies of *S. agalactiae*, *M. bovis* and *S. aureus* could be reliably detected with the bactotype Mastitis HP3 PCR Kits.

Comparison between the two DNA isolation methods showed that the performance of the DNeasy Mastitis Mini Kit correlated well with that of the MagAttract Mastitis Kit.

Comparing all pathogens detected by the new modular system, the modular mastitis detection method was shown to be better or equal to bacterial culture as well as another commercially available method.

**Conclusions:** The DNeasy Mastitis Mini Kit and MagAttract Mastitis Kit allow efficient and rapid isolation of DNA from the mastitis-causing bacteria in cow's milk.

The whole procedure (extraction and qPCR run) takes less than 3 hours. The use of a housekeeping gene as internal control in the bactotype Mastitis Kits appears to be a good indicator of the cell count in the milk sample and provides additional information when testing for mastitis.

The modular bactotype Mastitis PCR allows reliable detection of contagious and environmental mastitis causing bacteria. Due to the high sensitivity of the bactotype Mastitis PCR, individual

milk samples and pools of up to 50 milk samples can be tested. Screening of bulk milk samples from dairy herds, collected for quality or disease control monitoring can be an efficient and economical way of screening for mastitis bacteria of relevance in different countries and regions. To complement the existing bactotype Mastitis PCR portfolio, another assay detecting antimicrobial resistance genes will be available soon.

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UH-29

## The use of 'In Clinic' milk culture to determine prevalence of clinical mastitis in four dairying areas of New Zealand

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**Objectives:** As part of a larger field trial to access the merits of using individual milk culture results to prescribe treatment for clinical mastitis, we first sought to determine the prevalence of clinical mastitis in the herds enrolled.

As mastitis culture was performed by veterinary clinics with 'in house' laboratories we also sought to determine the practicalities of in house culture with reporting of results to farmers within 24-36 hours of case identification.

**Materials and Methods:** Five veterinary clinics representing the major dairying areas of NZ were invited to be involved in the study. Each clinic was provided with a still air incubator, Esculin Sheep blood agar and MacConkey selective media plates (Fort Richard, NZ) as well as catalyse (3% Hydrogen peroxide, Pharmacy Health NZ), Staph Rapid Latex (Mascia Brunelli, Italy), gram stain and Indole (Becton Dickinson, Mexico) test kits.

All clinics were provided with a total of 6 hours of training and supplied decision making trees and single page per organism identification charts to facilitate rapid identification of causative organism. Telephone support was provided by the study coordinator.

15,133 cows from spring calved seasonal herds, under the care of four of the veterinary clinics, were enrolled. Individual cows calved between June 2<sup>nd</sup> and November 17<sup>th</sup> 2017. The study period was the first 120 days post the individual herds planned start of calving.

Cows diagnosed by the farmers as having clinical mastitis (hot, painful, swollen udders or changes in the gland secretion such as clots, watery or blood) were sampled by the farmer immediately post identification. A 2-5ml sample was collected after the teat was cleaned with alcohol soaked wipes and delivered to the veterinary clinic within 2-15 hours of sampling.

Samples were plated onto both Esculin Sheep blood agar and McConkey selective media plates and incubated at 37°C for 12-16 hours. Morphological appearance and tests above were used to identify organisms. Samples were re incubated for a further 24 hours before final identification was made.

**Results:** One clinic was unable to complete the entire study



and was removed from analysis. The other four clinics found the task of culture and identifications relatively simple to learn, required less than 4 hours telephone support and have indicated they will continue to provide the service to clients on a commercial basis.

767 cows were diagnosed with a case of clinical mastitis. The average incidence within herds was 5.1% (range 0.58 to 15.05%). As a proportion of all cases, *Streptococcus uberis* (SU) was most commonly isolated (43.0% of cases), followed by *Staphylococcus aureus* (SA) (15.29%), Coagulase Negative *Staphylococcus* (CNS) (10.53%) and *E. coli* (7.52%). In 65 cases (8.47%) no causative bacteria were isolated.

First lactation (L1) cows (4.33%) were less likely to have a case of clinical mastitis than multiparous (MP) cows (5.27%,  $p=0.03$ ).

The proportion of cases caused by SU and CNS was higher in L1 animals (SU= 53.28%) (CNS= 18.85%) compared to MP cows (SU=41.12%,  $p=0.01$ ) (CNS=9.02%,  $p=0.001$ ). SA however, was no more likely to be the causative agent in L1 cows (16.39%) than MP cows (15.08%,  $p=0.711$ ).

A dichotomised analysis of cases by days in milk (DIM) showed that SU and CNS were more likely be the causative agents in the first 7 DIM (SU= 60.42%) (CNS= 63.417%) than after that period (SU= 39.58%,  $p<0.0001$ ) (CNS= 36.59%,  $p=0.0006$ ).

SA however was more likely be the causative agents after the first 7 DIM (72.46%) than the first 7 DIM (27.54%,  $p<0.0001$ ). This did not differ when comparing L1 (SA>7DIML1=69.57%) and MP animals (>7DIMMP=73.04%,  $p=0.7311$ ).

**Discussion:** This study shows that SU is still the most prevalent cause of clinical mastitis in NZ. CNS is also a significant cause. CNS has been considered a minor pathogen, implying that they result in little cellular damage or immune response, however as cases were included in this study on the basis of herd owners noticing changes in the gland or its secretion, CNS classification as minor pathogen may be questioned.

The above results will be used as part of the larger field trial to assess the cure rates of individual case prescribed treatments rather than the current "best guess" herd prescription based on perceived likelihood of causative organisms.

Ultimately this could result in more use of narrow spectrum antimicrobials medicines in commercial farm situation.

tween June and August of 2017, 650 quarter and composite milk samples submitted to Quality Milk Production Services (QMPS) for routine diagnosis, were selected based on result to ensure a meaningful distribution of important mastitis pathogens for the study. A sterile cotton swab was used to inoculate each milk sample onto a blood agar petri dish, ensuring that the swab had been saturated in the milk sample, and identified the plates with the corresponding sample. Plates were incubated aerobically at 37°C for 24 hours and, then, for identification of microorganisms by means of MALDI-ToF. Randomly, 20 samples were selected per day and re-cultured simultaneously on the test medias and incubated for 18 to 24 hours. The plates were interpreted independently by untrained readers and experienced QMPS technicians. All results were blindly compared with MALDI-ToF results. Interobserver agreement for variables of interest such as diagnosis of presumptive etiologic agent were assessed by ANOVA and kappa ( $\kappa$ ) statistic calculations. Sensitivity and specificity were determined in reference to the gold standard MALDI-ToF approach. Although laboratory based diagnostic tests, such as MALDI-ToF, have higher specificity and sensitivity of identifying microorganisms they still require expensive laboratory equipment and some specialized training and experience to interpret results. Furthermore, submitting milk samples to a diagnostic laboratory may not be feasible for each clinical case due to location or high cost. On farm culture systems are widely used as a diagnostic tool to make pathogen based treatment decisions in mastitis management. An outcome of this study will be the comparison of three commercially available systems in their sensitivity and specificity and whether untrained personnel are capable of interpreting culture results correctly, only relying on the manufacturer's instructions. The results of this study can be used to improve clinical outcomes, farm economics, and animal welfare as culture results would be available to farms that do not have access to a reference laboratory in a timely fashion, cows could be treated and appropriately; perhaps not needing antimicrobials.

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UH-30

## Evaluation of on-farm microbiology techniques for detection of pathogens in milk

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The objective of this study was to assess the accuracy of on-farm clinical mastitis diagnostic assays (AccuMast® Mastitis Culture System [chromogenic media in tripartite Petri dishes] and MN Easy™ Culture System [selective media in Bi-plates and Tri-plates]) by comparing them with the gold standard approach MALDI-ToF mass spectrometry and to compare accuracy of identification of microorganisms by untrained observers versus personnel with advanced microbiology training. Be-



YS-01

### Effect of an injectable trace mineral supplement containing selenium, copper, Zinc, manganese and chromium on mortality, morbidity and growth of dairy calves

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**Objectives:** This study was designed with approval from Massey University Animal Ethics Committee to investigate the effect of up to three sub-cutaneous injections of a multimineral preparation on the health and growth of spring born, dairy calves on four pastoral dairy farms in New Zealand.

**Materials and methods:** Calves were randomly allocated to receive injections within 24 hours of birth and /or at 35 days and / or at 70 days after birth. Each injection contained 40 mg zinc, 10mg manganese, 5mg selenium, 15mg copper and 5mg chromium per mL (Multimin®, Virbac New Zealand Ltd) administered at 1mL/50 kg body weight. Morbidity, mortality and growth rates were recorded on farm and calves were weighed and data collected every 35 days for the first 140 days of life.

**Results:** There were no differences in perinatal morbidity and mortality within 48 hours of birth for treated calves (1.8% (95%CI=0.60-3.10)) compared to control calves (3.2 (95%CI=1.69-4.66)),  $p=0.192$ .

Morbidity and mortality were highest in the period 3-35 days after calving with 7.5% (95%CI=5.00-9.99) of treated calves diagnosed as sick compared to 15.6% (95%CI=12.48-18.73) of control calves,  $p<0.001$ . Mortality was also lower with 4.4% (2.49-6.41) of treated calves dying compared to 10.4% (95%CI=7.78-13.03) control calves,  $p<0.001$ .

Between days 3 and 35, the relative risk of treated calves scouring was 0.46 (95%CI=0.29-0.75,  $p=0.001$ ) that of control calves and the relative risk of omphalitis was 0.51 (0.26-1.03,  $p=0.055$ ). The overall relative risk of mortality for treated compared to control calves over the same period was 0.43 (95%CI=0.26-0.71,  $p<0.001$ ).

To calculate the adjusted odds of the effect of treatment status on morbidity and mortality 3 and 35 days after birth, general estimating equations were used with an exchangeable correlation structure and robust standard errors to account for clustering within herds. Allowing for age, weight, dam parity and presence of active BVD virus in the calf cohort, the adjusted OR for treated calves of scouring between 3-35 days was 0.47 (95%CI=0.27-0.81,  $p=0.007$ ) for omphalitis 0.51 (95%CI=0.42-0.69,  $p<0.001$ ) and for overall mortality 0.40 (95%CI=0.25-0.66,  $p<0.001$ ). There was a significant but biologically small increase in the daily growth rate of treated calves (17g/day (95%CI=7.2-26.8,  $p=0.003$ ) between 3-35 days.

To calculate the adjusted odds of the effect of treatment status on morbidity, mortality and daily gain from birth to 140 days, general estimating equations were used with an autoregressive correlation structure and robust standard errors to account for repeat measures on the same calf with farm included as a fixed effect. Allowing for dam parity, farm and age, over the first 140 days of life, the model predicted that the probability of morbidity and mortality was approximately halved in the 35 days following injection for treated calves compared to control calves

( $p<0.001$ ), although there was no significant effect beyond 35 days ( $p=0.469$ ). Over the first 150 days there was no overall difference in the daily rate of gain between treated and control calves.

**Conclusions:** These results indicate that on these farms, injection with a trace mineral supplement was associated with a reduction in the morbidity and mortality for calves for approximately 35 days and that this effect was observed throughout the first 140 days of life. Given that the morbidity and mortality rates were highest in the first 35 days after birth, injection with a trace element supplement at birth was a simple and cost-effective way of reducing these losses.

YS-02

### Effects of active dried *Saccharomyces cerevisiae* on ruminal fermentation and the bacterial community during subacute ruminal acidosis in post-weaned Holstein bull calves

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**Objectives:** Active dried *Saccharomyces cerevisiae* supplementation improves ruminal fermentation and milk production in dairy cattle, as well as dry matter intake and growth performance in neonatal calves. Subacute ruminal acidosis (SARA) is a digestive disorder caused by feeding rapidly fermentable carbohydrates, resulting in depressed intake, poor body condition, and reduced production. However, the impact of yeast supplementation on SARA and the bacterial community has not been studied extensively in post-weaned Holstein calves. Therefore, we investigated the effects of *S. cerevisiae* supplementation on ruminal pH, fermentation, and the microbial community of ruminal liquid and solid fractions.

**Materials and Methods:** Five fistulated Holstein bull calves (132.3 ± 10 kg; 3.1 ± 0.3 months of age) were used in a replicated 2 × 2 Latin square design, and 0 (control group,  $n = 5$ ) or 2 g (SC group,  $n = 5$ ) of Levucell® SC containing  $1 \times 10^{10}$  CFU/g active dried *S. cerevisiae* strain CNCM I-1077 (Lallemand, Toulouse, France) was administered twice daily for consecutive 21 days. Calves were fed a high-forage diet during the first 14 days (days 1–14; pre-challenge), a high-grain diet for 2 days (days 15–16; SARA-challenge), and a high-forage diet for 5 days (days 17–21; post-challenge). Ruminal pH was measured continuously every 10 min throughout the experimental period. Ruminal fluid and content samples were collected once daily (08:00) on days 14, 17, 18, and 21 or twice daily (08:00 and 11:00) on days 15 and 16 to analyze the bacterial community, total volatile fatty acids (VFAs), VFA components, NH<sub>3</sub>-N levels, and lactic acid concentrations. Total bacterial DNA was extracted from ruminal liquid and solid fractions collected on days 14 and 17 for 454 pyrosequencing analysis.

**Results:** The 24 h and 1 h ruminal pH significantly decreased during the SARA challenge period in each group, although the changes were more severe in the SC group. Total VFA and lactic acid concentrations, and the proportions of propionic acid and butyric acid, significantly increased whereas the proportion of



acetic acid and the ruminal acetic acid-to-propionic acid ratio significantly decreased during the challenge period in both groups. Analyses of the liquid-associated bacterial community indicated that the relative abundance of the genus *Bifidobacterium* (phylum: Actinobacteria) significantly increased in the SC group (0.1 to 42.0%) during the challenge. Analyses of the solid-associated bacterial community indicated that the relative abundance of the genus *Prevotella* (phylum: Bacteroidetes) was significantly higher in the SC group than in the control group during the pre- and post-challenge periods. In addition, principle coordinates analysis plots showed a separation between the pre-challenge and post-challenge periods in both groups, whereas the plots of solid fraction in the control group were separate from others.

**Conclusions:** Among the predominant genera, *Bifidobacterium* species have health-promoting effects on the host, and *Prevotella* bacteria can dominate and thrive under a range of diets due to their capacity to use a variety of substrates. Therefore, the greater relative abundances of *Bifidobacterium* (liquid fraction) and *Prevotella* (solid fraction) in the SC group suggest that administration of active dried *S. cerevisiae* could have an important role in the establishment of ruminal fermentation ability or bacterial community in post-weaned Holstein bull calves.

YS-03

### Influence of metaphylactic use of antimicrobials on early gut bacterial colonization in Holstein calves during the first month of life

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In an attempt to prevent diarrhea and Bovine Respiratory Diseases in young calves, some farms use metaphylactic antibiotic treatment near birth. However, introduction of antibiotics during early development may negatively impact the development of intestinal microbiota. This study evaluated the effect of immediate use tulatromycin on gut bacterial colonization of Holstein calves in the first month of life. Holstein heifer calves (n=26) were divided into two groups based on whether prophylactic antibiotic was used (ATB+) or not (ATB-, tulatromycin at 2.5 mg per kg). It was delivered subcutaneously within the first 12 hours after birth. Fecal samples were collected in sterile vials within 12 hours after birth (less than or equal to 12h), and in the windows of D3-5, D7-9, D13-15, D20-23 and D27-30 after birth. Following collection, portions of the fecal samples were transferred to four separate tubes for storage at -80°C. Total DNA was obtained from the feces using the QIAamp DNA Stool Mini Kit (Qiagen) per the manufacturer's instructions. Quantitative real-time PCR assays were conducted using SYBR Green PCR Master Mix (GoTaq qPCR Master Mix, Promega Corporation) and standard commercial probes to detect *Bifidobacterium* spp., *Lactobacillus* spp., *Clostridium perfringens*, *Escherichia*

*coli* and the presence of all bacteria (based on 16S rRNA backbone probes). There was no difference observed between ATB+ and ATB- calves in the cycle number for total for bacteria (P=0.260), or the cycle number for *Clostridium perfringens* (P=0.221) during the study (Student's T test). ATB- calves had a higher copy number for *Bifidobacterium* spp. than ATB+ calves on D3-5 (ATB-= 1.61E+03; ATB+= 3.53E+00 copies/gram of feces, P=0.002) and D7-9 (ATB-= 1.12E+03; ATB+= 1.84E+02 copies/gram, P=0.018). In addition, the copy number for *Lactobacillus* spp. tended to be higher for the ATB- calves (4.23E+03 copies per gram) than ATB+ calves (6.48E+02 copies per gram) on D3-5 (P=0.097). In contrast, the copy number for *Escherichia coli* tended to be higher for the ATB+ calves than ATB- calves on D20-23 (ATB-=3.78E+01; ATB+= 4.95E+02 copies/gram, P=0.052), and D27-30 (ATB-= 3.78E+01; ATB+= 1.11E+03 copies per gram, P=0.072). The time series analysis by one-way ANOVA with post hoc Tukey assessment indicated a lower copy number for total bacteria (backbone 16S rRNA), *Lactobacillus* spp. and *Bifidobacterium* spp at the first assessment after birth (12H) in both groups of calves than at later points. All bacterial genus assessed had increased copy number over the course of the study (P=0.000). The peak for *Bifidobacterium* spp. was observed on D3-5 in ATB- calves (1.61E+03 copies per gram) and D7-9 in ATB+ calves (1.84E+02 copies/gram). The peak *Lactobacillus* spp. copy number was detected on D7-9 (6.25E+03 copies per gram) in ATB- calves and D20-23 (4.37E+03 copies per gram) in ATB+ calves. We observed peak copy number of *Escherichia coli* (ATB-=4.70E+03; ATB+=4.80E+03 copies per gram) and *Clostridium perfringens* (ATB-= 7.76E+02; ATB+=6.12E+02 copies/gram) in both group of calves on D3-5. The minimum copy number observed for *Escherichia coli* was detected on D27-30 in ATB- calves (3.44E+01 copies per gram) and D13-15 in ATB+ calves (5,83E+01 copies per gram). For *Clostridium perfringens*, the minimum copy number was observed on D20-23 for both groups of calves (ATB-=9.19E+00; ATB+=5.35E+00 copies per gram). The administration of tulatromycin at a metaphylactic dose of 2.5 mg per kg immediately after birth influenced the diversity of gut bacterial colonization. In this study, we observed a decrease in commensal bacteria (*Lactobacillus* and *Bifidobacterium*) and an increase in potential pathogens, specifically *Escherichia coli* in the ATB+ calves. From this data, we concluded that there may be long-term health effects associated with early metaphylactic use of tulatromycin in the microbiome mediated development of the immune system and physiological function

YS-04

### Distribution of *E. coli* K99, Rotavirus, Coronavirus and *Cryptosporidium* spp., in calves with diarrhoea from 17 regions of Mexico.

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**Objective:** The goal was to understand the distribution of *E. coli* K99, Rotavirus, Coronavirus and *Cryptosporidium* spp., in





a two-year transversal study in female dairy calves with diarrhoea from 17 regions of Mexico.

**Materials and Methods:** The study took place from 1/17/2016 to 11/30/2017 in 17 dairy producing regions in Mexico: Aguascalientes, Baja California Norte and Sur, Chihuahua, Durango, Estado de México, Guanajuato, Hidalgo, Jalisco, Puebla, Querétaro, San Luis Potosí, Sinaloa, Sonora, Tlaxcala, Torreón and Veracruz.

The sampled population included female calves from 1 to 135 days old with diarrhoea. A diarrhoea score was used, based on the Wisconsin calf disease App, to classify 3 types of diarrhoea: Semi-formed (D1), Pasty and runny (D2) and Watery (D3). An on-farm diagnostic test was used to identify the 4 pathogens of interest in calves with diarrhoea (Rainbow Test Bio-K156, Bio-X Diagnostics, Belgium).

A data base was made including result of the on-farm diagnostic, diarrhoea scores, ages of calves with diarrhoea, and region. Statistical analysis was made with JMP 13.1 and Minitab 18 in order to understand the distribution of the time of sampling, diarrhoea cases, pathogen distribution and correlations.

**Results:** 651 female calves were diagnosed with diarrhoea mainly during the neonatal period (93.5%). Diarrhoea cases by severity were: 133 calves with D1 (20%); 515 cases were D2 (79%); and 3 cases D3 (1%). Most of the cases were during the second week of life (360 cases), followed by the first week of life (161 cases), the third week (88 cases) and from 4 to 18 weeks of age (43 cases). The most prevalent pathogen was *Cryptosporidium* spp. with 410 cases, highly statistically different from other pathogens ( $p < 0.001$ ); then, 110 cases of Rotavirus, 87 of *E. coli* K99 and 29 of Coronavirus.

Most diarrheic cases were in Querétaro (79), Guanajuato (75), Jalisco (66), Estado de México (61), Aguascalientes (61) and Durango (60). Other regions had less than 50 cases. For 19.7% of the diarrhoea cases none of the 4 pathogens were diagnosed.

Age in days at onset was for: *E. coli* K99  $10.5 \pm 4.4$ ; Rotavirus  $12.7 \pm 13.4$ ; Coronavirus  $17.5 \pm 14.2$ ; and *Cryptosporidium* spp.  $10.9 \pm 7.7$ . No statistical correlation was found for *E. coli* K99 and Rotavirus by age. In the least square analysis for age, Coronavirus was statistically correlated ( $p < 0.05$ ), while *Cryptosporidium* spp. was highly correlated ( $p < 0.001$ ).

Overall prevalence of *E. coli* K 99 was 12.0%, being most prevalent in Veracruz (67%). A HSU's Multiple Comparisons with the Best (MCB) test showed that Veracruz was highly statistical different ( $p < 0.001$ ) from the other regions. Estado de México, Guanajuato and Querétaro were statistically significant different from the other regions ( $p < 0.05$ ).

Rotavirus prevalence was 17.1% overall, with the highest prevalence in Veracruz (67%) and Durango (35%), where the later was highly statistical different ( $p < 0.001$ ). Also, Veracruz and Estado de México were different from the other regions ( $p < 0.05$ ) by HSU's MCB.

Coronavirus was 5.5% prevalent overall, with the highest prevalence in Veracruz (33%) and Sonora (14%); when tested with HSU's MCB. Veracruz and Aguascalientes were statistically different ( $p < 0.05$ ).

*Cryptosporidium* spp. was the most prevalent pathogen with 410 cases (68.1%). Prevalence for Veracruz was 100%, San Luis Potosí, Baja California Norte and Sur were 90% and the prevalence in those regions was statistically significant different

from the others ( $p < 0.05$ ). When analysed with a multiple regression test, *Cryptosporidium* spp., was highly related to the diarrhoea score ( $p = 0.0087$ ).

**Conclusions:** Analyses made in 17 regions in Mexico on 651 female diarrheic dairy calves indicated that the most prevalent pathogen was *Cryptosporidium* spp. The prevalences of the other pathogens were by order of importance: Rotavirus, *E. coli* K99 and Coronavirus. Veracruz showed the highest number of cases for any pathogen related to diarrhoea. Although a relative small number of cases were sampled in this study, the results of *Cryptosporidium* spp. prevalence were similar to other studies in the country. In 19.7% of the cases, diarrhoea could not be related to the pathogens tested on-farm, either the timing for testing was not adequate, diarrhoea was non-infectious or another pathogen was involved. To our knowledge, this is the first study in Mexico indicating the prevalence of *E. coli* K99, Rotavirus and Coronavirus.

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YS-05

## Diagnosis of bacterial and viral infections in calves with Bovine Respiratory Disease

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**Introduction and Objectives:** The Danish Green Development and Demonstration Project - Diagnostic tests for veterinary practice (VetDiagnostics) aims to promote prudent use of antibiotics in cattle and pig production. As part of this, there is a need to develop improved diagnostic methods that can guide the veterinarian in the choice of prevention, treatment, and choice of drug.

In Danish slaughter-calf farms, calves are transported and mixed together from many dairy farms when calves are 2-4 weeks old. The most important herd health problem is Bovine Respiratory Disease (BRD) complex, and most of the antibiotic and vaccines used in the farms are used for controlling BRD

The current project aimed to demonstrate the benefit of using a quantitative qPCR method, the Pneumo 4 qPCR test, in diagnosis of Bovine Respiratory Disease (BRD) in calves.

**Material and methods:** The diagnostic material was tracheal lavage on slightly xylazine sedated calves. In order to avoid contamination from the upper respiratory tract, we used the endoscope iVet scope 2.0 in the oral cavity. When the epiglottis was presented and open, a catheter was passed through the endoscope and into the trachea and passed slightly down to the bronchia. 50 ml of an isotonic saline was flushed in the lungs and approximately 30 ml. tracheal fluid could after be aspirated.

The tracheal fluid was then tested by the Pheumo 4 qPCR bacteria test (detectes *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*) and Pheumo 4 qPCR Virus test (*Bovine coronavirus*, *Bovine parainfluenza*, *BRSV*). In parallel, bacteriological examination of samples were performed with culture on Blood agar plates. Bacterial isolates



were enumerated and confirmed by MalDI-ToFF technique. Antimicrobial resistance testing was performed using micro-broth dilution methods according to CLSI guidelines.

Three diagnostic studies were made:

92 tracheal fluids from 11 farms, In every farm, half of the samples were taken from sick calves and the other half from calf without symptoms of BRD. 65 tracheal fluids from 10 farms. In every farm half of the samples was taken in sick calves and the other half was from calves without BRD symptoms. 65 tracheal fluids from 8 farms in sick calves.

**Results:** All three studies showed that the traditional agents *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*, Bovine coronavirus and in some farms occasionally Bovine parainfluenza and BRSV (Denmark has eradicated BVD and IBR) can be detected from cases of BRD in slaughter calves in Denmark. The agents were found in both sick and healthy calves, but the mean CT value in *Manheimia haemolytica*, *Histophilus somni* and *Mycoplasma bovis* was lower in samples from sick calves indicating a higher infection.

Culture technique demonstrated a high number of other bacteria in tracheal samples from sick calves. Of note, the isolation of *Truperella pyogenes* were common in sick calves.

Antibiotic resistance testing shows that very few of the *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* isolates were resistant against the most common used antibiotic in Denmark.

**Conclusions:** Tracheal lavage in calves and laboratory use of the Pheumo 4 qPCR bacteria and virus test in private veterinary practice is a good diagnostic tool to get a quick and cost-effective lab result for veterinary herd health management. The results can be used to make decisions in optimal antibiotic treatments and use of vaccine.

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YS-06

### Randomised control trial of NSAID and antimicrobial treatments for pyrexia caused by bovine respiratory disease

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**Objectives:** Bovine respiratory Disease (BRD) is the one of the most important growth-limiting diseases of pre-weaning calves, with both short-term economic effects due to treatment costs as well as long-term effects on animal productivity. A randomised control trial was used to assess the efficacy of four different treatment methods for bovine respiratory disease (BRD), comparing antimicrobials with non-steroidal anti-inflammatory treatments. The BRD was detected via the occurrence of pyrexia (greater than or equal to 39.7°C that was sustained for a period of 6 hours) using external ear canal temperature probes (TempVerified FeverTags).

**Materials and methods:** Calves were randomly allocated to four treatment groups on detection of pyrexia caused by BRD. Upon pyrexia detection, the calves underwent a modified Wisconsin-Madison calf health scoring system to ensure that

BRD was the initiating cause of the pyrexia, and to record the presence of any clinical signs. Group 1 received 2mg/kg flunixin meglumine for three consecutive days. Group 2 received 6mg/kg gamithromycin. Group 3 received both flunixin meglumine for three days and gamithromycin. Group 4 were monitored for a further 48 hours, and if pyrexia was still present they were treated as per Group 3.

A convenience sample of five calves had deep nasopharyngeal swabs taken at the onset of pyrexia, and sent for PCR for Infectious Bovine Rhinotracheitis (IBR), Parainfluenza 3 (PI3), Respiratory Syncytial Virus (RSV) and *Mycoplasma bovis*. A convenience sample of nine calves had blood samples taken and tested for paired serology of antibody titres against IBR, PI3, RSV, bovine viral diarrhoea (BVD), coronavirus, *M.bovis* and *H.somni*.

**Results:** Three hundred and thirty-two pre-weaning calves from one dairy farm, were followed over a one year period. BRD prevalence was 57.7%, with mean age of onset of 29 days old. Mean growth rate was 0.76 kg/day, with no difference between the treatment groups. Bovine coronavirus was the only positively identified pathogen identified by a rising titre in the paired serology tests. However, the presence of bacteria pathogens can't be excluded, and the high success rate of the antimicrobial treatment would suggest their presence.

Detecting pyrexia through the use of FeverTags to identify BRD was a successful method as 76% of the Group 4 (wait 48hrs) calves maintaining their elevated temperature. Initial treatment with an antimicrobial (Group 2) had higher treatment efficacy ( $p < 0.01$ ) than using an NSAID or monitoring the calf for 48 hours, with no additional reduction in repeat BRD cases when an NSAID was used concurrently. NSAID treatment alone did allow 31.7% of calves to resolve their pyrexia, but they went on have significantly higher odds of requiring repeated treatments for BRD (OR 2.76,  $p = 0.022$ ), suggesting that any short-term reduction in antimicrobial usage may be followed by long-term increases.

**Conclusions:** Initiation of early BRD treatment is an important component of tackling the effects of lung infection, both for reduction in lung pathology and stopping the progression of clinical signs. This study supports the use of pyrexia as an indicator for the presence of BRD, with the FeverTags demonstrating their practical application and ability to detect both initial occurrence of pyrexia as well as providing continued monitoring. The study supports initial antimicrobial usage with or without NSAIDs as the most efficacious approach to BRD treatment on farms with minimal primary viral pathogens.

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YS-07

### Assessing the impacts of administration of a non-steroidal anti-inflammatory drug to beef calves after assisted calving on calf health and production

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**Objective:** Calf health and survival is crucial to successful cow-calf operations. Assisted calves are often born weak, injured, or oxygen deprived. A compromised calf may not ingest enough good quality colostrum, which may lead to failed transfer of passive immunity. Calves experiencing a difficult birth have a higher risk of morbidity, mortality, and poor performance during the pre-weaning period. Currently, there is no research assessing the effects of implementing a pain mitigation strategy for newborn beef calves after an assisted calving. Therefore, the objective of this study was to investigate the impact of the administration of a non-steroidal anti-inflammatory drug (NSAID) at birth to assisted beef calves.

**Materials and Methods:** Thirty-three primiparous dams requiring assistance at birth on 2 farms located in southern Alberta were enrolled. The calving difficulty of all assisted calves was classified as either an easy assist (one person pulling to deliver the calf) or difficult assist (two or more people pulling, or mechanical assistance). Within 10 minutes of birth, calves were randomized to a treatment group using a computer assisted randomization chart stratified by calving difficulty. Calves received a subcutaneous dose of meloxicam (Metacam®, 20 mg/ml, 0.5 mg/kg, Boehringer Ingelheim, Ingelheim, Germany) or an equivalent volume of placebo (sterile saline with 2% oxytetracycline to match the color of meloxicam). Cow-calf pairs were then placed in box stalls for observation.

Latency to stand and nurse were recorded. Blood samples were taken at 24 hours of age by jugular venipuncture and serum immunoglobulin (IgG) concentration was measured by radial immunodiffusion assay (RID) in the Quality Assurance Laboratory of the Saskatoon Colostrum Company Ltd. (Saskatoon, SK). Optimal passive immunity (OPI) was defined as serum IgG concentration greater than 24 g/L. Pre-weaning treatment and mortality information was collected. Calves were weighed at 7-10 days of age and at weaning to calculate average daily gain (ADG) in the first week and to weaning, respectively.

Data were analysed using STATA® 14.1 software (StataCorp LP, College Station, TX). Descriptive statistics and tests for normality were performed on all continuous variables. Multivariable linear or exact logistic regression models were performed to investigate the impact of treatment group on behavior (standing by 1 hour, suckling by 1 hour), passive immunity (serum IgG, OPI), health (preweaning treatment, mortality) and performance (ADG to 1 week, ADG to weaning) of assisted calves. Herd and calving difficulty were offered as covariates to all models, and serum IgG was offered to the health and performance models.

**Results:** Of the 33 calves enrolled, 11 were classified as easy assists and 22 calves were classified as difficult assists. Based on random assignment, 17 calves received meloxicam and 16 calves received a placebo. In calves treated at birth with meloxicam, ADG to 1 week was significantly higher ( $P < 0.05$ ) (mean = 0.9 kg; SD = 0.4) compared to placebo treated calves (mean = 0.6 kg; SD = 0.5). From birth to weaning, ADG was significantly higher ( $P < 0.05$ ) in assisted calves treated with meloxicam (median=2.5 kg; IQR: 2.2-2.7) compared to placebo treated calves (median=2.3 kg; IQR: 2.1-2.6). There were no significant effects of treatment with meloxicam at birth on standing or suckling by 1 hour, serum IgG concentration, OPI, or pre-weaning treatment and mortality ( $P > 0.1$ ).

**Conclusion:** Although this was a small study population,

meloxicam administration to assisted calves at birth influenced growth performance to 1 week and to weaning. This may indicate an important production management tool for improving calf health, production, and wellbeing in assisted calves by mitigating pain and inflammation. Future studies will investigate the impact of meloxicam on calf health in a larger size study population.

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YS-08

### Relationship between beef calf and dam serum L-lactate concentration at varying degrees of calving difficulty

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L-lactate is produced by the body when active muscles are deprived of oxygen. When calves experience a difficult birth, the oxygen supply from the umbilicus may be cut off in utero before they are able to breathe air. However, blood L-lactate in beef calves assisted at calving has previously been observed during our research as higher than expected from simple oxygen deprivation, suggesting another source of L-lactate. The objectives of this study were to compare blood L-lactate concentrations between newborn beef calves and their dams, and to investigate the impacts of calving difficulty on this relationship.

L-lactate concentration was measured using the STAT Profile pHox Ultra in serum samples from 44 cow-calf pairs collected within 10 minutes of birth. Cow-calf pairs were categorized based on the degree of calving difficulty: unassisted (calved on their own within 2 h of the observed onset of Stage 2 labour), easy assist (manually delivered by 1 or 2 people), or difficult assist (mechanically delivered using a fetal extractor, ie. calf jack). Median L-lactate concentration was compared between cows and calves overall and within category of calving difficulty using Wilcoxon rank-sum tests because the data was non-parametric. Median L-lactate was also compared among categories of calving difficulty for cows and calves separately using the Kruskal-Wallis rank test. The correlation between the calf and dam L-lactate concentrations was determined overall and within calving difficulty categories by calculating a Spearman Correlation Coefficient.

Median calf L-lactate concentration was 11.4 mmol/L (interquartile range: 8.4 - 14.2 mmol/L), which was significantly higher than the median cow L-lactate concentration of 5.0 mmol/L (3.5 - 6.6 mmol/L). Calf L-lactate levels were also significantly higher than cow L-lactate levels within each calving difficulty category. Median L-lactate concentration did not differ by calving difficulty among the calves, but was significantly greater for cows having a difficult calving (6.0 mmol/L; 4.9 - 9.2 mmol/L) compared to unassisted cows (3.6 mmol/L; 3.3 - 4.4 mmol/L) or easy assist cows (5.0 mmol/L; 5.6 - 11.5 mmol/L). Overall, there was a moderate, significant correlation of 0.39 between the L-lactate levels of calves and cows. However, when cate-



gorized by calving difficulty, there was no significant correlation between the levels of L-lactate among assisted cow-calf pairs, but a high, significant correlation of 0.70 between unassisted calves and cows.

Calves had higher concentrations of serum L-lactate than cows; however, only cow L-lactate levels were significantly impacted by calving difficulty. Calf serum L-lactate concentrations were not consistently related to the L-lactate concentrations of their dam, with a relationship being demonstrated among unassisted pairs but not among those assisted at calving. It is likely the variability of dystotic calvings eliminates the relationship between calf and cow L-lactate levels observed in unassisted calvings. As such, the previously observed high L-lactate levels in assisted calves is likely self-generated.

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YS-09

### Eligibility of a wireless pulse oximeter for monitoring of vital parameters in newborn calves

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**Objectives:** In human as well as in many areas of veterinary medicine, pulse oximetry is a well-established technique for monitoring of patients. In the farm animal sector, it could also be a useful tool for the detection of critical conditions with regards to the oxygen supply and the cardiovascular system. Among others, an innovative application could be the monitoring of the newborn during birth. This might help in identifying critical situations in a timely manner and support farmers and veterinarians in their decision to start obstetric or life supporting interventions. So far, however, its use in ruminant medicine is still limited to experimental applications.

The objective of this study was to evaluate the accuracy of the Radius-7 Wearable Pulse CO-Oximeter (Masimo Corporation, Irvine, USA) for monitoring vital parameters of newborn calves.

**Materials and Methods:** The sensor of the pulse oximeter was fixed in the interdigital space of a calves' front leg with a self-made hoof-shaped latex-cover. The pulsoximetric measured arterial oxygen saturation (SpO<sub>2</sub>) of 40 newborn calves was compared with the corresponding results (SaO<sub>2</sub>) from a portable blood gas analyzer (VetScan iStat1, Abaxis Inc., Union City, USA) which served as reference in this study. For this, an arterial blood sample was taken from the medial intermediate branch of the caudal auricular artery. Furthermore, the pulse rate was measured on 10 calves between 0 to 7 days of age with the pulse oximeter and simultaneously with a heart rate belt (Polar Equine Belt, Polar Electro Oy, Kempele, Finland) to determine their level of agreement.

**Results:** Spearman correlation coefficients for oxygen saturation was 93.8% for the pulse oximeter and the blood gas analyzer, and 97.7% for the pulse rate measured by the pulse oximeter and the heart rate belt. The pulse oximeter overestimated SaO<sub>2</sub> by 2.95 ± 6.39% and underestimated the pulse rate by 0.41 ± 3.18 beats per minute compared with the corresponding reference method.

**Conclusions:** In summary, the pulse oximeter is considered as a suitable tool for a continuous monitoring of the arterial oxygen saturation and the pulse in newborn Holstein Friesian calves.

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YS-10

### Maternally derived colostral immunoglobulins can replace maternal colostrum to achieve successful passive transfer of immunity in neonatal calves

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**Objectives:** Feeding the neonatal calf adequate immunoglobulins (IgG) via colostrum is one of the most important management decisions impacting the animal's life. Maternal colostrum is not always of acceptable quality and therefore alternatives are needed. The objectives of this study were to assess data from ten studies in which a colostrum replacer (CR) derived from maternal bovine colostrum was fed to 1) determine if the use of CR results in successful passive transfer and apparent efficiency of absorption (AEA) of IgG by the neonatal calf (> 10 mg/mL IgG in the serum), and 2) determine which factors impact the effectiveness of the CR used.

**Materials and Methods:** Data from ten studies (389 calves) were compiled where neonatal calves were fed CR (Zinpro Performance Colostrum, Eden Prairie, MN) within 1.5 hours of birth. Prior to first feeding, a blood sample was taken from the jugular vein of calves and a second blood sample was taken at 24 hours post-feeding to measure serum IgG levels and calculate AEA. Additional information (covariates) were collected: birth weight, total CR fed, total IgG fed, IgG content of product, dosage [defined as grams of IgG fed per unit of birth weight], location of study, gender, breed, and number of feedings of product.

Univariate and multivariable linear mixed-effects regression models were used to determine covariates' effect on serum IgG levels at 24 hours post-feeding and AEA. Serum IgG at 24 hours post-feeding was compared to an industry benchmark of 10 mg IgG/mL of IgG in serum to determine if the CR results in successful passive transfer. Total CR fed (g), total IgG fed (g), and IgG content (%) were analyzed as numeric and factor variables. All independent variables were centered around their means and Study ID served as a random effect. An error level of  $\alpha = 0.05$  was used to determine statistical significance. The random effect of Study ID was eliminated from the multivariable model if it was not significant using a log-likelihood ratio test. Backwards stepwise elimination was performed on the covariates until all remaining independent variables were significantly associated with the outcome. The Tukey's method was used to adjust the p-values for multiple comparisons when post-hoc contrasts were tested for significance. The statistical analysis was performed using R (version 3.4.2) and the lme4, effects, and lsmeans packages.

**Results:** Overall estimate of mean serum IgG levels at 24 hours post-feeding (17.59 mg/mL; SE 0.310) was found to be significantly greater than 10 mg/mL ( $P < 0.001$ ). Univariate



analyses found total CR fed, total IgG fed, IgG content, location of study, and number of feedings of colostrum replacer to not be significantly associated with serum IgG level. Univariate analyses found gender and birth weight to be significantly associated with serum IgG level, but those two variables were no longer significant once a multivariable regression model corrected for dosage. In the final multivariable linear regression model correcting for breed, the dosage coefficient was 4.075 (SE: 0.493,  $P < 0.001$ ). For every increase in dosage by 1 (i.e., an increase in 1 gram of IgG fed per unit of birth weight), serum IgG level increased by 4.075 mg/ml.

Univariate analyses found total CR fed, total IgG fed, IgG content of product, breed, gender, and number of feedings to not be significantly associated with AEA. Univariate analysis found birth weight to be significantly associated with AEA but was no longer significantly associated once a multivariable regression model corrected for dosage. In the final multivariable linear regression model correcting for location, the dosage coefficient was -5.455 (SE: 1.791,  $P = 0.002$ ). For every increase in dosage by 1, AEA decreased by 5.455 %.

**Conclusions:** The CR used in this study resulted successful passive transfer of immunity ( $> 10$  mg/mL IgG in the serum) in neonatal calves at 24 hours post-feeding. Both serum IgG levels and AEA of IgG were significantly associated with dosage rate of IgG (g IgG fed per unit birth weight). The CR used in this analysis can be used by producers with confidence in the place of maternal colostrum to achieve successful passive transfer by the neonatal calf.

ratories, Inc., USA). Data is presented as means, standard deviation (SD) and ranges. Pearson correlation was used to compare variables.

**Results:** Mean serum IgG was 10.72 mg/ml (1.8 - 24.2; SD 5.5) and saliva IgG 0.04 mg/ml (0.004 - 0.24; SD 0.03) with Elisa and 8.45% (6.1 - 10.1; SD 0.7) and 1.30% (0.7 - 2.4; SD 0.3) with Brix, respectively. Correlation between Elisa IgG saliva and serum was 0.51 ( $p < 0.01$ ), and 0.12 between Brix saliva and serum, respectively. Correlation between Brix saliva and Elisa IgG serum was 0.09 and between Brix serum and Elisa IgG serum 0.53 ( $p < 0.01$ ).

**Conclusions:** IgG serum concentrations with the studied 10 - 20 days old calves were at relatively low level. No statistically significant correlations were detected between saliva and serum IgG. We concluded that Saliva Brix measurement did not reflect the immune status of the calf.

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YS-11

### Comparison of serum IgG and saliva IgG content with Elisa test and Brix refractometer with young calves

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**Objectives:** Sufficient serum immunoglobulin G (IgG) concentration is important to young calf for maintaining good health. Determination is usually done from serum. However, recent preliminary results from newborn dairy calves indicated that also saliva could be used for determining maternal antibody level. Our aim was to compare saliva and serum IgG content of young calves in a commercial calf-rearing unit.

**Materials and Methods:** A total of 140 young bull calves, mean age of 16.5 days (10 - 30 days) were transported to calf-rearing unit. Calves were of Holstein ( $n = 57$ ), Ayrshire ( $n = 51$ ) and mix breeds (dairy and beef breed,  $n = 32$ ). We took jugular blood samples from calves one day after arrival and saliva samples with foam. Saliva samples were stored in a 4 °C and analyzed with digital Brix refractometer (ATAGO<sup>®</sup>) within 24 hours after sampling. Whole blood samples were centrifuged, and serum and saliva samples then frozen to -20 °C. Serum and saliva samples were analyzed 6 months after with Elisa test for IgG (Bovine IgG ELISA Quantitation Set, Bethyl Labo-



MT-01

## Hypoplasia of the omasal laminae in cattle: description of two cases

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ENVA

**Objectives:** The objective of this paper is to raise awareness among veterinarians about hypoplasia of the omasal laminae in cattle, a rarely observed miscellaneous condition, through the description of two bovine clinical cases which have been hospitalized at our facility.

### Materials and methods:

#### Case 1

A thirty-month-old Red Holstein primiparous cow was referred in November 2016 for weight loss associated with dysorexia and abdominal distension.

The clinical examination at arrival revealed a poor body condition, a moderate tachycardia associated with an extremely loud heart murmur, a moderate left-upper-sided and a severe right-sided abdominal distension, concomitant with a large area of tympanic resonance in the right cranial abdomen. Rectal examination exposed mucus-covered feces and was consistent with a free gas bloat associated with a right displaced abomasum.

An exploratory right-sided laparotomy was immediately performed. Abomasal volvulus was promptly dismissed, but the abomasum was indeed severely distended by gas and weighted by a coarse content to the point where it could not be exteriorized and drained.

Since no surgical correction could be achieved and considering the critical state of the patient, it was euthanized.

A necropsy was conducted the following day.

#### Case 2

A five-year-old multiparous Holstein cow was referred in December 2016 for weight loss associated with chronic diarrhea. The condition first started more than a year before the animal's hospitalization and underwent few or no changes and was non responsive to treatments according to the owner.

The clinical examination at entry revealed a very poor body score condition, diarrhea and a rumen atony. Rectal temperature, heart rate, and respiratory rate were within normal limits.

In the course of hospitalization, main causes of chronic diarrhea in adult cattle, including Johne's disease, infestation by gastro-intestinal parasites, and renal failure were excluded. No primary etiology could be identified.

Considering the chronicity of the condition and the absence of evolution during the week of hospitalization, the patient was euthanized and necropsied the same day.

### Results: necropsy

#### Case 1

A large ventricular septal defect was present. The fore-stomachs, including the omasum, were all distended by a similar content, coarse and gritty. The rumino-reticular mucosae showed no macroscopic abnormality, whereas both the omasal and abomasal mucosae were severely modified. The abomasal

mucosae was inflamed and a few chronic non-perforating ulcers were present. The opening of the omasum revealed a severe hypoplasia of the omasal laminae, associated with only one old star-shaped scar.

#### Case 2

The fore-stomachs appeared to be externally normal. The rumen and abomasal mucosae were normal although an old star-shaped abomasal scar was present. The reticular crests seemed rather small in height. Hypoplastic omasal laminae were observed with absolute certainty.

Without any macroscopic sign of active inflammation of the omasal mucosae, and considering the fact that congenital malformation of the laminae omasi had already been described, it seemed reasonable to assume that our two cases were also of congenital origin.

**Conclusions:** Only a few cases of such a malformation have been described throughout the past fifty nine years. It has been reported in juvenile animals and in young adults, with or without growth defect or digestive clinical signs.

Just like the description made by Dannacher and Benoit in 1958 in a 30 month-old Charolaise heifer – the first one ever recorded to our knowledge – and the one made later by Lombard and Goulard in 1961, our cases are not associated with any growth defect.

Because of the shared abdominal distension, case 1 resembles in some ways to the ones reported by Mendes *et al.* in 2014, and by Tagaki *et al.* in 2007. But, in each of these reports, the animals were juvenile steers, suffering from growth defect and chronic bloat of unknown origin, and the omasum were small at necropsy. None of these elements were found in our cases.

Case 2 constitutes, to our knowledge, the only reported case of this condition in a multiparous cow. It seems noticeable to report that there wasn't any growth defect and that it was associated with chronic diarrhea, even though this symptom could not be directly attributed to the condition.

MT-02

## How- and under what circumstances- can veterinary communication inspire farmer behaviour change? A qualitative investigation

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**Introduction:** Achieving herd health and welfare improvement increasingly relies on veterinarians to train and advise farmers (1,2), placing veterinary communication at the heart of knowledge exchange. Veterinarians recognise their influence and the need to be proactive advisors but struggle with acting upon this awareness in daily practice (3,4,5), reporting a need to enhance their advisory approach to influence farmer behaviour (6). Understanding the interaction between communication, advice and on-farm behaviour change is therefore critical.



**Objectives:** To examine factors influencing farmers' enactment of veterinary advice on the UK dairy farm to conceptualise how and under what circumstances- veterinary communication has the potential to support and inspire farmer behaviour change.

**Methods:** Fourteen UK dairy farms were recruited to take part in a qualitative study, involving research observation of a 'typical' advisory consultation between veterinarian and farmer (n=14) followed by an in-depth interview with the farmer(s) and their respective veterinarian (n=24). Interview data were organised using a template coding method and analysed thematically (7).

**Findings:** Data suggest three core elements underpin the enactment of veterinary advice on the UK dairy farm; farmer belief in veterinarian virtue, the perception of a shared understanding between veterinarian and farmer and the manifestation of advisory meaning at a local (farmer) level.

**Discussion:** Thematic analysis suggests that the enactment of veterinary advice can be conceptualised as the synergy of these three themes, within which desirable veterinary communication acts both as a necessary foundation and perceptual catalyst. A model of this advisory process will illuminate this interaction and promote discussion.

(1) Defra (2004) Animal Health and Welfare Strategy for Great Britain. <http://archive.defra.gov.uk/foodfarm/policy/animal-health/strategy/ahws.pdf>

(2) Farm Animal Welfare Committee (2011) Education, communication and knowledge application in relation to farm animal welfare. [www.defra.gov.uk/fawc](http://www.defra.gov.uk/fawc)

(3) Cannas da Silva J, Noordhuizen JPTM, Vagneur M, Bexiga R, Gelfert CC, Baumgartner W (2006) Veterinary dairy herd health management in Europe. Constraints and perspectives. *Veterinary Quarterly*. 28(1):23-32

(4) Jansen J, Steuten CDM, Renes RJ, Aarts N, Lam TJGM (2010) Debunking the myth of the hard-to-reach farmer: Effective communication on udder health. *Journal of Dairy Science*. 93:1296-1306

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(6) Ruston A, Shortall O, Green M, Brennan M, Wapenaar W, Kaler J (2016) Challenges facing the farm animal veterinary profession in England: A qualitative study of veterinarians' perceptions and responses. *Preventative Veterinary Medicine*. 127 (1): 84-93.

(7) Braun V and Clark, V (2006) Using thematic analysis in psychology. *Qualitative Research in Psychology*, 3 (2). pp. 77-101.

Knowledge of ethnoveterinary medicine is still widely used in different regions of South Africa. However, retention of this valuable knowledge is threatened by acculturation and availability of Western medicine. The aim of this study was to explore and document knowledge of EVM within the North West Province, as a contribution to the preservation of knowledge. A qualitative research approach was used in the study and target participants consisted of ethnoveterinary practitioners in Lokaleng, Mogosane, Lokgalong and Masutlhe villages. Data was collected through semi-structured interviews and analysed through thematic analysis. Knowledge of ethnoveterinary medicine that was documented included ethnodagnostic methods, medicinal plant use of thirty-one plant species, three non-plant remedies and nine procedures. The procedures included obstetrics, surgical, fracture reduction and four metaphysical procedures. Among the medicinal plants recorded, *Senna italica* (sebetebete) emerged as a plant of cultural importance in the study area, with the highest frequency in terms of recurrence and multiple animal treatment health indications. The most common animal ailments treated using EVM were *gala* and retained placenta. The study also determined consensus among participants in terms of culturally significant plants for the treatment of five ailments including coughs and diarrhoea. In addition, the study also revealed a concern for loss of ethnoveterinary medicine due to westernisation and also the high trust in ethnoveterinary medicine among participants.

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MT-04

### The effect of linseed meal on daily weight gain, dry matter intake, feed efficiency, Interleukin-1 and Resolvin E1 in pre-weaned Holstein dairy calves

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**Objectives:** Linseed meal is a by-product of the oil extraction from flax seed. It contains 36 to 39% of crude protein, 6 to 8% of ether extract and a high content of linolenic acid (omega-3 fatty acids [ $\omega$ -3 FA]). The  $\omega$ -3 FA have the property of modulating the immune response, resulting in anti-inflammatory effects. The objective of this study was to determine the effect of linseed meal as a component of the starter for Holstein dairy calves from 0 to 2 months of age on daily weight gain, feed intake, feed efficiency and serum resolving E1, an endogenous lipid mediator identified during healing post inflammation.

**Material and Methods:** The study was conducted in a commercial Chilean dairy farm. The cows were milked 3 times a day and fed a total mixed ration based on corn silage, alfalfa hay and concentrate. Dry cows were moved to a prepartum group 30 days before expected parturition. After calving, offspring was separated immediately from the mother. They received high quality pasteurized colostrum within the first 4 hours of birth. Then each calf was moved to a system of individual hutches and pasteurized discarded milk was fed (3 L AM and 3 L PM) plus water and starter beginning at 3 days old. Any health event was treated immediately according to established standard protocols. In order to determine a difference of 1.5 kg of live weight at weaning between a treated and a control group

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MT-03

### Knowledge of ethnoveterinary medicine within the North West Province, South Africa

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(SD  $\pm$  3.5 kg) with 95% confidence and 80% statistical power, a sample of 20 calves per group was calculated. The animals were randomly allocated at the time of delivery. Eligible animals had to come from cows that had a normal and standard drying period and delivery. Treatment group was supplemented with a starter with 25% dry matter basis of linseed meal. Control group received a similar concentrate, but replaced with canola meal, rich in omega-6 fatty acids. Both starters were isonitrogenous and isoenergetic. Calves were weighed at birth, at 30 and at 60 days of age. Feed (starter) intake was evaluated on a daily basis from 5 to 60 days-old. A blood sample was obtained at birth, 14, 28, 35, and 60 d of age. Serum Resolvin E1 and Interleukin-1 were assayed by a commercial ELISA kit. Daily gain, and feed efficiency from 0-30 days, 30 to 60 days and 0 to 60 days were analyzed by a GLM ANOVA. Feed intake, Interleukin-1 and Resolvin E1 were analyzed by mixed models ANOVA for repeated measures.

**Results:** Average daily gain from 0-30 d was 0.518 vs 0.564 kg/d; from 30-60 d was 0.842 vs 0.786 kg/d; and from 0-60 d was 0.680 vs 0.675 kg/d for control and treatment group, respectively ( $P > 0.05$ ). Feed intake was higher from 49 to 60 days for control compared to treated group ( $P < 0.05$ ); however, feed efficiency was similar between treated and control group (1.68 vs 1.65 kg of starter per 1 kg of gaining;  $P > 0.05$ ). Overall, average starter consumption through the entire period was 27.0 vs 27.8 kg/calf for treated and control calves, respectively. Serum concentration of IL-1 was only different at 28 d pp with a value of 1396.2 vs 1013.7 pg/ml (SEM 101.2) for treated and control, respectively ( $P < 0.05$ ). Serum concentration of Resolvin E1 at 28 d of age was 2047 vs 1741 pg/ml (SEM=188) for treated and control group, respectively ( $P=0.1$ ).

**Conclusions:** Calf starter with 25% of linseed meal had the same effect on weaning weight, average daily gain, and feed efficiency compared to a starter with 25% of canola meal. The concentrations of Resolvin E1 and IL-1 were higher for treatment than control group, suggesting that  $\omega$ -3 FA increased the synthesis of anti-inflammatory mediators in young calves, after a likely inflammation process.

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MT-05

### Current best estimates of adult dairy cattle weight in the United Kingdom

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**Objectives:** Knowledge of accurate cattle weights is crucial for both dosing of individual animals with medicine and for reporting medicine usage metrics, especially for antimicrobials. Average weights of dairy cattle in the UK are not well defined, and scientific papers, reports and guidelines present a wide range of adult dairy cattle weights. The most common weight for dairy cattle presented in the current literature is 600 kg, however this is not evidenced by data. This project aimed to provide an up-to-date estimate of the average weight of UK dairy cattle to better inform farmers, veterinarians and the scientific community.

**Materials and methods:** Data for approximately 2600 dairy cattle from 20 farms in the UK were collected; data were from

19 farms using Lely Automatic Milking Systems with weigh floors collected via Lely and one using a crush with weigh scales collected by a researcher. Calibration checks for the weigh floors were also carried out. Farms with different breed types were represented, including Holstein, Friesian, Holstein-Friesian and Jersey, as well as farms with dual purpose breeds and cross-breeds. Datasets from Lely were fully anonymised before they were received and contained the following relevant variables: *Animal Number, Robot, Date Time, Lactation No., Lactation days, Weight at Calving, Weight, Weight Avg., Weight Avg. Dev. and Day Production*. The variable "Weight" was the weight at last milking and "Weight Avg." was the mean weight for that animal over the last three milkings (from the current lactation). "Weight at Calving" was the very first weight recorded after calving for the current lactation. "Weight Avg." was used for all calculations. Data from one final farm is still to be collected by a researcher using a weigh crush. The following relevant variables will be collected from this farm: *Animal Number, Date, Lactation No., Lactation days and Weight*. Data were used to calculate a mean weight for dairy cattle by breed, and these breed weights were scaled by UK-specific breed proportions to generate a UK mean weight. Trends in weight by lactation number, day of lactation and production level were also explored using individual cattle-level data. To establish representativeness of the cattle used in this study to the UK herd, data were obtained from National Milk Records (NMR) for 500 UK herds on the proportion of heifers, mean lactation number, mean daily production and mean herd size. These national statistics were compared to the dataset.

**Results:** At the time of writing, data were available for the 19 farms collected via Lely; data collection for the final farm and analysis will be complete by the end of December 2017, and these results will be updated subsequently. For the preliminary data from 19 farms, mean weight for dairy cattle was 624 kg, although this varied across breeds, with dual purpose breeds being the heaviest with a mean of 663 kg, and Holstein-Friesian being the lightest, with a mean of 617 kg. The final data will come from a Jersey farm; this is anticipated to be the lowest breed weight. When scaled to UK breed proportions, the estimated mean UK dairy cattle weight was 618 kg. Overall, heifers (in their first lactation) weighed 9.4% less than cows. For cattle at peak production; mean weight was strongly positively correlated with production level. Data showed a decline in mean weight from calving to 30 days into lactation, and then a steady increase throughout the rest of lactation.

**Conclusions:** This study is the first to calculate a mean weight of adult dairy cattle in the UK based on on-farm data. Overall mean weight was higher than that most often proposed in the literature (600 kg). Evidence-informed weights are crucial, especially as the UK works to better monitor and report metrics for antimicrobial use that are useful to farmers and veterinarians to inform dosing decisions. The impact of having an evidence-based figure for the average weight will be marked. For example, for dosing, visual weight estimation of individual cattle will be easier and more accurate if an actual average is known in the first instance.



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## POSTER PRESENTATIONS

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Anti-Microbial Resistance [AR]  
Bovine Welfare and Cattle Comfort [WE]  
Buffaloes, Camelids and Wild Ruminants [BU]  
BVD [BV]  
Clinical Genetics [CG]  
Diagnostic Imaging [DI]  
E-learning and Continuing Education [EL]  
Emerging Diseases [ED]  
Epidemiology [EP]  
Herd Health Management [HH]  
Hoof Health and Lameness [HL]  
Immunology and Vaccines [IV]  
Infectious Diseases: Bacteriology [BC]  
Infectious Diseases: Parasitology [PA]  
Infectious Diseases: Virology [VR]  
Internal Medicine [IM]  
Nutrition and Metabolic Diseases [NU]  
Organic and Sustainable Production Systems [SP]  
Public Health and Food Safety [PH]  
Reproduction [RE]  
Reproductive Technology [RT]  
Small Ruminants [SR]  
Surgery [SU]  
Therapeutics and Pharmacology [TP]  
Toxicology [TX]  
Tropical Animal Diseases [TD]  
Udder Health and Milk Quality [UH]  
Young Stock [YS]  
Miscellaneous Topics [MT]



AR-P01

## Production animal antimicrobial use in New Zealand

### The first national analysis of antimicrobial use for production animals at a farm level.

\*Mark Bryan Skye Fruean Elena Knupfer

VetSouth Ltd

**Objectives:** The NZVA has stated its aspirational goal that 'by 2030, NZ Inc will not need antibiotics for the maintenance of animal health and welfare' (Anon<sup>1</sup>).

New Zealand already has one of the lowest rates of antimicrobial use (AMU) in the world, as determined from national sales data as (published in New Zealand by the Ministry for Primary Industries). However, there are few data available in New Zealand describing AMU in production animals to the level of detail required to both adequately monitor, and effect change, around AMU.

This paper reports on a pilot project to assess and analyse all production animal use (sales) data from six key clinics across New Zealand.

**Materials and Methods:** This project was co-funded by the NZVA and by XLVets in New Zealand.

Data were requested for all farm clients serviced by participating businesses during this period. A farm client was determined as a 'productive farm unit': ie, not including lifestyle blocks or hobby farms.

The data gathered were extracted from each clinic's proprietary sales database and included all antimicrobial sales for the period; all animals present on farm as at 1st July 2015; client post-code and region; client type (dairy, sheep, beef, deer or mixed); and ownership structure (for dairy only, classed as owner/operator, contract milker, lower order, sharemilker, manager, equity partnership, other).

Antimicrobial sales data was married with ACVM data and classified according to route of treatment, class, and active. The total mgs of active used for each product was calculated at a farm level and combined to give a total mg of AMU for each farm. Animal weight was calculated using industry recognised standard weights for each subclass of species, and total biomass was calculated for each farm.

Data were then combined to calculate mg active/kg PCU, for reference against existing data and for summary analyses.

Farms were not used in the final analysis where denominator data were unclear or unavailable, or where there were no AM sales for the period in question.

**Results:** Data were gathered from a total of 1462 dairy farms and 707 other (general) farms representing 623,430 adult milking cows, 27,399 mixed age beef animals, 706,035 mixed age ewes, and 12,107 mixed age hinds and stags.

Mean PCU for all dairy farms was 8.54 mg/kg. The lowest mean by clinic was 4.72 and the highest 11.91. Mean PCU for all general farms was 0.57 mg/kg; with a minimum by clinic of 0.32 and a maximum of 0.94.

For dairy farms, 56.3% of all AM used (by weight of active) was injectable, followed by intramammary dry cow (33.4%) and

then intramammary lactating cow (8.9%). The majority of active used was Penicillin (77.6%), followed by Macrolides (9.04%) and then Cephalosporins (5.1%).

For general farms, 59.0% of all AM used was oral, followed by injectable (39.2%). The majority of active was Tetracyclines (66.0%), followed by Penicillin (19.2%) and then Sulphonamides (7.1%).

**Conclusion:** These data confirm that the use of AMs amongst production animals in New Zealand is very small. The data also demonstrate that the most common routes of administration and actives are different in the red meat sector than in the dairy sector.

The data also confirm that the use of (3rd generation) cephalosporins and macrolides is very low amongst general farms and low amongst dairy farms. Fluoroquinolone use is virtually negligible across all species.

Penicillin – based actives represent the greatest AMU amongst dairy farmers- this is made up predominantly of dry cow antimicrobial therapy (DCAT) (Ampicillin, Cloxacillin); lactating cow intramammary (Cloxacillin, Ampicillin, Amoxycillin, Penicillin); and injectables (Penicillin, Penethamate, Amoxycillin). A significant portion of this is DCAT use.

Amongst general farm species, Oxytetracycline is the predominant AM used. This is largely used orally, and the greatest use is in calves or weaner deer.

The data presented here provide a far greater level of analysis than previously by providing for species used, active, PCU, region and route of administration. Also collected but not presented in this report are data on geography (post code) and on ownership structure (dairy farms only). Because all of the data is sourced by sales, it is also possible to drill down to product level if required. These data demonstrate that the veterinary profession is an effective source of AMU data for New Zealand.

AR-P02

## Reduction in antimicrobial usage following veterinary intervention in dairy herds

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**Objectives:** The aim of the study was to assess antimicrobial usage following implementation of a novel veterinary intervention programme designed to focus on stewardship of antimicrobial usage on dairy farms.

**Materials and methods:** 67 dairy farms located in the North and South Islands of New Zealand were enrolled in a two year prospective intervention study. In the initial 12 months of the study (Year 1), standard on-farm prescribing and treatment protocols were used by all farms. Total antimicrobial usage was calculated from records of sales from the servicing veterinary businesses, using the population corrected unit (PCU) approach. Usage (mg/kg live weight/year) was calculated as [total



mass of active antimicrobials sold]/[number of cows in the herd x 450 kg]. Within island, herds were ranked on antimicrobial usage, and within sequential pairs, randomly assigned to either a control group or a veterinary intervention group. Veterinarians servicing the herds attended a workshop outlining issues associated with antimicrobial resistance and antimicrobial usage, were provided with a series of open ended questions for use with each selected herd owner, and asked to undertake a 90 minute visit to each of the treatment herds. At the end of this visit, the herd owner and/or manager and the veterinarian were asked to define three or more specific goals around reducing antimicrobial usage on farm. Antimicrobial sales to the farms were monitored over the subsequent 12 month period (Year 2). The change in antimicrobial usage (that is Year 2 - Year 1) was analysed using linear regression with treatment group and farm location (Island level) as the main effects and with herd size as a covariate.

**Results:** Prior to the veterinary intervention the antimicrobial usage was 7.18 (95%CI=6.40-7.95) mg/kg live weight, and did not differ between groups ( $P=0.62$ ). Veterinary intervention was associated with a tendency ( $P=0.06$ ) for a reduction in antimicrobial usage, with the difference in usage being +0.94 (SE=0.37) vs -0.04 (SE=0.36) mg/kg live weight for Year 2–Year 1, for the Control vs Veterinary visit herds, respectively. There was no effect of farm location ( $P=0.14$ ), no Veterinary visit by Island interaction ( $P=0.11$ ) and no effect of herd size ( $P=0.23$ ) on change in antimicrobial usage.

**Conclusions:** We conclude that our program of veterinary intervention focused on promoting good stewardship of antimicrobials reduced their usage on dairy farms.

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AR-P03

## Validating the use of overboot environmental sampling compared to individual cow faecal sampling to monitor antimicrobial resistance prevalence on dairy farms.

### A practical and repeatable methodology

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**OBJECTIVES:** As the dairy sector responds to scientific, public and political pressures to make changes in the way that antimicrobials are prescribed and used, the effect of such changes on antimicrobial resistance (AMR) prevalence should be monitored so that evidence-based and effective management practices can be recommended.

Environmental sampling of farms could offer an applicable, economic and repeatable method by which AMR prevalence can be monitored, however the use of overboot sock sampling to assess the prevalence of AMR on dairy farms has not been fully validated.

### MATERIALS AND METHODS:

#### Part 1 – Environmental sampling of farm areas

A prospective cohort study was carried out on ten conventional dairy herds in South West England; eight overboot sock sam-

ples were collected per farm. One overboot sock sample was collected from each 'quarter' of the collecting yard, and a further four were collected from the whole collecting yard area, encompassing all previously sampled 'quarters' (W1, W2, W3, W4).

Agar dilution analysis was used to assess the prevalence of resistance in *E. coli* isolates within the samples. Six antibiotics were tested: ceftazidime, cefotaxime, ciprofloxacin, trimethoprim sulphonomide, tetracycline and amoxicillin.

Standard deviation from the mean of AMR prevalence for each of the six antibiotics for 2, 3 or 4 samples from either quarter or from the whole collecting yard was assessed in order to determine whether differences in the spatial area of the collecting yard sampled on each occasion would affect the estimated AMR prevalence. Whether or not variability in the estimated AMR prevalence could be reduced by sampling the same yard multiple times was also assessed.

#### Part 2 – Individual cow vs. environmental samples

Two overboot sock samples were collected from the collecting yard area of a further seven dairy farms, also in South West England. Individual cow faecal samples were simultaneously collected from rectal gloves after rectal palpation of cows presented to veterinary surgeons at routine fertility visits.

Overboot sock samples were processed and agar dilution analysis performed as in Part 1. Individual cow faecal samples were analysed for AMR using the Kirby-Bauer disk diffusion method. Resistance was assessed using CLSI breakpoints or, where these were unavailable, calculation of an epidemiological cut-off value.

Chi-squared tests with Yates correction (two-tailed) were used to assess whether AMR prevalence data from each of the seven farms differed between environmental samples processed using agar dilution compared with the mean prevalence found by individual cow faecal samples processed using disk diffusion.

### RESULTS:

#### Part 1

Repeated analysis showed that standard deviations of the means of AMR prevalence in *E. coli* isolates from overboot sock samples were not significantly different between quarters of a yard compared with repeated samples from the whole collecting yard on a dairy farm. Standard deviations were not significantly reduced by sampling yards more than twice.

#### Part 2

Both sampling and testing methods found resistance to all classes of tested antibiotics to be low except for amoxicillin. Amoxicillin resistance was found to be highest by both environmental overboot sock sampling and individual cow faecal sampling methods.

Overall the two sampling and testing methods did not differ in the prevalence of AMR detected except for amoxicillin resistance prevalence on all farms. On 5 of the 7 farms, agar diffusion method used on overboot sock samples detected higher levels of AMR than the mean AMR prevalence calculated from disk diffusion testing of individual cow samples; on the other 2 farms, the opposite was true.

**CONCLUSION:** Overboot sock sampling of the collecting yard of dairy farms is a practical and repeatable method to monitor



AMR prevalence. This sampling method is highly repeatable and can be performed by veterinarians, farmers or other lay personnel in order to analyse changes in AMR prevalence. Environmental sampling provides a representative sample of AMR prevalence in the adult dairy herd comparable to calculating the mean AMR prevalence from individual cow faecal sampling when AMR prevalence is low. Increased variability in both testing methodologies results in less consistency between the methods when resistance prevalence is higher, as with amoxicillin.

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AR-P04

### Formulating antimicrobial reduction strategies using farmer-led Participatory Development

#### Can we reduce antimicrobial use in food production animals by 20% within 2 years region-wide by using farmer initiatives?

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**Objectives:** Agricultural antimicrobial use (AMU) is recognized as being closely linked to the development of antimicrobial resistance (AMR) (Chantziaras et al. 2014). AMR is recognized as one of the greatest risks to human health globally (O'Neill, 2015), and agriculture uses the majority of antimicrobials globally.

The New Zealand Veterinary Association (Anon, 2015) has set an aspirational goal of reducing AMU to zero by 2030, and has embarked on a number of projects in an attempt to achieve this.

Participatory development (PD) has been used to help facilitate farmer projects previously (Reyher, 2016). This paper reports on the interim results of a three-year project using PD in farmer-led groups to help formulate AMU reduction strategies in New Zealand.

**Materials and Methods:** This project is currently active in the lower region of New Zealand. Farmers and veterinarians across the Southland and South Otago regions of the South Island have been invited to participate in 4 strategic farmer groups. These strategic groups comprise up to 12 farmers per group, from sheep, beef, deer (red meat) sectors, and from the dairy sector. They come from a range of backgrounds, from owner-operators, to managers; and they represent a wide variety of farm sizes, systems, terrains and types.

Each group has a facilitator, who is a trained agricultural consultant with a background in group facilitation. The groups are initially given a comprehensive AMR workshop, where they are introduced to the concept, to the risks and history, data around AMU and other background. This material is subsequently made available to them during the project in a variety of formats.

In addition, at each subsequent project meeting, a short summary is presented by veterinary epidemiologists, and a specific topic (actives, AMU calculations, etc) is explored in brief detail. The veterinarian is not involved beyond this other than to provide technical advice.

**Outputs:** A series of project meetings were held for each group throughout the year, during which the participants explored a range of options to reduce AMU, before alighting on 3 or 4 specific, deliverable strategies. These strategies were then worked up comprehensively to a format where they were able to be presented and delivered to farmers beyond the strategic group.

In year 2, 8 new farmer-led groups will be formed. These groups will then pick up some of the strategies developed by the original strategic groups and implement them on both their own, and other farms. AMU will be monitored and changes in AMU will be assessed. In addition, the ease of implementation of any strategies will also be gauged.

At the end of the second year, successful strategies at a farm level will be socialized further with a broader group of farms. The intent is to enroll up to 160 farms in various strategies.

**Conclusion:** This study intends to facilitate farmers to create a range of strategies for reducing AMU which they will feel ownership of, and then to test these strategies in the field, with regard to ease of implementation, level of effect, consequences and other impact.

From this, effective, simple strategies are hoped to be identified by farmer leaders and adopted by the farming community, because farmers will feel the ownership of these strategies themselves. The goal is to facilitate farmer uptake and effect reduction in AMU by farmer-led initiatives. Monitoring of AMU as part of this project will help determine the quantitative effect of the various strategies.

The ultimate goal is to reduce AMU on farm by 20% by 2020.

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AR-P05

### Monitoring animal welfare and antimicrobial use in calf rearing units in Finland 2017

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ETT ry/ Animal Health ETT

**Objectives:** Animal welfare and antimicrobial use in Finnish cattle production have become a more and more important issue. The consumer demands of animal welfare and responsible use of antimicrobial agents for production animals have increased. The dairy and beef industry need a tool for collecting data from the farm level to answer these challenges. The industry has set criteria for responsible production, which include indicators for animal welfare and antimicrobial use. The health service veterinarians have an important role. Animal welfare, responsibility of the production and use of antimicrobial agents are monitored during cattle health care visits. The data is stored and processed in the Centralized health care register for Finnish cattle herds called NASEVA.

**Materials and Methods:** Centralized Health Care Register for Finnish cattle herds was founded by ETT in 2005 and it is developed and maintained by slaughterhouses and dairy companies. NASEVA register has been built to follow up the voluntary national health care scheme for dairy and beef farms (7300) and calf rearing units (85). The register contains data from calf rearing units concerning management plan, health and welfare status, production and medication data of the animals on the



farms. The health service veterinarian visits the calf rearing unit at least once for every rearing batch. The animal welfare monitoring in NASEVA is based on the Welfare Quality® protocol, where evaluation is made mostly by observing the behavior and condition of the animals, not by exact measurement of the production environment. The management and production data are collected and used as indicators for responsible production concerning calf mortality, daily weight gain and use of antimicrobial agents. The usage of antimicrobial agents is supervised at the farm visits according to the legislation and the criteria for responsible use of drugs are discussed. The medication data from Finnish calf rearing units can be used for analyzing the treatments of the most important infectious diseases.

**Results:** The criteria for responsible production and responsible use of antimicrobial agents can be used to target special advisory activities to the farms which do not fulfill the limit values set by the industry. For animal welfare and the image of the dairy and beef production, the purpose of NASEVA register is to help farms, veterinarians and industry to improve the production conditions and the management on the farms. The aim monitoring is also to promote the responsible and prudent use of antimicrobial agents without increasing the risk of antibiotic resistance.

**Conclusions:** NASEVA is a tool for dairy and beef industry for documentation of the farm based quality in the production chain. It is the only register collecting medication data which follows the individual animal through its whole life. The demands of the consumers for animal welfare and responsible production can partly be fulfilled with this documentation.

AR-P06

### Investigation of antibiotics uses in dairy farms in Chiba Prefecture for a national action plan on antimicrobial resistance

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**Objective:** Following the adoption of the global action plan on antimicrobial resistance (AMR) by the World Health Organization in 2015, the national action plan on AMR was published by the Japanese cabinet office in April 2016. Based on the "one health" concept that recognizes the health of people, animals and the environment as one issue, the proper and prudent use of antibiotics in the veterinary medical field is strongly required more than ever. In order to understand the current status of resistant bacteria provide information for risk management, we investigated antimicrobial usage in dairy farms and analyzed the relationship between antimicrobial usage and the emergence of resistant bacteria.

**Materials and Methods:** Investigation of the antimicrobial usage in dairy farms in Chiba.

We evaluated 360 dairy farms in Chiba Prefecture consisting of 98 farms in 2014, 133 farms in 2015 and 129 farms in 2016. We calculated the antimicrobial usage required to produce 1kg of milk, the antimicrobial usage per parous dairy cow's milk

yield per year, and the total number of parous dairy cows.

Analysis of the relationship between antimicrobial usage and resistant bacteria.

For 892 samples of mastitis affected milk from 162 farms examined in 2016, we performed a logistic regression analysis of the antimicrobial usage and detection of methicillin-resistant *Staphylococcus* (MRS) or extended-spectrum  $\beta$ -lactamase producing bacteria at each farm. Data is reported as the mean  $\pm$  SD.

**Results:** The antimicrobial usage required for producing 1kg of milk was  $0.918 \pm 0.689$  mg, and the antimicrobial usage per parous dairy cow was  $7,527.8 \pm 5,296.7$  mg. In dairy farms where MRS was detected in milk samples, significantly higher doses of tetracycline ( $p < 0.01$ ) and cefuroxime ( $p < 0.05$ ) were used.

**Conclusion:** In this study, we revealed that an average of 0.918mg of antibiotics was used per 1kg of milk production per year. These are useful data for risk management and for understanding the status of resistant bacteria. The use of tetracycline and cefuroxime are possibly related to the emergence of resistant bacteria: therefore, it is advisable that we adhere to a more proper and prudent use of these antibiotics.

AR-P07

### Antimicrobial susceptibility of *Histophilus somni* isolated from diseased and clinically normal cattle and sheep.

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**Objectives:** Bovine respiratory disease (BRD) is one of the most economically important disease in beef cattle industry. *Histophilus somni* is a causative agent of BRD and also causes a variety of systemic diseases including thrombotic meningoencephalitis (TME). According to the sequence of major outer membrane protein (MOMP) gene, this bacterium grouped into at least nine clades (clade Ia to clade VIII), and most of the BRD isolates were divided into clades Ia, Ib, II and VII (manuscript in preparation). Antimicrobial agents including  $\beta$ -lactam, aminoglycoside, tetracycline, phenicol, macrolide and quinolone are used routinely to prevent and/or treat BRD in stock-raising site in Japan. Antimicrobial resistance monitoring is critical for proper and effective use of antimicrobials against BRD caused by *H. somni*, but there is no available information about that in Japan. The aim of this study is to elucidate the antimicrobial resistance of *H. somni*.

**Materials and methods:** This study investigated antimicrobial resistance among 252 isolates of *H. somni* isolated in Japan (175 isolates) and other countries (77 isolates including 21 sheep isolates). Cattle isolates were collected from cases of BRD, TME and others including myocarditis, abortion, etc. and also collected from clinically normal cattle (37 isolates). Minimum inhibitory concentration (MIC) of all isolates to 15 antimicrobial agents, namely, ampicillin (ABPC), amoxicillin (AMPC), cefazolin (CEZ), ceftiofur (CTF), kanamycin (KM), streptomycin



(SM), nalidixic acid (NA), enrofloxacin (ERFX), danofloxacin (DNFX), florfenicol (FFC), erythromycin (EM), tylosin (TS), oxytetracycline (OTC), sulfamonomethoxine (SMMX) and fosfomycin (FOM) were determined by using broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guideline. MIC breakpoints were taken from CLSI criteria or other studies on antimicrobial susceptibility. The quality control strains *H. somni* ATCC 700025 and *Actinobacillus pleuropneumoniae* ATCC 27090 were used for all testing.

**Results:** Among the 252 isolates, 143 (56.7%) were susceptible to the all antimicrobial agents used in this study except for SMMX. The all isolates showed resistance to SMMX. On the other hand, resistance or intermediate susceptibility to ABPC (6/252, 2.4%), AMPC (5/252, 2.0%), CEZ (1/252, 0.4%) KM (62/252, 24.6%), SM (18/252, 7.1%), NA (4/252, 1.6%), ERFX (1/252, 0.4%), DNFX (4/252, 1.6%), FFC (1/252, 0.4%), EM (53/252, 21.0%), TC (2/252, 0.8%), OTC (21/252, 8.3%) and FOM (15/252, 6.0%) were observed. Among the nine MOMP gene clades, multi-drug resistance ratio of clade Ib isolates (14/19, 73.7%) was significantly higher than that of the other clades. Focusing on the relation between antimicrobial resistance and diseases, BRD isolates tended to show multi-drug resistance, that is, 46/71 (64.8%) isolates showed resistance or intermediate susceptibility to more than two antimicrobials. In contrast, only 34/118 (28.8%) isolates isolated from other clinical signs showed multi-drug resistance. Furthermore, recent isolates tended to show resistance to high number of antimicrobial agents.

**Conclusions:** In this study, we showed the past and current situation of antimicrobial resistance of *H. somni*. Though more than half of the isolates showed susceptibility to all antimicrobial agents used in this study except for SMMX, many resistant isolates were detected especially in BRD and MOMP gene clade Ib isolates. The information is available for proper and effective use of antimicrobials against BRD caused by *H. somni* and continuous monitoring of the antimicrobial susceptibility will be important for the best choice of antimicrobial agent.

AR-P08

### Phenotypic susceptibility of *Streptococcus uberis* to selected veterinary and human antimicrobial agents

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**Objectives:** Mastitis is the primary cause for antimicrobial drug usage in dairy cattle and *Streptococcus (Sc.) uberis* intramammary infection (IMI) is a common disease that is treated with antibiotics. Veterinary treatment data collection in Austria revealed that  $\beta$ -lactams (intramammary penicillin, cefquinome, cefoperazone, kanamycin/cephalexin) were the most commonly administered antimicrobial class for therapy of acute clinical and subclinical mastitis. Antimicrobial susceptibility is a main prerequisite for therapeutic success. Penicillins are considered as first line agents for the treatment of streptococcal mastitis and it is commonly believed that *Sc. uberis* is highly sensitive

to this drug class. Recently a number of studies have shown that in some cases *Sc. uberis* demonstrates a shift towards resistance. It was one objective of this study to evaluate antimicrobial susceptibility of *Sc. uberis* strains to antibiotics used for mastitis treatment in order to improve bacterial cure rates.

Concurrently the use of antibiotics is considered to be a main factor for the development of antimicrobial resistance in general. From a public health and food safety perspective there is considerable concern about the use of antimicrobials in food-producing animals: Development and cross-species transmission of resistant bacteria or genetic elements from animals to humans (food or direct contact) occur in all settings. Although there is no consensus regarding the consequences that antibiotic use in food-producing animals has on antibiotic resistance in the human population, a "One Health" approach according to World Health Organization guidelines must be considered. Therefore a panel of antimicrobials used in human and veterinary medicine was included in this study with the objective to evaluate whether there is a relationship between the use of antimicrobials in veterinary medicine and the development of resistances towards important antibiotics.

**Materials and methods:** A total of 77 *Sc. uberis* isolates from bovine mastitis milk samples were confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility was assessed using the agar disk diffusion test. Testing for the antimicrobials norfloxacin, linezolid, rifampicin, vancomycin, tetracycline, trimethoprim/sulphonamide 1:19, erythromycin and benzylpenicillin was conducted according to the methods recommended by the EUCAST document version 7.1. Evaluation of susceptibility towards penicillin-novobiocin 1:3, cefoperazone, ceftiofur, kanamycin/cephalexin 2:1 and pirlimycin was based on the recommendation of CLSI VET01S. Production of  $\beta$ -lactamase was detected with color-based test disks (Cefinase, bioMérieux, France).

Zone diameters are interpreted and categorized as susceptible, intermediate or resistant according to the appropriate breakpoints. According to EUCAST notes, results were assessed as followed: The susceptibility to cephalosporins and to penicillins is inferred from the benzylpenicillin susceptibility. The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin. Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline and isolates susceptible to linezolid can be reported susceptible to tedizolid.

**Results:** None of the *Sc. uberis* isolates was resistant against penicillin-novobiocin, cefoperazone or ceftiofur. They were mostly susceptible to kanamycin/cephalexin (98.7%), norfloxacin (96.1%) and linezolid (93.5%). About 67 isolates were susceptible to vancomycin (87.0%) and trimethoprim/sulphonamide (88.3%) but 10.4% and 16.9% of isolates were resistant to pirlimycin and benzylpenicillin, respectively. Only 24.7% (n=19) were susceptible to tetracycline. More than two thirds demonstrated in vitro susceptibility to erythromycin (77.3%) and rifampicin (74.0%). None of tested isolates produced  $\beta$ -lactamase.

**Conclusions:** Although intramammary penicillins and third and fourth generation cephalosporins were the most frequently used antimicrobial groups for mastitis treatment, these antimicrobials seem to be still effective. With regard to penicillin resistance rates, we recommend carrying out a susceptibility testing to confirm treatment decisions. *Sc. uberis* isolates show almost



no resistance pattern against antimicrobial agents classified as highly or critically important except for tetracycline, erythromycin and rifampicin.

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AR-P09

### Antimicrobial susceptibility of pathogens isolated from cows with clinical mastitis across Germany

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Bayer Animal Health GmbH

**Introduction:** Mastitis of the dairy cow is one of the most costly diseases in the dairy industry and often antibiotic therapy is essential to cure the disease. The major pathogens involved are staphylococci, streptococci and coliform bacteria such as *Escherichia coli* and *Klebsiella* spp. Monitoring of antimicrobial susceptibility is important to ensure long-term efficacy of the antibiotics. Bayer Animal Health has established susceptibility monitoring programs in Germany and is part of various extensive programs performed across Europe by the veterinary pharmaceutical industry. Here, we present the results of the BAH monitoring program comprising isolates collected between 2006 and 2017 in Germany.

**Material and Methods:** Milk samples from animals with acute mastitis were collected across Germany between 2006 and 2017. Per farm and outbreak only one isolate was included. Isolates from pre-treated animals were excluded. The Minimum Inhibitory Concentrations (MICs) of amoxicillin/clavulanic acid, ceftiofur, enrofloxacin, gentamicin and tetracycline were determined by agar dilution methodology according to the Clinical and Laboratory Standards Institute (CLSI; VET01-A4, 2013 and previous versions). MIC range, MIC<sub>50</sub> and MIC<sub>90</sub> were determined for species comprising at least 10 isolates. Results were interpreted using CLSI clinical breakpoints, where available. The data were compared to other national (GERM-Vet) and European (VetPath) monitoring surveys.

**Results:** In total, 282 isolates were collected from diseased cattle: *E. coli* (n=66), *Staphylococcus aureus* (n=55), *Streptococcus (para)uberis* (n=53), *Klebsiella* spp. (n=24), *Streptococcus dysgalactiae* (n=19), coagulase-negative *Staphylococcus* spp. (CNS, n=18), *Streptococcus agalactiae* (n=15), *Trueperella pyogenes* (n=14), and various minor species (n=18).

For amoxi/clav resistance rates of 12.1% and 33.3% were found for *E. coli* and *Klebsiella* spp., respectively, while resistance was low for *S. aureus* (3.6%) and absent for CNS and streptococci.

Tetracycline resistance rates amounted to 19.7% for *E. coli* and 8.3% for *Klebsiella* spp. Around 11% of the tested staphylococci were resistant to tetracycline, while for streptococci high resistance rates of 43.4-71.3% were observed. Resistance to ceftiofur was low for *E. coli*, *S. aureus* and *S. dysgalactiae* (5.3-6.1%) and absent for *S. agalactiae*. In contrast, a moderate resistance rate was found for *S. uberis* (11.3%). For gentamicin, resistance was low for *E. coli* and *Klebsiella* spp. (4.5 and 8.3%) and absent for staphylococci. For enrofloxacin, for which no CLSI breakpoints are defined, susceptibilities were high. For *E. coli* the MIC<sub>90</sub> was 0.12 µg/mL, while for *S. aureus* and CNS

MIC<sub>90</sub> values of 0.25 and 0.5 µg/mL were found, respectively. With regard to streptococci and *T. pyogenes*, the MIC<sub>90</sub> was 1 µg/mL for enrofloxacin.

The data were comparable to other national (GERM-Vet) and European (VetPath) monitoring surveys, with exception of amoxi/clav, where the BAH monitoring revealed considerably higher resistance rates for Gram-negative bacterial species than the other programs.

**Conclusions:** The results of the resistance monitoring programs generally document the high antimicrobial susceptibility and the absence or low prevalence of antimicrobial resistance among the major pathogens isolated from cows with clinical mastitis. Exceptions are tetracycline resistance of *E. coli* and streptococci. In general, the different monitoring programs show comparable results and no clear trend over time could be observed. Besides the need to establish mastitis-specific clinical breakpoints, resistance testing is recommended whenever possible prior to treatment. Bayer Animal Health is committed to perform ongoing susceptibility monitoring studies on a national and European-wide level to generate quantitative and representative data to help veterinarians to choose the most effective antibiotic. The availability of a wide range of antibiotic treatment options and ongoing research is essential for curing clinical mastitis in dairy cows and, hence, for animal welfare reasons.

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AR-P10

### Antimicrobial susceptibility of bovine respiratory pathogens in Germany: Enrofloxacin resistance monitoring 2007-2017

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Bayer Animal Health GmbH

**Objective:** 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 bacterial pathogens recovered from bovine respiratory tract samples.

**Materials and Methods:** Samples from diseased animals were collected across Germany between 2007 and 2017. 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, MIC<sub>50</sub> (at least 5 isolates/species) and MIC<sub>90</sub> (at least 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, 224 isolates were tested. The most common species isolated from respiratory tract samples were *P. multocida*



da (n=100) and *M. haemolytica* (n=59). In addition, other species were found in lower numbers, e.g. *Pseudomonas aeruginosa* (n=21), *Trueperella pyogenes* (n=13), *Klebsiella* spp. (n=8), *Bibersteinia (Pasteurella) trehalosi* (n=6) and *H. somni* (n=5).

Determined MIC<sub>50</sub>/MIC<sub>90</sub> values (µg/mL; NA where not applicable due to low number of isolates) were as follows 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., 0.06/NA for *B. trehalosi* and 0.03/NA for *H. somni*. For *P. aeruginosa*, *T. pyogenes*, *Klebsiella* spp. and *B. trehalosi* 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 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 and VetPath (El Garch et al., 2015). In spite of this high susceptibility, prudent and responsible use of fluoroquinolones as well as susceptibility monitoring are mandatory.

AR-P11

### Penicillin Resistance of *Streptococcus agalactiae* isolated from bovine milk in Colombia

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**Objective:** The aim of this study was to determine the antimicrobial susceptibility of *Streptococcus agalactiae* (SAG) to penicillin (Minimum Inhibitory Concentration, MIC), and its association with the gene expression for resistance: *pbp1a*, *pbp1b*, *pbp2a* and *pbp2b*.

**Materials and Methods:** Milk samples (n = 2350) were collected from cows of dairy herds in Caldas, Colombia. In total, 241 isolations were confirmed as SAG by PCR (Phuektes et al., 2001). Chromosomal DNA was isolated from the positive SAG colonies using a commercial kit (Jena Bioscience Kit®. Jena, Germany). Penicillin resistance genes (*pbp1a*, *pbp1b*, *pbp2a* and *pbp2b*) were detected by PCR (Nagano et al., 2008). Samples were classified as 0 for gene absence, and 1 for gene positive. Minimum inhibitory concentrations of penicillin were determined by broth microdilution assay using the Sensititre® Mastitis plate (Trek Diagnostic Systems Ltd. East Grinstead, UK) following the manufacturer's instruction and the standards of Clinical Laboratory Standards Institute (CLSI, 2013). No bacterial growth at a concentration 0.12 µg/mL of penicillin was considered susceptible to the antibiotic (= S), while any growth at a concentration between 0.12 µg/mL and 1 µg/mL was considered as intermediate resistance (= I) and any growth at a concentration > 2 µg/mL corresponded to antibiotic resistance (= R). The Fisher's exact test was used to determine the association of gene expression and the MIC for penicillin. Statistical

analyses were done in Stata 15.1® (Stata Corp. College Station, TX, USA).

**Results:** The prevalence for the *pbp1a*, *pbp1b* and *pbp2b* genes in the analyzed isolates (n = 241) was 56%, 51.5% and 81.3%, respectively, indicating that the *pbp2b* gene was the most prevalent penicillin resistance gene in SAG isolates. The *pbp2a* gene was not detected among the strains we analyzed. Expression for one, two or three analyzed genes was found in 73 (30.2%), 168 (69.7%) and 76 (31.5%) isolates, respectively. Results of penicillin susceptibility are presented in the Table 1.

**Table 1.** Penicillin susceptibility in isolates of *Streptococcus agalactiae* (SAG) collected from milk samples in dairy cows of herds in Caldas, Colombia.

Susceptibility Gene expression

pbp1a pbp1b pbp2b

Negative Positive Negative Positive Negative Positive

Susceptible 46 63 46 63 21 88

Intermediate 48 59 53 54 22 85

Resistant 12 13 18 7 2 23

Our results indicated that the susceptibility to penicillin was associated with the expression of gene *pbp1b* (P < 0.05); while in the case of the gene *pbp1a* and *pbp2b*, there was no association with penicillin susceptibility (P = 0.81 and P = 0.37 respectively).

**Conclusions:** This study showed that the most prevalent resistance gene in SAG isolates was *pbp2b*, while *pbp2a* was not detected. Isolates with intermediate susceptibility and resistance to penicillin was found in milk samples from cows of dairy herds in Caldas, Colombia. The antimicrobial susceptibility of SAG to penicillin does not necessarily imply the presence or absence of a specific resistance gene.

AR-P12

### Random Amplification Polymorphic DNA analysis of genome diversity and antimicrobial resistance in *Streptococcus dysgalactiae* isolated from bovine mastitis in China

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**Objectives:** *Streptococcus dysgalactiae* is recognized as one of the mainly environmental pathogens causing bovine mastitis as well as some human diseases. To investigate the molecular epidemiology and population structure of *Streptococcus dysgalactiae* from bovine clinical mastitis and subclinical mastitis in different regions of China and to assess their antimicrobial resistance profiles.

**Materials and methods:** Ninety-three isolates of *Streptococcus dysgalactiae* were recovered from the milk of cows with bovine clinical and subclinical mastitis in six provinces (Beijing, Inner Mongolia, Hebei, Shandong, Guangdong and Shanghai) of China between 2014 and 2016. The identification of *Streptococcus dysgalactiae* was done by biochemical method and Polymerase Chain Reaction (PCR) with 16S rRNA gene se-





quence. A random primer: 5'-AGTCGGGTGG-3' was used to amplify the genome DNA of *Streptococcus dysgalactiae*, and then the amplification products were separated by gel electrophoresis and photographed under ultraviolet light. The random amplification polymorphic DNA (RAPD) profiles were compared on the basis of the presence or absence of each DNA band. The antimicrobial susceptibility of these isolates was tested against nine antimicrobial agents (ampicillin, clindamycin, cephalexin, ceftriaxone, erythromycin, gatifloxacin, meropenem, penicillin and tetracycline) by using minimum inhibitory concentrations.

**Results:** RAPD results showed that all the *Streptococcus dysgalactiae* isolates produced 4 to 8 different banding patterns and were divided into nine genotypes. A dominant genotype was found in different areas like in Beijing, Hebei and Inner Mongolia provinces. In addition, sixteen (17.2%) isolates were found susceptible to all the tested antimicrobials using the Clinical and Laboratory Standards Institute (CLSI) breakpoints. Thirty-one (33.3%) isolates expressed resistant to a single compound and forty-six (49.5%) isolates showed resistance to more than one antimicrobial agent. The antimicrobial agents to which antimicrobial resistance was demonstrated most frequently were erythromycin (50.5%), clindamycin (41.9%), cephalexin (32.2%) and tetracycline (31.2%). While, ceftriaxone (12.9%), penicillin (15.1%) and gatifloxacin (7.5%) were the antimicrobial agents with the lower level of antimicrobial resistance. None isolates expressed resistant to ampicillin and meropenem.

**Conclusions:** The diversity of *Streptococcus dysgalactiae* genotypes in areas of Beijing, Hebei and Inner Mongolia provinces indicated that environmental conditions may be an important factor. Furthermore, the prevalence of *Streptococcus dysgalactiae* and their antimicrobial resistance profiles strongly varied among dairy herds, demanding for antimicrobial susceptibility surveillance at the herd-level to ensure optimal therapeutic results.

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AR-P13

### Detection of polymyxin B-resistant *Escherichia coli* carrying MCR-1 gene in beef cattle in Heilongjiang Province, China

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**Objectives:** MCR-1 is the first plasmid-mediated polymyxin-resistance gene in *Enterobacteriaceae*, detected in livestock and intestinal bacteria. Polymyxin antibiotics are considered a last line of defense in the treatment of severe infections, and MCR-1 has attracted global attention. MCR-1 was found in *Enterobacteriaceae* from humans and pigs, but no reports on beef in-

fections existed. The objective of this paper is to screening MCR-1 in case of calf diarrhea, and analyze relationship among MCR-1, resistance genes, virulence genes, and pathogenicity.

**Materials and Methods:** Beef diarrhea fecal samples were collected from various beef farms in cities Harbin, Daqing, Zhaodong, and Suihua of Heilongjiang province. *Escherichia coli* (*E. coli*) strains were isolated from the fecal samples and undergone biochemical test and antibiotics sensitivity test. Antibiotics-resistant MCR-1 gene, 15 resistance genes, and 14 virulence genes in isolated *E. coli* were detected using PCR method.

**Results:** Among the 40 *E. coli* strains isolated, one strain from Daqing Yinlang beef farm was found carrying MCR-1 gene using PCR method. The identified sequence showed 100% similarity with MCR-1 gene sequences in NCBI database using blast analysis. *E. coli* carried the MCR-1 gene was resistant to polymyxin B and 14 other antibiotics, and carried virulence gene (FyuA) and 11 resistance genes (blaTEM, Sul1, Sul2, aac(3)-IIa, aadA1, tetA, tetB, floR, GyrA, GyrB, ParC). Animal pathogenicity test results showed that the strains carrying MCR-1 gene and the strains negative for MCR-1 induced death of experimental mice at more than  $1.0 \times 10^8$  CFU/ml. *E. coli* was isolated from the recollected samples from Daqing Yinlang beef cattle farm, but the MCR-1 gene was not detected.

**Conclusions:** Our study is the first to experimentally demonstrate that *E. coli* strain from the beef diarrhea fecal sample was found to be carrying MCR-1 gene, which provided an early warning for safe production and food hygiene of beef cattle.

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AR-P14

### Antimicrobial Resistance in Diarrheic Calves: Actual Status in southern Chile

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Neonatal diarrhea in calves is a multifactorial syndrome of global importance, and it is a major cause of death in those animals. Diarrheas that have bacterial etiologies are treated with broad-spectrum antibiotics, which have given rise to the phenomenon of bacterial resistance. The main microorganisms related to antimicrobial resistance are Enterobacteriaceae producing Extended Spectrum Betalactamases (ESBL), being able to inactivate penicillin, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins, among other antimicrobials. ESBLs are enzymes mediated by



plasmids with a high variability; among them we find: TEM, SHV, OXA and CTX-M and their characterization allows to know the resistance patterns and their diversity.

In order to know the antimicrobial status in diarrheic calves in southern Chile, here we summarize two studies, aiming to characterize: (1) the antimicrobial resistance observed in *Escherichia coli* strains and (2) the ESBL-producing enterobacterias, through specific microbiological cultures, antibiograms and conventional PCR from calf faeces.

Between January and October 2016, a convenience sampling was conducted in 20 dairies in the Regions of Los Ríos and Los Lagos, Chile. Ten faecal samples were collected in each dairy from 1-8 weeks old calves, where it was reported not to have been directly treated with antibiotics. All the samples were seeded in MacConkey agar, and later identified through biochemical batteries to detect and isolate *E. coli*, along with performing antibiograms using the disk diffusion technique to detect 7 of the most commonly used antimicrobials according to the National Agricultural Service. Furthermore, the same samples were seeded in a specific agar (Oxoid, ESBL Brilliance Agar®), which provides a presumptive identification of ESBL-producing enterobacteria such as *E. coli*, *Klebsiella*, *Serratia*, *Citrobacter*, among others. Once the ESBL-producing colonies were identified, each of them was isolated and then subjected to a DNA extraction protocol. To characterize the bacteria through the ESBL enzymes, seven pairs of primers were used, corresponding to the most common ESBL gene groups, producing the TEM, SHV, OXA and CTX-M enzymes. A conventional PCR was performed, and finally the products were processed by electrophoresis and visualized in a transilluminator.

Results showed that *E. coli* was isolated in 194 samples (97%) and 74% of the colonies presented multiresistance or extreme resistance. Resistance to amoxicillin predominated, while for the multiresistance and extreme resistance profiles, the constant repetition of the amoxicillin, oxytetracycline and sulfamethoxazole-trimethoprim patterns stands out, being those the most commonly used antimicrobials to treat diarrheic calves. Additionally, it was found that calves under 21 days had higher levels of resistance than the older ones.

Regarding the ESBL-producing bacteria characterization, 138 samples (65%) showed ESBL-producing colonies and 158 colonies were detected, being 145 (92%) of them *E. coli*, followed by *Proteus*, *Morganella* and *Providencia* with 4.4% and finally the KESC group (*Klebsiella*, *Enterobacter*, *Serratia* y *Citrobacter*) with 1.3%. Of the enzymes analyzed, the CTX-M group was the most frequent, followed by TEM. It was observed that some colonies presented reactivity to more than 1 group of enzymes.

*E. coli* isolates in diarrheic calves showed a high level of resistance. It is important to highlight that a large number of *E. coli* colonies were resistant to more than 3 antimicrobials, which makes it difficult to treat these infections. The most frequent ESBL detected was the CTX-M group, and also was observed that some colonies produced more than one group of enzymes.

In the short term, these baseline studies should continue and evolve to provide tools to evaluate the antibiotics administration in the dairy production and to determine the importance of this information in the calves' health.

In the Chilean dairy industry, it is estimated that on average 28.5% of all lactations will include a mastitis infection, which

are treated with broad spectrum antibiotics such as 4<sup>th</sup> generation cephalosporins; this fact could potentially explain the source of resistant bacteria in calves fed with discarded milk in our country.



WE-P01

## Tail damage in dairy cattle in New Zealand

### An analysis of the incidence of tail damage nationwide as part of a larger animal welfare assurance programme

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**Objectives:** Broken and damaged tails are an unacceptable feature of dairy farming. There is an obvious welfare impact, as well as a significant impact on the social licence of dairy farming to continue to operate as it does. Besides the obvious welfare impacts, the effect of such damage includes alteration of normal behaviour, such as feeding, with flow-on impacts on productivity.

Although rare, there have been several farmers investigated and prosecuted over recent years in New Zealand for deliberately breaking their cows' tails. However, the inconspicuous nature of broken tails in cattle (i.e. they require close inspection and palpation to identify) and the lack of objective data about their incidence mean the problem remains largely hidden from the industry. This leads to the assumption that this is not a ubiquitous nor significant problem and that these high profile cases are exceptions to the rule.

This paper reports the only New Zealand incidence data we are aware of that exists in this regard, which has been collected since 2013 through a national farm welfare assurance program.

**Materials and Methods:** Data were collected from dairy farms over a three year period. Tail scoring was performed once per season on each farm. Tails were scored as either normal (healthy, undamaged), broken (i.e. evidence of a fresh or past fracture), docked too short (i.e. higher than the last two or three vertebrae) (Animal Welfare (Painful Husbandry Procedures) Code 2005), or damaged (encompassing any other forms of tail damage other than fractures and inappropriate docking). Scoring was performed by trained veterinarians or veterinary technicians.

Broad benchmarking results were reported online through a secure dashboard (and available as pdf download) as the proportion of cows in the herd with normal tails, benchmarked regionally and nationally (all five regions represented collectively), for each farm.

Data for this paper were downloaded into a secure database and analysed using proprietary software programmes.

**Results:** A total of 267 whole-herd, tail scores were completed over the three seasons, representing 134,054 cattle individually scored. The average proportion of cows with normal tails was 0.85, ranging from 0.81-0.87 across seasons, and 0.73-0.93 across regions. Within-herd prevalence of abnormal tails ranged from 0 to 100% with 99% of all herds scored found to have some proportion of their herd with abnormal tails.

In all except one instance, individual regional results for each season showed that tail fractures were the most common form of tail damage recorded, with an average proportion of cows with broken tails of 0.08, ranging from 0.07-0.09 across seasons, and 0.06-0.13 across regions.

The proportion of cows with tails docked too short was fairly consistent across all seasons, averaging 0.05 (range 0.04-

0.05), but showed significant variation across regions, ranging from 0.0-0.11.

The remaining proportion of cows with damaged tails (from other causes) averaged 0.03, ranging from 0.01-0.06 across seasons and 0.0-0.08 across region.

There was a significant difference in the proportion of abnormal tails for each of the three seasons, with the greatest difference between 2014-15 and 2016-17 seasons (0.13 and 0.19). There was also significant regional variation, with Southland and South Otago region showing the greatest proportion of cows with abnormal tails, averaging 0.27 across all seasons (range 0.23-0.30). Herd size did not have a significant effect on the within-herd proportion of abnormal tails.

**Conclusion:** This paper reports the first known objective data on the prevalence of tail damage in dairy cattle in New Zealand. This data shows that, on average, 15% of dairy cattle in New Zealand may have experienced some form of tail damage during their lifetime, with almost every herd surveyed found to have some proportion of cows with damaged tails. The data suggests that broken tails are in fact a significant animal welfare concern throughout the national dairy herd and that a greater understanding of their cause and prevention is needed.

WE-P02

## Usefulness of human-conditioned sorting behavior in dairy cows for farm supplementation trials

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The cow-human relationship can influence and modulate group and individual behaviors in lactating dairy cows. To test the effectiveness of human sorting to separate subgroups of lactating dairy cows for farm studies and the level of conditioning to this activity, three sorting methods applied to the same cows were compared: 1) human active sorting (**AS**) at the pen gate, 2) human passive sorting (**PS**; human presence at pen gate), and 3) non-human gate sorting (**GS**). We hypothesized that after a training period cows became conditioned to human sorting allowing for an effective animal separation. One-hundred and seventy six Holstein cows (parity = 2.5 ± 1.3; mean ± SD) housed within the same lactating group were assigned to one of the two subgroups (treatment = 91 animals and control = 87 animals) to be separated subsequently by the three sorting method (1<sup>st</sup> AS, 2<sup>nd</sup> PS and 3<sup>rd</sup> GS). Each sorting method was observed for 5 d and the number of cows correctly allocated was recorded. The counts of correctly allocated cows and the error rate of each treatment group were compared by sorting method. Additionally, the individual error index rate was compared with treatment group, parity and general activity ratio (daily rumination/daily activity). When AS was applied, 99.8% of the animals were correctly sorted in their subgroup whereas PS had 94.8% of sorting accuracy. GS could not be assessed accurately because the cows lost the self-sorting behavior in the final assessment, overcrowding one side of the pen, making impossible the counting and data collection. The AS had a greater average of animal correctly placed when compared to



PS ( $175.2 \pm 1.3$  vs.  $166.6 \pm 3.5$ ;  $P = 0.004$ ) during the observation period. Cows that had longer walking distance to their subgroup had greater individual error rate especially when PS was applied. No association was found between parity and general activity ratio with the individual error index. Researchers observed a clear self-sorting behavior in response to the presence of a human sorter, regardless the use AS or PS. Therefore, after a period of training lactating dairy cows became operant conditioned to human sorting, which represents an opportunity to perform animal separation without an intense human labor or stressing practices for the animals.

WE-P03

## How do veterinary, advisory and farmer perceptions of the causes of poor welfare in a pasture-based dairy industry differ?

### Perceptions about cow welfare

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**Introduction:** Herd size increased following milk quota abolition (2015) in Irish pasture-based dairy farms. However, investment in farm infrastructure and changes in farm management may not have occurred concurrently due to low and volatile milk price. ProWelCow (DAFM RSF - A 14/S/890) is a desk-based project which aims to investigate risks to dairy cow welfare and to develop strategies to protect it. As a first step this study aimed to investigate the perceptions of key stakeholder groups on the main causes of poor welfare in Irish pasture-based dairy cows. Specifically, this study aimed to establish the changes in and opinions about such infrastructure/management issues on Irish dairy farms in the 3 years (2012-2015) which have potential implications for cow welfare in the future expanded dairy herds.

**Materials and methods:** A questionnaire was designed and piloted before use in the surveys. The survey was conducted with farmers (F;  $n=115$ ) at two national farming events and cattle vets (V;  $n=60$ ) at a national veterinary conference using a structured questionnaire, by interview. Teagasc dairy advisors were asked to complete the questionnaire themselves (A;  $n=48$ ) at a national training event. Results are expressed as % of group surveyed. The 223 respondents were asked to identify the main causes of poor welfare in cows from the following list: lameness (L), social stress due to overcrowding (SS), mastitis, metabolic disorders, infectious diseases, poor body condition score (pBCS), cold stress and calving difficulties. A Chi-Square Fisher test, (PROC FREQ,SAS), was used to investigate whether distributions of response frequencies differed for the 3 main causes of poor welfare between all respondents (% of all respondents) and between stakeholders groups (% of respondents within a poor welfare cause category).

**Results:** Three main causes of poor welfare of cows in pasture-based dairy systems differed in importance between all respondents (SS: 25.9%, BCS: 16.7%, L: 15.3%;  $X^2 = 7.5$ ; (df= 2)  $p=0.02$ ); SS, as a primary cause of poor welfare, was equally important for F, V and A (F: 7.9%, V: 8.3%, A: 9.7%;  $X^2 = 0.5$ ; (df= 2)  $p=0.8$ ). pBCS was rated as a primary cause by the ma-

ajority of F (F: 12%, V: 2.3%, A: 2.3%;  $X^2 = 24.5$ ; (df= 2)  $p < 0.0001$ ); while L was rated as a primary cause by the majority of V (F: 6.9%, V: 7.9%, A: 0.5%).

**Discussion:** Stakeholders agreed about the importance of SS as the main cause of poor welfare, which is perhaps surprising in dairy cows in pasture-based systems. There was a lack of consensus regarding pBCS and L. This probably reflects the differing focus and areas of expertise between the three stakeholder groups. However, all listed issues are important causes of poor cow welfare in expanding, low-cost, pasture-based systems.

**Conclusions:** Given these results, further research is warranted focused on these identified primary causes of poor welfare in Irish pasture-based dairy cows.

WE-P04

## Influence of the analgesic effect of carprofen administered to heifers in the postpartum period on animal welfare and milk production

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**Objectives:** Administration of non-steroidal anti-inflammatory drug (NSAID) to heifers and cows during first hours after parturition even if calving action seems to be physiological gives the positive effect on animal welfare. In addition this peripheral effect of NSAID produces also central analgesia via spinal and supraspinal mechanisms and reduces of stress and cortisol level which helps to improve the appetite of animals and thereby reduce the negative energy balance, which in turn results in higher milk production.

The aim of this study was to assess the effectiveness of a single administration of carprofen (Rimadyl® Cattle, Zoetis, Belgium) to the primiparous cows within the first 6 hours postpartum on their overall health and milk production.

**Materials and methods:** The Ethical Committee for Animal Experiments, Wrocław, Poland approved this study, and all owners provided informed consent prior to initiation of the study.

Observations of 140 heifers were carried out during perinatal period until the end of first lactation in a dairy herd of 630 cows. Animals had not access to the pasture and were fed a TMR feed, twice a day. During observation period heifers were divided into two groups:

Group (A) - 70 heifers; which were administered a single intravenous dose of 1.4 mg carprofen/kg b.w. - 1 ml / 35 kg b.w. of



Rimadyl® Cattle (Zoetis, Belgium), 2 to 6 hours postpartum.

Group (B) control - 70 heifers; which were administered placebo (0.9% NaCl) in a single intravenous dose of 1 ml/35 kg b.w., within 2 to 6 hours postpartum.

Statistical analysis was performed using Statistica v. 10 (StatSoft, USA) and Mann-Whitney U test ( $p > 0.01$ ).

**Results:** Milk production measurements during the first seven days of lactation showed statistically significant differences in favour of Group A (Table 1).

Table 1. The monthly milk yield of primiparous cows (kg/month) and the result of comparison by Mann-Whitney U test ( $p > 0.01$ ).

#### Months post partum

##### 1st month ( $M \pm SD$ )

Group A (n=70) - 763  $\pm$  110

Group B (n=70) - 657  $\pm$  146

Test result ( $p > 0.01$ )  $p < 0,001$

##### 2nd month ( $M \pm SD$ )

Group A (n=70) - 1000  $\pm$  129

Group B (n=70) - 874  $\pm$  166

Test result ( $p > 0.01$ )  $p < 0,001$

##### 3rd month ( $M \pm SD$ )

Group A (n=70) - 940  $\pm$  226

Group B (n=70) - 858  $\pm$  229

Test result ( $p > 0.01$ )  $p < 0,045$

##### 4th month ( $M \pm SD$ )

Group A (n=70) - 961  $\pm$  149

Group B (n=69) - 880  $\pm$  167

Test result ( $p > 0.01$ )  $p < 0,005$

##### 5th month ( $M \pm SD$ )

Group A (n=70) - 963  $\pm$  155

Group B (n=69) - 851  $\pm$  187

Test result ( $p > 0.01$ )  $p < 0,001$

##### 6th month ( $M \pm SD$ )

Group A (n=70) - 944  $\pm$  147

Group B (n=69) - 831  $\pm$  206

Test result ( $p > 0.01$ )  $p < 0,001$

##### 7th month ( $M \pm SD$ )

Group A (n=70) - 939  $\pm$  151

Group B (n=62) - 845  $\pm$  239

Test result ( $p > 0.01$ )  $p < 0,002$

##### 8th month ( $M \pm SD$ )

Group A (n=54) - 922  $\pm$  193

Group B (n=47) - 818  $\pm$  222

Test result ( $p > 0.01$ )  $p < 0,025$

##### 9th month ( $M \pm SD$ )

Group A (n=44) - 919  $\pm$  227

Group B (n=34) - 850  $\pm$  216

Test result ( $p > 0.01$ )  $p < 0,227$

In each month of lactation period the average milk production was higher in Group A (954.4 kg/cow) than in Group B (848.5 kg/cow). The difference was statistically significant ( $p = 0.001$ ).

**Conclusion:** Carprofen is a well known NSAID and is widely used in veterinary medicine. Drugs of this type bring relief to animals suffering pain which results in significant performance improvement of dairy cows. In our trial we observed significantly higher milk production in first lactation of primiparous cows what can be explained by analgesic action of caprofen bringing pain relief and stress limitation.

It can be stated that administration of one injection of carprofen to heifers in the first hours after calving had positive effect on animal welfare and resulted in better performance during first lactation period.

**Literature:** Lohuis J.A.C.M., Schukken Y.H., Verheijden J.H.M., Brand A., Van Miert A.S.J.P.A.M.: Effect of severity of systemic signs during the acute phase of experimentally induced *Escherichia coli* mastitis on milk production losses. *J Dairy Sci* 73, 333-341, 1990. Mainau E., Temple D., Manteca X.: Welfare of dairy cows in perinatal period. *FAVEC No 4/Jan.* 2013. Stilwell G., Schubert H., and Broom D.M.: *Short communication:* Effects of analgesic use post calving on cow welfare and production. *J. Dairy Sci.* 97:888-891, 2014. Weary D.M., Niel L., Flower F.C., Fraser D.: Identifying and preventing pain in animals. *App Anim Behaviour Sci* 100, 64-76, 2006.

WE-P05

### The use of cabergoline at dry-off reduced the udder pressure in commercial dairy herds around Europe

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Upon cessation of milking, a large quantity of milk is accumulated in the udder for several days due to the cow continuing to produce milk. As a result, the udder engorges and increases its volume so that the intramammary pressure increases considerably. This situation causes discomfort and pain in dairy cows, the lying time after dry-off (DO) is reduced and cows are prone to leaking milk. Contradictory reducing the milk production gradually i.e. intermittent cessation of milking or feed restriction in late lactation or immediately after dry-off, has been shown to result in metabolic challenge and welfare problems in cows.

**Objective:** The objective of the study was to investigate the effect of Cabergoline (Velactis® Ceva Sante Animale) a potent dopamine receptor agonist on D2 receptors, on udder pressure 20-24 hours, 30-34 hours and 48-52 hours after DO on commercial dairy farms around Europe.

**Materials and methods:** Six hundred eighty-one dairy cows in



19 farms from 8 European countries were involved in this multi-centric, randomized and blinded study. The individual cow was the experimental unit. Udder pressure was determined using a calibrated algometer immediately before and after the last milking and 20-24 hours, 30-34 hours and 48-52 hours after DO. The measurement consisted of applying force to the caudo-ventral side in the middle of the rear-left and rear-right half udders about 10 cm above the teat base. The measurements were obtained with a coefficient of variation (CV) < 10 after three repeated measurements per point. After the last milking, the cows received dry cow therapy according to the farms usual practice. Three hundred and thirty-eight cows received an intramuscular injection of 5 ml of a solution containing 5.6 mg of cabergoline (CAB) and the remaining 343 cows were considered controls (CTR). Mixed-effects linear regression analysis was performed, using STATA® (version 14.0) software.

**Results:** Udder pressure was lower in the CAB group than in the CTR group at all three visits after DO. Average udder pressure measured in Newtons-N were 15.9, 17.0 and 16.3 in CTR group compared to 11.6, 12.7 and 13.7 in the CAB group at 20-24 hours, 30-34 hours and 48-52 hours after DO respectively. Results were independent of the production 24h before dry-off.

**Conclusion:** The results showed that the treatment with a single intramammary injection of 5 mL of a solution containing 5.6 mg of cabergoline resulted in a significantly lower udder pressure after the DO when compared to the control group. This treatment may have a positive impact on animal well-being by reducing the discomfort due to udder engorgement after dry off.

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WE-P06

### A pilot study into the use of metabolomics to identify biomarkers for the assessment of pain in calves following disbudding

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**Objectives:** The aim of this pilot study was to develop a method to determine the feasibility of identifying specific metabolites that could be used to represent a pain response and potentially highlight a subsequent potential welfare issue in young stock.

**Materials & Methods:** Ten Holstein heifer calves were recruited aged 4-6 weeks on a single dairy farm, fulfilling pre-specified recruitment criteria. They were kept as one group to reduce potential confounders and were routinely disbudded by a trained operative. All calves received a cornual nerve block (6ml Adrenacaine® (Norbrook)) 15 minutes prior to disbudding. Before recruitment, a selection criteria for calves to receive meloxicam was developed based on horn diameter. Five calves that met the allocation criteria received 0.5mg meloxicam/kg bodyweight (Metacam®; Boehringer Ingelheim) by subcutaneous injection 3 hours prior to disbudding.

Physical examinations (including; temperature, heart rate, respiratory rate) to assess physiological changes of the calves was carried out prior to disbudding and for 12 hours post-disbudding. Behavioural assessment was also carried out post-disbudding via video recording from 0-48 hours post-pro-

cedure. The recordings of the calves were used to produce behavioural ethograms including the frequencies of the behavioural signs associated with pain. Blood samples were also collected for infectious disease screening and sent to Fera Science for metabolomics and cortisol analysis, alongside saliva swabs. Samples were collected before disbudding and 6 hours post-disbudding, and were immediately frozen prior to transportation to the lab.

The blood samples were submitted to Fera for metabolomic analysis allowing for comparison between pre- and post-procedure samples and meloxicam and control animals to determine any potential significant markers of stress/pain. Frozen serum samples were defrosted and diluted before being centrifuged. The subsequent supernatant was then analysed by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). MS acquisition was undertaken in two analytical runs to cover positive and negative ionisation models. Samples were analysed in a random acquisition order and an analytical quality control was also developed. Saliva samples were analysed using the same methodology.

Xcalibur software and Progenesis QI were used to assess the data in a non-targeted manner. Statistical analysis was carried out on all 20 samples comparing metabolite profiles pre- and post-disbudding and on the 10 samples after disbudding comparing meloxicam vs. control.

**Results:** There was a general trend in increasing body temperature from pre-procedure to 10 hours post-procedure. The body temperature of control calves were recorded consistently higher than the treatment, however no significant association was demonstrated. There were little consistent deviations seen in the heart and respiratory rates recorded. This helps to highlight the need for an objective measure of pain.

Ear flicking & head shaking were the most common behaviours seen, more frequently seen in the control group. Head scratching also commonly exhibited, but was seen more frequently in the treatment group. Feeding frequency was also slightly higher in the treatment group than control, but it did not appear to alter their drinking behaviours.

From the serum samples analysed, the mean cortisol concentration was seen to be lower in both groups post-procedure, however the mean adrenaline concentration was higher in both groups post-procedure, and considerably higher in the control group. Following non-targeted metabolite profiling, 5757 potential metabolites were found across all samples. After further analysis (ANOVA, T-tests), 53 masses of interest remained. After further data filtering between the pre- and post-procedure samples, 8 metabolites remained of interest.

**Conclusions:** This pilot study highlights how reliance on physical examination alone cannot be relied upon as a specific marker of pain/welfare. The metabolomic analysis highlighted 8 metabolic markers of interest, potentially highlighting markers of stress/pain. It was not in the scope of this study to identify these markers further. However further work to do this could potentially highlight an objective measure of pain/welfare.

**Acknowledgements:** This pilot study was supported by Boehringer Ingelheim.



BU-P01

**Molecular detection of *Babesia* sp. in buffaloes (*bubalus bubalis*) from Santarém, Amazon, Brazil**

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**Objectives:** The buffalo (*Bubalus bubalis*) is well adapted to the Amazon region, and Pará state accounting for 38% of the Brazilian herd. Despite the socioeconomic importance of buffaloes in the northern region of Brazil, little is known about the prevalence of hemoparasites transmitted by ticks, such as *Babesia* sp. that affect these animals. Thus, the aim of this study was to detect *Babesia* sp. in buffaloes in the municipality of Santarém, Pará, as well as to analyze the risk factors related to *Babesia* sp. infection.

**Material and Methods:** A cross-sectional study with two-level sampling was carried out including 60 farms and 621 animals. Whole blood samples were obtained from all the sampled animals, which were inspected for ectoparasite that, if present, were collected and identified. For molecular detection of *Babesia* sp. whole blood and ectoparasite samples were submitted to DNA extraction and then to PCR with the primers, BAB-143-167 forward and BAB-694-667 reverse. Chi-square tests were performed to investigate possible risk factors (obtained through an epidemiological questionnaire) for *Babesia* sp. infection. Analyses were performed at animal-level (considering the entire population sampled) and herd-level (considering a positive farm with at least one positive animal).

**Results:** *Babesia* sp. was detected in 2.09% (13/621) of blood samples with a positive associations with the age group, presence of ticks and farm site ( $p < 0.05$ ), with younger animals (1-12 and 13-24 months old) being more prevalent (3.9% and 5.4%, respectively) than adult (>24 months) animals (0.8%). Buffaloes raised in dry land had 4.455 (1.02-19.40 95% CI) more chance to had *Babesia* sp. than animals from floodplains. *Babesia* sp. was detected in eight of the 95 ticks analyzed (7.6%), five in samples of *Ripicephalus (Boophilus) microplus* and three in *Amblyomma cajennense*, reinforcing the importance of these tick species in the epidemiology of babesiosis. *Babesia* sp. was not found in any of the 320 specimens of *H. tuberculatus*. The occurrence of *Babesia* sp. at farm-level was 16.67% (10/60) and farms from dry land had 9.0 times more chance (1.06-76.46 95% CI) to had infected animals than farms located in floodplains.

**Conclusion:** Buffaloes raised in Amazon were infected by *Babesia* sp and its prevalence was influenced by the unique characteristics of the Amazon ecosystem. The floodplain environment, widely used for buffalo farming, contributes to a minor infestation of ectoparasites and lower prevalence of *Babesia* sp. in animals. Further studies are needed to evaluate the epidemiological role of lice in the transmission of *Babesia* in buffaloes; however, the results obtained here may indicate that *H. tuberculatus* has a limited participation in the *Babesia* transmission in this species.

BV-P01

**Determination of the fetal protection in pregnant heifers challenged with bovine viral diarrhoea type 1 virus twelve months after one administration of a live-attenuated vaccine**

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**Objectives:** Mucosiffa<sup>®</sup> (Ceva Santé Animale) is a modified-live vaccine (MLV) used for the control of bovine viral diarrhoea virus (BVDV) infection since 1982. In pregnant heifers experimentally challenged by BVDV-1, one administration of this MLV 126 days before challenge had been proven to prevent fetal infection (Meyer et al., 2012). Because current practices in cattle farms favors yearly vaccination schedules, a new experiment was carried out to evaluate the level of fetal protection obtained in pregnant heifers following a virulent challenge with BVDV-1 twelve months after one administration of the MLV.

**Materials and methods:** The study was performed according to the recommendations of EU pharmacopeia 04/2013:1952. Thirty-two Holstein heifers free of BVDV (antigen negative by PCR and seronegative for BVDV-1 and BVDV-2 by ELISA) were randomly allocated according to weight, breed and age to one of the following group at day 0: T1 (non vaccinated heifers, n=12) or T2 (heifers vaccinated with Mucosiffa<sup>®</sup>, n=20). All heifers were synchronized and received AI at day 276/277. On day 363, 15 pregnant heifers (5 from group T1, 10 from group T2) were challenged via the intranasal route with 10 ml of non-cytopathic BVDV-1f 22146/Han81 strain. At this time point, all the challenged heifers were between 60-90 days in pregnancy. Challenged heifers were humanely euthanized 28 or 29 days after challenge and their fetuses were harvested for the detection of the challenge virus in placenta and spleen (rt-PCR). Blood samples were collected from heifers at various times in the study to document their serological status (neutralizing antibody titers) and hematological parameters.

**Results:** The MLV administration was safe and no adverse events were observed related to the administration of the live-attenuated vaccine. All vaccinated heifers seroconverted 28 days after being vaccinated while control heifers remained seronegative. On the day of challenge, all the control heifers were seronegative (titer < 0.60 log<sub>10</sub> PD50) whereas all the vaccinated heifers were seropositive (mean titer = 2.82 ± 0.33 log<sub>10</sub> PD50). In control heifers, post-challenge period was marked by a peak of temperature (mean = 39.6°C) at day 367 and a significant decrease in leukocytes and lymphocytes counts (average value for lymphocytes at day 367 = 1.72 x 10<sup>9</sup> cells/L). On the opposite, rectal temperature and hematological parameters of vaccinated heifers remained unchanged during the post-challenge period (average counts for lymphocytes ≈ 6.1 x 10<sup>9</sup> cells/L). The efficacy of the BVDV-1f 22146/Han81 challenge was demonstrated by virus isolation from the fetuses of all five control heifers. The challenge virus was not detected in any of the fetuses from the vaccinated animals (100% fetal protection).

**Conclusions:** The MLV used in this study (Mucosiffa<sup>®</sup>) is safe and provides 100% fetal protection to breeding females for at least one year after one intramuscular administration.

**References:** Meyer, G., Deplanche, M., Roux, D., Moulignie,



M., Picard-Hagen, N., Lyazrhi, F., Raboisson, D., Mathevet, P., Schelcher, F., 2012. Fetal protection against bovine viral diarrhoea type 1 virus infection after one administration of a live-attenuated vaccine. *Vet. J. Lond. Engl.* 192, 242–245. <https://doi.org/10.1016/j.tvj.2011.05.011>

BV-P02

### Prevalence of positives animals for Bovine Viral Diarrhea Virus (BVDV) destined to exportation from Brazil to Turkei

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Bovine Viral Diarrhea Virus (BVDV) is the agent of Bovine Viral Diarrhea (BVD), a worldwide spread disease that causes economic losses in bovine breeding. BVDV is single strain RNA virus, belongs to the *Flaviviridae* family Pestivirus genus, which has high antigenic and molecular variability. The virus share close similarity with other important veterinary viruses from the same genus, like Classical Swine Fever and Border disease. In herds with BVDV infection, the more common clinical signs are abortion, stillborn and weak calf, less quantity and quality of milk production, less weight gain, diarrhea and respiratory diseases. Viral surveillance comes from BVDV ability of induce persistently infected (PI) animals. PI occurs when cows are infected with BVDV, between 40 and 120 days of gestation, and transfer the virus to fetus via transplacental. It can course with abortion or fetal immunotolerance (PI formation). PI animals will shed virus during whole life in all excretion and secretion, and it is the main maintenance of disease in herds. The aim of this research was to determinate the prevalence of animals positives for BVDV in a feedlot of calf from seven to 10 month old, males uncastrated, destined to exportation from Brazil to Turkey. Animals were housed in lots of 250 animal averages for 21 days of quarantine before shipping. A total of 7.374 animals were sampled by coccygeal venous puncture in 5ml tube without anticoagulant. Samples were centrifuge 10 minutes 1000 xg for serum separation and storage. Samples were tested for BVDV using an antigen capture commercial ELISA (BVDVag serum plus – IDEXX®) according to the label instructions. All positive animals were separated from animals destined to exportation. A total of 112 (1,51%) animals were tested positive for BVDV. According to international literature prevalence would be too high. It probably happens because most of the positive animals would be transitorily infected (TI) instead of PI. To confirm PI should be necessary a second positive test with, at list, 21 days after first test. Even we cannot confirm PI status from positive animal, we can consider the prevalence as a high risk of transmission and clinical/economic impact of BVDV spread in the Brazilian herd.

BV-P03

### Clinical Problems, Production Efficacy and Reproduction Efficacy in a Holstein Heifers and Cows Persistently Infected with Bovine Viral Diarrhea Virus

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The aim of this study was to evaluate the clinical problems, and the efficacy of production and reproduction in Holstein heifers and cows persistently infected (PI) with BVDV. A high producing commercial dairy farm (72,000 liters per day; with cow average of 38,5 litters per day) with a standing herd of about 3,700 animals was selected for this study. The PI screening was conducted using commercial ELISA antigen capture assays (Idexx) with tests done 30 days apart from ear notch samples. At screening, 25 PI animals were identified. Eight were heifers less than 12 months old; six were heifer between 13 and 24 months old; and eleven were animals from 25 to 36 months old. A control group composed of uninfected (NI) heifers and cows of similar age to the PI animals was randomly selected from the herd profile in Dairy Comp software. We observed an association between diarrhea score (P=0.012) and Bovine Respiratory Disease (BRD) score (P=0.004) between the PI and NI cattle using the Chi-squared test. We observed a higher frequency of diarrhea (P=0.074) and BRD (P=0.039) in PI than NI animals between 25 to 36 months of age. In aggregate, the total serum protein (TP) was lower in PI (6.53 g per dL) than NI cattle (6.99 g per dL) (P=0.021). With respect to age, we detected a significant difference in TP between PI and NI heifers less than 12 months of age (PI= 5.56; NI= 6.29 g per dL, P=0.022) and 13-24 months in age (PI= 6.57; NI= 7.20 g per dL, P=0.008). For haptoglobin, higher values were observed for PI (1.24 mg/dL) than NI (0.97 mg per dL) animals, 25-36 months of age (P=0.017). The analysis of milk yield indicated higher daily milk production for NI than PI cows during all phases of lactation (10 to 218 days in milk, P value less than or equal to 0.011). There was a difference of 10 to 19 litters between PI and NI cows over the course of lactation. Somatic cells counts (SCC) were higher for PI than NI cows across milk production (P value less than or equal to 0.066). The mean of SCC observed was 0,2-0,5 x10<sup>5</sup> (cells per mL) in NI group and 2,0-10,5 x10<sup>5</sup> (cells per mL) for PI cows. The age at first insemination (P=0.001) and number of insemination required for the first pregnancy was higher for PI than NI heifers (P=0.051). IN summary, PI heifers had more diarrhea, BRD, produced less milk, had high somatic cell counts and poorer reproductive performance than NI cattle. These findings are consistent with studies examining each of these factors in PI cattle individually.





BV-P04

### Innate and Humoral Immune Response in heifers and cows Persistently Infected with Bovine Viral Diarrhea Virus

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The aim of this study was to evaluate the innate and humoral immune response of Holstein heifers and cows persistently infected (PI) with BVDV. A high producing commercial dairy farm (~72,000 liters/day; with cow average of 38,5 liters/day) with a standing herd of about 3,700 cows was selected for this study. The PI screening was conducted using commercial ELISA antigen capture assays (Idexx<sup>®</sup>) with tests done 30 days apart from ear notch samples. At screening, 25 PI animals were identified. A control group composed of uninfected (NI) heifers and cows of similar age to the PI animals was randomly selected from the herd profile in Dairy Comp software. The absolute number and percentage of lymphocytes were lower in PI than NI cattle (PI=  $5.1 \times 10^3$ ; NI=  $6.7 \times 10^3$  per  $\mu\text{L}$ ,  $P=0.092$ ). In contrast, the percentage of monocytes was higher in PI than NI animals (PI=13.48%; NI=11.50%,  $P=0.014$ ). In aggregate, the percent of phagocytic cells and the average phagocytic avidity (expressed as mean fluorescence intensity, MFI) for *Staphylococcus aureus* and *Escherichia coli* were higher in PI than NI animals ( $P < 0.079$ ). The percentage of cells producing endogenous reactive oxygen species (ROS) activity was lower in PI than NI animals (PI=92.91%; NI= 94.86%,  $P=0.005$ ). A similar pattern was observed in the percentage of cells producing ROS after exposure to *Staphylococcus aureus* (PI=86.49%; NI=96.23%,  $P= 0.000$ ) or *Escherichia coli* (PI=85.30%; NI=97.85%,  $P= 0.000$ ). In contrast, the value for endogenous ROS per cell activity (MFI) was higher in PI than NI animals (PI=946.96; NI=526.05,  $P=0.011$ ). A similar pattern was observed for ROS MFI after stimulating with *Staphylococcus aureus* (PI=1559.08; NI=956.45,  $P= 0.011$ ) or *Escherichia coli* stimulation (PI=1582.66; NI=941.93,  $P= 0.013$ ). The median conventional SN titers for specific antibody recognizing BVDV (PI=  $\log_2 0.0$ ; NI  $\log_2 7.3$ ), BRSV (PI=  $\log_2 0.0$ ; NI=  $\log_2 5.0$ ) and BoHV-1 (PI=  $\log_2 5.0$ ; NI  $\log_2 8.0$ ) in serum were lower in PI than NI animals ( $P=0.000$ ). The antibody titers for BPIV3 (PI= 6.0; NI= 8.0) were similar between the groups animals ( $P=0,146$ ). Overall, there was a higher endogenous level innate cell reactivity in PI heifers and cows than NI animal. It appear that the PI animals were less efficient in clearance of antigens due to lower numbers of lymphocytes and related lower titers of specific antibody against respiratory viruses.

BV-P05

### Effect of BVDV and IBRV co-infection on virus replication and MDBK cells apoptosis

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**Objectives:** Bovine viral diarrhoea virus (BVDV) and infectious bovine rhinotracheitis virus (IBRV) are important viral diseases in dairy industry worldwide, and they both can lead to immunosuppression. There is a BVDV and IBRV co-infection in clinic. The objective of this paper was to study the effect of BVDV and IBRV co-infection on Madin-Darby bovine kidney (MDBK) cells apoptosis and replication of the two viruses, and lay the foundation for studying molecular mechanisms of viral infection and vaccine development.

**Materials and Methods:** 2 mL MDBK ( $5 \times 10^5$  cells/mL) cells were infected with three different concentrations (MOI 0.2, 1.0, 2.0) of cytopathic (cp) BVDV, reinfected with 0.1 MOI IBRV when 50% cytopathic effect was observed. The control group included MDBK cells infected with BVDV, IBRV, and no virus. We collected cells and extracted viral nucleic acids at 24h post-infection. Viral load was detected by real-time RT-PCR. Cell viability was detected by CCK-8. Virus localization was determined by indirect immunofluorescence. Apoptosis was detected by the TUNEL method and flow cytometry assays.

**Results:** There was no significant difference in IBRV copy number between co-infection group and IBRV infection group, when BVDV concentrations was 0.1 and 1.0 MOI. IBRV copy number of co-infection group was significantly lower than IBRV infection group, when BVDV concentration was 2.0 MOI. Indirect immunofluorescence test showed that BVDV and IBRV could co-infect MDBK and cause apoptosis. As the concentration of virus increases, cytopathic effect developed gradually. CCK-8 assay showed that cell viability was decreased as BVDV concentrations increases after co-infection, and cell viability of co-infection group was significantly lower than BVDV infection group. Apoptosis test results showed that co-infection rates was significantly higher than BVDV infection group, and BVDV could significantly influence IBRV replication.

**Conclusions:** BVDV and IBRV can co-infect MDBK cells, and both they can replicate in cells. High concentration BVDV affects IBRV replication by promoting cytopathic effect. Co-infection lead to more serious apoptosis than BVDV or IBRV infection alone.

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BV-P06

### Study of cross reactios between members of Flaviviridae Family: Hepacivirus and BVDV. Preliminary Data

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The bovine viral diarrhoea virus (BVDV) and novel bovine He-



pacivirus belong of *Flaviviridae* family. The first one is considered important pathogen in Brazilian cattle which has been detected as BVDV I, BVDV II and atypical BVDV (HoBi-like). The bovine Hepacivirus (BovHep) of *Flavivirus* genus presented rare data and is characterized by difficult to isolation and replication in cell culture, like human Hepacivirus (HCV) that is a threat in human health causing hepatitis, cirrhosis and hepatocellular carcinoma. For present study, it was analyzed 683 bovine blood samples from North region of Brasil for BVDV by RT-PCR. The viral RNA were obtained using Cador Pathogen 96 in automated extraction machine QIACubeHT (QIAGEN) and kepted at -20°C until analysis. For RT-PCR it was used a set of primers of 5'UTR region of BVDV that detected 1% (7/683) of positive animals. In contrast ELISA BVDV-Ag/Serum Plus (Idexx Laboratories HerdChek<sup>®</sup>) only detected 1 animal (1/7) positive for BVDV. The seven PCR positive samples were submitted to purification using Wizard SV Gel and PCR Clean UP System (Promega) and then to sequencing by capillar eletrophoresis (3500XL Genetic Analyzer/Applied Biosystems). From seven BVDV positive animals by RT-PCR, 6 animals presented 96% of identity with BovHep while just one animal had presented 100% of identity with atypical HoBi-like BVDV confirming the result obtained in ELISA BVDV-Ag. The analyze of 5'UTR BVDV set primers showed that reverse primer alignment with regions of BVDV and BovHep. The present study showed the necessity to improve knowledges about BovHep and implications for cattle industry.

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BV-P07

### Risk factors associated with the introduction of BVDV into a herd

#### *A review of European literature*

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In the Netherlands BVDV is endemic. Therefore herds that are BVDV free are subject to a considerable risk of (re)introduction of the virus. If BVDV is introduced into a free herd this will lead to economic losses for the farmer. Currently, voluntary programs for the control of BVDV are available and preparations are ongoing for a national plan for the eradication of BVDV. The Dutch cattle industry is evaluating different options for eradicating the disease. A veterinary plan has been developed and different scenarios are being considered to determine the effectiveness and economic consequences of a future national BVDV eradication program. These results will be the basis for decision making by the stakeholders concerning the approach to eradicate BVDV from the Netherlands. In the course of enrolling a BVDV eradication campaign in the Netherlands, communication with farmers, veterinarians and other professionals in the cattle industry is vital. There are different reasons for communication, for example explaining the program and sharing results so that participants will continue to be motivated. Another reason is to explain epidemiological aspects of a BVDV infection in an applied manner to educate farmers and their advisors on biosecurity measures to minimize the risk of introduction of BVDV into a herd. Knowledge of risk factors is therefore essential. The aim

of the study was to obtain evidence for risk factors for introduction of BVDV into herds through a literature review.

For this study, major scientific databases were systematically scanned for publications about risk factors for the introduction of BVDV at the herd level. Of the 224 unique studies found, 17 were selected. The requirements were European studies only, the language had to be English, Dutch or German with an English summary and the study had to quantify the risk factors for introduction of BVDV into cattle herds. Only European studies were included because they were considered representative for the Dutch cattle industry. Most studies quantified the risk factors with crude odds ratios (OR), the odds of a particular event occurring in an exposed group compared to the odds of occurrence of the event in a non-exposed group. The full text of 17 studies, concerning 12 different European countries i.e. the Netherlands, Belgium, England, Scotland, Ireland, Switzerland, Denmark, Norway, Italy, Spain, Lithuania and Croatia) were reviewed. One study used herds in both Denmark and the USA as a study population.

Results were classified in the following categories: herd characteristics (herd size, herd type, region/location, management system, age, sex), purchase related factors, other direct animal contacts (cattle shows, market, wildlife, sheep), grazing, neighborhood factors (e.g. distance, presence PI animals), risk of professional visitors and other risk factors, for example PI retention (retention of BVDV positive calves born in the previous calving season).

Our literature review confirmed that many risk factors play a role regarding the introduction of BVDV in a herd. Risk factors in one country may not have the same effect in another country. Relevant risk factors described were; management system, purchase related factors, grazing, neighborhood factors, other direct animal contacts, other animal species including wildlife and the risk of professional visitors.

Direct animal contacts are found to be the major risk factors for introduction of BVDV into cattle herds. Purchase of cattle of unknown BVD-status specifically when pregnant and contact with cattle from neighboring farms with an unknown BVD-status are important risk factors. Although contrasting results were found for biosecurity measures, it is advisable to take biosecurity measures for professional visitors that come in direct contact with cattle. Other animal species such as wildlife, sheep and goats can be considered of negligible risk for introduction of BVDV into cattle herds.

The different control scenarios for BVDV in the Netherlands should include risk mitigating actions for purchase of cattle as well as for the risk of neighboring herds.

**Acknowledgements:** We acknowledge the input from Prof. Mart de Jong and Prof. Miriam Nielen in the early stages of the study.

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BV-P08

### Epidemiologic survey and genetic analysis of bovine viral diarrhea virus (BVDV) type 1 in dairy herds of China

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**Objectives:** This study is to determine the prevalence and genetic typing of BVDV-1 infection in dairy herds of China.

**Materials and methods:** A total of 901 ear notches from less than 6 months old calves and bulk tank milk samples from 183 tanks were collected from 92 dairy farms of 19 provinces located in different regions of China in 2017. The bulk tank milk samples were directly analyzed by real time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) while all of the ear notch samples were first screened using IDEXX BVDV Antigen Test Point of Care (PoC) kit and the screened positive ear notches were retested to ascertain infection with BVDV by real time RT-PCR. A region of the 5'-untranslated region (UTR) was amplified by RT-nest PCR and the PCR products were sequenced. Phylogenetic analysis was performed based on the 5'-UTR sequences.

**Results:** A total of 80 of 183 (80/183, 43.7%) tank milk samples from 43 (43/92, 46.7%) of the 92 farms in 16 of 19 provinces were tested positive by real time RT-PCR while 10 (10/183, 5.5%) tank milk samples were defined as suspect. 21 positive ear notches from 10 (10/92, 10.9%) of the 92 farms were detected by the Antigen Test PoC kit and 20 (20/901, 2.2%) of them were confirmed positive by real time RT-PCR. Taken together, 5 farms were detected for BVDV in both ear notch and milk samples while 5 farms were only detected for BVDV in ear notches. Thus, BVDV was detected in a total of 48 of 92 (48/92, 52.2%) dairy farms located in 17 of 19 (17/19, 89.5%) provinces. 119 of 5'-UTR sequences were obtained from the amplicons by RT-nestPCR amplification by using two pairs of BVDV-1 specific primers. Phylogenetic analysis of the 5'-UTR regions revealed that the sequences classified into a variety of subgenotypes, including 1a (n=37, 31.09%), 1b (n=5, 4.2%), 1c (n=34, 28.57%), 1d (n=2, 1.68%), 1m (n=25, 21.01%), 1q (n=6, 5.04%) and two unknown subgenotypes (n=8 and 2, 6.72% and 1.68%, respectively). Subgenotypes 1a, 1c and 1m were the dominant strains with collectively accounting for 80.67% (96/119) of all sequences. The phylogenetic analysis based on selected Npro sequences confirmed the classification determined by the 5'-UTR sequences.

**Conclusions:** In conclusion, the high prevalence and increased genetic diversity of BVDV revealed a serious threat to dairy cattle health in China and highlights the need for BVDV control in Chinese dairy cattle.

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BV-P09

### Bovine viral diarrhea virus infection in Korean native goats

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**Background:** Infection with bovine viral diarrhea virus (BVDV) can occur in small ruminants, and clinical signs in these animals are similar to those in cattle. Field cases of BVDV in goats are typically characterized by reproductive failure, including abortion and poor viability of neonates. The population of Korean native

goats is increasing in the ROK, and their main products are meat and milk. Thus far, few studies on BVDV infection in Korean native goats have been reported. The objective of this study was to establish BVDV infection in Korean native goats.

**Methods:** Four goats were inoculated intranasally with the Korean noncytotoxic BVDV1b strain. Nasal swabs and blood were collected to detect viral RNA and BVDV antibodies. Extraction of RNA from the nasal swabs and blood samples was done using the PureLink Total RNA Blood kit. RT-PCR was performed using the 5'-untranslated region (UTR) and N-terminal protease region. The sequences were compared with the sequence of the virus used for experimental inoculations.

**Results:** The infected goats exhibited clinical signs of illness, such as coughing, nasal discharge, and pyrexia. Leukocytes counts were decreased on day 7 post-infection (pi), but leukopenia was not found. The depletion of lymphocytes was not observed in these goats. However, thrombocytopenia did occur in two goats on day 5 and was significant until days 9-12 pi ( $p < 0.001$ ). Viral RNA was detected on the nasal swabs on day 12 and in blood samples on days 14, 19, and 21 pi in two goats using the 5'-UTR and N<sup>pro</sup> regions. Antibodies to BVDV were detected in the same goats by enzyme-linked immunosorbent assay at days 16 and 21, but not on day 19. Sequence analysis on the nasal swabs and blood was confirmed as ncp BVDV1b.

**Discussion:** This study demonstrates that Korean native goats become infected with BVDV via the IN route and highlights the importance of surveillance in ungulates other than cattle. This study suggests that Korean native goats may be a potential reservoir for BVDV transmission in ungulates.

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BV-P10

### Comparative serology to subgenotypes BDV-1a and -1b in Italian dairy cattle operations

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**Objectives:** Bovine Viral Diarrhea Virus (BVDV) is widespread in Italian dairy cattle operations. BVDV infection involves over 70% of dairy herds. At the moment, three genotypes has been demonstrated for BVDV, namely BVDV-1, -2 and -3.

Molecular typing demonstrated seventeen subgenotype for BVDV-1, four subgenotypes for both BVDV-2 and BVDV-3. Viral isolation in BVDV outbreaks occurred in Italian dairy herds showed that BVD-1 subgenotype 1b followed by 1e are the most prevalent in Italian dairy cattle operations, while 1a has a low prevalence. BVDV-2 is scarcely demonstrated, not over 4%, of the examined cases. Vaccination and persistently infected (PI) animals detection and culling are the main tools to control-eradicate BVDV in Italy as worldwide. Since vaccine registered in Italy include only BVD 1a and 1b strains, to demonstrate the prevalence of the indicated viral subgenotypes overtime a comparative serological survey has been carried out on serum samples collected in the period 1995-2016.

**Materials and methods:** The study was set up in two steps. The first one included 419 serum samples from 36 dairy herds



and collected in 2015-2016; the second one, 200 serum samples from 25 dairy herds collected in the period 1995-2005. All the sera resulted seropositive for BVDV. The samples were kept frozen in a serum bank of the Department Veterinary Science – University of Parma. Most of tested animals belonged to dairy herds located in the Po Valley, Northern Italy and not submitted to vaccination for BVDV. In both the cases, each serum sample was tested for BVD 1a and 1b reference strains by serum neutralization test carried out in parallel.

#### Results:

**Step 1.** Serum neutralization test in parallel showed that 6% of serum samples were negative to both the subgenotypes, 32% were positive for BVDV 1b, 22% for BVDV 1a and for 41% was not possible to demonstrate the subgenotype responsible of the infection.

**Step 2.** The same test showed that 38% of serum samples were positive for BVDV 1a, 30% for BVDV 1b and for 33% was not possible to demonstrate the subgenotype responsible of the infection.

**Conclusion:** In many cases the test did not allowed to demonstrate the subgenotype responsible of the infection. Serum samples recently collected showed a higher positivity to BVDV type 1b. Conversely samples collected in 1995-2005 showed a higher positivity to BVDV 1a. We must underline that this serological survey did not allowed to demonstrate circulation of the other BVDV subgenotypes. Nowadays, on the basis of the obtained serological data, the vaccines containing BVDV 1b strain should be more efficient than 1a vaccines to control BVDV infection in our dairy cattle operations.

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**Objectives:** Acute bovine viral diarrhoea virus (BVDV) infections in non-pregnant sheep have been shown to be largely clinically in-apparent however infection of pregnant ewes has been shown to result in severe lambing losses, the development of persistently infected lambs and lambs born with developmental malformations. While the predominant Australian BVDV strain (BVDV-1c) has previously been reported to cause substantial lambing losses, the pathological outcomes in lambs, following gestational infection with BVDV-1c, have not been documented. As such the aim of this study was to characterise the wide range of pathological consequences seen in lambs born to ewes experimentally infected with BVDV-1c during early to mid-gestation.

**Materials and Methods:** Twenty two confirmed pregnant, merino ewes were experimentally inoculated with serum from a BVDV-1c persistently infected heifer by subcutaneous injection, between 59 and 69 days gestation. Ewes were left to lamb naturally and lambs remained with their mothers until death or euthanasia at 8 weeks of age. Fifteen aborted, stillborn, dead or euthanized lambs, from 10 ewes, were submitted for gross pathological and histological examination. The remaining 12 ewes were either scanned empty prior to lambing, were not observed to have lambed or aborted fetuses soon after infection.

**Results:** Six lambs were identified to be BVDV antibody positive and BVDV antigen negative while nine lambs were identified as BVDV antibody negative and BVDV antigen positive. A wide range of pathological lesions were identified in both subsets of lambs; anasarca, intrahepatic cholestasis, neurological malformations (hydrancephaly, porencephaly, cerebellar dysplasia) and skeletal malformations (arthrogryposis, facial asymmetry, brachygnathia) were most commonly observed. Anasarca in conjunction with a cholestatic hepatopathy was observed in four/nine antibody negative and antigen positive lambs. A hairy coat associated with primary follicular dysplasia, a developmental lesion normally identified with BDV infected lambs, was also identified in one lamb presenting with tremors and cerebellar dysplasia (“hairy shaker”).

**Conclusions:** This study details the gross and histopathological lesions identified in lambs, infected in utero by the predominant BVDV strain in Australia, BVDV-1c with many of the documented pathological outcomes in these lambs previously reported in calves infected with BVDV. Anasarca and neurological and skeletal malformations were the most common consequences following fetal infection although anasarca was associated only with lambs antibody negative and antigen positive for BVDV-1c. This study also reported primary follicular dysplasia in sheep infected with BVDV, a development normally identified with BDV infected lambs.

BV-P11

### Pathological outcomes observed in lambs born to ewes experimentally infected with BVDV-1c between 59 and 69 days of gestation

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BV-P12

### Acute BVDV infections in Australian alpacas

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**Objectives:** Bovine viral diarrhoea virus (BVDV), while predominantly a disease of cattle, has been reported to infect other ruminant and camelid species such as sheep and alpacas. While the clinical effects associated with acute BVDV-1c infec-



tions in Australian sheep have been recently documented, the effects following acute infections in alpacas have not. As such, the aims of this study were to determine if an acute BVDV infection would develop in naïve alpacas following co-mingling with a persistently BVDV-1c infected heifer; and in turn determine what, if any, clinical signs, haematological responses and selected biochemical changes can be seen due to an acute BVDV-1c infections in these animals.

**Materials and Methods:** A persistently infected heifer and four alpacas were co-mingled and housed together in a roofed 98m<sup>2</sup> pen, enclosed on three sides, for a period of two weeks. The four alpacas remained housed for a further 5 weeks upon removal of the PI heifer. Twice weekly clinical examinations were performed on the 4 alpacas for the full period of the study. Weekly blood samples were also collected and submitted for BVDV antibody and antigen testing (n = 4 alpacas) and blood biochemistry and haematology (n = 2 alpacas).

**Results:** Serum analysis by antibody ELISA indicated that all four alpacas were positive for BVDV-specific antibodies between 35 and 54 days post mixing with the persistently BVDV-1c infected heifer. Viral antigen was detected by antigen ELISA in two alpacas on Days 21 and 35 post initial mixing. In general all the physical clinical parameters measured were normal for all 4 alpacas.

Serum biochemical and haematological analysis for the 2 alpacas revealed marginally low sodium, chloride and elevated potassium concentrations, a lymphocytosis, a monocytosis and a neutrophilia at some point during the study period either in one or both of the alpacas. These marginal variations in blood biochemistry were transient and as such could be diet related.

**Conclusions:** This study has shown that following the co-mingling of a BVDV-1c PI heifer and naïve alpacas, acute BVDV-1c infections can develop in the alpacas. Results did also indicate that these acute infections are clinically mild and thus undetectable without serological testing.

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BV-P13

### Update on the epidemiology of bovine viral diarrhoea virus (BVDV) in Argentina: revising intervention strategies

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Bovine Viral Diarrhoea Virus (BVDV) is endemic in most regions around the world and has a detrimental effect on herds' performance. In Argentina, its high prevalence has been well established, although reports done almost 20 years ago showed quite heterogeneous distributions of prevalence values, going from 25 to 90% depending on the region. Strikingly, this feature was not studied in greater depth. Furthermore, the phylogenetic analyses described by the earliest reports in the 2000, differ significantly from recent findings. Bovine livestock production in Argentina has undergone, in the latest decades, a remodeling process due to the expansion of land used for crop.

This had a serious impact in structure of livestock production in the central area, as well as in the marginal territories. This process could have had a great effect on the dynamic of infectious diseases in the population. Even though vaccines are the main intervention strategy applied to control BVDV infection in the country, no control of vaccine matching is being implemented routinely. Purpose: The aim of this study was to assess the prevalence of BVDV in the main cow-calf productive region, and to compare it with historical reports. The serological profile in the population was assessed and interpreted, having into account the current phylogenetic information about BVDV isolates. Furthermore, a preliminary revision of the common management practices in concern to vaccine use was performed. Logic models framework for comprehensive monitoring and evaluation was applied in order to outline a systematic approach that allows to identify weaknesses and challenges. Methods: A cross-sectional study was performed in the Basin of Salado River. Samples were gathered from 100 herds located in 23 districts (the number of sampled herds was proportional to the animal stock per district), collecting an average of 25 samples per herd, making a total of 2750 samples. The samples were evaluated by virus neutralization (VN) for the presence of antibodies against three BVDV strains of different genotypes (1a, 1b, and 2). Endpoint titers were calculated using the Spearman-Kärber method of endpoint determination and then the VN results were compared by using the following formula:  $R = (3 \times A) / (A + B + C)$ . In this way, it was determined if the serological response from each animal was specific for each BVDV strain. The cut-off value to determine the specificity of the serological response was  $R > 0.2$ . In addition, a survey was completed on each farm to collect information that could be associated as herd-level risk factors for the disease. Information in concern to the use of vaccines that include BVDV in their formulation was also captured. Results: The results obtained show an individual prevalence of 64.6% (IC: 61.9% - 67.3%), and a 78% (70.3% - 85%) of positive herds, with a median intra-herd of 64.7%. The independent seropositivity against different genotypes tested was analyzed (1a, 1b, 2: 13.4-76.6-10%). Similar distribution of antibody titers were obtained against genotypes 1a and 2, while much more disperse distribution was detected for 1b. This result highlights the higher internal antigenic variability of the BVDV-1b isolates compared to the other ones. In concern to health management practices, 72% of the farms declared that they vaccinate against BVDV. Strikingly, 56% of the seronegative herds detected, declare to vaccinate, this could be due to the significant variability of vaccine protocols reported in this study. Conclusions: Preliminary results when analyzing the proportions of serologically positive animals against each strain show different frequency distribution among genotypes, which correlates with the phylogenetic results. Current vaccination practices could be jeopardizing the overall effectiveness. These results reinforce how essential is to have current and robust epidemiological information as a starting point to design rationally control plans for BVDV for any particular region. From this study also arouses new questions about the importance of estimating vaccine matching between a vaccine strain and the local isolates.



BV-P14

### Determination of antibodies by exposure to Bovine Viral Diarrhea Virus in Mexican cattle without vaccination

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Bovine viral diarrhea virus (BVDv) is classified into two species, namely, Bovine viral diarrhea virus 1 and Bovine viral diarrhea virus 2 within the genus *Pestivirus* and family *Flaviviridae*, together with classical swine fever virus and border disease virus. It's an important viral pathogen of cattle worldwide, resulting in significant economic loss. The infection in immunocompetent cattle ranging from subclinical or mild disease to a highly fatal form (Mucosal Disease). In addition, BVDv infection of susceptible pregnant cattle may result in fetal infection, with early embryonic death, abortion, or congenital malformations as possible outcomes, as well as the birth of calves immunotolerant to, and Persistently Infected (PI). Distribution data of biotype and genotype with clinical presentation is very important not only in controlling and understanding BVDv disease, but also in directing the choices for antigens to be utilized in vaccine and the correct treatment.

**Objectives:** In Mexico, there are few studies that reveal the prevalence or that mention the circulating strains present in cattle and in other species susceptible to BVDv infection. The purpose of this study was to determine the seroprevalence of BVDv in cattle from different productions (zootechnical purpose) and age located in the states of Oaxaca, Tabasco, Chiapas, State of Mexico and Mexico city through a commercial ELISA kit.

**Material and methods:** 549 samples of blood serum were collected from female and male cattle between 2 and 40 months of age, with no history of vaccination against BVDv or other viral agents (BHV-1 or IBR, BRSV, BPI-3), with suggestive BVDv (respiratory signs: cough, runny nose, rales). The procedure as well as the interpretation of the results was carried out according to the manufacturer's specifications. The ELISA test showed a sensitivity of 100% and specificity of 98.8%.

**Results:** The total seropositivity was 42.41%. By state were the following: State of Mexico (27.9%), Tabasco (26.21%), Chiapas (25.26%), Mexico City (52.56%) and Oaxaca (82.58%); by zootechnical purpose were the following: Milk (51.8%), Meat (4.54%), Dual purpose (16.88%) and Breeding stock (26.4%), and by age range were: 1-6 months (24.13%), 7-18 months (23.84%), 19-24 months (20.91%), 25-48 months (25.26%) and 49 or more months (3.84%).

**Conclusions:** It is concluded that there is seropositivity to BVDv, in addition to the production of specialized dairy cattle is where there is greater seropositivity (51.8%), while the animals most susceptible to disease by BVDv are those in the age range of 25- 48 months of age (25.26%) and 1-6 months of age (24.13%). The lack of vaccination associated with respiratory signs during the clinical examination and the presence of residual antibodies is an indication of an acute BVDv infection. As for the animals seronegative at diagnosis, Persistently Infected (PI) animals may be suspected, until other diagnostic tests (RT-

PCR, viral isolation) confirm it.

BV-P15

### Genetic diversity of Bovine Viral Diarrhea Virus and vaccine efficacy

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**OBJECTIVES:** The genetic diversity of bovine viral diarrhea virus (BVDV) between 2004 and 2011 in Poland indicated the predominance of BVDV-1b and BVDV-1d. In years 2012-2016 the most dominant subtype was BVDV-1f and BVDV-1d. Additionally BVDV-1e was identified on one farm in 2 animals and BVDV-2a was found in 4 animals from a single farm. However until recently only BVDV-1a based vaccines were used in Poland. Therefore the aim of the study was to identify herds where BVDV-1a based vaccination was not successful, leading to the birth of PI individuals due to genetic diversity of the field strains of the virus.

**MATERIALS AND METHODS:** The material used in the study came from 4 herds with clinical signs resembling BVD where vaccination against BVDV (subtype 1a) was introduced 2-3 years ago after identification and removal of PI animals. Clinical problems were observed mostly in calves and heifers and they included respiratory problems. Additionally, in one herd mortality rates reached 40% of affected calves. Serum samples from those herds were submitted for further analysis. Total RNA was extracted using TRI Reagent (Sigma-Aldrich, USA), according to the manufacturer's instructions and stored at -70°C until testing. Reverse transcription-polymerase chain reaction was carried out using the reagents from Transcriptor One-Step RT-PCR Kit (Roche, Germany) following the manufacturer's protocol. The complementary DNA (cDNA) was amplified using the set of primers specific for viral 5'-UTR region using Vilcek primers. The amplicons were gel-purified and sequenced in both directions using the same primers as for the RT-PCR with Big Dye Terminator v3.1 Cycle Sequencing Kit with a 3730XL Genetic Analyzer (Applied Biosystems). Subsequently, the DNA fragments were purified using a QIAquick PCR Purification kit (Qiagen), following the analysis in a 16-capillary sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and the results were analyzed using GeneScan Analysis Software (Applied Biosystems). The obtained sequences were aligned using CLUSTAL Omega software. Phylogenetic analysis was performed using the MEGA software (version 5.05). Bootstrap analysis was carried out on 1000 replicates. The nucleotide sequences were compared with each other using the identity matrix in BioEdit v.7.1.9 software and with the 5'-UTR regions of the reference strains available in the GenBank database using blastn maximum identity tool (NCBI). Phylogenetic trees were constructed using the neighbor-joining method.

**RESULTS:** Seven PIs were identified in 4 herds (1 animal, 1, 2 and 3 in each herd). All PIs were from 4 to 19 months old. Genetic typing within 5'UTR region identified single subtypes in each farm. Four animals were identified as subtype 1d and 3 animals were typed as BVDV-1b. None of the mothers of those PIs was persistently infected indicating recent introduction of the virus to the herds in question.



**CONCLUSIONS:** Despite previous removal of virus shedders and the implementation of prophylactic vaccination with killed vaccine, new PI animals were identified in 4 herds although good management practices and good veterinary care were common practice. It was impossible to identify the source of the virus in those herds. The use of a vaccine based on the BVDV subtype not present in those herds (1a) did not protect the foetuses from intrauterine infection and the establishment of the persistent infection. However, since none of the mothers of PI offspring was persistently infected it was concluded that fresh infections with BVDV field strains took place in those farms.

BV-P16

### Trends in detection of BVD virus in ear notch samples from aborted foetuses during the Irish BVD eradication programme

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**Objectives:** The current study was undertaken to assess the changing prevalence during the course of the programme between 2013 and 2016 and to compare the prevalence of infection in foetuses to that detected in neonatal calves in each of these years.

**Materials and methods:** The database of the network of Regional Veterinary Laboratories of the Department of Agriculture, Food and the Marine was analysed to identify aborted bovine foetuses submitted between 2013 and 2016 with full official identification numbers. The database of the national BVD eradication programme, provided by the Irish Cattle Breeding Federation was then queried to retrieve available BVDV test results for these foetuses.

**Results:** A full animal identification number was available for 8,137 of 9,805 (83.0%) aborted foetuses submitted between 2013 and 2016, with similar numbers of submissions received each year. The ICBF database contained BVDV test results for 6,215 (76.4%) of these foetuses. Submissions were seasonal, with the majority received between November and March, and predominantly (71.0%) from dairy herds. When 287 samples with empty or invalid results were discounted, 1.21% were positive overall. Analysis of these data by year showed a marked and steady decrease in prevalence each year, with 2.24%, 1.37%, 0.97% and 0.27% testing positive in 2013, 2014, 2015 and 2016 respectively. These levels were higher than the prevalence of PI calves born in each of these years, based on tissue tag testing of over two million calves per year within the national BVD eradication programme, when values of 0.66%, 0.46%, 0.33% and 0.16%, respectively, were reported.

**Conclusions:** The prevalence of BVDV in aborted foetuses declined in each year of the programme, reflecting progress towards eradication. However, the prevalence detected in foetuses each year was higher than that detected in neonatal calves during the same year, highlighting the pre-natal losses associated with BVDV and the value of foetal testing for surveillance purposes and the importance of encouraging submission and

testing of all aborted foetuses.

BV-P17

### Investigation of the potential for sera from cattle persistently infected with bovine viral diarrhoea virus (BVDV) to generate false-negative antibody results by indirect and competitive ELISA when included in serum pools containing varying proportions of seropositive and seronegative cattle

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**Objectives:** Serological screening (check testing) of groups of young stock for evidence of exposure to bovine viral diarrhoea virus (BVDV) is commonly as a surveillance tool used to provide evidence of freedom from infection. herd level. In Ireland, testing of 10 animals per separate management group has been proposed as one element of post eradication surveillance. Pooling of samples offers the potential to significantly reduce costs. However, the possibility exists that the presence of serum from a PI animal in a pool of sera containing one or more seropositive animals could result in a false negative antibody result and consequently an infected herd being wrongly categorised as non-infected. The primary objective of the current study was therefore to test the hypothesis that the inclusion of viraemic sera in pools of 10 sera with a variable seroprevalence (10-90%) does not result in false negative serology results.

**Materials and methods:** Twenty-three seronegative viraemic sera were assayed in the presence of artificially constructed pools containing varying percentages (zero to 90% in 10% increments) of antibody-positive sera. Each sample was tested by antibody using an indirect and a competitive ELISA and for BVDV antigen using an antigen capture ELISA.

**Results:** In all twenty three cases, a negative antibody result was obtained in the pool containing no positive serum. In contrast, all pools containing 10% or more positive sera, representing a single seropositive animal in a pool of ten samples, returned a positive result in both antibody ELISAs. The BVD antigen signal reduced as the proportion of antibody positive serum increased, becoming negative at between 20% and 50% levels for the viraemic sera tested.

**Conclusions:** It was concluded that concern over the impact of the presence of serum from a PI animal does not preclude the use of pooled serum samples in the context of serological surveillance using check tests of ten samples per management group, even when only a single seropositive animal is present in the sampled group. In contrast, false negative results would be anticipated if testing pooled sera for antigen.



BV-P18

### First ever commercially available vaccine against BDV targeted to immune system cells

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Bovine diarrhoea virus (BDV) is considered an important cause of economic loss within bovine herds worldwide. In Argentina, only the use of inactivated vaccines is allowed, however, the efficacy of inactivated BVDV vaccines is variable due to its low immunogenicity. The aim of this work is to present Vedevax®, the first ever commercially available targeted vaccine based on a truncated version of the structural protein E2 from BDV fused to a molecule named APCH, that target to antigen presenting cells (APCH-E2).

The APCH-E2 antigen is expressed efficiently in the baculovirus expression system (BEVs) using the single-use Wave bioreactor platform. The average yield of recombinant antigen APCH-E2 produced is enough to produce three doses of vaccine per milliliter. Vedevax® is formulated 50:50 in oily adjuvant and induce high antibody titers both in Guinea pigs and cows. The Guinea pig model is used in Argentina as a mandatory control for the approval of new BDV vaccine. Parentally immunized Guinea pigs with the first commercial batch of Vedevax® presented neutralizing antibodies (NA) titers of 3, significant higher than the cut-off of the test, that is 1.37. In addition, a field trial with the vaccine was performed and cows subcutaneously immunized with Vedevax presented NA titers significant higher than the control group.

Vedevax® is the first targeted vaccine against BVD commercially available and is able to stimulate very efficiently the immune system both in the Guinea pig model and in the natural host of infection. The results presented here demonstrate that Vedevax® is a useful tool for the prevention of BDV in herds.

BV-P19

### Rapid detection of Bovine Viral Diarrhoea Virus on ear notches: European validation of a new immunochromatographic test

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**Objectives:** Bovine viral diarrhoea (BVD) is a significant and global economic disease of cattle. Detection of persistently infected (PI) animals is critical to the management of the disease and requires diagnostic test kits which are proven to be reliable. The objective of the study was to evaluate the performances of a new immunochromatographic test for the detection of Bovine Viral Diarrhoea Virus (BVDV) Erns antigen in bovine ear tissue samples (WITNESS® BVDV, Zoetis LLC) and to demonstrate its suitability for PI detection and BVD control.

**Material and methods:** A total of 219 ear notch samples from two collections were used to estimate diagnostic sensitivity and specificity. The first collection contained 39 positive and 55 negative samples sourced from calves in France. The second collection contained 50 positive and 75 negative samples sourced from calves in Belgium. Ten samples from the 39 infected and one BVDV type 2 supernatant were selected for virus typing and to estimate inclusive analytical specificity. Culture supernatants of Bluetongue virus, Bovine Herpes Virus type 1, Bovine Herpes Virus type 4, Bovine Respiratory Syncytial Virus, Bovine Parainfluenza-3, Bovine Coronavirus, *Pasteurella multocida*, *Mycoplasma bovis* and *Haemophilus somnus* were tested to assess potential cross reactivity. One BVDV positive sample was serially diluted and assayed 5 times to determine the limit of detection (LOD) of the tests and the dose effect relation.

All tests were performed blindly at the Fougères site of Laboce, a multi-site diagnostic laboratory in Brittany region in France. The reference test used for qualification of the sample status was real time polymerase chain reaction (RT-PCR) routinely used in the laboratory. Test kits evaluated were a rapid immunochromatographic test (Kit A: WITNESS BVDV, Zoetis) and two additional comparative rapid tests (Kit B: SNAP® BVDV Antigen Test, IDEXX Laboratories Inc.; Kit C: IDEXX BVDV Ag Point-of-Care Test, IDEXX Laboratories Inc.). The extraction methods and assay protocols were performed per kit specific directional inserts.

**Results:** With all samples combined, estimates of diagnostic sensitivity for Kit A and C were 100% (95%CI: [97.2-100%]). The estimate of diagnostic sensitivity for Kit B was 98.9% (95% CI: [94.9-99.9%]), as one positive sample sourced from Belgium was misclassified. The diagnostic specificity determined for all three tests was 100% (95% CI: [98.1-100%]).

Analytical specificity, whether inclusive or exclusive, was 100% on all three kits. Cross-reaction with the viruses or bacteria known to be clinically and/or genetically close to BVDV was not observed with the test kits. All kits correctly detected the 4 BVDV type 1b, 6 BVDV type 1e and 1 BVDV type 2 strains.

Analytical sensitivity, also referred to as LOD, was superior in kit A: final dilution repeatedly positive were 1/400 for kit A, 1/50 for kit B and 1/8 for kit C.

**Conclusion:** This study assessed analytical and diagnostic sensitivity and specificity of a new rapid test using positive and negative ear notch samples sourced from France and Belgium. The assay performances demonstrate the suitability of the rapid test (WITNESS BVDV) for PI detection and BVD control particularly when a timely result on the farm is required.

**Acknowledgment:** We thank Dr C. Quinet, ARSIA, for access to the samples from Belgium.





CG-P01

## Evaluation of DNA chip card kit for Bovine Respiratory Disease Complex (BRDC)

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**Objectives:** The fast and simultaneous detection of the plural pathogens from the same specimen is required to diagnose multiple infections. Recently, Canon Medical Systems launched BRDC genome detection kit (Genelyzer™ KIT A/BRDC-C) by the Automated DNA Detection System (ADDS).

In this study, we examined the ADDS as simultaneous detection method for 9 of BRDC relation pathogens (bovine viral diarrhoea virus 1, bovine viral diarrhoea virus 2, bovine coronavirus, bovine herpesvirus 1, bovine respiratory syncytial virus, *Mannheimia haemolytica*, *Mycoplasma bovis*, *Mycoplasma bovirhinis*, *Mycoplasma dispar*) and evaluated the sensitivity and specificity.

**Materials and methods:** ADDS include DNA chip card kit (Genelyzer™ KIT A/BRDC-C) and gene analysis instrument (Genelyzer™ II). Each pathogen gene was amplified in DNA chip card and detected by Electrochemical DNA chip in Genelyzer™ II. Nucleic acid was extracted from the samples by use of a SepaGene (Eidia Co., Ltd.). The PCR was performed for evaluation the sensitivity and specificity of the DNA chip card kit. In addition, totally 128 clinical samples (nasal mucus) were analyzed.

**Results:** The serial dilution of the nucleic acid extraction product from a clinical control sample was tested by DNA chip card kit and compared with PCR. The sensitivity of DNA chip card kit was 10<sup>-2</sup> ~ 10<sup>2</sup>-fold compared to PCR at limit dilution analysis. The results of the DNA chip card kit and PCR showed a high rate of agreement (93.6%).

**Conclusions:** In this study, we confirmed sensitivity and specificity of the DNA chip card kit by ADDS system consist with PCR analysis. This kit compared favorably with PCR, and it has the advantage of being rapid and easily performed.

**Acknowledgements:** The study of the DNA chip card kit was funded by the Ministry of Agriculture, Forestry and Fisheries of Japan (Project No.25081C).

DI-P01

## The use of Diagnostic Techniques in Calves with Septic Arthritis – A Retrospective Study

### Arthroscopy in calves

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**Objective:** In the study, comparison of superiority in the clinical, synovial fluid analysis, radiographic, thermographic, ultrasonographic and arthroscopic diagnostic techniques were used calves with septic arthritis.

**Materials and Methods:** The study was performed at the Department of Surgery, Faculty of Veterinary Medicine. Thirteen joints (eight carpal-two of them bilateral), three genu, one tarsus and one cubiti from eleven calves with septic arthritis were used as a material. Respectively, in the radiographic, thermographic, ultrasonographic examinations were performed for all joints. During the arthroscopic examination, synovial fluid was obtained from the joint for routine biochemistry analysis.

**Results:** Physical examination of joint fluid has revealed three cases blurred, six cases yellow ship and four cases hemorrhagic were occurred. Thermography findings show number of the calves with arthritis 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 joints increase the heat of the joints (100%) while these changes have also been recorded during palpation examination. In 10 joints (76.9 %), was shown subchondral osteolysis, new periosteal bone proliferation, osteomyelitis or relative displacement (subluxation) of articular bones with or without widening of the intra-articular joint spaces was observed. Radiographs of the three affected stifle joint revealed increased width of the intra-articular joint space. Ultrasound examination of the all joints were shown increasing effusion revealed thick and moderate homogenous echoic structure (100 %). Arthroscopic examination were shown synovitis, erosion of cartilage (61.5%) 1, 5, 6, 7, 8 10, 11 and 12, and number 1, 5, 6 and 12 joints are displayed osteophytic formation (30.7%). AlkP U/L, Amy U/L, AST U/L, Chol mg/dl, CK U/L, GGT U/L, LD U/L, TP g/dl, UIBC ug/dl and Uric mg/dl levels were increased in arthritic cases compare with normal synovial fluids.

**Conclusion:** The diagnosis of all inspection methods such as synovial fluid analysis, radiographic, thermographic, ultrasonographic examination could be used for joint diseases. However, joint capsule lesions, extracapsular and intracapsular lesion information, the synovial membrane of a better view has to be revealed while arthroscopic examination. In a conclusion, diagnostic arthroscopy methods were more reliable and concluded to be necessary and advantageous than others in calves in the cartilage lesions and synovial hyperplasia and treatment.



DI-P02

### Non typical MRI from bovine brain abscess with two different anaerobic bacteria Infections

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**Introduction:** Brain abscess in dogs and cats with infectious encephalitis is typically known to show higher signals with T2-weighted images (T2WI), moderate high signals with T1-weighted images (T1WI) and non- to low-signals with fluid-attenuated inversion recovery (FLAIR) images, as well as which is shown in human beings. We have experienced a bovine encephalitis that was different from the typical signal pattern as mentioned above.

**Animal:** The case was a male, 20 days old and crossbreeding cattle with Holstein and Japanese black cattle (Wagyu). The cattle exhibited extension of the extremities with swimming motion and opisthotonus. However, sucking reflexion was remained, the animal showed confusion and reductions of light reflex and blink reflex. The blood biochemistry was normal but increased neutrophilia was detected. The cattle was considered to be no use for productivity and devolved to the university.

**MR and CT examination:** The animal was preinjected xylazine for sedation, thereafter kept for anesthesia with continuous intravenous infusion of pentobarbital during CT and MR examinations. CT and MR imaging were performed with 16-row multi-helical CT and 0.3 T permanent magnet system respectively. CT images represented asymmetric lateral ventricle size, and X-ray high-absorbance area was covered by the ventral of ventricles. On the other hand, T2WI and FLAIR MR images suggested defused high intense signal changes along the both lateral ventricles, the existence of pseudocyst which has a high-signal core surrounded by non-signal area and covered indistinct high-signal margin in a right temporal lobe and a left parietal lobe, and displayed the presence of amorphous, non- to low-intense solid component from left lateral ventricle to cerebral aqueduct and fourth ventricle.

MR images also showed asymmetric size and shape of lateral ventricles like in CT exam, especially for right ventricle enlargement. A rostral surface of the cerebellum was recessed by compression of swelling cerebrum and the slight cerebellar tonsillar herniation happened.

**Pathological examination:** One day after MR and CT examination, the animal was euthanized with general technique as deep anesthesia. Both lateral ventricles were asymmetrically expanded and had retention of cerebrospinal fluid and the existence of abscesses especially for the fourth ventricle. The cerebral aqueduct was embolized by an abscess and mesencephalic necrosis occurred. A portion of the right ventral temporal lobe was yellow colored and adhered to the dura mater. Both piriform lobes were protruded and thinning with the accumulation of purulent matter. An abscess was also recognized on a section of the cerebellum. The cerebellar tonsil compressed a cervical spinal cord. Hemorrhagic foci (3 cm diameter) and pus were from the medulla oblongata to cervical cord and adhered to the meninges. Each purulent matter displayed yellowish-white color and creamy. Two anaerobic bacilli, *Bacteroides stercoris* and *Porphyromonas asaccharolytica*, were isolated from the abscess by anaerobic culture.

**Discussion:** These two anaerobic bacteria were known as indigenous bacteria which induced opportunistic infection and also known as that genus *Bacteroides* could cause the brain abscess. The abscess in this report presented different and specific signal pattern, which was concentric non-signal cord around abscess core, from the common cerebral abscess in MR imaging. These non-signal areas were considered as rich moisture by grossly and microscopically observation. However, *B. stercoris* could produce the gas when coexistence with other facultative anaerobes, the non-intensity MR signals of gas findings was uneven observed above of fluidic purulent matter in an abscess. There was no incidence of the gas production and void in abscess confirmed by necropsy. These MR images were relatively characteristic signal pattern and considered to be difficult for interpretation but should be kept for the differential diagnosis.



EL-P01

## BioAwareism

### A Holistic Approach in Training Employees to Reduce Biological Risks on a Dairy

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**Objective and Background:** The National Milk Producers Federation, of which the majority of U.S. dairy producers are members, has made a concerted effort since 2008 to help standardize and document the care of dairy animals to satisfy demands of both the retailer and consumer. This led to the development of the FARM (Farmers Assuring Responsible Management) Program with the goal of continuous improvements in animal care, environmental and antibiotic stewardship on dairy operations. Additional employee training was needed which spawned the creation of the DairyCare365 series, with training modules in English and Spanish. More than 80 percent of non-family dairy employees in the U.S. speak Spanish. Dairy workers can be trained in low stress cattle handling, newborn calf management, care of non-ambulatory animals, and other topics. The newest DairyCare365 series, titled BioAwareism, provides a holistic approach to help employees and owners realize the importance of biosecurity, biocontainment, and hygiene. The training provides a roadmap for practical disease prevention measures to limit pathogen entry and spread on their dairy. Bioawareism also illustrates to retailers that food safety starts at the point of production.

**Materials and Methods:** Ideas were solicited (in 2008) from face to face meetings with milk cooperatives, dairy producers, and retailers to determine topics for the DairyCare365 series. Educational content was developed with experts based on prioritization of topics that aligned with the FARM Animal Care Program. The first modules released in 2011 addressed animal handling. The most recent series, BioAwareism, was developed in 2016 with a veterinarian from Iowa State University's Center for Food Security and Public Health who is a leader in this area. The content was split into two areas of focus: Biosecurity and Biocontainment. Module 1: Biosecurity explores potential threats from outside the farm gate (purchased animals, trucks, people, wildlife etc.), and describes using a hazard analysis to determine acceptable risks, which vary among farms. Module 2: Biocontainment looks behind the farm gate to determine practices to decrease pathogen load and to increase pathogen resistance of animals on the farm. These modules and accompanying Standard Operating Procedures (SOP) provide the tools necessary for dairy producers to develop a customized plan to protect their dairy and explain to employees why these procedures are important. The series was filmed on a commercial dairy, with realistic scenarios. Each module has an assessment. If 80% or greater is achieved, the user can print an individualized certificate to document training. The modules are available at no cost online at [www.DairyCare365.com](http://www.DairyCare365.com).

**Results:** Module 1 in the BioAwareism series was released in October 2017 and data on its usage is not yet quantifiable. As for its impact on individual dairies, it is difficult to put a value on

prevention, because if it works, outbreaks will not occur. Thousands of dairy workers from hundreds of farms have watched the other DairyCare365 modules and taken the assessments. The individualized certificates are used as positive reinforcement to the dairy employee as acknowledgement of completing formalized training. There are published reports where usage of other DairyCare365 modules (e.g. Managing Non-Ambulatory Cattle, Proper Euthanasia Methods, Moving cows to Milking Parlor) as part of the training has resulted in fewer accidents involving human animal interactions, and subsequently reduced workman's compensation claims.

**Conclusion:** Most people in the U.S. are three generations removed from production agriculture. It is reasonable that they are curious where their food comes from and, when this food is derived from animals, seek assurance that the animals are well cared for. DairyCare365 is filling a necessary training need in the dairy community. Without this education, the very people that we entrust to feed and care for our animals 365 days of the year may lack the understanding and motivation as to the importance of their role. Specifically, the BioAwareism series connects animal care to food security at the farm level and provides unique worker training.



ED-P01

## Monitoring of Schmallenberg virus antibodies in sentinel herds of Portugal

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**Objectives:** Schmallenberg virus (SBV) is a novel vector-borne virus that causes disease in ruminants in Europe. In 2012, Spain, the disease was reported in a newborn lamb. Despite the proximity to the Portuguese border, no reports of SBV infection in Portugal have ever occurred. In the present work, an SBV surveillance scheme was performed to evaluate the possibility of SBV emergence in the central region of Portugal.

**Materials and methods:** Sheep (n=168) from an autochthonous breed in Portugal (Serra da Estrela) were selected from 42 farms, located in 9 municipalities of the official geographical coverage of this breed. This autochthonous breed was selected for their geographical restriction to a single region, the central region of Portugal, a region known for providing harsh conditions for midges, and known to be a barrier for the northward spread of midge-associated diseases. Sera from the 168 selected sheep were collected both in 2015 and 2016 and screened for anti-Schmallenberg virus IgG antibodies (D Screen® Schmallenberg virus Competition Multi-species, ID-vet, France).

**Results:** From the 2015 and 2016 sera bank, 7 and 10 samples, respectively, were found to be positive for IgG anti-Schmallenberg virus. Results shows anti-SBV IgG prevalence of 4.2% in 2015 and 5.9% in 2016. Seroconversion in 4 animals was also detected, showing that the capable SBV vector circulates in Portugal. On the other hand, 1 initially seropositive animal showed to be seronegative in the 2016 screen. Schmallenberg virus specific antibodies are known to persist at least 12–24 months after natural infection, suggesting that in this animal, infection likely occurred early in the 2013-2014 season.

**Conclusions:** This study shows that SBV circulates in the centre of Portugal and seems to be emerging due to the increasing prevalence. For this reason the authors consider assertive to advise increased monitoring and detection of viral RNA in midges and stillbirths.

ED-P02

## Pathogenicity and teratogenicity of Schmallenberg and Akabane viruses in the embryonated chicken egg model

### SBV and AKAV in ECE

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**Introduction:** Schmallenberg virus (SBV) is a Simbu sero-

group Orthobunyavirus that emerged for the first time in north-western Europe during 2011. The virus is now sero-endemic in Europe (Collins et al., 2017a) with recent re-emergence (Collins et al., 2017b). Both SBV and Akabane virus (AKAV) have similar epidemiology, pathogenesis and clinical signs. *In vivo* research studies on teratogenic viruses in ruminants are expensive and can require a long time to complete. These challenges can be mitigated by using small animal models such as embryonated chicken eggs (ECE). Hence, the aims of this research were to investigate if chicken embryos are susceptible to experimental SBV infection and, if so, to what extent and to compare the pathogenicity and teratogenicity of SBV and AKAV infection in an ECE model.

**Material and methods:** The study design and methodology, including the age at which embryos were inoculated and virus inoculum doses used, were derived from previous studies which investigated the pathogenicity of Simbu serogroup Orthobunyaviruses in ECE models (McPhee et al., 1984). Two studies were conducted. In Study A, 0.2ml of undiluted cell culture-grown SBV ( $10^{6.4}$  TCID<sub>50</sub>/0.2 ml) was inoculated into the yolk sac of chicken embryos at 6 days[AA1] (n = 43) and 8 days (n = 41) of incubation. In Study B, groups of approximately 40 embryos were infected with SBV (n = 178) or AKAV (n = 177) at virus doses ranging between  $10^{2.0}$  and  $10^{6.0}$  TCID<sub>50</sub>/0.2 ml at 7 days of incubation. Control embryos in both studies were inoculated with 0.2ml of sterile phosphate buffered saline (PBS). Chicken embryos were incubated at 37°C until day 19 of incubation. when they were euthanised and submitted for necropsy examination. Embryos that died between day 7 and day 18 of incubation were also necropsied. Sterile plain swabs of brain tissue were collected from a sub-sample of 19 day old ECEs (both virus-inoculated and control embryos) at necropsy and tested for either SBV or AKAV RNA using quantitative real-time reverse transcription PCR (qRT-PCR). Chi-square statistical tests were used to compare the proportion of deaths, stunted growth, congenital defects and PCR-positive results between groups of embryos. Fisher's exact test was used when expected frequency values were below 5.

**Results:** Mortality was greater in embryos inoculated with SBV at 8-days (76%) compared to 6-days (47%), ( $P < 0.01$ ). The prevalence[AA2] [AC(3) of stunted growth (6-days: 37%; 8-days: 51%) and musculoskeletal malformations (6-days: 42%; 8-days: 41%), (arthrogryposis, skeletal muscle atrophy, contracted toes, distorted and twisted legs) did not differ between days ( $P > 0.05$ ), however, the prevalence of these findings was significantly higher in virus infected embryos compared to controls. [AA4] Mortality was greater in embryos inoculated with SBV (31%) compared to AKAV (19%), ( $P < 0.05$ ), suggesting that SBV was more embryo-lethal. However, embryos infected with AKAV had a significantly higher prevalence of stunted growth (SBV: 46%; AKAV: 76%;  $P < 0.05$ ) and musculoskeletal malformations (SBV: 18%; AKAV: 42%;  $P < 0.01$ ), suggesting that AKAV was more teratogenic in this model.

**Conclusion:** These studies demonstrate that SBV chicken embryos infected with SBV demonstrated gross abnormalities consistent with congenital Schmallenberg disease as reported in ruminants. When SBV and AKAV were compared, SBV appeared to be more embryo-lethal. However, a significantly higher proportion of embryos infected with AKAV had stunted growth and congenital defects. There was no statistical difference in the prevalence of stunted growth or congenital malformations between embryos inoculated at 6 days or 8 days of in-



cubation.

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ED-P03

## First evidence of significant re-emergence and re-circulation of Schmallenberg virus in previously exposed dairy herds in Ireland

### SBV re-emergence in Europe

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**Introduction:** In the years following the European Schmallenberg epidemic (2014-2016) there have been reports of SBV overwintering and continued virus circulation in a number of European countries including Ireland albeit at a considerably lower level when compared to the level of SBV circulation during the first European epidemic. The lack of significant virus re-circulation in the last number of years has resulted in a growing population of immunologically naïve animals which would be susceptible to SBV infection should the virus re-emerge in previously exposed and unexposed regions.

Currently, there are no published sero-surveillance studies available regarding the prevalence of SBV re-circulation in previously exposed regions in 2016. Hence, the aim of this study was to determine if there was evidence of SBV re-emergence and re-circulation in previously exposed dairy herds in Ireland in 2016, and if so, to what extent.

**Materials and methods:** A Schmallenberg virus sentinel herd surveillance study was set up in 2013 to monitor post-epidemic SBV circulation in 25 previously exposed Irish dairy herds between 2013 and 2015. In order to investigate whether there was evidence of SBV circulation in these herds between 2014 and 2016, a sentinel population of 1550 spring-2014-born immunologically naïve youngstock (range 25-118 per herd) were monitored prospectively for evidence of SBV infection for three years. In winter 2014 (after the 2014 vector active season) this population of animals (n=1550) was tested for SBV antibodies, of which 7 animals (0.45%) tested seropositive. In winter 2015, 1440 of these animals were re-tested for evidence of SBV infection during 2015; all animals, including the 7 animals which tested seropositive in winter 2014, tested seronegative. In order to determine if these herds were re-infected with SBV in 2016 a total of 366 of these seronegative spring-2014-born animals were blood sampled across the 25 study herds (average 15 animals per herd) between 3-10 March 2017 and tested for SBV antibodies.

A sample size of 366 animals was used in order to estimate animal-level SBV true seroprevalence in this population of animals with 95% confidence and 5% precision using a *priori*

prevalence estimate of 62.5%.

Serum samples were analysed for SBV-specific antibodies using a competitive ELISA (ID Screen Schmallenberg virus Competition Multi-species, IDVet, Montpellier, France) in accordance with manufacturer's guidelines.

Epidemiological analyses, including apparent prevalence (AP) and true prevalence (TP) estimates, and sample size calculations were completed using an online epidemiological calculator (EpiTools) (Sergeant, ESG, 2017).

**Results:** A total of 256 animals tested seropositive; an AP of 69.9% (95% CI: 65.1-74.4) and TP of 77.7% (95% CI: 72.3-82.8%) when correcting for imperfect test characteristics.

**Discussion:** These results demonstrate that a new epidemic of SBV circulation occurred in these previously exposed herds in Ireland in 2016. This is the first report of such re-emergence in previously exposed herds in Europe.

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ED-P04

## Selected vector born zoonoses in grazing cattle in Slovenia

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**Objectives:** The aim of this study was to detect the presence of *Anaplasma phagocytophilum*, *Babesia divergens* and *Borrelia burgdorferi* in grazing cattle in Slovenia. All three agents are transmitted with ticks and appear in a wide range of hosts, including humans. Infections with *A. phagocytophilum* and *B. burgdorferi* s.l. in cattle is asymptomatic or non-specific, so they are difficult to diagnose without special laboratory tests. *B. divergens* often causes a clinical illness and is therefore easier to recognize.

**Material and methods:** In total we examined the blood and serum of 291 cattle. We also collected 6 samples of cattle with a clinical picture that occurs in *B. divergens* infections. The collected samples were analyzed for haematological parameters and tested for the presence of specific IgGs with indirect immunofluorescence. The results were treated with descriptive statistics, ANOVA test, and a Hi-square test. The statistical significance was attributed to the results in which  $p < 0.05$ .

**Results:** Seropositive to *A. phagocytophilum* were 158 (54.64 %) of the 291 animals, 63/291 (21.65 %) of the animals were seropositive on *B. divergens*, 137/291 (47.08 %) of the animals were serologically positive for *B. burgdorferi*. 58/291 (19.93 %) of the animals were at the same time seropositive to *A. phagocytophilum* and *B. divergens*, 72/291 (24.74 %) of the animals were simultaneously seropositive to *A. phagocytophilum* and *B. burgdorferi*, and 25/291 (8.59 %) was serologically positive for all three pathogens simultaneously.

In the statistical analysis of the effect of serological positivity on hematologic parameters, we found significant effects on leukocytes, erythrocytes, hemoglobin, hematocrit and MCHC.



**Conclusions:** Influence of seropositivity on hematologic parameters could be connected to inflammation and erythrocyte destruction consequences of the infection by selected pathogens.

*Anaplasma phagocytophilum*, *Babesia divergens* and *Borrelia burgdorferi* are common in grazing cattle in different regions of Slovenia. Infections with *Babesia divergens* cause also clinical disease, while we were unable to prove clinical disease in cattle seropositive on *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. Coinfections are common and cattle can serve as sentinels for infection with these pathogens.

ED-P05

### **Culicoides species composition and abundance in relation to emerging arboviral diseases: bluetongue and Schmallenberg**

#### **Culicoides**

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**Introduction:** *Culicoides* biting midges have been implicated in the transmission of over 50 arthropod-borne viruses worldwide including BTV and SBV. The recent unprecedented emergence of arboviruses in northern Europe, such as SBV and multiple serotypes of BTV has highlighted Europe's susceptibility to exotic arboviruses transmitted by biting midges from distant geographic regions. The abundance of suitable *Culicoides* vector species in northern Europe facilitated the rapid spread of SBV across the continent in 2012 (EFSA, 2013). There have been no studies on the *Culicoides* species composition and abundance on Irish cattle farms since the outbreak of SBV in 2012. Hence, an in-depth *Culicoides* entomology survey was conducted on sentinel farms in 2014.

**Material and methods:** Ten herds in the south of Ireland which were part of a Schmallenberg virus sentinel herd surveillance study (Collins et al., 2016) were selected to cover as great an area of the south of Ireland as possible. Onderstepoort Veterinary Institute design ultraviolet light suction traps were used to collect insects; one trap was run overnight in the vicinity of livestock on each farm. Each site was sampled fortnightly over a period of 16 weeks (21<sup>st</sup> July – 5<sup>th</sup> November 2014) during the 2014 vector season, corresponding to eight catch collections per farm and a total of 68 night collections during the study period. Following collection, insects were frozen (-20°C) then stored in 75% ethanol. Collections were initially sorted into *Culicoides* and non-*Culicoides* spp. using a stereomicroscope. *Culicoides* were identified morphologically to species level using the keys of Campbell and Pelham-Clinton (1960) and reference wing images (The Pirbright Institute, UK). Female *Culicoides* were further classified according to physiological status into unpigmented, pigmented, gravid and blood-fed individuals.

**Results:** A total of 23,929 *Culicoides* were collected. *Culicoides* were found ubiquitously across all sites; however, there was a large variation in the number of *Culicoides* collected on

each farm (257 to 4,285 *Culicoides*). A total of twenty-one species of *Culicoides* were identified, including the first confirmed report of *C. clastrii* and *C. cameroni* in Ireland, constituting new Irish records. The most abundant species identified were members of the *Culicoides obsoletus* (*C. obsoletus/scoticus*; 38%, *C. dewulfi*, 36% and *C. chiopterus*; 5%) and *Culicoides pulicaris* groups (*C. pulicaris*; 9% and *C. punctatus*; 5%) comprising 93% of all *Culicoides* collected. The remaining *Culicoides* were principally *C. achrayi* (5.1%) and *C. festivipennis* (0.8%). The number of species identified at each site varied from 10 to 15 species (mean 13). The six major *Culicoides* arbovirus vector species from the *Culicoides obsoletus* and *Culicoides pulicaris* groups were present on all ten farms. The physiological status was determined for 98% (n = 19,458) of all female *Culicoides* collected. The majority of the female arbovirus vector species collected were unpigmented (46%) and pigmented (33%), followed by gravid (12%) and blood-fed (5%). For non-vector species, gravid *Culicoides* (33%) were the most abundant, followed by unpigmented (28%), pigmented (28%) and blood-fed (10%).

**Conclusion:** The most abundant *Culicoides* species identified in this study are the putative vectors of a number of arboviruses in Northern Europe. The presence and abundance of these species highlight that disease transmission could (re-)occur following a new incursion of SBV or other exotic *Culicoides*-transmitted arboviruses into Ireland.

**Acknowledgements:** We acknowledge the contribution of John Heffernan (Teagasc) in assisting with sample collection. We thank the farmers for access to their farms and their data.

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EP-P01

### Investigation of *Brucella abortus* infection in a Thai dairy herd

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**Objectives:** We aim to identify the infected animal using serology, milk culture, and polymerase chain reaction test and to set a surveillance and control strategy for disease control of the infected dairy herd.

**Materials and methods:** The bulk tank milk samples from 104 small dairy farms in Nakorn-Ratchasima province is included in our investigation. Only one farm in which both Milk Ring Test (MRT) and indirect ELISA (i-ELISA) are given the positive result. A positive dairy herd is intensively examination. All animal in herd are subjected to blood sampling (n=83) in order to determine serology status using rose bengal test, i-ELISA, and complement fixation test. All milking cow (n=40) are milk sampled for bacterial culture and polymerase chain reaction test for detecting *Brucella* spp.

**Results:** In this herd, we are able to detect seropositive animal according to their production status in which are 37.5% milking cows (15/40) and 10% heifers (2/20). However, only seven milking cows have been positively identified for *Brucella abortus* infection according to culture and PCR results.

**Conclusions:** Milk Ring Test is the efficient method for the dairy herd brucellosis screening in an active surveillance program for small dairy holders. MRT is able to represent the milking cow disease status in a herd. For the MRT positive herds, the following intensive tests need to confirm for establishing all animal disease status in order to disease control and/or eradication. We suggest culling all positive animal is the best advice. However, the advice for immediately conducting in farm quarantine and seropositive animal segregation are the important issue. The socioeconomic concerns including the cost of culling and zoonosis need to be considered.

EP-P02

### Association of farm location, time and other risk factors on FMD outbreak in a high density area of dairy cow population

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**Purpose:** The objective of this study was to determine the risk factor of foot and mouth disease (FMD) focusing on farm location, time of FMD occurrence, and prevention management factor at the farm level.

**Materials and Methods:** The study was performed in the high density area of dairy cow population in Jun Toung subdistrict in Nakhon Ratchasima province and Lumpraya Klang subdistrict in SaraBuri province. Two studies were performed. In the first study, data were collected to investigate the risk factors concerning farm location and time on FMD occurrence. The geo-location of farms, outbreak time, disease history, and animal population at the farm level were collected. Totally, 634 farms including 97 outbreak farms distributed over 2 subdistricts were in analysis. The logistic regression model was applied to identify the risk factor related with location of FMD. In the second study, case control study was performed. Data were collected from both of farm without the history of FMD and with the history of FMD that occurred between March 2014 and February 2016. Farmers were depth interviewed to provide information of the prevention management for FMD. The negative binomial regression model was performed at the farm level to identify the association between a number of sick FMD animal and risk factor.

**Result:** The results of spatio-temporal analysis indicated that the distribution of FMD at the farm level was sporadic pattern with 15% of total farm population affected. High density areas of FMD occurrence were identified for 3 temporal-spatial clusters. The result indicated the risk of a large farm, farm located in the lower land, and farms located closely to some milk cooperation associated with FMD occurrence. Moreover, a large farm lacking vaccinating, delaying to administrate vaccine to animal after receiving and a farm with a high number of sold animals were risky to FMD.

**Conclusion:** For above information on the sporadic distribution of FMD in this area and the risk of FMD outbreak mainly on vaccinating management, we conclude that the FMD prevention is able to achieve by increasing immunity in population with an enough number of vaccinating, an accuracy procedure and also reducing the risks of carrying virus into a dairy farm.

**Keywords:** Foot and mouth disease, dairy cow, risk factor, prevention, farm level

EP-P03

### Presence of antibodies against BVDV, BHV-1 and *Coxiella burnetti* in dairy cattle farms on eastern region of Poland – preliminary epidemiological study

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**Objectives:** The occurrence of BHV-1, BVDV and *Coxiella burnetii* in dairy cattle herds as well as the maintenance system affects animal health, and consequently animal reproduction and the presence of other, accompanying infections, that may also affect productivity of the herd. The aim of this study was to estimate the prevalence of antibodies against BHV-1, BVDV and *Coxiella burnetii* in dairy cattle farms from central-eastern region of Poland.

**Materials and methods:** The Ethical Committee for Animal Experiments (Wrocław, Poland) approved this study, and all owners provided informed consent prior to initiation of the study. Bulk tank milk (BTM) samples were collected in 211 dairy farms (January-December 2017), where the average of animals in the herds was 47 and 26 animals in lactation, respectively. Population of dairy cattle in investigated region amounted 949 000 cows, kept in 2524 dairy farms. Vaccination against described infections has not been carried out. Appointed veterinarians collected samples that were transported directly to the Diagnostic Laboratory EIP-VET (Faculty of Veterinary Medicine, Wrocław). The BTM samples were collected into 100ml container without preservatives. Milk samples were centrifugated at 16,000 x g for 4 min and the fat fraction was removed. The BTM skim milk samples were examined for the presence of antibodies against the gB protein of BHV-1, BVDV and *Coxiella burnetii* by an indirect ELISA test using a commercially available ELISA: HerdCheck BHV-1 gB, (IDEXX, Scandinavia AB, Sweden), IDEXX BVDV Total Ab test (IDEXX, Scandinavia AB, Sweden) and IDEXX Q fever Ab Test (IDEXX, Scandinavia AB, Sweden). Statistical analysis was performed using Statistica v. 10 (StatSoft, USA) and Mann-Whitney U test ( $p > 0.01$ ).

**Results:** Of the 211 dairy cattle farms investigated, 51 (24%), 199 (94.4%) and 102 (51.6%) were confirmed to contain antibodies against BHV-1, BVDV and *Coxiella burnetii*, respectively. A statistically significant correlation was observed between the occurrence of antibodies against BVDV and the etiological agent of Q fever. The group of herds with coinfections (BVDV and Q fever) was higher than herds with monoinfection (BVDV) (57.1% vs. 22.8%,  $p < 0.001$ ). Moreover, the statistically significant association was demonstrated between the occurrence of antibodies against BVDV and BHV-1. The percentage of herd, where at the same time antibodies against BHV-1 and BVDV have been detected was lower than % of herds, where antibodies against BVDV has been not found (80.5% vs. 67.7%;  $p = 0.041$ ). There was no statistically significant correlation between the occurrence of antibodies against BHV-1 and Q fever ( $p > 0.05$ ).

**Conclusion:** Our study showed that the coexistence of infectious diseases with the significant impact on the health status of dairy herds as well as on the milk production is common. This is of particular importance in regions where the stocking densities are high, in the absence or low rate of vaccination. The low level of biosecurity observed during sampling is an additional, enabling factor in the spread of reported infections between herds.

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EP-P04

### Issues to be known from questionnaires for farmers on bovine leukemia.

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In recent years, the sense of crisis against domestic bovine leukemia has become stronger, and the Ministry of Agriculture, Forestry and Fisheries issued guidelines showing the basic idea for this disease. However, there are no fundamental measures to prevent the spread. Under such circumstances, it is the farmer who actually responds to the epidemic prevention, and it is extremely important what kind of awareness the farmer has to this disease. So we learned the current situation and carried out a questionnaire for farmers to help useful measures. As a result, through testing of cattle leukemia, farmers who got information had abundant knowledge and sources of infection about sources of infection compared with farmers who have never tested ( $P < 0.05$ ). Furthermore, even at the rate at which infection control was implemented, the farmer who had tested was higher ( $P < 0.01$ ) than the farmer if he had never tested. Even if 70% or more farmers pay for it, they wanted inspection. In order to have farmers take infection control measures, it is necessary to inspect farmers who want to know the current state of their farm. Moreover, we found that it is necessary to implement a seminar to convey accurate information to farmers who want more information and farmers who want study group.

EP-P05

### Look past 'p-values' to the Confidence Intervals - to guide you as an evidence-based Buiatrician.

#### Assessment of outcomes by Evidence-based Buiatricians.

\*Mark Andrew Burgemeister

Thinking Cows Pty Ltd

Critical Appraisal of scientific information is a key skill for the modern, evidence-based Buiatrician – the presenter believes it to be one of the required skills for life-long learning and continuing development within our profession. The identification and measurement of the strength of an association or effect, and quantification of the level of doubt around this measure, is a vital component of this appraisal.

This presentation will display how evidence-based Buiatricians can look past the 'p-values' when they read scientific literature and sales company leaflets and information – seeking to interpret the Confidence Intervals. Especially for practical uses,





Confidence Intervals can provide a wealth of clinically useful information.

Hypothesis tests ('p-values') tell the reader only about statistical significance, where Confidence Intervals can tell the reader about statistical *and clinical* significance. This allows the Buiatrician to read much more from the data presented. They are not difficult to read and interpret with a small amount of training.

The presentation will highlight the key methods of reading Confidence Intervals, to drive Buiatricians to seek more than 'p-values' from information providers and to increase their literacy and comprehension in statistical discussions of uncertainty using Confidence Intervals.

A key tenet of this presentation is that Buiatricians have an opportunity as an advisory group to build a bridge between information developers and the information users (farmers). The continued development of the skills of interpreting Confidence Intervals (and requesting them from information providers) is a key part of building this position in our industry to further knowledge within our domain, and the effectiveness of the data which has been made available.

HH-P01

## Process related information management in 'Precision Dairy Farming'

### The interdisciplinary 'agriProKnow' project

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**Objectives:** Modern dairy farming often relies on the use of sophisticated technologies. In addition to already long-established milking and feeding technologies, various sensor systems (e.g. heat detection systems, climate logger, cow tracking systems) are available now, to assist the farmer in herd management. Applying these technologies e.g. targets in an improved health monitoring of the animals, in ensuring the quality of food as well as in increasing the work efficiency. Because of missing interfaces between different technologies and the high amount of generated data, the potential of an integrated data analyses facilitating herd management decision often remains unused by farmers and other stakeholders (e.g. veterinarians and feed advisors).

The interdisciplinary 'agriProKnow' project aims in creating a decision support platform, where collection, integration, and analysis from distinct sources of farm data will be managed. Hereby, new process knowledge and recommendations shall be created by using data mining and semantic data warehouse techniques. By using bovine ketosis as an example, the obtained data will be used to develop an algorithm allowing the prediction, and hence, the prophylaxis of this disease.

**Material and Methods:** In total 700 dairy cows were enrolled in this study. Data were collected between March 2016 and July 2017 from multiparous Holstein-Friesian cows from a commercial dairy herd, housing approx. 2,700 cows. The animals were equipped with ear-attached accelerometer (SMARTBOW, Smartbow, Weibern, Austria) allowing real time monitoring of an animals' activity, rumination, and localization. Additionally, health records of the animals, body condition, milk yield, feed, and climate data were considered and uploaded into the data warehouse. Ketosis was diagnosed based on the  $\beta$ -hydroxybutyrate concentration measured in blood with an electronic hand-held device.

**Results:** Data of distinct sources were successfully uploaded into the data warehouse. Based on the collected data, analyses of risk factors for the occurrence of ketosis are currently performed using a multi-stage model. Detailed results will be presented at the conference.

**Conclusion:** Extraction, transforming and loading (ETL) of data into a data warehouse was feasible under field conditions for further integrated analyses. Future research is required to evaluate the benefit of altering herd management decisions based on these complex analyses.



HH-P02

### Characterizing the relationship between rumination time and peripartum disease events in pastured Australian dairy cattle using an automated health monitoring system.

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**Objectives:** The rapid pace of development in sensor technologies has provided the ability to remotely monitor the health and reproductive status of dairy cattle. While the efficacy of these systems in identifying disease events has been demonstrated in housed dairy cattle, research in pastured herds such as those typically found in southern Australia is more limited. The objective of this study was to determine if significant relationships existed between sensor-derived measurements of rumination time and peripartum disease events in dairy cattle managed under Australian conditions.

**Materials and methods:** A prospective, observational cohort study was undertaken in which primiparous and multiparous Holstein cows (n=168) were subjected to physical examination by a veterinarian at 4-6 days in milk to determine the presence of post-partum health disorders. All cows were fitted with an automated remote monitoring device, the SCR HR-LD tag (SCR Engineers, Netanaya, Israel) by the herd manager, and two-hourly records of rumination and activity recorded from 10 days prepartum to 21 days post-partum. Multivariate analysis was performed on these data to determine if there were significant changes in pre and post-partum rumination time in cows diagnosed with subclinical ketosis, metritis, endometritis, retained foetal membranes and left sided displacement of the abomasum.

**Results:** Significant declines in rumination time were present in the five days prior to diagnosis animals affected by left displaced abomasum, metritis and subclinical ketosis. No significant relationship was observed between two-hourly or daily rumination time in cows affected by retained foetal membranes or endometritis. Declines in prepartum rumination time were also noted in cows that subsequently developed left displaced abomasum and subclinical ketosis.

**Conclusions:** Preliminary findings indicate that these technologies may have applications in pasture-based dairy systems for the timely identification of peripartum health disorders, and may be a tool for improving the management of early lactation cows in seasonally calving dairy herds. Rumination time in pastured dairy herds appears to have a similar relationship to disease events as is observed in housed cattle consuming a substantially different diet. Further analyses are being undertaken to characterize the relationship between sensor measurements of activity and peripartum disease events.

HH-P03

### Development of a new on-farm test system for determining blood non-esterified fatty acid and $\beta$ -hydroxybutyrate levels

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**Objectives:** Passive elevated levels of non-esterified fatty acids (NEFA) and  $\beta$ -hydroxybutyrate (BHBA) indicate a poor adaptation to negative energy balance (NEB) as dairy cows transition from late gestation to early lactation, which leads to an increased risk of detrimental health and production. For the past few years, handheld BHBA meters developed for human use have provided the dairy industry with on-farm testing for hyperketonemia (BHBA  $\geq 1.2$  mM) as a substitute. Nevertheless, monitoring the incidence of elevated NEFA is also important to determine a cow's energy status because an elevated blood NEFA level is the origin of the response to NEB and is applicable in various stages (e.g., the late gestation period). However, an on-farm testing system for NEFA has not yet been developed. The objectives of this study were to develop a new on-farm diagnostic assay system for cattle that could simultaneously determine blood NEFA and BHBA levels and evaluate them via comparison to the gold standard methods using plasma samples.

**Materials and Methods:** Sample collection was performed at 2 dairy farms that housed a total of 280 Holstein dairy cows, and 71 blood samples (-20 to 327 days in milk, average parity  $2.4 \pm 1.4$ ) were obtained. Coccygeal blood samples were taken from lactating cows just or 4 h after feeding, and from prepartum cows just before feeding. The blood samples were immediately placed in tubes containing EDTA and refrigerated until assayed. Aliquots of whole blood samples were measured using a newly developed device. The device was prepared by modifying a point of care testing device (L205 mm x W126 mm x H110 mm: 0.8 kg) for human use to fit the wavelengths analyzing NEFA and BHBA. After putting a drop of whole blood (50  $\mu$ L) on the newly developed cartridge and inserting it into the device, plasma was separated from whole blood and then transported to enzymatic reaction tanks for NEFA and BHBA. Thereafter, NEFA and BHBA concentrations were determined by colorimetric assays. To evaluate the data from the device, the residual whole blood samples were centrifuged and the plasma obtained was used for quantification. For this we employed commercial kits (NEFA-HR11 Wako test kit, Autokit-3HB Wako test kit for BHBA; Wako Pure Chemical Industries, Osaka, Japan) with an automated biochemistry analyzer (CA-90; Furuno Electric Co., Ltd., Hyogo, Japan). Both the newly developed system and the automated analyzer were simultaneously operated. Linearity was determined using simple linear regression with JMP (version 13 for Windows, SAS Institute Inc., Cary, NC, USA). For repeatability tests, coefficients of variation (CV) were calculated by 10 measurements using the same whole blood sample. The sensitivity and specificity for NEFA and BHBA were determined at 0.4 mEq/L and 1.2 mM.

**Results:** The prevalence of samples at the cutoff value or greater for NEFA ( $\geq 0.4$  mEq/L) was 9.9% and 9.9% as determined by the gold standard method and the new system, respectively. The prevalence of hyperketonemia (BHBA  $\geq 1.2$  mM) was 7.0% and 8.5%, respectively. The slope, coefficient of determination ( $R^2$ ), sensitivity, and specificity for the developed system analyzing NEFA compared to the gold standard plasma concentrations were 0.94, 0.97, 100%, and 100%, respectively. Those for BHBA were 0.95, 0.96, 100%, and 98.5%, respectively. For the repeatability test of the developed assay system, the CV for NEFA and BHBA were 6.9% and 5.0%. The times required for the reaction to reach the NEFA concentration at the cutoff value (0.4 mEq/L) and 1.2 mEq/L were 3 and 9 minutes, and those for



the BHBA concentration at the cutoff value (1.2 mM) and 5 mM were 2 and 3 minutes, respectively. These results suggest that the NEFA and BHBA assay systems can cover the concentration ranges of periparturient cows within 9 minutes.

**Conclusions:** These results demonstrated the possibility of an on-farm diagnostic system for NEFA and BHBA. We are planning to verify the accuracy of this assay system by continuous herd tests and to determine the running time and running cost.

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HH-P04

### A novel application for remote disease detection systems in lot fed cattle: a case report on acutely acidotic steers in a south east Australian feedlot

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**Objectives:** Ruminal lactic acidosis is a significant cost to beef feedlot production systems. Acute ruminal acidosis exists in a spectrum with subacute ruminal acidosis (SARA), which causes losses to feed intake and average daily gains (ADG) with minimally detectable clinical signs in the live animal. Acute acidosis is a profound clinical manifestation, including anorexia, diarrhoea, ataxia, recumbency and frequently death. SARA is typically associated with reduced production metrics and secondary conditions such as laminitis, and requires invasive diagnostics in the live animal or histopathological examination at necropsy to demonstrate its presence. Remote behaviour monitoring of lot-fed cattle could present an early diagnostic for acute episodes of rumen lactic acidosis.

This case report describes the use of a remote real time locational system (RTLS) to evaluate behavioural parameters of four animals deemed to have died from acute acidosis in a feedlot. Ante-mortem monitoring by the RTLS in the days before euthanasia, or spontaneous death, of these four cases is described.

**Materials and methods:** The behavioural data was obtained from a South-East Australian beef feedlot. All data was collected by onsite veterinarians, research students and trained animal health staff.

Two pens, Pen A and Pen B, were comprised of 300 Angus and mixed-breed steers weighing <380kg at feedlot entry. All steers received a wirelessly transmitting ear tag which relayed behavioural data to the RTLS. The RTLS recorded the following hourly behavioural parameters for each steer: DISTANCE TRAVELED (DT), defined as the mean distance walked (metres/hr); FEED BUNK TIME (FB), defined as the mean time spent in the feed bunk zone (mins/hr); WATER TROUGH TIME (WT), defined as the mean time spent in the water trough zone

(mins/hr).

One of the case subjects originated from Pen A (A1) and three of the case subjects originated from Pen B (B1, B2 and B3). The behavioural parameters for these individuals and the remaining group of animals in Pen A and Pen B were analysed. In the case subjects, a diagnosis of ruminal acidosis was made from gross pathology observations at necropsy. Specific study time periods were defined to compare the behavioural parameters of the case subjects to the mean behavioural parameters for each pen. All steers arrived at the feedlot between 5pm and 8pm. The study time period was defined as 28hrs for Pen A and 31hrs for Pen B from the time of arrival. This was determined by a trend existing in the amount of time spent in the feed zones in the four case subjects.

**Results:** Distance Travelled: There was no difference in distance travelled between the case subjects and the mean DT of Pen A (271.0m/hr; s.d. 27.8) and Pen B (289.6m/hr; s.d. 31.1).

**Feed Bunk Time:** In the first three hours post-arrival, all case subjects had an increased FB (2.12-23.85 min/hr) when compared to their respective pen mean. After this period to the end of the study time period, case subjects had a reduced FB (1.8-3.24 min/hr) compared to their respective pen mean FB (2.52 & 3.96 min/hr for pens A and B respectively).

**Water Trough Time:** There was no obvious trend in the mean WT and the WT for the case subjects was consistent with that of their respective pen (0.72min/hr for Pen A and 0.36min/hr for Pen B). From 1 to 3 hours after the initial spike in FB, all case subjects went to the trough and case subjects A1, B2 and B3 had a higher WT compared to their respective pen mean WT.

**Conclusions:** The behavioural data demonstrated that case subjects had greater FB in the first 3 hours after arrival at the feedlot, when compared to their respective group. All case subjects showed a decrease in FB after this time period. Compared to their respective pens, three case subjects had greater WT during the 1-3 hours after FB. These findings are consistent with the literature on the feeding behaviour and clinical pathophysiology of acidosis, wherein an initial starchy meal after a period off-feed (e.g. after transport) leads to an alteration of rumen properties resulting in inappetence and dehydration.

Further studies validating remote behavioural monitoring of feedlot animals are warranted for use as a potential diagnostic tool for SARA, to prevent mortalities and improve feed intake and ADG.

This Meat & Livestock Australia project was jointly funded by grain-fed levies and the Australian government in consultation with the Australian Lot Feeders Association.

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HH-P05

### Early Detection of Bovine Respiratory Disease (BRD) in Feedlot Cattle through Wearable Sensor Technology

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The objective of the study was to evaluate the effectiveness of



the Advex Management System (AMS), a wearable sensor device that monitors feeding activity, to detect BRD versus standard visual detection by feedlot cowboys (Cowboy) in confined cross-bred beef heifers at high risk of developing BRD. Nine hundred twenty (920) heifers in a commercial feedlot in Kansas, USA, were randomly assigned to an AMS (462) or a Cowboy group (458). Both treatment groups received an AMS monitor in each ear as well as identical processing at feedlot arrival. AMS is a proximity sensor that automatically records feeding activity data. A proprietary algorithm correlates such data to potential health issues. AMS was the sole method used to identify heifers requiring treatment for BRD whereas heifers in the Cowboy group were observed by feedlot personnel as per standard procedure for visual/behavioral signs of BRD. Upon a BRD diagnosis, heifers were treated with the same antibiotic product. If necessary, sick heifers received a second and a third antibiotic treatment before they were removed to a hospital pen. Morbidity, mortality and growth performance variables were analyzed using linear mixed models which included fixed effect of treatment group and random effect of block. No differences ( $P < 0.24$ ) were observed in the number of heifers treated once for BRD between AMS (32.0%) and Cowboy (35.7%) but heifers in AMS had increased first treatment cure rates compared to Cowboy (74.5% vs. 45.3%;  $P < 0.001$ ). Fewer heifers in the AMS group required second and third treatment than in the Cowboy group resulting in less treatments per heifer for AMS (0.43 vs. 0.67;  $P < 0.001$ ). Mortality due to BRD numerically favored AMS but was not significantly different than Cowboy (5.5% vs. 8.1%;  $P < 0.11$ ). Similarly, performance parameters such as average daily gain and feed to gain as measured at 60 days post feedlot arrival during favored AMS but were not statistically different. This study provides evidence that AMS is a viable means to accurately identify BRD within high risk populations. The use of AMS may lead to fewer treatments in high risk feedlot cattle with less labor and antibiotic use as a consequence.

HH-P06

### Biosecurity in the introduction of new animals in dairy herds: A focus for mammary Mycoplasmosis

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Bovine mastitis is a complex disease of multiple etiologies; is characterized by an inflammatory process of the mammary gland, mostly of them of infectious origin. Pathogens of the *Mycoplasma* genus are most frequent in large dairy herds. The objective of this study was to describe the occurrence of a mastitis outbreak by *Mycoplasma bovis* (*M. bovis*) in dairy cattle, after the introduction of new animals in the herd.

Milk samples from 64 clinical and 28 subclinical mastitis cases from cows in a dairy farm with average daily production of 22.000 liters of milk in an intensive free-stall system, located in southern Brazil, were analyzed. The responsible veterinarian reported that after the introduction of a new lot of animals, an

outbreak of keratoconjunctivitis, polyarthritis in anterior limbs, and pneumonia occurred in more than 6% of lactating animals and cases of mastitis with agalactia in multiple teats. The milk samples were sent for microbiological analysis. The research of *Mycoplasma* spp. was carried out in modified Hayflick culture medium (WHITFORD et al., 1994), and incubated at 37°C under in a 5% microaerophilic environment, with subsequent observation of the microbial isolation on days 3, 5, 7 and 10 after incubation. The polymerase chain reaction (PCR) were used in detection of Mollicutes class aim to identify product of 270bp (VAN KUPPEVELD et al., 1992) and for amplification of the *M. bovis* DNA, specific primers with 360 bp product were used (GONZÁLEZ et al., 1995).

Of the 64 clinical and 28 subclinical mastitis cases, 4.6% ( $n = 3$ ) and 17.8% ( $n = 5$ ) were positive for growing of *Mycoplasma* spp. respectively. The PCR of three samples from the bulk tank, were positive for the *Mycoplasma* spp. PCR with specific primers for *Mycoplasma bovis* of the isolates in the microbial culture were all positive for this agent and also for the samples of milk bulk tank.

The origin of the outbreak has been related with the introduction of new animals into the dairy herd, considering the clinical signs presented by the animals at the beginning of the outbreak, which usually occur in *Mycoplasma bovis* disease. Further research studies should be carried out to evaluate the dynamics of *Mycoplasma bovis* infection in dairy herds.

**KEYWORDS:** Mycoplasmosis, mastitis, bovine, outbreak.

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HH-P07

### Management of lactational mastitis in dairy herds from western Colombia

#### Grant funded by Colciencias and Universidad de Caldas.

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**Objective:** To study the preventive and management practices used to control lactational mastitis in dairy herds from western Colombia.

**Materials and Methods:** Between January/2013 and Novem-



ber/2014, 152 commercial dairy herds from three departments in western region of Colombia: Caldas (n=76), Risaralda (n=47) and Quindío (n=29) were visited. A questionnaire was administered to all the farmers on preventive and management practices to control lactational mastitis, and storage conditions for antimicrobials were inspected and recorded. Descriptive statistics and frequency tables were generated. Differences between frequencies of variables in the three departments were explored using Pearson  $X^2$  test or Fisher exact, as appropriate using Stata 15.1 (StataCorp, College Station, Texas, USA).

**Results:** From the 152 herds, 53 (35%) purchased lactating cows to expand the herd, four of them reviewed the history of mastitis of the cows, and only one conducted bacteriological culture for mastitis pathogens before introducing the cow to the herd. In 13 herds the California mastitis test (CMT) was used to detect mastitis upon the cows' arrival to the farm. For mastitis diagnose during lactation, CMT was used in 143 herds (95%). Also, the milk of forestripping was checked for clinical mastitis signs in 140 herds (94%). There were no statistical differences between departments in the frequency of these practices.

Regarding the treatment of lactating cows, 30 herd managers (20%) treat all the mastitis cases (clinical and subclinical), the other producers (n=122, 80%) treat only severe, chronic or recurrent cases. The treated cows were clearly identified with physical marking in 54 herds (35%), the remaining 98 herds (65%) did not identify the treated cows. The identification of the differed between regions ( $P=0.00$ ). Most of the herdsmen (n=124, 82%) did not segregate the treated cows in parlor or during milking from the non-treated ones, this practice was statistical different between departments ( $P=0.004$ ). The person in charge of applying the mastitis treatment to the cows had not received any training about that matter in 61 (40%) of 152 herds, being Quindío the department with less trained personnel (51%,  $P=0.001$ ). As a result, some mistakes were detected in the farms when using an intramammary antibiotic: the teat end was not disinfected before treating the cows in 41 herds (26%), most frequently found in Quindío (45%,  $P=0.036$ ) and the personnel did not use gloves when treating the cows in 89 herds (59%), without differences between departments.

In regard of the destination of the milk from treated cows with antibiotics, 82 herds (55%) discarded that milk, 57 herds (38%) use the milk from treated cows to feed the calves, and 10 herds (7%) use it for on-farm consumption. Dry cow therapy with antimicrobials was practiced in 146 herds (97%), usually as blanket dry cow therapy (n=103, 73%). The use of an additional antimicrobial in cows with clinical mastitis before drying off was a common practice (n=117, 79%). Most of the herds (n=126, 85%) had not defined clear policies about culling cows for mastitis, and one herd did not cull any cows for mastitis. Finally, in 101 herds (70%), the storage of the antibiotic was adequate: in a dry, clean room, the products were labeled and there were no expired antibiotics. However, 30% of the herds (n=43) did not meet these criteria, being more evident in Caldas (43%,  $P=0.01$ ).

**Conclusions:** This study identified some problems in the preventive and management practices of lactational mastitis in dairy herds from the western region of Colombia, related with biosecurity, identification of the cows, use of intramammary antimicrobial products, antimicrobial storage, discard of the milk from treated cows, dry cow therapy, and culling policies. It is necessary to establish training programs to farmers in Colombia about the correct prevention and management of lactational mastitis. A joint effort between government and private companies for training all the characters of the milk production chain

about the prevention and management of lactational mastitis would be ideal. The initiative should include farmers, milkers, veterinarians, pharmaceutical companies and the milk processors companies. Unified criteria for management of lactational mastitis and antimicrobials use should be taught, as well as sensitization of the economical and health impact of the bad use of antimicrobials. Additionally, it should include a strict control of sale and distribution of antimicrobials, in order to have a more responsible and competitive dairy industry.

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HH-P08

### Utilization of meat inspection data in detecting *Mycoplasma bovis* - positive dairy farms

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**Objectives:** *Mycoplasma bovis* causes economic losses to cattle farms and affects animal welfare, also increasing the use of antibiotics. Early detection of positive farms is beneficial in risk management and in hindering the spread of *M. bovis* to new farms. The objective of this research was to use meat inspection data as a tool of herd health management to identify *M. bovis* positive farms.

**Materials and methods:** All the meat inspection data was collected from one abattoir in Finland in the year 2015 from April to December. In this retrospective cohort study only dairy cows were included, study population being 20 401 cows in total.

Post mortem meat inspection data was collected from the routine meat inspection at the slaughter line. Data recorded for this study included inspected pneumonia (pneumonia and pneumonia with abscesses) and pleuritis cases. Due to practical reasons, mild chronic pleura lesions were not registered.

All the animals were categorized according to known *M. bovis* infection status in the farm slaughtering the cow. Animals sold to the abattoir from a known *M. bovis* positive farm were assigned to category 1 (n=124) and animals from a known contact farm with the *M. bovis* infection to category 2 (n=57). Contact farm is a farm with a known animal purchase from a *M. bovis* -positive farm. Some farms were tested positive during the study year 2015. Animals sent to slaughter from these farms or contact farms during the year 2015, before the positive laboratory results, were assigned to category 3 (n=65). Rest of the animals were assigned to the control group (n=20 155).

The risk of a farm in the different categories for having lung lesions was evaluated with mixed-effects logistic regression model. The farm was included as a random factor. The amount of slaughtered animals per farm in the years 2011 and 2015 reflecting the size of the farm and the age of the cow were included in the model as a confounders. Logarithmic transformation was used to normalize distribution with both continues variables. There was no interactions between variables. The final model consisted the amount of slaughtered animals per farm, age of the cow and *M. bovis* status category. Stata IC version



14 (Stata Corporation, Texas, USA) was used for statistical analyses.

**Results:** The total amount of lung lesions (pneumonia and pleuritis registered) among the cows was 2.7 % (n=553).

The odds for lung lesions in the category 3 was 6.6 times greater than in the control group ( $p < 0.001$ , CI 3.2–13.9). The odds for pleuritis was seven times greater in the known *M. bovis* positive farms compared to control ( $p < 0.01$ , CI 1.6–30.9). Pleura lesions were present together with pneumonia. This suggests that these cases were more severe than the others. There was a trend seen as a slightly elevated risk for lung lesions in the known *M. bovis* positive farms (OR =2.2,  $p < 0.06$ , CI 1–4.9).

**Conclusions:** *M. bovis* positive farms had a greater risk for pneumonia and pleuritis in post mortem meat inspection compared to other farms. The lung lesions seem to increase already before the positive laboratory results. Most probably being more numerous during the time the infection is active on the farm.

This knowledge can be used in herd health management in detecting farms with *M. bovis*. Identifying *M. bovis* positive farms as soon as possible, aids risk management on the farm and helps to avoid the spread of the infection to new farms. The amount of lesions, especially pleuritis, was small.

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HH-P09

### Variability in dry-off practices – results from France and Denmark

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Previous work have focused on udder health aspects<sup>1-3</sup> of the dry-off, but aside from that, there is a dearth of information of the impact on cow health and productivity of the applied dry-off procedure. Although some recommendations are given in the current literature<sup>4</sup>, there exist still a multiplicity of dry-off management procedures of the dairy cows across the different European countries (linked to the variety of milk production levels and applied management procedures).

**Objectives:** This work aims to assess the practices (feeding management and milking routine) applied to dry off cows in two European Countries; France and Denmark.

**Materials and methods:** Information on dry off management was collected at herd and animal level during a multicenter prospective cohort study involving 14 investigator sites including 558 dairy cows from 37 herds in France and 347 cows from 21 herds in Denmark. All dairy cows dried-off for either future lactation or for culling during the study duration were included. Information on disease events, production parameters, feeding and management practices at herd and cow level were collected. Abrupt dry-off implied no prior change in either milking frequency or feeding regimen. Gradual dry-off implied any change in these factors.

**Results:** The information on how cows were dried-off at herd

level (representing the overall management strategy at herd level) and at animal level (representing the actual included cows in the study) are as follows. In France 83,8% of herds employed an abrupt strategy at herd level. At animal level only 46,1 % were dried off abruptly. In Denmark 71,1% of herds dried cows off gradually, at animal level 67,4% of cows were dried off gradually.

Interestingly definitions of dry-off methodology were understood differently by farmers taking part in the study thus creating a large difference between the overall dry-off management at farm level and what was implemented at cow level.

**Conclusions:** This study demonstrated high variability in dry off methodologies applied at herd and animal level in France and Denmark. Additionally, definitions of dry off management does not seem to be agreed upon.

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HH-P10

### Comparison of 3 navel dip products on prevention of navel umbilical inflammation

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**Introduction:** Calves less than one week of age are most commonly afflicted by infections of the navel because the umbilical stump usually takes 7-10 days to dry up. The frequency of navel infections has been associated to cleanliness of calving environment, cleanliness of calf pen or hutch, adequacy of colostrum management and navel dipping. Several studies have consistently shown that about 2% of preweaned calf deaths are due to navel infections. Navel dip of newborn calves is commonly used to prevent umbilical issues.

**Objectives:** The objective of this study was to compare a novel navel dip formula compared to two commercial products on speed of navel drying and prevention of navel infections.

**Materials and methods:** All animals enrolled on the study were born in two separate facilities, but brought to the main one where



calves are raised after birth. They were managed equally. After birth, calf navel was evaluated for: diameter of navel stalk, diameter of umbilical cord, presence of inflammation, pinch test reaction (pain), if the navel was wet or dry outside, presence of secretion, hernia, blood. Also, calves were checked for general health status, including rectal temperature, eye and nasal discharge, ear drop or head tilt, cough or other signs to pneumonia and fecal consistency. After evaluation, the navels were dipped with either Product A (Bovadine Navel Dip, 2% iodine, DeLaval), Product B (salts, Super 7+, Vetericyn), or Product C (7% iodine tincture, Thatcher). Next, each calf received 4 L of colostrum (less than 1 hour after being born), and was housed in an individual hutch. At day 7 another evaluation using the same parameters of day 1 was performed. Logistic regression was used to evaluate if differences between test products.

**Results:** Approximately 120 calves were enrolled per treatment group. All newborn calves were eligible to be included in the study, unless they were unable to stand, or had other signs of illness or injury. Results showed that at day 7 after birth, Product A (2% iodine) had similar odds to Product B (salts; OR = 1.4; 95% CI = 0.79 – 2.50) or Product C (7% iodine; OR = 1.03; 95% CI 0.50 – 2.12) in navel inflammation at day 7. Overall, no differences were observed between the test products in navel inflammation ( $P = 0.70$ ) or navel dryness ( $P=1.00$ ). Data also showed that navels that were wet at day 1 were 1.7 times more likely (95% CI 1.10 – 2.70) to have an inflammation at day 7, but we observed no differences between products.

**Conclusions:** As a conclusion, navels that were wet at birth were 1.7 times more likely to have an inflammation at day 7. Furthermore, all products showed similar efficacy in preventing navel inflammation at day 7 after birth.

HH-P11

## Digestive disorders of dairy cow death: A retrospective study in a large dairy farm

### The causes of dairy cow due to digestive disorders

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High mortality among dairy cows constitutes a problem both finance and animal welfare. Several studies have evaluated causes of dairy cow deaths. However, the majority of studies describing causes of death were based on questionnaires with farmers or veterinarians. It is uncertain to what degree such information is sufficient and reflects the true cause of death or euthanasia. We evaluated the causes of death based on a thorough necropsy in a large dairy farm during 10 years. The major cause of death was digestive disorders and we conducted a retrospective study for the event of digestive disorders. The study was investigated with dairy cows death from 2001 to 2010 at National Institute of Animal Science (NIAS) in Chonan. All cows were raised in free-stall barns with standard Korean feeding practices for dairy cattle and fed a total mix ratio. All cows were milked twice daily. Farm managers and veterinarian recorded cow health and mortality. Data, including history, calving status, and disease information were collected from veteri-

nary records. Postmortem examinations of dairy cows in the period were performed according to standard routines. Each examined case was assigned a main diagnosis based on the postmortem macroscopic and histopathological findings, as well as the results of further laboratory analysis. Enteritis is the most common cause of death, and abomasum perforation (7 cases), bloat (3 cases), abomasum ulcers (2 cases), peritonitis (1 case), perforation of intestine (1 case) and rupture of intestine (1 case). The causes of death in enteritis were enterotoxemia and Johnes' disease. Over half the cases of abomasum perforation and ulcers occurred within 7 weeks of lactation. This result similar with abomasal ulcers can be seen any time during lactation, but are common in high-producing, mature dairy cows within the 1<sup>st</sup> 6 weeks after parturition. Abomasum perforation and ulcer were likely to have been treated without determination of etiology because cow carcasses are seldom subjected to postmortem in the field. This study showed that enterotoxemia and abomasum perforation were fatal dairy cows and good management need around parturition with high-yield milk producing cows. The study also indicated that necropsy examination findings with farm records in a complete postmortem evaluation would ideally provide a meaningful degree of detail when assessing causes of death.

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HH-P12

## Genomic Predictions to Improve Wellness in Holstein Cattle

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Impaired fertility together with painful conditions like lameness and mastitis are part of the welfare concern and responsible for around 70% of the culling of Holstein cows. Improving wellness traits through direct genetic selection presents a compelling opportunity for dairy producers to help reduce disease incidence and improve profitability when coupled with sound management practices. For that reason, Zoetis created a genetic evaluation to provide genomic predictions for dairy wellness traits - mastitis (MAST), metritis (METR), retained placenta (RETP), displaced abomasum (DA), ketosis (KETO), and lameness (LAME) to dairy producers. The objective of this study was to create genomic predictions for these wellness traits using a single-step methodology that combines phenotypic, genomic, and pedigree information in one analysis. The number of phenotypic records ranged from 2,432,181 for KETO to 4,919,527 for MAST obtained from 2,590,767 animals. The number of genotypes animals exceeded 440,000. The analysis was conducted using a univariate threshold animal model containing a fixed effect of lactation and random effects of herd x year x season of calving, animal, and permanent environment. The estimated heritabilities of the traits ranged from 0.059 (MAST and METR) to 0.081 (DA). The outputs were genomic predicted transmitting abilities (gPTA) where a higher value is associated



with a greater risk of the health event. Genomic predictions were obtained for 3,356,010 animals and the average of the gPTAs were -0.078, 0.005, -0.024, 0.004, -0.16 and 0.17 for MAST, METR, RETP, DA, KETO, and LAME, respectively. The average reliability for genotyped animals were 60%, 54%, 50%, 56%, 57%, and 55% for MAST, METR, RETP, DA, KETO, and LAME, respectively. For easier interpretation, the gPTA were expressed as standardized transmitting abilities (STA), with a mean of 100 and a standard deviation of 5, with higher values being more desirable. Animals with higher STA values have lower relative disease risk compared with herd mates with lower STA values. The results of this study indicate that wellness trait genomic predictions can be used to effectively select for improved health performance in dairy herds.

HH-P13

### Wellness Trait Genomic Predictions Effectively Identify Holstein Animals with Greater Genetic Risk for Transition Health Events

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Zoetis Genetics

The objective of this study was to demonstrate the effectiveness of Zoetis Wellness Trait genetic predictions in commercial herds of Holstein cows. Genomically enhanced standardized transmitting abilities (STA) for the Zoetis Wellness Traits including retained placenta (RETP), metritis (METR), ketosis (KETO), displaced abomasum (DA), mastitis (MAST) and lameness (LAME) were generated for more than 2,700 Holstein cattle using the Zoetis Wellness Trait Genetic evaluation. Cows were divided into equal sized genetic groups of four according to their STA value. Greater STA values are more desirable and as greater STA values are associated with lower relative genetic risk of the pertinent health event. Lactation records were collected from on farm herd management systems. Recorded health events were transformed into binomial observations (unaffected: 0, affected: 1) by lactation. All available lactations were included in the analysis. Statistical analysis was conducted using a general linear mixed model with a binomial distribution and a logit link. Genetic group and lactation were included in the model as fixed effects with animal and herd x year x season as random effects. The response variable was the binomial health event. Differences between genetic groups were considered to be statistically significant at the  $P < 0.05$  level. Significant differences were detected between all genetic groups for RETP, METR, KETO, DA, MAST and LAME. Difference in incidence for the best and worst genetic groups were 55% (RETP), 41% (METR), 44.5%, (KETO), 99% (DA), 45% (MAST) and 40% (LAME), respectively. These results clearly demonstrate that the Zoetis wellness trait predictions can effectively identify animals at greater genetic risk for these transition related health events and indicate that genomic data of young calves and heifers can be used to effectively predict future health performance.

HH-P14

### Estimating the lifetime total economic costs of respiratory disease in beef and dairy calves in the UK

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**OBJECTIVES:** Penetration of calf respiratory disease vaccination in the UK is low (estimated 25% of dairy calves and 35% of beef calves). Market research indicates that an important barrier to vaccination is a limited understanding of costs of calf respiratory disease across the lifetime of the animals. This project aimed to estimate the lifetime total economic cost of calf respiratory disease in beef and dairy cattle.

**MATERIALS AND METHODS:** Separate analyses were conducted for dairy heifer (DH), dairy-bred beef (DBB) and beef suckler (BS) calves. Analyses considered costs at the individual animal level from the perspective of the producer. Effects on expenditure (additional resources) and losses (decrease in production) were derived from the published literature. Contemporary (2015) valuations were applied using data mainly from Agriculture and Horticulture Development Board (AHDB).

**RESULTS:** The total cost included the immediate costs of calf respiratory disease and the subsequent impact on productivity across the animal's life course. In DH calves this included a 2-week delay to first calving, 4 and 8% reduction in first and second lactation milk yields respectively, and a lifetime reduction of 109 days in milk due to reduced longevity in the herd. In DBB and BS calves costs were compared to target to finish at 22 (DBB) or 16 (BS) months of age and included 72 and 202g reduction in daily liveweight gain for moderate and severe/chronic respiratory disease respectively, and impaired carcass quality. The costs of impaired lifetime productivity exceed the immediate costs of calf respiratory disease. The estimated total economic lifetime cost of respiratory disease was: £772 (DH); £131 (DBB moderate) £327 (DBB severe); £128 (BS moderate); £263 (BS severe).

**CONCLUSIONS:** Calf respiratory disease leads to significant immediate and subsequent lifetime productivity economic costs. These cost estimates have potential to inform partial budget models to assess the incremental net benefit of vaccine interventions.

HH-P15

### Assessing immune capacity of dairy heifers in a pasture-based production system

#### Associations between immune competence, stress responsiveness and production in Australian Holstein-Friesian heifers reared in a pasture-based production system

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The objectives of this study were to assess general immunity in first lactation Holstein-Friesian heifers reared under a pasture-based production system and to investigate associations between immune capacity and stress responsiveness, health and productivity. A commercial vaccine was used to induce measurable cellular and humoral immune responses, and animals were classified as High, Average and Low for each trait independently and in combination (general immune capacity. Cortisol responses to handling and yarding were used to assess stress responsiveness, worm egg counts to assess resistance to internal parasites, somatic cell counts to assess udder health and estimates of total daily milk volume, milk fat and milk protein to assess productivity. A total of 403 pre-joining heifers from two herds in a seasonally calving, pasture-fed production system were enrolled in the study and their performance monitored throughout their first lactation cycle. Correlations between humoral immunity and stress responsiveness were favourable. Favourable correlations were also observed between humoral immunity and resistance to internal parasites. No significant correlations were observed between immune capacity and milk yield, milk fat and milk protein content. Selection for enhanced immune capacity is expected to provide a genetic based strategy to improve dairy cow health, welfare and long term industry sustainability.

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HH-P16

### Monitoring synergy between neonatal diarrhea and pneumonia vaccination in heifer's health and growth until weaning.

Laura Elvira \*Carbonell Carlos Cristina Pousada

MSD AH

**Introduction:** Healthy, productive herd replacements are the result of a good management thought their rearing. Initially, quality and management of colostrum is the most important risk factor for dairy calf health. Numerous studies have associated failure of passive immunity transfer to both short term effects, like increased neonatal morbidity and mortality of scours and pneumonia. Additionally, different studies have shown how suffering scours increase the risk of suffering pneumonia later on in the calves. Moreover, neonatal diseases produce long term effects such as reduction in calf growth rate or decreases in future milk productivity.

By the other hand numerous studies have evaluate the improvement of scour morbidity and mortality through dam's vaccination during the last third of pregnancy, increasing the specific immunoglobulins produced against the main pathogens involved and transferring them through the colostrum. However, its potential positive effect for reducing not only scour morbidity and mortality, but also indirectly pneumonia in early age has never being evaluated to our knowledge.

**Objective:** The objective of the present study was evaluating the effect of vaccinating the dams for scours in the later total morbidity and mortality, not only for scour, but also for pneumonia. For a better pneumonia diagnosis, lung scanning ultrasound was used, being to our knowledge the first time that not only clinical but also subclinical pneumonia is monitored through this tool in this type of vaccine study.

**Material and methods:** The study was blind and randomized. Dams were randomly assigned to two study groups: vaccinated to prevent scour with Rotavec Corona® or Control negative not vaccinated; and blindly vaccinated by an administrator. Later on, the calves born were fed at least 2 liters of colostrum (mainly freshly milked from their dams, but otherwise the colostrum dam administered was registered) and the colostrum intake was monitor individually and indirectly measuring total proteins. Additionally, after 2 weeks of age the calves were vaccinated in groups to prevent pneumonia with Bovipast®. Sanitary follow up was performed as usual registering the appearance of disease and treatments. Additionally, an investigator evaluate individually all calves every 10 to 15 days in order to identify sick calves using McGuiirk scoring and lung scanning ultrasonography, and measuring body weight with a girth tape.

**Results and conclusion:** The study is still finishing and results are not complete, we will send them as soon as possible

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HH-P17

### Combined use of intranasal bovine rhinotracheitis and parainfluenza 3 vaccination and tulathromycin for the prevention of respiratory infections in calves arriving at a high-risk herd

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**Objectives:** This study examines the efficacy of an intranasal bovine rhinotracheitis and parainfluenza 3 vaccine (TSV-2, Zoetis Japan) used in combination with tulathromycin (Draxxin, Zoetis Japan) for the prevention of respiratory infections in high-risk calves.

**Materials and methods:** The study was conducted at a Japanese Black cattle farm that was experiencing frequent respiratory infection outbreaks in calves after transitioning from individual housing to the farm's robotic calf feeding facility. The farm had been previously infected with respiratory syncytial virus, which affected nearly all existing calves and required a long-term antibiotic therapy for control resulting in inefficacy of multiple first-line antibiotics and reduced herd immunity. Between November 2016 and January 2017, 73 2- to 3-week-old calves were either treated with a dose of intranasal vaccine (TSV-2) and 2.5 mg/kg of subcutaneous tulathromycin (n = 37) or untreated (n = 36) at the time of transition. Respiratory infection incidences were compared between groups and with the same period of the previous year.

**Results:** The incidence of respiratory infection was 29.7% (11/37) in treated calves. This figure was significantly lower than 77.8% (28/36) in untreated control calves. It was also significantly lower than 78.6% (55/70) observed in the same period of the preceding year. In untreated calves, respiratory infections mostly developed within the first two weeks of transition,



whereas they were generally observed after a month of transition in treated calves. The earliest infection onset was 21 days after transition in the treated group.

**Conclusions:** Combined use of intranasal vaccine (TSV-2) and tulathromycin is an effective approach for the prevention of respiratory diseases in calves transitioning into a high-risk herd. The efficacy observed in the present study was likely a result of synergy between the intranasal vaccine, which stimulates local immunity more rapidly than subcutaneous vaccination and also has an immunomodulatory action, and tulathromycin, which lasts up to 10 to 14 days at an effective concentration in the lungs.

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HH-P18

### The optimal replacement policies for Thai dairy cows

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**Purpose:** The objective of this study was to apply the developed economic simulation model to predict the optimal time for replacing cow of the large dairy farms in Thailand.

**Materials and Methods:** The dynamic stochastic modeling with one week step for 520 weeks of reproductive cycles and milk production of individual cow was created in Palisade @ Risk (Palisade Corporation, Ithaca, NY, USA) as the details in a previous study (Inchainri et al., 2011). The dynamic stochastic cow data and economic data were included as inputs in the stimulation model. The retention pay off (RPO) was calculated to determine the optimal time for replacing cow once when it was less than zero during lactation stage. To estimate the possible longest life time of a specific cow, the Palisade software with the function of Evolver (Palisade Corporation, Ithaca, NY, USA) was used to determine the specific situation of cow and economic input for the longest life.

**Result:** Our results indicated that a cow with low milk production (4000 kg per 305 days) or 53% of herd size should be replaced as soon as possible whenever a dairy farm could provide a replacement cow with a higher milk production. In comparison of non-pregnant cow and pregnant cow during lactation, the economic loss might reach to 15,000-32,000 baht per cow when the optimal decision for replacement was ignored. A cow was possible to prolong their replacement to 10 years when the milk price and discount rate was low.

**Conclusion:** The simulation model was advantage to support decision of a dairy farmer of when would replace an individual cow to gain more benefit. With our tool, the unnecessary loss of cow productivity would be able to avoid.

**Keywords:** dairy cow, retention pay off, replacement

HH-P19

### Usefulness of veterinary services in the agricultural insurance scheme in cattle farms: A case report in Japan

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**Objectives:** In Japan, farm animal mutual aid is a voluntary insurance scheme. It is intended to cover the cost of death, illness, and injuries of the animals. In veterinary hospitals belonging to each prefecture's agricultural insurance association across Japan, around 1,900 veterinarians treat insured farm animals. The agricultural insurance scheme covers over 4 million dairy and beef cattle. Japan's national government bears half of the mutual aid contributions. In Miyazaki Prefecture, veterinary practitioners in agricultural mutual aid associations make regular farm visits to monitor the health of insured animals. This study aimed to clarify the usefulness of veterinary services in the domestic animal mutual aid system by means of an average case of a beef farm for Japanese Black cattle.

**Materials and methods:** In 2016, the farm had 827 breeding cows and calves and 201 fattening cattle insured against illness or injuries and death. The total valuation of the animals was \$4,351,083, and the optional compensation proportion chosen by the farm's owner was 20%. The farm's owner paid a total of \$35,126 in mutual aid contributions; that included administration fees and \$5,606 for a herd health management service, which included regular farm visits and a reproductive examination every 2 weeks in a year. The first pregnancy diagnosis was performed using ultrasonography 30 days after artificial insemination; the second pregnancy diagnosis was made 4 weeks later in animals that were pregnant in the first diagnosis. An additional check (fresh check) was conducted 40 days or more after calving. Non-pregnant cows after the fourth insemination or more than 120 days postpartum underwent treatment. For early-weaning calves, 10 ml of rumen fluid from healthy cows was orally administered once every 2 weeks.

**Results:** After regular veterinary visits, the average number of days open was 14 days shorter than before such visits. Following such visits, production costs were \$28,778 lower based on the total number of cows (the calculation of production costs was based on \$7/day/cow). As a result of the visits, the number of calving animals increased by 48 even though the total number of rearing animals was unchanged. In addition, 91% of the breeding animals calved once a year. Based on the average market price of a calf (8–9 months old) of \$6,529, that resulted in an increase of \$313,393 in the farm's income. The rate of illness, injuries, and death among the 922 calves born decreased from 6.5% (before regular farm visits) to 3.5% (after the visits); the daily gain in body weight among 483 castrated calves (8–9 months old) increased from 1.01 to 1.07 kg/day. The difference between the average market price and the farm's average selling price before the visits was -\$78; the difference after the visits was +\$288. Thus, the farm's owner earned \$37,493 in additional income because that owner was able to sell 130 castrated calves in one year. For the veterinary hospital, income increased by \$21,799: \$19 of the contract charge per animal multiplied by 294 animals (\$5606); \$14,498 in medical treatment; and \$1695 for the first examination fee. This works out as an increase of \$74 per insured animal.

**Conclusions:** The financial effect for the farm was that it bore



costs of \$40,696 and received \$14,058 as cash compensation for the 22 deaths and \$44,573 in insurance as compensation for the 394 cases of illness and injury. Furthermore, it was determined that the farm earned around \$380,052 as a result of herd health management consulting. Thus, the domestic animal mutual aid scheme in Japan enables substantial improvement in farm productivity.

HL-P01

### **A modified claw trimming method shifting the load to the wall and bulb region – an ex vivo study**

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**Objectives:** Lameness in cows due to claw lesions continues to be a major problem in modern dairy farming. Flooring systems, mechanical factors and inappropriate claw trimming are among the predominant risk factors. Numerous studies focusing on the influence of stable flooring on claw biomechanics and claw trimming have been carried out using pressure measurement systems (Bergsten, Herlin 1996; Carvalho 2006; Zeiner et al. 2007). To objectively analyze the weight distribution, different methods have been developed to subdivide the sole in regions (Greenough, Vermunt 1991; Carvalho 2006). The “Dutch method” for claw trimming (Toussaint-Raven 1985; Fiedler et al. 2004; Greenough 2007) has been used many years but has been questioned recently by some authors due to development in dairy cow housing (Zeiner et al. 2007; Ouweltjes et al. 2009). Claws of cattle kept on pasture or soft rubber floors develop a prominent weight bearing margin around the tip and the abaxial wall of the claw (Benz 2002; Nuss et al. 2014). The hardest part of the claw is the wall horn which should therefore be the main weight bearing part (Franck et al. 2006). The aim of this study was to measure the pressure distribution under claws trimmed with a modified method imitating “pasture claws” on different floorings using a foil-based pressure sensor.

**Materials and methods:** 11 isolated distal hind limbs of Holstein Friesian dairy cows were obtained from a local abattoir and prepared to be fastened to a load applicator. Following functional trimming of all claws according to the “Dutch method”, an edge of 2 mm height was carved out around the tip and the abaxial wall of the claw. Metatarsi were arranged at a 90° angle towards the ground surface and a load of 150 kg was applied. In order to imitate in vivo conditions tendon strain on the deep digital flexor tendon and the digital extensor tendons were realized with 50 kg and 5 kg weight respectively (Riemersma et al. 1988). Pressure measurements were carried out on concrete and three different rubber mats (KARERA, KURA, profi-KURA; KRAIBURG Holding GmbH & Co. KG, Waldkraiburg, Germany) using a sensor foil (M3200E, Hoof™ System, Tekscan Inc., Boston, MA, USA). The sensor was positioned between flooring and claw and four measurements per floor type and claw were carried out. Maximum pressure, average pressure and contact area were recorded and analyzed. Additionally the pressure image was subdivided in five regions according to Carvalho (2006): region 1 - abaxial wall; region 2 - slope with typical sole ulcer area; region 3 – slope cranial of 2; region 4 – tip of the claw; region 5 – soft bulb. The localization of maximum pressure was recorded for each claw and allocated to one region.

**Results:** Results showed that overall contact area was 28.9±4.9 cm<sup>2</sup> on concrete flooring and up to 48.8±5.6 cm<sup>2</sup> on rubber mats with a clearly visible pressure pattern tracing the edge of the wall horn and the bulb. The overall average pressure was 49.8±9.0 N/cm<sup>2</sup> on concrete and up to 32.6±3.6 N/cm<sup>2</sup> max. on rubber mats. Average ratio of weight distribution



between lateral and medial claw was 1.7:1 on all floor types. Maximum pressure on rubber mats was mostly located at region 5 in both claws (56.0% of all measurements), on concrete at region 5 in the medial claw (49.0%) but at region 1 in the lateral claw (37.7%). Region 2, which resembles the typical area for developing sole ulcers, carried maximum pressure in 3.9% of performed measurements (concrete) and in 2.1% of measurements on rubber mats.

**Conclusions:** The described modified trimming method shows a considerable load shift in the claw. Most of the applied weight is located on the bulb area and weight bearing margin of the claw which relieves the sensitive parts of the sole. Concrete flooring is disadvantageous to claw health as it increases maximum and average pressure and decreases contact area (Oehme et al. 2017) as could be shown in horses as well (Hüpler et al. 2015). Subdividing the sole in regions enables objective analysis of clinically relevant pressure distribution patterns. Compared to results of van der Tol et al. (2002), who performed pressure measurements on functionally trimmed claws, the modified trimming method seems to shift more pressure on the weight bearing margin (region 1). Because van der Tol used a different subdivision of the sole in regions (Greenough, Weaver 1997) a comparison is only partially possible. Further investigations will include analysis of contact area, average and maximum pressure for each region.

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**References:** For detailed reference information please contact the presenting author.

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HL-P02

### Evaluation of the clinical and bacteriological efficacy of allyl isothiocyanate in dairy cattle with digital dermatitis

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Bovine digital dermatitis (BDD) is a polymicrobial infectious foot disease of cattle prevalent throughout the world. BDD often leads to lameness and decreases in body weight and milk production, causing economic loss and animal welfare problems in the dairy industry. Although treatment with antibiotics such as oxytetracycline and lincomycin results in rapid resolution of the lesions, such treatment may leave drug residues in milk and there is a risk that drug-resistant bacteria may emerge. Since allyl isothiocyanate (AITC), a natural spicy component found in wasabi and mustard, has a wide antibacterial spectrum and is used as a food additive, it may be applicable as an alternative antimicrobial agent in this context. Here we evaluated the clinical and bacteriological efficacy of AITC in dairy cattle with BDD. Seventeen dairy cattle with BDD were divided into an AITC group (n=9) and a control group (n=8) and treated with AITC and lincomycin, respectively. Lesion severity scores and lame-

ness scores were recorded before and 7 days after the treatment. Furthermore, the bacterial population in BDD lesions before and after treatment was examined using 16S-based metagenomics analysis with a next-generation sequencer. We further analyzed the population of selected BDD lesions at the species level using random clone libraries of the full-length 16S rRNA gene. Both groups showed significant improvement of the lesion severity scores after treatment, and lameness scores were improved significantly in the AITC group. Bacterial population analysis before and after treatment revealed that AITC was as clinically as effective as lincomycin specially in terms of eradication of *Treponema* spp., which are suspected to be the major causative agents of BDD, although more divergent microbes were detected after AITC treatment than after lincomycin treatment. The present data indicate the potential usefulness of AITC for treatment of BDD.

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HL-P03

### Effect of cow block on healing duration of claw lesions and lesion severities in lame cows in western part Thailand

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**Objectives:** The aim of this study was to determine the efficiency of cow block on claw lesion and lesion severities in lameness cows in dairy farms in Western, Thailand.

**Materials and methods:** In total 376 dairy cows were determined for their locomotion score by the veterinarian using a scale of 1 to 5 on the time of veterinary visit (1= normal; 5 = severely lame). Cows scoring 3 or greater were described as clinically lame. In sum, 134 clinical lame cows from 11 dairy farms from Kanchanaburi and Ratchaburi provinces were included in the analysis. Claw lesions were composed of white line abscess, bruise sole, sole ulcer, sole abscess, white line separate, double sole. 116 cows were used claw block as case, and 18 cows no claw block were treated as control. Cows were followed every week for healing process until two months unless cows were culled. Survival analysis with Kaplan-Meier Estimator and Cox Proportional Hazard Regression was modeled.

**Results:** The results were showed that the median healing times for lame cows with claw block and no block were 21 days and 36 days. After adjusting for the lesion severities, lame cows with no block have 3.08 (95%CI: 1.49-6.38) higher hazard ratio than those with claw block.

**Conclusions:** Treatment with claw block for those lame cows aimed to promote healing capacity of claw lesions or to mitigate the hazard of lameness after claw trimming.

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HL-P04

### Identification and characterization of treponemes from a case of bovine digital dermatitis in Korean dairy cattle

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**Objectives:** Bovine Digital dermatitis (BDD) is an infectious lameness in cattle caused by treponemes, responsible for considerable economic loss in farm. It was first described in Italy in 1974, but since then it has been reported in many countries. This disease is currently prevalent worldwide in most dairy herd and now also common in Korea and affect huge number of cattle population. In the study, the etiology of BDD was identified and characterized in Korean dairy cattle.

**Materials and Methods:** Tissue sample was collected from a clinical case of BDD, confirmed by its clinical lesions. Nucleic acid was extracted from tissue sample using a QIAmp Fast DNA tissue kit. The BDD treponeme specific PCR was performed for the confirmation of treponeme spirochete. Then group specific primer was used for the characterization of the treponeme species. The sequence of amplicon was analyzed and phylogenetic tree was constructed.

**Results:** A 400bp region of the 16S rRNA gene of the bovine treponeme was amplified using group 2 (*Treponema phagedenis*-like) treponeme specific primer. The sequence was 99% identity with a number of sequences (FJ004921) of 16S rRNA genes from treponeme isolated from a case of BDD in cattle from Japan. Phylogenetic relationship also represent that our treponeme isolates is 99% similar to some of other sequences from UK (KP859541, KP063160), but less similarities to *Treponema denticola* (KT192142).

**Conclusion:** This is first identification of group 2 treponemes infection in Korean dairy cattle DD lesions. Nationwide survey of BDD is needed further characterization of treponemes infection in Korean dairy cattle.

HL-P05

### Clinical aspects and incidence of bovine digital dermatitis in a herd of Holstein cows

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**Objectives:** The objectives of the study were to describe the clinical aspect of the digital dermatitis lesions and to assess the incidence of the disease in the herd.

**Materials and methods:** The study was conducted at the Ac-

tion Felix dairy farm in Sântandrei, Oradea between January 2014 and November 2014, on 240 Holstein dairy cows.

The clinical aspect of the lesions was based on clinical examination of the acropodium region, twice a year in the spring and autumn, in the same time the incidence of digital dermatitis was determined. Digital dermatitis aspect was described by macroscopic findings and measurement of the lesion.

**Results:** Based on clinical examination we described four clinical evolution forms of the disease in the herd:

- a small epithelial defect up to 1 centimeter in diameter, well circumscribed, of round or oval shape is observed.
- an ulcerative lesion of up to four centimeter in diameter, circumscribed, round or oval, red color with a serous exudate.
- an ulcerative, sometimes phlegmonous, red-gray lesion with a serum purulent exudate and the presence of a repulsive odor. The lesion exceeds 4 centimeters in diameter, extends over the heels, and is uncircumscribed.
- chronic (untreated) lesions of digital dermatitis, epithelial cauliflower-like proliferations are observed at inspection. The surface has long and thick hair yarns agglutinated by a serum-purulent exudate that emanates a repulsive odor. The size of the lesions exceeds 2 centimeters in diameter, with irregular shapes.

Regarding the incidence, in the spring of 2014 from the total number of cows (n=240) that were examined we found at least one digital dermatitis lesion on 172 cows (71,66%), that were cows that presented up to three lesions of digital dermatitis.

In autumn of 2014 from the total number of cows (n=240) that were examined we found at least one digital dermatitis lesion on 119 cows (49,58%).

Following the study, there was a noticeable decrease in the incidence of digital dermatitis due to better management in detection, treatment and prevention of digital dermatitis within the "Action Felix" dairy farm, and also a better training of the farm staff regarding the disease control.

**Conclusions:** Based on clinically aspects, we observed four clinically different digital dermatitis lesions.

Considering the high incidence we have encountered at farm level, we can say that digital dermatitis was the major lameness causing disease in the herd.

Improving hygiene conditions, regular controls, corrective and therapeutic trimming correctly performed, as well as active monitoring, lead to a decrease in the incidence of digital dermatitis.



IV-P01

### ***Staphylococcus aureus*-specific IgA antibody in milk suppressed the proliferation of *S. aureus* in infected bovine udder**

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**Objectives:** Bovine mastitis caused by *S. aureus* is extremely difficult to control and new methods for its prevention and management are required. This study sought to investigate whether induction of immunoglobulin A (IgA) in milk by nasal immunization could suppress proliferation of *S. aureus* in the bovine udder.

**Materials and methods:** Three Holstein cows were intranasally immunized three times (2 mL/dose) with a two-weeks interval in between each dose. Within four weeks following nasal immunization, cows were tested for experimental intramammary infusion with *S. aureus*. Six cows (three non-immunized cows and three nasal immunized cows) in the early stage of lactation were infused with the *S. aureus* BM1006 strain. Milk samples were collected from day 0 of pre-immunization every week for up to six weeks, and from day 0 of pre-infusion until one week after infusion with *S. aureus*. Samples were collected from each individual udder during milking every morning, and then, *S. aureus* counts were counted. For detection of specific IgA antibodies against *S. aureus* in milk, milk samples were analyzed by ELISA.

**Results:** Anti-*S. aureus*-specific IgA antibodies were significantly more abundant in the milk of immunized cows than in non-immunized animals ( $P < 0.05$ ). *S. aureus* counts in the udder were negative in both non-immunized and nasal immunized cows one week after mock infusion. Instead, in *S. aureus*-infused udders, *S. aureus* proliferation was significantly suppressed in immunized compared with non-immunized cows ( $P < 0.05$ ). Furthermore, a significant negative correlation was found between *S. aureus*-specific IgA antibodies and *S. aureus* counts in infused udders of both non-immunized and nasal immunized cows ( $r = -0.832$ ,  $P < 0.01$ ).

**Conclusions:** In conclusion, the present study demonstrates that *S. aureus*-specific IgA antibodies in milk successfully suppressed the proliferation of *S. aureus* in infected bovine udders. Although the exact mechanism explaining such suppressive effect remains to be elucidated, nasal vaccines that can induce humoral immunity may help prevent initial infection with *S. aureus* and the onset of bovine mastitis.

IV-P02

### **Apoptosis of bovine lymphocytes during experimental inflammatory response induced by peptidoglycan**

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**Objectives:** Peptidoglycan is a molecule naturally found in the cell wall of bacteria which is able to induce inflammatory response following intramammary infusion into the bovine mammary gland. The aim of this study was to determine whether apoptosis of bovine mammary gland lymphocytes is modulated during an inflammatory response of bovine mammary gland induced by peptidoglycan.

**Materials and methods:** The experiments were carried out on virgin clinically healthy heifers (Holstein x Bohemian Red Pied crossbred) aged 16 to 18 months. Lymphocytes were obtained by lavage of the mammary gland at 4 intervals (24, 48, 72 and 168 h) following stimulation with peptidoglycan. Apoptosis of lymphocytes was analysed by flow cytometry following simultaneous staining with Annexin-V and propidium iodide.

**Results:** Stimulation of mammary gland with peptidoglycan resulted in a significant increase in proportion of apoptotic lymphocytes in comparison to the control. The portion of apoptotic lymphocytes peaked at 48 hours following stimulation. In our preliminary experiments, there was demonstrated that apoptosis of bovine mammary gland lymphocytes is also induced during an experimentally induced infection of bovine mammary gland with lipopolysaccharide and muramyl dipeptide.

**Conclusions:** The results suggest that the cell wall components are able to modulate lymphocyte apoptosis during the process of mastitis.

IV-P03

### **Effect of probiotic *Bacillus Subtilis* C-3102 strain on function of dendritic cells in bovine peripheral blood before and after parturition**

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**Objectives:** Dendritic cells (DCs) are professional antigen presenting cell specialized in antigen uptake and processing, and play an important role in the innate and adaptive immune response. DCs are differentiated from premature progenitor cells in the bone marrow, and present antigens to T cells by major histocompatibility complex (MHC) class II molecule. Although DCs are identified at 0.1-0.7% of bovine peripheral blood mononuclear cells (PBMC), the phenotype and function of DCs remains poorly understood with regard to maintaining tolerance during the pregnancy. In recent years, *Bacillus subtilis* has been widely used in livestock as feed probiotics additives, but there were few reports studying on dairy cattle. We revealed that the number of DCs in bovine peripheral blood decreased in mastitis, and that the feeding of *Bacillus subtilis* C-3102 strain (BS) prevented the cattle from mastitis. However, the mechanism of mastitis prevention by DCs has not been described clearly in dairy cattle feeding a diet with BS probiotics. In this study, we first have established a novel method for the purification of DCs from bovine PBMC using Magnetic-activated cell sorting (MACS), and then investigated the effect of BS



probiotics on bovine peripheral blood DCs before and after parturition.

**Materials and methods:** Twenty Holstein cows were bred in Miyagi Prefecture Livestock Experimental Station. Each ten cows were fed a diet with or without BS probiotics (40 g,  $1.5 \times 10^9$  CFU/g) twice a day for two months before and after parturition, respectively. At one month before and after the parturition, total PBMC were separated from the jugular venous blood of cows using Lympholyte-H (1.007 g/ml). CD172a<sup>+</sup>/CD11c<sup>+</sup>DCs were purified by a two-step MACS method from PBMC. The expressions of surface markers such as MHC Class II, CD40, CD80 and CD86 on DCs were analyzed by flow cytometry, and the gene expressions of cytokines such as IL-12a, IFN- $\gamma$ , IL-4, TGF- $\beta$  and IL-6 of DCs were analyzed by qRT-PCR.

**Results:** The CD172a<sup>+</sup>/CD11c<sup>+</sup> DCs with high expression of MHC class II were purified at 84.8% purity from bovine PBMC by a novel method using MACS. The BS-feeding significantly increased the proportion of CD172a<sup>+</sup>/CD11c<sup>+</sup> DCs in the peripheral blood one month after parturition compared with that one month prior to the parturition. The proportion of CD80 positive cells of DCs in the BS-feeding group significantly increased after parturition. In contrast, there are no changes in the number and the CD80 expression of DCs in the control-feeding group. The TGF- $\beta$  expression of DCs in the control-feeding group after parturition was significantly higher than that before parturition. However, the TGF- $\beta$  expression in the BS-feeding group did not change after parturition, and then turned out to be significantly lower than that in the control-feeding group. In DCs of the BS-feeding group after parturition, we observed that there was a strong negative correlation between the expression of TGF- $\beta$  and the co-stimulatory factor CD80 molecule expression, and that there were positive correlations between the expressions of IL-12a and IL-6 and the CD80 expression.

**Conclusions:** These results suggested that the BS-feeding prevented the cattle from mastitis by increasing the proportion of DCs in bovine peripheral blood, and that the *Bacillus subtilis* C-3102 strain changed the surface markers and the cytokine production in DCs. These data also indicate that DCs of the BS-feeding group may have the ability to lead naive T cells to cell-mediated immunity involved in the removal of bacteria.

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IV-P04

### Changing the paradigm of stress and immune suppression

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Recent advances in measuring immunologic responses in cattle has allowed for re-evaluation of the immune system during biologically stressful periods. These advances have been coupled with the ability to study local immune responses and clinical outcomes from systemically administered immunomodulators in stressed cattle. New studies indicate that local homing of the immune system has shown two separate local immune systems: gastrointestinal and respiratory/reproductive. Other published studies have indicated higher local immune responses in cattle undergoing high biological stress than in unstressed

cattle. This has been shown with non-specific, humoral and cell-mediated immune responses. Since all previous studies have measured post-stress immune suppression on the systemic immune system, these studies are suggesting that a portion of immune suppression that has been seen following stress may involve immunologic shifting to at-risk sites. While our understanding of the implications of these findings is still developing, a fresh look at the postpartum dairy cow and the post-stress beef calf is warranted. More research will need to be done to fully understand immune function following stress in cattle. However, as we learn more about potential immune shifting, new opportunities for immune modulation and vaccination will arise to take advantage of these immunologic phenomena.

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IV-P05

### Tick Glutathione S-transferases: vaccine targeting multiple tick species

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**Introduction:** Ticks cause high losses to the livestock industry globally. In most cattle production systems, tick control is principally through intensive acaricide application, an approach that is threatened by increasing tick resistance to acaricides. This calls development of novel control strategies. Our research group focuses on characterizing immunogenic tick proteins to develop a vaccine against these ectoparasites. Previously, it was shown that the *Haemaphysalis longicornis* glutathione s-transferase (GST-HI) induces a partial protective immune response against *Rhipicephalus microplus* infestation, suggesting GST has potential for inclusion in a broad-spectrum anti-tick vaccine against multiple tick species. We are currently evaluating different tick GST antigens against economically important ticks.

**Objectives:** Analyze the effect of vaccines composed by GST-HI against *Rhipicephalus appendiculatus* and *Rhipicephalus sanguineus* infestations, as well as new *R. appendiculatus*, *Amblyomma variegatum* and *Rhipicephalus decoloratus* GSTs against *R. sanguineus* infestation.

**Materials and methods:** GST ORFs from *R. appendiculatus*, *A. variegatum*, and *R. decoloratus* were amplified by PCR and cloned in pET plasmids. *H. longicornis* GST ORF was cloned previously. Recombinant GSTs were expressed in *Escherichia coli*, purified through glutathione-affinity chromatography and analyzed by SDS-PAGE. Purified rGSTs were used to immunize rabbits. Cross-reactivity between GSTs was evaluated by western-blot probing the recombinant proteins with heterologous sera obtained from GST immunizations. For vaccination experiments, treatment group was inoculated with recombinant GST-HI antigen, while the control group was received adjuvant only. Rabbit groups were challenged with *R. appendiculatus* and *R. sanguineus* ticks. Group of cattle vaccinated in the same way were challenged with *A. variegatum*, *R. appendiculatus* and *R. decoloratus*. Ticks that detached spontaneously



were collected and biological parameters were analyzed. Vaccine efficacy was estimated from comparing feeding success (days to detachment, engorgement weights, number of engorging ticks) and fecundity (oviposition rates and egg hatchability) among vaccinated and control animals.

**Results:** Western-blot assays showed strong cross-reactivity of GSTs with sera raised against GSTs from different tick species. Vaccination with rGST-HI decreased the number, weight, and fertility of *R. appendiculatus* engorged in rabbits and *R. appendiculatus*, and *R. decoloratus* in cattle, with an overall vaccine efficacy of approximately 60% in both host species. In contrast, rGST-HI vaccination was ineffective against *R. sanguineus* and *A. variegatum*. Ongoing work is evaluating the efficacy of the immunization with GSTs derived from *A. variegatum* and *R. decoloratus* ticks.

**Conclusion:** The results provide evidence that tick GSTs have potential use in future cross-protective anti-tick vaccines. However, there is need to clarify the differential protection obtained with some of the tick species through further vaccine studies.

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IV-P06

### Serological response of calves vaccinated with 1 dose of a gE-/tk- deleted MLV IBR vaccine

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HIPRA

**OBJECTIVE:** HIPRABOVIS® IBR MARKER LIVE is a double deleted (gE-/tk-) modified live viral (MLV) marker vaccine against Infectious Bovine Rhinotracheitis (IBR). The objective of this study was to describe the dynamic of the humoral response against Bovine Herpesvirus 1 (BHV-1) induced by a 1-shot administration programme of this vaccine (off-label) and two other commercialized gE- MLVs up to the revaccination period.

**MATERIAL AND METHODS:** Calves between 6 and 7 months of life were randomly allocated into 4 groups and vaccinated intramuscularly with 1 dose of either HIPRABOVIS® IBR MARKER LIVE (G1 n=15) or two other licensed gE- MLVs (G2 n=15 and G3 n=14) or not vaccinated (G4 n=12). Animals were bled to obtain sera at 0, 42, 198 days post vaccination (dpv). BHV-1 gB/gE antibody responses were tested by using commercially available blocking ELISA kits. Data were normalized by bootstrapping 1000 folds. G1 was compared with the other vaccinated groups by non-inferiority tests. For this purpose, the confidence interval of the differences between groups was estimated by ANOVA. Non-inferiority margins for each reference group (G2-G3) were calculated taking into account 75% of their effect when compared with G4.

**RESULTS:** Vaccinated calves were all seropositive against BHV-1 gB at 42 dpv and they maintained this status up to the end of the trial. The magnitude of the antibodies immune response against BHV-1 gB in G1 was non-inferior compared to the other vaccinated groups during the entire study. Finally, all Animals maintained a seronegative status against BHV-1 gE throughout the study and thus they were probably not exposed to wild-type BHV-1.

**CONCLUSIONS:** The serological response against BHV-1 gB induced by the double deleted IBR MLV showed non-inferiority in comparison with other protocols, when just one dose of the product was administered. Moreover, this was maintained up to the revaccination period. These results suggested that one dose of the double deleted IBR MLV might have immunogenic property similar to single deleted IBR MLVs.

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IV-P07

### Peripheral blood lymphocyte proliferative responses in dairy cows supplemented with an immunomodulatory feed additive and under polyvalent vaccination

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This study evaluated the effect of dietary OmniGen-AF® supplementation on lymphocyte proliferation in dairy cows under vaccination. Here, we used thirty-two Holstein x Gir dairy cows. Blood samples from all dairy cows were collected to determine leptospiral antibody titers, and samples from sixteen dairy cows were collected for lymphocyte proliferation. On day -6, cows were uniformly divided into dairy cows that receive or not with 56 g/cow daily as-fed basis of OmniGen-AF® from day 1 to day 56. Then, dairy cows were vaccinated at day 56 and 70 with a commercial polyvalent vaccine. Lymphocyte proliferation was measured by flow cytometry using 5-(6-) carboxyfluorescein diacetate succinimidyl ester dilution dye at day 56 and 77. At these same times, the antibody titers were determined by leptospiral microscopic agglutination test. Overall, we only observed that dairy cows supplemented with OmniGen-AF® during lactation showed an increase in the spontaneous lymphocyte proliferation and a lower B cells proliferation stimulated with *L. heat-killed Leptospira borgpetersenii* serovar hardjo after 21 days of vaccination (day 77). Indeed, no significant difference immediately before vaccination was observed. Furthermore, no alteration of leptospiral antibody titers was found in dairy cows that received OmniGen-AF® compared with control ones. Thus, the present study pointed out that supplementation with that OmniGen-AF® did not affect robustly the lymphocyte proliferation and the levels of antibodies induced by vaccination in healthy dairy cows.





IV-P08

### Impact of integrated use of anti-tick vaccine and acaricides in progressive control of the cattle fever tick, *Rhipicephalus microplus*, in Puerto Rico

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The cattle fever ticks, *Rhipicephalus microplus* and *R. annulatus*, are considered the most economically important ectoparasite of livestock worldwide because it is a blood-sucking parasite and a vector of the infectious agents causing bovine babesiosis and anaplasmosis. Zoetis Inc has developed an anti-tick vaccine for the control of these ticks that consists of rBm86 antigen that was originally discovered by Australian scientists and a unique proprietary Zoetis adjuvant. Vaccination of cattle with the anti-tick vaccine induces an antibody response that is taken up by the ticks when feeding and targets the Bm86 protein located in the mid-gut of the tick destroying its integrity and ability to complete a life cycle. This effect decreases the tick population through time when the entire cattle herd is vaccinated, which also impairs the vector competence of *R. microplus* for bovine babesiosis and anaplasmosis. This vaccine was tested in a private-public collaboration that included Zoetis Inc., the USDA-ARS and USDA-APHIS-VS and supported by the dairy and beef cattle producers in Puerto Rico. Dairy and beef cattle were vaccinated with the Zoetis anti-tick vaccine and treated with acaricides. Integrating vaccination extended the need for acaricide treatment, and decreased the overall use of acaricides during the course of the study. Over a period of six months, there was a significant reduction in applications of acaricides. This reduction ranged from 50% to 80% across 5 farms with 813 beef and 525 dairy breed cattle enrolled for the evaluation. Results from this field research in Puerto Rico confirmed reports from other tropical countries where the integrated use of other Bm86-based vaccines and acaricides not only controlled *R. microplus* and mitigated the risk for the development of acaricide resistance, but also decreased mortality and morbidity in cattle due to bovine babesiosis. In conclusion, this field data provides evidence that an integrated vaccination/acaricide regimen for the control of these ticks is an efficient strategy and should be pursued in other endemic regions of the world.

IV-P09

### The IBR-BRSV-PI3 intranasal vaccine overrides maternal antibody and induces a 6 month duration of immunity against IBR Disease

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Zoetis Inc has been developing an evolving life-long vaccination program to prevent bovine respiratory diseases in cattle from birth through to the end of the production cycle. The challenge for early immunization of young calves is the interference of maternal antibodies on the response to vaccination given via the parenteral routes. A modified live virus vaccine against three key viral respiratory diseases caused by IBR, PI3 and BRSV is administered intranasally to override maternal antibodies and stimulate mucosal immunity in calves during the first week of life. In this highly stressed and vulnerable age group, the intranasal vaccine developed by Zoetis Inc is shown to be extremely safe. The USDA has approved efficacy claims of prevents BRSV pneumonia and aid in prevention of IBR and PI3 respiratory diseases for the other two fractions. In addition, this intranasal vaccine has been shown to override maternal antibodies for the IBR and BRSV fractions, and duration of immunity of 180 days has been demonstrated for the IBR fraction. The role of this intranasal vaccine against IBR-BRSV-PI3 in the development of calfhood immunity will be discussed.

IV-P10

### The Role of an Intranasal Vaccine against IBR, BRSV and PI3 in Developing a Healthy and Productive Calf Population in North America: Lack of Interference between IBR, PI3 and BRSV MLV Fractions

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Zoetis Inc has been developing an evolving life-long vaccination program to prevent bovine respiratory diseases in cattle from birth through to the end of the production cycle. A modified live virus vaccine against three key viral respiratory diseases caused by IBR, PI3 and BRSV is administered intranasally to override maternal antibodies and stimulate mucosal immunity in calves during the first week of life. Interference between fractions of multivalent vaccines are not uncommon. Herein data will be presented to demonstrate that there is a lack of interference between the IBR, PI3 and BRSV fractions present in the vaccine. Due to these unique properties of efficacy and safety, this intranasal vaccine has become an important vaccine in developing a healthy dairy and beef calf population in North America. This has given rise to a series of prime-boost vaccine programs using the IN and parenteral routes for optimal stimulation of immunity providing life-long immunity against viral diseases that commonly cause calf respiratory diseases and predispose to bacterial pathogens.



BC-P01

## Paratuberculosis - what new can we learn using social network analysis?

### Paratuberculosis SNA

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**Introduction:** Traditionally in Ireland, the approach to paratuberculosis/Johne's disease (JD) was based on passive surveillance of clinical cases with non-responsive chronic diarrhoea, largely by submission of a faecal sample to the Veterinary Laboratories Service (VLS-DAFM) and subsequent control by voluntary removal of the affected animal. In most cases, the infection was largely well established in the herd by the time *Mycobacterium avium* paratuberculosis (MAP) was detected. In recent years, a different and more proactive approach aimed at estimating disease risk and earlier detection of infection has been adopted by many farmers (repeated serology testing). The movement and trade of live animals from farm to farm, plays a primary role in the introduction and spread of infectious agents, especially MAP. Social Network Analysis (SNA) provides a methodology for the analysis and illustration of the relationship between the movements of animals and the transmission of a contagious pathogen associated with those movements. Hence the objectives of this study were to: 1) describe the demographics of confirmed JD positive (JD +) animals in Ireland, 2) to construct and characterise the connections made between herds by the movements of JD + animals through different premises (JD network), 3) to increase our understanding of the latent spatio-temporal herd-to-herd transmission of the disease and 4) to provide a disease surveillance framework for the identification of those herds (nodes) more likely to facilitate the spread of infection.

**Material and methods:** Laboratory data covering 11 years and consisting of faecal mycobacterial culture results of animals with persistent non-responding diarrhoea were organised in an adjacency matrix formed by a collection of nodes (herds) and an array of directed arcs (movements) linking the nodes (JD network). Once the database was tidied, selected datasets were extracted and graphed, and then the relationships between herds were explored and analysed by their connections (movements). Three programs for large network analysis and visualization were used: UCINET, Pajek, and R.

A network is a collection of units of interest or nodes (herds, animals, etc.) that may or may not be connected (arcs/edges) to each other through a relationship of some sort (movement, lineage, etc.). A movement event was defined as the change from an identified premise of origin (Herd No.) to other identified premise of destination (Herd No., factory, knackery, etc.). Since each animal movement implied a certain direction, the graph produced was a directed network. The dataset contains 1220 confirmed (faecal culture) JD+ animals and 1089 herds and a total 1413 movements, including movements to factories, knackeries and 728 movements excluding them. Since infection with MAP normally happen early in life, a herd in which an JD + animal is born (herd of origin) was classified as 'source herd'. Quantification of how many 'source herds' are connected directly or indirectly between, within each component, represents a measure of disease transmission. An assumption

was made that premises sharing JD+ animals are potentially capable of spreading JD. Descriptive statistics of the network and nodes were calculated (infection chain, centrality indicators, geodesic distance, density, etc.). Inferential statistics were attended by comparing the JD network with random network model (Erdos-Renyi random network) with similar network characteristics.

**Results:** The network was fragmented into 415 components (sub-networks where nodes are connected within, but do not have ties with, other sub-networks) ranging from 1 to 50 nodes. Out of the 258 components with two or more nodes, 59 had two or more connected 'source herds' nodes, representing 23% of these subset and 14% of the total number of components. In addition, herds with high ingoing contact chain and in-degree showed strong association (asymmetric Rajska 0.63 and 0.60 respectively) with those source herds. Preliminary results show a significant association between source herds and disease transmission by the movement of JD+ animals. It was also found that 179 JD+ animals (110 of beef breed and 69 dairy; 163 females and 16 males) were connected by lineage.

**Conclusion:** SNA analysis provided a robust approach to assess disease transmission and identify those premises more likely to facilitate the spread of JD (high ingoing contact chain and degree centrality), thus providing a framework for the development of a risk-based Johne's surveillance program.

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BC-P02

## Identification and phase inversion of *Salmonella* flagellar antigens

### Re-examination of disc immuno-immobilization method

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*Salmonella enterica* has traditionally been classified by serological identification of specific somatic and flagellar antigens. Traditional serotyping of flagellar antigens in most cases requires a strain phase reversal, which is time consuming and skill-intensive. Disc immuno-immobilization is a simple method for typing the flagellar phase of *Salmonella enterica*. We re-examined this method using commercial antisera, which contains the preservative sodium azide. Originally prepared motility agar activates bacterial motility and renders *S. enterica* resistant to sodium azide, resulting in the formation of immuno-immobilization lines around reactive immuno-discs. Though disc immuno-immobilization serves both serotyping and phase inversion, this method is insufficient for the strains in which phase variation rarely occurs. Here, we devised a novel immuno-disc phase inversion method. Spot-inoculated *S. enterica* on motility agar was overlaid with a reactive immuno-disc. Another immuno-disc was separately placed. During growth, phase inverted cells appeared and starts migration, resulting to reach another immuno-disc. Bacterial cells, which were not phase inverted, also starts to migrate after cell counts exceeded the titer of the antibody; however, they were immobilized by another immu-



no-disc. Disc immuno-immobilization, combined with immuno-disc phase inversion, is simple to interpret and all *S. enterica* strains tested in this study possessed flagellar antigens, which is consistent with the results obtained using conventional methods. These methods would drastically simplify the task of *S. enterica* typing in clinical laboratories.

BC-P03

### ***Klebsiella pneumoniae* infection secondary to bovine viral diarrhoea in neonatal calves of premature birth**

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**Objectives:** *Klebsiella pneumoniae* is a gram-negative, aerobic, nonmotile bacillus of the family Enterobacteriaceae. *K. pneumoniae* can cause pneumonia, meningitis, peritonitis, cystitis, and septicemia in both Old and New World primates, although it also is reported to constitute normal alimentary tract flora of a number of mammals including livestock. This case report describes *K. pneumoniae* infection in neonatal calves of premature birth.

**Materials and methods:** Two calves were affected on a farm keeping 184 adult Hanwoo. Two calves were born at the fetal age of 252 and 262 days, respectively. The animals were weak and showed nervous signs such as depression and inability in standing. They died 4 days after birth, and were submitted to the Animal and Plant Quarantine Agency for postmortem examination. After necropsy, representative tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Tissue sections were stained with hematoxylin and eosin and Gram stain. Brain, lungs, pleura and peritoneal effusion were collected for bacterial culture. Also, polymerase chain reaction was performed to detect the viral genome (bovine viral diarrhoea virus, herpesvirus, Akabane virus, Chuzan virus, Aino virus, bovine ephemeral fever virus).

**Results:** Grossly, yellowish fibrinous materials were attached to the serosa of the internal organs. In the brain, loss of definition of gyri, meningeal cloudiness and thickening were observed. Histopathologically, severe diffuse meningoencephalitis characterized by infiltration of neutrophils and lymphocytes with numerous bacilli was observed. Fibrinopurulent inflammation was also found in several visceral organs. Numerous gram-negative bacilli were found into the meningitis lesions. *K. pneumoniae* was isolated from the brain, lungs, pleura and peritoneal effusion. Also, BVDV was detected from the most of internal organs by PCR method.

**Conclusions:** In conclusion, this is the first report of *K. pneumoniae* meningoencephalitis in calves. Bacterial polyserositis including meningoencephalitis might occurred secondary to systemic BVDV infection. *K. pneumoniae* has zoonotic potential, further studies will be needed to accumulate knowledge of the virulence factors and pathogenesis.

BC-P04

### **The course of *Mycoplasma bovis* infection in dairy herds**

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**Objectives:** *M. bovis* is an important pathogen causing substantial economic losses and welfare effects in cattle industry worldwide. *M. bovis* was first detected in Finland in 2012. Cattle industry runs a voluntary control program for *M. bovis*, and less than 1 % of dairy herds are estimated to be infected. In this study we analyzed dynamics of *M. bovis* infection in recently infected dairy herds to improve the testing scheme in the control program.

**Materials and methods:** A total of 17 recently infected dairy herds were included in the study. In all farms the infection was detected for the first time from clinical mastitis by PCR. The number of *M. bovis* mastitis cases varied from 1 to 8 per herd. The herds were sampled 4 times during 1.5 years. Each sampling consisted of a set of blood and respiratory samples (nasal swabs, NS and deep nasopharyngeal swabs, NP) from calves, and blood samples from cows. All clinical and subclinical mastitis cases were analyzed for *M. bovis*. A total of 2727 serum, 1211 NS and 342 NP samples were collected. *M. bovis* antibodies were examined by MiIA elisa. NS were analyzed by PCR and NP by enrichment culture followed by PCR. To control the infection, the farmers were advised to cull *M. bovis* mastitis cows, to group and separate the calves from older cattle and not to purchase new calves.

**Results:** The herds were classified into 6 groups according to the presence/absence of *M. bovis* in NS/NP and in clinical mastitis samples. In S0 herds no other positive animals were detected after the first mastitis cases. In S1 herds *M. bovis* was detected only at first sampling; in S1S2 herds in first two samplings, etc. The number of herds in each group was S0 n=2 herds, S1 n=3, S1S2 n=2, S1S2S3 n=4, S1S2S3S4 n=3. In the remaining 3 herds *M. bovis* was detected intermittently. A total of 73 % and 84 % of samples from calves and cows, respectively, were antibody positive. In all groups 70-96 % of the cows were antibody positive in the last sampling (S4), regardless of the PCR findings in the herd. However, herd group related differences were observed among calves. In S0 group the percentage of antibody positive calves decreased from 93 % to 11 %, and in S1 group from 84 % to 10 %. In contrast, in S1S2S3 and S1S2S3S4 groups 60 % and 97 % of calves remained seropositive in the last sampling.

**Conclusions:** The antibodies can be detected in the cows for a long time even though the agent cannot be detected in calves or clinical disease. In the eradication of *M. bovis*, it is crucial to prevent the spread of infection among calves. Antibody levels in dairy cows do not indicate how the infection circulates among calves. Therefore, PCR and antibody testing of NS, NP, and serum samples from calves, and from all clinical cases, is the best scheme to indicate the progress of infection control in the herd.



BC-P05

### The Evaluation of MLVA Genotyping Assay of *Mycoplasma bovis* by Whole-Genome Sequencing

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**Objectives:** *Mycoplasma bovis* (*M. bovis*) is a causative agent of bronchopneumonia, arthritis, mastitis, and other clinical conditions. *M. bovis* has high infectivity and has spread broad in Japan. Nevertheless, there is little epidemiological study because of lack of classification method of genotypes and serotypes. Recently, multiple-locus variable-number tandem repeat analysis (MLVA) has been reported as one of the methods of molecular biological typing of *M. bovis*. Although it seems that MLVA is relatively convenient and cost-effective typing tool for surveillance of *M. bovis* epidemiology, it is necessary to confirm its reliability. Hence, in the present study, we performed genotyping based on MLVA and whole-genome sequence analysis, and then evaluated the correlation between these genotyping assays. Further, we conducted a serological analysis in order to compare with MLVA genotyping assays.

**Materials and Methods:** One hundred twenty one *M. bovis* strains were isolated from 2007 to 2017 in Japan. All the strains were genotyped using the MLVA assay using 6 pairs of PCR primers targeting the regions of tandem-repeat loci. Two strains each for selected 7 MLVA genotypes were fully sequenced. In order to determine the serological characteristics, antisera against 7 strains were used in metabolic inhibition tests.

**Results:** Overall 39 genotypes were obtained in this study. Almost all the strains were distant from the reference strain PG-45. The diversity of the MLVA genotypes was related to epidemiological surveillance in several farms. Further, an analysis of whole-genome sequence revealed that the MLVA genotypes were correlated with variant frequency on genomes of *M. bovis*. Besides, serological characteristics were not determined correlations with the MLVA genotypes. It was possible that there were some regions which determine serological characteristics at highly variable regions on *M. bovis* genome.

**Conclusions:** In this study, the MLVA assay revealed genetic diversity of *M. bovis*, and so it is an effective method for epidemiological surveillance.

Fic motif found in each of approximately 400-residues direct repeat 1 (DR1) and 2 (DR2) domains in the *lbpA* is known to catalyze adenylation of a significant tyrosine residue of Rho-GTPase and inactivate this signal switching molecule, which causes disturbance of rearrangement of actin cytoskeleton, thus cell rounding and phagocytosis inhibition of host macrophages and cell retraction of alveolar epithelial cells. No cytotoxic effect on bovine macrophage-like FBM-17 cells was observed for a mutant strain, 2336.ΔDR1DR2, created by in-frame deletion of DR1 and DR2 region in *lbpA* gene from the *H. somni* virulent pneumonic strain 2336. In order to investigate the role of the *lbpA* cytotoxicity in the pathogenesis of *H. somni* pneumonic infection, virulence of the mutant strain 2336.ΔDR1DR2 and the parent strain was compared in an intra-bronchial calf challenge model in the present study.

The challenge dose at  $2.5 \times 10^8$  CFU for both the mutant strain 2336.ΔDR1DR2 and the parent strain was inoculated intra-bronchially to the caudal part of left cranial lobe of 8 to 12-week-old holstein calves. Euthanasia and necropsy for the infected calves were done at seven days after challenge. Each of the clinical signs including temperature, respiratory rate, cough, apathy, anorexia and nasal discharge monitored twice a day for the infected calves was assigned a numerical value 0, 1, 2, or 3 according to a set scale for each parameter and the total value from all monitoring points for each calf was indicated as the clinical score. Both dorsal and ventral surface images of lungs from the necropsied calves were photographed and the pneumonic lesion and whole lung areas were measured by an image analysis software (Image J) to determine the ratio of total involved area to total lung area. Tissue samples collected from the inoculated lung lobes and the other lung lobes were homogenized, serially diluted and then inoculated to blood agar plates to measure the bacterial numbers.

The mean total clinical score for five calves infected with the mutant strain 2336.ΔDR1DR2 was significantly lower than that for five calves infected with the parent strain ( $11.0 \pm 3.6$  vs  $69.4 \pm 18.5$ ;  $P = 0.015$ ). The mean ratios of total pneumonic lesion area to total lung area was also significantly lower for calves infected with the mutant strain than for calves infected with the parent strain ( $6.0 \pm 2.3\%$  vs  $31.8 \pm 7.3\%$ ,  $P = 0.0096$ ). The mean bacterial numbers of *H. somni* isolated from the inoculated lung lobes and from the other lung lobes were lower for calves infected with the mutant strain, but the differences were not significant. These results clearly demonstrated that the mutant strain 2336.ΔDR1DR2 lacking *lbpA* cytotoxicity showed a reduced capacity to induce clinical signs and lung damage in the bovine host.

BC-P06

### IbpA protein is contribute to virulence of *Histophilus somni* in experimental calf pneumonia model

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The immunoglobulin binding protein A (IbpA) of *Histophilus somni* is one of newly recognized Fic family virulence proteins.

BC-P07

### Flunixin Transdermal as therapy in the treatment of bovine respiratory disease

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**Objectives:** Flunixin has anti-inflammatory, anti-pyretic and analgesic effects. This molecule is commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature. A 50 mg/ml flunixin



transdermal formulation was developed by MSD Animal Health and is now the first NSAID registered to be administered as a pour-on. Two studies were conducted to determine the effectiveness of flunixin transdermal, with or without antibiotic, in the treatment of bovine respiratory disease (BRD), under field conditions in Europe (EU) and United States (US).

**Materials and methods:** Two different studies, one in EU with antimicrobial, another in US without antimicrobial, were performed to evaluate the treatment of cattle with 3.3 mg/kg flunixin transdermal (Finadyne®/ Banamine® Transdermal) administered topically along the dorsal midline on day 0.

Cattle were evaluated for evidence of BRD, characterized by a rectal temperature  $>40.3^{\circ}\text{C}$ , a depression score  $> 2$  (on a scale 0 to 4) and a respiratory score  $> 2$  (sum of the presence of individual sign such as polypnea, dyspnea, cough and mucopurulent nasal discharges) to be included in the study.

The EU study included 197 animals in France, Germany and Spain, and was conducted in comparison with a positive control, 1.4 mg/kg carprofen (Rimadyl®; Zoetis), administered subcutaneously on day 0. Both treatment groups received an antimicrobial, cefquinome (Cobactan® LA 7.5%; MSD Animal Health), administered subcutaneously on days 0 and 2. The animals were observed for clinical signs of disease at 6 hours after treatment initiation and daily thereafter for 5 consecutive days.

The US study included 235 animals in California, Kansas, Nebraska and Texas, and was conducted in comparison with a negative control, saline with red dye, administered topically along the dorsal midline on day 0. The animals were observed for clinical signs of disease at 6 hours after treatment initiation.

**Results:** The rapid reduction of rectal temperature was defined as the primary criterion to demonstrate effectiveness as this is one of the main aims in the BRD treatment. In the EU study, the decrease in temperature was significantly greater with flunixin transdermal ( $-1.3^{\circ}\text{C}$ ) compared to injectable carprofen ( $-0.9^{\circ}\text{C}$ ). In the US study, the decrease in temperature was shown to be  $-2.04^{\circ}\text{F}$  ( $-1.3^{\circ}\text{C}$ ) with flunixin transdermal. In both studies, flunixin transdermal was effective to reduce pyrexia, and the difference versus the positive or the negative control product was statistically significant ( $p < 0.0001$ ).

Other clinical signs such as depression and respiratory signs were evaluated in the EU study. A significant decrease ( $p < 0.0001$ ) in the severity of these signs was observed in animals receiving flunixin transdermal.

Animals treated with flunixin transdermal had a reduction of mean depression score from 2.02 to 1.02 at six hours after treatment. The animal general attitude improved over the time. On day 5, 79% of animals returned to normal attitude and did not show any more signs of depression.

Similarly, the mean respiratory score dropped from 2.68 to 1.44 in animals treated with flunixin transdermal at six hours after treatment. The animal respiratory health improved over the time. On day 5, 79% of animals presented only one respiratory sign and 34% did not show any more sign.

In the EU study, nasopharyngeal swab samples were collected from each animal enrolled, and the outbreaks could be attributed to *Mannheimia haemolytica* (51.78%), *Pasteurella multocida* (41.12%), *Mycoplasma bovis* (34.01%) and *Histophilus somni* (1.52%). No microbiological sample was collected in the US study.

**Conclusions:** Both EU and US studies demonstrated that prompt treatment of sick cattle with flunixin transdermal reduces fever, and improves depression and respiratory scores. This adjunct antimicrobial treatment used in the EU study might contribute also to additional benefits such as controlling bacterial replication, and reducing the likelihood of permanent lung lesions. The single transdermal application of flunixin is a safe and effective therapy to alleviate the clinical signs of inflammation associated with BRD.

BC-P08

### Biofilm formation by *Candida albicans*, *Escherichia coli* and *Streptococcus dysgalactiae* isolated from bovine mastitis and their relation with antibiotic resistance

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The purpose of this work was to investigate biofilm production by *Candida albicans* (CA), *Escherichia coli* (EC), *Streptococcus dysgalactiae* (SD) and their combinations isolated from bovine mastitis clinical cases, as well as to establish their relationships with antibiotic resistance.

The milk samples were collected from cows with clinical and subclinical mastitis from a dairy herd located in Querétaro, México. Milk samples were aseptically obtained and transported at 4 C to the laboratory. The samples were cultured in blood agar medium and Sabouraud Dextrose agar medium and incubated at 37 C during 48 hrs.

Identification was made by microscopic morphology and Gram stain. For CA, fermentation tests of maltose, dextrose, galactose and lactose were performed, as well as germinal tube formation by inoculating 500  $\mu\text{l}$  of fresh bovine serum with a fresh culture and incubating at 37 C during 2 hours. EC identification was performed by means of biochemical tests and SD was identified using catalase and CAMP tests.

Biofilm formation was assessed in 96 polystyrene well plates for tissue culture, inoculating 8 wells with 198  $\mu\text{l}$  brain heart infusion and the next combinations: 2  $\mu\text{l}$  of CA, 2  $\mu\text{l}$  of CA + 2  $\mu\text{l}$  of EC, and 2  $\mu\text{l}$  of CA + 2  $\mu\text{l}$  of SD, incubating for 24 hours at 37 C. After the incubation, the content was removed by gently rinsing the plates. Biofilm was stained with crystal violet 0.1%. Optical density of stained adherent bacteria was determined with a micro ELISA reader at 492 nm. Experiment was performed by triplicated.

The results obtained were analyzed with Variance Analysis Test and Tuckey test with a significance level at 95% to determinate difference among groups.

The susceptibility test to antimicrobial was performed inoculating Müller Hilton medium with next combinations: CA + EC y CA + SD, in both cases BioRad Multidisc with 13 antibiotics were used, after every inhibition halo was measured.

The 75% analyzed samples of CA presented biofilm formation



with 498 nm average absorbance, same thing happened with the combinations CA + EC 0.722 nm y CA + SD 502 nm. However, the amount of biofilm formation of CA+ EC was significantly higher.

In the antibiotic resistance test, both CA+EC and CA+SD were resistant to the 13 antibiotics used.

The combined infections with yeasts and biofilm-forming bacteria, can origin bacterial resistance to the antibiotics, due to the protection provided by the slime produced by microorganism-forming biofilm.

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BC-P09

### Herd prevalence of *Enterobacteriaceae* producing CTX-M-type and CMY-2 $\beta$ -lactamases among Japanese dairy farms

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**Objectives:** Since 2000, *Escherichia coli* and other *Enterobacteriaceae* species producing CTX-M-type extended-spectrum  $\beta$ -lactamases (ESBLs) (CTX-M) have been commonly isolated from community-acquired extraintestinal infections in humans, companion and food-producing animals, and retail meats of chicken, beef, and pork worldwide. The CTX-M has been derived in five CTX-M clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25) from base sequence homology. The CTX-M confers resistance against penicillins, oxyimino-cephalosporins, and monobactams. The aims of this study were to determine the herd prevalence of *Enterobacteriaceae* producing CTX-M among 381 dairy farms in Japan.

**Materials and Methods:** Between 2007 and 2009, we screened 897 faecal samples using BTB lactose agar plates containing cefotaxime (2  $\mu$ g/ml). Positive isolates were submitted for CLSI ESBLs confirmatory tests. Isolates with a positive ESBLs confirmatory test were screened for metallo- $\beta$ -lactamases using the sodium mercaptoacetic acid (SMA) double-disc synergy test (SMA-test).

The ESBL-positive, and the SMA-test-negative, *Enterobacteriaceae* isolates were identified using ID 32 E API system. The MICs of 23 antimicrobials were determined by the CLSI broth microdilution. These isolates were analyzed by multiplex PCR for the presence of  $bla_{\text{CTX-M}}$  genes, and plasmid-mediated AmpC  $\beta$ -lactamase genes. The CTX-M types of the CTX-M-positive isolates were identified by bidirectional sequencing using group-specific PCR primers for  $bla_{\text{CTX-M-1 group}}$ ,  $bla_{\text{CTX-M-2 group}}$  and  $bla_{\text{CTX-M-9 group}}$ . AmpC-positive isolates were analyzed using type-specific PCR primers (e.g.,  $bla_{\text{CMY-1}}$  and  $bla_{\text{CMY-2}}$  genes), and  $bla_{\text{TEM}}$  and  $bla_{\text{SHV}}$  genes were analyzed and bidirectionally sequenced.

For CTX-M-producing *E. coli* isolates, pulsed-field gel electrophoresis (PFGE) was conducted according to the PulseNet standardized protocol, and multilocus sequence typing (MLST) was conducted according to standard protocols using the *E. coli* MLST website. The *E. coli* isolates were O- and H- serotyped.

**Results:** The incidence of *Enterobacteriaceae* producing

CTX-M-15 ( $n = 7$ ), CTX-M-2 ( $n = 12$ ), CTX-M-14 ( $n = 3$ ), CMY-2 ( $n = 2$ ), or CTX-M-15/2/14 and CMY-2 ( $n = 4$ ) in bovine faeces was 28/897 (3.1%) faecal samples. These genes had spread to *E. coli* ( $n = 23$ ) and three genera of *Enterobacteriaceae* ( $n = 5$ ; *Klebsiella pneumoniae*,  $n = 3$ ; *Citrobacter freundii*,  $n = 1$ ; *Enterobacter cloacae*,  $n = 1$ ). Herd prevalence was found to be 20/381 (5.2%) dairy farms. The 23 *E. coli* isolates showed clonal diversity, as assessed by MLST and PFGE. The pandemic *E. coli* strain ST131 producing CTX-M-15 or CTX-M-27 was not detected.

**Conclusions:** Three clusters of CTX-M (CTX-M-15, CTX-M-2, CTX-M-14) had spread among Japanese dairy farms. The 23 *E. coli* isolates showed clonal diversity. This is the first report on the prevalence of multidrug-resistant CTX-M-15-producing *E. coli* among Japanese dairy farms.

**Reference:** M.Ohnishi et al. 2013 J. Appl. Microbiol. **115**:282-289.

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BC-P10

### Antimicrobial susceptibilities, bacteriological characteristics, and genetic relatedness of *Pseudomonas aeruginosa* and *Serratia marcescens* isolates from bovine mastitis and *Stenotrophomonas maltophilia* isolates from a mastitis outbreak

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**Objectives:** The presence of metallo- $\beta$ -lactamase (MBL)-producing and multidrug-resistant *Pseudomonas aeruginosa* (MDRP) strains among bovine isolates of Gram-negative bacilli, and O-serotypes of bovine *Serratia marcescens* and *P. aeruginosa* isolates have been reported rarely. The aims of this study were to (1) elucidate antimicrobial susceptibilities and O-serotypes of *P. aeruginosa* and *S. marcescens* isolates from bovine mastitis and the presence of MBL-producers and MDRP strains among them, (2) evaluate their relationships to human isolates, and (3) evaluate the antimicrobial susceptibility and genetic relatedness of 11 *Stenotrophomonas maltophilia* isolates from an outbreak of bovine clinical mastitis in one herd, and two isolates from two separate mastitis cases in two other herds.

**Materials and Methods:** We investigated the MICs of 24 antimicrobials by CLSI broth microdilution and O-serotypes for 116 *P. aeruginosa* and 55 *S. marcescens* isolates in Japan, primarily in 2006. Thirteen *S. maltophilia* isolates were obtained from milk samples from 11 cows from three dairy herds in Japan during 2008. We tested their susceptibility to 14 antimicrobials by the CLSI broth microdilution and identified their genotypes by enterobacterial repetitive intergenic consensus 2 (ERIC2)-PCR.

**Results:** A total of 171 isolates exhibited high antimicrobial susceptibilities with the exception of a partial drug. *P. aeruginosa* isolates exhibited high susceptibilities of 95.7% or more to ciprofloxacin, imipenem, meropenem, piperacillin, ceftazidime, cefepime, cefoperazone/sulbactam, amikacin, tobramycin, and gentamicin; however, they exhibited a susceptibility of only 69.8% to aztreonam. They exhibited substantial resistances to



ceftriaxone, enrofloxacin, cefotaxime, and moxalactam. *S. marcescens* isolates exhibited high susceptibilities of 90.9% or more to kanamycin, ceftiofur, sulfamethoxazole-trimethoprim, and the 15 aforementioned drugs, but exhibited resistance to minocycline. Neither MBL-producers nor MDRP strains were detected among the 171 strains.

The dominant serotypes of *P. aeruginosa* isolates were OG, OA, OB, OI, OF, OE, and OK; those of *S. marcescens* isolates were O6 and O5. Every *S. marcescens* isolate was pigmented.

Every cow affected by mastitis caused by *S. maltophilia* had acute mild mastitis (slightly watery foremilk with flakes) without systemic symptoms and all resolved within 3 to 5 weeks of diagnosis. Eleven of the 13 isolates derived from nine cows in one herd over a seven month period exhibited a closely related ERIC2 type (A). The remaining two isolates derived from two cows from two other herds exhibited two distinct ERIC2 types (B and C). Most of the 13 isolates exhibited susceptibility to trimethoprim-sulfamethoxazole, chloramphenicol, minocycline, and levofloxacin; however, they were resistant to four  $\beta$ -lactams, kanamycin, gentamicin, and oxytetracycline. They were intermediate to enrofloxacin.

**Conclusions:** (1) These findings suggest that bovine *P. aeruginosa* and *S. marcescens* isolates differ from human isolates from both antibiogram and phenotypic perspectives, and could help to evaluate differences in bacteriological characteristics between bovine and human isolates. (2) Eleven closely related *S. maltophilia* isolates were involved in a herd outbreak of mastitis to some extent. Bovine *S. maltophilia* isolates exhibited resistance to many classes of antimicrobials. This is a rare report of a herd outbreak of bovine mastitis involving closely related *S. maltophilia* isolates.

**Reference:** (1) M.Ohnishi et al. 2011 Vet. Microbiol. **154**:202-207.

(2) M.Ohnishi et al. 2012 Lett. Appl. Microbiol. **54**:572-576.

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BC-P11

## Diagnostic methods of brucellosis in buffaloes from Argentina

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**Introduction:** Brucellosis is an endemic disease in many regions of Latin America. The production of buffalo for dairy production is currently under development in Argentina and Latin America, and it is praised for the elaboration of premium products, such as cheese and ice cream. Buffaloes are frequently infected by brucellosis and serological tests are currently used for detection of this disease. Buffered Plate Agglutination Test (BPAT) has been used in Argentina for many years as a screening test in the diagnosis of brucellosis in cattle. Fluorescence polarization assays (FPA) has been developed in the 90s for serological diagnosis of brucellosis, and has been used since then as a confirmatory test. This test is simple to perform,

gives rapid results, and reduces the errors of the conventional agglutination tests. At the same time, Complement Fixation Test (CFT), a confirmatory and reference technique recognized worldwide, is also carried out on suspected serological results. Here we show comparative performance among these serological tests for diagnosis brucellosis in buffaloes.

**Material and Methods:** Blood samples were taken from a herd of 50 adult unvaccinated animals, [where abortions and estrus repetition were reported. Sera were frozen at -20° C until processing. BPAT, FPA and CFT were simultaneously performed according to the Manual of Standards for Diagnostics Test and Vaccines (OIE). FPA was carried out in a SENTRY 100® (Diacchemix) fluorescence polarization instrument] using a 1/100 serum dilution. CFT was done through micro method, titrating the complement to 50% of hemolysis. The same cut-offs (FPA: 105 mP; CFT: 41 UIFC) were used as in cattle for the determination of positive and negative animals. Isolation of *Brucella* spp. was attempted from milk samples by inoculation in selective medium with antibiotics and 5% of equine serum, and at 37° C incubation. No isolation attempt could be performed on abortions as all samples were in bad conditions

**Results:** 22 samples were positive not only to the BPAT but also to both confirmatory tests (FPA and CFT). All sera that were positive for BPAT showed strong agglutination. FPA results obtained in the positive sera were in the range between 170 and 250 mP while the average measurement of the negative control was 75 mP. CFT was positive at very high serum dilutions in all confirmed samples. No false positive results for BPAT were obtained in any sample. FPA and CFT confirmed exactly the same results that BPAT anticipated. No isolations compatible with *Brucella* spp. were recovered.

**Discussion:** Serological diagnosis for brucellosis in buffaloes is sometimes controversially. These results suggested that FPA might be used as a confirmatory test for buffalo brucellosis, due to the same performance relative to CFT, its adjustable cut-off useful in different epidemiological situations, its reliability and ease of performance. CFT is a very useful test, as long as proper equipment and trained laboratory professionals are available. We show here a very precise methodology using BPAT as screening test and FPA as confirmatory one. A larger study with a more comprehensive design is needed in order to obtain a cut-off that respond to the current epidemiological situation of buffaloes in Argentina.

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BC-P12

## Development and optimization of an enzyme-linked immunosorbent assay (ELISA) for detection of specific anti- *Leptospira* spp IgG in cattle

### Leptospirosis in cattle

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**Introduction:** Leptospirosis is one of the most widely distributed zoonosis in the world and is enzootic in Argentina. The early detection of animals with symptoms compatible with leptospirosis provides a considerable contribution to the control measures of the disease.

We describe a development and optimization of an enzyme-linked immunosorbent assay (ELISA) for detection of specific anti-*Leptospira* spp IgG in cattle.

**Material and Methods:** Six protein extracts were obtained from the OIE Reference Laboratory of Leptospirosis at INTA. Strains used were *L. interrogans* serovar Pomona Pomona, *L. borgpetersenii* serovar Castellonis Castellon 3, *L. interrogans* serovar Wolffi 3705, *L. interrogans* serovar Hardjo Hardjoprajitmo, *L. interrogans* serovar Copenhageni M 20 and mix of them.

The technique was developed and optimized after testing diverse conditions (different coating reagents, serum dilutions, secondary antibody, KPL Cat. Nº 14-12-06 alicuotes, and several protein extracts). Forty (40) samples of bovine sera were evaluated by MAT previously using a battery of *Leptospira* *interrogans* serovar Pomona Pomona, *L. interrogans* serovar Canicola Hond Utrecht IV, *L. borgpetersenii* serovar Castellonis Castellon 3, *L. interrogans* serovar Wolffi 3705, *L. interrogans* serovar Hardjo Hardjoprajitmo, *L. interrogans* serovar Copenhageni M 20, *L. interrogans* serovar Tarassovi Perepelicin and *L. kirschneri* serovar Grippotyphosa Moskva V. as antigen.

**Results:** No significant difference was observed between coating reagents; therefore buffer coating with 2% skimmed milk was chosen. The best protein extract was obtained from the strain *L. interrogans* serogroup Icterohaemorrhagiae, serovar Copenhageni M20. Two and a half µg / ml of the protein extract was used at a 1: 2500 dilution of the secondary antibody, meanwhile a serum dilution was 1/200

**Discussion:** Using the ELISA test, immunoreactivity was observed 100% of the sera positively confirmed by the MAT. None of the negative serum to MAT showed reactivity using the ELISA assay. We understand that this assay could be an important tool for diagnosis of bovine leptospirosis. In Argentina, there is a need for the development of a more practical techniques for the detection of the disease.

PA-P01

### Besnoitiosis as an emerging and exotic disease – new clinical features in breeding bulls

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A pure 6 year old limousine bull was presented for clinical examination because of loss of appetite, hyperthermia (>40.7°C) and severe depression. The animal was first treated with an antibiotic and a non-steroidal-anti-inflammatory drug (flunixin-meglubine). A week later the bull continued with the same clinical signs plus thickening of the skin, limited to the ventral part of the abdomen and thorax and scrotum. The skin was hard, thick, and wrinkled. The scrotum skin was completely dry and detached from inner tissue (skin fragments broke on touch). Secondary bacterial infection was present in skin folds and cracks. Painful swellings were also evident on the limbs. Movement and defecation (very dry faeces) elicited pain. No scleral cysts were observed. No similar lesions were seen on the dorsal part of the body, neck or head so that at the distance the animal seemed to have a delimitation line crossing along its body. For humanitarian reasons the bull was euthanized. Necropsy was performed but no internal lesions were evident macroscopically. The testes were atrophied and the parenchyma was orange. Skin fragments from ventral and dorsal areas were sent for histopathology that confirmed the presence of typical *Besnoitia besnoti* crescent-shaped bradyzoites. A necrotizing orchitis was also confirmed. Other cases in two breeding bulls from the same farm did not show so exuberant clinical signs, but all lesions were also limited to the ventral parts of the body. A concomitant investigation was developed to establish the antibody prevalence in this farm (with almost 2,000 beef cattle). Preliminary results (454 blood samples) show a prevalence of 17% in adult animal and 5% in young stock.

EFSA has recently appointed besnoitiosis as an emerging disease in the EU as there is evidence of an expansion in cattle herds in some European countries. However, most aspects of this disease remain unclarified, including prevalence, routes of transmission and risk-factors associated to infection. Arthropods such as horseflies, wildlife, rodents and several ungulates may be responsible for besnoitiosis spreading. There is no known treatment for this disease.

Although the clinical disease is well defined, there are characteristics that are still puzzling. It is well established that there are two distinct stages of the disease: an acute oedema/hyperthermia stage associated with proliferation of endozoites in blood vessels, and a chronic scleroderma stage associated with cyst formation in the skin and other tissues. Permanent infertility of bulls is usual. We show in these clinical cases an unreported feature of the disease: lesions limited to the ventral skin areas of the body. We propose that the parasite has a tropism for areas less exposed to sunlight and heat. This would explain why, in the several cases that we have followed, the lesions are mostly on the underbelly, scrotum, limbs and lower part of the neck.

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PA-P02

### Effect of population size in livestock neosporosis - a seroprevalence study in bulk milk samples in the north and centre of Portugal

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**Objectives:** Since its first recognition in 1984 and subsequent description of the coccidian parasite *Neospora caninum*, neosporosis has emerged as a serious disease of cattle and dogs worldwide. The severity of the economic impact is related to abortions and neonatal mortality in livestock. The aim of this work was to study the effect of livestock herd size in the seroprevalence of antibodies against *Neospora caninum* in the north and center of Portugal.

**Materials and methods:** A total of 105 milk farms located under the influence area of the Association of Milk Farmers in the region of Center and North of Portugal (PROLEITE) were recruited. These farms gave their permission to collect and test bulk milk, every 4 months for a period of 7 years. Farms were categorized by population size (52 farms with size less than 100 cows, 38 farms with size between 100 and 200 cows, and 15 farms with size higher than 200 cows). Milk sera were tested by ELISA for anti-neospora caninum IgG by a commercial assay.

**Results:** Results revealed that anti-neospora caninum seroprevalence decreased with increasing population size (from 30% to 15%). Smaller farms frequently reported having free-roaming dogs for guard. Throughout the 7 years, anti-neospora caninum IgG in milk sera in this regions decreased from 75% to 65%.

**Conclusions:** Decreasing seroprevalence with increasing population size is likely related with higher sanitary conditions and increased biosafety measures in high population farms. The presence of dogs is a known factor for disease due to their role in the parasite life cycle and in the transmission of *Neospora caninum*.

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PA-P03

### Changes over time in *Eimeria* oocyst excretion in dairy cattle before and after parturition and the underlying factors

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**Objectives:** Neutrophils have been reported to be involved in the defense mechanism of cows against coccidiosis. Meanwhile, the function of neutrophils is decreased in late pregnancy in cows due to metabolic and endocrine changes. In the present study, changes over time in fecal OPG values in dairy cattle were determined before and after parturition, and blood parameters related to excretion of oocysts were investigated.

**Materials and Methods:** A total of 41 pregnant Holstein cows bred in Notsuke County, Hokkaido, and 24 liveborn calves were used. Feces were collected from the rectum and blood from the jugular vein at five time points: approximately 4 weeks and 2 weeks before the delivery day, from 3 days before the delivery day to the delivery day, and 3 days and 2 weeks after the delivery day. *Eimeria* oocysts per gram (OPG) of feces was calculated by the O-ring method, and *Eimeria* species were estimated. TP, Alb, AST, GGT, BUN, Ca, NEFA, and BHBA were determined as blood parameters. In addition, feces were collected from calves 20 and 30 days after birth for calculation of OPG and estimation of *Eimeria* species.

**Results:** Eight types of *Eimeria* oocysts were detected during the study period: *E. bovis*, *E. zuernii*, *E. ellispsoidalis*, *E. auburnensis*, *E. canadensis*, *E. cylindrica*, *E. alabamensis*, and *E. wyomingensis*. Excretion of oocysts was seen at least once during the study period in 35 cows (85.4%) and 11 calves (45.8%). Mean OPG in these cows at each time point was 44, 660, 665, 352, and 59, respectively, with the highest OPG being seen from 3 days before the delivery day to the delivery day. NEFA levels in cows that excreted oocysts from 3 days before the delivery day to the delivery day and those that did not were  $0.700 \pm 0.100$  and  $0.45 \pm 0.05$  (mean  $\pm$  standard error), respectively, with the former being statistically high ( $P < 0.05$ ). No correlation was seen between NEFA levels and OPG. Of the 24 pairs of cows/calves from which feces were collected from both mother and child, oocyst excretion was found in both the mother and child in 11 pairs. Oocyst excretion was seen in neither mother nor child in three pairs, and oocyst excretion was seen in the mother only in 10 pairs.

**Conclusions:** Transitory excretion of a large number of oocysts was found in cows with a high NEFA level from 3 days before the delivery day to the delivery day.

PA-P04

### Effects of Lasalocid addition in diet for the control of *Eimeria* spp. parasitism in naturally infected Nelore calves in tropical area of Brazil.

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This study aimed to evaluate the efficacy of lasalocid (Taurotec®, 1 mg.kg<sup>-1</sup>) administered over four months along the protein and energy supplementation in creep-feeding in the control of *Eimeria* spp. parasitism in males and females calves naturally infected, raised on pasture, and clinically asymptomatic. This work also evaluated animal weight gain under the two different treatment schemes. Four- to six-months old Nelore calves were randomly assigned to an untreated control group (T01; n=138) with a mean of 690.9 oocysts of *Eimeria* spp. per gram of feces (OoPG) or to a Lasalocid-treated group (T02; n=142) with a mean of 692.2 OoPG. Control group calves (T01) were fed with a protein and mineral supplement (150 grams/animal/day) daily for four months starting at two months prior to weaning. Calves in the treatment group (T02) received the same protein and mineral supplement with the addition of 1.3 grams of lasalocid for every 200 kg of body weight during the same period. To minimize the effect of gastrointestinal nematodes, calves from both groups were treated with moxidectin (200 µg/kg) every 45 days. Calves were weighed and fecal samples were collected for the quantification of OoPG by the McMaster technique monthly. Mixed model procedure was used for the statistical analysis. Regardless of the age of the animals, Lasalocid-treated calves showed a significant (P<0.05) reduction of oocyst shedding in feces in comparison to the untreated control group. This reduction was observed in all dates post-treatment. An efficacy rate above 95% (arithmetic means) was observed four months after initial treatment (at 60 days post-weaning). Furthermore, calves fed Lasalocid had significantly increased weight gain (7.2 kg; P<0.05), in comparison with the control animals by the end of the study period. In conclusion, Taurotec significantly reduced the environmental shedding of *Eimeria* spp. as early as one month following treatment onset. Such an effect was consistently observed throughout the four-month study period. The control of these protozoan infections provided a significant increase in weight gain in comparison to untreated animals. This protocol may be recommended as a management tool to reduce the environmental dispersion of oocysts of *Eimeria* spp., minimize the risks of re-infection, and promote healthy weight gain.

PA-P05

### Evaluation of metaphylactic efficacy and safety of Diclazuril 0.25% (w/w) oral suspension against coccidiosis in calves exposed to natural infection. A clinical field trial in Japan.

\*Masaya Naito Tomohide Kamiya

Shokukanken Inc.

**Objective:** Metaphylactic efficacy and safety of "Vecoxan" (diclazuril formulation) for bovine coccidiosis following single oral administration to calves in 2 coccidiosis-positive farms in Japan were compared with those of an approved formulation "Baycox for Cattle" [Bayer Yakuhin, Ltd.].

**Material and method:** A total of 200 calves from 2 farms (80, 80 and 40 for the test article, negative control and positive control groups, respectively) were included in the study. The test article group received single oral dose of "Vecoxan" (diclazuril formulation) at 0.4 mL (1.0 mg diclazuril) per kg body weight. To the positive control group, single oral dose of commercially available "Baycox for Cattle" [Bayer Yakuhin, Ltd.] was administered at 0.3mL (15 mg as toltrazuril) per kg body weight. No treatment was performed for the negative control group. For 6 weeks thereafter, clinical observation, fecal sample collection and body weight measurement were performed and occurrence of coccidiosis was observed for efficacy and safety evaluation. Criteria for onset of coccidiosis were (1) OPG of >10<sup>5</sup>, (2) fecal consistency score of >2 and OPG of >10<sup>4</sup> within 1 week, or (3) fecal consistency score of >1 for >5 consecutive days and OPG of >10<sup>3</sup> within 1 week after the fecal consistency score becoming >1.

**Result:** 7 species of *Coccidia* including *Eimeria zuernii*, *E. bovis* and *E. ellipsoidalis* were detected in this study. Some concomitant infection were also observed.

Regarding efficacy, incidence during the study period was 0/80 (0%), 32/80 (40.0%) and 0/40 (0%) in the test article, negative control and positive control groups, respectively. No coccidiosis occurred in the test article and positive control groups, and the incidence was significantly lower than the negative control group (P<0.01). Geometric mean OPG values in the test article, negative control and positive control groups were 128.1, 126.5 and 134.7 prior to administration, 116.9, 364.9 and 139.0 at Week 2, 122.2, 476.1 and 134.3 at Week 4 and 159.9, 336.2 and 179.5 at Week 6. At Week 2, 4 and 6, it was significantly lower in the test article and positive control groups as compared to that in the negative control group (P<0.05). Treatment rates were 8/80 (10.0%), 33/80 (41.3%) and 8/40 (20.0%) in the test article, negative control and positive control groups, respectively, significantly lower in the test article and positive control groups as compared to the negative control group (P<0.01, P<0.05).

Regarding safety, there was no abnormal clinical sign considered attributable to administration of the test article "Vecoxan" (diclazuril formulation) and no serious adverse event occurred for 6 weeks after administration. In addition, there was no significant difference in mean body weight gain among study groups.

**Conclusion:** The field clinical study demonstrated that single oral administration of "Vecoxan" (diclazuril formulation) is effective for prevention of bovine coccidiosis at least for 6 weeks after administration and no clinical or production safety issues were reported.

PA-P06

### Prevention of bloody diarrhea caused by coccidiosis in beef calves using toltrazuril 5%

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**Objectives:** Parasitic diseases are a great challenge for beef



producers in Brazil. In rainy seasons, diarrheas caused by coccidiosis are an important cause of economic loss in young calves. This trial aims to measure the incidence of bloody diarrhea caused by coccidiosis in beef calves born from Fixed Timed Artificial Insemination (FTAI) programs after a prophylactic oral administration of a toltrazuril 5% based product (Baycox®) on the day of birth.

**Materials and Methods:** A total of 307 beef calves were allocated into two experimental groups: 161 calves in control group (untreated) and 146 calves in treated group. The groups were randomized using three parameters: calf progenitor (bull used in the FTAI program), breed (Nelore or Brangus) and gender of the animal. All the calves were identified with earrings, weighed and had the navel treated on the day of birth. At the same moment, the calves of the treated group were orally medicated with toltrazuril 5% (Baycox®) in the dosage of 3 ml/10 kg of body weight. The calves were kept in homogeneous groups (about 100 to 130 calves with their respective mothers/yards), accounting for about 50% of the animals in each group/yard due to the pasture management of the farm. All calves were daily observed for clinical symptoms of diarrheas and other diseases. The sick animals were treated according to the veterinary protocol of the farm. For the statistical analysis, the chi square test was performed with significance of 5%.

**Results:** After 70 days of age, eight animals presented cases of bloody diarrhea. The treated group had one case and the control group had seven cases of bloody diarrhea. A statistical difference ( $p=0,04$ ) was observed between both groups.

**Conclusions:** The profilactic treatment with Baycox® in beef calves from FTAI programs on the first day of life was effective to reduce bloody diarrhea caused by coccidiosis in calves up to 70 days of age. Possibly, there was a great environmental contamination due to the large number of animals in the same group and the fact that the season of birth coincides with the beginning of rainy season, which increases the parasitic challenge.

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PA-P07

### Evaluation of treatment efficacy and safety of Diclazuril 0.25% (w/w) oral suspension against coccidiosis in calves exposed to natural infection. A clinical field trial in Japan.

\*Masaya Naito Tomohide Kamiya

<sup>1</sup>Shokukanken Inc.

**Objective:** Treatment efficacy and safety of "Vecoxan" (diclazuril formulation) following single oral administration to calves with coccidiosis in 2 coccidiosis-positive farms in Japan were compared with those of an approved formulation "Daimeton Soda" [Meiji Seika Pharma Co., Ltd].

**Material and method:** A total of 90 calves from 2 farms (60 and 30 for the test article and control groups, respectively) satisfying the criteria for onset of coccidiosis (fecal consistency score of >1, total clinical score of >4, OPG of >10<sup>3</sup> and coccidial infection was determined as the primary cause) were included in the study. The test article group received single oral dose of "Vecoxan" (diclazuril formulation) at 0.4 mL (1.0 mg di-

clazuril) per kg body weight. To the control group, "Daimeton soda" [Meiji Seika Pharma Co., Ltd] was orally administered at 60 mg sulfamonomethoxine sodium/kg body weight for 3 days.

For 14 days thereafter, clinical observation, fecal sample collection and body weight measurement were performed and recovery from coccidiosis was observed for efficacy and safety evaluation. To determine efficacy rate, improvement rate was calculated first from the individual total clinical score prior to administration, and on Day 3, 7 and 14 after administration, and the cases with improvement rate of >70% were regarded as effective cases.

**Result:** 7 species of Coccidia including *Eimeria zuernii*, *E.bovis* and *E.ellipsoidalis* were detected in this study. Some concomitant infection were also observed.

Efficacy rates on Day 3, 7 and 14 after administration were 76.7%, 91.7% and 95.0% in the test article group, and 73.3%, 86.7% and 86.7% in the control group, respectively. The rates were higher in the test article group as compared to the control group. Geometric mean OPG values in the test article and control groups were 17,927.3 and 18,358.8 prior to administration, 602.6 and 594.6 on Day 3, 367.6 and 789.1 on Day 7 and 337.8 and 1,137.0 on Day 14. It markedly decreased after administration in both groups as compared to that prior to administration. On Day 7 and 14, it was significantly lower in the test article group as compared to the control group ( $P<0.01$ ).

In addition, the incidence of coccidiosis recurrence for 14 days after administration was 0/60 (0%) and 4/30 (13.3%) in the test article and control groups, respectively, significantly lower in the test article group ( $P<0.05$ ). Regarding safety, there was no abnormal clinical sign considered attributable to administration of the test article "Vecoxan" (diclazuril formulation) and no serious adverse event occurred for 7 days after administration. There was no significant difference in mean body weight gain between study groups.

**Conclusion:** The field clinical study demonstrated that single oral administration of "Vecoxan" (diclazuril formulation) is effective for treatment of bovine coccidiosis in calves with coccidiosis and clinical or production safety issues were reported.

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PA-P08

### The influence of Geographical Indication disciplinaries on parasitic burden and epidemiology.

#### Case-study of the Italian dairy production system.

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**Objectives:** Parasite load and epidemiology are highly influenced by breeding systems and intensive cattle farming is usually supposed to be gastro intestinal (GI) parasite free. With the present study we aimed to assess the burden of parasitic infections on dairy cows and heifers from intensive dairy cattle farms from three areas of Northern Italy: Parmesan, Grana Padano and Piedmont, characterized by different breeding systems. In



the Parmesan area, grass and hay must represent 50% of ratio, corn silage is not allowed and cattle are kept indoor. In the Grana Padano area cattle are also kept indoor but corn silage is allowed. In Piedmont, cattle are fed with fresh grass or allowed to graze on pasture.

**Materials & Methods:** Fecal samples were collected from 1304 animals from 45 intensive stables of Northern Italy. A mean number of 15 dairy cows and 14 heifers was sampled from each farm. Farms were evenly distributed among the three productive areas: Parmigiano Reggiano (n=15), Grana Padano (n=15) and Piemonte Region (n=15). Fecal Egg Count (FEC) was performed using MINI-FLOTAC on each specimen to detect and quantify *Coccidia* oocysts, GI Nematode eggs and Cestoda oncospheres. Baermann technique was used to detect first-stage larvae of pulmonary strongyles and for species identification of GI Nematodes. Parasite load on pasture was monitored from an entire vegetative season, from April to November 2016. Larvae quantification was performed on 1 m<sup>2</sup> of freshly collected grass. Results were normalized on grass dry weight and expressed as larvae/kg of dry weight. Pasture contamination was monitored monthly and compared to parasite load of animals from the same farm. Generalized linear mixed models were used to assess risk factors for parasite infections.

**Results:** *Coccidia* were recorded with an overall prevalence of 23.54% (CI95% 21.32-25.92) from 97.78% of the sampled farms (44/45). Heifers were significantly more infected than cows at all sites (p<0.001) while higher prevalence was recorded in the Grana Padano area compared to Parmigiano and Piemonte (p<0.001). GI Nematodes were recorded with a prevalence P= 10.35% (CI95% 8.81 – 12.12%) in 18 of the sampled farms. Mean parasite load was 2.52 epg (eggs per gram) (median=0; sd=15.27). Significant differences existed between the three study areas. GI Nematodes were less prevalent in Parmigiano Reggiano area while higher frequency of infection was recorded in Piemonte Region. Cows and heifers from Parmesan area were found significantly more infected with *Moniezia* sp. than in the other two study sites (p<0.01) with a prevalence of P=1.99% (CI95% 1.36-2.91). Larvae seasonal trend on pastures showed a peak in mid-June (93.05 larvae/kg dry weight) and again in mid-October (73.04 larvae/kg dry weight).

**Conclusions:** This study has revealed new evidence that even on intensive dairy farms GI parasite can be presents even with low prevalence and burden. Different breeding systems show specific parasite risks linked with the use of fresh grass or hay and give helpful indications to farmers and veterinarians on how to maximize efficiency and efficacy of parasite management.

PA-P09

### Presence of *Ostertagia ostertagi* antibodies in bulk tank milk (BTM) from dairy cattle herds in the Central Eastern region of Poland – preliminary study

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**Objectives:** Gastro-intestinal nematodes are common cattle parasites that can be found in all cattle farms. Cattle can be infected with one of several nematode species, especially members of the *Trychostrongylidae*. *Ostertagia ostertagi* is considered the most widespread nematode type, with the highest health and milk yield impact. In highest frequency, infected dairy cows present the decrease in milk production.

This work presents serological evidence of *Ostertagia ostertagi* infection in dairy cattle farms in the Central Eastern region of Poland.

**Materials and methods:** The Ethical Committee for Animal Experiments (Wrocław, Poland) approved this study, and all owners provided informed consent prior to initiation of the study. Bulk tank milk (BTM) samples were collected in 211 dairy farms (January-December 2017). Population of dairy cattle in investigated region amounted 949 000 cows, kept in 2524 dairy farms. Appointed veterinarians collected samples that were transported directly to the Diagnostic Laboratory EIP-VET (Faculty of Veterinary Medicine, Wrocław). The BTM samples were collected into 100ml container without preservatives. Milk samples were centrifugated at 16,000 x g for 4 min and the fat fraction was removed. The BTM skim milk samples were stored at -20°C until testing.

The *Ostertagia ostertagi* antibody levels in BTM were determined using a semi-quantitative indirect ELISA (Svanovir® O.ostertagi-Ab, Svanova, Uppsala, Sweden) following the manufacturer instructions. The test results were expressed as optical density ratio (ODR) values. According to the manufacturer, ODR > 0.5 could be linked to milk yield reduction.

Statistical analysis was performed using STATISTICA v.12 (StataSoft, Inc., Tulsa, USA). The Shapiro-Wilk test, nonparametric Mann-Whitney U-test, and Pearson's chi-square test were used for statistical evaluations.

**Results:** The research was performed in 211 dairy cattle farms. The presence of ODR average values and their range shows - see below.

#### No. of herds

211

#### Medium ODR

0.462

#### ODR < 0.5

119 (56.4%)

#### ODR > 0.5

92 (43.6%)

In statistical analysis we noted correlation between ODR>0.5 and number of cows in lactation only.

**Conclusion:** In the present study, the authors could verify that farmers and breeders in this region do not seem to consider gastrointestinal nematode infections as a potential problem in their dairy herds.

The obtained results indicate the spread of these parasites in



the dairy cattle in region, where concentration of animals per 100ha is high.

Studies on *Ostertagia* infection require further continuation, including analysis of livelihood and milk production in seropositive herds.

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PA-P10

### Prevalence of gastro-intestinal parasitic infections in Leizhou Black Goats of China

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Leizhou black goat is Chinese Guangdong local popular goat breeds, which are well-known for meat and skin production, also easily available to consumers and its meat are export in all parts of the country and also in Hong Kong and Macao markets. Currently, there are about 300,000 goats. In goats parasitism can cause decreased fertility, abortion, unthriftiness, increased susceptibility to disease, and death. However, investigation on intestinal parasites in Leizhou black goats is scarce. The aim of present study was to determine the prevalence of the intestinal parasites including worm and protozoan in three farms with 500 goats from Leizhou city of Guangdong province, China. A total of 200 feces were collected for egg count analysis and coccidial oocyst count by McMaster's method. The results showed that the average incidence of intestinal parasites on worm and protozoan in three farms were 95.8%. While, the most frequently infected goats feeding pens with net rearing were 100%, and semi-intensive feeding to half pasturing with net rearing were 94.6%, the full grazing was 87.5%. Majority of the infections parasites were nematode worm (61.13%), tapeworm (23.14%) and coccidian (15.73%). In addition, the incidence caused by *trichostrongylidae* was the highest (53%), *moniezia spp.* (47%), *Eimeria christensenii* (34.3%) and *Eimeria caprina* (17.6%). This study indicated that gastro-intestinal helminths and protozoa infections are prevalent in goats of Leizhou of Guangdong, the infection intensity was obviously related with the feeding condition. The infection rate of feeding pens with net rearing was significantly higher than grazing system.

**Keywords:** Leizhou Black Goats, gastro-intestinal parasites, egg count.

PA-P11

### Pharmacokinetics of eprinomectin in lactating ewes following administration of a subcutaneous formulation of eprinomectin

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**Objectives:** Because of a limited excretion in milk and broad spectrum antiparasitic activity, eprinomectin is a good option for helminth control in lactating small ruminants. Eprinomectin is currently available for small ruminants as a topical product with a dose rate of 1 mg/kg (Eprinex<sup>®</sup> Multi, BI) but parenteral administration seems preferable given a higher bioavailability (Lespine et al., 2003) and substantial less variation in drug exposure that could be associated with suboptimal treatment and increased risk of resistance (EMA, 2017). Recently a subcutaneous formulation of eprinomectin that has been registered in cattle (Eprecis<sup>®</sup> 2%, Ceva) at a dose rate of 0.2 mg/kg without milk withdrawal period. In order to precise the optimal dose rate of this new formulation in sheep, a pharmacokinetic study was performed in lactating ewes at 0.2 and 0.4 mg/kg. Additionally, milk residue profile associated with the optimal dose rate was determined.

**Materials and methods:** Sixteen healthy lactating Lacaune ewes (mean age = 6.1 years, mean BW = 70.4 kg) were randomly allocated to two treatment groups. Half of the ewes received eprinomectin subcutaneously (Eprecis<sup>®</sup> 2%) at 0.2 mg/kg while the other half was administrated 0.4 mg/kg. Milk and blood were sampled at defined intervals after treatments for 7 days. Plasma and milk samples were assayed for eprinomectin b1a using LC/MS–MS methods. Pharmacokinetic parameters were determined and compared to the minimal effective concentration required for optimal antiparasitic activity against endoparasites (1 to 2 µg/L, (Lifschitz et al., 2004).

**Results:** Eprinomectin plasmatic concentrations following subcutaneous administration of eprinomectin at either 0.2 mg/kg or 0.4 mg/kg were above the efficacy threshold (>2 µg/L) at all time during the study. Consequently, it can be anticipated that the optimal dose rate for this eprinomectin formulation in sheep is 0.2 mg/kg. At this dose rate, 96% of the milk samples did not have detectable eprinomectin residues and the 4% of eprinomectin positive samples (5 samples out of 128) presented concentrations widely below the published maximum residue level (MRL). Finally, in comparison with the topical formulation dosed at 1.0 mg/kg (Eprinex<sup>®</sup> Pour-on, Merial), the subcutaneous formulation dosed at 0.2 mg/kg showed a higher *AUC*<sub>last</sub> (73.3 vs 48.8 day\*ng/mL) and *C*<sub>max</sub> (19.5 vs 6.2 ng/mL), demonstrating a greater bioavailability with 5 times less active ingredient.

**Conclusions:** According to this pharmacokinetic study, the optimal dose rate in sheep for this subcutaneous formulation of eprinomectin (Eprecis<sup>®</sup> 2%) is 0.2 mg/kg. Bioavailability with this formulation at this dose rate was found to be higher compared to the topical formulation dosed at 1 mg/kg. In conclusion, the subcutaneous formulation of eprinomectin is expected to be efficacious in sheep for the control of endoparasites at 0.2 mg/kg with a zero day withdrawal period in milk.

**References:** EMA, 2017. Reflection paper on anthelmintic resistance. EMA.



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PA-P12

### **Efficacy of an exclusive doramectin 1% formulation on the weight gain of Nelore heifers raised in pasture compared to other commercial products**

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**Objectives:** Helminth parasitism is a disease that greatly affects cattle productivity in many regions of the world due to the weight loss. In Brazil, animals raised on natural pastures are exposed to infection by gastrointestinal nematode larvae, particularly *Cooperia*, *Haemonchus*, *Ostertagia*, *Strongyloides*, *Trichostrongylus* and *Oesophagostomum* genus. The present study aims to evaluate the weight gain in Nelore heifers treated with an exclusive doramectin 1% formulation (TRUCID® - Bayer Animal Health) compared to other commercial products. The trial was conducted in the city of Lins, state of São Paulo - Brazil, in a field challenge.

**Materials and Methods:** Eighty Nelore heifers in adequate nutritional status, aged less than 24 months, were allocated into four groups, each composed of 20 animals. During the study, the animals were maintained in pasture supplemented with corn silage. The animals were randomized according to the daily weight gain between D-30 and D0. On D0, all the animals were separated in groups according to the randomization method: 20 heifers were treated with saline solution (Control Group), 20 heifers were medicated with the exclusive doramectin 1% formulation (TRUCID® Group), 20 heifers were medicated with another doramectin 1% product (Doramectin Group) and 20 heifers were medicated with a high concentrated ivermectin product (Ivermectin Group).

For this study, all the recommendations of the manufactures were followed: subcutaneous administration above the subscapular region, at the dose of 1 ml/50 kg of body weight. The animals were weighed on D7, D14, D21 and D28 after the treatments.

**Results:** The mean body weight gain in D28 was 32.73 kg (Control Group), 40.24 kg (TRUCID® Group), 31.59 kg (Doramectin Group) and 31.05 kg (Ivermectin Group).

The TRUCID® Group presented superior weight gain compared to Control Group (7.51 kg), to Doramectin Group (8.65kg) and to Ivermectin Group (9.19 kg).

**Conclusions:** Heifers treated with TRUCID® presented superior weight gain compared to animals that were not treated or treated with other commercial products, which suggest that TRUCID® promotes a better helminth control due to its exclu-

sive formulation.

PA-P13

### **Anthelmintic efficacy of oral trichlorfon 97% against cattle gastrointestinal nematodes in feedlot**

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**Objectives:** Nematoda phylum has a large number of species that parasite bovines and cause important economic loss. Due to the intensive use of parasiticides in cattle, the helminth resistance to drugs rose in the last years and has been reported by different researchers in Brazil. The present study had the purpose of assessing the anthelmintic efficacy of a metrifonate 97% product (Neguvon®) compared to other commercial product in bovines in feedlot and its impact on weight gain. The trial was conducted at Ipiranga do Norte city, in Mato Grosso state, Brazil, in a field trial.

**Material and methods:** a total of 180 zebu male bovines, aging 24 to 36 months, were used at the trial. The animals were in a feedlot, with a diet based on corn silage and concentrated, and were allocated in three experimental groups: 60 animals were not treated (Group 1), 60 animals were treated with ivermectin 1% (Group 2) and 60 animals were treated with metrifonate 97% (Neguvon®; Group 3). Group 2 received a subcutaneous injection over the right subscapular area (dosage 1 ml/50 kg of body weight). In Group 3, the tested product was administered at the dosage of 10 g/200 kg of body weight. The total dose was divided in three supplies (D0, D1 and D2), once a day, diluted in water and applied over the food disposed in the animal feeder. All the animals were weighed on days 1, 21 and 113. The clinical examinations were performed at days -3, 0, 1, 2, 7, 14 and 21. Stool samples were collected for eggs per gram (EPG) counting at days -3, 7, 14 and 21.

**Results:** During the trial, no animal presented signs of intoxication during clinical examination. Two genus of helminth were identified in the exams: *Haemonchus* spp. (81%) and *Cooperia* spp. (19%).

The mean EPG counting on day -3 (before treatment) was 215. Group 3 had a mean EPG count of 11, 24 and 13 on days 7, 14 and 21, respectively, corresponding to an efficacy rate of 94.17%, 85.43% and 95.92% when compared to Group 1. Conversely, Group 2 had a mean EPG count of 46, 41 and 28 on the same days, corresponding to an efficacy rate of 76.67%, 74.87% and 52.11%, when compared to Group 1. Group 1 had a mean EPG of 200, 165 and 118.

At day 113, Group 3 presented the mean weight gain of 157 kg, significantly higher than Group 2 (138 kg;  $P < 0.05$ ) and Group 1 (123 kg;  $P < 0.05$ ).

**Conclusion:** The use of metrifonate 97% (Neguvon®) administered in feed for bovine in feedlot resulted in an effective and safe method to control the main gastrointestinal parasites, presenting higher weight gain than ivermectin 1%. Considering that helminth parasitism greatly jeopardizes weight gain in cattle and that controlling the infections has become a challenge



for farmers due to drug resistance, Neguvon® can be considered an interesting option to optimize beef cattle production in feedlots.

PA-P14

### Molecular detection of tick-borne pathogens in Holstein cattle in the Republic of Korea

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**Background:** Global warming increases the incidence and risk of tick-borne diseases in domestic animals and humans in the Republic of Korea (ROK). In this study, we investigated tick-borne pathogens in 12 Holstein cattle in the ROK using specific PCR assays.

**Methods:** Holstein cattle used in this study were raised on the farm located 400 meters above sea level in the city of Sangju in the Gyeongbuk province, ROK. The cattle, aged 11-54 months, were allowed to graze on grass from May to October. Twelve blood samples were randomly collected to detect the TBPs, *Anaplasma* and *Theileria*. PCR products were purified using the Accupower PCR Purification Kit (Bioneer, Daejeon, Korea) and was then used for direct sequencing (Bioneer). A phylogenetic tree was constructed based on nucleotide alignments using the neighbor-joining method. Bootstrap analysis was conducted with 1000 replicates using MEGA version 7.

**Results:** The prevalence of *Theileria orientalis* and *Anaplasma phagocytophilum* was 58.3% (7/12) and 16.7% (2/12), respectively. Of the seven cattle infected with *T. orientalis*, Chitose, Ikeda, and co-infection of Chitose and Ikeda were detected in one, three, and three animals, respectively. Mixed infection of *T. orientalis* and *A. phagocytophilum* was observed in two animals. To the best of our knowledge, this is the first report to identify *A. phagocytophilum* infection in Holstein cattle. Our findings showed that the 16S rRNA gene was the most sensitive assay for *A. phagocytophilum* detection in cattle. Phylogenetic analysis revealed that *A. phagocytophilum* sequences identified in Holstein cattle might be less pathogenic.

**Discussion:** These findings suggest that *T. orientalis* is prevalent in the ROK, but *T. orientalis* infection is not necessarily associated with anemia, while mixed infection with *T. orientalis* and *A. phagocytophilum* reduced the host's RBC count. Further studies are necessary to investigate the genetic diversity and pathogenicity of *T. orientalis* and *A. phagocytophilum*.

PA-P15

### A survey of bovine Chorioptic mange in Japan Concomitant infection with *Chorioptes bovis* and *C. texanus*

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**Introduction and Objectives:** Bovine mange is causes severe clinical symptoms such as depilation and crust formation accompanied by severe pruritus, and is a form of allergic dermatitis initiated by the feeding or excreta of mites of the genus *Chorioptes*, *Psoroptes* or *Sarcoptes*. In Japan, bovine mange is caused by infection of only Chorioptic mites, and Psoroptic and Sarcoptic manges have not been reported in dairy and beef cattle. Until 1980's, it has been believed that only *C. bovis* causes mange in cattle by veterinarians, but after confirming the infection with *C. texanus* were firstly reported from cattle in Japan in 1992, major cause of bovine mange has been estimated as *C. texanus*. *C. bovis* and *C. texanus* are morphologically identical in all stages except for the adult male, in which differences occur in the lengths of the opisthosomal setae.

We re-recognized *C. bovis* of cattle kept in several areas of Japan, and also confirmed the concomitant infection cases in Hokkaido, firstly in the world [Fukumoto et al. (2007), *Jpn.J. Animal.Hyg.* 33, 41-45].

In the present opportunity, we tried to investigate the infection status of bovine Chorioptic mites in Japan.

#### Materials and methods:

1) Cattle examined: More than 200 heads of Japanese black cattle or Holstein cows from 8 prefectures, i.e., Hokkaido, Yamagata, Chiba, Okayama, Mie, Shimane, Nagasaki and Okinawa prefecture and Nagasaki with typical skin lesions, depilation and crust formation, were examined for mites.

2) Detection and identification of mites: the adult males were detected under an anatomical microscopy and magnified using a microscope. In the classification of the genus *Chorioptes*, attention is paid to the morphology of a structure called a lobed projection around the tail of a male mite and the length of five bristles derived from the periphery, and distinguish between *C. texanus* and *C. bovis*.

**Results:** In this survey, *C. texanus* was detected from cattle in Hokkaido, Okayama, Chiba, Mie, Shimane, Nagasaki and Okinawa prefectures, and *C. bovis* was confirmed in Hokkaido, Chiba and Okayama prefectures. And also, concomitant infection case with both species were found from cattle kept in Hokkaido and Chiba prefectures.

Recently, most of reports recognized only *C. texanus* infection from cattle in Japan. According from the result of our investigation *C. texanus* also might be major species of cattle widely distributed in Japan, but *C.bovis* is also widely distributed in Japanese cattle.

**Conclusions:** We confirmed the prevalence of *C. texanus* and *C. bovis* from cattle in Japan, and also found concomitant infection with both species from different area in Japan. We are continuing to investigate the distribution and diagnosing the two species of the genus *Chorioptes* in cattle also with molecular biological technique.



PA-P16

### Insecticide efficacy of a new 1% fipronil-based Pour-on formulation against horn fly (*Haematobia irritans*) in Brazil.

#### A field trial.

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**Objectives:** *Haematobia irritans*, the horn fly (HF), is a livestock pest of key economic impact on beef and dairy cattle industries for its direct impact on foodstuff production, its wide geographical distribution, and its invasive nature.

Since HF is an obligate blood-feeding pest, it is considered a constant threat to cattle. HF's typical painful bites may result in both food intake decrease and production losses. In addition HF is a potential carrier of a number of parasites, and constitutes a known risk factor of mastitis. In areas where HF is active throughout the year, pest control is essential to ensure profitability of cattle operations. Control is mostly achieved through the use of topical insecticides. The aim of this study was to assess the ability of a brand new 1% fipronil-based pour-on formulation to control HF natural infestations in cattle in Brazil.

**Materials & Methods:** For this study, 30 male crossbreed animals *B. indicus* x *B. taurus* (Gir x Holstein) from 120 to 320 kg have been selected from the experimental herd of the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária of Universidade Federal Rural do Rio de Janeiro (UFRRJ, Seropédica, RJ, Brazil).

HF were enumerated on both sides of animals on D0 (immediately before the treatment), and the study population was stratified with consideration to the total number of flies observed. Animals were then alternatively assigned to either the control group (C, n=15) or the treatment group (T, n=15), in descending order of parasitic load. On day 0, 1 mL/10 kgBW (1 mg/kg) of a brand-new 1% fipronil-based medicine was poured on the back of group T animals, from the withers to the base of the tail. Animals in Groups T and C were kept in two different locations 5 km apart, and fed on pasture (*Brachiaria* spp.).

On the morning of days +1, +3, +7, +14, +21, and +28, HF were enumerated on animals, always by the same investigator. For the statistical analysis, transformation by natural logarithm (1+logN) was applied to fly counts.

According to the Brazilian regulation for insecticide treatments, a new medicine is deemed effective where the reduction of the fly population on animals is greater than 80%.

**Results:** In the pre-treatment period, the average fly counts were 113.26 and 112.6 flies/animal in groups T and C, respectively. Post-treatment levels of infestation decreased significantly (p<0.05) for all time points between D+1 and D+28. In treated animals, HF population decreased by 99.2, 99.6, 94.2, 89.5, 81.3 and 62.53% at days D+1, +3, +7, +14, +21, and +28, respectively. The HF population did not significantly fluctuate in control animals. Reduction of the parasitic burden was greater than 80% from D+1 to D+21.

**Conclusion:** In this study, this new 1% fipronil-based pour-on insecticide (EFFIPRO BOVIS, Virbac do Brasil, São Paulo, Brazil) was found to be effective against the horn fly with a reduction of fly counts by more than 80% for 21 days after the treatment.

PA-P17

### Strategic control of *Rhipicephalus microplus* with topical Fluazuron and systemic macrocyclic lactones reduces environmental and animal infestation in pasture-raised Angus heifers.

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The aim of this study was to assess the efficacy of a parasite control program comprised of an association of topical fluazuron (Tackzuron®, 2.5%) and systemic macrocyclic lactones (doramectin or moxidectin) against *R. microplus* ticks compared to traditional interventions based on the combined use of topical organophosphate (clorpirifos) and synthetic pyrethroids (cipermetrin). The study was conducted in Piratini, Rio Grande do Sul state, Brazil. Cattle included were 13-month old Angus females weighing 265 kg in average. Heifers were maintained under the same conditions, in a region of high environmental concentrations of *R. microplus*. Seventy eight heifers were blocked based on tick counts and live weight and randomly assigned to one of three treatment groups (n=26 per group). Treatment groups were further split in two blocks (n=13) and assigned to six independent pasture areas to account for field variation. Heifers were assigned to receive a combination of topical Fluazuron and subcutaneous Moxidectin (FLU+MOX Group), or to receive a combination of topical Fluazuron and subcutaneous Doramectin (FLU+DOR Group), or to an untreated control group. Groups FLU+MOX and FLU+DOR were treated according to the manufacturer's instructions. For a full year following treatment (day 0), heifers were submitted to a tick count of the entire left side of the body every 14 days for a total of 28 assessments. Whenever the mean count for a group was over 30 ticks per heifer, all animals in that group was pulverized with a combination of clorpirifos and cipermetrin as per manufacturer's label per standard practice (designated as tactical interventions). Parameters evaluated included the number of tactical interventions required for each group during the experimental period (day 0 through day 364) as well as the average number of ticks per animal between day 185 (estimated as the length of Fluazuron activity) and day 364 following initial treatment. The latter parameter was deemed to be a measure of the environmental infestation following cessation of Fluazuron activity. Data were compared using Statistix 9.0 and EpiInfo 7. The number of tactical interventions required during the experimental period were as follows: 2 interventions for the FLU+MOX group, 8 interventions for the FLU+DOR group, and 16 interventions for untreated controls. Statistically, FLU+MOX required less tactical interventions than the control group, representing a protection factor (OR=0.093, 0.02-0.43; P<0.01). The average number of ticks per animal throughout the entire experimental period (day 0 through day 364) was lower in the





FLU+MOX group (8.7 ticks/animal/assessment) compared to the FLU+DOR (13.6 ticks/animal/assessment) and Control (40.6 ticks/animal/assessment) groups ( $P < 0.01$ ). Furthermore, in regards to this same parameter, the FLU+DOR group had a significantly better performance than the Control group ( $P < 0.01$ ). When the number of ticks per animal from different assessments were tabulated, heifers in the FLU+MOX group had significantly lower ( $p < 0.01$ ) tick count on 16/28 assessments compared to the Control group and in 10/28 assessment days compared to the FLU+DOR group. Conversely, FLU+DOR had lower tick counts ( $p < 0.01$ ) on 17/28 and on 5/28 assessments when compared to the Control and FLU+MOX groups, respectively. Environmental infestation (i.e., tick count per animal after the residual Fluzuron effect on day 185 through day 364) was significantly reduced ( $P < 0.01$ ) for the FLU+MOX group ( $n = 137.6 \pm 42.1$ ) compared to the FLU+DOR ( $n = 196.2 \pm 61.6$ ) and Control ( $n = 218.7 \pm 92.6$ ) groups. No statistical differences in environmental infestation were observed between FLU+DOR and Control groups. Topical Fluzuron based treatments promoted a sharp reduction in the environmental infestation of *R. microplus* for a full year following initial treatment. The association of Fluzuron and Moxidectin was the most efficient treatment to reduce tick populations on the animal during the expected period of activity for Fluzuron (day 0 through day 185) and for the subsequent tick environmental control following cessation of activity (day 0 through day 364). Furthermore, the association of topical Fluzuron and Moxidectin reduced the need for tactical interventions when compared to the control. Adoption of such a strategic program may increase convenience to producers and reduce potential environmental concerns associated with organophosphate and synthetic pyrethroid interventions to control tick infestation in pasture-raised cattle.

PA-P18

### **Efficacy of a new 2% eprinomectin-based injectable formulation on experimental infestation by *Rhipicephalus (Boophilus) microplus* in cattle.**

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**Objectives:** In tropical and subtropical regions. *Rh. microplus* (tick) causes serious economical losses, and control tick infestation is one of the most important veterinary challenge. Periodical application of topical acaricide are routinely used to control ticks, however concerns have raised about the emergence of resistance as well as environmental consequences of such strategies. Injectable macrocyclic lactones has been demonstrated to be effective to eliminate and to prevent tick infestation, and they have a more limited impact on environment than topical treatments. The objective of this study was to test the efficacy of a single injection of 0.2 mg/kg of eprinomectin on the elimination as well as the prevention of tick infestation in cattle maintained in controlled conditions.

**Materials & Methods:** The trial has been carried out at the Estação para Pesquisas Parasitológicas W.O. Neitz of UFRRJ

(Seropédica, RJ, Brasil). Twelve cross-breed (*B. taurus x B. indicus*) male cattle aged 10 to 24 m.o. were enrolled in this study. All animals were free of acaricide treatment ( $> 30$  d), and maintained in individual boxes from Day-34 to D+45 (D0 being the treatment day). Presence of ticks was then assessed daily over the study period.

Every second day from D-25 to -1, animals were infested with  $\pm 2.500$ , 14 days-old larvae of ticks (poured on the back line). In order to assess the preventive effect of the treatment, animals were infested on D+7, +14, +21, +28, +35 & +42 with 10.000 larvae.

On D0, the study population was stratified with consideration to the average number of engorged females collected on D-3, -2 & -1, and animals were alternatively assigned to the control group (C) or the treatment group (T), in descending order of the infestation level. On the same day, group T animals were dosed with 0.2 mg/kgBW (1 mL/100kg) of an injectable commercial solution of eprinomectin (NEOPRINIL 2%, Virbac).

The curative efficacy of the treatment was calculated comparing tick counts post-treatment to the average tick counts on D-3, -2 & -1. In order to calculate the preventive efficacy, tick counts in group T were compared to those of group C.

Finally reproductive performances of engorged ticks was evaluated, based on tick weight, mass of eggs laid, and hatching rate was calculated in 10% of ticks collected on D+1 to +45.

**Results:** From D-3 to D-1, and from D+1 to D+23, average number of engorged ticks collected on animals were 70.94 and 50.96, or 70.94 and 7.46 for group C or T, respectively. Post-treatment prevalence of ticks differed significantly ( $p < 0.01$ ) between groups. In group T animals tick population decreased by 7.46% on D+1 to a maximum of 99.66% on D+7. From D+5 to D+23 on one hand, and D+6 to D+23 on the other hand, average acaricide efficacy rates were 97.43% and 97.49%, respectively.

Average number of engorged ticks collected in periods D+25 to D+31, then D+32 to D+38, and finally D+39 to D+45 was never lower in group T than in group C.

Average weight of engorged ticks, as well as average clutch weight and hatching rate were always numerically lower in ticks collected on treated animals. Consequently, reproductive performances were lower in ticks collected from Group T animals than from Group C.

**Conclusion:** From day 5 and up to day 23 following a single injection of this new 2% eprinomectin-based solution in artificially infested cattle, the acaricide efficacy was greater than 97%. The product failed to prevent re-infestation of animals, as it was expected with this kind of active. Interestingly reproductive performances of ticks engorging on treated animals was seriously impaired.

PA-P19

### **Efficacy of a new 2% eprinomectin-based injectable formulation against *Rhipicephalus (Boophilus) microplus* in cattle under field conditions in Brazil.**

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**Objectives:** In tropical and subtropical regions, *Rh. microplus* (tick) causes serious economic losses, thus making the control tick infestation is one of the most pressing veterinary challenges. Although periodical application of topical acaricide is routinely used to control ticks, concerns have been raised about the emergence of resistance as well as the potential environmental implications of such strategies. Injectable macrocyclic lactones have been demonstrated to be effective in tick control, and they have lower impact on environment than topical treatments. The objective of this study is to test the efficacy of a single injection of 0.2 mg/kg of eprinomectin on the elimination of tick infestation in cattle under Brazilian conditions.

**Materials & Methods:** For this study, 20 male or female cross-breed animals *B. indicus* x *B. taurus* (Gir x Holstein), 350-400 kg live weight have been selected from the experimental herd of the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária of the Universidade Federal Rural do Rio de Janeiro (UFRRJ, Seropédica, RJ, Brazil). Before the study, animals had been kept on pastures, without parasiticides treatment and were very likely to become naturally infested by (not exclusively) either gastro-intestinal nematodes, *Dermatobia hominis* (berne), *Haematobia irritans* (horn fly) or *Rh. microplus*.

On D0, the study population was stratified with consideration to the average number of engorged females ( $\emptyset$  4.5 to 8 mm), which had been collected by the same investigator before 9 a. m. on D-3, -2 & -1. Animals were then alternatively assigned to the control group (C) or the treatment group (T), in descending order of infestation level. On the same day, group T animals were dosed with 0.2 mg/kgBW (1 mL/100kg) of an injectable commercial solution of eprinomectin. Group T and group C animals were kept in two different locations, and fed on pasture (*Brachiaria* spp.), with mineral supplementation and good-quality water.

The body surface of the experimented animals was carefully observed, again, by the same investigator on early morning on days D+1, +3, +7, +14, +21, and +28, and ticks were counted.

The curative efficacy of the treatment was calculated by comparing the geometric means of tick counts after treatment to the average tick counts on D-3, -2 & -1. According to the Brazilian regulation for ixodicide treatments, a new medicine is deemed to be effective if the reduction of the tick population on animals, based on geometric means, is greater than 95% on D+7 and D+14 after treatment.

**Results:** In the pre-treatment period, the average tick counts were 79.0 and 79.8 ticks ( $\emptyset > 4$  mm) in group T and C, respectively. The post-treatment level of infestation decreased significantly ( $p < 0.05$ ) for all time points between D+3 and D+28. In treated animals, the tick population decreased by 47.19, 82.41, 97.70, 98.64, 96.36, and 72.74% on days D+1, +3, +7, +14, +21, and +28, respectively. The tick population fluctuated a little (-39.8% at D+28; +29.9% at D+14; n.s.) in control animals. Reduction of the parasitic burden was greater than 95% from D+7 to D+21.

**Conclusion:** From day 7 up to day 21 following a single injection of this new 2% eprinomectin-based solution (NEOPRINIL 2%, Virbac do Brasil, São Paulo, Brazil) in naturally infested

cattle, the acaricide efficacy was greater than 95%.

PA-P20

### Efficacy of a new 1% fipronil-based pour-on formulation against *Rhipicephalus (Boophilus) microplus* in cattle under field conditions in Brazil.

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**Objectives:** In regions of the intertropical band, *Rhipicephalus (Boophilus) microplus* (tick) causes serious economic losses, thus making the control of tick infestation one of the most pressing veterinary challenges. Although periodical application of topical acaricide is routinely used to control ticks, concerns have been raised about the emergence of resistance. The objective of this study was to test the efficacy of a single topical application of 1 mg/kg of fipronil on the elimination of tick infestation in cattle under Brazilian conditions.

**Materials & Methods:** For this study, 20 male crossbred animals *B. indicus* x *B. taurus* (Gir x Holstein) have been selected from the experimental herd of the Laboratório de Quimioterapia Experimental em Parasitologia Veterinária of Universidade Federal Rural do Rio de Janeiro (UFRRJ, Seropédica, RJ, Brazil). The animals were not subjected to parasite control for a month or longer before the start of the study. The animals were kept in pastures in order to become susceptible to natural infestation by (not exclusively) either gastro-intestinal nematodes, botfly, horn fly, or ticks.

On D0, the study population was stratified with consideration to the average number of engorged females ( $\emptyset \geq 4.5$  mm) collected on D-3, -2 & -1, before 9:00am by the same investigator. Afterwards, animals were alternatively assigned to the control group (C) or the treatment group (T), in descending order of infestation level. Then, animals were alternatively assigned to either the control group (C, n=10) or the treatment group (T, n=10), in descending order of parasitic burden. On the same day, 1 mL/10 kgBW (1 mg/kg) of a brand-new 1% fipronil-based medicine was poured on the back of group T animals, from the base of the horns to the base of the tail.

On the morning of days D+7, +14, +21, and +28, ticks ( $\emptyset \geq 4.5$  mm) were enumerated on animals, always by the same investigator.

According to the Brazilian regulation for *Rh. microplus* control, a new medicine is deemed effective where the reduction of the tick population on animals is greater than 95%.

**Results:** In the pre-treatment period, the average tick counts were 24.8 and 24.7 ticks in group T and C, respectively. The post-treatment level of infestation decreased significantly ( $p < 0.05$ ) for all time points between D+7 and D+28. In treated animals, tick population decreased by 96.9, 96.6, 92.6, and 86.9% at days D+7, +14, +21, and +28, respectively. Tick counts did not significantly fluctuate in control animals. Reduction of the parasitic burden was greater than 95% from D+7 to D+14.



**Conclusion:** In this study, this new 1% fipronil-based pour-on insecticide (EFFIPRO BOVIS, Virbac do Brasil, São Paulo, Brazil) was found to be effective against *Rhipicephalus (Boophilus) microplus* with a reduction of tick counts by more than 95% for 14 days after the treatment.

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PA-P21

## **Bovine Neosporosis in Chile: Pathology and Epidemiology**

### **A review**

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*Neospora caninum* is an intracellular coccidian parasite that has been implicated as a major cause of infectious bovine abortion. There are no therapies or effective vaccines against it and it is a prevalent disease worldwide. Neosporosis is considered an emergent disease in cattle production in Chile. According to information originated from a seroprevalence study in the late 90's, the estimated prevalence rate varies between 15% and 30%, but there is a gap of information in the last 20 years, since there are no research lines or control policies focusing on this disease in the country. The main clinical manifestation in cattle is abortion, and therefore it is important for the production indexes and economical parameters, since it is responsible for significant economic losses in livestock production.

The aim of this study was to provide actualized information about the pathology and epidemiology of the bovine neosporosis in Chile, through the analysis of: (1) seroprevalence and risk factors of *Neospora caninum* infection in bovines of central Chile and (2) pathology diagnostic review of bovine abortions cases observed in the south part of the country, where the dairy industry is based.

The epidemiological study was performed in the central Chile region, analyzing small dairy farms in central Chile. Sera samples were collected from milking cows and analyzed by a commercial ELISA kit. Additionally, a questionnaire was applied to farmers to identify risk factors through logistic regression.

The pathology review of bovine abortions was made through the retrospective analysis of data from 1991 to 2016, including all causes of abortion and the neosporosis cases description, as well as the development of diagnostic tools to improve the quality of the final diagnostic.

Epidemiological results showed that the farm level prevalence was found to be 67%. Regarding the risk factors related: abortion history ( $p=0.037$ ,  $OR=5.09$ ), dogs feed source ( $p=0.0429$ ,  $OR=6$ ), cattle drinking water source ( $p=0.034$ ,  $OR=4.5$ ) and abortions management ( $p=0.017$ ,  $OR=7.43$ ) were found as significant factors for infection.

The pathological review results showed that neosporosis in the southern part of Chile is an important cause of bovine abortion,

being in the 2<sup>nd</sup> place, after bacterial and viral causes. Until 2013 the diagnostics at the Institute of Pathology at the Universidad Austral de Chile were based only on compatible histopathological findings. This study shows an increase in fetuses diagnosed by this cause after 2004, which is similar to other authors for whom the presence of *N. caninum* is considered the greatest cause of abortions in places where brucellosis is being controlled. The diagnostic of fetuses aborted by this agent were more frequent in the middle of gestation, with an average of gestational age of 5.7 months (range of 3 to 8 months). The most important histopathological lesions were observed in the central nervous system (brain, cerebellum, brain stem and optic nerve) with multifocal necrosis, glial and mononuclear infiltration. Also, the histopathological features in other organs were the infiltration of mononuclear cells in the myocardium and skeletal muscles, and non-suppurative hepatitis.

There is a high *N. caninum* seroprevalence in small dairy farms central Chile. Neosporosis is an important cause of abortion in southern Chile too. A possible bias may occur when underestimating the actual occurrence of the infection, mainly due to the difficulty of making a histopathological diagnosis in fetuses that are not adequate to the analysis. The lesions observed and the characterizations of the abortion cases correspond to reports from other countries.

These studies showed that more information is required in order to understand the important and relevance of this disease in Chile. Also, it highlights the need of improving *N. caninum* surveillance in areas where the livestock industry is relevant. Finally, the development of country policies in order to control and prevent the disease is needed, in order to avoid the economic losses in livestock production related to neosporosis.



VR-P01

### Relationship of IL-12, TNF $\alpha$ , IFN $\gamma$ , IL-4, IL-10 and TGF $\beta$ expression with persistent lymphocytosis in bovine leukemia virus infected cows

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**OBJECTIVES:** Development of persistent lymphocytosis (PL) is an outcome of bovine leukemia virus (BLV) that represents a polyclonal expansion of target B cells which are IgM<sup>+</sup>, CD5<sup>+</sup>, CD11b<sup>+</sup>, MHCII<sup>+</sup>, sharing phenotype with B<sub>reg</sub> cells. It is regarded that BLV promotes immune suppression. The aim of the present work was to relate expression of IL-12, TNF $\alpha$ , IFN $\gamma$ , IL-4, IL-10, and TGF $\beta$  with the observation of PL in order to determine basis of BLV immune suppression.

**MATERIAL AND METHODS:** Experimental design.

The current study was done in a dairy production complex with high BLV seroprevalence: Trial 1. Eighty cows were randomly sampled from one production unit to select 34 cows as follows: BLV<sup>+</sup>/PL<sup>+</sup> (n= 11), BLV<sup>+</sup>/PL<sup>-</sup> (n= 12) and BLV/PL<sup>-</sup> (n= 11). The cytokine expression of IL-12, IFN $\gamma$ , IL-4, and IL-10 of 2 monthly samplings was determined by qPCR). Trial 2. One hundred fifty cows were haphazardly sampled from different production units across the dairy complex to select 60 cows as follow: 16 BLV<sup>+</sup>/LP<sup>+</sup>, 14 BLV<sup>+</sup>/LP<sup>-</sup> y 30 BLV/LP<sup>-</sup>. The cytokine expression of IL-12, TNF $\alpha$ , IFN $\gamma$ , IL-4, IL-10, and TGF $\beta$  of 3 monthly samplings was measured by qPCR. The peripheral blood mononuclear cells (PBMC) were obtained using density gradient to extract ARN using commercial kits. The retrotranscription was done in 20  $\mu$ l reactions using Oligo (dT)20 primer. The qPCR was performed in 20  $\mu$ l reactions containing 50 ng/ $\mu$ l of cDNA template, 20 pM of each primer and SYBR Lo-Rox master mix. Each sample was done in triplicate. The cytokine expression was determined by relative quantification using hypoxanthine phosphoribosyltransferase. The data was statistically analyzed by ANOVA and Tukey test.

**RESULTS:** In trial 1, the expression of IL-12, IFN $\gamma$ , IL-4 and IL-10 was strikingly higher in the BLV negative group compared to the BLV<sup>+</sup>/PL<sup>+</sup> and BLV<sup>+</sup>/PL<sup>-</sup>, being statistically significant for IL-10 and IL-12 whereas trial 2 the IFN $\gamma$  and IL-10 the BLV group was statistically significant higher than BLV<sup>+</sup>/PL<sup>+</sup>. The IL4 expression of BLV<sup>+</sup>/PL<sup>+</sup> was lower but no statistical significant than the other groups. The TGF $\beta$  expression of BLV<sup>+</sup>/PL<sup>+</sup> was statistically significant higher than BLV<sup>+</sup>/PL<sup>-</sup> and BLV<sup>-</sup> groups. The expression of TNF $\alpha$  was not statistically significant.

**DISCUSSION:** The cytokine expression variations between trials might indicate the trial 1 production unit management and the overall situation of the dairy complex. The herein data strongly suggests that polyclonal target B cell proliferation may enrich a population of phenotypically similar B<sub>reg</sub> cells. That proliferation might promote a severe immune suppression in BLV<sup>+</sup> animals since trial 1 data showed lower expression of IL12,

IFN $\gamma$ , IL4 and IL10 in BLV infected cows than a BLV<sup>-</sup> group while findings of trial 2 have provided some insights regarding TGF $\beta$ -induced immune suppression since it was found was higher in BLV<sup>+</sup>/PL<sup>+</sup> group. The above sustains the hypothesis of a TGF $\beta$ -induced immune suppression that switch off Th1 and Th2 responses and IL10 secretion, being more evident in trial 1. Likely, IL10-secreting Br1 cells promote T<sub>reg</sub> cell differentiation which in turns induces a negative feedback on Br1 cells with a consequent decrease of IL-10. In this scenario, expression of IL10 might portray progression and/or disease severity, explaining discordant findings of IL-10 among different reports. On the other hand, induction of T<sub>reg</sub> cell differentiation might be regulated by TGF- $\beta$ -secreting Br3 cells from B cell polyclonal expansion. Concerning TNF- $\alpha$  expression, it has been suggested that TNF- $\alpha$  might influence proliferation of B cells due to a TNF- $\alpha$  receptor high expression on CD5<sup>+</sup>, IgM<sup>+</sup> cells. In the current work, the TNF- $\alpha$  expression data does not agree with such assumption. Further studies must be performed to provide more information regarding the role of TGF $\beta$ -induced immune suppression and characterize the putative B<sub>reg</sub> cell population.

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VR-P02

### Relationship of BLV viral load and BLV antibody titers with lymphocyte counts in a bovine leukemia high prevalence dairy herd.

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**OBJECTIVES:** Bovine leukemia virus (BLV) promotes persistent lymphocytosis (PL) which used as a susceptibility trait. BLV viral load directly correlates with antibody titers and PL. In Mexico, the prevalence of BLV is high, fluctuating from 66% to 72%. The aim of this work was to determine the BLV viral load and BLV antibody titers associated with high lymphocytes counts that might be used as predictive values to determine severity of disease.

**MATERIAL AND METHODS:** Experimental design.

The current study was done in a dairy production complex with high BLV seroprevalence: Trial 1. Eighty cows were randomly sampled from one production unit to select 34 cows as follows: BLV<sup>+</sup>/PL<sup>+</sup> (n= 11), BLV<sup>+</sup>/PL<sup>-</sup> (n= 12) and BLV/PL<sup>-</sup> (n= 11). The sera from two monthly samplings were used to determine BLV antibody titers by indirect ELISA test to relate with lymphocyte counts. Trial 2. One hundred fifty cows were haphazardly sampled from different production units across the dairy complex to select 60 cows as follow: 16 BLV<sup>+</sup>/LP<sup>+</sup>, 14 BLV<sup>+</sup>/LP<sup>-</sup> y 30 BLV/LP<sup>-</sup>. The BLV proviral load of 3 monthly samplings was mea-



sured by qPCR to relate with lymphocyte counts. The BLV antibody titers was determined by serial dilution (1:50-1:6400). Peripheral blood mononuclear cells (PBMC) were obtained using 1.077 density gradient to extract ADN with commercial kits. The qPCR was performed in 20 µl reactions containing 50 ng/µl of ADN template, 20 pmol of each gene *pol* specific primers and SYBR Lo-Rox master mix in a real-time thermocycler. Each sample was done in triplicate. The relative quantification of BLV viral load was done using hypoxanthine phosphoribosyltransferase (HPRT) as constitutive gene. According to BLV antibody titers and viral load data, the cut-off values were determined and the cows were grouped to relate with normalized lymphocyte counts as follows: Group 1) High antibody titer (HA) or High proviral load (HV), Group 2) Low antibody titer (LA) or Low proviral load (LV), and Group 3) Negative (N) group. The results were statistically analyzed by ANOVA and Tukey test.

**RESULTS:** In both trials, the lymphocyte counts of BLV<sup>+</sup>PL<sup>+</sup> group were statistically higher than BLV<sup>+</sup>LP<sup>-</sup> and BLV/LP<sup>-</sup>. The BLV antibody titer and BLV proviral load of BLV<sup>+</sup>PL<sup>+</sup> were higher than BLV<sup>+</sup>LP<sup>-</sup> but this finding was only statistical significant for the viral load. Concerning lymphocyte counts, both the antibody titers and the viral load showed a statistically significant direct relationship with lymphocyte counts. The HA and the HV groups depicted a statistically significant difference solely with the N group. The cut-off values for HA and HV were of 200 and 10<sup>2.11</sup>, respectively.

**DISCUSSION:** In the present work, the lymphocyte counts were significant higher in both HA and HV groups than BLV cows, indicating that both parameters have a direct effect on blood lymphocyte increases. The high lymphocyte counts related to HA and HV groups are representative of strong induction of humoral response as well as increased severity of disease, as previously described. Conversely, low BLV antibody titer and BLV proviral load were linked with low PL to normal lymphocyte counts. However, the BLV proviral load reported herein is interpreted to have a better predicted value for disease severity than antibody titers since the increases of BLV proviral load were gradually increasing along with lymphocyte counts while the antibody titers showed an abrupt cut-off, restricting its usefulness as a marker connected with lymphoma development. Nevertheless, BLV antibody response must be taken as an indication of susceptibility rather than protective immunity since it is associated with PL.

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VR-P03

### Identification of novel antisense RNAs in the genome of bovine leukemia virus (BLV)

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Human T-lymphotropic virus type 1 (HTLV-1) and bovine leukemia virus (BLV) are close relatives of the cancer retroviruses that are known to naturally infect human and cattle, respective-

ly, and both of which are highly endemic in Japan. Although the genomic architecture of the two retrovirus resemble each other, HTLV-1 causes the T-cell hematological malignancy, called adult T cell leukemia/lymphoma (ATLL) after a long latency period, and genetic alterations of HTLV-1-infected T-cells, induced by expression of HTLV-1 viral proteins, including Tax and antisense strand-encoded protein termed HTLV-1 b-ZIP factor (HBZ), play important roles in transformation of HTLV-1-infected T-cells and persistent proliferation of ATLL cells. The infection of cattle with BLV causes lymphoproliferative disorders of mature B-cells, B-cell lymphocytosis and enzootic bovine lymphoma (EBL). Both diseases are major causes of economic losses in the cattle industry, with estimate approximately 40% and 30%, respectively, of beef cattle and dairy cattle having infection with BLV in Japan, and the cases of BLV-positive cattle and BLV-induced lymphoma have been reported to be increasing in Japan, pointing to an urgent need for effective strategies for eradicating BLV. Although the BLV Tax protein has been shown to activate viral genes as well as cellular genes, the mechanisms of BLV-induced leukemogenesis are still largely unknown. Here, we performed detailed analysis of the gene expression from the antisense strand of BLV in BLV-transformed fetal lamb kidney cell line FLK-BLV by a rapid amplification of cDNA ends (RACE) method, which can comprehensively isolate transcripts expressed in the cell. We found that, similarly to HTLV-1, BLV produces spliced and unspliced antisense RNAs, plausible counterparts of HTLV-1 HBZ, transcribed from the 3' long terminal repeat (LTR). In the case of HTLV-1, HBZ functions both as RNA and protein forms by regulating the activity of cellular proteins as well as viral proteins including Tax. We confirmed that an antisense transcript spliced at positions similar to those occurred in HTLV-1 is produced in BLV, as reported previously. Unexpectedly, however, some of these antisense RNAs were found to contain additional alternate exons that are not described in HTLV-1. These results suggest that patterns of BLV-antisense RNA expression might be more diverse and complex than HTLV-1 does, and may have some unique roles in the development of BLV-induced pathogenesis. We are currently assessing the occurrence of these phenomena under natural conditions using EBL-derived B-lymphoid cell lines and also testing the possibility the existence of protein products derived from these BLV-antisense RNAs.

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VR-P04

### Analysis of molecular diversity of 5' Untranslated Region of Bovine Viral Diarrhea Virus isolates from cattle in Mexico

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Bovine viral diarrhea virus (BVDv) genome is a positive-stranded molecule of RNA, approximately 12.3 kb in length. The genome contains a single Open Reading Frame (ORF) flanked by a 5 and a 3 Untranslated Region (UTR). The 5 UTR of almost 400 nucleotides is the most conserved part of the Pestivirus genome it has been proposed that sequences from this region



can be used to differentiate among the members viruses and has allowed for the genetic typing of BVDv isolates into two genotypes, BVDv-1 and BVDv-2. RT-PCR has been commonly used as a diagnostic method and, it was used for genotyping strains, which allows distinguishing genotypes and subgenotypes.

**Objectives:** The aim of this study was to determine the presence of BVDv and determine the genotype and subgenotype present in samples from cattle of Mexico through the analysis of the 5' UTR region.

**Material and methods:** Blood samples were obtained with heparin and bovine ear biopsies from different regions of Mexico (Chiapas, Mexico city, Guerrero, Hidalgo, Jalisco, Mexico and Tabasco) with respiratory signology. Blood samples with heparin were processed for RNA extraction by using extraction kits according to the manufacturer's specifications (Qiagen), subsequently analyzed by end point RT-PCR of the 5' UTR of the samples. From BVDv positive samples we selected 1 to 3 samples from the same herd to carry out viral isolations in Mardin Dabin Bovine Kidney cells (MDBK) free of BVDv. The isolations were analyzed again by RT-PCR (specific to 5' UTR), sequenced and products were edited (Lasergen Seqman program). The phylogenetic analysis were obtained using the neighbor-joining method (in software MEGA-6). Also, an RT-PCR test was standardized for direct genotyping of strains, identifying the genotypes by means of the product size.

**Results:** 536 samples were screened by RT-PCR for the 5' UTR region of BVDv and the presence of BVDv was demonstrated in dual purpose herds beef herds. The percentage of positive samples for BVDv was 31.7% by RT-PCR. It was observed that 20.7% of the herds were vaccinated against BVDv, of which 41.4% were positive in RT-PCR. The genotypic analysis of viral isolates showed that BVDv-1a and BVDv-2a are currently circulating in the country.

**Conclusions:** The results showed that genotypes 1 and 2 circulate widely in the country. The genetic composition of the samples analyzed suggests that there is currently more than one subgenotype of BVDv circulating in the country. The data obtained from the analysis of samples, as well as the medical records collected, show us that vaccination is not as effective as expected, on the other hand, given that there is not enough data in the country to show which are the circulating strains, it is necessary to continue doing studies that collaborate to know the state of the disease in the country.

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VR-P05

### Identification of epitopes /mimotopes against to Bovine Viral Diarrhea virus antibodies using Phage Display libraries.

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Bovine viral diarrhea virus (BVDv) is classified as a member vi-

rus of the genus *Pestivirus* within the *Flaviviridae* family, present in populations of cattle that causes considerable economic losses to the dairy and beef industries worldwide. The diagnosis is based on virus isolation, nucleic acid detection (RT-PCR) or antibody detection (ELISA). Currently ELISA test is most commonly used, however, requires the production and purification of viruses or antibodies whose limitation is the cost and specificity. One alternative is the use of Phage-display technology which provides an effective and powerful way of identifying peptides that can mimic the BVDv epitopes.

**Objectives:** Select specific peptides that mimic epitopes for the identification of BVDv-specific antibodies through the Phage Display technique.

**Material and methods:** We use two commercially libraries of linear heptapeptide and a loop-constrained heptapeptide with hyperimmune rabbit sera against BVDv genotype 1 NADL (ATCC, VR-1422), and using a library of linear heptapeptide with hyperimmune rabbit sera against BVDv genotype 2 (Mexican strain). After 3th rounds of biopanning, we selected phage clones who showed high specificity and reactivity to the target.

**Results:** 10 phage clones to hyperimmune rabbit sera against BVDv NADL, 6 phages clones to the hyperimmune sera anti-BVDv-2 Mex and one non-reactive clone. Each phage clones were purified and sequenced. The binding activity of selected phage clones in cattle serum was determined by ELISA: phage clone anti-BVDv-NADL showed high reactivity with BVDv-1 and BVDv-2 positive sera; phages clones anti-BVDv-2 Mex showed similar reactivity with BVDv-2, BVDv-1 and co-infections sera. Meanwhile, non-reactive clone showed low reactivity for BVDv positive serum, co-infections serum and negative serum.

**Conclusions:** Our results suggest that selected phage-peptides mimic important immunological characteristics that can be used to develop diagnostic techniques, which involve lower cost than commercial kits, thus allowing the detection of antibodies against BVDv-1 and BVDv-2 in serum from cattle to control the virus within herds in our country.

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VR-P06

### Sero-survey of BVDV, BEFV, IBAV in cattle in Northern Vietnam

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**Objectives:** Cattle husbandry, including both dairy cows and beef herd, is a developing area in Vietnam. To prevent diseases, Pasteurellosis and Foot and Mouth disease (FMD) are the most commonly used vaccines for cattle. Since epidemiological data is limited, other vaccines are not circulated in Vietnam. Hence, there is a demand for more insight information on viral diseases in Vietnam, for better disease control and prevention.

In the scope of this study, we will focus on 3 pathogenic viruses: bovine viral diarrhea virus (BVDV), bovine ephemeral fever virus (BEFV), Ibaraki virus (IBAV). BVDV is one of the most common viral pathogens in cattle, widespread all over the world, often responsible for significant economic losses in husbandry. BEFV and IBAV prevail periodically and also make



economical impairing. An epidemiological survey of these three viruses in the Northern Vietnam is conducted by viral neutralization test (VNT) to provide some insights into their distribution and prevalence in Vietnam.

**Materials and methods:** The material used in this study consists of 118 cattle blood samples obtained from April 2017 to May 2017. The cattle are from 26 small farms (from 1 to 11 cattle) and a medium-sized farm (45 cattle), in 3 areas in Northern Vietnam, namely: Trung Mau (n=48), Phu Dong (n=30), Ba Vi (n=40). Most cattle are female Holstein Friesian (n=103), some are Red Sindhi hybrid (n=13) and other breeds. All cattle are from a wide range of age (from 4 months to 10 years) and were not vaccinated with any targeted viruses. The collected sera were tested by viral neutralization test in cell culture free from all targeted viruses. BVDV neutralization test was performed by inoculating the sera against BVDV Nose T1 strain, genotype 1a, on bovine testis cell (BT), and BEFV, IBAV neutralization test was conducted in the same method, against BEFV YHL-KB strain and IBAV No.2-KB strain on hamster lung cell (HmLu). All these strains are registered vaccine strain in Japan. The cells were observed for specific cytopathogenic effects (CPE) in 7 days.

**Results:** The VNT results show that about 13% of samples were antibody positive against BVDV, they appear in all age groups and have similar frequencies all 3 regions (from 8% to 20%). Among 27 farms, 18 farms are antibody-carrier free, while 9 farms have antibody positive cattle. BEFV also has positive samples distributed equally in 3 regions, however, the positive proportion is far higher than BVDV's (from 67% to 70%), in which, 11 farms have more than 80% of samples are antibody positive. IBAV is only virus with imbalanced distribution, in which, Ba Vi and Phu Dong have 97% and 94% of the samples are antibody positive, while Trung Mau has about 57% of samples are antibody positive. 17 farms have IBAV antibody positive samples at more than 80%.

**Conclusions:** The results indicated that BEFV and IBAV are significantly prevalence in the Northern Vietnam. Since vaccination of all three diseases was not carried out in Vietnam, the result of antibodies titer is anticipated only due to viral infection. This result is interesting because BEFV and IBAV are not well-known diseases in Vietnam, which mean, more studies are required to provide a measurement to control them. The results also show only more than 10% of cattle are BVDV antibodies carriers, however, the experiment only tested one type of BVDV, and not capable of detecting persistently infected (PI) cattle, hence, a further survey with a wide range of strains is required in order to achieve more completed results. Comparing positive cattle across three diseases shows no correlation between them.

**Keywords:** Bovine, virus, disease, BVDV, BEFV, IBAV, neutralization test, detection, Vietnam.

VR-P07

## Prevention of Bovine Herpesvirus-1 Respiratory Infection and Clinical Disease Following a Secondary Intranasal Vaccination Three Weeks Pre-Weaning or the Day After Weaning

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**Objective:** Bovine respiratory disease (BRD) during the post-weaning period remains the greatest cause of morbidity and mortality in beef calves despite the availability of numerous vaccines targeting both viral and bacterial respiratory pathogens. Strategic use of these vaccines has been limited, however, by restricted access to calves prior to weaning. Our earlier studies confirmed a primary IN vaccination of 6-8 week old calves induced immune memory but this immune memory was protective only after a secondary intranasal (IN) vaccination at the time of weaning. The purpose of the current study was to determine whether timing of the secondary IN vaccination, relative to the day of weaning, significantly altered protection against a bovine herpesvirus-1 (BHV-1) respiratory infection in fall-weaned calves.

**Materials and Methods:** Beef calves received either an IN dose of diluent (placebo) (n=30), an IN dose of a modified-live viral vaccine (MLV) (Nasalgen-IP, MSD Animal Health, Madison, NJ, USA) (n=30), or an intramuscular (IM) modified-live viral vaccine (MLV) (Bovi-shield IBR/PI3, Zoetis, USA) (n=30) when 6 to 8 weeks old. Serology performed at 5 months of age revealed IN vaccination with MLV had significantly ( $P < 0.05$ ) elevated BHV-1 antibody titres relative to the placebo and IM group. A subset of calves (n=15 from each group) with no detectable BHV-1 serum neutralizing antibody titres were selected for secondary vaccination to ensure subsequent disease protection could not be attributed to residual maternal antibody. A bovine herpesvirus-1 (BHV-1) respiratory disease challenge model was then used to compare disease protection in fall-weaned beef calves receiving a secondary IN vaccination with the MLV, either three weeks pre-weaning (pW) or the day after weaning (W). Calves in the IM group were vaccinated three weeks pre-weaning (pW). All calves were aerosol challenged with BHV-1 four days after weaning and then monitored for signs of clinical disease (fever, weight loss) and shedding of infectious virus in nasal secretions.

**Results:** A secondary IN vaccination given either pre-weaning (pW) or the day after weaning (W) provided equivalent and significant ( $P < 0.05$ ) protection against both clinical disease (fever, weight loss) and virus infection when calves were challenged with BHV-1. Naïve control calves (n = 15) shed virus in nasal secretions on average for 7.4 days. In contrast, IN vaccinated calves shed detectable levels of virus on average for 0.67 days (n = 15; W) and 0.93 days (n = 15; pW). IM vaccination with MLV (n = 15; pW) also significantly ( $P < 0.05$ ) reduced clinical disease and virus shedding (mean = 2.4 days). Elevated interferon (IFN)- $\alpha$  and - $\gamma$  levels in nasal secretions and increased serum haptoglobin levels were observed in Naïve Controls following BHV-1 infection but these responses were not detected in any of the vaccinated groups. All vaccinated calves also displayed significantly ( $P < 0.05$ ) elevated BHV-1-specific serum antibody responses following infection when compared to naïve controls (placebo).

**Conclusions:** The results of this study demonstrate:

1/ Combining a primary IN vaccination of young calves, given interference of maternal antibodies, with a secondary IN vaccination at 5 to 6 months of age provides an effective strategy to prevent post-weaning BHV-1 respiratory infection and clinical



disease.

2/ Equivalent protection against both clinical disease and virus infection was achieved when the secondary IN vaccination was given either three weeks pre-weaning (pW) or the day after weaning (W).

3/ Rapid onset of a protective immune response within four days after calves receive a secondary IN vaccination at weaning provides a strategy to prevent respiratory disease while minimizing animal handling prior to weaning.

VR-P08

### Natural occurrence of interspecies recombinant viruses between bovine herpesviruses 1 and 5

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**Objectives:** *Alphaherpesvirinae* subfamily members are numerous viral agents of great relevance for ruminant health and production. The large diversity of this subfamily could have been caused by recombination as important mechanism. It can also be hypothesized that this mechanism could be involved in the acquisition of “new” properties in a viral strain, e.g. a strain with low virulence could acquire characteristics that modify its capacity to induce severe infections. From a viral evolution point of view, recombination can be considered like a driving force essential that would increase the chances to “channel” rare but advantageous mutations within a viral species. Homologous recombination is based on a high degree of sequence similarity. In the case of ruminant alphaherpesviruses, their degree of genetic similarity has allowed to classify them within a phylogenetic group, where bovine herpesvirus 5 (BoHV5) and bubaline herpesvirus 1 (BuHV1) are closely related to BoHV1, followed by elk herpesvirus 1, cervid herpesviruses 1 and 2, and more distantly to caprine herpesvirus 1. However, even a low degree of similarity between BoHV1 and BoHV5 (82.3% average similarity) allowed, under experimental conditions, the production of the interspecies recombinants BoHV1/5. Recently our group has reported the natural recombination between BoHV1 and BoHV5 viral species based on phylogenetic analysis, multiple PCR sequencing assays and sequence and genetic recombination analysis using a sliding-window genetic diversity plot (Simplot). We found three recombinant field isolates between BoHV1 and BoHV5. Our findings indicate that BoHV5 subtype b, whose current classification by restriction enzyme analysis is based on the analysis of the three examined field isolates, should rather be considered as a natural recombinant of BoHV5 subtype a and BoHV1 subtype 1.2b. We further detected two recombination breakpoints in the open reading frame of the UL27 gene. We showed here the identification of other homologous recombination points between BoHV1 and BoHV5 in these field isolates previously reported.

**Materials and methods:** The complete sequencing of recombinant viral genomes was performed by Next Generation Sequencing (NGS) specifically with the MiSeq technology of Illumina. High-throughput sequencing (HTS) was performed

following the manufacturer’s protocol for DNA library construction.

Raw sequence reads were trimmed with the aid of Geneious software (version 9.0.5). Assembly and annotation of the viral genome were done using template-assisted assembly, in which trimmed sequences were mapped to the BoHV5 SV507/99 reference genome (accession number NC005261) using Geneious software. The genetic recombination was analyzed 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 previously described three recombinant viruses where BoHV1 glycoprotein B gene sequence was present in a BoHV5 genetic background. The complete genome sequencing of two recombinant virus reveal further recombination events. Two additional recombination breakpoints were located in the long unit (UL) genomic region. Based on the BoHV5 genome (GenBank accession number YP003662497.1) one recombination breakpoint is on nt 82881 and the other on 87867 nt giving a recombination fragment of 4986 base pairs showing 100% nucleotidic identity with the BoHV1.2b subtype genome. Our results support the hypothesis that the occurrence of a first recombination event positively influences the occurrence of a second recombination event in a neighboring region.

**Conclusions:** We identify recombinant ruminant alphaherpesviruses and provide evidence of the existence of natural recombinants and recombination events within distinct viral species in the subfamily *Alphaherpesvirinae* infecting ruminants. Recombination should be now investigated with regards to its impact on differential diagnosis methods, the risk to escape vaccine-induced immunity and pathogenesis of infections.

VR-P09

### Evaluation of BoHV1-free certification through bulk milk sampling

#### Eradication progress in the Netherlands

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In the Netherlands, bovine herpesvirus 1 (BoHV1), the causative agent of infectious bovine rhinotracheitis (IBR), is endemic. Farmers can voluntarily participate in two IBR control programs: ‘IBR-free’ certification and ‘IBR-unsuspected’ certification. For monitoring of dairy herds, both programs rely on monthly bulk milk sampling. In this study we analyzed herds that switched from ‘unsuspected’ to ‘free’ certification. The aim was to look at characteristics of BoHV1 positive animals and herds with positive animals in order to improve the existing programs. Information concerning on- and off-farm movements from the national identification and registration database was used to determine herd size and whether farmers purchased cattle.

The IBR-unsuspected status can be obtained after an initial se-





seronegative bulk milk sample. The gE antibody test in bulk milk is negative at an estimated within-herd prevalence of less than 10%. Consequently, a small number of latently infected cows can still be present in a dairy herd with a seronegative bulk milk sample. Monitoring of the herd status for BoHV1 relies on monthly bulk milk sampling. When unsuspected herds purchase cattle from non-free herds, the farmer is notified and advised, but not obliged, to test that cattle serologically. After at least two years of IBR-unsuspected status (i.e. minimum of 18 consecutive negative bulk milk samples) a herd has the possibility to qualify for the IBR-free status by testing all animals older than six years of age and purchased cattle for gE antibodies in serum (i.e. 'program switch test'). This in contrast to the regular method to directly obtaining the IBR-free status, which is by initial serological testing of all animals over one year of age in a closed (last year) herd and subsequent culling of seropositive animals if present. After this intake procedure, the main tool for monitoring is also monthly bulk milk sampling.

Results of individual blood tests of 691 switched dairy herds were available over the years 2014 (277) and 2015 (414). For each herd the proportion of BoHV1 positive cows was determined. In herds that switched from unsuspected to IBR-free, 93% did not find any cows with BoHV1 antibodies in the program switch test. Thereby indicating that no old infection was present in the herd and these herds did not have to cull cattle to become IBR-free.

In 7% of the herds one or more seropositive animals were found. In 25 out of 46 herds these animals were born on the farm and where typically the oldest animals in the herds. In the other 21 herds the seropositive cows were bought in and were likely infected prior to purchase (and did not reactivate since then). Purchase was an important risk factor for the presence of seropositive cows in the program switch test, 72% of herds with seropositive cows had purchased cattle over the last three years (Dutch average 43%).

In herds with seropositive cows, the mean number of cows tested was 19 (range 4 to 80) animals. In the herds that qualified directly for the IBR-free status, 20 cows (range 1 to 129) were tested. In the positive herds, on average 1.4 (range 1 to 4) cows had gE antibodies. Out of 46 herds with seropositive animals, 33 herds had just one cow with antibodies. Possibly some false positive test results contributed to this, but mostly they were either the oldest cow present or bought in. The average age of seropositive cows was 9.3 years (range 6.0 to 17.1 years).

Certifying herds free for IBR after a minimum of two years of consecutive negative bulk milk samples is easy and cost-effective. The chances of obtaining the status IBR-free without culling of seropositive animals is high. Other studies showed that once unsuspected herds become IBR-free, the probability of (re)introduction of BoHV1 is similar to herds that became IBR-free through the faster route of blood testing all cattle older than one year. A shift to compulsory testing of purchased cattle from non-free herds soon after arrival will contribute to preserving the IBR-unsuspected status. The results can be used for future modelling and communication purposes. In the beginning of 2018 the analyses will be repeated on all individual blood samples in the program switch tests performed in 2017 and the latest results will be presented at the conference.

VR-P10

## Estimation of the basic reproductive ratio ( $R_0$ ) of BoHV1 on field data

### Transmission in Dutch dairy herds in past and present

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In the Netherlands, bovine herpesvirus 1 (BoHV1), the causative agent of infectious bovine rhinotracheitis (IBR), is endemic. A mandatory BoHV1 eradication program has been in place for a short period from 1998 to 1999 and was cancelled due to vaccine contamination issues. Since then, farmers can voluntarily participate in an IBR control program. A new national plan for mandatory BoHV1 eradication is in preparation. The basic reproductive ratio ( $R_0$ ) of an infection is the number of cases one infectious individual generates on average over the course of its infectious period, in a fully susceptible population. The  $R_0$  of BoHV1 is an important parameter for the design of future control programs.

The aim of this study was to estimate the  $R_0$  of BoHV1 on recent field data of dairy herds with a BoHV1 outbreak and to compare it to earlier estimates of the nineties. Since the nineties, the Dutch dairy industry has gone through a substantial change. The average herd size doubled and when earlier the whole herd was housed in one building, nowadays the subgroups within a herd (e.g. calves, heifers, dry cows, lactating cows) are often housed in separate units or even in other herds. There is a growing number of young stock raisers that focus exclusively on the raising of replacement heifers.

For this study, all lactating cows in eight dairy herds that lost their IBR-free status in 2014 (either because of positive BoHV1 PCR on nasal fluid or bulk milk samples containing BoHV1 antibodies) were sampled in individual milk for antibodies (IDEXX IBR gE Ab Test, IDEXX Laboratories, Inc.) in the beginning of 2015. Information from the national identification and registration database was used to determine herd size and cattle movements of the herds. The proportion of positive BoHV1 samples in each herd was used to estimate the  $R_0$  of BoHV1 with a deterministic final size model.

The selected herds had on average 118 milking cows, which was significantly larger than the average Dutch dairy herd with 98 milking cows. In the year before the outbreak, 50.0% of the herds purchased cows and 87.5% grazed their cows, which was slightly higher than the Dutch average of 47.3% and 75.0%, respectively.

In all outbreak herds, the percentage of infected animals was high and varied from 74.5% to 99.3%. BoHV1 seemed to spread fast within a herd and most lactating animals became infected. Nevertheless, these large outbreaks often were sub-clinical. The low number of seronegative animals were mostly young heifers that were added to the milking herd after the epidemic had ceased. The average time between the outbreak and sampling was 8.2 months and varied from 4.4 to 14.2 months. A longer period may have resulted in an underestimation of the herd prevalence on farm level because some seropositive cows might have been replaced by naïve heifers. The



infection seemed often limited to the lactating herd and did not spread to young stock.

The median  $R_0$  in this study was 3.0 and varied from 1.8 to 5.1. This number was comparable to earlier scientific work on the basic reproductive ratio of BoHV1 about twenty years ago. It is likely that the initial spreading of IBR within an uninfected population is not different in small or large herds. However, persistence of the infection in large herds may be higher, especially when young stock was not part of the initial epidemic and thus forms a susceptible group for reactivating cattle. The current  $R_0$  can be used for future modelling and communication purposes.

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VR-P11

### Seroprevalence of bovine herpesvirus 1 infections in dairy cattle herds in Eastern Poland

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BHV1 infections are distributed globally however, significant differences in regional incidence and prevalence were found. In Poland bulls at the national semen production centres are free from BHV1 infections, however no official IBR eradication program was established in dairy herds.

The aim of the study was to estimate seroprevalence of BHV1 infections in dairy herds in Eastern Poland.

To determine BHV1 infection status of the farms 1790 individual blood samples from milking cows from 28 herds located in Eastern Poland were collected. The size of dairy farms ranged from 24 to 186 milking cows of the Holstein-Friesian breed. Eight of the 28 herds were vaccinated with BHV1 gE deleted marker vaccine. In unvaccinated herds serum samples were tested with IDEXX IBR gB blocking ELISA while in vaccinated herds IDEXX IBR gE ELISA test kit was used.

From a total of 1790, 1105 (61.7%) cows had gB antibodies and 681 sera out of them (61.6%) reacted positively in IBR gE ELISA. All the cows from 8 vaccinated herds (n=424) had gB antibodies only. Therefore, the true prevalence of BHV1 infection in cows from unvaccinated herds was estimated at 49.3% (95%CI: 46.7-52.0%), since 681 out of 1366 animals originating from unvaccinated herds had both gB and gE antibodies. Out of 20 unvaccinated herds, BHV1 seropositive milking cows were found in 13 (65%; 95%CI: 43.3-81.9%) herds. The true herd BHV1 prevalence was estimated at 64.6% (95%CI: 43.5-85.8%). All the vaccinated herds were gE negative indicating no BHV1 infections.

The results of our study show that cattle population in Eastern Poland is endemically infected with BHV-1. The eradication of BHV1 in some dairy herds will be feasible by lowering the prevalence of infection through the use of marker vaccines.

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VR-P12

### Preparation of a Monoclonal Antibody against gD Protein of Bovine Herpesvirus 1 and epitope mapping

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The two DNA fragments encoding predicted main antigenic regions, aa 20-160 and aa 257-344 on gD protein of bovine herpesvirus-1 (BoHV-1) were tandem cloned into the vector pE-T28a. The recombinant His-tagged  $\Delta$  gD1- $\Delta$  gD2 was expressed in *Escherichia coli* BL21(DE3)pLysS by induction with 0.5 mM IPTG and western blot analysis demonstrated that the recombinant His-tagged  $\Delta$  gD1- $\Delta$  gD2 reacted with the positive serum against BoHV-1. A monoclonal antibody (MAb), designated as 2B6, was prepared by fusion of SP2/0 myeloma cells with splenocytes from female 8-week old BALB/c mouse immunized with purified His-tagged  $\Delta$  gD1- $\Delta$  gD2 protein with aids of Freund's adjuvant. The titer of ascitic fluid triggered by hybridoma cells secreting MAb 2B6 was  $1:2.5 \times 10^8$  and the subtype of MAb 2B6 was IgG 2a/k. MAb 2B6 recognized positively with BoHV-1 infected-MDBK cells by an indirect fluorescent antibody assay (IFA). Moreover, MAb 2B6 showed 1:160 viral neutralizing activity using 60% plaque reduction assay. Through expression of overlapping fragments of truncated gD protein in a bacterial system, western blot, the epitope on gD protein of BoHV-1, which was recognized by MAb 2B6, was mapped. Furthermore, a linear B-cell epitope within gD glycoprotein of BoHV-1 identified in this study demonstrated a wide conservation in the members of Herpesvirus type I. Therefore, this work suggested that MAb 2B6 a potential of diagnosis and development of novel subunit vaccine candidate against bovine respiratory disease complex associated with BoHV-1, and function analysis as well.

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VR-P13

### Polycrylamide Gel Electrophoresis and Silver staining for detection of Rotavirus in Goat fecal samples

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**Objective:** Rotavirus diarrhea assumes a special importance in developing countries where malnutrition is common in young animals and children as severe dehydration following rotavirus diarrhea leads to high rate of mortality as well as the significant economic loss to farmers of domestic livestock. The present study was undertaken to detect the presence of Rotavirus in non-diarrhoeic goat fecal samples by RNA-PAGE and silver staining.

**Material and Methods:** Around 70 fecal samples from non-diarrhoeic goats, aging between 2 months to 2 years were collected from Dindori, Madhya Pradesh in sterile screw-capped containers. The fecal samples were suspended in phosphate buffer saline (PBS) pH 7.2 to make 10% w/v suspension. From this suspension, 500 µl of the sample was taken and RNA extraction was done using phenol: chloroform method. RNA pellet was suspended in nuclease-free water for RNA-PAGE analysis. RNA-PAGE was carried out for all the samples for detection of goat Rotavirus on the basis of 11 RNA segments and their typical migration pattern on PAGE after silver staining.

**Result:** The etiology of a diarrheal syndrome is quite complex, involving many infectious agents like rotavirus, *E. coli*, *Salmonella* etc. In the present study, the survey was conducted for detection of rotavirus in non-diarrheal goats. All the 70 fecal samples were subjected to PAGE after nucleic acid extraction, of which, none of the samples was found to show 11 segments migration pattern typical of Rotavirus.

**Conclusion:** The study indicates the absence of Rotavirus in the studied goat population. The absence of the virus may also be due to the selection of non-diarrhoeic fecal samples. Rotaviruses are generally associated with neonatal diarrhea, and hence sample collection during the first month after birth is crucial in establishing their role in causing diarrhea. As the samples in the present study were collected from goats aged above 2 months, this also might have resulted in negative results. However, further study is required including larger sample size from the diarrhoeic and non-diarrhoeic goats aged below one month. The technique for detection of the rotaviral double-stranded RNA genome in stool specimens by PAGE in combination with silver staining is simple and inexpensive and can be established in small diagnostic laboratories.

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VR-P14

### Sero-epidemiological survey and detection of bovine rotavirus in cattle in Northern Vietnam

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**Objectives:** Bovine rotaviruses (BRVs) are recognized as a major cause of severe neonatal diarrhea in calves worldwide. There is no specific treatment for diarrhea caused by BRVs; hence, it can become economically devastating to the farmer. Therefore, vaccination is one of the most effective tools to protect calves against this disease. In Viet Nam, neither vaccine nor sufficient information of BRVs is available. It is necessary to estimate the occurrence and diversity of rotavirus infection in this country. Thus, the aim of this study was to investigate the prevalence of BRVs, their distribution in Vietnam to explicate

their relevance for control and prevention strategies.

**Materials and methods:** The material used in this study consists of 117 blood samples obtained from cattle (30 cattle less than 1 year old, 56 cattle from 1 to 3 years old and 31 cattle more than 3 years old). All cattle were unvaccinated with BRVs. And samples were collected from 26 small farms (less than 11 cattle) and a medium-sized farm (45 cattle) in 3 areas, namely: Ba Vi district (40 samples), Phu Dong commune - Gia Lam district (30 samples) and Trung Mau commune - Gia Lam district (47 samples) in Northern Vietnam. Those cattle belong to four breeds (Holstein Friesian, Red Sindhi Hybrid, Belgian blue, Vietnamese). The serum neutralization test was performed with Gunma8701 strain, genotype G6P[1] and Shimane9501 strain, genotype G10P[11] on Monkey African Green foetal kidney cell (MA104).

From December 2016 to July 2017, 37 fecal samples were collected from less than 9-month old calves with diarrhea from three districts: Kim Dong district (2 samples), Ba Vi district (34 samples), and Gia Lam district (1 sample). Fecal samples were tested, using a commercial immuno-chromatographic kit, Dipstick ROTA (Eiken Chemical, Eiken, Japan).

**Results:** Neutralization test results showed 90% (105 samples), and 69% (81 samples) of samples were positive against Shimane9501 strain and Gunma8701 strain, respectively. The antibody positive samples were found in all 3 regions; in which, Trung Mau has the highest percentage of anti-Shimane9501 strain samples (96% of samples), while Phu Dong has the highest percentage of anti-Gunma8701 strain samples (80% of samples).

The antibody positive cattle against both strains appeared more common in older age group, with the oldest group of more than 3 years old cattle has the largest proportion, 100% for anti-Shimane9501 strain and 94% for anti-Gunma8701 strain.

The Rotavirus detection results showed that 27% of fecal samples (10 of 37 samples) were positive. One specimen (14%) was positive in calves less than 1 month old, 7 specimens (30%) were positive in calves from 1 to 3 months old, and 2 specimens (29%) were positive in calves more than 3 months old.

**Conclusions:** The result from the BRVs detection study showed that BRVs are prevalent in Northern Vietnam. On the other hand, in our serological survey suggested that G6 and G10 BRVs are prevalent. Since Rotaviruses are widely varied in serotype, and G6, G8 and G10 are the most prevalent in the world, further serological studies with G8 strain and genotyping of viruses are needed in order to clarify the diversity of the prevalence in Vietnam.

**Keywords:** Rotavirus, Bovine, Dipstick Rota, Gunma8701, Shimane9501, MA-104, Neutralization

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VR-P15

### Phylogenetic analysis of glycoprotein gene of bovine respiratory syncytial virus detected from Korea

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**Objectives:** Bovine respiratory syncytial virus (BRSV), a member of the genus *Pneumovirus*, from the family *Paramyxoviridae*, is a major cause of lower respiratory tract disease in young cattle, as the closely related Human respiratory syncytial virus is a common threat for young infants. In Korea, BRSV have been reported in relation to clinical respiratory diseases in cattle and serological studies against to BRSV. However, there is no report on the genomic analysis of BRSV. This study was conducted to detect the prevalent BRSV in Korea and to analyze phylogeny of BRSV circulating in Korea.

**Materials and methods:** The deep nasal swab samples were collected from 181 calves under 6 months old age of 16 farms showing respiratory diseases from October, 2016 to April, 2017. The nucleocapsid gene of BRSV was detected by quantitative real-time RT-PCR. The partial nucleotide sequence of glycoprotein gene was sequenced by direct sequencing with the PCR products. The nucleotide sequence was compared with those of BRSV of other countries. And percent identity and phylogenetic analysis of nucleotide and deduced amino acid sequence were performed using Meg-Align (DNA-STAR, USA).

**Results:** Only one strain (0.6%) of BRSV was detected from a total of 181 calves affected with respiratory disease. This strain was sequenced and nominated as KYBRSV5. In nucleotide analysis, KYBRSV5 strain showed higher degree of homology with BRSV strains detected from Netherlands (Y08717, 89.4%), Sweden (JN619446, 87.2), and Belgium (U24714, 87.2%). Phylogenetic analysis conducted here allowed us to allocate BRSV strains in within the six genetic groups proposed by (Valarcher et al, 2000) and additionally to identify those groups in the subgroups A (group III), AB (intermediary; group II, IV, V and VI, and B (group I). However, Korean KYBRSV5 strain is located on the totally different lineage outside the six genetic groups.

**Conclusions:** This study identified the nucleotide sequence of glycoprotein gene of BRSV and genetic relationship of KYBRSV5 strain with those of other countries. This information is useful for understanding for epidemiological studies and for establishing further preventive measures and vaccination programs against BRSV.

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VR-P16

### Seroepidemiologic profile of viruses associated with Bovine Respiratory Disease (BRD) in steers at feedlot entry in Brazil

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BRD is the leading cause of feedlot cattle morbidity and mortality worldwide. Animals often become sick in the first 21 days following feedlot entry. BRD is multifactorial and ultimately determined by interactions between the host, the environment, and viral and bacterial pathogens, but viruses often act as the primary agent. Under Brazilian conditions, feedlot cattle are often believed to be at risk of developing BRD due to the presence of multiple risk factors: wide age range at confinement entry, long-distance transport, need for cattle unaccustomed to feeding facilities to quickly adapt to their new environment and diets, and challenging environmental conditions. Nonetheless, the serological profile for the main BRD-causing viruses in

feedlot cattle in Brazil is largely unknown. Therefore the objective of this study was to determine the rate of seropositive cattle as well as the antibody titers for the main respiratory virus at feedlot entry. The study was carried out in the central-west and central regions in Brazil which make up for the largest share of feedlot cattle in the country. In the central-west region 1,012 animals from 23 feedlots located in three states were included. In the central region, 259 animals from 8 feedlots located in two states were evaluated. To be included, cattle had to have travelled at least 500 km prior to feedlot arrival and be deemed to be at high risk for developing BRD. A total of 1,271 blood samples were collected from an average of 41 animals per feedlot. Steers were 24 months of age in average (range = 7 to 36 months) and were not vaccinated for BRD prior to this study. Samples were tested for anti-bovine alphaherpesvirus 1 (BoHV-1), bovine parainfluenza virus 3 (BPIV-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhoea virus (BVDV) in accordance to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2015). Animals seropositive for the four viruses were identified in all of 31 feedlots evaluated. The total rate of seropositive animals for BoHV1, BPIV-3, BRSV, and BVDV were 92.2%, 86.7%, 77.7%, and 51.8%, respectively. Particularly in regards to BVDV, it was observed that a large proportion of animals were seronegative and potentially susceptible to infection from seropositive pen mates. Considering the immunosuppressant characteristic of BVDV and the frequency and intensity of risk factors present at the beginning of confinement, authors suggest this virus may be an important primary etiology of BRD in Brazilian feedlots. Most seropositive animals for BoHV-1 and BPIV-3 had high titers of neutralizing virus antibodies while seropositive animals for BRSV and BVDV showed a predominance of low antibody titers. The mean age of the animals evaluated in this study - which could be considered high in comparison to feedlots operations in the northern hemisphere (US and Europe)- may explain the high rate of seropositive animals for the viruses included in the analysis. In the one feedlot were evaluated animals were of younger age (6 to 8 months-old), the rate of seronegative animals for the four tested viruses were the highest and the antibody titers were the lowest, demonstrating greater susceptibility to infection at arrival. It is important to highlight that particularly because tested animals had never been vaccinated for BRD, these results indicate a high prevalence of natural infections in beef cattle operations in Brazil. We conclude that given the high prevalence of BRD viruses allied to the presence of additional risk factors, most Brazilian feedlots are at significant risk for developing respiratory viral infections that predispose animals to secondary bacterial infections. Measures to mitigate risks associated with the host and environmental factors should be combined with vaccination programs against the main BRD-associated viral agents to improve the health and welfare of feedlot cattle under Brazilian conditions.

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VR-P17

### Molecular epidemiological survey and phylogenetic analysis of bovine respiratory coronavirus in Japan

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Bovine respiratory disease (BRD) is one of the most commonly diagnosed causes of morbidity and mortality in the cattle industry. Co-infection with several viruses and bacteria, such as bovine respiratory syncytial virus, bovine viral diarrhoea virus, *Mycoplasma bovis*, and *Pasteurella multocida*, causes the development of BRD in cattle. Despite the use of antibiotics and several commercial vaccines against these pathogens, BRD remains the most common and costly problem in the cattle industry. Bovine coronavirus (BCoV), which is a member of the *betacoronavirus* genus, is an etiological agent of respiratory disease and diarrhoea in cattle. This virus is mainly considered important for cause of diarrhoea in calves and winter dysentery in adult cattle. However, several metagenomic analyses have suggested that this virus plays an important role as an inducer of BRD. There is no available BCoV vaccine to aid in the prevention of BRD, and a large-scale molecular-epidemiological survey has not been conducted in Japan. To assess the viral contribution of BCoV to BRD in Japan, we conducted a molecular-epidemiological survey of respiratory-diseased and healthy cattle and performed a phylogenetic analysis.

Nasal swab samples were collected from a total of 164 healthy and 158 respiratory-diseased cattle from 26 farms in the Aichi (6 farms:  $n=30$ ), Mie (3 farms:  $n=37$ ), Kagoshima (4 farms:  $n=36$ ), Kumamoto (2 farms:  $n=30$ ) and Miyazaki (11 farms:  $n=189$ ) prefectures in Japan from January 2016 to February 2017. All the farms had at least one or more respiratory-diseased subjects. After RNA extraction and reverse transcription from nasal swab samples, qPCR was performed to detect and quantify the BCoV transmembrane (M) gene. To determine the partial sequence (411bp) of the polymorphic region of BCoV spike (S) gene, sequencing of this region from qPCR-positive samples was performed. The molecular phylogenetic trees were constructed from the viral genome sequences that were determined in our study and the available sequences that we retrieved from GenBank using the neighbor-joining method with the MEGA7 software.

Twenty-four of the 158 (15.1%) cattle with respiratory symptoms were PCR positive, with the medium value of viral copies of 81.8 ( $Av.=4,701.6$  copies). On the other hand, 20 of the 164 (12.2%) healthy cattle were also PCR positive, with the medium value of viral copies of 14.8 ( $Av.=797.1$  copies). Although the infection rates were no significant differences, the viral copies of the cattle with respiratory symptoms were significantly higher than those of the healthy cattle ( $P<0.05$ , Wilcoxon signed-rank test). Based on the partial sequence of BCoV S gene, all the epidemic viruses were clustered into genotype 4 with the Japanese BCoV strain in 2004-2008 and different from available BCoV diarrhoea vaccine strain in genotype 1. The epidemic viruses were divided into two subgroups in genotype 4 and separated from the Japanese strain in 2004-2008. However, there were no regional specificities in the subgroups.

The results suggest that BCoV possibly contribute to BRD and the genotype 4 is still predominant lineage in Japan. However, the genetic distance was gradually separated from the past epidemic strains.

VR-P18

### Investigation of the occurrence of Schmallenberg virus in bovine foetus from Brazil

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Schmallenberg virus (SBV) is RNA virus, segmented, single strand, negative polarity, 90 to 100 nm diameter and enveloped. It belongs to the family Peribunyaviridae genus Orthobunyavirus. Infection is mainly reported in ruminants and transmission in animals is by insect vectors (*Culicoides* spp) and vertically. Congenital and stillborn malformations and positive PCR results for SBV have been reported in several European countries, but the occurrence and impact of SBV in Brazilian animals is unknown. The objective of this study was to validate and optimize RTqPCR for detection of SBV and investigate the presence of SBV in samples of aborted bovine foetus from Brazil. Was analyzed 245 samples of aborted bovine foetus from several Brazilian states and referred to the Biological Institute Animal Health Research and Development Center, from January 2011 to July 2017. The nucleic acids were extracted using cador Pathogen 96 QIAcube HT (QIAGEN) according to the manufacturer's manual. Reverse transcription followed by quantitative polymerase chain reaction (RTqPCR) was optimized and validated using GoTaq Probe 1-Step RTqPCR System kit (PROMEGA) and primers and probe targeting the gene S of SBV genome. For positive control gBlocks Gene Fragments (Integrated DNA Technologies-IDT) of segment S (length 823 bp) encoding nucleocapsid protein (strain BH80 /114 GenBank HE649914.1) were designed. This fragment was cloned into pTZ57R plasmid (Thermo Fisher Scientific) after digestion with EcoRI / BamHI restriction enzymes, and introduced into *E. coli* (strain JM109). The recombinant plasmid was confirmed by PCR and sequencing using M13 universal primers, extracted with GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific) and submitted to MEGAshortscript T7 Transcription Kit (Thermo Fisher Scientific). RNA was quantified by QuantiFluor RNA System (PROMEGA). All samples were amplified in triplicate in RTqPCR. It was possible to optimize and validate the RTqPCR, that presented analytical sensitivity of 1 copies of RNA per microliter (Cq 38, Slope -3,359 and Efficiency 1,985). Of the samples analyzed, none were positive for SBV (0/245). Brazil is a highlight in bovine farming with large effective herds. Despite having favorable conditions for the dissemination of SBV (presence of vector, favorable climate for its dissemination and great bovine herd), no SBV was detected in the samples analyzed, possibly indicating the non-occurrence of aborted bovine foetus from Brazil, which is of fundamental importance has seen the damage that the disease causes both direct losses due to abortion and indirect losses in milk production as well as from international health barriers. Also, a rapid, sensitive and specific diagnostic technique for SBV surveillance in Brazil was established, making it suitable for certifying herds in relation to SBV, as well as for the country to meet the requirements of international markets.



VR-P19

### Endometrial carcinoma in cows

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The bovine papilloma virus-1 and 2 (BPV-1,2) DNA have been found in uterine lavage cows, indicating that the virus infects the feminine reproductive tract, but studies need to study its pathogenicity. It was investigated the BPV-1, 2 infection and their association to macroscopic and microscopic lesions in tissues of the reproductive tract of adult cows (dairy cattle and cross bred). Tissue samples from 80 animals (ovaries, tubas, horns, body and cervix) and total blood in EDTA anticoagulant were collected in abattoirs in São Paulo state, Brazil. Materials for histopathology were fixed in 10% buffered formalin; total blood and tissue samples for nested-PCR were kept frozen -20 Celsius until analysis. Cervical swabs collected for cytological study were fixed into glass slides by polyethylene glycol spray. Different diagnostic techniques were performed: macroscopy and histology (hematoxylin and eosin staining - HE); immunohistochemistry (IHC) for oncogenic p16 and Ki-67 markers, and estrogen receptors (ER); cytological analysis of smear cervix by the Papanicolaou technique; molecular biology by nested-PCR for the L1 gene of BPV viral capsid using the primers FAP59/ FAP64, and Delta Epsilon F/ Delta Epsilon R. The macroscopy showed in 80 animals 5.0% metritis and 5.0% endometritis; in histopathology (HE) 25.0% samples showed non-specific endometritis. The cytology found atypia in 25.0% of (20/80) samples: karyomegaly, binucleation and multinucleation. IHC and nested-PCR were performed in order to check for association between lesions and BPV infection. The nested-PCR and IHC p-16 were negative in all samples. 20 animals with cellular atypia (Papanicolaou) were analyzed by HE confirming unspecific endometritis; ki-67 and ER IHQ detected positivity in 17.5% (14/80) samples by both biomarkers, identifying endometrial adenocarcinoma. It was demonstrated that, instead BPV-1, 2 the estrogen was the risk factor for this neoplasia.

IM-P01

### Retrospective evaluation of clinical outcomes among cattle evaluated with a computer-aided lung auscultation system at the time of bovine respiratory disease diagnosis

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**Objectives:** The objective of this observational study was to assess the value of combining computer-aided lung auscultation (CALA) technology (Whisper<sup>®</sup> Veterinary Stethoscope) and rectal temperature on multiple clinical outcomes, in aggregate and across different weight classes, to assess the value in the refinement of the bovine respiratory disease (BRD) case definition.

**Materials and Methods:** Feedlot BRD treatment records comprising 85,160 crossbred beef calves with CALA readings from 22 US feedlots in Kansas, Nebraska, and Colorado (collected from 2012 to 2016) were utilized in this study. All records were originally hand-entered into animal health software systems and downloaded to a commercial spreadsheet program. All calves in this study would have been recognized as potentially sick and pulled out of their home pen, then brought to a chute for restraint and further evaluation including rectal temperature and CALA.

The CALA system records sounds from the thorax of a calf and processes them with a proprietary machine-learning algorithm. A score from 1 to 5 is generated which reflects the severity of BRD (1=minimal lung pathology; 5=severely compromised lung tissue) for the individual calf. For the purposes of this study, the CALA scores were categorized into CALA = 1 or CALA = > 2. Likewise, rectal temperature (RT) was collected on all calves and categorized as < 40 °C or > 40 °C.

We used two general statistical modeling approaches, both with individual animal as the unit of analysis. In model one, the effects of CALA score (i.e. 1 or > 2) and rectal temperature category (i.e. < 40 °C or > 40 °C) were combined into 4 groups: 1) CALA=1, RT < 40 °C; 2) CALA=1, RT > 40 °C; 3) CALA > 2, RT < 40 °C; and 4) CALA > 2, RT > 40 °C. These groups were then compared against one another but also to cattle observed but not treated for BRD (respiratory observes). These comparisons were evaluated within a generalized linear mixed model with a random effects structure to account for lack of independence within feedlot, year, and the incoming weight class of the cattle. For the purpose of this study, incoming weight class data was categorized into 45.4 kg increments beginning at 181.4 kg up to 408.2 kg.

Model two evaluated the CALA and temperature categories by weight classes (described above), with a random effects structure to account for feedlot, year, and antimicrobial category used at first BRD treatment.

In both models, gender (i.e. male/female) was utilized as a covariate, and the primary outcome was BRD case-fatality. An alpha of 0.05 was considered significant across all analyses and was adjusted for multiple comparisons.

**Results:** In the first set of models, significant differences



( $p < 0.05$ ) in BRD case-fatality were observed across all CALA+RT groups: CALA=1, RT < 40 °C (3.62%; 95% confidence interval [95%CI]; 3.05%, 4.28%); CALA=1, RT > 40 °C (7.60%, 95%CI; 6.61%, 8.73%); CALA > 2, RT < 40 °C (5.53%, 95%CI; 4.85%, 6.29%); and CALA > 2, RT > 40 °C (10.96%, 95%CI; 9.74%, 12.31%). Similarly, the risk of BRD case-fatality was significantly increased ( $p < 0.05$ ) in each of these four groups as compared to the respiratory observes (2.31%, 95%CI; 1.62%, 3.29%).

The second modelling approach demonstrated that BRD case-fatality was significantly associated ( $p < 0.05$ ) with all three main effects: CALA score (CALA=1, 5.65%; CALA > 2, 8.02%), RT (RT < 40 °C, 4.77%; RT > 40 °C, 9.43%), and incoming weight class (> 181.4kg to < 226.8 kg, 9.66%; > 226.8 kg to < 272.2 kg, 7.41%; > 272.2 kg to < 317.5 kg, 6.27%; > 317.5 kg to < 362.3 kg, 5.11%; > 362.3 kg to < 408.2 kg, 5.07%). However, none of the interaction terms (i.e. CALA x RT, CALA x incoming weight class, RT x incoming weight class, and CALA x RT x incoming weight class) were statistically significant ( $p > 0.05$ ).

**Conclusions:** Based on the outcomes of this retrospective cohort study, both RT and CALA classifications were shown to detect differences in the risk of BRD case-fatality within this population. However, by combining both pieces of information (i.e. CALA + RT), further differentiation in the risk of BRD case-fatality may be possible. By utilizing both pieces of information, additional management opportunities may arise for veterinarians and producers regarding improved BRD diagnostic accuracy and enhanced strategic BRD therapy decisions.

IM-P02

### Clinical case study of four calves with wheezing

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An overview of report on four calves with wheezing. These animals were diagnosed disorder of respiratory disease from the clinical presentation and all cases have poor appetite.

First case, Japanese black cattle in approved mild stridor that endoscopy revealed extensive inflammatory image in throat head. This case was cured by antibiotics and corticosteroid administration. Second case, Holstein calf revealed polyps in the small intestine large side opening tear cartilage. Autopsy tumor left arytenoid tear cartilage at the base on either side, were found in histological nodule bacteria clumps. Third case, Japanese brown calves of severe to paralysis, dyspnea with tracheostomy tube by tracheostomy. Dyspnea and wheezing are temporarily good, but then have died due to tracheal secretions due to airway obstruction. Fourth case, Difficulty in breathing are severe wheezing was present in the calves, pneumonia was diagnosed, has died. Endoscopy examination showed severe inflammation and structures arytenoid tear cartilage at the base. Bacteria test results from *a-Streptococcus* spp and *Escherichia coli* was observed. However as the bacteria was unclear.

Discussion, these documented cases with mild to severe

wheezing, throat head inflammation was observed by endoscopy. All patients showed organizational structures became severe inflammation and tumor were killed. Breathing difficulties due to increased bacterial infections due to mucosal damage and mass by the likely cause. Further increase the patient, must be considered.

**Key word:** calves, dyspnea, endoscopy, respiratory disease, wheezing

IM-P03

### Potential application of pulse oximetry for diagnosing bovine respiratory disease

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**Objectives:** In farm animals, respiratory diseases were found to account for 40% of all bovine diseases and were often associated with poor prognoses. Auscultation, imaging, and arterial blood gas analysis to identify hypoxemia are recommended for making a pathological diagnosis. However, pulmonary imaging of adult cattle is difficult, and arterial blood gas analysis requires special sampling techniques and rapid measurements, making it impractical for routine use. In humans and small animals, pulse oximetry is commonly used to measure percutaneous arterial oxygen saturation (SpO<sub>2</sub>). Therefore, we investigated the application of pulse oximetry in cattle.

**Materials and methods:** Measurement methods and location

Three clinically healthy Japanese Black fattening cattle (11–18 months old) were included. A pulse oximeter (Rad-5V, Masimo Japan, Tokyo, Japan) was used, and SpO<sub>2</sub> was measured by attaching a clip-on probe to the ear apex, tongue apex, and labia. A reflectance probe was attached to the base of the tail.

Comparison with arterial oxygen saturation

A total of 25 cattle, including 15 clinically healthy Japanese Black fattening cattle (11–28 months old), nine cattle exhibiting respiratory symptoms (10–32 months old), and one cow exhibiting heart murmur (19 months old), were included. Arterial blood was sampled from the ear artery, and arterial oxygen saturation (SaO<sub>2</sub>) was immediately measured using a portable blood analyzer (i-STAT CG8+, Fuso Pharmaceutical Industries, Ltd, Osaka, Japan). SaO<sub>2</sub> was simultaneously compared to SpO<sub>2</sub> obtained via the tail-mounted pulse oximeter.

**Results:**

1) We were unable to measure SpO<sub>2</sub> from the ear apex, tongue apex, or labia using the clip-on probe. However, a valid measurement (99–100%) was obtained from the base of the tail using the reflectance probe.

2) There was a significant positive correlation between SaO<sub>2</sub> obtained from the arterial blood taken from the ear artery and SpO<sub>2</sub> obtained blood taken from the base of the tail ( $r = 0.95$ ,  $p < 0.01$ ).

**Conclusions:** There is a previous report on pulse oximeter use to measure SpO<sub>2</sub> in newborn calves with hypoxemia; however, there are almost no studies describing the application of pulse oximetry in adult cows or for diagnosing bovine respiratory dis-



ease. Our results revealed that it is possible to measure SpO<sub>2</sub> in adult cattle using a reflectance probe on the base of the tail. Further, the measured values reflected SaO<sub>2</sub>. Moreover, this measurement method is easy to perform and non-invasive. Thus, our results suggest that this technique is feasible for continuous pathological monitoring and determining the prognosis of respiratory diseases.

IM-P04

### Effect of Fecal microbiota transplantation to refractory diarrhea in calf

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**Introduction:** Fecal microbiota transplantation (FMT), where healthy feces are transferred to a sick patient, shows remarkable curative effects in cases of recurrent *Clostridium difficile* infection in human medicine. Since then, it has been widely applied to various types of refractory diarrhea. It is suspected that the pathology of refractory diarrhea in cattle is similar to human pathology. Here, we examine the effects of FMT on refractory diarrhea in the calf.

#### Materials and methods:

- 1) Examination period and number of cases: 03/03/2015 to 06/21/2017; eight heads, nine cases
- 2) Recipient cattle: seven Holstein cows (32–137 days old) and one Wagyu cow (41 days old). General diarrhea symptoms remain after treatment, and the cattle lack the ability to form stable fecal condition.
- 3) Donor cattle: six Holstein calves (14–451 days old) and two Wagyu calves (167 days to 5 years old) without parasites, pathogenic *Escherichia coli*, BVD, Rotavirus or coronavirus, raised in a salmonella- and johne disease-free farm.
- 4) FMT method: After mixing 100 g of donor feces with saline, filter the mixture through cotton gauze. Insert catheter into recipient cow rectum and pour in all filtered liquid.
- 5) Gram stain of smeared feces (cases 3 and 4, and 6 through 9): dilute 1 g of feces from donor, and 1 g of feces from recipient, pre- and post-FMT, with 2 mL of distilled water. Filter the mixture, then smear 10 µL of filtered liquid on a slide. Gram stain and examine by microscopy. Calculate the area of Gram-positive and -negative staining, using Image J (Wayne Rasband(NIH)). Compare the area of pre- and post-FMT Gram-positive and -negative staining, using the chi-square test.
- 6) Judgment of cure rate: classify recipient fecal score (microscopy, culture, and sensitivity, MCS) into grades 1–4. Define a case as cured when it improves to >3 on the MCS scoring and retains that score for >5 days.

**Result:** Cure rate was 88.9% (8/9). The area of Gram-positive and -negative staining in recipient feces was significantly changed, and largely approximated that of the donor feces. It remained stable 5 days after FMT in three of five cases.

**Conclusion:** FMT resulted in a high cure rate in cases of refractory diarrhea in the calf, indicating that FMT provided stable intestinal flora. As a result, the intestinal metabolism and overall immune system were able to recover. FMT is an easy operation; further, it is cheap, appears to be harmless, and provides a stable treatment result, thereby reducing the duration of antibiotic treatment and reducing the emergence of drug-resistant bacteria. In the future, we will evaluate standards for donor selection, and will research the effects of FMT on the intestinal flora, metabolism, and immune system.

IM-P05

### A case of Hepatic fibrosis accompanied with biliary abnormalities in Holstein calf

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**Introduction:** The most common causes of liver fibrosis in ruminants are congested heart failure, toxicity (alkaloids, aflatoxins, and copper intoxication), and parasite infection. In cows, biliary disease per se is comparatively rare and, as such, liver fibrosis caused by biliary disease is very uncommon. We examined and treated a case of liver fibrosis accompanied with intra and extrahepatic biliary abnormalities and have summarized our findings in this case report.

**Case:** A 3-month-old female Holstein calf was raised in a common farm in Kanagawa prefecture. She had anorexia, fever and diarrhea. No improvement in the symptoms was observed despite systemic administration of antibiotics and application of anti-inflammatory drugs by first veterinary consultation. Therefore, the calf was admitted to Azabu University Veterinary Teaching Hospital for further diagnosis and treatment. On admission, the calf weighed 113 kg and had a body temperature of 39.9°C. Though her general condition was good, there was some abdominal distension. Moreover, a solid cartilaginous tissue was palpated to the right of the xiphoid process.

**Progress:** Blood tests at admission showed high values for red blood count (1,661 million/µl) and white blood count (18,500/µl), and a markedly high value for gamma-glutamyl transferase activity (2,498 IU/l). A diagnostic laparotomy was performed immediately after admission and a seriously hardened liver and mildly enlarged gall bladder were confirmed. The liver tissue was examined by biopsy. Marked liver fibrosis associated with serious pseudo-bile duct proliferation was diagnosed. After the surgery, the symptoms worsened gradually, and on day 7 of hospitalization, the calf lost her appetite and was given a poor prognosis.

The prognosis was poor and thus, the heifer was euthanized on day 8. The calf was offered for pathological autopsy, and the autopsy revealed that the entire liver had turned white and had seriously hardened. Cholangiography of the excised liver revealed that two extrahepatic bile ducts opened into the duodenum. The bile duct entered the liver at a site 12 cm from the gall bladder. At 5.5 cm from there where the main bile duct had entered, a sinus-like structure 2.5 cm in diameter had formed. Histologically, a marked amount of diffuse connective tissue was present in the liver. In addition, between the connective





tissue, various large and small pseudo-bile ducts had formed, hepatocytes that had transformed to cholangioles were frequently noted, and pseudolobules were also observed. Moreover, in areas of mild fibrosis, mild neutrophil invasion was observed, and some areas had abscessed. Long gram-negative rods were noted in liver tissues from the periphery. Parasites such as *Fasciola hepatica* were not detected.

**Discussion:** The hepatic lesions observed in this case, i.e., marked diffuse liver fibrosis, as well as the abnormal course and shape of the intra and extrahepatic bile ducts and extensive small bile duct formation, are characteristic. Similar diseases have been reported as congenital liver fibrosis due to bile duct dysplasia in the embryonic stage in cows. The histological findings of this case were consistent with those of previously reported cases in many respects. However, there are also differences, such as age of onset, abnormality of the major intra and extrahepatic bile ducts, and the presence of inflammation. Further investigations are needed in the future to understand the confirmed diagnosis and onset of pathology.

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IM-P06

### Effect of Prevention about Ivermectin on *Theileria orientalis* Infection in Grazing Cows

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**Objective:** This research is to identify the effects of grazing cows on hemolysis and *Theileria orientalis* infection and using pour-on ivermectin as a preventive method on these hemolytic effects and *T. orientalis* infection.

**Materials & Methods:** Grazing cattle farms in the Republic of Korea (ROK) was selected for this research to identify the effects of grazing on hemolysis and *T. orientalis* infection. Whole blood was collected from 48 cattle (group A) in the spring season of 2014 and 2015 (before grazing) and from 38 housing cattle (group B) and 28 grazing cattle (group C) in the summer season of 2014 and 2015. Hematocrit (Hct) was examined by Procyte Dx Hematology Analyzer and compared by each group. Also, *T. orientalis* was detected by polymerase chain reaction (PCR) and the infection rate of each group was compared. After identifying the effects of grazing, ivermectin (IMEC-POURON, SF Co. Ltd., Korea) was treated as a preventive method for the hemolytic effects and *T.* infection in grazing cattle. In 2016 and 2017, 68 cattle were selected and divided into 2 groups. Group D (17 cattle) was not treated with ivermectin and group E (51 cattle) was treated before grazing. Whole blood was collected to compare Hct and *T. orientalis* infection rate before and after grazing. Statistical analysis was performed using the SPSS 23.0 software package (SPSS, Chicago, Illinois, USA).

**Results:** Hematocrit and *T. orientalis* infection rate was compared; group A to group B, and group A to group C. The average of Hct (%) in group A, group B and group C was 32.78, 32.47, and 30.46, and *T. orientalis* infection rate (%) in group A, group B and group C was 2.08, 3.57 and 15.79, respectively. We found statistical significance in the decrease of the average rate of Hct in group A to group C ( $p < 0.01$ ) and the infection rate of *T. orientalis* in group A to group C showed 9 times more than that in group A to group B. The decreasing rate of average Hct and the increasing rate of *T. orientalis* before and after grazing were compared between group D and group E to identify the preventive effects of ivermectin on hemolysis and *T. orientalis* infection. The decreasing rate of average of Hct before grazing and after grazing in group D and group E was 10.21% (3.51/34.38, 34.38 to 30.87), and 5.48% (1.75/31.93, 31.93 to 30.18), respectively ( $p < 0.01$ ). The increasing rate of *T. orientalis* infection rate in group D and group E was 300.17% (17.65/5.88, 5.88 to 23.53) and 17.65% (1.96/5.88, 5.88 to 7.84), respectively.

**Conclusion:** Raising cattle through grazing is known to be good for animal welfare. However, the harmful effects on hemolysis and *T. orientalis* infection were identified in grazed cows from this research. This research proved that ivermectin is one of the effective ways to prevent these effects. Also, other effective ways and their effects on cattle health in grazed cows should be further researched in terms of animal welfare.

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IM-P07

### Relationship between Thymus Thickness and Physical Features in the Healthy Holstein Calves

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The aim of this study was to clarify the relationship between the thymus and physique in the calves. Thymus thickness and body mass index [BMI, body weight (kg) / body height (meter)<sup>2</sup>] were analyzed as physical features. Sixty-three clinically healthy Holstein calves from birth to 12 weeks old were used in this investigation, and the calves between 3 to 12 weeks of age were divided into two groups; the calves with lower body weight than the Japanese Feeding Standard for dairy cattle (Underdeveloped Group, N=30), and calves with normal body weight (Control Group, N=20). But we couldn't find lower body weight animals in the thirteen calves between birth to 3 weeks of age (Neonatal Group, N=13). Peripheral leukocyte population and RNA levels of immune factors in the peripheral blood mononuclear cells (PBMC), and thymus thickness were analyzed. The average of thymus thickness in the Underdeveloped Group was lower than that in the Control Group ( $P < 0.05$ ). Positive correlations of the thymus thickness with BMI, numbers of CD4<sup>+</sup>CD45R<sup>-</sup> and numbers of CD8<sup>+</sup>CD45R<sup>-</sup> cells were found ( $r = 0.553$ ,  $r = 0.427$ , and  $r = 0.422$ , respectively;  $P < 0.05$ ). In the Underdeveloped Group, IL-17A RNA level was significantly higher than that in the Control Group. These results suggested that the thymus development is associated with increase in BMI and development of immune system.



IM-P08

### Hypocalcemia and acetonemia are highly correlated in dairy cows until the fourth lactation, then the table turns

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Of all diseases in adult dairy cattle, 75% occurs within the first three weeks post partum. Hypocalcemia and acetonemia are two metabolic diseases which can occur after parturition and are associated with other post partum diseases like metritis, endometritis, ruminal acidosis, laminitis, mastitis and abomasal displacements. To study the potential correlation between calcium concentration and ketone body concentration, we took blood samples from 445 mainly Holstein Friesian cows on eight commercial dairy farms in The Netherlands. For the calcium concentration the blood sample was taken within 48 hours after parturition. Ketone body concentration was determined on site with an hand held ketone body concentration meter. Ketone body concentration was measured twice per cow, the first sample was taken between day 3 and 9 post partum and the second sample was taken between day 9 and day 16 post partum. Of every cow, the highest ketone body concentration was used for analysis. First of all, obviously, calcium concentrations were strongly correlated with the parity of the cow. Lower calcium concentrations resulted in higher ketone body concentrations indicating a higher risk for acetonemia after suffering hypocalcemia. The most interesting part was the effect of parity on the correlation between calcium concentration and ketone body concentration. Up to the fourth lactation, ketone body concentrations are negatively correlated with calcium concentrations: lower calcium concentrations lead to higher ketone body concentrations. From the fifth lactation, ketone body concentrations are slightly positive correlated with calcium concentration. Combined with lower calcium concentrations per higher parity, older cows suffer less from acetonemia as a consequence of hypocalcemia. Of course, selection is one of the most important reasons to explain this effect: not all cows become old enough to finish fourth lactation and higher. These results provide important insights in the metabolism of ageing dairy cows.

IM-P09

### Sense of sensors: several behavioral parameters in the dry period are associated with calcium concentrations within 48h post partum

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lands

Despite all efforts of farmers, feed advisors and veterinarians, hypocalcemia, unfortunately, is still common in modern dairy practice. Nowadays, innovations like the use of sensor technology, make it possible to monitor dairy cattle 24/7. The use of sensor technology can possibly open new doors to understand the origin of hypocalcemia post partum or to see the different kinds of behavioral signs between healthy cows, cows with subclinical hypocalcemia and cows which suffer from clinical hypocalcemia. In the multiannual study "Sense of Sensors in Transition Management" we study behavioral parameters using two sensors (Nedap, Groenlo, The Netherlands) attached to the neck collar and attached to one of the front legs of the cows respectively. The Nedap Smarttag Neck measures eating time, number of eating bouts, duration of eating bouts, ruminating time, number of ruminating bouts and duration of rumination bouts. The Nedap Smarttag Leg measures number of steps, lying time, number of lying bouts and duration of lying bouts. On eight commercial dairy farms in The Netherlands, we have equipped all cows with both sensors. From more than 600 dairy cows, we were able to take a blood sample within 48 hours after parturition to measure the calcium concentration. Several behavioral parameters are correlated with calcium concentrations already weeks before parturition, indicating that there is more than just an inadequate ration in the dry period leading to hypocalcemia.

IM-P10

### Effects of different dosages of propylene glycol in cows according to calving

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**Objectives:** The application of propylene glycol (PG) supporting the cows energy metabolism during periods of negative energy balance has already been used since the 1950s. Although PG has been used over decades the literature provides controversial information about dosages, application methods and differences in efficacy of PG at different periods of lactation. The aim of the study was to investigate the metabolic effects in dairy cows at different times around the transition period giving different doses of PG.

We hypothesized that the temporary insulin resistance during the transition period may cause significant differences in the efficacy of PG at the four sampling periods (dry cows, close up cows, fresh cows and lactating cows) and that in some cases this effect will be dose dependent (100 ml, 300 ml and 500 ml, per os, one application per day).

**Material and Methods:** A convenience sample (animals which were in a suitable stage of pregnancy at that period before starting their 2nd to 5th lactation) of 7 pregnant cows was se-



lected for the study. The cows entered the study according to their pregnancy status. Eighty four sampling sets were generated by studying the 7 multiparous Holstein cows repeatedly at 4 sampling periods of 3 days length (dry cows: days 40, 39 and 38 *ante partum*; close up cows: days 10, 9 and 8 *ante partum*; fresh cows: days 3, 4 and 5 *post-partum*; lactating cows: days 38, 39 and 40 *post-partum*). On each of these days three hours after morning feeding propylene glycol was drenched in different dosages of 100, 300 or 500 ml once per day (cross over study). The different doses were applied in an alternating order (Latin square). Blood samples were taken before, every 30 min up to four hours, after six hours and twelve hours after PG application. Following parameters have been measured: insulin, non-esterified fatty acids (NEFA), betahydroxybutyrate (BHB), bilirubin, cholesterol, potassium, aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH). Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was calculated.

**Results:** It was found that glucose, insulin, NEFA, BHB, bilirubin and potassium concentrations were influenced differently by the three defined dosages of propylene glycol at the four different sampling periods. Whereas RQUICKI, cholesterol, AST and GLDH did not differ between the sampling periods and treatments. The major results of the study are that the effect of PG is dose-dependent and that the effect of PG is depending on the time of application according to calving. It can be concluded that in fresh cows higher dosages are necessary to provoke similar effects in comparison to dry, close up and lactating cows. Although the present study did not compare to PG used as topdressing or mixed in the TMR there is strong evidence that the bolus application of a certain volume PG (depending on lactation status) is necessary to provoke an insulin peak which results in the major effect on the metabolism.

**Conclusions:** Summarizing the study showed that for the major metabolic parameters the impact of PG application is dose dependent and that the effect of PG is also dependent on time to or from calving. Fresh cows require higher dosages of PG to provoke similar effects in comparison to dry, close up or lactating cows. These findings might explain some of the conflicting reports about efficacy of PG application volume of 500 ml PG for post calving cows seems the most appropriate whereas during dry period or later in lactation dosage of 100 or 300 ml are sufficient. The required dosage of PG has to be given as bolus to generate the desired effects on metabolism.

IM-P11

### Serum potassium disorders in hospitalized calves under 6 months of age

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**Objectives:** The main objective of this retrospective study was to determine the serum potassium concentration disorders in

hospitalized calves less than 6 months of age and its association with different clinical entities.

**Material and Methods:** A ten-year retrospective study was conducted at the Large Animal Hospital, Faculty of Veterinary Medicine and Zootechny of the National University of Colombia. The study involved all the medical records of calves less than 6 months of age that were admitted to the hospital during that period. The inclusion criteria in the study were that the calf had to be less than 6 months of age and serum potassium levels were measured before any therapeutic intervention. On each medical record signalment, main complaint, working diagnosis, biochemical profile, hemogram and working diagnosis were collected. In all the calves that met the inclusion criteria, a venous blood sample was collected into serum test tube during the first 5 years of the study and K<sup>+</sup> was measured using a chemical analyzer (Vitros® 350 Chemistry System (Ortho-Clinical Diagnostics-Johnson & Johnson company ®). In the last 5 years of the study, the venous blood sample was collected into Na-heparin blood gas syringe and analyzed for blood gases, electrolytes including K<sup>+</sup>, using ion-selective electrode (EPOC® BGE- Test Cards).

**Results:** Seventy of 92 calves admitted to the Large Animal Hospital met the inclusion criteria, 68.57% (n=48) of these calves were considered neonates (<30 days old) and 31.43% (n=22) were between 31 days and 6 months of age. The calves were of a great array of dairy and beef cattle breeds. 65.71% of calves (n=46) were normokalemic (reference values: 3.9-5.2 mmol/L), 21.43% (n=15) were hypokalemic (<3.9 mmol/L) and 12.86% (n=9) were hyperkalemic (>5.2 mmol/L). The potassium concentration range in the hypokalemic patients varied from a lower serum concentration value of 2.7 mmol/L to 3.8 mmol/L. The serum potassium concentration levels in the hyperkalemic calves varied from a lower value of 5.3 mmol/L to 9.5 mmol/L. Fifty percent of the hypokalemic calves were mainly associated with respiratory diseases, followed by 20% of the calves with digestive diseases and 20% with sepsis; the remaining ones included muscular problems and very few with undetermined diagnosis. Hyperkalemia was observed in 44.44% with sepsis, in 22.22% with respiratory diseases, in a same percentage (22.22%) with digestive disease and in 11.11% with unknown diagnosis.

**Conclusions:** Serum potassium concentration disorders are commonly observed in hospitalized calves. These serum potassium disorders are mainly associated with digestive, respiratory and septic diseases.

IM-P12

### Right-side displacement of the abomasum in an adult zebu bull

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**Objectives:** Left displaced abomasum (LDA), right displaced abomasum (RDA) and abomasal volvulus (AV) are common conditions in high producing cows and constitute the most common reason for abdominal surgery in dairy cattle. However, there are a few case reports of zebu cattle with LDA, RDA, or



AV. The aetiology includes abomasal hypotonia and aggregation of gas related with gastro intestinal atony, concurrent diseases, early post-partum or abrupt changes in diet. Clinical signs for RDA include moderate to severe abdominal distention, tachycardia and a ping detectable over a large area on the right side. The aim of this paper is to report a case of right displacement abomasum in an adult gir bull in Colombia.

**Materials and methods:** A 3 1/2-year-old gir breed bull, 800 kg of body weight, was presented with a history of 2 days of apathy, anorexy and signs of abdominal pain after having been transported for 10 hours. The bull was treated with oral fluids, oral mineral oil and flunixin meglumine. The diet includes concentrate, grass silage and hay. At the clinical examination the animal showed lethargy, dehydration, tachycardia, tachypnea, ruminal atony, a ping area (simultaneous auscultation and percussion) on the right flank including the last intercostal spaces and a distended gas filled viscus palpable during rectal examination on the abdominal right side. Fibrinogen, AST, creatinine, glucose and lactate were increased, whereas hyponatremia, hypocalcemia and mild metabolic acidosis were observed. Based on this findings RDA was rolled out as diagnosis and a laparotomy via right paralumbar fossa, decompression of the abomasum, repositioning, omentopexy and pyloropexy were carried out. Treatment consisted of parental fluid therapy, flunixin meglumine, calcium gluconate, oxytetracycline, ranitidine and oral ruminal fluid.

**Results:** The bull has improved progressively from 2 days of surgery and treatment, with return of appetite and rumen motility. The patient was discharged on day 10.

**Conclusions:** Right displaced abomasum is a condition neither frequently reported in bulls nor in zebu cattle. However, it is important to characterize the clinical and pathological findings as well as the therapy and the evolution of the sporadic cases. This case revealed that a variety of risk factors like stress due to transport, change of diet and environment could be associated with the occurrence of RDA in zebu bulls. As in cows, it would be assumed that all factors that lead to a decreased rumen volume and inhibition of abomasal motility could predispose to abomasal displacement. An early recognition of the disease is necessary because in contrast to LDA, the course of RDA is severe and rapid, showing more signs of a general impaired condition.

vitis caused by *M. agalactiae* in a young goat.

**Material and methods:** A three-month-old female Saanen goat weighting 5.8 kg was presented in the Clinic for Bovines and Small Ruminants, School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil, showing corneal opacity in left eye. During physical examination, animal presented heart rate of 200 bpm; respiratory rate of 150 rmpm and rectal temperature of 39.2°C. The young goat was from a caprine arthritis encephalitis virus positive farm and cases of respiratory disease and arthritis were recorded in other animals, but keratoconjunctivitis was detected in this young goat, only. Ocular swabs were collected and one immediately conditioned in transport media for mycoplasmas and the other conditioned in Stuart transport medium. Both were kept in ice during the transport to the laboratory. The detection of aerobic bacteria was performed by plating sample in sheep blood agar and McConkey agar. Biochemical tests were used to confirm the species presented in sample. Clinical sample was also analyzed for DNA detection for small ruminant mycoplasmas. Conventional PCR was used to detect *Mollicutes* as a screening method. If positive, sample was analyzed again to detect important mycoplasma species for small ruminants such as *M. agalactiae*, *M. mycoides* subsp. *capri*, and *M. conjunctivae*.

**Results:** No aerobic bacteria were detected. The presence of *Mollicutes* microorganisms was confirmed. After specie-specific conventional PCR, only *M. agalactiae* was detected. Afterwards, Oxytetracycline (20 mg/kg; IM) was administered during two days, and tylosin (10 mg/kg; IM) was administered during 13 days. After the period of treatment, animal recovered from keratoconjunctivitis, and *M. agalactiae* was no longer detected.

**Conclusion:** The detection of *M. agalactiae* in a keratoconjunctivitis case alert us to improve sanitary management to prevent the spread of this microorganism and cases of contagious agalactia.

IM-P13

### Keratoconjunctivitis by *Mycoplasma agalactiae* in a young goat – case report

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**Introduction:** *Mycoplasma* spp. is part of the *Mollicutes* Class and they are important pathogens for ruminants. *M. agalactiae* is responsible for contagious agalactia, a syndrome characterized by mastitis, agalactia, keratoconjunctivitis, and pneumonia.

**Objectives:** The present abstract describes a keratoconjuncti-



NU-P01

### Non-invasive blood calcium measurements using electrocardiography in postpartum Jersey cows

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Jersey cows easily develop milk fever and have difficulties with recovery. Clinical symptoms are not always immediately obvious despite low blood calcium (Ca) levels after parturition. Therefore, early detection and treatment of hypocalcemia is particularly important for Jersey cows because delayed treatment can result in various complications. We previously developed a non-invasive method of measuring blood Ca levels in Holstein cows on-site using electrocardiographic (ECG) waves and calving number, based upon a close positive correlation between blood Ca concentrations and the inverse of the ST peak interval corrected according to the Bazett formula (STc): ST peak interval/SS peak interval<sup>0.5</sup>. The present study aimed to estimate blood Ca concentrations in 69 Jersey cows within three days after parturition using this method.

The ECG findings from these cows were recorded for 30 seconds using a base-apex lead and STc intervals were measured. Calving number, the condition of the cows, rumen movement, pupillary reflex, ear temperature, skin temperature, limb temperature, and other items were also investigated to diagnose milk fever. Correlations between blood Ca concentrations and these items were investigated using multiple regression analysis and quantification theory type 1.

The blood Ca concentrations of all 69 cows ranged from 2.4 to 15.2 mg/dL. Twelve cows were unable to stand. Another 12 cows were mobile despite having blood Ca concentrations < 6.0 mg/dL. As with Holstein cows, blood Ca concentrations closely correlated with STc<sup>-1</sup> in the Jersey cows ( $r = 0.79$ ,  $p < 0.001$ ). The correlation coefficient further increased when calving number was added as a variable ( $r = 0.80$ ,  $p < 0.001$ ). Although these correlation coefficients were slightly lower in Jersey cows than Holstein cows, groaning, the absence of rumen movement, pupillary reflex, and warmth at the root of the ear, or an ST peak interval/SS peak interval (> 0.5) could also discriminate hypocalcemia.

We estimated blood Ca concentrations in Jersey cows based on STc intervals and calving number. However, some cows have estimated blood Ca concentrations that are much higher than the measured values. Therefore, total judgment should consider other items in addition to ECG findings and calving numbers. We will continue to increase the number of samples and improve measurement accuracy so that ECG can serve as a tool for diagnosing peripartum hypocalcemia in Jersey cows.

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NU-P02

### Continuous monitoring of ruminal pH and changes in blood metabolic parameters in subacute ruminal acidosis cows

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**Objectives:** In this study, we monitored ruminal pH continuously by using the wireless transfer system (pH sensor) and measured plasma concentrations of hormones and metabolites related to energy metabolism in cows suffering from subacute rumen acidosis (SARA) with different severity. The objective of this study is to evaluate the relationship between ruminal pH and plasma parameters as diagnostic markers for SARA.

**Materials and methods:** 11 head of Holstein-Friesian delivered cows were orally administered pH sensors, and location of sensors were checked by a metal detector. In pH measured every 10 minutes, when ruminal fluid pH<5.6 or reticulum fluid pH<6.3 continued for more than 3 hours a day, we defined it SARA. Cows were divided to 3 groups, A, B, C according to frequency of SARA in 30 days after parturition. Group A (n=5): 29 and 30 days SARA positive; Group B (n=3): 22 to 28 days SARA positive; Group C (n=3): Under 21 days SARA positive. Groups were compared using Student's t-test. Statistical significance was set at <0.05.

We obtained blood samples from tail vein at 2 weeks before, 1, 2, 4 and 8 weeks after each parturition (-2w,1w,2w,4w,8w). We measured concentrations of total cholesterol (TC), free fatty acid (FFA), glucose (GLU), insulin, adiponectin, and activities of MDH (malate dehydrogenase) and LDH (lactic acid dehydrogenase) in plasma. ML ratio (M/L) as a useful indicator for energy usage is calculated as MDH activity divided by LDH activity. Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) is calculated as the following formula. RQUICKI is considered to be a useful indicator to estimate insulin sensitivity.

$$\text{RQUICKI} = 1 / (\log_{10}(\text{Gb}) + \log_{10}(\text{Ib}) + \log_{10}(\text{FFAb}))$$

Gb: plasma glucose (mg/dL); Ib: plasma insulin ( $\mu\text{U}/\text{mL}$ ); FFAb: plasma FFA (mmol/L)

An individual milk yield, milk fat percentage, milk protein percentage in peak period and adjusted milk yield were picked out from data of herd test. Individual delivery intervals were picked out from a breeding ledger.

#### Results:

1. The correlation between anterior stomach fluid pH and blood properties

Pearson's coefficient of correlation between average daily anterior stomach fluid pH at the day of sampling (in the following, pH) and blood properties were calculated. There was negative correlation between pH and adiponectin. There were weak negative correlations between GLU and NEFA, NEFA and M/L, GLU and adiponectin. There was weak positive correlation between pH and M/L. There was positive correlation between TC and M/L.

2. The relationship between frequency of SARA and blood properties, milk production, reproduction performance



The average adiponectin of Group C was significantly lower than that of Group B in 1w, 8w ( $p < 0.05$ ). That was also significantly lower than that of Group A in 4w ( $p < 0.05$ ). The average M/L of Group C increased as time proceeds after parturition. In 8w, both average M/L of Group A and B were lower than that of Group C. Both average RQUICKI of Group A and B were lower than that of Group C in 4w, 8w. The average TC of all groups increased as time proceeds after parturition. The average FFA of Group A and B were higher, and the average GLU of Group A and B were lower than that of Group C in 1w, 4w, and 8w.

Both average milk fat and milk protein percentage of Group A, B were lower than those of Group C in peak period. The average milk yield in peak period of Group C was the lowest. On the other hand, the average adjusted milk yield of Group A was the lowest.

The average delivery intervals became shorter in order of Group A, B, and C.

**Conclusions:** Adiponectin improves insulin resistance by reducing gluconeogenesis in the liver and promoting sugar uptake in skeletal muscle. M/L is reported to be an index of energy metabolism. Since anterior stomach fluid pH correlated negatively with adiponectin, and correlated positively with M/L, these markers passively reflect ruminal fermentation conditions.

Group A tended to show lower RQUICKI and showed significantly higher plasma adiponectin concentrations compared with those in Group C. However, M/L of Group A was lower. In addition, milk fat and milk protein percentage in peak period of Group A were lower, and delivery interval of Group A was longer than other groups. These results suggest that SARA may cause compensatory reaction for insulin resistance, but energy metabolism inside the cell declines in the long run, and that may lead to decreased milk protein percentage and extended delivery intervals.

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NU-P03

### The relationship between the concentration of selenium in the blood and the herd health in the Czech Republic

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**Objectives:** Selenium is an important biogenic element of great significance for the health, performance and reproduction. Cattle herds in the Czech Republic often show selenium deficiencies which adversely influence the animal health, performance and reproduction. Although the prevalence of selenium deficiency in dairy cattle has been lower in recent years, the issue is still important. From 1995 to 2000, we diagnosed selenium deficiencies in dairy cows affecting from 53% to 75% of the herds. The situation has been improving gradually, but there are still farms where the selenium status is marginal, es-

pecially in calves, heifers and fattening bulls. Our further observations showed that selenium deficiencies occurred in 80% and 90% of the calves and heifers monitored, respectively. Currently, the highest prevalence of selenium deficiency is present in beef herds in submontane and mountain regions. A lack of selenium is a matter of geographic location. The selenium content of feed is determined by its content in the soil. In the Czech Republic, the selenium concentrations in soils are low, therefore the feeds have low selenium levels. The dietary supplementation with highly available sources of selenium is necessary to meet the requirements of cattle, particularly of dairy cows.

The aim of this study was to determine the occurrence of selenium deficiency in 10 Holstein herds during the first 100 days of lactation and assess the impact of selenium deficiency on the health status of dairy cows.

**Material and methods:** In order to assess the health status of cows in the herds under study, the concentrations of selenium in whole blood were measured using the hydrid method of AAS with the SOLAR 939 device. Selenium concentrations were measured in 120 selected cows from the 10 herds. The herds were monitored for the occurrence of retained placenta, metritis, mastitis, and for milk somatic cell counts. In the herds with marginal and optimal whole blood selenium the levels of selenium in colostrum and milk were measured.

**Results:** The results show that in 6 dairy herds (60%) the concentrations of selenium in whole blood were ranging from 80 µg/L to 136 µg/L, in 3 herds whole blood selenium levels were between 62 and 76 µg/L and in 1 herd only 56 µg/L. The differences in whole blood selenium among the herds were also related to the health status. While in the herds with selenium concentrations above 80 µg/L the incidence of retained placenta and metritis was minimal and the puerperium was uncomplicated, in the herds with low selenium status retained placenta and metritis were common. Somatic cell counts in milk were also varied among the herds. The cows with optimum selenium status had SCC from 116 to 225 thousand/ml, whereas the cows with the marginal selenium status averaged 378 thousand somatic cells per ml of milk. In this herd the prevalence of clinical mastitis was significantly higher.

**Conclusion:** The study has confirmed that the occurrence of selenium deficiency on dairy farms is a pressing issue and has a negative impact on the health status, performance and the course of puerperium. Of the 10 dairy herds monitored, only in 6 the good selenium status was found. The 4 herds showed a suboptimal selenium status. Selenium deficiency had a negative impact on the health, performance and puerperium.

The study was performed within the projects NAZV QJ1530058 and MZERO717.

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NU-P04

### Selenium Deficiency in beef and dairy calves

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**Objectives:** Selenium is an important anti-oxidant. It is important for health, immunity and growth. In herds in the Czech Re-



public is relatively high prevalence of selenium deficiency in calves.

The aim of the study was to monitor the incidence of selenium deficiency in beef and dairy calves in several cattle herds in the Czech Republic.

**Material and methods:** In 2012 30 (15 females and 15 males) beef calves of Aberdeen Angus (AA) breed from 3 farms were repeatedly examined at 10 to 20 days, 90 to 120 days and 160 to 180 days of age. The calves were born from February to May 2012 and were kept at pasture, along with their mothers. During the entire study period the calves suckled from their mothers and up to 90 days of age they received *ad libitum* a calf starter with 0.3 mg selenium (Se) per kg. Another 30 calves (15 females and 15 males) of two Holstein (H) herds were examined at similar ages. The calves were housed in outdoor individual calf hutches till 8 weeks of age. For the first 5 days of life they received colostrum and then milk replacer till of the 8<sup>th</sup> week. From 10 days of age the Holstein calves were provided *ad libitum* a calf starter of the same composition as the one given to the AA calves. After the weaning the calves received total mixed ration that consisted of clover-grass silage, meadow hay and concentrate. All the calves had free access to water. The calves were withdrawn blood samples from the jugular vein and analysed for whole blood selenium levels by the AAS hydride method. The calves were weighed when handled for blood collection 80ug/l was regarded as a threshold blood selenium concentration value.

**Results:** The highest incidence of suboptimal blood selenium values was found in the AA calves between 10 to 20 days of age. All the calves showed blood selenium levels lower than 80 ug/l, with 20% of calves being under 60ug/l. 90 to 120 days old calves had the highest blood selenium levels. However, 20% of those were under 80ug/l. Towards the end of the study period a proportion of calves with marginal Se values reached about 50%. However, blood selenium levels below 60 ug/l were not found. The health status of the calves under study was satisfactory. Some neonatal calves showed short episodes of diarrhoeic syndrome. In older calves no serious health disorders were diagnosed and there were no deaths either. The Holstein calves showed generally higher blood Se levels than the AAs. From 10 to 20 days of age 50% of H calves had lower blood Se levels; from 90 to 120 days the Holsteins showed similar blood Se levels as the AAs. Towards the end of the period under study 20% calves had suboptimal blood Se levels. Average daily weight gain (ADWG) in the AA males and females was 1302 g and 1175 g, respectively. 6 calves (4 females and 2 males) with long-lasting low blood selenium levels had significantly lower ADWG (946 g on average). There were not any significant differences between blood selenium values between the herds under study. Pasture quality was high and all the calves got the same calf starter feed. Mean ADWG values were lower in the Holstein calves than in the AA ones (982 g and 912 g in males and females, respectively). The health status was good in all the calves. Some neonatal calves showed scours of dietary origin. Neither respiratory diseases nor deaths were observed.

The results of monitoring suggest that in neonatal calves selenium deficiency is most pronounced and the situation improves only upon calf starter intake. Towards the end of the study period when concentrate was not given any more, the AA calves showed a greater reduction in blood Se level than the Holsteins.

**Conclusion:** In beef herds both subclinical and clinical selenium deficiencies occur quite often. This study revealed the highest frequency of Se deficiency occurrence in neonatal calves. Suboptimal blood Se levels were diagnosed in all the calves under study from 10 to 20 days of age. The older calves that received the calf starter feed with 0.3 mg Se showed much higher blood selenium levels. The Holstein calves showed suboptimal blood Se levels less frequently than the AA ones, even at neonatal ages. The consumption of calf starter feed improved the selenium status of calves. In the end of the study period 90% of calves showed blood selenium levels above 80 ug/l.

The study was supported by the institutional research fund of the Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno.

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NU-P05

### **Isoflupredone acetate (Predef® 2X) induces hypertension, hypernatremia, hypokalemia, kaliuresis, alkalemia, sodium retention, and plasma volume expansion in lactating dairy cows**

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**Objectives:** Intramuscular isoflupredone acetate is a common treatment for clinical ketosis in lactating dairy cows. Isoflupredone has both glucocorticoid and mineralocorticoid activity, and hypertension is a well-documented response to isoflupredone administration in sheep. We hypothesized that isoflupredone administration would increase mean arterial blood pressure in dairy cattle.

**Materials and methods:** Fifteen healthy dairy cows in early lactation received two intramuscular 10 mg injections of isoflupredone acetate (9-alpha fluoro-prednisolone acetate; Predef® 2X) 24 h apart. Systolic, diastolic, and mean blood pressures were determined indirectly using an oscillometric technique and a cuff on the coccygeal artery, with the cuff width ranging from 25 to 40% of the tail circumference. Blood pressure measurements were adjusted for the distance between the scapulohumeral joint and the center of the cuff. Heart rate was determined electrocardiographically. Cardiovascular measurements and blood, milk, and urine samples were obtained periodically up to 7 days after the initial injection.

**Results:** Systolic, diastolic, and mean blood pressures were increased ( $P < 0.001$ ) 24h after the second injection of isoflupredone, with mean arterial blood pressure increasing from  $97 \pm 13$  to  $113 \pm 13$  mmHg. Plasma potassium concentration, hematocrit and urine sodium-to-creatinine ratio were decreased at 48h after the first injection, whereas blood pH, plasma sodium, bicarbonate, and glucose concentrations, and urine potassium-to-creatinine ratio, were increased at 48h.

**Conclusions:** Isoflupredone acetate administered at the labelled dose rate to healthy lactating dairy cows produced typical mineralocorticoid effects including hypertension, hypernatremia, hypokalemia, kaliuresis, alkalemia, sodium retention, and plasma volume expansion. Isoflupredone acetate has glu-



corticoid and mineralocorticoid activity in cattle.

NU-P06

### Effects of dexamethasone and insulin alone or in combination on energy and protein metabolism, reproduction and milk production in dairy cows in early lactation

#### dexamethasone and insulin on metabolism and performance in dairy cows

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This study investigated the effects of dexamethasone and insulin on energy and protein metabolism, reproduction and milk production in dairy cows. Two hundred Holstein cows were enrolled in a randomized controlled clinical trial. The cows were randomly assigned to receive 1 of 4 treatments at 3 or 10 days in milk: control group, 10-mL i.m. injection of sterile water, group insulin, s.c. injection of 100 units of insulin, group dexamethasone, i.m. injection of 20 mg of dexamethasone, group insulin plus dexamethasone, i.m. injection of 20 mg of dexamethasone and 100 units of insulin. Serum samples obtained at the time of enrollment, time of treatment and at 2, 4, 7 and 14 days after intervention. The sera were analyzed for  $\beta$ -hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), glucose, cholesterol, albumin, urea, and aspartate amino transferase (AST). To assess reproductive performance, the following outcomes were measured:

interval from calving to first insemination, calving to conception interval, first service pregnancy risk (%), pregnancy rate at 120 days in milk, and cumulative pregnancy risk (%) till 9 month. All analyses were performed using SAS software. Data were analyzed using a repeated measures mixed model that accounted for the effects of parity, body condition score, dystocia, retained placenta, metritis and the random effect of cow. Kaplan-Meier survival function estimates were used to calculate crude associations of treatment with median time to first breeding and pregnancy, and to generate graphs of cumulative pregnancy risk over time. There was no significant interaction of group of treatment and time of intervention (day 3 or 10 post-partum) on serum components. Cows that received insulin or dexamethasone alone or in combination, had significantly ( $P < 0.05$ ) lower BHBA 2 days after treatment compared with control cows, whereas concentrations of NEFA, were unaffected suggesting that glucocorticoids lipolytic effects do not appear to be important in healthy cows. AST activities significantly reduced in cows that received dexamethasone with or without insulin at 2 and 4 days after treatment. Albumin and urea concentrations 2 days after treatment were higher for cows that received dexamethasone only or dexamethasone plus insulin compared with control and Ins received cows. There were no treatment effects on test-day milk production, milk fat and protein percentages. Dexamethasone treated cows had significantly lower days to pregnancy than controls. In addition, dexamethasone treated cows had a significantly greater pregnancy rate at 120 days in milk than other treatment groups and controls. The odds of the development of pregnancy at 120 days

were 2 times greater in cows that received dexamethasone in comparison to controls ( $P = 0.02$ ). In conclusion administration of glucocorticoids in early lactation resulted in short-term improvement of metabolism in postpartum dairy cows in biochemical terms and a single dose of dexamethasone injection in early lactation, might improve reproductive performance.

NU-P07

### Changes in the behaviour responses of beef cattle with experimental subacute ruminal acidosis (SARA)

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USP

**Objectives:** To understand better the clinical picture, that is scarce in the SARA, an experiment was carried out to study the behavior responses of cattle before and after the induction of subacute ruminal acidosis.

**Material and methods:** Twelve Nellore heifers ( $\pm 400$  kg) were previously rumen cannulated and fed during 30 d a basal diet (75% of coast-cross grass hay and 25% of concentrate), offered on a 2.5% live weight basis. The animals were kept in individual barns and food was offered twice daily. Water was offered *ad libitum*. SARA was induced by sudden rumen administration of pelleted citrus pulp (PCP) according to individual live weight ( $Y = \text{Live Weight}^{0.75} \times 54.7$ , where  $Y = \text{g}$  of PCP). On the day before and for three consecutive days after the induction the following variables were recorded: time that the animals remained idly laying down or standing, ruminating, eating and drinking. These observations were carried out with 5 min intervals. Food intake was monitored during the whole experiment. SARA was diagnosed when the rumen pH was between 5.8 to 5.2, for at least 5h continuously.

**Results:** All heifers developed clinically SARA. The rumen pH ranged between 5.20 to 5.79; the average pH  $5.56 \pm 0.14$  and the duration of acidosis lasted  $547 \pm 215$  min. The lower the minimum rumen pH, the longer the duration of SARA ( $r = -0.71$ ;  $P = 0.01$ ). Food consumption was significantly depressed in the 1<sup>st</sup> ( $3.4 \pm 1.9$  kg DM) and 2<sup>nd</sup> d ( $5.2 \pm 3.0$ ) post-induction, but was restored at day 3 ( $8.82 \pm 1.9$ ;  $P = 0.004$ ). The reduction of food consumption was influenced by the lower mean rumen pH ( $r = 0.609$ ;  $P = 0.035$ ) and the longer duration of acidosis ( $r = -0.712$ ;  $P = 0.009$ ). The lower the mean rumen pH on the 1<sup>st</sup> day of acidosis, the greater the reduction of the feed intake in the following days ( $r = -0.647$ ;  $P = 0.04$ ). Although there was no difference between the time spending for eating between the basal period ( $316 \pm 43$  min) and the 1<sup>st</sup> and 2<sup>nd</sup> days of acidosis ( $326 \pm 105$ ) ( $P = 0.725$ ), SARA increased significantly the time spending for consumption of 1kg of dry matter in the first two days of acidosis (Basal  $32 \pm 4$  min; SARA  $90 \pm 31$ ;  $P = 0.035$ ). SARA did not interfere in the time spending for drinking ( $P = 0.90$ ), but reduced the time of rumination ( $P = 0.001$ ) in the 1<sup>st</sup> ( $187 \pm 63$  min) and 2<sup>nd</sup> day ( $277 \pm 87$ ) days as compared to the basal period ( $450 \pm 68$ ) and the 3<sup>rd</sup> d ( $356 \pm 66$  min). The idle time spending laying down was longer in the 1<sup>st</sup> ( $590 \pm 61$ ) and 2<sup>nd</sup> d ( $528 \pm 115$ ) of acidosis than in the basal period ( $380 \pm 60$ )





( $P=0.005$ ). The longer the idle lying down time, the lower the feed consumption (0.715;  $P=0.009$ ). The lower the mean rumen pH during SARA, the longer the idle lying down time ( $r=0.730$ ;  $P=0.011$ ).

**Conclusions:** SARA deeply interferes with behavior responses, mostly on the 1<sup>st</sup> and 2<sup>nd</sup> days after the induction, causing depression in the feed intake and temporary capricious appetite by provoking a longer time to eat the same amount of food. This disease also caused a reduction in the time of rumination, and a longer time of idle lying down. The severity of the symptoms in SARA is potentialized when the mean rumen pH is lower and the time of duration of acidosis is longer. These findings will help the clinician to recognize and establish the diagnosis of SARA.

The protocol of this experiment was previously approved by the Committee for Ethical Use of Animal of the School of Veterinary Medicine and Animal Science São Paulo, BRAZIL – Number 5774270116 approved in Sep. 1<sup>st</sup>, 2016.

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NU-P08

### Colostrum characteristics in cows treated with cabergoline at dry-off

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Colostrum quality is of vital importance for the protection of newborn calves against infectious agents during their first days and weeks of life. There are many risk factors potentially associated with lower colostrum production (CP) and IgG content (Ig). It is well known that CP and IgG play an important role in the success (or failure) of transfer of passive immunity. A low colostrum Ig concentration can lead to hypogammaglobulinemia or failure of passive transfer (FPT). This may end up in increased risk of infectious diseases in the calf and impaired performance.

**Objectives:** The objectives of the study were to investigate the quantity and quality of the colostrum in Holstein dairy cows treated with cabergoline (Velactis® Ceva Sante Animale, France) at dry-off. Cabergoline, is a potent dopamine receptor agonist on D2 receptors and inhibits prolactin secretion and consequently, induces a reduction of milk production

**Materials and methods:** Sixteen primiparous and 4 multiparous lactating cows at the moment of dry-off were involved in the study. After the last milking, 10 out of 20 cows were treated intramuscularly with 5 ml (5.6 mg of cabergoline (CAB)) and the rest ( $n=10$ ) with 5 ml of saline solution (CTR). Immediately after calving, colostrum production was recorded for each individual cow and IgG content and total protein were determined. IgG content was determined by radial immunodiffusion and total protein by kjeldahl using a conversion factor of 6.38. Data were processed with an analysis of variance with treatment as a main effect.

**Results:** The treatment of cabergoline after drying-off cows abruptly had no effect on colostrum production, IgG content, or

protein contents with  $P=0.77$ ,  $P=0.34$ , and  $P=0.17$  respectively. On average, colostrum production in CTR cows was  $13.3\pm 1.86$  kg whereas in CAB cows was  $14.1\pm 1.96$  kg. IgG content was  $39.4\pm 1.91$  mg/ml in CTR cows and  $37.1\pm 1.82$  mg/dl in cows on CAB had. Protein content in colostrum from CTR cows was  $14.0\pm 1.16\%$  and in CAB cows was  $16.3\pm 1.10\%$ .

**Conclusion:** The use of cabergoline, a prolactin inhibitor, to reduce the milk production at the moment of dry-off has no effect on colostrum quantity and quality (as assessed by IgG and protein concentration) in the treated cows, after calving.

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NU-P09

### Dynamics of dry-off method on cure rate and new intramammary Infections in the dry period in the Netherlands

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The dry period is a suitable moment to cure preexisting intramammary infections (IMI) with the application of antibiotic dry cow therapy at dry off. However, it is also a period with high susceptibility for acquiring new IMI. The level of milk production before drying off and milk leakage incidence during the days after the last milking are known to be risk factors for new IMIs during the early dry period and may negatively affect cure rates during the dry period. In recent decades there has been a dramatic increase in average milk yield. With the objective of reducing milk production before the dry-off (DO), methods such as reducing milking frequency or feeding regimen are used.

**Objectives:** The objectives of this study were to investigate the cure rate (CR) and the incidence of new IMI (Infection Rate, IR) when applying two methods of drying-off cows, defined as: "gradual" DO (when there was an intervention to reduce milk production through e.g. reducing milking frequency and/or feeding regime) and abrupt DO (with no obvious changes in either milking frequency or feeding regimen).

**Materials and methods:** Data from 42,633 dairy cows from 656 Dutch commercial herds were analyzed to investigate the impact of different methods at DO on CR and IR during the dry period.

Discrimination between gradual and abrupt DO was obtained by analyzing daily Milk Meter data (yield and frequency of milking) in the last 60 days before DO. A Multi-Layer Perceptron classification algorithm was trained using an expert-based labelling approach and subsequently run over the 42,633 animal dataset to predict the respective DO method. The algorithm was validated on a separate dataset to have classification accuracy = 0.85.

Using the afore mentioned methodology for classification of DO management, the impact on cure rate and IR was studied in 28,566 cows with eligible Milk Recording (MRO) data. This was done by comparing the last somatic cell count (SCC) recording before DO and the first SCC recording after calving (ranged from maximum 30 days before date of DO to maximum 30 days after subsequent calving) at cow level. Cows were classified as infected if the level of SCC was equal or more than 200.000



cells/mL.

CR was calculated as the proportion of non-infected cows after calving of the infected cows at DO. IR was calculated as the proportion of infected cows at calving of the non-infected cows at DO.

**Results:** The study showed that 32% of the animals involved in the analysis underwent a gradual DO and the remaining 68% an abrupt DO. Around 26 % of herds applies the same dry-off management to all cows while in the rest the DO management varied at cow level. Higher producing herds were associated with a greater share of gradually dried off animals.

The CR and IR were 71.1 % and 18.9% respectively in animals with gradual dry-off and 72.5 % and 17.1% in animals with abrupt DO. In both cases the IR increased with parity and the CR decreased.

**Conclusions:** Reducing the milk production at dry-off gradually (e.g. by reducing milking frequency and/or feeding regime) resulted in higher IR and did not increase CR during the dry period compared to animals dried-off abruptly.

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NU-P10

### Non-steroid anti-inflammatory drugs in the treatment of acute laminitis induced by oligofrutose in Nellore cattle

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**Objective:** The treatment of laminitis is based on the use of drugs that decrease the inflammatory chain reaction, triggered in the foot region. Among the drugs used, the non-steroidal anti-inflammatory drugs (NSAIDs) are preferred. NSAID treatment protocols are variable according to the intensity and the course of the disease, and multiple applications are recommended for at least three days after diagnosis of the disease. The present study aimed to evaluate the efficacy of three NSAIDs (meloxicam, ketoprofen and flunixin meglumine) in the treatment of acute laminitis induced by oligofrutose in Nellore cattle.

**Material and Methods:** We used 29, 3-years-old, rumen-cannulated Nellore heifers, weighing  $474.5 \pm 58.5$  kg. For laminitis induction, all heifers were challenged with oligofrutose (10.71 g/kg of body weight). Around seven percent (0.765 g/kg) of the main dose was given twice daily for 3 days before the main overload. Oligofrutose was administered direct into the rumen through the cannula. After induction locomotion scoring and hoof-testing were performed every six hours during the initial 24 hours, and the every 12 hours up to 72 hours. *Sprecher* locomotion score was used assigning values from 1 to 5 (1 absence of lameness and 5 severe lameness). When the animals had two positive reactions in the hoof testing and locomotion score greater or equal to 2, it was considered positive for laminitis. Positive animals were randomly assigned to four groups receiving the following medications for three consecutive days (intravenous): Control (8 ml of isotonic saline; n=6), Flunixin meglumine (Desflan, Ouro Fino, 1.1 mg/kg; n=7), Ketoprofen

(Ketofen, Merial, 3 mg/kg; n=7) and Meloxican (Maxican, Ouro Fino, 0.5 mg/kg; n=7). After the beginning of NSAID treatment the animals were evaluated every 12 hours for up to 96 hours and underwent a clinical examination including locomotion scoring, hoof-testing, appetite, attitude (station or decubitus) and tarso-crural joint evaluations, as well as the collection of blood for cortisol and pepsinogen dosage.

**Results:** From the 29 animals used, 27 developed laminitis between 24 and 72 hours after oligofrutose overload in at least two limbs. From the positive animals, eight heifers (29.6%) developed polysynovitis in the tarso-crural joints. The three NSAIDs reduced cortisol concentration in relation to the control group ( $P < 0.05$ ) but no differences were observed in the pepsinogen concentration. Meloxicam was effective in reducing foot sensibility, improving in the same way as ketoprofen the locomotion score. These two drugs stimulated a return of appetite and the improvement in attitude.

**Conclusion:** A non-steroidal anti-inflammatory drug should be used in the treatment of cattle laminitis, with preference given to meloxicam, followed by ketoprofen.

The protocol of this experiment was previously approved by the Committee for Ethical Use of Animal of the School of Veterinary Medicine and Animal Science São Paulo, BRAZIL – Number 2545300714 approved in Feb. 25, 2015.

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NU-P11

### Diagnosis of metabolic disorders in transitional dairy cows based on changes in characteristic blood biochemical indicators

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**Objective:** According to blood biochemical indicators, ketosis in cows may be diagnosed when the following values match the clinical signs: beta-hydroxybutyrate (BHB)  $> 1.2$  mmol/l, glucose  $< 2.5$  mmol/l, and triglycerides (TG)  $< 0.12$  mmol/l, and blood values: non-esterified fatty acids (NEFA)  $> 0.7$  mmol/l and aspartate-transaminase (AST) activity above 100 U/l, which is indicative of hepatic lipodosis. On that account, objective of this experiment is to diagnose the metabolic disorders of late pregnant and early lactation dairy cows based on changes in characteristic blood biochemical indicators.

**Materials and Methods:** This experiment was conducted in a dairy Simmental herd diagnosed with a number of metabolic and reproductive disorders. Clinically healthy late pregnant (n=15) at 25 to 1 ( $13.7 \pm 9.3$ ) days to partus and early lactation cows (n=15) in the first month of lactation ( $16.1 \pm 9.2$  days) were selected. Blood plasma of BHB, NEFA, glucose, TG concentrations and AST activity were determined by different colorimetric techniques using spectrophotometers (Cobas Mira and Gilford Stasar). Data were subjected to statistical analysis using the GLM model and t-test for difference of means between two independent groups (software: Statgraphic Centurion, Stat-



point Technologies Inc. Warrenton, Va, Virginia, USA). The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

**Results:** Cows in early lactation had significantly higher ( $p < 0.05$ ) levels of serum BHB, NEFA and AST, and lower ( $p < 0.05$ ) glycemia and TG compared to late pregnant cows. High lipomobilization (NEFA  $> 0.4$  mmol/l) was detected in 6 (40%) of early lactation cows but in none of late pregnant cows, while subclinical ketosis (BHB  $> 1.2$  mmol/l) was detected in 14 (94.4%) of early lactation cows and 4 (26.6%) of late pregnant cows. AST activities above 100 U/l were detected in 2 early lactation cows and in none of late pregnant cows. TG levels below 0.12 mmol/l and glucose levels below 2.5 mmol/l were found in 7 (44%) and 10 (66.6%) of early lactation cows, respectively, and in none of late pregnant cows.

**Conclusions:** Results on blood levels of glucose, TG, BHB, NEFA and AST in early lactation cows suggest metabolic disorders associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. These serum biochemical indicators may have a key role in diagnosing metabolic disorders in late pregnant and early lactation dairy cows.

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NU-P12

### The correlations between serum enzyme activities in blood and milk in the different stage of lactation in Holstein dairy cows

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**Objective:** The objective of this study was to determine correlation between serum blood and milk enzyme activities of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and alkaline-phosphatase (ALP) in the different stage of lactation in dairy cows as markers for diagnosis subclinical metabolic diseases.

**Materials and Methods:** Three groups of clinically healthy Holstein dairy cows were chosen from the herd. Group 1 consisted cows in the start of lactation ( $n = 12$ ) in period of  $5 \pm 3$  days after calving; Group 2 -consisted of early lactation cows ( $n=12$ ) in the first month of lactation ( $22 \pm 15$  days), and Group 3 included mid lactation cows ( $n=12$ ) between 90 to 150 days of lactation ( $133 \pm 75$  days). Blood and milk samples were taken simultaneously from each lactating cow during morning milking. Blood samples (10 ml) were taken by jugular veni puncture into a sterile tube from each animal, and the blood serum was separated by centrifugation at room temperature (1,800g, 15 min). Milk samples were collected in sterile tube and centrifuged at 12,000g for 30 min at 4 C and the supernatant was transferred into the new sterile tubes. Blood plasma and milk were stored at -20 C until being used for biochemical measurements. The blood and milk serum activities of aspartate-transaminase (AST) alanine-aminotransferase (ALT) and alkaline-phosphatase (ALP) were measured in the biochemical laboratory OXUS (Kragujevac, Serbia) by spectrophotometric techniques using a

BT 1000, (Biotechnica Italia) and the corresponding commercial kits (DIALAB, YUNICOM). The statistical analysis of the obtained data was carried out by ANOVA-procedure. Correlation coefficients were calculated using linear regression models to study relationships between variables (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA). The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

**Results:** Statistically significant higher ( $p < 0.01$ ) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation group of cows. No significant difference ( $p > 0.05$ ) was observed in milk serum value for AST between the three groups of cows. In the period of mid lactation (Group 3) we measured the highest ( $p < 0.01$ ) activity of ALT in blood serum compared to other groups of cows. No significant difference ( $p > 0.05$ ) was observed in milk serum value for ALT between the three groups of cows. The higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid lactation cows, but without statistical significance ( $p > 0.05$ ) as consequence high individual variabilites. Significant correlations among AST, ALT and ALP activities in blood and milk serum are not determined ( $p > 0.05$ ) in this study.

**Conclusions:** On the basis changes in blood AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration. No significant correlations among AST, ALT and ALP activities in blood and milk serum are determined ( $p > 0.05$ ) and shows that activity of these enzyme in the milk are not used as markers for early diagnosis of subclinical metabolic disease.

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NU-P13

### Metabolic monitoring of German Simmental cows during early lactation based on milk infrared spectroscopy

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**Introduction and objectives:** Due to significant changes in the metabolic system, early lactation is a crucial time for high-yielding dairy cows as it predisposes the animals to metabolic disorders, such as ketosis. During the first period of lactation, ketosis is prevalent in up to 59% of dairy cows. Especially subclinical ketosis is hard to diagnose and can lead to other health problems and substantial economic losses.

The aim of the present study was the development and implementation of a cost-effective and systematic monitoring tool for metabolic disorders, in particular ketosis. The risk of ketosis was evaluated based on the milk infrared spectra, which are generated during routine milk recordings.

**Materials and methods:** Twenty-six German Simmental dairy herds with an automatic milking system were visited once a



week over a period of seven weeks. Cows between day 5 and day 50 in milk were clinically examined; additionally blood and milk samples were taken. Blood beta-hydroxybutyrate (BHB) concentration  $\geq 1.2$  mmol/l was defined as the threshold for ketosis. The infrared spectra were limited to those areas which contained the relevant chemical information. Milk infrared spectra of healthy cows were compared with those of ketotic cows to detect significant differences. Linear discriminant analysis was performed to develop the monitoring tool based on these differences in the infrared spectra. 10-fold cross validation was used to estimate the quality of the model. To increase clarity and accuracy, a "traffic light system" with three stages (green = low suspicion of ketosis, yellow = average suspicion of ketosis, red = high suspicion of ketosis) was developed.

**Results:** A total of 1078 examinations taken from 358 animals were used for analysis. The prevalence of hyperketonemia in the study population was 10%. A total of 20.1% of the study animals had at least one ketosis event.

Significant differences between the infrared spectra of healthy and ketotic cows were detected. Based on these differences, a monitoring tool with a hit rate of 85% was generated. In respect to the blood BHB concentration, reports labelled as "green" (low suspicion of ketosis) by the tool were free of ketosis 95% of the time, whereas 68% of the animals with a "red" report (high suspicion of ketosis) suffered from ketosis according to the blood beta-hydroxybutyrate concentration. "Yellow" reports were rare and were observed in 29% of animals with ketosis and 71% of animals without ketosis.

**Conclusions:** In conclusion, the assessment of metabolic health based on milk infrared spectra appears promising. The developed model seems to be an essential tool for ketosis monitoring. The practical application as part of routine milk recording is scheduled for mid-2018.

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NU-P14

### An Innovative Management Approach to Ketosis Treatment in Dairy Cattle

#### Reducing energy demand instead of increasing energy intake to more effectively treat ketosis in lactating dairy cows

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Subclinical ketosis affects approximately 40% of dairy cows in North America. Current recommended treatments for ketosis focus on providing cows with more energy. However, the most effective treatments, such as oral propylene glycol, only resolve ketosis approximately 50% of the time. The objective of this research was to evaluate the effect of decreasing milking frequency from two milkings/day, to one milking /day for two weeks; in conjunction with a standard treatment of five days of oral propylene glycol in cows diagnosed with ketosis. Ketosis was defined as a blood beta-hydroxybutyrate concentration of greater than or equal to 1.2mmol/L. From November 2016 to

September 2017, 104 ketotic cows from the University of Guelph Livestock Research Innovation Centre were studied., 55 cows were randomly allocated to the once/day milking group, and 49 were enrolled in the twice/day milking group. All cows inhabited the same pen during their time on trial, and all cows were milked in a DeLaval VMS robot equipped with Herd Navigator. Blood, milk, and urine samples were collected in a 21-day period to analyse ketones over time in the cows. Disease occurrence was recorded up to 60 DIM and reproductive performance, examining days to first breeding, and pregnancy on the first breeding were explored. CanWest DHI herd recording data was collected on a weekly basis, for a period of 15 weeks, examining milk production, milk component data, and SCC. Results indicate that both heifers and cows milked once/day for a two-week period are significantly more likely to recover from ketosis than heifers and cows milked twice/day during treatment ( Fisher's exact test;  $P < 0.005$ ).

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NU-P15

### Energy profile in relation to number of lactation in dairy cows

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In the study we compared parameters of energy profile (glucose - GL, non-esterified fatty acids - NEFA,  $\beta$ - hydroxybutyrate - BHB, total cholesterol - TCH, triacylglycerols - TG and total lipids - TL) of Slovak Pied dairy cows with number of lactation (L.1 – 6 cows, L.2 – 5 cows, L.3 – 6 cows, L.4 – 5 cows). Blood samples were collected 20 days a.p. and 20, 40, 60, 80 days p.p. There were observed lower concentrations of glucose and BHB in groups of cows L.1 and L.2 compared with cows of groups of L.3 and L.4. The highest concentrations of glucose was recorded in cows L.4 20 days a.p. ( $4.32 \pm 0.09$ ;  $P < 0.01$ ). NEFA, TL were increased with a.p. period. TCH values in groups L.3 and L.4, were lower than in groups of cows L.1. and L.2 ( $P < 0.01$ ;  $P < 0.05$ ). In the assessment of concentration of TG was found the highest concentrations in group of cows L.1 during *ante partum* ( $0.22 \pm 0.03$ ;  $P < 0.05$ ). Cows during *a.p.* had significantly higher TG concentrations compared to cows in postpartal period ( $P < 0.05$ ). These results showed dynamic changes in the energy profile during *a.p.* and *p.p.* which reflect the physiological response of the organism to the variation of metabolic functions occurring from gestational to a lactating state in dairy cows. Our results indicate that older cows have higher GL, BHB and NEFA levels, which proves that dairy cows with higher number of lactations have a better adaptation to the metabolic challenge, for example to milk production, in terms of maintenance of glycemia.

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NU-P16

### Blood n-3 fatty acid concentration and metabolic responses of dairy cows fed perilla seed oil extract during the transition period

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Chisato Minagawa Shigefumi Tanaka Takakazu Nishikawa

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**Objectives:** Fatty liver syndrome is a serious problem in dairy cows during the transition period. Previously, it has reported that to improve health condition of the cows, flaxseed is provided as a supplemental source of fat and protein to lactating dairy cows because of its rich content of the essential n-3 fatty acid  $\alpha$ -linoleic acid (ALA). Perilla seed oil is commonly produced and consumed in Asia and is also rich in ALA. However, the effect of perilla oil as a supplemental source for cows is unknown. The objectives of this study were to examine the effects of supplementation with perilla seed oil extract on metabolic responses during the transition period in dairy cows.

**Materials and methods:** Sixteen pluriparous cows (parity: 2-5) were used. The experimental period was from 28 days before the expected date of calving to 28 days after parturition. The cows were divided into four groups: A) supplementation with 500 g/day perilla extract (nearly 30 g / day ALA) throughout the entire transition period from 21 days before the expected due date to 21 days after calving; B) supplementation with perilla seed oil extract 500 g/day from 21 days before the expected date of calving; C) supplementation with perilla seed oil extract 500 g/day for 21 days after calving; D) no perilla extract supplementation as a control. Blood samples were collected from the tail vein 28, 14, and 7 days before the expected date of calving, and 0, 7, 14, and 28 days after calving. Blood concentrations of non-fatty acid, albumin, total cholesterol, gamma-glutamyl transferase (GGT) and beta-hydroxybutyrate (BHBA) were measured. Blood ALA was also analyzed using gas chromatography.

**Results:** In the control group, blood ALA was  $6.24 \pm 0.43$  % (in average  $\pm$  SD) at the 28 days before due date. After parturition, it was declined from  $5.60 \pm 0.78$  % to  $3.79 \pm 0.90$  % and  $4.3 \pm 0.60$  % at the day of calving, day 7 and day 14. The same tendency was observed at group A; transition group and group B; close up group, but not in group C. The effect of perilla extract supplementation on the blood ALA concentration during the transition period was not clear. Blood non-fatty acid concentrations tended to be higher in the group A than in the other groups, but no differences were observed in blood albumin, total cholesterol, GGT, or BHBA concentrations within other three groups.

**Conclusions:** Supplementation with 500 g/day perilla seed oil extract during the transition period in dairy cows tended to increase blood non-fatty acid concentrations, with no effect on ALA or other metabolic parameters.

**Objectives:** Periparturient diseases in dairy cows including hypocalcemia, retained placenta, ketosis, metritis and displaced abomasum, reduce the productivity resulting in a huge economic loss in dairy farms. If the occurrence of these diseases can be predicted, productivity losses can be prevented before the diseases develop clinically. Therefore, this retrospective study is aimed to investigate whether the preparturient blood biochemical parameters can be used to predict the occurrence of metabolic diseases in the early lactating dairy cows.

**Materials and methods:** Clinical data were obtained from 91 Holstein periparturient cows raised in University Animal Farm in Seoul National University for about 3 years. Physical examination and blood analysis were taken from 8 weeks preparturient until the uterus recovered (4 to 6 weeks after parturition) at 2-week intervals. Through periodical health check during the transition period, we diagnosed the occurrence of diseases and provided optimal treatments. Incidences of periparturient diseases including subclinical/clinical hypocalcemia, retained placenta, subclinical/clinical ketosis, metritis and displaced abomasum were calculated and odds ratio analysis was performed to confirm the association between risk factors and the outbreak of diseases. For all statistical analyses, SigmaPlot 12.5 was used.

**Results:** Among the 91 heads, 46 parturient cows had postparturient diseases: 8 subclinical hypocalcemia (8.8%), 5 retained placenta (5.5%), 35 subclinical ketosis (38.5%), 1 clinical ketosis (1.1%), 1 left displaced abomasum (1.1%), and 3 metritis (3.3%). In most cases, the disease occurred in single, but in some cases accompanied by other diseases. One preparturient parameter did not show any significance, but the combination of preparturient total cholesterol (T-chol) and triglyceride (TG) concentration correlated with the occurrence of diseases. To identify this correlation more precisely, the standards were determined separately by the parity (TG < 20 mg/dL or T-chol < 95 mg/dL in primiparous cows; TG < 25 mg/dL or T-chol < 110 mg/dL in multiparous cows). Then, the Odds ratio value for the criterion and disease occurrence was 5.929 (95% CI: 2.353-14.940,  $P < 0.001$ ).

**Conclusions:** The standard values determined through this study (TG < 20 mg/dL or T-chol < 95 mg/dL in primiparous cows; TG < 25 mg/dL or T-chol < 110 mg/dL in multiparous cows) can be used as an indicator to predict the occurrence of periparturient diseases. However, lipid parameters such as TG and T-chol are significantly affected by feeding environment. Since the current reference value is obtained from a single farm, more data will be needed to establish reference values that can be applied to other farms.

NU-P17

### Utilization of preparturient total cholesterol and triglyceride concentration as indicators for predicting postparturient metabolic diseases in dairy cows

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NU-P18

### Incidence of subclinical and clinical ketosis in grazing dairy cattle in Colombia

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**Objectives:** The main objective of this prospective cohort study was to determine the incidence of subclinical (SCK) and clinical ketosis (CK) in pasture-grazed dairy cows in Colombia within the first six weeks of lactation, using an electronic cow-side



test.

**Materials and methods:** A purposive sample of 150 lactating dairy cows from 10 commercial dairy farms in the Andean plateau of Bogotá in Colombia were enrolled at the first week of lactation. Cows were tested weekly using an electronic cow-side test (Precision Xtra meter, Abbott Laboratories, Abbott Park, IL) for  $\beta$ -hydroxybutyrate (BHB) during the first 6 weeks of lactation. SCK was defined as BHB > 1.2 up to 2.9 mmol/L and CK as BHB >3 mmol/L. Parity, days in milk (DIM), body condition score (BCS), dry period length, breed, milk production and peripartum diseases (hypocalcemia, retained placenta, metritis) were recorded for each cow and management practices were determined using a survey for each farm at the beginning of enrolment. Weekly incidence, cumulative incidence and prevalence were calculated. The time of a negative test after a positive one was described as well as the number of positive test for each animal.

**Results:** The cumulative incidence of SCK was 25.33% and 3.33% for CK. The SCK weekly incidence from the 1st to the 6th postpartum week was 4%, 7.33%, 3.33%, 8%, 1.33% and 1.33% respectively. The peak incidence was 8% at week 4 postpartum for SCK and 2.6% at week 2 for CK. The overall prevalence of SCK was 42.6% and CK prevalence was 6%. The peak prevalence of SCK and CK was 10.6% at week 4 and 2.6% at week 2 postpartum, respectively. Just one positive test was observed in 45.9% of the animals, whereas 54.1% had two or more positive tests, none of the animals were positive for all 6 weeks of the study and 13.5% of the positive cows to SCK were also positive to CK. The 32.3% of the animals had a positive test in non-consecutive weeks and only one primiparous cow was positive to SCK.

**Conclusions:** The results of this study show that the incidence of ketosis is significant in dairy herds in Colombia during the first six weeks of lactation affecting multiparous more than primiparous cows, but the clinical stage of the pathology is less common, and its low incidence agrees with previous studies in different countries. Most of the animals were positive for two or more weeks in the study. To the authors knowledge this is the first study of bovine ketosis in Colombia. The development of monitoring programs and early treatment of positive animals would be necessary to avoid economic losses due to this disease.

NU-P19

### Comparative study on different field test of Subclinical and Clinical Ketosis by using blood, milk and urine in Dairy cattle

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**Objectives:** Subclinical ketosis (SCK) is a common metabolic disease during the transition period in dairy cattle, characterized by increase concentration of circulating ketone bodies without the presence of clinical sign of ketosis. In North Ameri-

ca, the incidence of SCK is 40-60%, which is much higher than the incidence (2-15%) of clinical ketosis (CK). Several field tests are frequently used for quick diagnose of Ketosis, but there is limited information are available about the comparative efficacy of this test by using blood, milk and urine samples. In the study, the diagnostic performances were evaluated among different field tests.

**Material and Methods:** Animals having SCK and CK were selected as a part of this study as well as normal individual. Blood, milk and urine samples were taken from each cow simultaneously for the accuracy of test results. Blood sample was collected and tested for detection of  $\beta$  - hydroxybutyrate (BHB) by using portable ketone test kit (Abbott, UK). All milk samples were tested for BHB using three different commercial test kits. Urine samples were collected aseptically by using urinary catheter and then analyzed it by urine stick. Milk and urine samples were tested for SCK are based on color reaction of ketone bodies.

**Results:** Determination of level of BHB in blood was considered as "gold standard" for identifying SCK and CK. A cutoff value of 1.2 to 2.9 mmol/l of BHB in blood samples is used to distinguish between cows with and without SCK. On the basis of BHB level in blood, Animals were categorized into three states, such as normal, subclinical and clinical state of ketosis. Milk test kit showed similar type of result like blood test kit and clearly differentiates between SCK and CK. However, normal state cattle frequently showed the SCK response in milk test kit. Urine test kit clearly identified the CK, but SCK was rarely detected in Urine test Kit.

**Conclusion:** Generally, the methods used in the field are suitable for detection of CK. However, the SCK data interpretation should be cautioned due to the low specificity of SCK detection in Milk test and low sensitivity of SCK detection in Urine test.

NU-P20

### Metabolic profile and survey of bovine reproductive conditions in communal farms of Ngaka Modiri Molema District of the North West Province in South Africa

#### Cattle reproductive conditions

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**Abstract:** Inability to account for the nutritional requirements to maintain development, production and reproduction in livestock still remain a challenge due to the imbalanced feed supply. To develop a physiological profile of bovine reproductive conditions (downer cow syndrome, dystocia, retained placenta, vaginal prolapse and abortion) in order to establish a reference tool for animal care practitioners. A total of 108 blood samples were collected from 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 cows in the final trimester of pregnancy (n=31) reported to the North-West University teaching hospital. Structured questionnaires were also used to collect data from farmers in the study area. Data were analysed in SAS (version 20) using the analy-



sis of variance techniques (ANOVA). The data from completed questionnaires were coded, captured and analysed using Statistical Package for Social Sciences (SPSS) version 21. The results obtained showed significant differences in the incidences of reproductive conditions and ages, it was as well noted that breed differences were significantly difference in cows encountering reproductive condition ( $P < 0.05$ ). Significant levels of calcium and magnesium were seen in aborting Afrikander cows. On the other hand calcium levels in downer cow syndrome and dystocia were significantly high in cross breed cows. Age and breed of cows do have an influence on the incidences of reproductive conditions.

The incidences of reproductive conditions contribute to production loss, consequently affecting the economy of communal farmers.

**Key words:** Reproductive conditions, nutrition, animal health, production

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NU-P21

### NEFA and beta-hydroxybutyrate in dairy cows with claw diseases

#### NEB and lame cows

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**Objectives:** Lipid mobilisation and ketosis generally occur in dairy cows as a result of negative energy balance. This usually occurs due to a rapid increase in milk production after calving in the absence of compensation by a rapid rise in feed intake, or can be due to a simple decrease in feed intake as a result of any impairment of health condition. One of the most frequent health disorders of dairy cows – claw diseases – were found to significantly alter the resting behaviour of cows during transient period, resulting in an elevated risk for negative energy balance in dairy herds. The objective of this study was to examine the effect of claw diseases on serum concentrations of NEFA and beta-hydroxybutyrate (BHB) in dairy cows.

**Materials and Methods:** 652 dairy cows housed in free stalls were included in the study; farm feeding management was based on TMR. Average yearly milk yield was 8,000 kg. The dairy cows were examined during claw trimming in the crush. The udder and milk of each cow were examined for possible inflammation, this included the California mastitis test. The cows were also free of any inflammation of the genital tract. Blood samples were obtained from the jugular vein immediately after the animals entered the crush. Serum NEFA and BHB were assayed with the kits supplied by Randox Laboratories Ltd. on spectrophotometer Alizé (Lisabio, France). For testing of effects of claw diseases on lipid mobilisation the dairy cows were selected into two groups: healthy cows (control) and cows with claw diseases (CD). Group differences in serum NEFA and BHB concentrations were tested by Student's *t*-test.

**Results:** Claw diseases were found in 87 (13.3 %) dairy cows which were free of mastitis and metritis/endometritis (CD). Main pathological claw disorders were digital dermatitis, sole ulcer, toe ulcer, bulb ulcer, and white line abscess. No claw diseases, mastitis or metritis/endometritis could be observed in 168 (25.8

%) dairy cows (control). Mean concentrations of NEFA were  $0.51 \pm 0.33$  and  $0.54 \pm 0.37$  mmol/L in control and CD cows, respectively while BHB concentrations were  $0.56 \pm 0.35$  and  $0.62 \pm 0.41$  mmol/L in control and CD cows, respectively. The statistical analysis did not find a significant difference in NEFA and BHB between control cows and cows with claw diseases.

**Conclusions:** Results of our observation show that the claw diseases in dairy cows are not associated with negative energy balance. The majority of the cows with claw diseases seem to be able to meet their energy demand by a sufficient daily feed intake.

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NU-P22

### Effect of one- or two-phase dry cow feeding on dry matter intake, milk yield and animal health in German Holstein Cows

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**Objectives:** Feeding of dairy cows during the dry period and transition period has a marked influence on the performance and health in the following lactation. In the light of this, there are many studies and recommendations on the optimal feeding of dry cows. It was the aim of this study to compare two feeding strategies (one phase vs two phase) with regards to dry matter intake, milk yield and animal health during the dry period and early lactation.

**Material and Methods:** One hundred German Holstein dairy cows were included into the study which lasted from the beginning of the dry period until Day 49 of lactation. The cows were randomly divided into two groups of 50 animals each. Group 1 received the diet of the lactating cows mixed with straw (5.95 NEL/kg dry matter [DM]). Group 2 received a diet consisting of grass and corn silage, straw and minerals until Day 14 ante partum (ap) (5.55 NEL/kg DM). From Day 14 until parturition a mixed ration enriched with concentrates (6.6 NEL/kg DM) was fed. After calving, all cows received the diet of the lactating cows (6.95 NEL/kg DM). All diets were offered ad libitum. During the dry period, individual DM intake was recorded daily, and body weight as well as body condition (BSC) weekly. During lactation, DM intake, body weight and milk yield were recorded daily. Weekly blood samples were collected from 6 weeks ap until 6 weeks post partum (pp) for the determination of serum concentrations of non-esterified fatty acids (NEFA) and  $\beta$ -hydroxybutyrate (BHBS). Calving ease and the birth weight of calves were also noted. During lactation, diseases were documented. The statistical analysis was performed using SAS and applying mixed general linear models. Means are least significant means (LSM).

**Results:** Mean daily DM intake during the dry period was high-



er in Group 1 (13.5 kg/day) than in Group 2 (11.1 kg/day) ( $P < 0.0001$ ). DM intake in Group 1 slowly declined towards calving. In contrast, in Group 2 during Phase 1 DM intake was lower and during Phase 2 it was slightly higher than in Group 1. Mean body weight and BCS did not differ between groups during the dry period. Calves from cows of Group 1 were heavier than calves of cows from Group 2 (47.6 kg vs 44.1 kg,  $P < 0.05$ ) without any differences concerning dystocia. During early lactation, daily DM intake and milk yield did not differ between groups. However, mean body weight was lower in Group 1 (641 kg) than in Group 2 (663 kg). Whereas the mean concentration of NEFA and BHBS did not differ between groups during the dry period, during early lactation, mean NEFA and BHBS were higher in Group 1 than in Group 2 (750 vs 638  $\mu\text{mol/l}$  ( $P < 0.05$ ) and 0.723 vs 0.635  $\text{mmol/l}$  ( $P < 0.1$ ), respectively). Frequency of cows with BHBS concentration  $> 1.2 \text{ mmol/l}$  at least once during early lactation indicating ketosis was higher in Group 1 (35.4 %) than in Group 2 (18.4 %) ( $P < 0.1$ ). The frequency of other disease was low and did not differ between groups.

**Conclusions:** The one phase diet used in this study did not differ from the two phase diet with regards to milk yield and dry matter intake pp. However, the overall higher DM intake ap in Group 1 apparently led to a higher risk of fat accumulation ap followed by increased lipid mobilization pp which might explain the higher body weight loss and higher incidence of ketosis pp compared with Group 2, even though overall animal health was not affected. This apparent ketogenic stress might be prevented by avoiding very long dry periods or choosing a lower energy level for the one-phase diet.

#### NU-P23

### Prevalence of Subclinical Ketosis and Production Diseases during Early Lactation in Dairy Cows in Central and South America, Africa, Asia, Australia, New Zealand and Eastern Europe

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**Objectives:** Subclinical ketosis (SCK) and other periparturient diseases considerably account for economic and welfare losses in dairy cows. The majority of scientific reports investigating the prevalence of SCK and production diseases in early lactation are predicated on empirical studies conducted in countries of Western Europe and North America.

The objective of the present study was to survey the prevalence of SCK and production-related clinical diseases in early lactating dairy cows in various countries across the world beyond North America and Western Europe.

**Materials and Methods:** Twelve countries of South and Central America (Argentina, Brazil, Chile, Colombia, Mexico), Africa (South Africa), Asia (Thailand, China), Eastern Europe (Russia, Ukraine), Australia and New Zealand were assessed, and data from a total of 8.902 cows kept at 541 commercial dairy farms were obtained. A minimum of five cows per farm was investi-

gated once after parturition up to day 21 of lactation. Plasma concentration of  $\beta$ -hydroxybutyrate (BHBA) was measured and the presence of production-related diseases such as retained placenta, milk fever, metritis, mastitis, displaced abomasum, claw disease and lameness, and clinical ketosis was recorded. The threshold for classifying cows with SCK was set at an BHBA concentration of 1.2  $\text{mmol/l}$ .

**Results:** More than 95% of all cows were examined in their second week of lactation. Across all investigated countries, the mean SCK prevalence was 24.1%, ranging from 8.3% (Colombia) up to 40.1% (New Zealand). The prevalence of production-related diseases detected during the first 21 days of lactation was relatively low (retained placenta 4.0%, milk fever 4.3%; metritis 5.3%, mastitis 3.4%; displaced abomasum 0.3%; claw diseases and lameness 1.7%; clinical ketosis 0.7%). Calculated odds ratios [OR; using TESTIMATE (version 6.5, IDV Datenanalyse & Versuchsplanung) according to following formula:  $\text{OR} = (p1/(1-p1)) / (p2/(1-p2))$ ] did not indicate an elevated risk for production diseases in cows with SCK.

**Conclusions:** Despite differences in production systems across countries and variation between individual farms within a region, the present data on SCK prevalence agree with observations in Western European and North American dairy herds. At the time of sampling and clinical examination, which was early in lactation, the occurrence of common production diseases was minor. Results might have been different at a later stage of lactation.

#### NU-P24

### Effect of injectable supplementation with copper and zinc on weight, hematological parameters and immune response in pre-weaning beef calves

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Cow-calf operations may be affected by trace mineral deficiencies, particularly copper (Cu) and zinc (Zn) deficiency, which may decrease the calf daily weight gain and modified hematological and immunological parameters. Salado's river basin is the most important area of Argentine devoted to cow-calves operations, and there are antecedents of Cu and Zn deficiencies. Both are clinically different at an advanced grade of deficiency, but cause similar consequences at the beginning, such as low weight gain, hematological changes and immune failure. Although the diagnosis of both deficiencies in the herd is based on the assessment of plasma Cu and Zn concentrations, there are discrepancies regarding data interpretation. To evaluate these possible consequences Aberdeen Angus calves ( $n=40$ , average BW  $99 \pm 8 \text{ Kg}$ ) clinically healthy were selected. They were kept as cow-calf pairs through the trial, started when calves had 3 months of age and finished at weaning time (7 months of age). The calves were assigned in two groups ( $n=20$  per group), and they were treated with a subcutaneous injection at d 0, d 40, d 80 and d 120 of the trial. At the same days, blood samples were taken and body weight (BW) of the animals was registered. The supplemented group (SG) was treated with copper edetate (0,3 mg/kg) and zinc edetate (1





mg/kg) (Suplenut® Biogénesis Bagó- Argentina), while the control group (CtlG) received a saline sterile solution. On d 40 and d 80 of the trial, all calves were vaccinated with inactivated bovine herpesvirus 1 (BHV1-Bioqueratogen Air® Biogénesis Bagó- Argentina). The vaccines were subcutaneously administered according to label directions. Blood samples were used to measure plasma Cu and Zn concentrations (n=20 per group) and hematological parameters (n=10 per group). At d 40, d 80 and d 120 BHV1 titers (n=10 per group) were evaluated by serum virus neutralization (SN) assay, and titers were reported as log<sub>10</sub> transformation of the reciprocal of the average greatest dilution that observed no cytopathic effect. Data were analyzed as a complete randomized design with repeated measures using PROC MIXED (SAS 9.1). Treatment increased plasma Cu but not Zn concentration (P <0.05 and >0.1, respectively), maintaining the Cu values higher than 60 µg/dL (adequate range) in the SG and lower than 20 µg/dL (severe deficiency range) in the CtlG. Zn plasma concentration did not show differences between groups and remained higher than 90 µg/dL, lower threshold limit value proposed as adequate range for several authors. Similarly, calves in SG had higher packed cell volume, mean corpuscular hemoglobin and mean corpuscular volume (P <0.05), and tended to increased hemoglobin concentration (P= 0.07). The BHV1 titers were significantly higher (P <0.05) in the SG on d 80 and d 120. Body weight, also was different in SG (Time by treatment interaction P <0.05) being 184 and 172 (±3.2) kg for the SG and CtlG respectively on d 120 of the trial. Cu deficiency, under conditions of this trial, caused less body weight at weaning time and immunological consequences, with early hematological changes; while Zn deficiency was not present. On the other hand, Cu and Zn plasma concentration were good indicators of risk. Finally, frequent Cu supplementation was necessary to prevent deficiency consequences in Salado River basin area.

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NU-P25

### Copper and Zinc Parenteral Supplementation in Pre-weaning Calves

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Beef cattle production represents the main economic activity of the Salado River basin (SRB), in the province of Buenos Aires, Argentina. This area covers 5.5 million hectares and produces two million calves a year. Animals are raised under an extensive system based on naturalized grass as the main source of nutrients. The economic benefit of the region resides on selling calves weaned at 6-7 months of age. Different authors have reported copper (Cu) and zinc (Zn) deficiency in the SRB, together with related effects such as decreased daily weight gain and hematological changes. Although the diagnosis of both deficiencies in the herd is based on the assessment of plasma Cu and Zn concentrations, there are discrepancies regarding data interpretation. Here we discuss whether plasma Cu and Zn concentrations are modified after parenteral Cu, Zn and Cu+Zn supplementation of pre-weaning calves, thereby altering daily weight gain and hematological parameters. A total of 40 clinically healthy Aberdeen Angus calves were used. They were

kept as cow-calf pairs since 0 day of the trial (three months of age) until weaning (month seven; 120 day of the trial). Calves were assigned to one of four homogeneous groups according to weight, sex and age (n= 20 each group), and treated as follows: Cu group (0.3 mg/kg Cu edetate); Zn group (1 mg/kg Zn edetate); Cu+Zn group (same doses of Cu and Zn edetate - Suplenut®, Biogénesis Bagó-); and control group (supplemented with saline sterile solution). The animals were subcutaneously injected every 40 days from November 2015 to March 2016 within 0, 40, 80 and 120 days, when plasma Cu and Zn concentrations, weight and hematological parameters were recorded. A completely randomized 2x2 factorial treatment design was used and data were analyzed with a mixed model for repeated measures over time. In groups with Cu supplementation (Cu and Cu+Zn groups), plasma Cu concentration increased after the second sampling (Cu x time interaction, p < 0.01). We also found Cu x Zn interaction (p = 0.09), being plasma Cu concentration higher in the Cu+Zn than in the Cu group (76.2 vs 73.4 µg/dL). In turn, plasma Cu concentration was lower in the Zn than in the control group (46.2 vs 50.7 µg/dL). In the case of plasma Zn concentration, it increased after Zn supplementation (Zn and Cu+Zn group, p= 0.02). In terms of weight, time differences were observed in the Zn-treated group, finding higher body weight after the second sampling (p < 0.02). Differences in weight gain (Zn x time interaction; p < 0.01) were observed in the Zn but not in the Cu group (p > 0.1). Regarding hematological parameters (erythrocytes, leukocytes and enzymes), no differences were detected in any of the four study groups. Cu plasma concentration was a good indicator of risk, since it remained into a marginal range (between 20 and 60 µg/dL - in unsupplemented Cu groups), indicative of depletion of the liver copper store, but not lower enough to provoke lower weight gain. Zinc plasma concentration was higher than expected in unsupplemented Zn groups, and with a narrow difference with supplemented groups (96.3 vs 103.9 µg/dL). Our results show that Zn supplementation improved body weight in pre-weaning calves raised in the SRB. Further research showing the importance of herd risk diagnosis based on plasma Zn concentration could contribute to preventing a lower body weight at weaning time.

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NU-P26

### Effects of oral calcium formate supplementation in peripartum dairy cows

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**Objectives:** The objectives of the study were to evaluate the effect of calcium formate supplementation (Calfon Oral®; Bayer Animal Health) on calcium concentration in blood, subclinical hypocalcemia (SHC), ketosis, displacement of abomasum (DA) and milk production in early-lactation dairy cows.

**Materials and Methods:** The study was carried in two commercial dairy farms in Castro county, Parana State, Southern Brazil, 264 Holstein cows (164 multiparous and 100 primipara-



rous) were blocked by herd, parity, and total calcium (tCa) status 6 h after calving. Blood samples were analyzed for group allocation (normal and hypocalcemia groups) using 8.7 mg/dL as the cutpoint (IDEXX VetTest® Chemistry Analyser, Inc., Westbrook, ME). Within each block, fresh cows were randomly allocated to treatment (T; n= 130) and control (C; n=134) groups, with treated-cows being supplemented twice, 6 and 31 hours after parturition, with 350 ml of 14.3% (w/w) calcium as a 48.6% aqueous suspension of calcium formate (Calfon Oral®, Bayer Animal Health). Six blood samples from each animal were collected (in the medium time of 6, 17.5, 30, 53, 77, and 100 h after calving) for determination of tCa and ionic calcium (iCa). Blood samples for BHBA analysis were collected on days 3, 5 and 7. The displacement of abomasum was diagnosed by examining the left flank of the cow, through by auscultation of the metallic "ping" sound on trough the percussion with the finger. Total calcium concentration was determined in laboratory of Universidade Federal do Paraná, through an automated biochemical analyzer, using the colorimetric method (Arzenazo III, Dialab®). For iCa the method used was gasometry by selective ion electrode (RAP-IDLab® 348 EX Blood Gas Analyzer, Siemens Healthineers). Milk production was measured daily after parturition was through Delpro herd management software (DeLaval®). The cut point for the determination of subclinical hypocalcemia through laboratory analysis was tCa < 8.0 mg/dL (2.0 mM). The cut point in IDEXX VetTest® (8.7 mg/dL) was established through the optimum sensitivity (54.8) and specificity (86.8) with the analyse of tCa in the laboratory, using receiver operator characteristic (ROC), with MedCalc software (version 14.8.1). The results were submitted to analysis of variance and the mean values were compared by the Tukey test, using the GLM method of the SAS program (v.9.4). Data was analyzed using MIXED procedure of SAS with a model containing the effects of treatment, time, and treatment\*time interaction as fixed effects and cow within treatment as a random effect. Differences with  $p < 0.05$  were considered significant and 0.05

**Results:** Hypocalcemia incidence rates were 39% using on-farm tCa from VetTest (< 8.7 mg/dL), 64% using tCa (< 8.0 mg/dL) and 76% using iCa (concentration < 4.0 mg/dL), with the lowest iCa values being observed at 17.5 h postpartum. Serum iCa values were higher ( $P=0.02$ ) in oral Ca formate-treated cows; 3.68 vs. 3.52 ± 0.01 mg/dL for the controls. Subclinical ketosis (serum BHBA >1.2 mmol/L) incidence rate was 23.8% (63/242). Estimates of BHBA on day 5 were lower ( $P<0.01$ ) for treated cows; 0.70 vs. 0.91 ± 0.06 mmol/L for the non-treated ones. The incidence rate of DA was 5.7% (15/264), with the highest occurrence in group C (12/15) compared to the T group (3/15) ( $P < 0.05$ ). In the average milk production in the first 100 days of lactation, there was a trend of higher production for the multiparous of the T group in relation to C (T = 44.8 kg vs. C = 44.1 kg of milk;  $P = 0, 08$ ).

**Conclusions:** The results for subclinical hypocalcemia in both farms were high. The oral calcium formate supplementation had shown modest, but beneficial effects in early-lactation dairy cows, with an increasing ionic Ca, reducing BHBA concentrations, incidence the displacement of abomasum and effect in milk production. Important goals to control metabolic disorders in dairy farms.

NU-P27

### Impact of Management Practices on blood calcium levels in dairy cows at dry off – results from France and Denmark

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Different authors<sup>1-3</sup> have evaluated the incidence and interactions of hypocalcemia at calving. Despite of the increasing milk production at the time of dry-off, an interaction with Ca levels in association with dry-off management practices around dry-off has not been evaluated. Normal blood calcium levels at calving are debated but suggested to be normal when >2,2 mmol/L, as subclinical hypocalcemia (SHC) in the range of 1,5 – 2,2 mmol/L and clinically low when <1,5 mmol/L<sup>1</sup>. Cows with SHC have no clinical signs but may be more susceptible to disease both at calving and dry-off.

**Objective:** This study aims to assess the subclinical health status of dairy cows at drying-off by recording the levels of biochemical parameters (calcium and others) after the last milking in dairy cows in France and Denmark. Additionally, to identify risks factors related to the drying-off management procedures leading to the emergence of hypocalcemia at this stage of the lactation cycle.

**Materials and methods:** This is a multicenter prospective cohort study involving 14 investigator sites including 381 dairy cows from 37 herds in France and 345 cows from 21 herds in Denmark. Biochemical parameters (total Calcium and others) were assessed by two blood samplings; one immediately (BS1) after the last milking and another 8 - 12 hours later (BS2). All dairy cows dried off either for future lactation or for culling during the study duration were included. Information on disease events, production parameters, feeding and management practices at herd and cow level were collected. Specifically, the interaction between dry-off methodology and incidence of SHC at BS2 was assessed. Abrupt dry off implying no prior change in either milking frequency or feeding regimen. Gradual implying any change in these factors.

**Results:** Calcium levels at BS1 and BS2, absolute and relative variation between BS1 and BS2 and evolution in classes at cow level (Decrease, Stable and Increase) will be shown in the poster.

The overall percentage of cows that were in SCH at BS2 was 11,8% (45/381) in France and 15,6% (53/339) in Denmark. Risk of SHC at BS2 was affected by dry off method. Cows dried off gradually in France had an incidence of SHC at BS2 at 17% (35/201), in Denmark 18% (32/175). Cows dried off abruptly had a lower incidence of SHC at BS2 in France 5,5% (10/180) and in Denmark 13,6% (21/154).

**Conclusions:** This study demonstrates that reduction of Ca levels after dry-off occurs with varying incidence across countries, and that a gradual dry off increases the likelihood that cows will have subclinical hypocalcemia in the hours after dry off.

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NU-P28

### **Influence of the placental parathyroid hormone-related protein for parturient disorders in dairy cows.**

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**Objectives:** The concentration of parathyroid hormone-related protein (PTHrP) in the blood of healthy mammals is extremely low or below the detection limit. However, circulating PTHrP concentrations are elevated during lactation, and it has been suggested that PTHrP contributes to Ca homeostasis of mothers<sup>[1]</sup>. It is not clear whether circulating PTHrP in dairy cows increase during lactation and influence parturient disorders. To answer these questions, this experiment investigated whether the placental PTHrP reaches the maternal circulation.

**Materials and methods:** Umbilical cord blood (arterial and venous) of fetus and jugular vein blood of Holstein cows were obtained at the operations of Caesarean section from dystocia (n = 12). The isolated serum and plasma were employed as samples, and PTHrP, Ca, iP and trace minerals were measured. The placenta tissues were collected and PTHrP mRNA expressions were evaluated by quantitative real-time PCR.

**Results:** Maternal plasma concentrations of PTHrP were under the detection limit of the assay. Plasma PTHrP concentrations of umbilical cord artery and vein were higher than those of maternal jugular vein. Serum Ca and iP concentrations were statistically higher in fetus than those in dams. PTHrP mRNA expression of placenta was higher in maternal side than that in fetus side.

**Conclusions:** Our previous studies identified PTHrP derived from the lactating mammary gland did not detect in the maternal circulation<sup>[2]</sup>. Present experiment demonstrated that PTHrP gene expression was high in the placenta and PTHrP detected in the circulation of fetus, not of dam. These results suggested that placental PTHrP does not influence to the parturient disorders like retained placenta and milk fever in dairy cows.

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NU-P29

### **Post-partum calcium levels in pure Holsteins and crossbred dairy cows after Vitamin D injection**

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The periparturient period of the dairy cow is dominated by huge metabolic challenges, needed to support high milk yield. Calcium metabolism is one of the most affected, resulting in a high proportion of cows experiencing a reduction in their ability to maintain physiological plasma calcium levels. Several methods have been applied to prevent post-partum hypocalcemia, but none has proved to be 100% effective. The administration of vitamin D<sub>3</sub> just before calving is an easy and cheap method that deserves further studying. For this study 153 cows, with more than one lactation and belonging to three intensive dairy farms were divided into two groups – 83 cows received one injection of vitamin D<sub>3</sub> between 2 and 8 days before calving and 70 cows received a placebo at the same moment. Two farms (A and B) raised only pure Holstein cows, and one of these (Farm B) used anionic diets during the last stage of the dry period. The third farm (C) only had crossbred cows (Holstein, Montbéliard and Swedish Red).

Blood samples for total calcium measurement were collected six to twelve hours after uneventful calving and before any medication was applied. Milk yield at 100 days in milk (DIM), days to first insemination, number of inseminations for conception and prevalence of diseases such as metritis, placenta retention, mastitis, ketosis and abomasum displacement were recorded. Calcemia was significantly higher in treated groups, overall (7.98 ± 1.12 mg/dl vs 7.23 ± 1.21 mg/dl) and in each farm. Calcemia was significantly higher in treated and control groups in farm B (8.30 and 7.82 mg/dL) and C (7.98 and 7.17 mg/dL), compared with the corresponding groups from farm A (7.59 and 6.78 mg/dL).

It was also shown that sub-clinical hypocalcemia and the positive effect of treatment with vitamin D<sub>3</sub>, increases with age. No difference between groups was found in milk yield at 100 DIM and in reproduction measures. Probably because of very low prevalence in these well-run farms, no differences in post-partum diseases between groups were shown, contrary to what is mentioned in the literature.

In summary it was demonstrated that the use of vitamin D<sub>3</sub> will help sustain higher calcium blood levels post-partum, especially in herds using anionic diets during the last stage of the dry period. It was concluded that the use of vitamin D<sub>3</sub> may be particularly useful in farms where postpartum infectious or metabolic diseases, for which hypocalcemia has proven to be a predisposing factor, are frequent.

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SP-P01

### Cattle reproductive performance in different production systems in North Spain

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**Objectives:** Organic farming is highly dependent on local resources and is very conditioned by a strict regulation related to the use of chemical products and allopathic treatments. These factors may necessary to breed animals properly adapted to organic conditions. Last decades selection of dairy cattle nearly exclusively focused in Holstein-Friesian for milk production resulting in cows with a low reproductive performance. The present study aimed to evaluate reproductive performance (number of services per conception, calving interval, calving to first service interval, calving to conception interval, age at first service, age at first conception and age at first calving) of Holstein-Friesian cows in organic farming in Northern Spain compared with conventional systems (pastured-based and zero-grazing) and other breeds or crosses organically reared.

**Material and Methods:** Data on which this paper is based were collected in a subset of representative organic (n=10), conventional pasture-based (n=5) and conventional zero-grazing (n=5) farms in Northern Spain. American Holstein-Friesian was the only breed present in all the conventional and in 3 organic farms. The other 7 organic farms had different breed composition including Holstein-Friesian, pure-breeds or crosses. Data related to reproductive parameters were collected from 2013 to 2017 during visits to farms or using Milk Recording System; farmers included in this study were also asked some questions related to reproductive management. Statistical analysis was performed using Linear Mixed Models for comparing reproductive performance of different systems and breeds.

**Results:** Farmers were asked about reproductive management. The principal differences between systems were the use of reproduction treatments and estrous detection. On one side organic farmers do not use reproduction treatments, with the exception of cows in severe illness, and pasture-based conventional farmers use few or none reproduction treatments. On the other side, zero-grazing conventional farmers use many treatments to absolutely control the reproduction by programming or synchronizing reproduction. Other important point where production systems differ is the management of heat detection. Organic and zero-grazing systems have a clear pattern to detect estrous whereas in pasture-based systems farmers try to detect estrous while doing other activities.

Respect to Holstein-Friesian performance in different production systems, statistically significant differences have been found for calving to first service and calving to conception, being shorter in organic managed cattle compared to pasture-based conventional cattle, zero-grazing conventional cattle showing values in between both. With regard to breed performance Holstein-Friesian cows showed significantly higher calving interval, higher calving to first service and to conception intervals than other breeds (either pure or crossed).

**Conclusions:** Our results indicate that in Northern Spain Hol-

stein-Friesian cows in organic systems show a reproductive performance similar to those in zero-grazing conventional systems, although clearly better in terms of estrous detection than those in pasture-based conventional systems. When Holstein-Friesian cows are compared with other breeds or crosses under the same organic management, worse results were obtained only for calving interval, calving to first service and calving to conception intervals when compared with crosses, which indicate again that the estrous detection should be carefully managed in the farm to improve reproductive performance. Apart from this, no reproductive parameter evaluated in our study indicates that Holstein-Friesian cows performed worse from a reproductive point of view than other breeds or crosses in the organic farms, or that their counterparties in the conventional systems.

SP-P02

### Evaluation of trace element status of organic dairy cattle

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**Objectives:** Organic farming promotes the use of local resources and limits the application of chemicals, including inorganic mineral supplements (Council Regulation (EC), 2007). In organic farming, animal nutrition is therefore highly dependent on local geographical conditions and mineral deficiencies may occur in certain areas due to the low availability of some trace elements. The present study aimed to evaluate trace mineral status of organic dairy herds in northern Spain and the sources of minerals in different types of feed.

**Material and Methods:** Blood samples from organic and conventional dairy cattle and feed samples from the respective farms were analysed by inductively coupled plasma mass spectrometry to determine the concentrations of the essential trace elements (cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), iodine (I), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn)) and toxic trace elements (arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb)). Multivariate chemometric analysis was conducted to determine the contribution of different feed sources to the trace element status of the cattle.

**Results:** Overall, no differences between organic and conventional farms were detected in serum concentrations of essential and toxic trace elements (except for higher concentrations of Cd on the organic farms), although a high level of inter-farm variation was detected in the organic systems, indicating that organic production greatly depends on the specific local conditions. The dietary concentrations of the essential trace elements I, Cu, Se and Zn were significantly higher in the conventional than in the organic systems, which can be attributed to the high concentration of these minerals in the concentrate feed. No differences in the concentrations of trace minerals were found in the other types of feed. Concentrate samples were mainly associated with Co, Cu, I, Se and Zn (i.e. with the



elements supplemented in this type of feed). However, pasture and grass silage were associated with soil-derived elements (As, Cr, Fe and Pb) which cattle may thus ingest during grazing.

**Conclusions:** The study identified two main sources of trace elements: (i) concentrate feed as the main source of trace elements included in the mineral supplements at concentrations higher than in the other feedstuffs (mainly, Co, Cu, I, Se and Zn), thus preventing mineral deficiencies, and (ii) ingestion of soil (during grazing or consumption of soil contaminated forage) as the main source of trace elements that are not supplemented (Cr, Mo and Ni), trace elements supplemented but present at higher.

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SP-P03

### Dairy cow nutrition in organic farming systems

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**Objectives:** Adequate provision of suitable feed is one of the primary objectives of farmers to ensure the health of their livestock. In conventional dairy farming, the nutritional requirements of cows, even highly productive cows, are usually covered, because the diets are generally supplemented with concentrate feed that provides high amounts of energy and protein. However, in organic dairy farming the aim is to optimize available resources rather than maximize production, so that maximum use must be made of forage. The present study aimed to evaluate the composition of diets used on organic dairy farms and to establish the dietary energy-protein balance. Exhaustive information about diets was obtained from organic (ORG), conventional grazing (GRZ) and conventional no-grazing (CNG) farms in northern Spain.

**Material and Methods:** Information about the types and quantities of the different feedstuffs consumed was obtained from each farm. Feed samples (duplicate samples of each type of feed from ORG (n=244), GRZ (n=52) and CNG farms (n=50) were analyzed using near infra-red spectroscopy (NIRS). Multivariate analysis was used for comprehensive assessment of the differences between types of farm based on the data set including all cases considered in the study.

**Results:** Overall, milk yields and dry matter intake were lower on the ORG farms than on the conventional farms. More than 80% of the ration used on ORG farms was composed of forage, whereas the corresponding proportion was significantly lower on CNG farms (63%). On the ORG farms, forage was mainly provided by pasture, whereas on CNG farms maize silage was the main type of forage provided. The ration used on ORG farms had significantly higher percentage of ADF and lower organic matter content than the rations used on both conventional types of farms, indicating that the diets were less digestible. Although the protein concentration of the diets used on the grazing farms (ORG and GRZ) was higher than on CNG farms, the protein intake was similar. Due to the low level of energy provided by the ORG diets, the results showed an imbalance

between energy and protein: higher **PDIN** (protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-degraded dietary nitrogen) than **PDIE** (protein digested in the small intestine supplied by rumen-undegraded dietary protein and by microbial protein from rumen-fermented OM). The efficiency of protein use was lower for the diets used on ORG farms than for those on both conventional types of farm, yielding less milk per kg of CP intake.

**Conclusions:** The diets used on organic and conventional farms are very different, especially regarding the ingredients used, which allowed the three systems to be differentiated in the multivariate analysis. The DMI was lower on the organic farms and energy intake was therefore also lower, because of the lower percentage of concentrate and maize silage used in the diets. Although the protein intake was similar in both conventional and organic systems, the protein use efficiency was lower on organic farms, probably because of the lower amounts of energy provided by the diets.

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SP-P04

### Simulation models for the evaluation of the carbon footprint of alternative production systems to the traditional cow-calf system

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**Objective:** The aim of this study was to evaluate the carbon footprint of two alternative production systems for the traditional cow-calf system (TCCS) used in the South region of Chile, through of use of simulation models.

**Materials and methods:** Two alternative systems were proposed, the first of which corresponds to the single-calf heifer system (SCHS). The second system is the All Heifer No Cow system (AHNCS) which was proposed by Seidel and Whittier (2015) as an alternative to minimize the inefficiency of traditional meat production. SCHS consists of not keeping cows for breeding, which is achieved through the retention and rearing of all heifer calves born and the purchase of heifers to complete a stable number of animals during the time of parturition, the sale of all steer calves and the sale of heifers after the first calving. AHNCS corresponds to an improvement of the SCHS. This system incorporates the synchronization of estrus, artificial insemination and the use of sexed semen for the production of heifer calves.

The cradle-to-farm-gate methodology (FAO, 2010) used by Toro-Mujica *et al.*, (2017) was used to estimate the system's Carbon Footprint (CF). Using this methodology and taking as a base the model proposed by Catrileo *et al.* (2009) to evaluate management modifications in TCCS on temperate pastures in Chile, two simulation models were developed. In TCCS, the products correspond to heifer calves and steer calves of 6-8 months of age (180-200 kilos) and culled cows of between 400-500 kg. For the representation of SCHS and AHNCS, a model was developed granting flexibility to the use of artificial insemination and sexed semen. In both systems, the products that the system generates correspond to first-calf heifers and steer



calves about 3 months old (Seidel and Whittier, 2015; Sell et al., 1988). The pastures used to simulate with the model corresponded to pastures of fescue with subterranean clover (Rojas and Romero, 1990), besides, intake of supplements in strategies stages was considered. The emission factors used by Toro-Mujica *et al.*, (2017) and/or derived from the methodologies proposed for Tier 2 by IPPCC (2006) were used to estimate methane emissions, nitrogen oxide emissions, emissions associated with supplementary feed and emissions associated with pastures fertilization. To estimate the CF of heifer purchase an average value of 37.5 kg CO<sub>2-eq</sub> per kg of live weight of heifer was used (Nijdam et al., 2012). Soil carbon sequestration was assumed to be 10% of the carbon deposited in the soil by fertilization (Petersen *et al.*, 2013). In the case of organic matter deposited in the soil by senescence of the pasture, it was assumed that 15% of the organic matter is converted to organic matter in soils (Lal, 1997). The functional unit used to estimate the CF was defined as kg CO<sub>2-eq</sub> /kg of live weight (LW) at the farm gate (Florindo *et al.*, 2017). Scenarios with modification of stocking rate and level of supplementation were simulated. Runs with and without carbon sequestration were conducted.

**Results:** An ANOVA test showed significant effects of the production system, stocking rate and consideration of carbon sequestration over the carbon footprint ( $p < 0.01$ ). Mean differences between the three systems studied were observed. Averaged across all scenarios, the production system with the lowest carbon footprint was AHNCS (11.8 kg CO<sub>2-eq</sub> /kg of LW) followed by the SCHS (17.1 kg CO<sub>2-eq</sub> /kg of LW) and then by TCCS (28.1 kg CO<sub>2-eq</sub> /kg of L. Carbon sequestration decreased the carbon footprint in AHNCS (4.1%) and SCHS (5.1%) and increased the CF in TCCS (8.2%). The increase of stocking rate increased the CF in TCCS, but in SCHS and AHNCS clear effect was not observed. Not considering the carbon sequestration attenuate the effect of stocking rate.

**Conclusions:** The alternative systems decreased the CF of meat production systems. The AHNCS is the one with the better results. The effect of increasing the stocking rate is important in the TCCS, especially when the carbon sequestration was considerate. It showed that the currently used level of stocking rate is in equilibrium with the pastoral resources.

PH-P01

## Regulatory and Non-regulatory Tissue Residue Levels for Feedlot Cattle Fed Ractopamine with Terminal Withdrawal Times of 12 Hours, 2, 4 and 7 Days

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In the United States, cattle fed in confinement can receive ractopamine hydrochloride for the last 28 to 42 days on feed with 0 days (12 hours from last feeding to slaughter) of withdrawal time. Liver and muscle tissue are the official tissues tested to ensure that any residual ractopamine is below established safe levels. The amount of ractopamine in other edible tissues is assured to be within safe limits when ractopamine in these "regulatory tissues" is below tolerance. Many other "non-regulatory" edible tissues are exported with the importing countries sometimes testing these tissues directly and using regulatory tissue tolerances as standards for acceptance. As a precaution against having their products potentially declared in violation, some U.S. cattle producers have extended ractopamine withdrawal times to 2 or more days. From a scientific perspective, there is very little published data on the impact of extending withdrawal times on the level of residual ractopamine in "regulatory tissues" and virtually no data on the level of ractopamine in "non-regulatory" tissues even at the 0 day withdrawal time.

The objective of this research was to determine the concentration of ractopamine and total ractopamine related residues (ractopamine + ractopamine metabolites) in tissues from animals fed ractopamine hydrochloride at the highest approved dose and duration after 12 hours, 2 days, 4 days, and 7 days of withdrawal. Commonly exported tissues with no previously described regulatory levels were sampled and analyzed as well as liver and muscle (regulatory tissues).

Tissues Collected: liver\* (a U.S. regulated tissue), muscle\* (a U.S. regulated tissue), rumen/reticulum, omasum, abomasum, small intestine, large intestine, heart, tendon, tongue

\*Residue data for tissues were not collected from the 0-day (12 hour) withdrawal group because data for these tissues at this withdrawal time are available.

**Design:** In-life: The study design for the in-life phase was run in two identical phases, consisting of 6 pen blocks (30 pens) of yearling steers totaling 1,200 to 1,500 head in each phase. One phase began in August 2017 and one began in October 2017. Tissues from animals from the phase starting in October 2017 were collected and analyzed for this study.

**Sample Collection:** A set of 10 samples (from each of the tissues listed above) were collected from 2 randomly selected animals in each pen of treated animals (for a total of 12 animals per treatment group times 4 treatment groups - 48 animals) and 1 animal per pen for untreated animals (for a total of 6 animals).

**Residue Analysis:** Tissues were cryogenically homogenized and tissue homogenate was extracted. Samples were analyzed twice, once for ractopamine (the marker residue for which regulatory levels have been set) and once for total ractopamine related residues (ractopamine + metabolites). Samples were analyzed by LC-MS/MS using a system comprised of a Waters



Synapt G2-Si with a standard flow ESI source coupled to a Waters Acquity I-Class UPLC equipped with a reverse phase 1.0 mm x 50 mm Waters Acquity UPLC HSS T3 column (1.8  $\mu$ m particles). The results for ractopamine concentration and total ractopamine concentration in each tissue type were analyzed separately using a general linear mixed model. The ractopamine concentration and total ractopamine concentration were log transformed [Ln(x)] prior to analysis. The analysis of variance model included fixed effects of tissue, withdrawal time, and the random effect of block.

**Results:** To be presented at WBC.

PH-P02

### Effects of Different Ractopamine Withdrawal Periods Pre-harvest on the Performance and Carcass Merit of Yearling Steers

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In the United States, ractopamine hydrochloride (Actogain™) is approved to be fed to cattle during the last 28 to 42 days of the confinement period with a 0 day (12 hours from last feeding to slaughter) withdrawal period pre-harvest. The benefits of Actogain include improved rate of weight gain, improved feed efficiency, and increased carcass leanness. To minimize potential residue concerns when marketing beef and beef products to foreign markets, some U.S. cattle producers have considered withdrawing ractopamine from rations for 2 days or longer. However, the potential effects of various extended withdrawal periods on cattle performance and carcass merit have never been reported.

The objective of this study was to compare the potential effects of different ractopamine withdrawal times on performance and carcass merit in yearling feedlot steers. Approximately 3,000 yearling steers were weighed and randomly assigned to one of five treatment groups upon feedlot arrival by blinded feedlot personnel. Diets including Actogain were formulated to contain 24.6 g/ton of ractopamine (90% dry matter basis) with a targeted consumption of 250-275 mg/head/day.

Treatment groups were fed Actogain for 33 days but with different withdrawal periods prior to harvest. Group 1: Actogain feeding was initiated at 33 days and was removed from the diet 12 hours prior to harvest. Group 2: Actogain feeding was initiated at 35 days and was removed from the diet 2 days prior to harvest. Group 3: Actogain feeding was initiated at 37 days and was removed from the diet 4 days prior to harvest. Group 4: Actogain feeding was initiated at 40 days and was removed from the diet 7 days prior to harvest. Group 5: No Actogain was included in the diet (negative control). The confined feeding period lasted approximately 100 days. Data was analyzed as a randomized-block design.

Results will be reported for performance (i.e., average daily gain, feed efficiency, final body weight) and carcass metrics (i.e., weight, grading). This study will provide nutritionists and cattle producers with options regarding the use of different withdrawal periods after feeding ractopamine hydrochloride to

feedlot cattle.

PH-P03

### Detection method of diclofenac sodium residues in bovine edible tissues

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The HPLC-ESI<sup>+</sup>-MS/MS quantitative detection method of diclofenac sodium in the bovine edible tissues was established by using <sup>13</sup>C isotope markers of diclofenac sodium as internal standard, organic solvent to extract residual markers, n-hexane to eliminate fat. The result showed that the LOD and LOQ of diclofenac sodium were 0.3 and 0.3  $\mu$ g/kg in the muscle, liver, kidney and fat tissue, and concentration range were 0.5 ~ 500  $\mu$ g/kg in muscle, liver and kidney and 0.5 ~ 100  $\mu$ g/kg in fat. Moreover, it has good linear relationship and the correlation coefficient is greater than 0.99. In muscle and liver, diclofenac sodium concentrations were added as 0.5 (LOQ), 2.5 (1/2 MRL), 5.0 (MRL), 10.0 (2 MRL) and 200.0  $\mu$ g/kg, and the sample recovery rate was between 90% ~ 120%, coefficient of variation between batch and batch are less than 10%. In kidney, diclofenac sodium concentrations were added as 0.5 (LOQ), 5.0 (1/2 MRL), 10.0 (MRL), 20.0 (2 MRL) and 200.0  $\mu$ g/kg, and the sample recovery rate was between 95% ~ 115%, coefficient of variation between batch and batch are less than 10%. In fat, diclofenac sodium concentrations were added 0.5 (LOQ, 1/2 MRL), 1.0 (MRL), 2.0 (2 MRL), 5.0 and 50.0  $\mu$ g/kg, and the sample recovery rate was between 90% ~ 110%, coefficient of variation between batch and batch of less than 10%. The results showed that the method is stable and reliable, simple to meet inspection requirements of diclofenac sodium residues (MRL was 5, 5, 10 and 1  $\mu$ g/kg, respectively) in the bovine edible tissues (muscle, liver, kidney and fat). It can be used for determination of diclofenac sodium residues in bovine edible tissues, and after diclofenac sodium injection was used, residue elimination regulation of diclofenac sodium in bovine edible tissue was studied with this method.

PH-P04

### Perception, attitude and demeanour about biosecurity by dairy farmers in Chile

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**Objective:** Biosecurity in animal production has become increasingly important, since the efficiency in different areas of the agricultural sector, such as animal health, food safety, environment and animal welfare depend on the correct implementation and implementation of biosecurity protocols. In Chile, biosecurity procedures in dairy farms are less advanced than more intensive systems such as the poultry and swine industries. For producers, biosecurity measures may or may not be



relevant, and this depends on their knowledge and interest in the subject. Therefore, the objective of this study was to know the perception of dairy farmers of central Chile regarding biosecurity, evaluating their attitude and demeanor in relation to this important topic, and discussing their potential interventions.

**Material and Methods:** The study was carried out in 30 dairies from central Chile, within an area of 100 km around the coordinates 33.7 ° S; 70.6 ° W. All dairies met the requirements of being intensive, with permanent confinement and with more than 100 lactating cows. The area is characterized by a temperate climate with 4 well defined seasons. A survey based on a questionnaire consisting of 18 questions was implemented as the primary analytical technique. The questionnaire was based on individual interviews carried out on farms. The target population was the owners of dairy farms, or in their absence to representatives of the dairy in the field, such as managers or administrators.

**Results:** The median number of lactating cows per farm was 272, and the number of workers was 12. Regarding the respondents, 53% were administrators, 33% were managers, and only 13% were owners. Ninety-three percent were men and 7% were women. The median age was 47 years, (28 to 83 years). In terms of the education level, the highest was a technical degree, ranging from middle school to some type of specialty in dairy business. Of the 30 respondents, 77% (23/30) replied that they had heard of the concept of biosecurity. Of the 23 individuals, 61% (14/23) indicated that they understood the concept but did not have the ability to fully explain it. This majority was followed by 26% (6/23) who indicated that they had heard of the concept but did not know what they were referring to, 9% (2/23), which indicated that they understood the concept but did not have the ability to explain it or mention some of its action measures, and 4% (1/23) that claimed to understand the concept perfectly, could explain it and mention its main measures of action. Most respondents associated biosecurity with environmental management and animal welfare. Respondents placed the environmental management as the least important within the dairy. Regarding the perception of biosecurity within their own dairy, 53% (16/30) answered that their biosecurity level was "good", and 33% (10/30) considered it "regular". Fifty percent (15/30) had a "good attitude" towards biosecurity, followed by 23% (7/30) who had "very good" and "regular attitude". Overall, 73% of the respondents had a "very good attitude" or "good attitude" toward building biosecurity programs. Eighty-three percent (25/30) indicated that they had made changes focused on improving the biosecurity of their farm. Only 50% (15/30) believed that the veterinarian had an active role in biosecurity issues. However, 27% (8/30) indicated that veterinarians had a passive role because they only reported on biosecurity when asked about the issue and therefore were not proactive.

**Conclusions:** This study provides important information on what Chilean managers believe about biosecurity. It is a first approach to focus not only on facilities, good practices or animals, but also those who direct and make decisions on the farm.

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**Objectives:** Post-mortem examinations provide necessary information pertinent to the wholesomeness of meat. In terms of food safety, it is important to determine threat levels posed to humans by individual organs and categories of ruminants. The aim of the study was to evaluate the results of veterinary examinations of cattle carried out at a selected slaughterhouse in the Czech Republic and to determine the risk of detection of patho-anatomical changes in internal organs.

**Materials and methods:** The monitoring was carried out at a selected slaughterhouse in the Czech Republic in the period from 2009 to 2016. Data on numbers of slaughtered animals and individual findings were obtained from records made by an official veterinarian operating at the slaughterhouse and carrying out bovine post-mortem examinations. For the purposes of the study, changes in lungs, gastrointestinal tract, liver, kidneys, genital organs, heart, spleen, central nervous system, and other changes in internal organs have been assessed and classified into one of three categories: acute, chronic, parasitic. All data were analysed using the statistical package Unistat v. 6.5. (Unistat Ltd., GB). Statistical comparisons between frequencies of the categorical variables of interest were performed with the Chi-square test within the Contingency table procedure.

**Results:** During the monitored period, 4,307 cows, 620 heifers, 3,469 bulls and 633 calves were slaughtered. Between individual categories of cattle, statistically significant ( $P < 0.05$ ) differences were found in the numbers of patho-anatomical changes detected in internal organs. The most frequent findings in cows were changes in liver (22.87%), lungs (12.14%) and kidneys (4.02%), these changes were predominantly of chronic origin. In heifers, the most frequent findings were changes in lungs (6.45%), liver (2.58%) and kidneys (2.58%) with prevailing chronic changes. Post-mortem examinations of bulls most frequently revealed changes in lungs (6.08%), liver (3.92%) and kidneys (1.24%), predominantly of chronic origin. In calves, the most frequent findings were changes in lungs (43.44%), liver (5.82%) and heart (2.37%) - predominantly chronic, followed by changes in gastrointestinal tract (1.90%), namely both acute and chronic changes in intestines, and changes in kidneys (1.42%) with chronic changes being prevalent.

**Conclusions:** Post-mortem examinations of bovine internal organs most frequently revealed changes in liver, lungs and kidneys, and in calves also changes in gastrointestinal tract and heart. In the vast majority of cases, these were chronic changes indicative of disorders arising from conditions on farms. The results are important for those involved in veterinary examinations of internal organs of cattle at slaughterhouses in the Czech Republic in terms of the practical veterinary determination of the risk of detection of patho-anatomical changes in cattle and its possible reduction by measures adopted on farms and in transit.

PH-P05

### Patho-anatomical changes in organs determined during bovine post-mortem examinations in the Czech Republic





PH-P06

## Musculoskeletal system findings in cattle slaughtered in the Czech Republic

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**Objectives:** The results of animal and meat inspection at slaughterhouses indicate possible risks due to unsafe meat obtained from bovine carcasses at slaughterhouses. The aim of the study was to assess the results of veterinary inspection carried out at a selected slaughterhouse slaughtering cattle and to determine the risk of detection of patho-anatomical changes during veterinary examinations of carcasses.

**Materials and methods:** The monitoring was carried out at a selected slaughterhouse in the Czech Republic in the period from 2009 to 2016. Data on numbers of slaughtered animals and individual findings were obtained from records made by an official veterinarian operating at the slaughterhouse and carrying out bovine post-mortem examinations. For the purposes of the study, changes in limbs, torso, head and skin (classified into one of three categories: acute, chronic, traumatic) and also occurrence of abscesses, emaciation, cysticercosis and other changes have been assessed. All data were analysed using the statistical package Unistat v. 6.5. (Unistat Ltd., GB). Statistical comparisons between frequencies of the categorical variables of interest were performed with the Chi-square test within the Contingency table procedure.

**Results:** In the period from 2009 to 2016, 4,307 cows, 620 heifers, 3,469 bulls and 633 calves were slaughtered at the monitored slaughterhouse. Between individual categories of cattle, statistically significant ( $P < 0.05$ ) differences were found in the numbers of patho-anatomical changes detected in the musculoskeletal system. In cows, the most frequent findings during veterinary examinations at the slaughterhouse were changes in limbs (1.95%) and torso (1.74%), these were predominantly chronic changes. Emaciation was detected in 1.16% of cows. In heifers and bulls, the most frequent findings were changes in limbs (1.94% and 1.21, respectively) with chronic changes being prevalent in both categories. In calves slaughtered at the monitored slaughterhouse, the most frequent findings were changes in torso (1.42%), predominantly of chronic origin. Emaciation was detected in 26.22% of calves.

**Conclusions:** The most frequent patho-anatomical changes in musculoskeletal system in cattle were found in limbs; in cows and calves also changes in torso and emaciation were frequently detected. Changes of chronic origin were predominant and indicative of possible welfare problems on farms. In calves, the dominant finding was emaciation (26.22%). Emaciation affects the quality of meat and carcass and poses a threat to the health of the consumers as it could indicate serious underlying health problems.

RE-P01

## Relationships of plasma insulin-like peptide 3, testosterone, inhibin and insulin-like growth factor-I concentrations with scrotal circumferences and testicular weights in Japanese Black beef bull calves

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(Kitagawa and Sakase contributed equally in this study.)

**Objective:** This study was conducted to clarify relationships of plasma insulin-like peptide 3 (INSL3), testosterone, inhibin and insulin-like growth factor-I (IGF-I) concentrations with scrotal circumferences and testicular weights in Japanese Black beef bull calves from the birth to pre-pubertal period.

**Materials and methods:** Blood samples were collected monthly from Japanese Black beef bull calves ( $n=20$ ) from 0 to 7 months of age. Scrotal circumferences of the calves were measured monthly from 1 to 7 months. The calves were castrated at 7 months and testicular weights were recorded. Plasma INSL3 and inhibin concentrations were assayed by TRFIA and testosterone and IGF-I concentrations were measured by EIA.

**Results:** The plasma INSL3 concentrations progressively increased from 0 to 7 months (0 vs 1 month, 1 vs 3 months, 3 vs 7 months,  $P < 0.05$ ), thus the increment of INSL3 seems relatively constant during the whole period. On the other hand, plasma testosterone concentrations did not change significantly from 0 to 4 months but increased ( $P < 0.05$ ) from 0 to 5 months and from 5 and 7 months, indicating no clear rises of testosterone until 4 months. Plasma inhibin concentrations decreased ( $P < 0.05$ ) from 0 to 3 months and from 3 to 6 and 7 months. The change of inhibin concentrations implies a constant decline of the hormone during the whole period. Plasma IGF-1 concentrations rose ( $P < 0.05$ ) from 0 to 1 month then did not change from 1 to 2 month, and the concentrations at 3 and 4 months did not differ significantly from 0 month, indicating presence of a transient small increase of IGF-I at 1 and 2 months of age. The IGF-I concentrations subsequently increased again from 4 to 6 and 7 months ( $P < 0.05$ ). Scrotal circumferences were positively correlated with the three hormonal concentrations (INSL3, testosterone and IGF-I) and negatively correlated with the inhibin concentrations from 0 to 7 months ( $n=136$ ). Their coefficients were highest for INSL3 ( $R^2=0.419$ ,  $P < 0.0001$ ), followed by testosterone ( $R^2=0.357$ ,  $P < 0.0001$ ), then inhibin ( $R^2=0.205$ ,  $P < 0.0001$ ), and the lowest for IGF-I ( $R^2=0.160$ ,  $P < 0.0001$ ). When calves were classified by the testicular weights at 7 months into large ( $>60$  g) or small ( $<60$  g), plasma INSL3 concentrations were lower ( $P < 0.05$ ) throughout a period from 3 to 7 months, but testosterone concentrations were lower ( $P < 0.05$ ) at 5 and 7 months, for the calves with smaller than the calves with larger testes. The plasma inhibin concentrations were lower ( $P < 0.05$ ) from 1 to 4 months in the calves with smaller than the calves with larger testes. The plasma IGF-I concentrations were lower ( $P < 0.05$ ) only at 6 months in the calves with smaller than the calves with large testes.



**Conclusions:** Blood plasma INSL3 concentrations constantly increased for 7 months from birth to pre-puberty while testosterone concentrations clearly rose only in the latter phase in Japanese Black beef bull calves. Inhibin concentrations declined constantly for 7 months after birth. There was a transient rise of IGF-I concentrations for the first 2 months after birth, followed by a steep increase after 4 months of age. The correlation coefficients between those hormonal concentrations and scrotal circumferences from birth to 7 months of age was the highest for the INSL3. Bulls with larger testes (>60 g) at 7 months of age had higher INSL3 and inhibin concentrations than those with smaller testes (<60 g) from 3 to 7 months and from 1 to 4 months, respectively. These results suggest that secretions of those testicular hormones are associated with testicular development in the beef bull calves. Also blood INSL3 concentrations might be a best indicator among those hormones for testicular size in the pre-pubertal bull calves.

RE-P02

### Peripheral Anti-Müllerian hormone as biomarker in evaluating function of intra-abdominal testes in cattle

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**Objectives:** Intra-abdominal testicles are seen in cases such as cryptorchidism which is the failure of one or both testes to descend into the scrotum during the first trimester of pregnancy, and male pseudohermaphroditism which has intra-abdominal testes but otherwise phenotypically resemble females. Fattening with intra-abdominal testes in the later growing stages usually results with a stout body type because of their functional testes synthesizing and secreting testosterone (T). In addition, the aggressive bulling in such cases results in a lowered quality of beef. The endocrinological function of the testes is usually examined by the changes in peripheral T levels before and after human chorionic gonadotropin (hCG) stimulation. However, the profiles of blood T levels after an hCG stimulation have not been shown to be consistently similar, especially among prepubertal or cryptorchid male calves.

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein belonging to the transforming growth factor  $\beta$  family and is exclusively expressed by Sertoli cells in males. In humans with non-palpable testis or intersexual disorders, circulating AMH level is a measure of the structural integrity of the gonads; this helps to delineate the cause of the disorder.

This study was carried out to clarify the efficacy of peripheral AMH biomarker for evaluating intra-abdominal testes in bovine.

**Materials and methods:** Eighteen Japanese Black male calves were enrolled in the current study, and classified in the four groups: pre-pubertal intact male group (IM, 6 month-old, n = 5), bilaterally castrated calves group (CA, 6 month-old, n = 5), Unilateral cryptorchid calves group (UC, n = 5), Unilateral cryptorchid calves whose testes showed histological structure similar to testicular regression syndrome (TRS) in human (UC-trs, n = 2), and bovine male pseudohermaphroditism (PH, n = 1). In UC and UC-trs groups, the descended testis in scrotum had already been castrated before the first bleeding. In UC, UC-trs and PH groups, after the final bleeding, the intra-abdominal testes were extracted by laparotomy and had a confirmed parenchyma and epididymis.

To characterize the testicular capacity to synthesize and secrete AMH and T, peripheral blood samples were collected from the jugular vein into heparinized tubes. Samples were taken just before intramuscular administration of 3,000 IU of hCG (Day 0), and additionally on Days 5 and 7. The plasma samples collected at Day 0 were assayed for AMH and T., and the other samples were assayed only for T.

**Results:** The plasma AMH levels were detectable in IM, UC and PH groups, whereas, not to detectable in CA group. Interestingly, in UC-trs group, the plasma AMH level in the one case was detected, and in the other case was not detected. Moreover, in histological findings, Sertoli cells were remained in a small part of the intra-abdominal testis in former case, on the other hand, these were not showed in the latter case.

The plasma T levels in all cases of IM group were increased at Day 5, whereas in all cases of CA and UC-trs groups were not detected at all sampling points. In UC group, the changes differed from one individual to the next. In PH, the plasma T level decreased once, after then increased.

**Conclusions:** It was concluded that AMH level at single blood sample was able to evaluate the existence of intra-abdominal testis with more efficiency than T levels which requires multiple blood sampling. Interestingly, in two cases similar to TRS, the blood AMH levels were different with or without Sertoli cells in the each intra-abdominal testis. Therefore, if the Sertoli cells in testis are disordered, blood AMH levels might be reflected, and expect the degree of degeneration.

RE-P03

### Effect of subclinical hypocalcemia on the fertility of grazing dairy cows

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**Introduction:** The effect of subclinical hypocalcemia (SCH) on reproductive performance in dairy cows has recently been eval-



uated, with inconsistent results. Some found negative effects whereas others reported no effect. So, the objective of the present study was to assess the effect of SCH on the fertility of grazing dairy cows.

**Material and methods:** A prospective observational study was carried out, in a commercial dairy farm from Argentina, where all the cows calving between Oct 15, 2016 and Apr 15, 2017 were enrolled. Sample size ( $n = 126$ ) was estimated as the number of cows needed to detect a difference of 15 d (with a pooled SD of 30 d) between means in the interval from calving to conception, with 80 % of power and 95 % of confidence. Cows were tail bled at parturition and total calcium (Ca) concentration was measured by Atomic Absorption Spectrometry (Laboratorio de Nutrición Mineral, FCV-UNLP). Subclinical hypocalcemia was declared as calcium concentration  $<8.0$  mg/dL ( $<2.07$  mM). The effect of SCH on the odds for conception at first service (C1AI), and of pregnancy by 100 (P100) were evaluated with Binomial distribution and Logit link function in Proc GLIMMIX, its effect on the number of services per conception (SPC) was evaluated with Poisson distribution and Log link function in Proc GLIMMIX, and finally, its effect on the hazards of being inseminated and of becoming pregnant were evaluated with proportional hazard regression models in Proc PHREG. These models also included the fixed effect of parity and diseases (subclinical ketosis, metritis and endometritis). Modeling was performed by the backward elimination method with an exclusion criterion set at  $P > 0.2$ . Statistical significance was set at  $P < 0.05$  and a tendency was declared at  $P < 0.10$ . Finally, a receiver operating characteristic (ROC) analysis was run in SIGMAPLOT 11.0, with a pre-test  $P = 0.4$  and a cost ratio = 1, to determine a critical threshold for blood Ca to predict the cows pregnant at 1st AI.

**Results:** Because of premature culling, the odds of C1AI and of P100 were assessed on 118 and 104 cows, respectively. A total of 26 cows were right censored before 150 days in milk (end of study period) in the analysis of the hazard of conception. SCH was detected in 27.3% (34/126) of cows at parturition. No cow was diagnosed with milk fever. The odds for conception was 3.8 times lower for cows with SCH compared to normocalcemic cows (odds ratio [OR] = 0.261, 95% confidence interval [95%CI] = 0.069-0.987,  $P=0.048$ ) given that C1AI were 17 vs. 33%, respectively. The odds for pregnancy was 3 times lower for cows with SCH compared to normocalcemic cows (OR = 0.321, 95%CI = 0.089 - 1.163,  $P = 0.082$ ) given that P100 were 42 vs. 70%, respectively. Cows having SCH needed 0.85 more SPC compared to normocalcemic cows (2.95 vs. 2.10,  $P = 0.024$ ). The hazard of first service was not affected by SCH (HR = 1.270, 95CI = 0.784 - 2.057,  $P = 0.332$ ). Finally, the hazard of conception was 2.81 times lower in cows with SCH compared to normocalcemic cows (HR = 0.356, 95%CI = 0.144 - 0.881,  $P = 0.026$ ) and had a median calving to conception interval 32 d longer (105 vs. 73).

The determined cutoff of blood Ca to predict pregnancy outcome at 1 AI was 9.15 mg/dL (2.28 mM) with a Sensitivity = 51.61% and a Specificity = 68.66% (AUC =  $0.601 \pm 0.06$  and  $P = 0.091$ ).

**Conclusions:** Subclinical hypocalcemia impairs the odds for pregnancy and the hazard of conception, it increases the services per conception and the interval from calving to conception, and therefore, it is associated with reduced fertility in grazing dairy cows.

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**Key words** 4 subclinical hypocalcemia, pregnancy rate, fertility, grazing dairy cow.

RE-P04

### Associations of lunar cycle and climate factors with frequency of mature and premature births in Japanese Black cows

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In cattle production, it is essential for producers to predict the onset of calving because many calf mortalities occur at peripartum period. Therefore, producers have to supervise the calving and treat a cow and calf at an appropriate time in case unusual or abnormal conditions are encountered. Several exogenous factors including weather conditions and lunar cycle have been reported for possible associations with calving conditions, but only limited information in this regard is available to date, especially no study has investigated these associations in beef cattle. In Japan, the Japanese Black (also known as "Wagyu") is the most common beef cattle breed. Therefore, the objective of the present study was to determine the effect of weather conditions and lunar cycle on calving frequency and an occurrence of premature birth in Japanese Black cows.

The study was undertaken using data from farms in the suburban areas of Miyazaki city, located on the southeastern coast of Kyushu, Japan. Calving records were collected from 905 farms including 41,116 calvings in 15,378 animals between April 2006 and March 2010. Weather conditions and lunar cycle were obtained from the Japanese Meteorological Agency. Daily weather data collected were average, maximum, and minimum temperature, precipitation amount, barometric pressure and relative humidity. Temperature-Humidity Index (THI) was also calculated. The present study conducted two studies, study 1 and study 2. Study 1 investigated the effect of weather conditions and lunar cycle on daily calving frequency (DCF) that defined as the sum of calving event in each day. The observational unit was each day. Since there was large variation of DCF among calving month, adjusted DCF was calculated by the number of DCF in each day subtracting average value of DCF in the calved month. Study 2 investigated the effect of weather conditions and lunar cycle on an occurrence of premature birth. The observational unit was calving. Premature birth was measured by whether or not a cow calved before 280 days of gestation, lower 10th percentile of gestation length, and by whether or not a cow calved before 289 days of gestation, median of gestation length. The data were analyzed by general linear model in study 1 and by a mixed-effects logistic regression model in study 2 to declare the effects of weather conditions and lunar cycle on frequency of mature and premature births.

In study 1, average, maximum and minimum temperatures were positively associated with adjusted DCF in all cows, but these associations were insignificant. The other weather condi-



tions, diurnal temperature variation THI, precipitation amount, barometric pressure and relative humidity, were also not associated with adjusted DCF. Similarly, adjusted DCF in primiparous cows and multiparous cows was not associated with weather conditions. As well as weather conditions, lunar cycle was not associated with adjusted DCF in all cows, primiparous cows and multiparous cows. In study 2, an occurrence of premature birth was associated with all weather conditions except for precipitation amount ( $P < 0.05$ ). Average, maximum and minimum temperature, THI and relative humidity were positively associated with an occurrence of premature birth. In contrast, diurnal temperature variation and barometric pressure were negatively associated with an occurrence of premature birth. Lunar cycle was not associated with an occurrence of premature birth.

In conclusion, there was no effect of lunar cycle on both calving frequency and an occurrence of premature birth, but high temperature and THI conditions may increase an occurrence of premature birth.

RE-P05

### Effect of trace minerals and vitamin supplementation on pregnancy rate with fixed-time artificial insemination in beef cattle

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Fixed-time artificial insemination (FTAI) is a technique widespread throughout the world to improve genetic quality and concentrate calving in beef cattle. Normal pregnancy rate per FTAI is around 50 percent. However, it is considered that conception rate is much higher, but embryonic losses reduce pregnancy rate. Among the factors causing this decline stands out oxidative stress. For this reason, the supplementation with minerals trace and vitamins with antioxidant capacity could improve the pregnancy percentage obtained by FTAI. In Argentina, there are extensive areas devoted to natural pasture-based extensive grazing beef cattle. Offering free-choice trace mineralized salts are unusual in these areas, and producers prefer to perform parenteral supplementations at specific times. With the objective of evaluating the effect of parenteral supplementation with antioxidants, 20 trials of supplementation were performed in beef cattle herds submitted to estradiol/progesterone-based FTAI protocols, fifteen of them in Heifers (n: 2190) and five in cows that have had one or more births (n: 580). In each trial were formed two homogeneous groups in body condition and cyclicity. The supplemented group (SG) was injected subcutaneously with 5 mL of trace mineral containing Cu (0.15 mg/mL as edetate), Zn (0.50 mg/mL as edetate), Mn (0.10 mg/mL as edetate) y Se (0.05 mg/ml as selenite) (Adaptador MIN®, Biogénesis Bagó, Argentina) and with 5 mL vitamin solution containing vitamin A (35 mg/mL as palmitate) and vitamin E (50 mg/mL as acetate) (Adaptador VIT®, Biogénesis Bagó, Argentina). The control group (CON) was injected with 10 mL of isotonic saline solution. Treatments were carried out at along with the insertion of the intravaginal device. Pregnancy diagnosis was performed by ultrasonography on days 35±5. The experiment was performed as a completely randomized block design, with farm as blocking cri-

terion and the data were analyzed by logistic regression with binomial distribution and Logit arrangement, using the statistical package SAS (9.1). Pregnancy rate was higher in heifers than in cows [53.5% (1171 / 2190) and 47.9% (278/580), respectively; ( $P = 0.04$ )]. Pregnancy rate in heifer was 55.1% and 51.8% in SG and CON, respectively (OR: 1.1485, SE: 0.0985; confidence limits: 0.9708-1.3588;  $P = 0.11$ ). Pregnancy rate in cows was higher in SG (52.9%) than in CON (42.8%) (OR: 1.50; SE: 0.2510; confidence limits: 0.819-2.0836;  $P = 0.01$ ). Considering that treatment with trace minerals and vitamins with antioxidant capacity helped to both categories, although it became statistically significant in cows, could be assumed that this category has greater requirements for antioxidants. Heifers probably have a better antioxidant capacity as coming from a rearing in extensive grazing systems. Fresh forage grasses are an adequate supply of vitamins A and E, which are able to be deposited in the body. On the other hand, lactation had probably produced increased antioxidant consumption in cows. These possibilities should be evaluated by measuring antioxidant and oxidative damage indicators in the near future

RE-P06

### Effect of ovulation synchronization on Japanese Black cow bred at farms using cowshed feeding and grazing

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Efficiency in calf production is essential in breeding management of Japanese Black cows. Ovulation synchronization and subsequent timed artificial insemination (AI) are effective approaches used in clinical reproduction. In this study, we investigated the effectiveness of a protocol of ovulation synchronization that involves CIDR-Ovshynch and estradiol benzoate (EB) administration for fertility promotion in the cow herd bred at our institute. Furthermore, we examined the effect of introducing this protocol in two Japanese Black cow breeding farms with cowshed feeding and grazing, respectively. To elucidate the effectiveness of the protocol for enhanced fertility, 34 cows from the breeding cow herd were studied at our institute. The protocol consisted of the following steps. A controlled internal drug-releasing (CIDR) device was inserted into each of the cows approximately 40 days postpartum (Day 0), along with EB administration (1 mg). After 7 days (Day 7), the CIDR devices was removed from the cows and 0.5 mg of chroprostenol (PGA) was administrated. On Day 8, the cows were administrated 1 mg of EB, and on Day 9, 0.1 mg of gonadotropin-releasing hormone agonist (GnRHA) was administered. AI was performed on the evening of Day 9 in cases wherein standing estrus behavior was confirmed on the morning of Day 9 or on the morning of Day 10 in cases wherein the standing estrus behavior was confirmed on the evening of Day 9, and not on Day 9. As a control group, 21 cows that conceived by without hormonal treatment were investigated at their postpartum fertility. The effectiveness of the protocol was evaluated on the basis of the conception rate by AI performed using the protocol and number of days open. To determine the effect of introducing the protocol in the farm where cowshed feeding is followed, the embryo transfer (ET) results of eight cows were assessed. The



same protocol of ovulation synchronization as that used for the animals at the institute was followed for these cows and this was followed by rectum examination to observe the condition of the corpus luteum (CL) and uterus 7 days after GnRHA administration (Day 16). If good condition of the CL and uterus was observed, appropriate for ET, the embryo was transferred into the cow. The effect of introducing the protocol was evaluated by estimation of the conception rate of ET by using the protocol described. To assess the influence of the introduction of the protocol into the farm that fed its cows by grazing, the postpartum fertility of a total of 17 cows was evaluated. The cows received the same protocol as that utilized in the experiments carried out at the institute, except for the initiation point (CIDR insertion and first EB administration). The protocol of ovulation synchronization for the cows of this farm was initiated on days  $95.6 \pm 40.2$  (ranging from 47 to 189 days) postpartum. The impact of protocol introduction was evaluated by examining the conception rate achieved through AI by the protocol and the number of days open. A total of 31 of 34 cows (91.2%) of the institute herd demonstrated clear estrus behavior. The conception rate obtained following AI performed using the protocol described was 58.8% (20/34), and the mean of number of days open was  $69.3 \pm 22.1$  days. Furthermore, the number of cows that conceived within 80 days postpartum was 25 (73.5%). In contrast, the conception rate of AI by confirming natural estrus behavior, mean of number of days open and number of cows conceived within 80 days postpartum were 42.9% (9/21),  $76.6 \pm 39.0$  days, and 13 cows (61.9%), respectively. The postpartum fertility of cows for which the protocol of ovulation synchronization was followed showed a tendency for improvement compared with that of the cows that conceived by without hormonal treatment. In the Japanese Black cow breeding farm that fed cows in cowshed, six of eight cows were selected for ET by rectum examination. Of these, two cows were diagnosed with a poor CL shape or uterine inflammation with nebula mucus. Four of six cows (66.7%) were conceived by ET. Clear estrus behavior was observed in all cows in the Japanese Black cow breeding farm where the cows were allowed to graze, and 10 of 17 cows (58.8%) were conceived by AI using the protocol. The number of days open in a total of 17 cows was  $131.5 \pm 54.0$ , whereas that for the 10 cows that were conceived by AI using the protocol was  $99.8 \pm 43.4$ . In this farm, the mean delivery interval of the last year was 595.9 days, whereas that of current year was 468.3 days. The delivery interval was successfully shortened by using the protocol of ovulation synchronization. In conclusion, this protocol of ovulation synchronization may be effective for improving the postpartum fertility of cows.

RE-P07

### Effect of CIDR-Ovsynch plus equine chorionic gonadotropin on pregnancy rate in subfertile dairy cows

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Lactating dairy cows (milk yield range : 8,500-10,800 kg / 305 days) received fixed time artificial insemination (AI) or embryo transfer (ET) with or without 500 IU of equine chorionic gonadotropin (eCG) at the same time of PGF<sub>2a</sub> treatment in process

of CIDR-Ovsynch during July in Kumamoto prefecture, Japan. The cows with Corpus Luteum (CL) received GnRH injection and progesterone device (CIDR) insertion on Day -10. They were removed the CIDR and treated PGF<sub>2a</sub> on Day -3, and received 2nd injection of GnRH 2 days later. Subsequently, 53 cows received AI on Day 0 (16-20 hours after 2nd GnRH) and 7 cows received ET on Day 7, respectively. All cows were determined pregnancy diagnosis on Day 32 by transrectal ultrasonography and Day 60 by rectal palpation for all cows. In the results of eCG treated group, 71.4 % (10/14) of primiparous and 21.1 % (4/19) of multiparous cows became pregnant by AI. In addition, 75.0 % (3/4) of multiparous cows became pregnant by ET. On the other hand, no cows became pregnant in without eCG group (number: 20 and 3 cows received AI and ET, respectively). Therefore, evident difference was showed between the eCG with and without group. Besides, significantly difference was showed between primiparous and multiparous cows by AI in eCG group (P=0.004). In conclusion, 500 IU of eCG treatment, when the timing of PGF<sub>2a</sub> injection of CIDR-Ovsynch protocol prior AI and ET, improved fertility of dairy cows during summer season. Especially primiparous cows received AI showed marked elevation of conception rate. It is suggest that, eCG affect luteinizing and recover of CL dysfunction, furthermore improve pregnancy rate of subfertile cows by milking and/or heat stress for both of AI and ET.

RE-P08

### Delaying progesterone releasing intravaginal device removal by 24 h during a 5-day PRID-SYNCH protocol decreased estrus expression before timed AI without affecting fertility in Holstein heifers

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**Objectives:** 25-45% of synchronized heifers with a 5-day PRID-Synch protocol for 1<sup>st</sup> AI are detected in estrus and need to be inseminated (AI) before the scheduled Timed Artificial Insemination (TAI).

The objective of the study was to evaluate the effect of delaying progesterone release intravaginal device (PRID) removal during a 5-day PRID-synch protocol on expression of estrus before TAI and pregnancies per AI (P/AI). Our hypothesis was that delaying by 1 day PRID removal would reduce the % of heifers in estrus before TAI without affecting P/AI.

**Methods:** Nulliparous Holstein heifers (n=434) at ~14 months of age were randomly assigned to receive their 1<sup>st</sup> AI after synchronization with: 5-day PRID-synch protocol with PRID (PRID® Delta, Ceva Sante Animale) removal on D5 (PRID5: D0, GnRH (Ovarelin®, Ceva Sante Animale) + PRID; D5, PGF (Enzaprost T®, Ceva Sante Animale) -PRID; D6, PGF; D8, GnRH + TAI), or 5-day PRID-synch protocol with PRID removal on D6 (PRID6: D0, GnRH + PRID; D5 PGF; D6 -PRID PGF; D8, GnRH + TAI). Heifers detected in estrus from PRID removal to the scheduled TAI where inseminated, whereas heifers not observed in estrus received TAI as scheduled on D8. Pregnancy



diagnosis was performed using transrectal ultrasonography 32 and 67 d after AI. Data were analyzed by logistic regression using the GLIMMIX procedure of SAS.

**Results:** More PRID5 heifers received AI to estrus before the scheduled TAI than PRID6 heifers (12% vs. 1%  $P < 0.01$ ). Overall, P/AI did not differ between treatments 32 (55.7% vs. 55.6% for PRID5 vs. PRID6 heifers, respectively  $P = 0.99$ .) or 67 (54.8% vs. 54.3% for PRID5 vs. PRID6 heifers, respectively  $P = 0.92$ ) days after AI. Pregnancy loss between 32 and 67 d after AI was not different between treatments (1.6% vs 2.3% for PRID5 vs. PRID6 heifers, respectively  $P = 0.65$ ).

**Conclusions:** Delaying PRID removal by 24h during a 5-day PRID-synch protocol decreased the incidence of estrus before scheduled TAI without affecting P/AI thereby decreasing the need for estrus detection in heifers during the synchronization protocol.

RE-P09

### Effect of a progesterone-based estrus synchronization program for timed AI (TAI) on reproductive performance in a seasonal pasture-based dairy production system

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**Objective:** The aim of the study was to investigate the effect of PRID-based timed artificial insemination (TAI) programs on fertility in seasonal-calving pasture-based dairy herds.

**Methods:** A total of 1421 lactating dairy cows on 4 spring-calving farms were stratified based on days in milk (DIM) and parity and randomly allocated to one of 3 treatments: Control: no hormonal intervention, inseminated at detected estrus; PRID-Ovsynch: cows received a 7-Day progesterone releasing intravaginal device (PRID Delta ®, Ceva Sante Animale) with 100 micrograms of GnRH analogue (Ovarelin ®, Ceva Sante Animale) at PRID insertion, 25 mg injection of PGF2alpha (Enzaprost T ®, Ceva Sante Animale) at PRID removal, GnRH at 56h after device removal and TAI 16h later; PRID-Ovsynch+eCG: same as PRID-Ovsynch, but cows received 500 IU of equine chorionic gonadotropin (eCG; Synchrostim ®, Ceva Sante Animale) at PRID removal.

At d10 days before planned start of breeding (PSB), all cows that were >35 DIM were examined by transrectal ultrasonography to assess presence/absence of corpus luteum (CL) and body condition score (BCS) was also recorded. Pregnancy diagnosis was performed by transrectal ultrasonography 30-35 days after insemination. Data were analyzed using SAS. The effect of synchronization treatment on binary traits was determined using GLIMMIX procedure.

**Results:** Overall conception rate was not different between groups (50.9%, 49.8% and 46.3% for Control, PRID-Ovsynch and PRID-Ovsynch+eCG, respectively) but the 21-Day pregnancy rate was increased by the use of synchronization (35.0%, 51.7% and 47.6%, respectively  $P < 0.05$ ). Compared to

the Control group, synchronization significantly reduced the interval from PSB to conception (34.6, 23.0 and 26.5 days, respectively) and consequently reduced that average days open (98.0, 86.0 and 89.0 days). Across all treatments groups, DIM at the start of synchronization had a significant effect on conception rate (42.3%, 49.5% and 53.9% for <60, 60-80 >80 DIM;  $P < 0.03$ ), but neither parity (46.5%, 50.4% and 48.4% for parity 1, 2 and >3;  $P = 0.6$ ) neither BCS (44.0%, 49.4% and 58.6% for <2.50, 2.75-3.25 and >3.5,  $P = 0.2$ ) had significant effects on the likelihood of pregnancy per AI.

**Conclusions:** In conclusion, the use of PRID-Synch TAI programs accelerated pregnancy establishment for dairy cows in a pasture-based dairy production system by reducing days open.

RE-P10

### Comparative field trial in dairy cows fitted with progesterone releasing intravaginal devices having two different tail types

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**Objectives:** Progesterone releasing intravaginal devices (PRID) are extensively used for the control of reproductive management in cattle. PRIDs are composed of a polyamide spin-coated with Progesterone and tail for removal. Two types of devices are mostly used according to their shape: Delta and T-shape devices.

The objective of this study was to assess retention rate, easiness of removal and vaginal discharge score of a Delta-Shape device (PRID® Delta, Ceva Sante Animale) built with two different tails (Nylon cord vs. plastic Grip tail).

**Methods:** A total of 205 lactating Holstein cows housed in a herd located in Tulare (California, USA) were selected for this study. Cows were 124.1±12.3 DIM, BCS 3.0±0.2 and producing 37.2±4.1 L/day. Animals were randomly assigned to one of the following groups: N-Group (n=102 PRID® Delta with Nylon cord) and G-Group (n=103, PRID® Delta with plastic Grip tail). All devices were applied and evaluated by the same operator. Devices were placed according to label recommendations, and checked daily for 7 days to assess retention rate (% of cows that remained with the device), ease of device removal (score 1 = easy to 3 = hard), vaginal discharge (score of 1 = little discharge to 3 = plenty of discharge). Data were analyzed by the proc GLIMMIX procedure in SAS.

**Results:** At the end of the 7-days period, the retention rate was similar ( $P > 0.10$ ) between the two experimental groups, although numerically greater for the G-Group (98.1% vs. 97.3%). In contrast, easiness of removal was significantly better ( $P < 0.05$ ) for G-Group (1.1 vs 1.6). The grade of vaginal discharge was also lower ( $P < 0.05$ ) for the G-Group (1.8) than for cows in the N-Group (2.3). None of the studied variables were affected by BCS, DIM or parity of the cows.

**Conclusions:** The use of (PRID® Delta) with plastic Grip tail produced outstanding retention rates, demonstrate a significant improvement in the easiness of removal of the PRID device as



a well as less vaginal discharge at device removal.

RE-P11

### Synchronization with a 5-day progesterone releasing intravaginal device SYNCH protocol for first AI decreased the age at first calving and the rearing costs in Holstein dairy heifers

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**Objectives:** The objective of this study was to compare the reproductive performance and the rearing costs of Holstein dairy heifers after synchronization of ovulation and timed AI (TAI) for first service and natural service for subsequent breedings or natural service (NS) for all breedings.

**Methods:** Nulliparous Holstein heifers (n=366) at ≈14 months of age were randomly assigned to two groups TAI-Group: all the heifers in this group received synchronization with 5-day PRID-synch protocol (D0, GnRH (Ovarelin®, Ceva Sante Animale) and PRID insertion (PRID® Delta, Ceva Sante Animale); D5, PGF (Enzaprost T®, Ceva Sante Animale) and PRID withdrawal; D6, PGF; D8, GnRH + TAI) for first service and natural service for the following breedings (bulls were allowed with the heifers only 10 days after TAI) or exclusively NS for all the services (NS-Group). A partial budget analysis was developed to calculate the economic differences between the reproductive programs using specific inputs for each heifer. The structure of the economic evaluation included the expenses of the hormones for synchronization, labor associated with the hormone administration and AI, semen and AI supplies, costs of bull, and feed costs, as well as, the genetic gain associated with the use of AI sires of greater genetic potential. Data were analyzed by ANOVA and logistic regression using the MIXED and GLIMMIX procedures of SAS.

**Results:** Age at first calving was less for TAI than NS heifers (23.7 vs 24.9 months  $P<0.01$ ). In addition, more TAI than NS heifers calved with <24 months [77% (141/183) vs. 44% (81/183)  $P<0.01$ ]. Considering all the economic inputs, rearing costs were 67.5 € less for TAI than NS heifers (1430.9 € vs. 1498.4 €;  $P<0.01$ ).

**Conclusions:** In conclusion, the use of a 5-Day PRID synch timed AI program for first service reduces the age at first calving and significantly decreases the rearing costs for Holstein dairy heifers.

RE-P12

### Estrous Characteristics of Dairy Heifers Treated at Different Stages of the Estrous Cycle with two Prostaglandin Formulations

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**Objectives:** Prostaglandin (PG)  $F_{2\alpha}$  is commonly used to synchronize the estrus of dairy heifers, but its effectiveness is dependent on the phase of the estrous cycle (PEC) at treatment. Cloprostenol sodium (CLO) is a synthetic analogue of  $PGF_{2\alpha}$ , which may have differential effects on luteolysis and estrus behavior compared with natural  $PGF_{2\alpha}$  molecules [i.e. dinoprost tromethamine (DIN)]. We hypothesized that treatment of heifers in early diestrus (ED; 4 to 6 d) with CLO would improve estrous detection rate, shorten the interval from treatment to onset of estrus, and enhance estrous characteristics compared with DIN treatment.

**Materials and methods:** Holstein heifers (n = 1,019) were enrolled in the experiment at 11 months of age, when they were fitted with a collar containing a microphone and accelerometer (SCR Ltd., Netanya, Israel). The device recorded activity and rumination in 2 h intervals and determined the occurrence of estrus according to changes in activity and rumination. One month after enrollment, heifers were paired according to PEC [ED; mid-diestrus (MD) = 7 to 17 d; proestrus (PE) = 18 to 26 d] and assigned randomly to the  $PGF_{2\alpha}$  treatment (PGFTRT: CLO and DIN). Heifers were serviced (inseminated or embryo transferred 5 to 9 d after estrus) upon detected estrus and heifers not serviced were treated with  $PGF_{2\alpha}$  every 14 days until serviced using the same  $PGF_{2\alpha}$  formulation. Using the Data-Flow2 (SCR Ltd., Netanya, Israel) software, estrous characteristics (onset, duration, activity peak, rumination nadir, and heat index) were recorded. A subgroup of heifers (n=80) had blood sampled on the day of  $PGF_{2\alpha}$  treatment and on the day of estrus to determine progesterone (P4) concentrations. Continuous variables were analyzed by ANOVA using the MIXED procedure. Binary variables were analyzed by logistic regression using the LOGISTIC procedure. Hazard of estrus was analyzed by the Cox proportional hazard ratio using the PHREG procedure. A non-parametric procedure (Kruskal-Wallis) was used to analyze non-normally distributed data using the NPAR1WAY procedure. Statistical significance was considered at  $P<0.05$  and tendency at  $0.05<P<0.10$ .

**Results:** Within 7 days after the initial  $PGF_{2\alpha}$  treatment, percentage of heifers in estrus was ( $P<0.01$ ) affected by the interaction between PEC and PGFTRT. Among ED heifers PGFTRT did not affect the percentage of heifers in estrus within 7 d of treatment (CLO = 40.7 vs. DIN = 37.2%), but among MD and PE heifers CLO treatment increased the percentage of heifers in estrus compared with DIN (95.3 vs. 86.2%; 66.7 vs. 53.9%). Hazard of estrus detection was greater for CLO than DIN (AHR=1.36, 95%CI = 1.18-1.56;  $P<0.01$ ) independent of PEC. Duration of estrus ( $P=0.88$ ), interval from onset of estrus to peak activity ( $P=0.66$ ), and rumination nadir on the day of estrus ( $P=0.82$ ) were not affected by PGFTRT. Likelihood of heat index > 80 was ( $P=0.02$ ) affected by  $PGF_{2\alpha}$  treatment. Progesterone concentration on the day of  $PGF_{2\alpha}$  treatment was not ( $P=0.27$ ) different between CLO and DIN heifers. On the day of estrus, CLO heifers had ( $P<0.01$ ) lower P4 than DIN heifers (0.04 vs 0.11 ng/mL) and a larger ( $P<0.01$ ) percentage of CLO heifers had  $P4 < 0.009$  ng/mL (81.0 vs. 50.0%).

**Conclusions:** Treatment of dairy heifers with CLO increased the hazard of estrus within 7 d after treatment and reduced P4



concentration on the day of estrus compared with DIN treatment but it did not affect estrous behavior.

RE-P13

### Effect of adding a second dose or doubling the dose of prostaglandin during resynchronization of ovulation and TAI in lactating dairy cows

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**Objectives:** Incomplete luteal regression is a limiting factor for fertility in dairy cows receiving timed artificial insemination (TAI). Recent research on resynchronization programs has shown that addition of a second prostaglandin (PGF) treatment 24h after the first PGF increases luteal regression and Pregnancy per AI (P/AI). The administration of the second PGF treatment requires that cows are handled one additional time. Thus, doubling the dose of PGF administered could potentially have a similar effect to the administration of a second dose of PGF 24 h later on luteal regression and P/AI. The aim of this study was to evaluate the effect of a second PGF or doubling the dose of PGF on P/AI during a resynch protocol with a progesterone releasing intravaginal device (PRID Delta<sup>®</sup>).

**Methods:** Lactating Holstein primiparous and multiparous cows (n=884) at a weekly 35-d post-AI pregnancy diagnosis (range of d 35 to 41), found non-pregnant (D0) were evaluated for presence or absence of corpus luteum (CL; NO CL), and then randomly assigned to 3 different 7-Day PRID based resynchronization protocols: 1) 7D1PGF: D0 progesterone releasing intravaginal device insertion (PRID<sup>®</sup> Delta, Ceva Santé Animale) and GnRH 1 (100 mcg of Gonadoreline diacetate i.m., Ovarelin<sup>®</sup>/Cystoreline<sup>®</sup> Ceva Santé Animale); at D7 PGF (25 mg of Dinoprost i.m., Enzaprost T<sup>®</sup> Ceva Santé Animale), on D8 PRID was removed; D9.5 GnRH 2 (100 mcg of Gonadoreline diacetate i.m., Ovarelin<sup>®</sup>/Cystoreline<sup>®</sup>) and 16 h later all the cows were TAI. 2) 7D2PGF D0 progesterone releasing intravaginal device insertion (PRID<sup>®</sup> Delta) and GnRH 1 (100 mcg of Gonadoreline diacetate i.m., Ovarelin<sup>®</sup>/Cystoreline<sup>®</sup>); at D7 PGF (25 mg of Dinoprost i.m., Enzaprost T<sup>®</sup>); on D8 PRID was removed and the cows received a second injection of PGF 2 (25 mg of Dinoprost i.m., Enzaprost T<sup>®</sup>); D9.5 GnRH 2 (100 mcg of Gonadoreline diacetate i.m., Ovarelin<sup>®</sup>/Cystoreline<sup>®</sup>) and 16 h later all the cows were TAI. 3) 7DdoublePGF the same as 7D1PGF except that at D7 the cows received (50 mg of Dinoprost i.m., Enzaprost T<sup>®</sup>). All the cows were pregnancy diagnosed at 38±3 and 67±3 days after TAI by transrectal ultrasonography. The Data were analyzed by logistic regression using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC).

**Results:** At 38 days after TAI, P/AI did not differ among treatments [31% for 7D1PGF, 35% for 7D2PGF and 32% for 7DdoublePGF], as well as pregnancy loss from 38 to 67 days was 10% for all the groups (P = 0.49). There was no effect (P = 0.38) of presence or absence of a CL at protocol initiation on P/AI. For cows NOCL, adding a second PGF2 treatment 24 h after the first within a resynch protocol for TAI increased P/AI (7D2PGF=45%) compared to a single dose of PGF (7D1PGF=27%) or double dose of PGF2α at a single time (7Ddou-

blePGF=30%).

**Conclusion:** The addition of a second PGF treatment 24 h after the first within a resynchronization protocol for TAI increases P/AI only for cows without a CL at the initiation of the protocol (NOCL). Furthermore, the use of a progesterone releasing intravaginal device (PRID Delta<sup>®</sup>) in a resynchronization protocol for TAI in cows lacking a CL (NOCL) guarantees fertility results similar to the ones achieved in cycling cows (CL).

RE-P14

### Effect of using CIDR in first step of double ovsynch programs in high producing dairy cows with anovulatory follicle

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The Objective of this study was to investigate the effect of CIDR using in first ovsynch part of double Ovsynch synchronization program in high producing dairy cows with anovulatory follicle. 40 Holstein high yielding (> 40 liter milk) dairy cows 1 - 5 lactation and after the voluntary waiting period (50 days post-partum) which have anovulatory follicle were selected as experimental group. the cows were examined by ultrasonography for presence of follicles <18 mm and lack of corpus luteum and the animals undergoes to double ovsynch program with the use of CIDR(progesterone, Eazi-Breed, Zoetis, 1/9 g) at the initiation of first ovsynch of the program. the double ovsynch program were performed chronologically as below :

(GnRH and CIDR 7 days later PGF2α 3 days later GnRH 7 days later GnRH 7 days later PGF2α

56 h later GnRH 16 h later timed insemination)

Control group NO 79 cows received double ovsynch protocol without using CIDR. Diagnosis of pregnancy was performed at 30 day after TAI by ultrasonography examination.

Pregnancy per AI (conception rate) on d 30 were in CIDR group (treatment) 45 % and in control group 41/7 % with no statistically difference (p>0/05). Pregnancy per AI in subsequent insemination were in CIDR group 45 % and in control group 47/7 % which was not significant different (p>0/05). It seems that the double ovulation- synchronization programs may enhance ovulatory mechanisms and reset normal function of ovaries.

In conclusion using CIDR in first part of double Ovsynch synchronization program in anovular high producing dairy cows had not significant effect on conception rates in our finding.

RE-P15

### Effect of the 5-day Co-synch with CIDR protocol on pregnancy rate in lactating dairy cattle

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## Agricultural Mutual Aid Associations

The 7-day CO-Synch with controlled internal drug release (CIDR) protocol is a popular timed artificial insemination (AI) program to improve reproductive performance in cattle. Recent research indicates that the 5-day CO-Synch with CIDR protocol (CIDR-5d) improves pregnancy in beef cows and dairy heifers. The objective of this study was to evaluate the effect of the CIDR-5d on pregnancy rate in lactating dairy cattle.

The experiment was conducted in 2017 on a farm in Hokkaido, Japan. Group 1 (CIDR-5d, 17 cows), received a CIDR on d 0, followed by CIDR removal with 25 mg PGF $_{2\alpha}$  i.m. on d 5. And they were inseminated 72 hours after CIDR removal (on d 8), concurrent with injection of 100  $\mu$ g GnRH. Group 2 (ESTRUS, 33 cows), received AI based on detected estrus. Pregnancy was diagnosed from Days 43–56 by transrectal ultrasonography.

The overall pregnancy rate was 29.4% and 36.4% for the CIDR-5d and ESTRUS groups, respectively, and there were no significant differences. One cow in the CIDR-5d group did not receive AI because a corpus luteum was detected by rectal palpation on d 8. The rate of twin pregnancy was 60.0% and 8.3% for the CIDR-5d and ESTRUS groups, respectively, and there tended to be higher in the CIDR-5d group ( $p < 0.1$ ). Pregnancy loss was not occurred in both groups.

The use of CIDR-5d in lactating dairy cattle seems to give acceptable pregnancy results, though the risk of twin pregnancy might get higher.

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RE-P16

### Conception rate after different models of treatment in non pregnant cows

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**Introduction:** In order to resynchronise the oestrus cycle in non-pregnant cows after TAI, various and often complex hormonal programs are used. Such proceedings aim to address the issue thoroughly and are carried out mainly in dairy cows on large farms. Cows maintained in smaller groups are subject to simpler hormonal programs, often only when the need requires their use. The purpose of the study was to compare several simple programs of ovulation induction and synchronisation in non-pregnant cows.

**Material and Methods:** 1601 non-pregnant cows were subject to analysis. They were tested for pregnancy using ultrasound imaging (iScan, Draminski, the 7.5 MHz probe) between day 32 and 46 post-insemination. The ovaries of all the females were examined clinically as well as with the ultrasound. Based on the diagnosis, females were divided into two groups. 58% (930, group A) of cows had a corpus luteum, while the remaining 671

(42%) of cows had non-enlarged ovaries or had no large functional structures on them (the so-called group B). The 704 A1 group of cows with a corpus luteum were given an intramuscular injection of cloprostenol - CLO (500  $\mu$ g). The 83 A2 group of cows were also given CLO, but later were inseminated at the set TAI time – that is, 80 hours after the prostaglandin – PG - injection. Additionally, 143 cows were examined using ultrasound imaging (iScan, Draminski). The results of the ultrasound test allowed for the cows to be divided into two groups – the A3 group (68 animals with a corpus luteum over 20mm in diameter and a follicle over 15 mm in diameter) and the A4 group (the corpus luteum over 20 mm in diameter, the follicle under 5 mm in diameter). The animals from A3 and A4 groups were administered 500mg injections of CLO. 48 hours later they were also given 105  $\mu$ g of buserelin (Receptal, Intervet). The insemination at a set time (TAI) was carried out either 16-23 or 24-30 hours after the GnRH injection. Females from group B were treated with one of the following hormonal programs. The B1 group animals were subject to OVS (406 animals, GnRH, cloprostenol 7 days later, GnRH again 48 hours after the cloprostenol administration, TAI after 24 hours). The B2 group animals were given a single intramuscular injection of buserelin. Finally, the B3 group animals were inserted with CIDR (Zoetis) or PRID-Delta (Ceva) for 7 days. The results were evaluated using the SAS program.

**Results:** The conception rate was following: group A1-A4 – 26.2 ( $n_{A1}=704$ ), 43.9 ( $n_{A2}=83$ ), 47.0 ( $n_{A3}=68$ ) and 36.0% ( $n_{A4}=75$ ) ( $P < 0.01$ ), respectively, group B1-B3 – 26.7 ( $n_{B1}=406$ ), 6.3 ( $n_{B2}=157$ ) and 51.3% ( $n_{B3}=72$ ) ( $P < 0.001$ ), respectively. The pregnancy rate was lowest in group B2. Additionally, as few as 14% of cows subject to therapy displayed signs of oestrus in 7 days after being administered the hormone.

**Conclusions:** The results indicate that the highest pregnancy rate can be achieved by placing the progesterone releasing device inside a cow's vagina, injecting it with 500  $\mu$ g of cloprostenol and 105 $\mu$ g of buserelin 48 hours later. The method of lowest efficiency seems to be the one in which the animal is administered one intramuscular injection of buserelin.

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RE-P17

### Progesterone devices and Ovsynch – efficacy in anestrus and non-pregnant dairy cows – retrospective study

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**Objectives:** The Ovsynch protocol with time oriented insemination (TAI) and progesterone devices are commonly used to treat anestrus and non-pregnancy in cows. The aim of this study was to compare pregnancy results after using the Ovsynch protocol and progesterone devices (CIDR or PRID-Delta) in anestrus and in non-pregnant dairy cows.

**Materials and methods:** 1082 results of hormonal interventions were analysed. Before hormonal treatment all cows were



clinically examined. Only females with small ovaries without a palpable structure of corpus luteum were used for the analysis. Generally, 1082 cows were enrolled in the experiment. The Ovsynch protocol (TAI) was conducted on 382 cows (group I) whereas 700 cows (group II) were treated with progesterone. On day 0 cows from group I received i.m. GnRH injection of 105 µg busereline acetate (Receptal, Intervet), 7 d. later 500 µg of cloprostenol (Estrumate, Intervet) and two days later (48 h.) 105 µg of busereline acetate. 16-24 hours after the final GnRH injection the animals were artificially inseminated. Intra-vaginal progesterone devices were inserted on 7 days (start of program 0 d.). One day before removing the devices the animals were injected with 500 µg of cloprostenol. PRID-Delta (Ceva) and CIDR (Zoetis) contains 1.55 g, the latter 1.38 g. of progesterone respectively. Based on the visual observation the cows were artificially inseminated.

**Results:** Signs of heat were shown by 73.5% cows in anestrus and 81.5% non-pregnant cows. The general percentage of pregnant cows after TAI and CIDR/PRID was 25.6 and 48.4% resp. ( $P < 0,01$ ). The pregnancy rate was 64.7% for cows with CIDR and 32.1% for females with PRID. In group I – in anestrus and in non-pregnant cows it was 24.1 and 27.1%, respectively. In group II the pregnancy rate was 29.5 and 62.9%, for cows in anestrus and non-pregnant females, resp. During the study, 10 females lost progesterone devices (1,4%). They were excluded from the next stages of the proceedings.

**Conclusions:** The difference between the pregnancy rate for the Ovsynch and CIDR / PRID indicates that progesterone devices seem to be a more effective therapy of anestrus and non-pregnancy in cows than the Ovsynch protocol.

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RE-P18

### The influence of GnRH and prostaglandin F2alpha analogues on the morphology of corpus luteum in dairy cattle

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**Objectives:** Corpus luteum (CL) in cattle has two morphological forms – with or without a cavity. The cause and implications of the formation of cavity in CLs is still not clear (Rizzo, 2016). The aim of the study was to examine the influence of the exogenous hormones routinely used in dairy cows reproduction management for the presence of different forms of corpora lutea in the cycles which follow the hormonal injections.

**Materials and Methods:** The studies included 333 estrous cycles (natural and induced) in 162 Polish Holstein – Friesian artificially inseminated cows. All of the cows were from a single herd and during the examinations were in the same environmental conditions. Cows were examined for the presence and echostructure of CLs at weekly intervals with the use of a portable ultrasound instrument (Draminski iScan, Poland) with a linear probe (7.5 MHz). Ultrasonograms of the corpora lutea were evaluated for the presence of the cavities. The corpus lu-

teum with a cavity was defined as an echodense structure with a non-echodense cavity smaller than or equal to 20 mm in diameter (Hatvani, 2013). Every estrous cycle in which at least once inside the CL cavity occurred was considered as a cavitary cycle, all the rest as noncavitary cycles. Estrous cycles were divided into three groups: Control Group – natural cycle, without any use of exogenous hormones; Group P – induced with the use of synthetic prostaglandin analogues; Group G – induced with the use of GnRH analogues (as a simple doses or in Ovsynch protocol – GnRH, 7 d later PGF2alpha, 2 d later GnRH 9 d, and 24 h later AI).

**Results:** Hormonal induction of heat were conducted in 114 cycles (34.2%) – 39 and 75 after prostaglandin and GnRH analogues, respectively, – while 219 were natural (65.8%). Generally, corpus luteum with a cavity occurred in 116 cycles (34.8%), while the solid luteal structure in 217 cases (65.2%). In natural estrous cycles cavity occurred in 80 CLs (36.5%) and the compact CLs in 139 (63.5%) ( $p < 0.001$ ). In Group P cavitary cycles to noncavitary were 16 (41%) to 23 (51%), respectively ( $p = 0.2757$ ). In the cycles of Group G cavity occurred in 20 CLs (26.7%), whereas 55 CLs were noncavitary (73.3%) ( $p < 0.001$ ).

From all of the cycles ( $n = 333$ ) 189 CLs were on the right ovary (56.8%), 128 (38.4%) on the left ovary and 16 (4.8%) on both ovaries at the same time. For the right ovary natural cycles 46 (35.9%) in compare to 82 (64.1%), were cavitary CLs to compact CLs, respectively ( $p = 0.0028$ ), whereas for the left ovary 31 (36%) cycles were with cavitary CLs and 55 (64%) with non-cavitary CLs ( $p = 0.0143$ ). Hormonal induction in Group P resulted in 10 (45.5%) noncavitary and 12 cavitary (54.5%) cycles for the right ovary ( $p = 0.6787$ ) and 10 (71.4%) in compare to 4 (28.6%) noncavitary to cavitary cycles for the left ovary ( $p = 0.1668$ ). Heat induction in Group G resulted in 29 cycles (74.4%) with noncavitary CLs and 10 (25.6%) with cavitary ones for the right ovary ( $p = 0.0094$ ) and 19 (67.9%) in compare to 9 (32.1%) for the left ovary, respectively ( $p = 0.0844$ ). For the cycles with both ovaries luteal activity cavity occurred in 3 cycles (18.8%).

From all 333 cycles, 130 (39%) ended with pregnancy, 78, 47 and 5 for the right, left and both ovaries, respectively. Pregnancy occurred in 32 (41%), 17 (36.2%) and 2 (40%) cycles with cavitary CLs, for the right, left and both ovaries, respectively.

**Conclusions:** The results present the effect of hormonal treatment on the morphological type of the corpus luteum in dairy cows. Heat induction and synchronization protocols with the use of GnRH analogues do not generate more cavitary corpora lutea. Their percentage is low and close to the percentage of cavitary corpora lutea formed after spontaneous ovulation. The use of protocols based on PGF2alpha changes the ratio between compared morphological forms of the corpus luteum increasing the convergence between the percentage of compact CLs and cavitary CLs. Cavitary corpora lutea have no negative effect on the pregnancy rate in dairy cows.

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RE-P19

### Effect of the prolonged preovulatory period in a short synchronization protocol on the conception rate after timed embryo transfer in dairy cows

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**Objectives:** In most of the estrus synchronization programs, the intervals between the last prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) and gonadotropin-releasing hormone (GnRH) injections are determined to be 48 hours in order to synchronize ovulation. However, induction of early ovulation has a potentially negative effects on the subsequent luteinization. Good luteinization is considered to be important for embryo transfer (ET). The objective of the present study was to compare the luteinization and timed ET (TET) outcomes of the modified short synchronization (Shortsynch) protocol (Day -3, Confirmation of the presence of an active CL and PGF<sub>2α</sub>; Day 0, GnRH; Day 7, TET), in which the interval between administering PGF<sub>2α</sub> and GnRH is prolonged to 72h, with the Shortsynch protocol (Day -3, Confirmation of the presence of an active CL and PGF<sub>2α</sub>; Day -1, GnRH; Day 7, TET).

#### Materials and Methods:

##### *Survey on ovulation and luteinization*

26 lactating Holstein-Friesian cows in two commercial dairy farms were used to evaluate the morphology of the corpus luteum (CL) and follicles and the plasma progesterone (P<sub>4</sub>) concentrations. The follicle diameter at GnRH administration and the CL diameter on Day 7 were measured by transrectal ultrasonography. To estimate the time of ovulation, ultrasonography was performed on Day 0 and at 18, 24, 42, 48 h after Day 0. Plasma P<sub>4</sub> concentrations were measured on Day 7. In addition, 15 lactating Holstein-Friesian cows in four commercial dairy farms were used to evaluate the vascularity of the CL. The blood flow area of the CL on Day 7 was measured by transrectal color Doppler ultrasonography.

##### *Survey of TET outcomes*

47 lactating Holstein-Friesian cows in two commercial dairy farms were used. Cows with a CL greater than 15 mm in diameter received embryo transfer on Day 7. Pregnancy diagnosis was conducted by ultrasonography on Day 30. The selection rate, which is the proportion of cows received embryo, conception rate, and pregnancy rate (= selection rate × conception rate) were calculated.

#### Results:

##### *Survey on ovulation and luteinization*

In the Shortsynch group, all cows ovulated within 24 h after Day 0. However, in the modified Shortsynch (MSH) group, the ovulation time slightly varied, and 21.4% of the cows ovulated within 24 h after Day 0 and 78.6% ovulated from 24 to 42 h. There was no difference between the two groups with regard to the follicle diameter at GnRH administration. Although there was no difference regarding the CL diameter, the P<sub>4</sub> concentrations were significantly higher in the Shortsynch group than in the MSH group (8.40 ± 4.27 ng/ml vs. 4.43 ± 2.54 ng/ml; P < 0.05). In contrast, the blood flow area of the CL on Day 7 tended to be larger in the MSH group than in the Shortsynch group (1.40 ± 0.45 cm<sup>2</sup> vs. 2.03 ± 0.81 cm<sup>2</sup>; P = 0.09).

##### *Survey of TET outcomes*

The selection rate, conception rate, and pregnancy rate were 76.2%, 31.3%, and 23.8%, respectively, in the Shortsynch group and 84.6%, 59.1%, and 50.0%, respectively, in the MSH group. The conception rate and pregnancy rate tended to be

high in the MSH group (P = 0.09, P = 0.07).

**Conclusions:** The ovulation time in the MSH group was delayed by approximately one day compared with the Shortsynch group. This was considered to be the reason why the P<sub>4</sub> concentrations on Day 7 were lower in the MSH group. However, the blood flow area of the CL on Day 7 was larger in the MSH group than in the Shortsynch group. A previous study reported that the blood flow area of the CL on Day 7 was significantly larger in pregnant cows than in non-pregnant cows after ET. In the present study, the high vascularity of the CL in the MSH group might also contribute to the increased TET conception rate. In conclusion, the prolonged preovulatory period in Shortsynch protocol might improve the vascularity of the resultant CL and increase the conception rate after TET.

RE-P20

#### Comparison of two different timings of GnRH injection and subsequent artificial insemination post progesterone insert removal in treated non-cycling dairy cows

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**Objectives:** The study was conducted under approval from Kaiawhina Animal Ethics Committee to compare the reproductive performance of non-cycling cows treated with a 9 day or 10 day anoestrus treatment program.

**Materials and methods:** 2,226 cows from 19 seasonally calving dairy farms in New Zealand (NZ) were randomly assigned to one of the two treatment programs. Treatment started nine days before the herd's planned start of mating (PSM). At enrolment cows had been calved more than 24 days and had not been detected in standing heat by the farmer in the preceding 26 day period.

All cows were treated with an intravaginal progesterone releasing device (Dib-h, AgriHealth NZ) and 100µg gonadotrophin releasing hormone (GnRH) (Gonasyn, AgriHealth NZ). The device was removed seven days after insertion and cows were concurrently injected with 500µg cloprostenol (Cyclase, AgriHealth NZ) and 400IU equine chorionic gonadotrophin (eCG) (Novormon eCG, AgriHealth NZ).

Cows were observed once only for signs of standing heat at 48 hours after device removal (heat check, day 9). Cows were randomly assigned to either a nine day or ten day program which differed as follows:

Cows in the nine day program were injected with a second dose of GnRH 48 hours after device removal and underwent fixed-time artificial insemination (FTAI) 10-16 hours later.

Cows in the ten day program that were detected in standing heat at heat check were immediately artificially inseminated. Those cows not in standing heat at that time were injected with a further dose of GnRH 10 hours later and FTAI 16-20 hours after the injection.

Cows were tested for pregnancy via transrectal ultrasonography twice, being 80-90 days and 120-140 days post PSM.



**Results:** 24 nine day and 15 ten day cows were lost to follow up leaving 1,086 and 1,101 respectively for analysis ( $p=0.1414$ ).

Data was checked for potential bias caused by industry recognised factors (average age, BCS or calving to mating interval) with no statistical difference between the two groups ( $p=0.6882, 0.5162, 0.2073$ ).

Cows treated with the nine day program had a 4.09% (95%CI= 0.1278% to 8.0473%) better first service conception rate (FSCR) (33.79% vs 29.70%,  $p=0.0398$ ) and conceived 5.2 days earlier (Kaplan Meier mean days to conception 37.89 days (95%CI= 35.73 - 40.05 days) than those in the ten day program (43.19 days (95%CI= 40.97 - 45.41), log rank  $p=0.0036$ ).

19.66% of cows were observed in heat at the heat check. These cows had an 18.41% (95%CI= 13.18% to 23.66%) higher conception rate than those cows not detected in heat (46.56% vs 28.15% respectively,  $p<0.001$ ) regardless of which treatment program they received. For cows detected in heat, there was no statistical difference in the FSCR between cows treated with the 9 day program compared to the 10 day program (48.54% vs 44.78%,  $p=0.4325$ ).

Cows that were not detected in heat on the morning of day 9 had a 4.6% (95%CI= 0.30 to 8.88) better conception rate if they were treated in the nine day program compared to the ten day program (30.44% vs 25.84% respectively,  $p=0.033$ ).

**Conclusions:** This study showed reproductive performance was better for the nine day program compared to the ten day program. This may be because the window for insemination was more practical for FTAI technicians. It was observed that many of the ten day cows were inseminated towards the latter end of the recommended 10-20 hour window, this may have contributed to the poorer conception result. There may be better opportunity for compliance to the recommended timing of insemination in the nine day program.

Additionally, the nine day program eliminates the need to undertake heat detection and thus reduces the risk of sub-optimal heat detection having a negative effect on conception rate, especially in non-cycling cows that had not been previously detected in heat.

The nine day program thus reduces the impact that heat detection inaccuracy and suboptimal insemination timing can have on the chance of conception in cows that are treated for having not been observed in heat prior to the PSM for the herd.

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RE-P21

### Application of ELISA for early pregnancy detection in Limousin beef cows.

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**Objectives:** In mature beef cows, when energetic needs for production are increased, the energetic restriction results from larger body size and associated increased requirements for maintenance. This reduction in reproductive effectiveness represents an indirect influence on efficiency, considering reproductive efficiency as the number of cows that produce a calf each year. Therefore the potential for a cow getting and maintaining pregnant during a breeding season is an important goal in a beef cattle breeding programs. Pregnancy is traditionally diagnosed manually or by ultrasonographic examination per rectum, which is difficult to perform in pasture-mating system. Alternatively, immunological detection of pregnancy-specific substances in maternal serum such as Pregnancy-Associated Glycoproteins (PAG) may be conducted. PAG are secreted by the binucleate giant cells of the cattle placenta and enter maternal circulation during placentation. Detection of PAG by way of RIA or ELISA in

maternal serum has been shown to be a useful as early pregnancy detection method in dairy cows as early as 22 days post mating. Wherefore PAG concentration in maternal serum has been recognized as an indicator of pregnancy, which may be useful in the reproductive management of beef cattle, especially maintained in pasture-mating system.

**Materials and methods:** The aim of this study was to establish an ELISA applicability to detect pregnancy in beef cows maintained in the most extensive system. The Limousine beef cows ( $n=95$ ) were maintained in classical pasture-mating system with and without breeding programs, relying solely on bull breeding. The percentage of cows bred only by bull breeding over breeding season in 2016 was 94.2 %, whereas the percentage of heifers was 79.2%. Cows underwent pregnancy examination (manual or ultrasonographic examination per rectum) and the blood samples were collected between 1 and 7 month after mating over breeding season in 2016. After 12 h, at room temperature, the blood was centrifuged at 1500 x g for 10 min to obtain serum. Serum samples obtained at various stages of pregnancy were analyzed with ELISA in own laboratory. Heterogeneous two species-competitive-indirect capture-assay with the double antibody approach and pre-incubation of the specific antibody was used in order to enhance an assay sensitivity at low concentrations and reduces the amount of tracer needed while providing for higher specificity. Pregnancy status of open and pregnant cow corresponded to serum SN values of  $<0.30$  and  $\geq 0.30$  respectively.

**Results:** Pregnancy in beef cows with previous bull exposure was confirmed with manual (88 cows) and ultrasonographic examination (90 cows) per rectum as well as by serum PAG ELISA (86 cows). Significant differences between pregnant and non-pregnant cows were manifested from day 22 after mating and at 31–35 days after mating, overall reliability for the identification of pregnant and non-pregnant animals was over 94%. For manual examination per rectum sensitivity was 97.8%, specificity was 100.0% and the positive and negative predictive values were 100.0% and 71.4%, respectively. For ultrasonographic examination per rectum all established parameters (sensitivity, specificity, positive predictive values, negative predictive values) were 100.0%. For serum PAG ELISA performance of the test was sensitivity of 95.6% and specificity of 100% with positive and negative predictive values at the level of 100% and 55.6%, respectively.

**Conclusions:** With the ELISA being more efficient than the RIA, the practicability of the PAG test has great possibilities to be developed. Therefore, we stated the serum PAG ELISA is



accurate in predicting pregnancy status and may be useful for breeding management in beef cows husbandry systems based on bull exposure in pasture-mating.

RE-P22

### Effect of bovine leptospirosis vaccination on reproduction, mastitis and limb diseases in a dairy farm

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Ehime P.F.A.M.A.A.

**Objectives:** Bovine leptospirosis is a zoonotic disease primarily caused by *Leptospira borgpetersenii* serovar Hardjo-bovis, for which cattle are the maintenance host. In cattle, the disease is characterized by reproductive loss such as lower conception rates, failure to conceive and embryonic and fetal deaths. The objective of the study was to investigate whether hadjo-bovis vaccination would improve reproductive performance and reduce mastitis and limb disease incidences in a dairy farm experiencing increased early abortions associated with Hardjo infection.

**Materials and methods:** The study was conducted in a dairy farm in Imabari City, Ehime, Japan. Eighty-three Holstein cattle including 76 milking cows were raised on a total mixed ration in a free-barn system. An average of from 3 to 7 cows per month turned negative for pregnancy around Day 60 after artificial insemination(AI), after they tested positive between Day 30 and 35 post-AI. *Leptospira* Hardjo ELISA (Linnodee Animal Care, Northern Ireland) on bulk tank milk showed a medium positive result indicating the presence of a mixture of infected and susceptible animals. A monovalent vaccine containing inactivated *Leptospira borgpetersenii* serovar Hardjo strain 181 ( $>1 \times 10^9$  cells/2ml) was subcutaneously injected to all the cattle twice one month apart. Herd performance data, breeding records and disease incidences were collected for one year, starting before and ending after the vaccination.

**Results:** After vaccination, the mean numbers of nonpregnant days, AI services and abortions in the first trimester all decreased significantly ( $P < 0.01$ ,  $P < 0.05$ , and  $P < 0.01$ , respectively). Although statistically insignificant, the conception rate also improved. The mean annual milk yield per haed also increased significantly ( $P < 0.01$ ). Furthermore, there were significant decreases in disease incidence ( $P < 0.05$ ), occurrence of peracute and acute mastitis ( $P < 0.05$ ) and the number of cows with high somatic cell count ( $P < 0.05$ ). The incidence of limb disease such as arthritis, papillomatous digital dermatitis, sole ulcers also decreased significantly ( $P < 0.05$ ).

**Conclusions:** Herd vaccination using an inactivated leptospirosis vaccine resulted in significantly improved reproductive performance and reduced occurrence of peracute and acute mastitis and limb diseases. These effects were thought to be exerted through reduced colonization of *Leptospira* in the kidneys and enhanced Th1 immunity via the activation of T cells.

RE-P23

### Profiles of hair cortisol concentrations in relation with health, nutrition and reproductive parameters in postpartum dairy cows

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Management of stress is an important component in improving the welfare, health and performance of livestock animals. Recently, measurement of the cortisol concentration in hair has been used as an index of chronic stress in several species. The objective of the present study was to investigate the profiles of hair cortisol concentrations in postpartum dairy cows in association with health, nutrition and reproductive parameters. Samples were collected from 25 Holstein dairy cows that were delivered July or August in 2016 on 13 commercial dairy farms in Tokyo, Japan. Hair was collected from the tail switch  $-19.2 \pm 11.4$  (mean  $\pm$  SD),  $44.8 \pm 11.9$ ,  $103.0 \pm 9.9$ ,  $168.0 \pm 9.7$  days postpartum (Dry, L1, L2, and L3, respectively). Body condition scores (BCS), hoof and hock health scores were also evaluated. Blood samples were collected together with the hair samples at Dry, L1, and L2, for the determination of blood glucose, non-esterified fatty acid (NEFA), b-hydroxybutyric acid (BHBA), and other metabolites. BCS were correlated with BHBA ( $r = -0.31$ ), glucose ( $r = 0.28$ ), and hair cortisol concentration ( $r = -0.26$ ). Hock health scores were correlated with hoof health scores ( $r = 0.32$ ), BHBA ( $r = 0.24$ ), glucose ( $r = -0.21$ ), and hair cortisol concentrations ( $r = 0.27$ ), and cows with hock injuries had higher hair cortisol concentrations than cows with non-hock injuries ( $6.8 \pm 4.5$  vs.  $3.8 \pm 3.4$  pg/mg,  $P < 0.01$ ). Hair cortisol concentrations during postpartum period showed different patterns according to the time of first AI and fertility. Cows that were submitted to first AI by 86 days postpartum ( $61.8 \pm 34$  days postpartum) showed a peak hair cortisol concentration at L1, whereas cows with delayed first AI ( $125.2 \pm 25.5$  days postpartum) had a peak at L2. The cows with delayed first AI had increased NEFA concentrations at Dry and increased BHBA concentrations at L1. When cows were divided into two groups by days open, hair cortisol concentrations of low fertile cows ( $>147$  days open) increased significantly at L1 and L2 than at Dry ( $5.5 \pm 5.5$ ,  $4.8 \pm 2.7$ ,  $2.4 \pm 1.6$  pg/mg, respectively). In contrast, hair cortisol concentrations of fertile cows ( $\leq 147$  days open) were not different significantly among the sampling time. In conclusion, these results suggest that hair cortisol concentrations change in association with nutrition and health status. Cows with nutrition and health problems caused by inadequate management appear to experience greater chronic stress, which may influence their reproductive function.

RE-P24

### Standardized comparative analysis of the reproductive performance in large commercial Hungarian dairy herds

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**Objectives:** Profitability is fundamentally influenced by the reproductive performance in the dairy herds. The evaluation of reproductive performance varies greatly among the dairy farms in Hungary, but many conventional reproductive indices are widely used, such as productivity, calving interval (CI), services per conception (SPC) and the percentage of pregnant cows (PP), at the same time the heat detection rate (HDR), the conception rate (CR) and the pregnancy rate (PR) are quite rarely applied. The aim of this survey was to assess the average values of the most commonly used reproductive indices in the Hungarian dairies, to introduce more powerful, novel reproductive parameters and to examine their practical applicability.

**Materials and methods:** The authors surveyed the major reproductive indices on 21 large commercial dairy farms from six Hungarian counties between February and May 2015. Altogether the individual data of 12,723 cows were collected from the farm management software RISK (Systo Ltd., Hungary). Associations between the major reproductive parameters were analysed by Spearman's rank correlation. Data were managed in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were performed in R version 3.4.0. (R Core Team, 2016).

**Results:** Average values of the conventional reproductive indices were: calving-to-conception interval (CCI) – 160.1 days, services per conception (SPC) – 4.3 and first service conception rate (CR1) – 22.3%. The CCI, SPC and CR1 were much poorer than the former reference values even in the best herds. It was proven that pregnancy rate (PR) and the corrected pregnancy rate (cPR – pregnancy rate within 200 DIM) are applicable in Hungarian dairy farms due to their strong correlation ( $p < 0.05$ ) with other reproductive parameters (CCI, SPC, CR1, PP, productivity, calving-to-conception interval within 200 DIM, percentage pregnant within 200 DIM, open cows beyond 285 DIM), therefore these parameters are advised for routine use in dairies. In this study corrected pregnancy rate (cPR) was introduced as a novel parameter that is destined for overcoming inaccuracies stemming from Hungarian culling policy. In the surveyed herds PR was 9.6% and cPR was 17.3%, on average.

**Conclusions:** PR and cPR should be brought to the fore instead of productivity, since these parameters better reflect the reproductive performance of the herd. SPC should not be over-emphasized, because it is much less relevant from an economic point of view, e.g. compared to CCI. Reproductive performance must always be evaluated taking several indices into account. The use of some relevant parameters (PR, cPR, CR1, CCI) is enough for the daily routine, but in-depth analysis is required when the reproductive performance is diminishing.

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RE-P25

### Effects of Increased of Lipopolysaccharide Level in the Rumen due to Feeding with Increased Corn Grain in Concentrated Feed on the Reproductive Organs of Dairy Cows.

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**Objectives:** To compensate for the energy shortage of high-lactating cows, the amount of concentrated feed is increasing year by year. Endotoxin (lipopolysaccharide[LPS]) is known to be released in rumen supernatant in large amounts by feeding of concentrated feed, and the LPS concentration in peripheral blood also increases. In this study, we investigated the effects of ruminal LPS concentration on the reproductive organs by using cows with concentrated feed diet.

**Materials and methods:** Four Holstein dairy cows (nonpregnant, dry cows) were used for comparison between the control and concentrated feed diet groups, respectively. The control group was fed with the Timothy-based feed. The concentrated feed diet group showed decreased roughage concentration stepwise weekly (4 weeks, 40% to 30%, 25%, and then 20%) with corn and soybean meal-based feed. The test cows were ovulation synchronized by the CIDR synch program, and blood samples were collected on days -9, -2, 0, and 1, with day 0 of the second gonadotropin-releasing hormone (GnRH) administration. Progesterone ( $P_4$ ), 13,14-dihydro-15-keto-prostaglandin  $F_{2\alpha}$  (PGFM), haptoglobin (Hp), and tumor necrosis factor (TNF)- $\alpha$  concentrations were measured. LPS and LPS-binding protein (LBP) concentrations were measured during day 1 of blood collection. Furthermore, on day 1, rumen fluid ( $n = 3$ ), follicular fluid, uterine perfusate, hepatic portal vein, and hepatic venous blood ( $n = 1$ ) were collected, and their LPS concentrations were measured. Volumetric fatty acid concentration was also measured for rumen fluid.

**Result:** Hp significantly increased after 4 weeks ( $P < 0.05$ ) as compared with that before concentrate feeding. The acetic acid-to-propionic acid (A / P) ratio significantly decreased ( $P < 0.05$ ) in the concentrated feed diet group as compared with the control group. The mean LPS concentration in the rumen changed from 12 EU / l in the control group to 30 EU / l in the concentrated feed group. When the A / P ratio was low, the Hp concentration tended to increase ( $P < 0.05$ ), and a positive correlation ( $P < 0.05$ ) was observed between Hp and peripheral blood LPS concentrations. On the other hand, no obvious relationships were found among the LPS concentration in the rumen and follicular fluids, uterine perfusate, hepatic portal vein, and concentrations of hepatic venous blood LPS,  $P_4$ , PGFM, TNF- $\alpha$ , and LBP.

**Conclusions:** A decrease in A / P ratio due to concentrated feed diet suggested a change in bacterial flora in the rumen. Therefore, we inferred that Hp increased owing to inflammation when the LPS concentration in

the rumen increased. The results did not suggest that ruminal LPS concentration invariably affects peripheral blood, hepatic portal vein, and reproductive organs directly in nonpregnant, dry Holstein dairy cows with less absolute amount of concentrated feed in feed.

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RE-P26

### Relationship between endometritis in dairy cows and peripheral blood lipopolysaccharide and haptoglobin concentration

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**Objectives:** Half of high-lactating cows were reported to develop inflammatory uterine diseases such as metritis and endometritis. The conception rate in endometrial cattle is reduced by 20%; as a factor for this, when bacterial infection occurs in the uterus, lipopolysaccharide (LPS) liberated from bacteria acts on the endometrium. The secretion of prostaglandin (PG) reportedly switches from PGF<sub>2</sub>α to PGE<sub>2</sub>, and LPS indirectly affects ovarian function via the hypothalamus-pituitary system. In this study, we examined the relationship between the diagnosis of endometritis by vaginal discharge scoring (VDS) and endometrial cytology, and peripheral blood LPS and haptoglobin (Hp) concentrations.

**Materials and methods:** Blood was collected weekly, for 8 weeks after parturition, from 15 cows who delivered normally; blood biochemical properties, peripheral blood LPS, Hp, and 13,14-dihydro-15-keto-prostaglandin F<sub>2</sub>α (PGFM) concentrations were measured. In addition, at 4 and 8 weeks after parturition, VDS using the Metrichick device (Simcrotech, Hamilton, New Zealand) and endometrial cytology using cytobrush technique were performed; uterine perfusion with 50 ml of physiological saline was also performed, and the LPS concentration in the uterine perfusate was measured. From the diagnosis based on VDS and endometrial cytology, the normal (n = 5), endometritis (n = 5), and endometritis recovery groups (endometritis at 4 weeks postpartum, it recovered, n = 5) were divided into subgroups.

**Results:** Peripheral blood LPS concentration was significantly higher in the endometritis group than in the normal group at 4 and 8 weeks postpartum (4 weeks:  $0.48 \pm 0.11$  vs  $0.77 \pm 0.13$  EU/ml,  $p < 0.05$ , 8 weeks:  $0.43 \pm 0.10$  vs  $0.73 \pm 0.03$  EU/ml,  $p < 0.05$ ). Peripheral blood Hp concentration was significantly higher in the endometritis group than in the endometritis recovery group ( $p < 0.05$ ) at 4 weeks after parturition. No clear differences were found in the blood biochemical properties, and PGFM concentration and LPS concentration in uterine perfusate.

**Conclusions:** The above-mentioned results showed that the endometrial cows had a higher LPS concentration in peripheral blood than the normal cows. Moreover, we can infer that in individuals who had high Hp and persistent inflammation 4 weeks after parturition, the uterus took longer to recover.

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RE-P27

### Differences in endometrial mRNA expression of important genes related to fertility in repeat breeder and fertile cows

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**Objectives:** An altered uterine environment produced by changes in the endometrial mRNA expression of key genes may be responsible for reduced fertility in repeat breeder cows (RBC). The aim of the present study was to compare the endometrial gene expression of epidermal growth factor receptor (EGFR), nodal growth differentiation factor (NODAL), prostaglandin-endoperoxide synthase 2 (PTGS2), estrogen receptor 1 (ESR1), and progesterone receptor (PGR) in RBC and fertile cows (FC) during diestrus by RT-qPCR.

**Materials and methods:** This study was conducted in commercial dairy farms located in Castelli, Brandsen and Lobos, province of Buenos Aires, Argentina. A total of 82 grazing Holstein dairy cows (RBC and FC) were evaluated to select the cows that were included in the study. We considered a RBC as a cow without any clinical disease that had equal or greater than 3 unsuccessful artificial insemination (AI), and a FC as a cow without any clinical disease and that became pregnant at first or second AI. Cows were evaluated by endometrial cytology to exclude cows with subclinical endometritis (SCE) and a blood sample from the coccygeal vein was obtained to exclude cows with less than 1 ng/mL of P4. Endometrial samples were collected from each RBC and FC included in the study by cytobrush technique for RT-qPCR analysis. Samples were stored in a tube with RNA stabilizing solution at -20°C. The RNA isolation was performed using Trizol (TriReagent, Molecular Research Center Inc., Cincinnati, OH, USA). Complementary DNA was synthesized using a reaction mixture containing 1 µg of total RNA, random hexamers (Promega, Madison, WI, USA) and Moloney murine leukemia virus reverse transcriptase (Invitrogen Corp., Carlsbad, CA, USA), following the procedures suggested by the manufacturer. Reactions of qPCR for bovine genes (ESR1, PGR, EGFR, NODAL and PTGS2) were carried out using specific primers. The amplification conditions consisted of an incubation at 95°C for 3 min and 40 cycling repeats of: denaturation at 95°C for 20 s, annealing for 20 s at indicated annealing temperature and an elongation step at 72°C for 20 s. A dissociation curve was generated at the end of the amplification to confirm that a single product was amplified. To analyze the relative level of expression of each mRNA, the 2-ΔΔCT method was used. Significance difference of endometrial gene expression between RBC and FC was analyzed by general linear model procedure.

**Results:** From a total of 31 RBC sampled, 6 were positive to SCE and 9 were not in diestrus (P4 less than 1 ng/mL); and were not included in the study. Furthermore, from a total of 51



FC sampled, 8 were positive to SCE, 18 were not in diestrus (P4 less than 1 ng/mL), 15 were not pregnant within the two first insemination and one was outlier; and were not included in the study. Therefore, a total of 16 RBC and 9FC samples were included in the study. Our results demonstrated that the mRNA expression of EGFR (RP= -1.098; 95% IC= -1.66 – -0.54; P less than 0.01) and NODAL (RP= -3.227; 95% CI= -4.15 – -2.30; P less than 0.01) were higher in RBC than in FC, while the mRNA expression of PTGS2 (RP= 0.439; 95% CI= 0.39 – 0.49; P less than 0.01) was lower in RBC than in FC. On the other hand, there were no significance differences in the mRNA endometrial expression of ESR1 and PGR (RP= 0.229; 95% CI= -1.13 – 1.59; P=0.73; RP= -0.517; 95% IC= -1.27 – 0.23; P=0.17, respectively).

**Conclusions:** In conclusion, there are significance differences in the mRNA endometrial expression of EGFR, NODAL and PTGS2, but there are not in the mRNA endometrial expression of ESR1 and PGR between RBC and FC during diestrus. This study provides important information that could be used as a base to future studies to elucidate the causes of reduced fertility in RBC.

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**Key Words:** Subfertility, RT-qPCR, dairy cow, gene expression.

RE-P28

### Relationship of reproductive management and performance in primi- and multiparous cows on large commercial Holstein-Friesian farms

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**Objectives:** Reproductive performance of a dairy herd has huge impact on the total milk production, moreover, both milk yield and fertility are related to the age of the cow. Therefore, age distribution of the herd influences the productivity and profitability. The aim of this study was to analyse the associations among management practices and reproductive performance by parity in dairy cows.

**Materials and methods:** Personal interviews were performed between 22 May and 6 November 2015 in order to survey the reproductive management practices on 34 Holstein-Friesian large commercial dairy herds in Hungary. Individual data of 23,781 cows that calved between 1 January 2014 and 31 December 2014 were also gathered from the farms participating in the survey. The associations of the management practices and reproductive performance by parity were analysed retrospectively by mixed effects models. Data were managed in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were performed in R version 3.4.0. (R Core Team, 2017).

**Results:** Primiparous cows performed better in terms of breeding interval (42.2 vs. 43.2 days,  $p < 0.001$ ), calving to conception interval (152.3 vs. 161.8 days,  $p < 0.001$ ), first-service conception risk (24.8 vs. 17.3%,  $p < 0.001$ ) and probability of pregnancy at 200 days in milk (65.2 vs. 55.4%,  $p < 0.001$ ) compared to multiparous cows, however, no differences between parities were found regarding days to first service (75.7 vs. 75.6 days,  $p > 0.05$ ). Lack of a voluntary waiting period was linked to larger improvement in calving to conception interval ( $p < 0.05$ ) and probability of pregnancy at 200 days in milk ( $p < 0.001$ ) in multiparous cows. Primiparous cows experienced larger improvement in days to first service ( $p < 0.001$ ), breeding interval ( $p < 0.05$ ), calving to conception interval ( $p < 0.01$ ) and probability of pregnancy at 200 days in milk ( $p < 0.001$ ) than their multiparous counterparts when estrus synchronization was used (vs. not used). Early pregnancy diagnosis (by transrectal ultrasonography or pregnancy-associated glycoprotein tests) and pregnancy recheck were associated with larger advantage in primiparous compared to multiparous cows regarding breeding interval (both  $p < 0.01$ ), calving to conception interval ( $p < 0.01$  and  $p < 0.001$ , respectively) and probability of pregnancy at 200 days in milk (both  $p < 0.001$ ).

**Conclusions:** Primiparous cows generally experienced larger improvement in reproductive parameters when estrus synchronization, early pregnancy diagnosis and pregnancy recheck were applied. The associations between reproductive performance and management were different by parity.

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RE-P29

### Associations of management and reproductive performance of cows on large commercial Holstein-Friesian farms

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**Objectives:** As a result of the increase in herd size and the intensification of production, the complexity of reproductive management has been growing in dairy herds. The aim of our study was to examine the associations of management practices and reproductive performance in Holstein cows on large commercial dairy farms.

**Materials and methods:** Management practices applied to cows were surveyed between 22 May and 6 November 2015 in 34 large Holstein-Friesian dairy herds in Hungary. Individual data of 23,784 cows having calved between 1 January 2014 and 31 December 2014 in the surveyed herds were gathered. Associations between the management practices and the re-





productive parameters were analysed by mixed effects models. Data were managed in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were performed in R version 3.4.0. (R Core Team, 2017).

**Results:** We found that heat abatement with ventilation and sprinklers was associated with the shortest breeding interval, the shortest calving to conception interval ( $p < 0.001$ ), and the highest odds of being pregnant by 200 days in milk ( $p < 0.01$ ), whereas solely ventilation showed similar results to lack of heat stress protection. Lack of a well-established voluntary waiting period (VWP) or a VWP shorter than 50 days was associated with reduced days to first service after calving ( $p < 0.01$ ), shorter breeding interval ( $p < 0.001$ ) and calving to conception interval ( $p < 0.05$ ), as well as higher odds of carrying a calf by 200 days in milk ( $p < 0.01$ ) compared to those using a VWP of at least 50 days. Applying estrus synchronization protocols was associated with reduced days to first service ( $p < 0.01$ ) and higher odds of pregnancy by 200 days in milk ( $p < 0.05$ ). Performing early pregnancy diagnosis (by transrectal ultrasonography or pregnancy-associated glycoprotein tests) was linked to shorter breeding interval ( $p < 0.05$ ), reduced calving to conception interval ( $p < 0.05$ ) and higher odds of pregnancy by 200 days in milk ( $p < 0.01$ ) compared to rectal palpation.

**Conclusions:** Our study highlights the management practices most closely related to improved reproductive performance, which are, therefore, suggested to be applied on dairy farms, considering the local circumstances of the individual farms.

The research was supported by the Hungarian Ministry of Human Capacities [grant number 12190/2017/FEKUTSTRAT]. The project was also supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.1-16-2016-00024, project title: Innovations for Intelligent Specialisation on the University of Veterinary Science and the Faculty of Agricultural and Food Sciences of the Széchenyi István University Cooperation).

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RE-P30

## Reproductive management and performance of replacement dairy heifers in Hungary

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**Objectives:** Heifer raising represents 15-20% of the total milk production costs, but the management of replacement heifers is often neglected. The goal of replacement heifer programmes is to reduce raising costs, while maximizing future profitability. The aim of our study was to survey the reproductive management practices and the reproductive performance of replacement heifers in large commercial dairy herds in Hungary.

**Materials and methods:** Reproductive management practices were surveyed using a questionnaire between 22 May and 6 November 2015, and altogether 34 large-scale Hungarian dairy herds were involved. Questions regarding estrus detection, insemination, culling policy, pregnancy diagnosis, housing and

feeding were raised to the farm manager or the veterinarian in each herd. Individual heifer data from the farms participating were gathered for 50,396 heifers first inseminated between 1 January 2011 and 31 December 2014, and these were used for the calculation of the major reproductive parameters. Data were managed in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

**Results:** Mean ( $\pm$  standard deviation) age at first service, age at first calving and mean first-service conception risk were  $15.53 \pm 1.59$  months,  $25.61 \pm 2.22$  months and 47.10%, respectively. 8.6% of the inseminated heifers was culled prior to first calving,  $246.25 \pm 107.10$  days after first insemination, at  $23.94 \pm 3.95$  months of age, on average. Heifers were grazed on 35.3% of the surveyed farms. Body weight was regularly measured on 47.1%, body condition was regularly scored on 8.8% and estrus detection aids (e.g. pedometers, tail chalking) were used on 14.7% of the farms. Sexed semen was applied in 94.1% of the herds, mainly for the first and second inseminations (43.8%). Early pregnancy diagnosis (by transrectal ultrasonography or pregnancy-associated glycoprotein tests) was performed in 38.2% of the herds. Most commonly, pregnancy diagnosis was performed weekly (34.4%) or monthly (25.0%).

**Conclusions:** The use of labour-intensive and costly management measures was infrequent, therefore, there is room for the uptake of intensive management practices in the reproductive management of heifers. In order to minimize losses stemming from the prolonged non-productive period, farm managers and veterinarians should dig deeper than monitoring average AFC and conception risk only.

The research was supported by the Hungarian Ministry of Human Capacities [grant number 12190/2017/FEKUTSTRAT]. The project was also supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.1-16-2016-00024, project title: Innovations for Intelligent Specialisation on the University of Veterinary Science and the Faculty of Agricultural and Food Sciences of the Széchenyi István University Cooperation).

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RE-P31

## Factors affecting pregnancy loss in dairy cows

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**Objectives:** The objectives of this study were to examine possible relationships between pregnancy loss (described as the loss of pregnancy occurred after a positively diagnosed pregnancy -days 28-40- and before day 110 of pregnancy) and different factors such as synchronization protocol used, parity, number of Artificial Insemination (AI), days in milk at AI, age at AI (in the case of heifers) and the fact of having had a previous pregnancy, in dairy cows managed under intensive milk production systems

**Material and methods:** A total of 12978 AIs from seven different farms were included in the study. The number of AIs from each farm were 812/12978 from farm 1 (6.3%), 710/12978 from farm 2 (5.5%), 1865/12978 from farm 3 (14.4%), 4104/12978 from farm 4 (31.6%), 1530/12978 from farm 5 (11.8%), 589/12978 from farm 6 (4.5%) and 3368/12978 from farm 7 (26.0%). A total of 5119 were first AIs and 7859 were second or more AIs (from them 1729 were 5<sup>th</sup> or more AIs). The AIs were performed in animals with different lactation orders, with 1586 AIs having been in heifers (only from farms 4 and 7), 3983 in primiparous cows, and the rest in multiparous cows (with lactation order up to 9<sup>th</sup>). Synchronization protocols recorded were observed estrus, G6G, Double Ovsynch, Presynch, Ovsynch (with or without intravaginal dispositive), 5dCosynch (with or without intravaginal dispositive). A total of 10186 AIs was performed during the cool season (September to May) and 2792 during the hot season (June, July and August). Straightforward stepwise Wald logistic regression analysis was used to study the influence of the different factors, including the factor farm as covariable in the model.

**Results:** Average conception rate for all AIs was 36.7%, and average pregnancy loss was 13.9% (664/4764). The range among farms oscillated from 9.5% (28/294) to 16% (194/1209), with the factor "farm" being a significant one affecting pregnancy loss ( $P < 0.0001$ ). When all AIs were included into the model (controlled including the factor "farm" in the model), significant factors affecting pregnancy loss were season with the hot season increasing the risk of pregnancy loss (11.6 vs. 14.5%; OR, 1.539; 95% CI, 1.215–1.949;  $P < 0.0001$ ); the number of AIs, with second or more inseminations showing an increased risk (14.2 vs. 10.5% for first and second or more AIs, respectively; OR, 0.696, 95% CI, 0.581–0.834;  $P < 0.0001$ ), the parity using the stage of heifer (nulliparous) as reference value, and both primiparous and multiparous increasing the loss risk when compared to the heifers (7.8 vs. 9.9 vs. 14.7% for heifers, primiparous and multiparous, respectively; OR, 1.854, 95% CI, 1.330–2.585;  $P < 0.0001$  for primiparous cows, and OR, 3.057, 95% CI, 2.248–4.157;  $P < 0.0001$  for multiparous cows). The fact of having had a previous pregnancy loss was demonstrated as a "protective factor (OR, 0.445, 95% CI 0.278–0.713;  $P < 0.0001$ ). Synchronization protocol did not affect significantly pregnancy loss. When heifers were separately studied ( $n=1586$  AIs), in order to explore the effect of age at insemination a significant effect of this factor was (OR, 1.026, 95% CI, 1.016–1.035;  $P < 0.0001$ ), besides the significant effect of number of AI observed with second or more inseminations showing less rates of pregnancy loss (OR, 0.305, 95% CI, 0.120–0.774;  $P=0.012$ ). In the case of primiparous cows separately analyzed ( $n=3982$  AIs), besides farm ( $P < 0.0001$ ) and season (OR, 1.855, 95% CI, 1.252–2.747;  $P=0.002$ ) no other factor was kept in the model as significant factor; and in the case of multiparous cows ( $n=7409$  AIs) season (OR, 1.647, 95% CI, 1.221–2.221;  $P=0.001$ ), second or more insemination (OR, 0.640, 95% CI 0.513–0.799;  $P < 0.0001$ ) and having had a previous pregnancy loss (OR, 0.340, 95% CI 0.189–0.610;  $P < 0.0001$ ) influenced

significantly pregnancy loss. Synchronization protocol and days in milk at insemination did not significantly affect the risk of having pregnancy loss in adult cows ( $P > 0.05$ ).

**Conclusions:** Our study suggests that the cool season, a higher number of insemination, a younger age of the dam in the case of heifers and having previously suffered pregnancy loss are protective factors for pregnancy loss. However, these factors seem to affect in a different way depending if the inseminations are performed on heifers, primiparous or multiparous cows, which highlights the different reproductive characteristics of the dairy cattle.

RE-P32

### Risk factors for late embryo loss in grazing dairy cows

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**Introduction:** The fertility of high-producing dairy cows has decreased during the last decades, concomitantly with an increase in milk yield. This lower fertility has partly been associated with pregnancy losses. Late embryonic losses (LEL), diagnosed by ultrasonography 30–44 days post-AI, represent a big component of these losses. Therefore, defining the risk factors for LEL would be very important to later develop strategies to control this problem.

**Objective:** The objective of the present study was to assess the risk factors for LEL in grazing dairy cows.

**Material and methods:** A data set of a commercial dairy farm having productive, reproductive and health records of dairy cows calving between Jan 1, 2011 and Dec 31, 2015 ( $n=13,551$ ) was used in this study. Pregnancy was diagnosed by ultrasonography at 30–44 days post-AI. At this time, cows showing lack of a heart beats, membrane detachment, disorganization and echoic floating structures including embryo remnants were defined as having LEL (CASE). Standard disease definitions were used for diagnosis. Cows having retained fetal membranes, metritis, clinical endometritis and/or pyometra were classified as uterine disease (UD). Cows having clinical mastitis and/or clinical lameness were classified as non-uterine disease (NUD).

A case-control study was carried out with a temporal matching design to assess the risk factors for LEL. Four cows were randomly selected from non-case records (CONTROL, positive pregnancy diagnosis on the same date of each case) per every CASE of LEL included. The logistic model (Proc GLIMMIX, SAS) included the fixed effect of year of LEL (2011 through 2015), season of LEL (summer, fall, winter, spring), parity (1, 2,



3 plus), UD, NUD, anestrus, daily milk yield (MILK) and days in milk (DIM) to the event. Statistical significance was set at  $P < 0.05$  and a tendency at  $P < 0.10$ .

**Results:** A total of 642 cases of LEL were reported during the 5-year period. From 13,551 lactations started in this study period, 10,149 had a positive pregnancy diagnosis and were defined as non-cases, among which 2,568 were finally included in the study as controls. From all the included records, 6.3, 20.5, 27.2 and 14.7 % of cows suffered from LEL, UD, NUD and anestrus, respectively. Year and season had no effect on the odds for LEL ( $P=0.509$  and  $0.870$ , respectively). Parity had an effect on the odds for LEL given that cows from parity 2 (Odds Ratio [OR]=1.165, 95% Confidence Interval [95%CI] =0.897–1.512,  $P < 0.001$ ) and parity 3+ cows (OR=1.581, 95%CI=1.236–2.023,  $P < 0.001$ ) had higher odds of LEL than primiparous cows. The UD had no effect on the odds for LEL (OR=1.090, 95%CI=0.886–1.341,  $P=0.415$ ); whereas, NUD increased the odds for LEL (OR=1.298, 95%CI=1.044–1.616,  $P=0.019$ ). Anestrus had no effect on the odds for LEL (OR=0.931, 95%CI=0.704–1.231,  $P=0.615$ ). An increase of 1 SD in milk was associated with a 28% higher odd for LEL (OR=1.281, 95%CI=1.144–1.435,  $P < 0.001$ ). Finally, DIM also increased the odds for LEL (OR=1.008, 95%CI=1.007–1.010,  $P < 0.001$ ) given that the chance increased 0.8% per day.

**Conclusions:** The odds for LEL increased with parity, with the presence of NUD, with daily milk yield, and with DIM in grazing dairy cows. Conversely, the odds for LEL were not associated with year, season, and presence of UD or anestrus.

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**Key words:** late embryo loss, risk factors, milk yield, fertility, grazing dairy cow.

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RE-P33

### Risk factors for pregnancy losses between 30 and 210 days of gestation in grazing dairy cows

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**Objectives:** The objective of this study was to assess the risk factors of pregnancy loss (PL) between 30 and 210 days of gestation in grazing dairy cows in a large commercial dairy farm in Argentina.

**Materials and methods:** A retrospective case-control study including a total of 3,504 records of lactations with 1, 2 or 3 plus PL (CASE) were used. Also, 3,779 records of lactations that did not lose the pregnancy during lactation were included as controls (CONTROL) for comparison. All cows calved from January

1, 2010 to December 31, 2015. Pregnancy diagnosis (PD) was performed every two-weeks between 30-44 days post AI. Pregnancies were recorded as single or double during each diagnosis. Pregnancy losses after confirmation of pregnancy ( $n=3,504$ ) were classified as: 1) cows that had a dead embryo at PD with ultrasonography 30-44 days after, 2) cows that were diagnosed not pregnant at the PD reconfirmation between 60 and 90 days post AI, 3) cows that returned to estrus and were diagnosed not pregnant at the next examination after detected in heat, and 4) cows that were diagnosed pregnant and returned to estrus 30 days after PD and were inseminated. The odds of PL was explained with a Logistic Regression Model that included year of PL (YR; 2010-2016), season of PL (SEAS; spring, summer, autumn, winter), parity (PAR; 1, 2, 3+), number of pregnancy lost (PREG; 1, 2, and 3+), body condition score (BCS;  $\geq 2.5$  and  $\leq 3.0$  [Slim],  $< 2.5$  [Thin],  $> 3.0$  [Fat]), as categorical predictors (fixed effects); and days in milk (DIM) and daily milk production closest to PL (MILK) as continuous predictors. Statistical significance was set at  $P < 0.05$ .

**Results:** Cows in spring, autumn and winter had less chances of losing pregnancies compared to summer (Odds ratio [OR], 95% Confidence Interval [95%CI]; 0.58, 0.46-0.65; 0.56, 0.46-0.64; 0.58, 0.47-0.67;  $P < 0.001$ ). Cows of PAR2 and PAR3+ had higher chances of PL compared to PAR1 cows (1.34, 1.18-1.53; 2.76, 2.39-3.19;  $P < 0.001$ ). Thin cows had more chances of PL compared to slim cows (1.38, 1.43-1.67,  $P < 0.001$ ). Fat cows had similar chances of PL than slim cows. Cows with daily milk production of 1 standard deviation (SD = 7 kg/d) over the mean (36.6 kg/day) had more chances of PL than cows of average milk production (29.6 kg/day; 1.08, 1.02-1.14;  $P < 0.001$ ). Finally, the odds of PL increased with DIM (1.082 [per one day of increase over the mean of DIM], 1.123-1.145;  $P < 0.001$ ).

**Conclusions:** In conclusion, PL is more likely in summer, in PAR2 and PAR3+, and in thin cows. In addition, PL is associated with greater daily milk yield and with higher DIM.

**Key Words:** pregnancy losses, dairy cows.

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RE-P34

### Influence of mineral levels, body condition score and gestation length on retained placenta in cows with stillbirths in Polish dairy herds

#### Association of mineral status and gestation length with placenta retention

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**Objective:** Separation of fetal membranes depends on multiple factors including macromineral disturbances, calving difficulty, body condition score (BCS), gestation length, twin calving, infectious diseases and immune system function. Failure to expel fetal membranes within 24h of calving is defined as retained placenta. The aim of study was to evaluate influence of selected macromineral levels, body condition, gestation length on placenta retention.

**Material and methods:** The study was carried out on 110 cows with stillbirth (death after gestation length 260d or longer). The



material was collected from November 2013 to June 2015 in 29 Polish Holstein-Friesian dairy herds (1-1031 cows/herd, 1-35 stillbirth/herd). Mean intercalving period was 415 d, average milk yield 8884 kg/cow/305 DIM. The collected data included: gestation length, time of placental expulsion, pregnancy number and single/multiple calving. Plasma blood samples were collected between 2 to 6 hours after calving and body condition (scale 1-5 points) was scored. In plasma samples calcium, magnesium and phosphorus concentration were evaluated. All cows subjected to study were classified to 3 mutually exclusive groups, according to time of placenta expulsion: 1 - before/with calf, 2- expulsion 30 min after calving and within 24 hours, 3 - retained placenta (manually removal or expulsion after 24 hours).

**Results:** From 110 cows 52 were primiparous (P) and 58 were multiparous (M). In one M cow placental expulsion wasn't observed. In P cows the number of animals in 1, 2 and in 3 group were 8, 33 and 11 respectively. Twin pregnancies were observed in 6.1% in 2 group, 27.3% in 3 group, and were not observed in 1 group. In M cows group the number of animals in 1, 2 and in 3 group were 10, 28, and 19 respectively. The twin pregnancies were 28.6% in 2 group, 21.1% in 3 group, and were not observed in 1 group. The mean (SD) gestation length in 1, 2, 3 groups were 275.1 (5.2); 279.1 (5.5); 273.2 (3.3) in P cows and 283.7 (12.9); 278.1 (8.0); 271.2 (7.2) in M cows, respectively. The gestation length was significantly shorter in the 3 M group than in 1, 2 M and 2 P groups ( $P<0.05$ ). Gestation length in 3 P cows was significantly shorter than in 1 M group ( $P<0.05$ ). The BCS in 1, 2 and 3 groups of primiparous and multiparous were: 3.1 (0.4) ; 3.2 (0.5); 3.0 (0.4) and 3.3 (0.5); 3.2 (0.6); 3.2 (0.4), respectively. The concentrations of inorganic phosphorus (in 1, 2, 3 group sequence) in P cows were: 1.7 (0.5); 1.6 (0.4); 1.6 (0.5) and in M cows were: 1.7 (0.4); 1.8 (1.3); 1.7 (0.6), respectively. The magnesium concentrations in P cows were: 1.0 (0.1); 1.0 (0.3); 1.0 (0.2), whereas in M cows were: 1.1 (0.4); 1.1 (0.4); 1.0 (0.3) respectively. The levels of calcium in primiparous group were: 2.2 (0.1); 2.4 (0.4); 2.1 (0.2) and in multiparous group were: 2.3 (0.9); 2.1 (0.5); 2.1 (0.6), respectively. The average concentration of serum inorganic phosphorus, magnesium, calcium and BCS not differ significantly between analysed groups.

**Conclusions:** Retained placenta was significantly associated with gestation length in multiparous, but not in primiparous cows in cases of perinatal mortality. The BCS, serum phosphorus, magnesium and calcium concentration didn't have significant impact on placenta status in cows with perinatal mortality.

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RE-P35

### Endometrial mRNA expression of growth factors related with subfertility in high and medium producing dairy cows

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**Introduction:** There is much debate about possible antagonism between high milk production and reproductive performance. Ovarian steroid hormones primarily regulate uterine function by stimulating growth factors and their receptors playing an important role in the regulation of uterine functions.

**Objectives:** The aim of this study was to evaluate the effect of milking production level on the endometrial expression by RT-qPCR of vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor 1 and 2 (VEGFR1 and VEGFR2), nodal growth differentiation factor (NODAL) and epidermal growth factor receptor (EGFR) during diestrus in dairy cows.

**Materials and methods:** This study was conducted in two commercial dairy farms located Buenos Aires province, Argentina. Grazing Holstein dairy cows >50 days postpartum without any clinical disease and with BCS =>2.50 were included in the study. Cows were randomly select and allocated in three groups: 1) High milk production cows (HMP, ~10.000 kg/305d., 2) Medium milk production cows (MMP, ~7.500 kg/305d., 25 l/day, 3) Anestrus cows (ANE, follicles <8 mm, without CL). Endometrial cells samples (q-PCR) were obtained from endometrium by using cytobrush technique for RT-qPCR analysis and to evaluate and discard cows with subclinical endometritis. From all cows, a blood sample was obtained and levels of plasma P4 were measured by quimioluminescence (Roche, Mannheim, Germany). Cell samples from two cytobrush samples per animal were transferred into cryotubes and placed in liquid nitrogen and stored at -80C until subsequent RNA extraction and reverse transcription. Quantitative analysis of the mRNA expression of the selected factors (VEGF, VEGFR1, VEGFR2, Nodal, and EGFR) in endometrial epithelial cells were performed using real-time PCR (Rotor Gene Q, Qiagen, Germany). For mRNA quantification, a dilution series with known quantities of the purified specific amplicon was amplified simultaneously with the samples as a standard (GAPDH). The RNA isolation was performed using Trizol (TriReagent, Molecular Research Center Inc., Cincinnati, OH, USA). Complementary DNA was synthesized using a reaction mixture containing 1 µg of total RNA, random hexamers (Promega, Madison, WI, USA) and MMLV transcriptase (Invitrogen Corp., Carlsbad, CA, USA), following the procedures suggested by the manufacturer. Reactions of qPCR for bovine genes (VEGF, VEGFR1, VEGFR2, EGFR and NODAL) were carried out using specific primers. A dissociation curve was generated at the end of the amplification to confirm that a single product was amplified. To analyze the relative level of expression of each mRNA, the 2- $\Delta\Delta$ CT method was used. Samples from anestrus cows were used as calibrator. The results are reported as the mean fold change of gene transcription levels in samples from HMP over the levels detected in samples from MMP. Significance difference of endometrial gene expression between groups was analyzed by general linear model using R software. This study was approved by the Graduate School and the Laboratory Animal Care and Use Committees of the Faculty of Veterinary Sciences at National University of La Plata.

**Results:** After discarding all samples from cows not in diestrus (P4<1 ng/mL), with low amount of RNA and with outlier results,



a total of 6 HMP and 6 MMP samples were analyzed and included in this study. Our results showed no significance differences in the mRNA endometrial expression between HMP and MMP cows in EGFR (MD= 0.2026, 95% CI= -0.313158, 0.718358; P=0.7037), NODAL (MD= 0.0933, 95% CI= -0.171699, 0.358299; P=0.7329), VEGF (MD= 0.2977, 95% CI= -0.097427, 0.692827; P=0.4702), VEGFR1 (MD= 0.4464, 95% CI= -0.341128, 1.233928; P=0.5846) and VEGFR2 (MD= 0.0089, 95% CI= -0.491824, 0.509624; P=0.9862).

**Conclusions:** In conclusion, the results from this study showed no significance differences in mRNA endometrial expression of the selected growth factors and receptors between HMP and MMP. However, the results obtained are very important for future research related to subfertility in dairy cows.

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**Key Words:** growth factors, milk production, RT-qPCR, endometrial expression.

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RE-P36

### Analysis of two clinical endometritis antibiotic treatment plans in Polish native cattle breeds: preliminary results

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**Objectives:** The aim of the study was to compare clinical endometritis intrauterine treatments by two antibiotics in Polish Red and Lowland Black-and-White (PR and LBW) breed cows. There is lack of research regarding this native cattle breeds, though results may differ significantly in comparison with Holstein-Friesian cows.

**Materials and methods:** The study covered PR and LBW cows (n=52) from the herd in north-eastern Poland kept in semi-intensive system. Cows were milked twice daily and were fed with PMR combined with pasture. They were between second and fifth lactation and were inseminated with frozen semen. The diagnosis of clinical endometritis was based on a character of vaginal discharge (purulent or mucopurulent), a cervical diameter >7.5 cm, presence of hyperechogenic fluid in uterus during ultrasound scan. Cows were between 21 and 55 days postpartum. They had no metabolic, infectious and reproductive disorders other than clinical endometritis. All groups were injected with PGF2 $\alpha$  after finding corpus luteum, and intrauterine infusions were done 48 hours later. Groups A (n=19) and B (n=18) cows were treated once by intrauterine cephalosporin and ceftiofur, respectively. The control group C (n=15) did not get any intrauterine antibiotics. All cows were checked 20 days after the intrauterine treatment. The days open (DO) and the number of services per conception (NSC) were also recorded.

**Results:** There were no signs of clinical endometritis after

treatment in groups A, B and C in: 14 cows (73.68%), 9 cows (50%), 5 cows (33.33%), respectively. Mean DO and NSC were as follows: 109.10 and 1.68; 110.33 and 1.66; 131.66 and 2.4, respectively. Although there was no statistical difference between groups ( $p > 0.05$ ), there was a trend for better efficacy after cephalosporin therapy. Considering DO and NSC, there was a trend towards decreasing DO and NSC in experimental groups, however there was no significant difference between the cephalosporin and ceftiofur treated cows.

**Conclusions:** Results indicate, that both antibiotics are effective and in long term perspective of reproduction cephalosporin and ceftiofur treatments are giving similar results in PR and LBW cattle. It may indicate that cephalosporin is more effective towards clinical endometritis, but subclinical form persists, which could cause similar long term reproductive performance to ceftiofur treated animals. Further studies are needed on a larger population, moreover they should include diagnosis of subclinical endometritis.

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RE-P37

### Use of a bentonite clay as tetracycline drug carrier

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The dairy industry is based on the number of births that a cow has per year. So, it is necessary, for these animals, being efficient in reproducing to guarantee a continuous milk production. During postpartum process, due to the environmental conditions of labor, cows may suffer from intrauterine infections, which compromise the animal's ability to get pregnant again, leading to financial losses for the owner. In Brazil, intrauterine infections in cows are treated with two types of treatments: antibiotics and hormones. Antibiotic treatment used to be expensive because the veterinarian must to administer all the doses. This situation makes farmer decides to give just one antibiotic dose, which lead to antibiotic bacterial resistance. Clay minerals that have applications in many areas such as health, which is usually used as pharmaceutical excipient. Many studies evidence that clays can interact with drugs like antibiotics, characteristic that permit them to become drug carriers in nanocomposites materials. The aim of this work was to study the incorporation of tetracycline in a bentonitic clay and its release. There was prepared a 4% aqueous clay dispersion and the antibiotic was added up to 0.25 g/g clay by mechanical agitation. X-ray Diffraction and Thermogravimetry showed that there was an effective incorporation of the tetracycline in the bentonite. Antibiogram test using E. Coli was used to verify if the antibiotic could release from the clay, its result showed a halo formation. Those results indicate that a bentonite-tetracycline system has a potential use in the controlled delivery of that antibiotic in veterinarian medicine.



RE-P38

### The effect of peri-estrous vaginal discharge on the conception in Holstein dairy cows

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**Objectives:** Purulent vaginal discharge (PVD) or urovagina of postpartum cow has been reported to have a negative impact on subsequent reproductive performance. Vaginal mucus becomes less viscous as ovulation approaches. Thus, evaluation of vaginal discharge around estrus might be important for successful AI. The present study aims to score the vaginal discharge around estrus such as urovagina, purulent discharge and viscosity and to determine the association of the scores with conception rate (CR) in Holstein dairy cows.

**Materials and methods:** The present study analyzed 653 times of spontaneous estrus from 431 Holstein-Friesian cows in 40 commercial dairy herds. Estrus was claimed by farmers and confirmed by the presence of palpable dominant follicle and regressed corpus luteum. The vaginal discharge was evaluated by gloved hand method and speculum immediately before AI. Vaginal discharge score (VDS) ranging from 0 to 4 (where 0 = no material, 1 = translucent mucus, 2 = mucus containing flecks of white pus, 3 = less than 50% white pus, and 4 = greater than 50% white pus) was diagnosed by gloved hand method. PVD was designated the VDS ranging from 2 to 4. Urovagina was classified into mild or moderate/severe according to the volume of uterine inside the vagina judged by speculum (mild = small amount of urine-mixed mucus only on the floor of the vagina, moderate = appreciable amount of urine-mixed mucus covering up to half of the external cervical os and severe = large amount of urine-mixed mucus covering at least half external cervical os). The viscosity of vaginal discharge was classified into 3 categories, such as no material, watery or sticky/starchy. Body condition score (BCS) was also recorded. The contribution of PVD, urovagina and viscosity of mucus on insemination rate (IR) and CR were evaluated. The effect of vaginal discharge on the conception was also evaluated by logistic regression analysis.

**Results:** The PVD was observed in 15.1% of cows. Mild and moderate/severe urovagina were found in 5.9% and 4.4% of cows, respectively. In the examination for viscosity of vaginal discharge, cows with no material and sticky/starchy vaginal discharge were 3.0% and 9.6%, respectively.

Overall IR was 85.6%. The IR for PVD cows (64.4%,  $P < 0.01$ ) was significantly lower than that of normal cows (90.3%). No significant difference in the IR was found between mild urovagina (91.7%), moderate/severe urovagina (85.2%) and normal cows (87.1%). The IR of cows with sticky/starchy viscosity (44.8%,  $P < 0.01$ ) was lower than that of cows with watery viscosity (90.4%). The IR of cows with no material (77.8%) is not significantly different from that of cows with watery viscosity.

Overall CR was 35.4%. There was no significant difference in CRs between PVD (32.1%) and normal cows (35.8%). Statistically significant decreasing trend in CR of urovagina was observed (37.2% in normal cows; 21.9% in mild urovagina; 13.0% in moderate/severe urovagina, Cochran-Armitage trend test  $P$

$< 0.01$ ). The CR in no material (15.4%) or sticky/starchy viscosity cows (15.4%) was not significantly different from that in watery animals (36.9%).

In logistic regression analysis, moderate/severe urovagina (OR = 0.26,  $P < 0.05$ ) and sticky/starchy viscosity (OR = 0.26,  $P < 0.05$ ) were selected as explanatory variables associated with the conception against none of urovagina or watery viscosity respectively, along with BCS.

**Conclusions:** Moderate/severe urovagina and sticky/starchy vaginal discharge around estrus have a negative impact on the conception in dairy cows, indicating the importance of evaluating vaginal discharge before AI to increase the CR in dairy cows.



RT-P01

### Influence of Genotype, Diluents and Storage Time on Bovine Semen Characteristics Stored at 5° C in Nigeria

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**Objectives:** The objectives of this study are: To assess the effect of genotypes, diluents and storage time at 5° C on Bovine semen characteristics in Nigeria.

**Materials and Methods:** The research was conducted at Live-stock Investigation Division (LID) of National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. Three genetically different mature bulls as follows: 100% Friesian; 75% Friesian x 25% Bunaji; and 50% Friesian x 50% Bunaji weighing between 400 and 650 Kg, 5 to 7 years old and with a mean scrotal circumference of 36.6 cm were used as semen donors for the experiment. The experiment consists of four diluents: Tris-based diluent (TBD), Egg - yolk citrate (EYC), Skimmed-milk diluents (SMD) and Phosphate buffer solution (PBS). Semen samples were collected once in a week during early hours of the day using artificial vagina. The semen from each bull was split into 4 and diluted with each of the 4 diluents and stored at refrigeration temperature at 5° C for 72 hours. The whole process was repeated for 12 weeks. All bulls were maintained under uniform condition of feeding, housing and management: concentrates 1.5 % and hay 2.5 % body weight per head per day were provided.

**Results:** The overall mean for genotype on volume, colour, pH, percentage motility, concentration, percentage live and normal spermatozoa were 8.30±1.96 ml, 1.18±0.52, 6.91±2.43, 76.90±16.45%, 578.60±518.24 x10<sup>6</sup>/ ml, 89.01±7.05 % and 93.40±4.38 % respectively. The semen characteristics observed (with the exception of pH) differed significantly (P<0.01) with genotype. The 50% Friesian x 50% Bunaji bull had higher semen volume, motility, percentage live and normal sperms as compared to 75% Friesian x 25% Bunaji and 100% Friesian. The 100% Friesian bull had the highest sperm concentration which was the lightest in colour. Sperm concentration, percentage live, and volume were lowest in 75% Friesian x 25% Bunaji while colour was best in this genotype. Only motility and concentration differed significantly (P<0.01) among diluents. The TBD had highest mean sperm motility (86.56±8.99%) and concentration (683.30±490.86x10<sup>6</sup>/ml) followed by EYC. On the other hand, PBS had the lowest sperm motility (64.88±13.93 %) and concentration (526.29±531.37 x10<sup>6</sup>/ ml); while SMD had moderate motility mean value of 75.049±21.61% and concentration of 527.59±532.06 x10<sup>6</sup>/ ml. The per cent difference in motility was 5.32 between days 0 and 1 and 9.53 between days 1 and 2. There was significant (P<0.01) interaction effect of diluent x day on percentage motility. However, values for SMD and PBS decreased with storage. Specifically, values on days 2 and 3 for SMD (67.67±7.53 and 68.58±8.52) were similar but statistically lower than those on days 0 and 1 (83.17±8.24 and 80.78±38.86 %) respectively. The interaction effect of diluents x day on sperm motility of 100% Friesian was also significant (P<0.01). The TBD and EYC did not show variation in motility with storage. Most of the correlation coefficients

between bovine semen characteristics were low and negative.

**Conclusions:** The use of Tris-Based and Egg-yolk Citrate as diluents for Bovine semen best maintained motility and concentration of sperm cells in Nigeria. Semen characteristic parameters were better in the 50% Friesian x 50% Nigerian Bunaji bulls than in the other genotypes. Quality parameters of diluted Bovine semen stored at 5° C declined with increased storage period.

RT-P02

### Objective high-throughput measurement of bovine spermatozoa motility

#### Using differential dynamic microscopy (DDM) in an "on-farm" setting.

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**OBJECTIVES:** Bull Breeding Soundness Examinations are routinely carried out on farms across the UK by veterinarians. As part of this process semen is assessed using subjective microscopy for gross as well as progressive motility. Using this technique results can vary between operators. It is possible to collect a sample and assess motility using computer assisted semen analysis (CASA) systems, however this equipment is expensive and cumbersome – requiring permanent situation in a laboratory and a highly trained technician to assess samples. This makes it unsuitable for on-farm assessments. The aim was to establish a high-throughput, objective assessment system for bovine semen using differential dynamic microscopy (DDM) that can be used by technicians in an "on-farm" setting.

**MATERIALS / METHODS:** The DDM system was validated using frozen bovine semen (produced for standard artificial insemination). The DDM system consisted of a purpose-built microscope system and a computer with specially designed software to process videos. After validation, samples of fresh semen collected on farm were assessed using the novel system. Differential Dynamic Microscopy measures fluctuations in density with time at a given length scale, quantified by the correlation function  $g(q, \tau)$ .

**RESULTS:** It was successfully demonstrated that DDM is a portable high-throughput characterisation of bovine semen motility that can be applied to both fresh and frozen (thawed) samples on-farm and in-lab respectively. The technique was validated with particle tracking. DDM could be used to measure & control the effect of diluting a semen sample, an unavoidable constraint of current techniques of progressive motility assessment.

**CONCLUSIONS:** DDM can be used alongside established techniques for objective assessment of bovine semen, including swimming speed, head amplitude, head frequency and % motility. Currently the bull pre-breeding examination includes subjective visual microscopy. CASA provides objective measurements in-lab but is not routinely used on-farm. DDM could offer an objective bull-side evaluation in pre-breeding examinations and evaluations of thawed AI semen on-farm, as it is pos-



sible to use a portable setup to take measurements. In the future it might be interesting to investigate the use of DDM for performing measurements on samples where analysis is difficult with CASA, e.g. AI semen extended in milk before freezing. Future studies could investigate a link between DDM measures of motility and parameters already associated with semen quality (e.g. those provided by flow cytometry) as well as to field fertility outcomes.

SR-P01

### **Occurrence of the main viral agents that cause respiratory diseases in sheep in the State of São Paulo and Rio de Janeiro - Brazil**

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The respiratory diseases of small ruminants constitute a serious problem for breeders due to economic losses that include the costs of treatment, prevention, lower meat and milk production and animal death. It is a multifactorial disease and there are several etiological agents responsible for the illness. Bacterial respiratory diseases are as most commonly described, however, sheep suffer from diverse viral disease. Acute pneumonia is associated with parainfluenza virus type 3 (PI-3), adenovirus and respiratory syncytial virus, affecting more lambs. However, chronic pneumonia is more common in adults and is related to Maedi-Visna, herpesvirus, bovine viral diarrhea virus, among others.

**Objective:** The aim of this study was to determine the main virus present in the lower respiratory tract of healthy and sheep with respiratory disease.

**Materials and methods:** Were used 99 sheep (male and female, two months to three years old) from 6 farms of Rio de Janeiro State and 4 farms of São Paulo State – Brazil. To classify animals as healthy and unhealthy, the evaluation of vital parameters and a specific examination of the respiratory tract were performed. Blood was collected from the external jugular vein, obtaining 8 ml of blood using a vacuum system without anticoagulant (BD Vacutainer® Plus Serum). The samples were centrifuged for 10 minutes to obtain the serum and transferred to microtubes with a capacity of 0.5 mL and stored until the moment of the analysis. The detection of antibodies against PI-3, herpesvirus, respiratory syncytial virus and bovine viral diarrhea virus was performed the virus neutralization test. In the case of Maedi-Visna, the agar gel immunodiffusion test (AGID) was realized.

**Results:** Of the animals studied, 62 were classified as healthy and 37 as unhealthy. With the tests performed, neither group presented positive samples for the virus studied.

**Conclusion:** With the increase in the commercialization of by-products from sheep farming, it was necessary to concentrate more animals in less space to intensify production. This type of breeding was reflected in a high occurrence of respiratory viruses. Because of this, it is necessary to know the responsible agents to create adequate prevention and control management, reducing the occurrence and losses inherent to viral respiratory disease. Although no sample was positive for the agents studied, further research should be carried out to better understand which viral agents are linked to respiratory disease in the Brazilian regions.





SR-P02

### Schmallenberg virus antibodies surveillance in bulk milk samples in Portugal

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**Objectives:** Schmallenberg virus (SBV) is a novel virus of the Bunyaviridae family, genus Orthobunyavirus causing disease in ruminants with significant economic impact. A recent study in Portugal showed evidence of sheep exposure to the virus, however SBV has not yet been detected and characterized. In the present work a bulk milk tank study was performed to detect evidence of SBV spread, in the mountainous region of Serra da Estrela, Portugal, between 2015-2016.

**Materials and methods:** A total of 78 sheep milk farms from 46 parishes within the mountainous region of Serra da Estrela, Portugal, were recruited to perform a prospective longitudinal study, in order to monitor the expected spread of SBV between 2015 and 2016. All farms provided a bulk milk sample in 2015 and 2016. Bulk milk sera were tested for SBV IgG by using an indirect ELISA (ID Screen Schmallenberg virus Milk Indirect; ID.vet; Montpellier, France), previously described to be a useful method for detecting herd level exposure to SBV. None of the participating farmers used SBV vaccine during the course of the study.

**Results:** Of the 78 bulk milk sera from 2015, 73 (93.5%) showed to be positive for IgG anti-SBV, while in 2016, only 63 of those farms (80.8%) showed anti-SBV IgG presence in bulk milk sera. Of the 2015 seronegative farms, only 2 became seropositive while of the seropositive farms, 13 became seronegative. Analysis of the monthly average temperature in the region showed that in 2015, the temperature reached values of 7°C which might have contributed to the decrease in vector activity and hence the decrease in seropositivity.

**Conclusions:** This study shows that SBV circulates in the mountainous region of Serra da Estrela, Portugal and seems to be decreasing the prevalence at the herd level. The weather conditions may have assisted in reducing the number of viable vector midges, thus preventing the infection from spreading.

SR-P03

### Surveillance of Bluetongue virus circulation in sheep herds of Portugal

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**Objectives:** Bluetongue is a disease of domestic and wild ruminants caused by bluetongue virus (BTV), a non-contagious vector-borne *Orbivirus*. BTV causes hyperpyrexia, leukocyte decrease and mucosal ulcerative inflammation changes, ultimately causing fatal clinical signs in sheep. The aim of the present work was to detect and monitor the circulation of the

virus in central region of Portugal, to study the surveillance of BTV circulation in Portugal.

**Materials and methods:** Four municipalities of the region of Beira Interior, Central Portugal, were selected, considering the high number of sheep heads. In February 2016, a total of 27 farms were visited and 102 individual sheep blood samples were collected by venepuncture. In June 2016, blood from the same sheep and tanks was again collected so as to observe changes in seropositivity after one period of vector activity. A commercial ELISAs (ID Screen® BlueTongue Competition kits) was used to screen samples for the detection of antibodies against the BlueTongue virus VP7 protein.

**Results:** In the first period, anti-BTV IgG antibodies in sheep sera were detected (4.63%), as well as in the second period (3.13%). The decrease in the seroprevalence of IgG antibodies against BTV likely suggests that BTV is decreasing its circulation in Central Portugal.

**Conclusions:** The present study suggests that BTV is decreasing its circulation the Beira region of Portugal. This sets the need of further screenings, with increased number of animals, to provide a clearer picture of BTV in this region.

SR-P04

### Phenotypic antibiotic resistance pattern and presence of *mecA* in *Staphylococcus aureus* isolated from caprine mastitis

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Emergence of antibiotic resistance in *Staphylococcus aureus*, and in particular community associated Methicillin-resistant *S. aureus* (MRSA) is one of the most serious problem. In the present study, 55 isolates of *S. aureus* from cases of subclinical mastitis were evaluated for phenotypic antibiotic resistance patterns. A total of 27 antibiotics belonging to 12 groups of antibiotics were tested. Cloxacillin, ceftriaxone + sulbactam, clindamycin, chloramphenicol, teicoplanin and bacitracin showed high activity while penicillin, amoxicillin + clavulanic acid, ampicillin, amoxicillin, ceftazidime and cefaperazone exhibited least sensitivity. Multidrug resistance (MDR) was observed in 41 of the studied isolates, and of these 5 exhibited extreme drug resistance (XDR) and one isolate demonstrated resistance to all the antibiotics (pan drug resistance, PDR). Though phenotypic methicillin resistance was observed in 21 isolates, *mecA* was present in only 4 isolates. The antibiotic resistance is mainly attributed to acquisition of resistance genes by genetic exchange. However, the present study revealed that there may be some other mechanisms associated with methicillin resistance in *S. aureus*.



SR-P05

### Observational study on a novel management system involving 10 lambings per year in one high-yield dairy ewe farm

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**Objectives:** In order to reduce non-productive days in dairy ewe production, the “accelerated sheep mating systems” can be used. One example is the STAR system, which involves up to 5 lambings/ewe in 3 years (1.2 to 1.4 lambing/ewe/year) through 5 successive annual breeding and lambing seasons (5LY). Despite its feasibility, a major disadvantage to this system is an irregular distribution of the workload throughout the year. For instance, the farm staff capacity is easily overwhelmed during the lambings periods, resulting in a deficient care of the newborns. In an attempt to distribute the workload more evenly, a high-yielding Lacaune farm transitioned from the 5LY system to a novel one consisting on 10 lambing periods per year (10LY). The present study examines the efficiency, productivity and workload concentration in this farm under both systems. Its goal was to determine the effects of a more intensive management system on the farm’s productivity, animal welfare and workload distribution.

**Material and methods:** This observational study was performed in a high-yielding Lacaune dairy ewe farm (“Granja Cerromonte”, Spain). The 5LY system was performed from 2010 to 2012, and the 10LY system from 2014 to 2015. Years 2009 and 2013 were considered transition years. During this period, 27,415 lactations were recorded from an average of 3,746 ewes/year.

**Results:** Daily production ( $1.70 \pm 0.62$  l/d vs  $1.73 \pm 1.66$ ;  $P = 0.038$ ), interlambing period ( $302 \pm 44$  vs  $283 \pm 50$  d;  $P < 0.0001$ ) and lambings/ewe/year ( $1.30 \pm 0.01$  vs  $1.42 \pm 0.01$ ;  $P < 0.05$ ) slightly worsened in the 10LY system compared to the 5LY one. In contrast, milk yield/lactation ( $370 \pm 156$  vs.  $349 \pm 185$  L), lactation length ( $218 \pm 75$  vs  $192 \pm 75$  d) and dry period length ( $53.5 \pm 38.3$  vs  $69.1 \pm 34.8$  d) (all  $P < 0.0001$ ) were better in the 10LY system. Most importantly, the workload distribution improved by the 10LY system implementation. During 2010–2012, an average of  $889.19 \pm 158.47$  lambings/lambing period (range, 664 to 1 291) was recorded, resulting in an average of  $1,502.73 \pm 267.82$  live newborn lambs/lambing period and  $20.80 \pm 18.73$  lambings/day (range, 1 to 110). There were 500.6 lambs and 296.6 lambings per worker during lambings periods. Extra work hours were necessary at peak lambings/day. However, during 2014–2015, an average of  $443.66 \pm 253.18$  lambings/lambing period (range, 85 to 937) was recorded, resulting in  $709.85 \pm 405.10$  live newborn lambs/lambing period and  $15.16 \pm 7.83$  lambings/day (range, 1 to 51). There were 354.5 lambs and 221.5 lambings per worker during lamb-

ing periods. Since the lambing and lamb related workload was better distributed in the 10LY-period, the workload/worker fell by 50%, needing only two workers/lambing season to take care of the lambings and lamb pens in the 10LY system (354.5 lambs and 221.5 lambing per worker), compared to the three necessary in the 5LY one (500.6 lambs and 296.6 lambing per worker). Further, culling rate decreased ( $35.39 \pm 0.53$  vs  $42.51 \pm 7.51$  %  $P = 0.294$ ) and higher-order lactations increased ( $24.17$  vs  $16.08$ %,  $P < 0.0001$ ) which could be the result of better ewe and lamb management. Another possible benefit could be the increase in the worker’s welfare, since the workload does not concentrate so drastically in certain days.

**Conclusions:** Our study suggests that a 10LY herd management system can be compatible with good profitability and productivity and, more importantly, better animal welfare due to lack of animal overcrowding and better care of lambs and ewes during lambing. Furthermore, it could also mean better working conditions for the farm staff, since the workload is more uniformly distributed throughout the year.

SR-P06

### Effects of maternal factors on the metabolic profile throughout pregnancy in dairy sheep

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**Objectives:** Physiological states as pregnancy and lactation modify metabolism in sheep. The aim of this study was to evaluate the influence of pregnancy features (parity and type of pregnancy) on the maternal metabolism in high yielding Lacaune dairy sheep.

**Material and methods:** The study was carried out in a single commercial farm on 334 Lacaune dairy sheep, classified by age (187 mature and 147 maiden ewes), parity (145 multiparous vs. 42 primiparous) and type of pregnancy (161 single vs. 173 multiple). Fasting plasma samples were collected at two points during pregnancy mid and late-pregnancy ( $74 \pm 5$  and  $141 \pm 3$  d, respectively) and *postpartum* ( $52 \pm 5$  d after delivery). The following metabolic parameters were considered: plasma  $\beta$ -hydroxybutyrate ( $\beta$ -OHB), nonesterified fatty acids (NEFA), glucose, lactate, urea, cholesterol, triglycerides and fructosamine. Differences among groups and interactions were evaluated with ANOVA and Kruskal-Wallis test when non-normal distributed.

**Results:**  $\beta$ -OHB was higher during gestation in mature than in maiden ewes ( $0.58 \pm 0.20$  vs.  $0.37 \pm 0.11$  mmol/L;  $P < 0.0001$ ) at mid pregnancy, ( $0.67 \pm 0.34$  vs.  $0.55 \pm 0.21$  mmol/L;  $P < 0.0001$ ) and late pregnancy, but did not differ with age at *postpartum*



( $0.78 \pm 0.25$  vs.  $0.74 \pm 0.23$  mmol/L;  $P > 0.05$ ). During gestation  $\beta$ -OHB was not affected by type of pregnancy, although, *postpartum*  $\beta$ -OHB was lower in ewes that had carried out single gestation than in those with multiple pregnancy ( $0.59 \pm 0.27$  vs.  $0.65 \pm 0.31$  mmol/L;  $P < 0.01$ ). At mid pregnancy NEFA were lower in mature than in maiden ewes ( $0.46 \pm 0.22$  vs.  $0.57 \pm 0.17$  mmol/L;  $P < 0.0001$ ); whereas NEFA were higher in mature at late pregnancy and *postpartum* ( $0.67 \pm 0.29$  vs.  $0.55 \pm 0.18$  mmol/L;  $P < 0.0001$  for late pregnancy); ( $0.50 \pm 0.18$  vs.  $0.44 \pm 0.16$  mmol/L;  $P = 0.004$  for *postpartum*). At late pregnancy, ewes carrying a single pregnancy showed lower NEFA than those with multiple fetuses ( $0.57 \pm 0.25$  vs.  $0.67 \pm 0.25$  mmol/L;  $P < 0.0001$ ). Glucose levels during gestation did not differ with age, however, at *postpartum* mature ewes had lower levels than maiden ewes ( $67.90 \pm 6.44$  vs.  $74.63 \pm 7.54$  mg/dl  $P < 0.0001$ ). Glucose during gestation was higher in ewes carrying a single gestation at mid ( $65.45 \pm 15.48$  vs.  $60.44 \pm 8.82$  mg/dl;  $P < 0.004$ ) and late pregnancy ( $71.84 \pm 14.62$  vs.  $67.37 \pm 12.53$  mg/dl;  $P < 0.003$ ) but did not differ *postpartum*. Lactate was lower in mature ewes at mid pregnancy ( $16.34 \pm 8.73$  vs.  $18.85 \pm 10.13$  mg/dl;  $P < 0.003$ ), and *postpartum* ( $11.13 \pm 6.90$  vs.  $12.07 \pm 5.64$  mg/dl;  $P < 0.01$ ), but it was similar at late pregnancy and did not change by type of pregnancy. Urea was higher in mature ewes at mid pregnancy ( $57.44 \pm 11.23$  vs.  $39.95 \pm 7.86$  mg/dl;  $P < 0.0001$ ) and *postpartum* ( $67.56 \pm 15.01$  vs.  $58.18 \pm 13.53$  mg/dl;  $P < 0.0001$ ), but lower at late pregnancy ( $41.44 \pm 10.70$  vs.  $42.31 \pm 8.81$ ;  $P < 0.048$ ). Urea was higher at late pregnancy in ewes carrying a single gestation ( $43.29 \pm 10.15$  vs.  $40.45 \pm 9.50$  mg/dl;  $P < 0.004$ ). Cholesterol was higher during gestation in mature than in maiden ewes ( $P < 0.0001$ ). However, at *postpartum* cholesterol was higher in maiden ewes ( $108.79 \pm 21.76$  vs.  $100.44 \pm 21.74$  mg/dl;  $P < 0.001$ ) and it was not affected by type of pregnancy. Triglycerides were higher in mature ewes ( $23.51 \pm 8.04$  vs.  $19.29 \pm 7.33$  mg/dl;  $P < 0.001$ ) at mid pregnancy. At *postpartum*, triglycerides were higher in maiden ewes ( $17.73 \pm 8.62$  vs.  $15.07 \pm 4.37$  mg/dl;  $P < 0.001$ ) and did not differ with type of pregnancy. Fructosamine was higher at mid gestation in mature ewes ( $310.77 \pm 31.63$  vs.  $275.47 \pm 30.58$  umol/L;  $P < 0.0001$ ) and at the same time, it was affected by type of pregnancy ( $289.75 \pm 36.95$  vs.  $300.30 \pm 33.90$  umol/L for single and multiple pregnancies respectively;  $P = 0.013$ ). The daily average milk yield during the lactation was not different between primiparous and multiparous ( $312.42 \pm 130.68$  vs.  $339.6 \pm 142.9$  L;  $P > 0.05$ ); before getting pregnant, primiparous produced less than multiparous ( $1.71 \pm 0.62$  vs.  $2.07 \pm 0.67$  L;  $P < 0.05$ ), and after conception primiparous produced the same as multiparous ( $1.11 \pm 0.38$  vs.  $1.07 \pm 0.46$  L;  $P > 0.05$ ). Milk yield was not affected by type of pregnancy.

**Conclusions:** The present data reveal interactions among maternal factors (age and type of pregnancy) physiological status (pregnancy and lactation) and time of pregnancy on the metabolism of dairy ewes, which be related to health and productivity.

SR-P07

### Seasonal lambing distribution of the Romanov breed in northwestern Croatia during five consecutive years

#### Seasonal lambing distribution of Romanov breed in Croatia

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**Objectives:** The aim of this study was to investigate the lambing distribution of the Romanov breed in northwestern Croatia during five consecutive years. Most European breeds of sheep are seasonal breeders in the moderate climate region; however, the Romanov breed is a meat breed that is aseasonally polyoestrous. Romanov sheep are considered to be highly fertile, with a fecundity rate of 230% or more. In Croatia, the lambing season is primarily in the winter and spring for seasonal breeders.

**Materials and methods:** During five consecutive years (December 2011 to November 2016) at eight medium-scale sheep farms in northwestern Croatia, there were 5379 matings with 5046 successful conceptions *i.e.* lambings. Ewes were kept at pasture, which primarily provided area for exercise, with access to stables during the night, in a semi-intensive environment. According to standard farming practices, animals had free access to good quality meadow hay (about 1.8 kg per doe daily), adequate concentrate and drinking water.

**Results:** Fertility was 93.81%. The seasonal distribution of lambings in this study was: 47.64% of ewes delivered in winter ( $n=2422$ ), 23.37% in spring ( $n=1179$ ), 18.82% in summer ( $n=950$ ) and 9.81% in autumn ( $n=495$ ). The winter season refers to the period of December to February. Sexual activity was lowest was during spring and early summer (March to June) with a peak of sexual activity from August to October. Litter size was greater during spring and winter than in other seasons ( $1.67$  vs.  $1.36$ ) though birth weight was lower in larger than in smaller litters ( $2.64 \pm 0.65$  vs.  $2.87 \pm 0.61$ ).

**Conclusions:** More lambs during lambing season and a higher percentage of multiple births (triplets, quadruplets, etc.) was expected during the optimal breeding season, as seen in most European sheep breeds. Despite being aseasonally polyoestrous, the distribution of mating and lambing was not uniform through the seasons for the Romanov breed.

SR-P08

### Expert knowledge elicitation for ranking hazards affecting dairy goat welfare in Italian farms

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**Objectives:** The opinion of 14 Italian veterinarians was gathered using an expert knowledge elicitation in order to charac-



terize a set of management and housing hazards potentially associated with negative welfare outcomes in dairy goats kept in loose housing systems (intensive, semi-intensive and semi-extensive farms) in Italy. This was the first step for the development of a feasible and simplified protocol for the on-farm assessment of dairy goats welfare to be used at national level.

**Materials and methods:** Following the methodology described by Bertocchi *et al.* (2018), 14 veterinarians, expert in dairy goat farming and welfare, were asked to score 21 hazards (10 management hazards and 11 housing hazards) that could affect the welfare of dairy goats. The experts were asked to weigh the potential negative impact of each proposed hazard in relation to 5 welfare categories: i) udder health, ii) metabolic needs, iii) locomotion and foot health, iv) integument alterations and v) behavior. Experts were asked to estimate the magnitude (scoring scale from 0 – none – to 3 – high) and the likelihood (from 0% to 100%) of the negative welfare consequence that could be associated with the exposure of the animals to each of the 21 hazards. Experts were also asked to rate their certainty in relation to the likelihood value they provided (scoring scale: high, medium, low).

Using the experts' ratings, an overall impact score (IS) was determined for each hazard as in Bertocchi *et al.* (2018). Then, management and housing hazards were ranked based on the obtained ISs.

**Results:** The 3 management hazards that were characterized by the highest ISs on dairy goat welfare were found to be: i) presence of dirty and not correctly managed pens, with no bedding material or with dirty bedding material (IS = 6.77); ii) no accurate grouping strategy that takes into account the specific animal needs in the different life stages: all the animals are kept together (young stock, adult dairy goats and bucks) (IS = 5.74); iii) absence of biosecurity programs or implementation of inadequate biosecurity measures (IS = 5.43).

The top 3 housing hazards were determined to be: i) number of feeding places < 100% of the total number of adult goats or feeding places with wrong dimension and design (< 35 cm/goat) (IS = 6.24); ii) bucks kept in pens with wrong dimension and design (space availability < 3.5 m<sup>2</sup>/buck, feeding place dimension < 60 cm/buck, no drinkers, absence of bedding material) (IS = 4.90); iii) kids kept in pens or boxes with wrong dimension and design (space availability < 0.3 m<sup>2</sup>/kid, feeding place dimension < 20 cm/kid and less than one dummy for 15 kids) (IS = 4.79).

**Conclusions:** The obtained results underlined the importance of both space and resource availability for the welfare of dairy goats, together with an accurate management of pen hygiene, an attentive grouping strategy and the implementation of measures for the reduction of the risk of transmission of infectious diseases within the herd.

Bertocchi L. *et al.*, 2018. *Characterization of hazards, welfare promoters and animal-based measures for the welfare assessment of dairy cows: Elicitation of expert opinion. Prev. Vet. Med.* 150, 8-18.

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SR-P09

## Dermatosparaxis in lambs

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Dermatosparaxis, also known as cutaneous asthenia, is a disease caused by a deficiency in the conversion of p N-collagen to collagen, causing skin fragility in affected animals. Similar disease was related in humans, cattle, horses, mink, dogs and cats. This disease is a recessive inheritance, in the composition of collagen, in humans a similarly disease is Ehlers-Danlos syndrome type VII. The recessive homozygous causes tearing of the skin, hyperextensibility and fragility of skin.

In sheep, this disease was first related in White Dorpers in the 1980s in The Republic of South Africa, with reports of the occurrence of this disease in four other breeds of sheep: Border Leicester – Southdown, Finnish-Merino cross, Norwegian Dala, and Romney. In Brazil, the disease was described in White Dorper. This case report describes the occurrence of dermatosparaxis in crossbred Santa Inês lambs with White Dorper, as well the clinical and hematological findings of this disease. The lambs were the offspring of crossbred ewe (White Dorper vs Santa Inês) with your father, a White Dorper sheep. The two lambs (1 female and 1 male) were referred in 2015 to Hospital Clinical Didactic Unit of the University of São Paulo, in Pirassununga, with various wounds on the body. On physical examination, both animals have a fever; no significant abnormalities were identified in heart rate, respiratory rate, ruminal movement and body temperature. The hematological exam of the male lamb were: red blood cells 8.23 (x10<sup>6</sup>); hemoglobin 8.0 (g/dL), packed cell volume 24.1 (%), MCV 29.4 (fL), MCH 9.7 (pg), MCHC 33.1 (g/dL), RDW 24.2 (%), platelets 278 (x10<sup>3</sup>/uL) and WBC 15.1 (x10<sup>3</sup>/uL). The differential of leucogram were: neutrophil 7.24 (x10<sup>3</sup>/uL), lymphocyte 7.70 (x10<sup>3</sup>/uL), monocyte 0.15. The biochemistry analysis were: urea 122.97 (mg/dL), creatinine 1.2 (mg/dL), total protein 3.98 (mg/dL), albumin 1.8 (g/dL), globulins 2.18 (g/dL), GGT 41 (U/L), AST 69 (U/L), CK 115.85 (U/L), cholesterol 48.39 (mg/dL). This results show that animals suffer fever because by the lacerating wounds, causing an intense inflammatory reaction in the skin. The hematological exam show a reduced value of packed cell volume, and higher value of urea, and lower values of total protein, albumin and cholesterol which may have occurred due to intense inflammation, associated with the inappetence presented by the animals. The animals did not respond to antimicrobial, anti-inflammatory and hydro-electrolytic therapy, being the injuries incompatible with the life, causing the death of the first lamb with 16 days of live, (1 day after the hospitalization in the Veterinary Hospital and second lamb died with 19 days of life (4 days in Veterinary Hospital).

The disease was confirmed by PCR test using blood DNA sample and specific primers to amplify the single nucleotide polymorphism (SNP) (c.421G>T) in the ADAM metalloproteinase with thrombospondin type 1 motif, 2 (ADAMTS2) gene was optimized in order to perform molecular diagnosis of this disease. The direct sequencing of the PCR products evidenced the mother and the father of the lambs were heterozygous and the lambs homozygous for the mutation responsible for der-



matosparaxis. The lambs were consanguineous, sons of the father with the daughter, being that the father and mother was heterozygous, elucidating the origin of the homozygosis. The results of the observations of these clinician cases shows that the recessive gene responsible for dermatosparaxis is spreading in the Brazilian herds, affecting including crossbred animals of the Santa Inês breed. This disease can causes many economic problems due to lack of control in sheep herds in Brazil, such as in South Africa, the USA, New Zealand and Australia, where the disease is prevented in almost all herds.

SR-P10

### Effect of organic selenium and zinc methionine on feedlot and carcass traits of hair sheep

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In order to evaluate the effect of the supplementation of zinc methionine (ZM) and organic selenium (Se) in the finishing diet of male lambs intended for meat production, 24 lambs (average initial weight of 25±3.4 kg) of Pelibuey and Pelibuey x Dorper breeds were housed in individual cages under a completely randomized design and divided in 3 groups of treatments (8 lambs per treatment); the three treatments were: (T0) control group, no supplementation at all; (T1) group supplemented with 0.3 mg/kg DM of ZM; and (T2) group supplemented with 0.04 mg/kg DM of Se. The experimental period lasted 45 d, and the response variables recorded were daily weight gain (DWG), dry matter intake (DMI), feed conversion (FC), and partial feed utilization efficiency (PFUE). Also, the carcass traits measured were back fat (BF) and rib-eye area (REA). The analysis of variance showed that there were no differences in the performance variables DWG, DMI, and FC. However, the variable PFUE was higher ( $P < 0.05$ ) in T2 (0.52 ppm) compared to T0 (0.22 ppm) and T1 (0.33 ppm). The carcass traits variables BF and REA showed no change by effect of treatment. In conclusion, addition of selenium or zinc methionine to the finishing diet of hair male lambs did not improve their performance characteristics or some carcass traits.

SR-P11

### Ovarian Doppler ultrasonography in Santa Inês ewes superovulated with different doses of exogenous FSH

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The most important factors that affect embryo transfer (ET) in ewes are the high variability of the superovulatory response and the premature regression of corpora lutea, which may be correlated to the high doses of exogenous FSH used in superstimulatory protocols. The aim of this study was to study blood flow rates of the ovarian arteries (through Doppler ultrasonography) in Twenty-nine ewes treated with different doses of exogenous porcine FSH (Folltropin-V®, Bioniche, Belleville, Canada) in gonadotrophic overstimulation. The animals were treated with a progesterone intravaginal device (CIDR®, Pfizer, Hamilton, New Zealand) on day 0, which was removed on day 8, following 0.5 ml of a PGF2α (Sincrocio®, Ouro Fino, Cravinhos, Brazil) analogue at the same days. Gonadotrophic treatment started 48 hours before CIDR® removal (D6), when the animals were randomly divided into three experimental groups according to the total dose of exogenous porcine FSH: G200 (n=9) - 200 mg; G133 (n=10) - 133 mg; e G100 (n=10) - 100 mg. At day 6 (1st injection of Folltropin®-V), animals received 300 IU of eCG (Novormon®, Shering-Plough S. A., Syntex S.A., Buenos Aires, Argentina). Ultrasound evaluations were performed during the luteogenesis period, from day 11 to day 15 (day corresponding to embryo collection). Statistical analysis was performed using software R®, and a value of  $P < 0.05$  was considered statistically significant. Resistivity index (RI) was higher ( $p=0.02$ ) on day 15 (5-6 days after ovulation), than on day 12 in all groups, and in G200 ( $p=0.02$ ) than in G100 throughout the study. The RI was higher ( $p < 0.01$ ) on days 14 and 15 than on the other days evaluated ( $p < 0.01$ ). Peak systolic velocity (PSV) and end-diastolic flow velocity (EDFV) were similar between treatments and moments ( $p=0.5$ ,  $p=0.05$ ). We concluded that the lower dose of exogenous FSH promoted greater ovarian perfusion, which may be related to a sufficient super ovulatory response, providing a lower cost in the gonadotrophic treatment of embryo donor females.

SR-P12

### Effect of follicular status at the time of estrus synchronization upon estrus response and time to ovulation in anestrus goats

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**Objectives:** In goats, reproductive function has been described as seasonal, a situation that generates in turn a seasonal supply of both milk and meat commodities. Therefore, it is of particular importance to design reproductive strategies to decrease or evade seasonal reproduction. The aim of this study was to determine the effect of the follicular status at the time of estrus synchronization on estrus response and time to ovulation in anestrus goats.



**Materials and methods:** Multiparous anestrous mixed-breed goats (n=37) were synchronized with 20 mg of progesterone (P4, im) and 24 h later were injected with 100 UI of human chorionic gonadotropin (hCG, im). At the time of hCG injection, goats were submitted to an ultrasonography scanning and depending on their follicular status, goats were classified in three groups: Non-dominant follicle (NDF, n= 7), dominant follicle that become ovulatory (DFO, n= 13) and dominant follicle that don not become ovulatory (DF-NO, n= 17). From day 0 to day 5, goats were observed twice a day, in order to determine the onset of estrus and were daily scanned to determine the time and diameter of follicle at ovulation.

**Results:** No differences (P>0.05) were detected regarding both estrus and ovulation response (34/37). The time to estrus onset was similar (P>0.05) among groups (NDF, 61.7 ± 10.8 h, DFO, 52 ± 9.0 h and DF-NO, 57 ± 10.3 h; P=0.13). The time to ovulation was also similar (P>0.05) in both the DFO (90 ± 10.9 h) and in the DF-NO (94.5 ± 6.0 h) groups, with the largest time to ovulation observed in the NDF group (104 ± 12.4 h; P=0.02). No differences (P>0.05) occurred with respect to follicular diameter at ovulation (NDF, 8.7 ± 1.2 mm, DFO, 8.1 ± 0.8 mm and DF-NO, 8.4 ± 1.0 mm; P>0.05).

**Conclusions:** In summary, results of this study demonstrate that the follicular status at the onset of estrus synchronization (P4 + hCG) affected the time to ovulation in goats. The last could be of importance in time fixed artificial insemination programs in goats while could be of interest to other animal industries.

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SR-P13

### Effect of the use of half or complete intravaginal sponges impregnated with medroxyprogesterone upon the induction of estrus and ovulation in Dorper ewes during the anestrus season

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Sheep are seasonal polyestrous animals whose sexual activity is mainly modulated by photoperiod. While most sheep breeds are sexually active during late summer, fall and winter, they are sexually inactive during spring and early summer. The use of intravaginal sponges impregnated with progestogens is a way of estrus synchronization in small ruminants although the cost of such devices could be a restriction for most sheep breeders, mainly under marginal production systems. The aim of this study was to compare the effect of either a complete or a half of a sponge impregnated with medroxyprogesterone in order to induce estrus activity and ovulation in anovulatory Dorper sheep. The study was carried-out in the Comarca Lagunera, northern Mexico (26°N), during the anestrus season (April). Anovulatory Dorper ewes (n=20) were fed with the leftovers of a completely mixed dairy cattle ration (14.5% CP, 2.78 Mcal/EM kg-1 DM). The ewes were randomly divided into 2 groups (n=10 each) homogeneous regarding body weight and body condition score. The first group of females received a complete intravagi-

nal sponge impregnated with 60 mg of medroxyprogesterone acetate, while the second group received half of the intravaginal sponge. The intravaginal sponges were in place for a 6 d period. At the moment of withdrawal, both groups received 300IU i.m. eCG. The female estrus activity was registered during the following 5 days after eCG administration using a sexually active male which was exposed to females twice a day (0800 and 1700 h) during 15 min. If females remained still during the mounting process they were considered in estrus. The ovulation percentage (females with the presence of a corpus luteum) was evaluated 10 d after mating by transrectal ultrasonographic scanning. Both estrus activity and ovulation percentage in both groups were analyzed by a Chi-square test. The statistical analyses were performed using the statistical package SYSTAT 10 (Evenston, ILL, USA, 2000). No differences (P>0.05) between groups occurred regarding estrus response and ovulation (Table 1). Results of the present trial demonstrate that half an intravaginal sponge impregnated with medroxyprogesterone is as effective regarding the use of a complete sponge in order to promote estrus induction of estrus and ovulation in female Dorper sheep during the natural anestrous season.

Table 1. Estrus response and ovulation of anovulatory sheep treated with half or a whole sponge impregnated with medroxyprogesterone.

#### Treatments

#### Variables

Half (n=11) Whole (n=9)

Females in estrus (%) 100 (11/11)<sup>a</sup> 88.9 (8/9)<sup>a</sup>

Ovulating Females (%) 82 (9/11)<sup>a</sup> 89 (8/9)<sup>a</sup>

Values with different letters within variable, differ (P<0.05)

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SR-P14

### Effect of varying concentrations of egg yolk in cryopreservation of Gaddi goat semen

#### Varying concentrations of Egg yolk in Gaddi goat semen cryopreservation

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**Objectives:** Goats are important livestock species of India. Gaddi breed of goats are the mainstay of a large portion of nomadic and hilly tribes of Himachal Pradesh. The sensitivity or resistance to cryopreservation varies among different species as also between animals of same species. To mitigate the effects of cryopreservation, the components of extender have been subjected to hit and trial method culminating into most viable options. Egg yolk is an important constituent of semen extender providing cryoprotection to the sperm cells and has been used in varying concentrations in goats. Due to variability in reports regarding best egg yolk (EY) concentration in extender, a varying number of ejaculates in present study were utilized to establish most promising egg yolk concentration (5, 10, 15 and 20 percent) for Gaddi goat semen cryopreservation.



**Materials and methods:** Study was conducted at livestock farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur (32.6°N, 76.3°E, altitude 1290.8m) from September 2016 to January 2017 and again from September 2017 to December 2017. Eleven adult Gaddi bucks aged between 1.1 to 4.5 years (2.16±0.36 years), weighed 31-57 kg, (39.1±2.82 kg) were selected. Semen collection was done twice weekly by artificial vagina using induced estrus female as teaser. A total of 180 ejaculates were collected during the period of study. Absence of any gross abnormality in semen colour, mass motility of greater than 3 and initial progressive motility of greater than 70 percent were considered as main criterion for a sample to be fit for further processing. The ejaculates were extended in Tris citrate egg yolk extender containing 6 percent glycerol with varying concentrations of egg yolk to maintain a concentration of  $150 \times 10^6$  sperms/straws. Filled and sealed straws were equilibrated at 5°C for 4 hours followed by vapour freezing of straws for 7 minutes at 4 centimeter above the liquid nitrogen and finally plunged into liquid nitrogen. The representative straws from each ejaculates were thawed at 37°C for 30 seconds, 24 hours post incubation to compare the progressive motility, viability, morphological abnormalities and HOST reactive sperms in between different egg yolk concentrations along with per cent change due to the processing. The data was analyzed using package R version.

**Results:** The perusal of the results for varying concentrations of egg yolk revealed the average post thaw progressive motility was higher (35.18±0.87) in the extender containing 10 percent egg yolk which was followed by 32.14±1.60 in 5 percent, 29.52±1.64 in 15 percent and 25.09±2.70 in 20 percent egg yolk. The percent change due to processing was also minimal (52.03) for 10 percent egg yolk. In addition to the progressive motility, semen viability was also maximum (45.26±1.32) with a least percent change due to processing (40.12) in extender with 10 percent egg yolk, which was numerically higher than other egg yolk concentrations and ranged from 39.85±2.15 to 41.8±3.04, respectively. In terms of absolute average values of morphological abnormalities, 10 percent egg yolk was superior to 20, 15 and 5 percent egg yolk. The corresponding average values being 7.93±0.28, 11.42±0.67, 10.84±0.53 and 8.39±0.35, respectively. Whereas, it was inferior to 15 percent egg yolk, in terms of percent change (8.83 versus 20.15) for 15 and 10 percent egg yolk, respectively. The absolute average values of HOST does not differ between 15 per cent (59.96±1.93), 10 percent (52.48±1.43) and by 5 percent (59.07±2.18) egg yolk, all of which were higher ( $P < 0.05$ ) than 20 percent (42.57±4.20) egg yolk concentrations. Accordingly, the percent change at different egg yolk concentration for HOST reactive sperms was minimal for 15 percent egg yolk (22.08) followed by 5 percent (24.91), closely followed by 10 percent (26.26) but was maximum for 20 percent (41.62) egg yolk concentration.

**Conclusions:** Considering the above findings for absolute average values and percent change in the seminal attributes in post-thaw semen in Gaddi goats 10 percent egg yolk was considered as best, at least with respect to progressive motility and viability and therefore best for Gaddi goat semen cryopreservation.

SU-P01

### Clinical findings and surgical outcomes of Japanese Black calves with perinatal rib fracture : A review of eleven cases

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**Objectives:** We had earlier reported that partial costectomy had provided favorable outcomes for tracheal collapse and stenosis associated with perinatal rib fracture of calves. However, the criteria for surgical management has still been undetermined among the calves. In this study, we reviewed the clinical findings and surgical outcomes of our cases with rib fractures. The purpose of this study is to discuss the criteria for surgical management among calves with the disease.

**Materials & methods:** Medical records of eleven Japanese Black calves with rib fractures were reviewed in this study which were presented to Veterinary Teaching Hospital, University of Miyazaki, for their diagnosis and treatment from 2011 to 2017. The sex, body weight, parturition condition, clinical sign, image finding, surgical treatment and the prognosis were reviewed in each case. The respiratory sign in the calves were classified into three categories on the basis of the severity of the condition, as followed; Grade 1: no wheezing after excitement Grade 2: wheezing only after excitement, Grade 3: wheezing at rest. The prognosis was informed by referral veterinarians and owners.

**Results:** The calves consisted of ten male and one female in this study. All calves had history of dystocia and received excessive traction during their birth, and 10 of the calves showed the breech presentation and excessive fetus size. According to our classification, three calves were classified as Grade 1, five were as Grade 2 and three as Grade 3, respectively. Three calves of Grade 1 had no stridor after excitement, despite the presence of callus formation after rib fracture and tracheal collapse and stenosis. Calves in Grade 2 (n=3) and Grade 3 (n=5) received the surgical treatment : partial costectomy. Unilateral partial costectomy was performed in 5 calves (Grade 2 n=3, Grade 3 n=2), and bilateral costectomy was performed in 2 calves of Grade 3. Three calves of Grade 1 received no surgery, and one calf of Grade 2 had died suddenly while waiting for the surgery at farm. In Grade 1 and Grade 2, all calves had favorable outcomes. In Grade 3, one calf had recurred the dyspnea in spite of receiving the surgery.

**Conclusions:** The result of this study suggested that the perinatal rib fractures were associated with dystocia due to excessive fetus size and breech presentation as similar to previous articles. In addition, the partial costectomy would be recommended to the calves with symptomatic respiratory sign of the disease and unnecessary for the cases with asymptomatic sign. The number of surgeries required will depend on the severity of the fracture.



SU-P02

## Surgical repair anal atresia in a calf associated with the vaginal fistula of Rondônia region North of Brazil: case report

### Congenital defect, bovine, recto-vaginal fistula.

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**ABSTRACT:** Brazilian cattle raising is an important source of income for the country, representing approximately 30% of Brazilian agribusiness besides occupying a prominent position for presenting the largest commercial herd in the world. The state of Rondônia in the north of Brazil contributes to these values, with a herd of around 14 million cattle. However, the occurrence of congenital pathologies is important in this production and may interfere with the economy of the cattle raising sector. The incidence of congenital anomalies at birth may represent 15% of deaths in the first 48 hours of life. Between March and December 2017, 4 animals with anal atresia were attendend at the University Luterana of Brazil/Centro Universitário Luterano de Ji-paraná. Anal atresia is a congenital malformation consisting of non-development of the anal opening and may be associated with the recto-vaginal fistula. Being classified, according to the literature in four types, based on the various degrees of dysgenesis or agenesis of the rectum and anus, its etiology is not completely elucidated by the literature, in some circumstances, it suggests that the disease is conditioned to heredity and, in others, to environmental causes. The clinical signs most commonly presented are non-elimination of feces, absence of anal orifice, abdominal distension, becoming depressed and anorexic animals, in association with rectovaginal fistula, observed, elimination of feces through the vulva, vulvar irritation and tenesmus. The diagnosis is by the clinic and radiographic examination can be used to confirm it and help in the classification of the atresia. The treatment consists of opening the anal opening and repairing the vaginal fistula.

**Objective:** This study aims to report the case of a calf of approximately nine months old, diagnosed with type III anal atresia associated with the recto-vaginal fistula, where the surgical correction of the same was performed.

**Materials and Methods:** For sedation, xylazine at a dose of 0.05 mg / kg was used which was administered intramuscularly. Trichotomy and antisepsis were performed in every perianal region. At the incision site, 15 mL of lidocaine with diffusely vasoconstrictor was administered to all layers, divided into regions (muscular, subcutaneous and skin). After the 10 cm incision, the tissue was divided to the rectum carefully until the membrane was found in the rectum, where it was isolated and drawn for fixation of the rectum at four points of fixation in the skin. The first was correction of the fistula rectal access with nylon 2.0 thread and separated simple points. Subsequently to this the correction was made by vaginal access, where it was exposed to fistula and sutured with wire catgut 3.0, separated simple point. Finally, suture was passed through the attachment points on the skin. The yarn used for this procedure was nylon 2.0 for 11 day removal. The immediate postoperative period was the application of a bezilpenicillin G based antibiotic at

the dose of 20,000 IU / kg just one day, flunexin-meglumin anti-inflammatory at the dose of 2.2 mg / kg for three days and for analgesia sodium dipyron at the dose of 50 mg / kg just one day. At the end, the surgery was cleansed with 2% chlorhexidine and 10% iodopovidone, a healing ointment based on zinc oxide 15 g and cresylic acid 2 g (Unguento), and a silver-sulfadiazine-based spray, 1 g, aluminum - 5.0 g, cypermethrin - 0.4 g. After the animal was released to the picket together with the other animals of the university.

**Results:** As a result, satisfactory reconstruction of the anal orifice and relapse of the vaginal fistula were obtained. The patient was sold soon after the removal of the stitches, not allowing the follow-up of the stitches for a longer period of time.

**Conclusions:** However, was concluded that new studies are fundamental to unravel the a etiology as well as the improvement of surgical techniques, and to know more the incidence of these congenital diseases in the northern region of Brazil, important in the cattle raising commerce.

**Keyword:** congenital defect, bovine, recto-vaginal fistula.

SU-P3

## Trial of Femoral Head Ostectomy for hip joint disease of F1(Wagyu and Holstein) calf

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We performed treatment by closed reduction of an F1 calf that presented with astasia immediately after birth and was diagnosed with left coxofemoral luxation. However, the calf remained invalid. Thus, we attempted femoral head ostectomy which enabled the calf to standup and place weight on the bone, one day after surgery. At 153 days old, CT image reveals that the coxofemoral joint has held its normal position, and shows enlargement of the muscular layer. The calf grew to 263 kg (DG:1.02 kg) by 2-3 years old. Our case shows that femoral head ostectomy is valid for coxofemoral luxation.

**Materials and Methods:** Animal: a F1 calf (born at September 7, 2016) with astasia diagnosed as left coxofemoral luxation and unresponsive to closed reduction.

**Observation period:** From September 2016 to April 2017

**Process of diagnosis:** Hearing of crepitation sound in hip joint by passive motion test, blood biochemistry, ultrasonography, and postoperative X-ray and CT imaging.

**Treatment procedure:** Under sedation, incise vertically for 20cm from sacral spine to great trochanter, and exfoliate skin from subcutaneous tissue. Bluntly exfoliate three layers of gluteal muscle to exteriorize great trochanter and incise joint capsule avoiding ischiatic nerve. Over pulling an affected limb cranially, guide wire saw around femoral neck. Not to break muscle starts from greater trochanter, pull wire saw to dorsal, cut bone head and remove. Finish by suture each muscle and skin.

**Result:** This procedure does not need special equipment





,needs only 3 surgeons and takes only 30minutes. Patient got be able to stand up in 1 day after surgery, recovered to better locomotion in 30 days. At this moment, an abscess occurred and it disappeared by antibiotics administration and drainage. 90 days after surgery, upright posture recovered to symmetrical and to be able to place weight normally. After 220 days after surgery ,body weight gained to 263kg(DG 1.02kg/day).

**Conclusion:** We try femoral head ostectomy for coxofemoral luxation. As a result, it's enabled standing up and weighting from day after surgery. It means femoral head ostectomy is valid for coxofemoral luxation. CT imaging is important diagnosis method for coxofemoral luxation. In the future,we will consider the investigation of daily gain.

#### SU-P4

### Effects of Propolis extract a new reinforcement material in improving bone healing in rat

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Propolis has antibiotic and antiseptic properties and may also have antiviral and anti-inflammatory effects. It also was known for its antioxidant, immune response modulating, and wound healing effects. 36 healthy male rats were randomly divided into 6 groups (n= 12). The groups included autograft, defect or untreated group, chitosan, DBM, chitosan and propolis (chitosan-propolis), and DBM and propolis (DBM-propolis). A critical gap was created in the radius of all rats. The healing process of bones was evaluated using radiography, gross morphology, histopathology, and biomechanics. The DBM-propolis group, showed better structural and biomechanical properties compared to the untreated, DBM, chitosan and chitosan-propolis groups. The defect site in the chitosan and untreated groups were mainly restored by fibrous connective tissue while the lesions in the autograft group were mostly filled by cartilage and a lesser amount of woven bone. The woven bone, followed by hyaline cartilage was the main constituents of the newly formed tissues in the DBM-propolis group. The results of this study showed that percutaneous injection of diluted aqueous propolis extract in the bone defect can improve bone formation in critical radial bone defect of rat. Since there was no significant difference between the autograft and DBM-propolis group, probably this therapeutic strategy has high potential in augmentation of autologous bone grafting.

#### TP-P01

### Clinical study for the determination of plasma bioavailability of fenthion and its metabolites in bovines submitted to topical treatment with two different commercial products

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**Objectives:** Pharmacokinetic parameters are evaluated by plasma bioavailability studies of veterinary products through clinical and laboratory tests. They are necessary to measure the distribution of the active principle in the plasma of treated animals. These procedures are used in order to determine efficacy through correlation with minimum inhibitory concentrations or pharmaceutical bioequivalence. Tiguvon®15 Spoton® is a systemic larvicide, especially suitable for large herds, consisting of fenthion 15%. It is rapidly absorbed through the skin and distributed through the bloodstream throughout the animal's body, eliminating larvae like *Cochliomyia hominivorax* (myiasis). This study aimed to determine the plasma bioavailability of the Tiguvon®15 Spoton® formulation (Bayer Animal Health) compared to a competitor product based on fenthion 15%.

**Materials and Methods:** Twelve animals were separated into two groups (six animals each): Tiguvon®15 Spoton® and a competitor product based on fenthion 15%. The animals were male and female (*Bos taurus taurus* X *Bos taurus indicus*), aged 12 to 24 months, with body weight between 150 and 250 kg. No animals were treated with organophosphates within 90 days prior to the study. The two investigated products were applied with a graduated syringe in a single point over the skin of the animals between the scapulas in the dosage of 7 mL/100 kg of body weight. The animals were kept in covered pens for 24 hours. Plasma samples were collected for the quantification of the active fenthion at 10, 24, 48 and 72 hours after treatment (AT). All samples were analyzed in laboratory by a chromatography method considering the active fenthion and all its metabolites.

**Results:** Based on the evaluated parameters (analytical measurements of plasma from cattle treated with fenthion products), the product Tiguvon®15 Spoton® had availability of fenthion of 81.93 µg/L 10 hours AT, 56.05 µg/L 24 hours AT, 41.00 µg/L 48 hours AT and 16.15 µg/L 72 hours AT. The competitor product based on fenthion 15% had availability of 43.72 µg/L 10 hours AT, 43.98 µg/L 24 hours AT, 21.04 µg/L 48 hours AT and 8.97 µg/L 72 hours AT. The final average AUC<sub>0-t</sub> (µg/L) was 3835.3 for Tiguvon®15 Spoton® group and 2450.2 for competitor group. High concentration observed resulted in statistical difference between the groups 48 hours after the administration of the products.

**Conclusions:** Tiguvon®15 Spoton® is more effective than the compared competitor based on fenthion 15% due to its higher plasma availability. The higher concentration in the first hours after administration is fundamental for the effectiveness of the product in the control of wound infestations with larvae of *Cochliomyia hominivorax* already developed in the animals.



TP-P02

### Effects of leukoreduction on cattle whole blood conservation in plastic bags.

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**Objectives:** Considering the difficulty to produce hemocomponents for large animals and the clinical importance of blood transfusion in cattle, we propose to validate the use of a plastic blood bag with leukocytes filtration under field conditions, and to compare the cattle blood conservation after leukocytes removal.

**Materials and methods:** We used ten healthy Nelore cows, weighing around 500 kg, from a commercial cattle farm in the Santarém municipality. From each animal a total of 900 g of whole blood were collected, 450 g of which were stored in CPD / SAG-M triple bags (Composampler, Fresenius Kab, São Paulo, Brazil) and 450 g in CPD / SAG-M quadruple bag with in-line leukocyte filter (CompoFlow Flexible, Fresenius Kabi, São Paulo, Brazil). Both bags contained a preservative solution composed of sodium citrate (1.66 g), citric acid (188 mg), sodium phosphate (140 mg) and dextrose (1.61 g) in the primary bag, and sodium chloride (877 mg), mannitol (525 mg) and adenine (17 mg) in the satellite bag. Blood collection was performed on the external jugular with manual restraint. In the triple bags, the additive solution from the satellite bag was transferred to the main bag immediately after blood collection and then the bag was homogenized and refrigerated. In the quadruple bag, manual filtration for leukocyte removal was performed after one hour of the blood collection, by transfer of whole blood to a secondary bag passing through a filter. After filtration, the additive solution from the satellite bag was added and the blood was homogenized and refrigerated. In both triple and quadruple blood bags, one additional satellite bag (for centrifugation purpose) was not used. The filtering procedures were carried out at field condition and the filtration time ranged from 15 to 30 minutes. The 20 bags were stored under refrigeration at 2 to 6 ° C (mean of 3.5 ± 0.5 ° C) for 42 days and homogenized on alternate days. Samples of blood stored in the plastic bags were collected immediately after blood collection (D0) and after 7, 14, 21, 28, 35 and 42 days (D7, D14, D21, D35, D42, respectively). At all times, complete hematological and blood gas evaluations were performed and biochemical variables (total protein, albumin, lactate, cholesterol, sodium and potassium) were determined. A microbiological test was performed at D0 and D42 to certify absence of contamination during the study. The data were submitted to a two-way repeated measure analysis of variance and post-hoc tests.

**Results:** The quadruple bag with leukocytes filter proved to be efficient, with removal of 99.3 % of white blood cells in cattle blood, and the entire filtration process was performed in the field. Microbiological contamination were absent. It was observed a reduction in the number of red blood cells (P <0.001) in both bags from D14 onwards, with a decrease of 19.7% and

17.1% at D42 for the triple and quadruple bags, respectively. The concentration of total hemoglobin decreased from D7 onwards in both bags (P <0.001). Increases (p <0.05) of pO<sub>2</sub>, pCO<sub>2</sub>, sO<sub>2</sub> and potassium and decrease (p <0.05) of sodium, bicarbonate, base excess (BE) and pH were observed during storage. The globular volume remained stable. There were differences between the blood bags from D7 onwards in pH and pCO<sub>2</sub>, from D14 onwards in pO<sub>2</sub>, from D21 onwards in BE and sO<sub>2</sub> and at D42 in albumin, and the quadruple bag presented results more compatible with a better blood conservation when compared to the triple bag. Bovine whole blood stored in blood bags underwent discrete hematological, biochemical and blood gas alterations during storage and remained viable for transfusion when stored at 2-6 ° C for up to 42 days.

**Conclusions:** The quadruple bag proved to be efficient for leukocyte removal and feasible to be used under field conditions. The leukocytes removal produced discrete alterations in cattle blood preservation, noted only in blood gas analysis and albumin, however presenting results more compatible with a better blood conservation. Considering the deleterious effect of leukocytes during whole blood transfusion, the quadruple bag with filter can be indicated for use in cattle.

TP-P03

### The effect of rumen fluid on itraconazole stability in dairy cattle - pilot studies

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**Objectives:** Itraconazole is an antifungal drug commonly used in humans, but up to date it has not been used in bovine. Meanwhile, systemic and cutaneous mycosis are serious problems in dairy cows. The lack of effective therapy often leads to involuntary culling of the affected animals. Due to the anatomical and physiological differences, it is not possible to simply transfer the treatment from other species. The purpose of this study was to preliminary determine the effect of the rumen environment on the itraconazole stability.

**Material and methods:** The samples of rumen fluid was taken from the Holstein-Friesian cow using probe. It was transported in a container with thermal insulation. The liquid was double filtered through a cellulose filter. Itraconazole at doses of 250ng, 500ng, 100ng and 2500ng was added to filtered samples, then they were incubated in a shaker in a water bath at 37.5°C for 3 seconds, 5 minutes and 180 minutes. The concentration of itraconazole was determined by liquid chromatography with a dual mass spectrometer (LC-MS/MS).

**Results:** Itraconazole concentration has changed during the incubation. Although itraconazole is considered a very stable substance, a non-linear decrease of concentration was observed. The itraconazole concentration changes rate during first 30s of incubation (0-30s) was 1.1035min<sup>-1</sup> (half-time period - t<sub>1/2</sub>= 0.63min) and for period 0-5min the rate was 0.1059min<sup>-1</sup> (t<sub>1/2</sub>=6.03min). The area under the curve (AUC) for the period 0-5min was 1039.43ngxmin/mL in relation to the theoretical (no effect of rumen fluid on the itraconazole metabolism)



$AUC_{(0-5min)}=2083.5ngxmin/mL$ .

**Conclusions:** Itraconazole is not stable in rumen fluid environment. The kinetics of concentration changes during incubation in rumen fluid is non-linear – it depends on the dose of the drug. The kinetics of changes in itraconazole concentration in the rumen fluid environment caused it to become saturated over the time of 5 minutes.

TP-P04

### Effects of an oral *Echinacea purpurea* (L.) MOENCH alcoholic spissum extract in calves - a placebo controlled, randomized, double-blinded study

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Antimicrobial resistance resulted in international accordance to strengthen the research to investigate novel treatment options including medicinal plants. Medicinal plants bear a potential as alternative or additional treatment also in livestock. Based on versatile effects of their plant specific multi-component-compositions, medicinal plants can potentially act as multi-target drugs. To investigate the effect of two dosages of a hydro-ethanolic *Echinacea purpurea* (L.) MOENCH spissum extract (EP) on performance, clinical health, antibody production, red and white blood cell count and expression of immunological parameters in calves, a double-blinded placebo-controlled clinical trial has been performed focusing on gastrointestinal and respiratory tract diseases. The trial was approved by the cantonal veterinary inspection office and was conducted according to the Swiss law on animal welfare. The calves were enrolled randomly in three groups (each group n=9): placebo (1) and two doses of EP (0,3 g EP/days:2; 3 g EP/day: 3) one day after birth (day 1). They received the treatment per os twice daily for 4 weeks in a double-blinded manner (day 7-35). To investigate EPs effects on the immune system, the animals were vaccinated twice with a bluetongue virus (BTV) vaccine, serotype 4 on day 14 and 21. On day 28 the calves were brought to a new farm to simulate transport stress. Body weight (weekly), clinical health and milk intake (daily) were recorded. Blood samples were analysed with ELISA for BTV antibodies (day 14, 21, 28 and 56), white and red blood cell count (weekly) by flow cytometry and for the mRNA abundance of a variety of inflammatory factors via real time quantitative PCR (directly before and 24 hours after vaccinations).

Preliminary findings based on least square means (using linear mixed effect models and Tukey's post-hoc analysis in the R environment version 3.2.5) show that EP reduced the days of diarrhoea in group 2 compared to 1 significantly ( $p=0.03$ ; 1: 13.6 days, 2: 7.5 days, 3: 10.1 days) and increased the mean body temperature over the whole experiment by trend in group 3

compared to 1 ( $p=0.08$ ; 1: 38.93 °C, 2: 39.05 °C, 3: 39.05 °C). No effect was found in red and white blood cell counts, milk intake, weight gain, incidence of bovine respiratory disease and drug consumption when the EP groups were compared to placebo. In addition, no differences in the mRNA abundance in blood cells of interleukin 1 beta, interleukin 8, tissue necrosis factor alpha and cyclooxygenase II were found between groups. However, the mRNA abundance of the prostaglandin-synthase was significantly increased in group 2 and 3 compared to 1 after the first vaccination (linear regression analysis and Tukey's post-hoc analysis,  $p<0.05$ ).

The measured effects might be due to immune modulation of EP known from the literature. Diarrhoea reducing effects might be explained by stimulation of the local enteric immune system of calves by orally administered EP. The quantification and serotyping of specific BTV antibodies by serum neutralization test is important for a conclusive evaluation of the potential of EP in calves, and is currently under way. There is a need for further research focused on the effects of EP in bovines in particular in broad field trial settings to draw final conclusions.

TP-P05

### Comparison of clinical and economical efficacy of anti-inflammatory: meloxicam (Loxicom) to ketoprofen for treatment of diarrhoea in neonatal calves

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The aim of this study was to evaluate the efficacy of different anti-inflammatory (NSAID) products on a farm with suspected sanitary challenges in feeding.

24 Holstein calves of 1 month age were enrolled in the study at the naturally occurring onset of diarrhoea, defined as the first occurrence of a fecal score greater than 2 on a 5-point scale. Calves were randomly divided in two groups, 12 calves each group. For the treatment of diarrhoea all animals received antimicrobial (tylozin, 50 mg per 10 kg b. w. intramuscularly, 5 consecutive days) and rehydrated therapy.

As supportive therapy and antisecretory agent animals from Group 1 received ketoprofen 3 mg/kg b.w. intramuscularly during 3 consecutive days since Day 1 and animals from Group 2 received single subcutaneous injection of meloxicam 0.5 mg/kg b. w. (Loxicom® produced by "Norbrook" Ireland) at Day 1.

Clinical diarrhoea score, clinical index score, respiratory rate score for each animal were evaluated daily by the 1-5 point system for assessing the severity of diarrhoea proposed by Heinrichs&Kehoe (2009), where 1 point = normal, 5 = the most severe disorders. A final assessment was performed by summing up clinical diarrhoea score, clinical index score and respiratory rate score of the groups 1 and 2 (the threshold value = 6 points). Rectal temperature was daily evaluated on a quantitative basis.

Both groups of animals have almost the same average clinical diarrhoea score at the Day 1: 3,3 and 3,4 in calves from Group 1 and 2 respectively. Calves from Group 2 witness more faster



improvement during first three days of treatment: the average clinical diarrhoea score on the day 3 was 2,0 compare to Group 1 - 3,0. At the Day 5 animals from both groups had normal stool and achieved 1,0 and 1,1 points in Group 1 and 2 respectively.

Improvements in rectal temperature were significantly faster and more pronounced in Group 2 since Day 3 till Day 5 (with statistically significant difference). At the onset of diarrhoea at Day 1 the average rectal temperature was almost similar in both groups – 39,5° and 39,7° in Group 1 and Group 2 respectively, on the Day 2 rectal temperature became lower in Group 1 for 0,6°C and in Group 2 for 1,5° C. Rest days of the study the rectal temperature in both groups were within the normal range but it was lower in Group 2 and at the Day 5 it was 37,8°C compare to 38,6°C in Group 1.

Group 2 had more severe clinical index score and respiratory rates score at the onset of diarrhoea at the Day 1: 3,1 and 2,1 respectively compare to 2,2 and 1,2 in Group 1. Despite this difference animals from both groups achieved clinical normal rates at the day 5: in Group1 - 1,1 point and in Group 2 - 1,0. Significant improvement of the breathing function in Group 2 was observed in the Day 4 – 1,2. At the Day 5 breathing function in animals from both groups came to the normal – 1,2 in Group 2 and 1,1 in Group 1.

Summing up data of clinical diarrhoea score, clinical index score and respiratory rate of the both groups, it is obvious that Group 2 had more severe diarrhoea at the beginning of disease – 8,6 points compare to 6,7 in group 1. Nevertheless both groups achieved sum of points below threshold value of 6 points at the Day 3 and at the end of the study at Day 5 both groups demonstrated almost the same results – 3,2 points in group 1 and 3,3 in group 2.

Price for ketoprofen 10% is around 10 Euro (with VAT) per 100 ml and for Loxicom® 20% is around 14 Euro (with VAT) per 100 ml. Total sum spent for anti-inflammatories during the treatment for calves with 60 kg body weight were 0,5 Euro per head in Group 1 and 0,2 Euro per head in Group 2, thus meloxicam usage was 2,5 cheaper.

Measuring the effect of the treatments scheme using the system for assessing the severity of diarrhoea by Heinrichs&Ke-hoe (2009) we can resume that animals from both groups had the same clinical effect after treatment however meloxicam provided better recovery in the reason of faster rectal temperature improvement and clinical diarrhoea score. Using a meloxicam (Loxicom®) as anti-inflammatory proves to be 2,5 times cost-effective in comparison to ketoprofen usage.

TP-P06

### Comparative efficacy of a single administration of a combination of florfenicol and meloxicam with florfenicol alone in naturally occurring cases of respiratory disease in young cattle

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**Objectives:** Bovine respiratory disease (BRD) is still a major health and economic issue for cattle farmers around the world.

Treatment of acute BRD classically rely upon administration of a systemic antimicrobial targeting Gram-negative bacteria but addition of a nonsteroidal anti-inflammatory drugs to the treatment plan has proven to mitigate the inflammatory response and to rapidly improve the clinical condition of affected animals. Recently, a florfenicol and meloxicam combination (FMC) was developed (Zeleris®, Ceva). In order to assess the efficacy of this combination in the field and to precise the benefits of combining meloxicam with florfenicol, a comparative field trial with a florfenicol-based product (Nuflor® 300, MSD) was carry out.

**Materials and methods:** 329 young cattle (mean age = 20 weeks) suspected of BRD were enrolled through a randomized and blinded field trial performed according to Good Clinical Practice standards in five different farms in Germany, Hungary and Portugal. Evaluation of the clinical condition of calves was based on behavioral and respiratory scorings, with each score ranging from 0 (normal) to 3 (severe). Calves were included whenever they displayed a rectal temperature (RT) superior or equal to 40°C and a sum of clinical scores (CS) > 2. Right after inclusion, calves were randomly allocated to one of the two treatment groups (FMC, n = 164 or florfenicol, n = 165). Following allocation and treatment, calves were observed for 30 days and evaluated to monitor their respiratory and behavioral scores as well as their rectal temperature. Local reactions at injection sites were also recorded.

**Results:** At inclusion, clinical signs displayed by the calves of this study were consistent with those commonly observed for BRD. Calves showed moderate respiratory distress, mild to moderate depression, coughing or nasal discharge and their mean RT was 40.4°C. No difference was observed between the two treatment groups at that time. 6 hours after treatment, calves receiving FMC had a significantly lower RT compared to animals treated with florfenicol alone (39.4°C vs. 39.9°C, P<0.001) whatever the farm considered. 53% of the calves in FMC group had a normal rectal temperature (RT < 39.5°C) while 83% of cattle treated with florfenicol were still affected by fever (P<0.001). Superiority of FMC over florfenicol in reduction of rectal temperature was maintained until 48 hours after treatment administration (P=0.044). Additionally, a higher percentage of calves treated with FMC clinically improved compared to calves receiving florfenicol alone as soon as 6h after treatment and up to day 3 (P<0.05). 7 days after treatment, 93.9% of animals treated with FMC were cured compared to 88.5% of animals treated with florfenicol (P=0.119). Percentage of animals with local reactions at injection sites were lower in cattle receiving FMC (26.8%) than in cattle receiving florfenicol (34.5%) but this difference was not statistically significant (P=0.15).

**Conclusions:** In field conditions, FMC (Zeleris®) achieved significant better performances than florfenicol alone (Nuflor® 300) for the control of fever (until 48h after treatment) and for the clinical recovery (until 72h after treatment). These results are in line with current recommendation to take in consideration inflammation for the treatment of BRD.

TP-P07

### Comparative efficacy of a combination of florfenicol and meloxicam with a combination of florfenicol and flunixin meglumine in young calves experimentally challenged with Mannheimia haemolytica



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**Objectives:** Bovine respiratory disease (BRD) continues to be a major health and economic issue for farmers raising young cattle around the world. Treatment of acute BRD rely on the administration of a systemic antimicrobial targeting Gram-negative bacteria such as *Mannheimia haemolytica* together with a nonsteroidal anti-inflammatory drugs to mitigate the inflammatory response and therefore to rapidly improve the clinical condition of affected animals. Florfenicol, meloxicam and flunixin are commonly used by bovine practitioners in the field for BRD treatment with documented efficacy and recently, a florfenicol and meloxicam combination (FMC) was developed (Zeleris®, Ceva). In order to evaluate the efficacy of this new combination and to precise the benefits of meloxicam over flunixin meglumine, an experimental study was performed that compare the efficacy of FMC with a florfenicol and flunixin meglumine combination (FFC, Resflor®, MSD) and with a control group.

**Materials and methods:** A randomized and blinded challenge study was performed in calves according to the principles of Good Clinical Practice. Assessment of the clinical condition of calves was based on rectal temperature (RT) and a combined total clinical score (TCS) aggregating evaluation of 4 different clinical parameters associated with respiratory disease (de-meanor, nasal discharge, coughing, respiration pattern). At day 0, 90 young calves (mean age = 40 days) were challenged by intra-tracheal deposition of 300 mL of *M. haemolytica* M7/2 diluted broth culture with a challenge dose concentration of  $2.33 \times 10^9$  colony forming units per calf to the bifurcation of the main bronchus by means of a fiber-optic bronchoscope. Eighty-four calves were finally enrolled based on a rectal temperature (RT) > 39.5 °C and a combined total clinical score (TCS) > or equal to 3. Calves were then randomly allocated to one of the three study groups and promptly treated with FMC, FFC or saline. Following allocation and treatment, calves were clinically observed for 4 days. Calves were considered 'cured' if the sum of the clinical signs where < or equal to 1 and rectal temperature (RT) < 39.5°C. Calves were considered 'in relapse' if cured on a specific day and then not cured on the following day. Calves were observed for 4 days. At the end of the study, all calves were humanely euthanized and the lungs of each calf were removed, assessed and scored for the presence of lesions and/or consolidation based on a standard scoring system (Jericho and Langford, 1982).

**Results:** RT and TCS of calves treated with a FMC or FFC were consistently lower than RT and TCS of calves belonging to the control group (P<0.01). Moreover at the end of the study, a high clinical cure rate was observed in calves treated with FMC (100%) or FFC (96.6%) whereas cure was limited for calves receiving saline (29.6%). Lung lesions scores were significantly lower for calves treated with FMC (6.7%) or FFC (7.2%) compared to calves in the control group (23.5%, P<0.0001). Interestingly in this study, calves treated with FMC presented higher rates of clinical cure without relapse compared to calves treated with FFC (P<0.05) and lower risk of clinical relapse due to pyrexia (3.6% vs 24.1%, P=0.05). Additionally, a tendency was observed for a higher bodyweight gain at the end of the study for calves in the FMC group compared to calves in the FFC group (+2.3kg vs. +1.2kg respectively,

P=0.06).

**Conclusions:** In this experimental study, the florfenicol and meloxicam combination (Zeleris®) was found to be highly effective for the treatment of BRD (cure rate = 100%). Calves treated with this new combination were moreover at lower risk for clinical relapse and presented a higher bodyweight gain compared to calves receiving the florfenicol and flunixin meglumine combination. These results are in line with the pharmacokinetic properties of florfenicol and meloxicam.

**References:** Jericho, K.W., Langford, E.V., 1982. Aerosol vaccination of calves with pasteurized *haemolytica* against experimental respiratory disease. Can. J. Comp. Med. Rev. Can. Med. Comp. 46, 287–292.

TP-P08

### Comparison of the syringeability of a fixed combination of florfenicol and meloxicam with florfenicol-based products commonly used in bovine respiratory disease (BRD)

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**Objectives:** Syringeability is a direct measurement of the performance of a formulation for any injectable product. It is a key-product criterion strongly connected to compliance and user safety. For calves with BRD, a better syringeability can also positively contribute to reduce the stress associated with the administration of the product. On the other hand, some florfenicol-based products are known for having a poor syringeability. This study was therefore designed to compare the syringeability of a new fixed combination of florfenicol and meloxicam: Zeleris® (florfenicol 400 mg/mL and meloxicam 5 mg/mL, Ceva) with three florfenicol-based veterinary products commonly used in BRD: Resflor® (florfenicol 300 mg/mL and flunixin meglumine 16.5 mg/mL, MSD), Nuflo® 300 (florfenicol 300 mg/mL, MSD), and Florkem® (florfenicol 300 mg/mL, Ceva).

**Materials and methods:** To determine the syringeability of each product, a volume of 15 mL was withdrawn in a 20 mL glass syringe set up with a 1.2 mm diameter steel needles at 5°C and at room temperature. A mass of 1 kg (equivalent to 1 Newton force) was applied to the piston and the time needed to empty the last 10 mL of solution was recorded. This procedure was repeated six times for each product. The fastest time necessary to empty the syringe characterize the product with the highest/best syringeability.

**Results:** Results are provided as the mean ± standard deviation.

For the 1.2 mm needle at 5°C, the times needed to empty the syringe were: "Resflor®"(MSD, florfenicol 300 mg/mL and flunixin meglumine 16.5 mg/mL): 84 seconds ± 3.4, "Nuflo® 300" (MSD, florfenicol 300 mg/mL): 76 seconds ± 3.3, "Florkem®" (Ceva, florfenicol 300 mg/mL): 42 seconds ± 2.3, "Zeleris®" (Ceva, florfenicol 400 mg/mL and meloxicam 5 mg/mL): 24 seconds ± 1.2.

For the 1.2 mm needle at room temperature, the times needed



to empty the syringe were: "Resflor®"(MSD, florfenicol 300 mg/mL and flunixin meglumine 16.5 mg/mL): 71 seconds  $\pm$  6.4, "Nuflor® 300" (MSD, florfenicol 300 mg/mL): 48 seconds  $\pm$  0.8, "Florkem®" (Ceva, florfenicol 300 mg/mL): 24 seconds  $\pm$  0.6, "Zeleris®" (Ceva, florfenicol 400 mg/mL and meloxicam 5 mg/mL): 19 seconds  $\pm$  0.8.

**Conclusions:** This study clearly demonstrated the high syringeability of the fixed combination of florfenicol and meloxicam (Zeleris®). Syringeability of this combination was better than others florfenicol-based products dedicated to BRD treatment both at at 5°C and at room temperature. The superior performances of this product may be explained by the various excipients used in its formulation. These results along with efficacy studies suggest that this new combination of florfenicol and meloxicam is a reliable and practical treatment of BRD in the field.

TP-P09

### Comparative Efficacy of Tulathromycin versus Tildipirosin for First-line Treatment of Acute Bovine Respiratory Disease (BRD) in Feedlot Heifers

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The objective was to compare the efficacy of two macrolide antimicrobials when used for the initial first-line treatment of naturally occurring acute BRD in feedlot heifers. 791 Angus-cross heifers averaging 235 kg in weight arrived at a feedlot in Texas, USA after being transported for approximately 15 hours. The heifers were processed within 24 hours of arrival according to standard feedyard procedures: vaccinated with Bovi-Shield GOLD® 5 and Ultra Choice® 7, treated for internal and external parasites with Dectomax®, implanted with Synovex® C, and administered Lutalyse® to abort any pregnancies. Beginning the day after arrival processing (day 0) and continuing for 10 days, heifers that exhibited clinical signs of BRD were assigned a clinical appearance score (CAS) that ranged from 0 to 4 (with 0=normal and 4=moribund). Heifers were considered to have BRD and enrolled in the study if they had CAS of 1 and rectal temperature = or > 40°C or a CAS = or > 2 regardless of rectal temperature. A total of 600 clinically ill heifers were pulled for enrollment, weighed, and randomly assigned to either one of two treatment groups for first-line BRD treatment: one group was treated with Draxxin® (2.5 mg tulathromycin/kg BW; n=300) while the other group received Zuprevo™ (4.0 mg tildipirosin/kg BW; n=300) as a single-dose subcutaneous injection in the left lateral neck. Following antibiotic treatments, a 10-day post-treatment interval (PTI) was observed during which no other BRD treatment was provided. After the initial PTI period elapsed, heifers in both treatment groups that continued to demonstrate signs of BRD (based on the same enrollment criteria as for the first treatment) were pulled and retreated with a different class (non-macrolide) antibiotic. General and generalized linear mixed models for performance and health variables, respectively, were used for all analyses using SAS Glimmix.

**Results:** First-line treatment success rate was greater for Draxxin compared to the Zuprevo treatment group (67.2% ver-

sus 59.3%; P = 0.05). The percentage of heifers requiring re-treatment tended (P=0.09) to be reduced for heifers treated with Draxxin as compared to heifers treated with Zuprevo (28.0% versus 34.4%). Mortality due to BRD was significantly decreased by treating heifers with Draxxin as compared to Zuprevo (5.0% versus 10.3%; P=0.02). As first-line therapy for naturally-occurring BRD in feedlot heifers, Draxxin significantly improved health outcomes compared to Zuprevo. This approach could potentially reduce the overall use of antibiotics in BRD treatment protocols for feedlot cattle.

TP-P10

### A randomized positive-controlled field trial comparing a new formulation of a cloxacillin-based to a long-acting intramammary mastitis treatment.

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**Objectives:** The objectives of this study were to test the hypothesis that a new formulation of a cloxacillin-based intramammary mastitis treatment is non-inferior to an existing long-acting cloxacillin-based intramammary treatment.

**Materials and methods:** The trial design was a randomized positive-controlled non-blinded design and was conducted following the standards of Good Clinical Practice (GCP). A total of 1,017 udder quarters (906 lactating cows) from 44 farms across Southland and Otago regions of New Zealand were enrolled in the trial between August 2016 and February 2017. Quarters were enrolled on the day they developed clinical mastitis if they satisfied all other inclusion and exclusion criteria. The first quarter enrolled on each farm was allocated at random to either the investigative veterinary product (IVP, Ultraclox 24, Bayer New Zealand Ltd.) or control product (CP, Orbenin L.A., Zoetis New Zealand Ltd.) group and each subsequently enrolled quarter was allocated systematically to either group. Allocation of treatment was at a cow level. Enrolled quarters were treated for a full course (3 treatments at 24h and 48h intervals for IVP and CP, respectively).

**Results:** A total of 971 quarter cases were eligible and completed the study. Analysis of the secondary efficacy criterion (clinical cure) was performed on this dataset. A further 356 cases were excluded from analysis of the primary efficacy criterion (bacteriological cure) for not meeting one of the per-protocol case definitions (e.g. due to no growth or contamination), leaving 615 cases for analysis of bacteriological cure. Milk samples for bacteriological culture were collected aseptically by trained veterinary technicians on the day of enrolment. All quarters with a positive pre-treatment bacteriological culture result were sampled again for bacteriological culture on three consecutive days starting 16 (+/- 2) days after completion of treatment. The crude bacteriological cure proportion for the IVP group was 84.1%; and for the control group was 85.0%. For both treatment groups the lowest cure proportion was for *Staphylococcus aureus* (37.3% and 39.2%; IVP and CP, respectively); and the cure proportion for all major pathogens was 83.0% and 84.5% for IVP and control, respectively. The crude clinical cure proportion for the IVP group was 81.3%; and for the CP group



was 82.3%. The clinical cure proportion for all major pathogens was 80.3% and 81.6% for the IVP and CP groups, respectively. Bacteriological cure proportion was assessed for each of *Staph. aureus*, *Streptococcus uberis*, *Streptococcus* spp., and for all major pathogens (*Staph. aureus*, *Strep. uberis*, *Streptococcus dysgalactiae*). For all outcome groups, the IVP was demonstrated to be non-inferior to the CP, with respect to bacteriological cure. The adjusted (least square means) cure proportion was lowest for *Staph. aureus* (36.0% and 37.0% for IVP and CP, respectively); and highest for *Strep. uberis* (95.9% and 94.6% for IVP and CP, respectively). The adjusted clinical cure proportion for IVP was demonstrated to be non-inferior to the CP.

**Conclusions:** This large scale field study demonstrated that the IVP, the newly formulated cloxacillin-based product (Ultra-clox 24, Bayer New Zealand Ltd.) was non-inferior to the CP (Orbenin L.A., Zoetis New Zealand Ltd.) for both bacteriological cure proportion and clinical cure proportion for clinical mastitis in commercial dairy herds in New Zealand. The IVP achieved this with a shorter treatment period than the reference product, (3 x 24h versus 3 x 48h), yet without any concomitant reduction in bacteriological cure proportion. This was found for all major pathogens, including *Staph. aureus*, where duration of activity has previously been considered an important treatment consideration.

This trial was approved by the Invermay Animal Ethics Committee (study 13951) and funded by Bayer New Zealand Ltd.

TP-P11

### Study on the treatment of diclofenac sodium injection in dairy cow clinical mastitis

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The incidence of clinical mastitis in dairy cows is about 25%. It leads to a decrease in milk production, disappearance of milk and severe lactation, gangrene and atrophy in the milk area, a permanent loss of lactation ability. Moreover, the elimination of dairy cattle from clinical mastitis accounts for about 10% of the total elimination rate of dairy cows. At present, the treatment of clinical mastitis is mainly based on antibiotics, but antibiotic residues, bacterial resistance and other issues have become increasingly prominent. Using anti-inflammatory drugs combined with antibiotics to control inflammation, which rapidly relieve clinical symptoms and reduce the use of antibiotics, has become a new way of treatment of mastitis in dairy cows. In order to verify the efficacy of diclofenac sodium injection combined with ceftiofur sodium in the treatment of dairy cows with clinical mastitis, 50 dairy cows with clinical mastitis were evenly divided into 5 groups such as high dose (3.3mg·kg<sup>-1</sup> diclofenac sodium), the middle dose (2.2mg·kg<sup>-1</sup> diclofenac sodium) and low dose (1.5mg·kg<sup>-1</sup> diclofenac sodium) group and positive (2.2mg·kg<sup>-1</sup> analgin) and negative (0 mg·kg<sup>-1</sup> diclofenac sodium) control group within 3 days, and all groups were given with 2.2mg·kg<sup>-1</sup> ceftiofur sodium. The test results showed that the cure rates for clinical mastitis in high dose, middle dose and low dose group and positive and negative control group were 70%, 70%, 60%, 60%, 50%, respectively, while their effective

rate for clinical mastitis were 80%, 80%, 70%, 70%, 60%, respectively. Therefore, from effective and economy, 2.2mg·kg<sup>-1</sup> can be selected as the recommended dose of diclofenac sodium injection for curing dairy cow clinical mastitis. Moreover, the effective rate of diclofenac sodium was better than analgin. It can also shorten the course of treatment and reduce antibiotic residues. This clinical mastitis cure method provides a new idea for effective treatment of clinical mastitis in dairy cows and further promotes the healthy development of dairy industry.

TP-P12

### Clinical efficacy of a single subcutaneous injection of meloxicam as adjunctive therapy of clinical mastitis in lactating cow in Japan

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**Objective:** The objective of this study was to investigate the efficacy and safety of Metacam 20 mg/ml solution for injection (0.5 mg meloxicam/kg BW, single administration, SQ) when used in combination with cefazolin for the management of clinical signs associated with clinical mastitis in lactating cow. A clinical trial was conducted in field condition in Japan.

**Materials and methods:** Overall 122 lactating cows with clinical signs of mastitis from twenty six dairy farms in Japan met the inclusion criteria. Sixty two animals were randomly allocated to the Verum group, and sixty to the Saline group as negative control, respectively. Due to the difference in primary packaging format for Verum and Saline, a "dispenser-investigator approach" was chosen to ensure the blindness of the study. Clinical examinations were performed on Day 0 prior to the treatment, 24 hours (Day 1), 48 hours (Day 2) and 120 hours (Day 5) after treatment to evaluate clinical efficacy. Either Verum or Saline was administered on Day 0. Cows in both treatment groups also received a concomitant treatment of cefazolin sodium given systemically and intramammarily for 3 consecutive days from Day 0 till Day 2. A "Clinical Sum Score" (CSS; min 0, max 21) was defined to assess the severity of general and local signs associated with clinical mastitis. It consisted of the sum of scores given to the following parameters: Feed intake, General condition, Swelling, Induration, Heat, Palpatory pain, Somatic cell count (from 0 to 3 for each). In addition, secondary parameters such as milk appearance, milk yield, plasma prostaglandin E2 (PGE2) were recorded. In order to assess field safety, adverse events including reaction at injection site were monitored throughout the study period. Efficacy was defined as a reduction of either CSS or "Combined scores of Heat and Palpatory pain" at Day 1 or Day 2 in comparison to the control group.

**Results:** 105 cows were kept for the statistical analysis (Verum; 55, Saline; 50). The CSS of the Verum group on both Day 1 and Day 2 was significantly lower than that of the Saline group (p=0.0021 for Day 1, p=0.0071 for Day 2). In addition, the reduction of "Combined scores of Heat and Palpatory pain" at Day 1 and Day 2 for meloxicam-treated cows was signifi-



cantly larger when compared to that of the control cows ( $p < 0.0001$  for Day 1,  $p = 0.0032$  for Day 2); thus, all four primary endpoints have shown statistical significance in favor of meloxicam in the reduction of systemic and local pain and inflammatory parameters when compared to Saline. No significant difference among the treatment groups during the study period was detected for the secondary parameters. No adverse reaction including injection site reaction was reported.

**Conclusions:** This study has demonstrated the clinical benefit of adding meloxicam to a combination of systemic and local antibiotic treatment with cefazolin for the treatment of clinical mastitis in dairy cows. The results support the effect of meloxicam on the reduction of comprehensive clinical scores as well as local udder pain and inflammation scores.

TX-P01

### Cyanogenic toxicosis in cattle: clinical case in Mexico

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**Abstract:** In cattle, the plant toxicosis is dangerous and may cause important economic losses due to mortality or sick animals.

The objective of this work was to describe a clinical case of cattle toxicosis.

During the dry season 16 out of 120 grazing cattle died suddenly in the highlands of Mexico. Other four animals were alive but showed breathing problems. Clinical signs included accelerated and deep breathing with mouth open, weak irregular pulse, increased salivation and muscular tremors. Cattle began showing hyperacute signs as soon as 15 minutes after grass ingestion.

At the necropsy, cherry red blood colour with lack of clotting, dark muscles and petechial haemorrhages in pericardium were observed.

Based on the clinical and necropsy findings, especially the dyspnea with an intense red colour of the blood, as well as by the observation of the grazing land, which was poor in grass but with bad weed; an acute poisoning by cyanhydric acid was presumed. A picric acid paper test was performed with some suspicious plants confirming the diagnosis.

Additionally, some plants were sent to the toxicology laboratory, where San Gregado (*Jatropha cuneata*) and Chapulixtle (*Dodonaea viscosa*) plants were identified as the ones that contain the highest levels of cyanogenic precursors.

In livestock species, the most frequent cause of acute and chronic cyanide poisoning is the ingestion of plants that constitutively contains cyanogenic glycosides or plants that produce cyanogenic glycosides and cyanolipids as a protective response to environmental conditions (plant stress cyanogenesis). When ruminants eat those substances they convert them in cyanide, which prevents energy production by the cells (adenosine triphosphate or ATP). This effect is due to the inactivation of an enzyme called cytochrome oxidase. Although the blood is saturated with oxygen, the inactivation of cytochrome oxidase avoids cells from using the oxygen, so cells die rapidly.

The consumption of toxic plants by grazing cattle occurs frequently during the dry season, when the availability of forage is scarce. Usually bad weed is attractive because they look fresh and with an intense green colour. Nonetheless, many of those are toxic plants, further if they contain cyanogenic glycosid precursors. It is very important to distinguish this toxicosis from nitrite poisoning because the treatment is very different. In the last case the blood looks brownish because the lack of oxygen due to the methemoglobinemia.

In the case described above there was no opportunity to give the treatment (based in intravenous solution with sodium nitrite), the only possible action was to avoid cattle from grazing in those pastures. Once this was done, the problem stopped





and the animal's health was recovered one week later.

**Conclusions:** The consumption of toxic plants during the dry season in the highlands is a common cattle dead cause. Nevertheless there are only a few reports that support the diagnosis for the practitioners. The readily identification of toxic plants will permit to take appropriate measures to avoid this kind of incidents happening again.

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TX-P02

### **Solanum linnaeanum (Apple of Sodom) toxicity.**

#### **Seasonally associated mortality in beef cattle in NSW.**

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**Objectives:** To determine the aetiology of acute death in a group of suckler beef cows in NSW, Australia.

**Materials and Methods:** The clinical investigation was undertaken on a suckler beef farm in NSW, Australia. One hundred and thirty Aberdeen Angus cows plus followers were kept on the property and the owner had sought veterinary attention after acute death in two adult animals, whilst one more was exhibiting neurological signs including wandering, depressed mentation and colic-like symptoms including kicking at the abdomen between periods of recumbency. Hypersalivation was also a feature of the cow's presentation. The owner reported previous unexplained deaths in cattle over the past two years, occurring in the same paddock, at the same time of year (March). The current herd had just been returned to this pasture one week previous.

On clinical examination the affected animal was aggressive and hyperaesthetic. She was pyrexia (temperature 40.1°C), had a facial skin tent >3 seconds, a reduced menace response bilaterally, was tachycardic (124 beats per minute), Tachypnoeic (39 breaths per min), had a CRT of >3 seconds and her mucus membranes and vaginal mucosa were injected. A bilateral mucoid nasal discharge was noted and hypersalivation was present. Rumen fill was poor and no rumen contractions were audible. Scant, mucoid faeces were present on rectal examination and urinalysis was unremarkable.

Blood samples for full haematology and biochemistry were taken and submitted to the University Veterinary Teaching Hospital Camden (UVTHC) pathology department.

The animal was administered flunixin meglumine (2mg/kg), oxytetracycline (10mg/kg) and 3 litres hypertonic saline (7.2%) via a 16G intravenous jugular catheter.

A field necropsy was undertaken on both recently deceased animals and samples submitted to the UVTHC pathology department for histopathological examination.

Based on clinical presentation, blood and necropsy findings and farm history, a paddock walk was instigated to check for possible exposure to toxic plants.

**Results:** Haematological findings revealed mild haemoconcentration and hyperproteinaemia, attributable to dehydration, neutrophilia ( $1.1 \times 10^9/L$ ) with left shift and toxic changes. Bio-

chemistry revealed an elevated CK (1312 U/L) and a moderate elevation in AST (168 U/L) and creatinine (181  $\mu\text{mol/L}$ ).

Necropsy findings included a necrotising abomasitis, severe haemorrhagic jejunitis and enlargement of the mesenteric lymph nodes. The kidneys were pale with focal areas of haemorrhage on the cortex and the liver had multifocal, pale areas of necrosis. However, mild autolysis was noted in both animals.

Histopathology revealed multifocal areas of marked acute haemorrhage in the ileum, with focal MALT necrosis. Moderate inflammation was present in the submucosa mainly consisting of neutrophils, lymphocytes and plasma cells. The superficial layers of the abomasum were autolyzed, but the deeper layers revealed a mild to moderate, acute, multifocal, lymphoplasmacytic abomasitis and multifocal acute haemorrhages in the adipose tissue adjacent to the abomasum. The liver had marked, acute, multifocal centrilobular necrosis and the kidneys had evidence of multifocal, moderate congestion.

The paddock walk revealed the presence of a number of *Solanum linnaeanum* (apple of Sodom) shrubs (of the nightshade family) with ripened fruits, scattered throughout the paddock. There was evidence that both the plant and ripened fruits had been eaten by cattle.

**Conclusion:** *Solanum linnaeanum* toxicity is consistent with the clinical presentation. The plant contains glycoalkaloids (solanoside and solanoside), which are cholinesterase inhibitors, resulting in abnormally increased stimulation of nicotinic and muscarinic receptors by acetylcholine. Chemical irritation of the gastrointestinal tract occurs through an as yet unknown mechanism and accounts for the gastrointestinal pathology seen in the necropsied animals. The entire plant is toxic, with ripened fruits the most toxic portion. Its consumption can account for the clinical syndrome seen and the history of seasonally associated deaths in this paddock.

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TX-P03

### ***Aspergillus flavus* and aflatoxins in dairy food chain a case in Mexico**

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Aflatoxin (AF) are produced by *Aspergillus spp.* fungi. AF exhibit mutagenic, carcinogenic, immunosuppressive, hepatotoxic and nephrotoxic effects. AF might be present in animal and human diet and excreted through milk or urine as Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>). The aim was summarized our data on the *A. flavus* and AF occurrence in dairy feed and dairy products in Central Mexico. Samples of feedstuffs, milk (raw, pasteurized and ultrapasteurized), and white cheese were obtained in dairy farms and local markets. Samples of breast milk and urine were obtained of mother (8-10 days post-partum) in a local public hospital. The fungi were isolated and identified in dairy feedstuffs via optical and SE microscopy and PCR; AF were tested by HPLC and ELISA. Dairy feedstuffs samples were AF contaminated (99%) and exhibit aflatoxicogenic *A. flavus* isolation (17%).



Dairy feed exhibit AF contamination (12.4 +1.0 microg/kg); dairy cows were exposed (627+0.43 microg/cow/d), and AFM<sub>1</sub> was eliminated in milk (30-60 ng/L) in rate of 0.93% of original AF amount ingested. All milk brands and cheese samples had AFM<sub>1</sub> (12.9+5.4 ng/kg). In human milk and urine samples AFM<sub>1</sub> was detected (40 and 72%); AFM<sub>1</sub> levels were not show correlations with dairy-eating patterns ( $r^2=0.1$ ;  $P>0.05$ ), suggesting other main exposure sources. These results suggest that occurrence of *A. flavus* and AF in Mexican dairy food chain, is relevant to Human and Animal Health in order to reduce the risk of exposure and damage.

TX-P04

### Fumonisin and Aflatoxins contamination in Mexican corn (*Zea Mays L.*)

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Climate change affects temperature and humidity in agricultural fields around the world, causing the proliferation of fungi with mycotoxigenic capacity. The maize grain is an important resource in animal nutrition, however this feeds can be contaminated by mycotoxins. The aim of this research was to identify the mycotoxicological contamination in post-harvest maize grain from two regions in Mexico. A 42 total analysis of aflatoxins and fumonisins were made in 26 maize grain, using immunoassay flow lateral method, and HPLC confirmation. 16 genotypes from region 1, with a temperature average 32 °C and 900 mm rain annual, and 10 genotypes from region 2, with a temperature average 20 °C and 600 mm rain annual, and two maize seed analyzed by UHPLC and MS. The results showed in grain maize an aflatoxins contamination in region 1, similar to region 2 (14.8 µg/kg vs 13.4 µg/kg). The fumonisin contamination in both regions are similar (428.6 µg/kg vs 342.5 µg/kg). In two samples of maize seed were detected fumonisins and aflatoxins, too. In conclusion all samples of grain and seed contained mycotoxins, aflatoxins outside the limit established by the European Union. Fumonisin and aflatoxins are contaminants that cause DNA damage in liver cells and a potential risk in human health.

TD-P01

### Complex bovine parasitic sadness in the state of Rondônia North of Brazil: case report

#### Babesiosis, Anaplasmosis, vectors.

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**ABSTRACT:** Brazilian cattle raising is an important source of income for the country, representing approximately 30% of Brazilian agribusiness besides occupying a prominent position for presenting the largest commercial herd in the world. The state of Rondônia in the north of Brazil contributes to these values, with a herd of around 14 million cattle. However, the occurrence of complex bovine parasitic sadness is important in this production and may interfere with the economy of the cattle raising sector. Bovine parasitic sadness is a complex of diseases caused by *Babesia* spp. and *Anaplasma* spp. Babesiosis is a disease caused by a protozoan of genus *Babesia* being subdivided into species *Babesia bovis* and *B. bigemina*. The anaplasmosis caused by a rickettsia of the genus *Anaplasma* spp., being the species *Anaplasma centrale* and *Anaplasma marginale*. Transmission occurs mainly through mechanical vectors (hematophagous flies) and biological vectors (tick *Rhipicephalus Boophilus microplus*). For the diagnosis of bovine parasitic sadness, epidemiological data, clinical signs, necropsy lesions and, in particular, laboratory tests should be considered. The treatment has as a function to destroy the host parasites with administration of drugs, the substances that can be used are: diminazene aceturate and imidocarb dipropionate associated with oxytetracycline.

**Objective:** The objective of this study is the describe the diagnosis and treatment for complex bovine parasitic sadness.

**Materials and Methods:** The was attended at the Veterinary Hospital (CEULJI / ULBRA), located in the municipality of Ji-Paraná, in the state of Rondônia a female bovine, eight months old, without breed, weighing 110 kg, with an entire reproductive status. The main complaint was depression, anorexia, blackened diarrhea, dermatitis, large infestation of ectoparasite (tick) throughout body extension, bruises and presence of crusts on the skin. During the clinical examination, the body condition score was 1.25 (evaluated from 1 to 5), rectal temperature of 40.3 °C, whitish mucosa, with severe dehydration. Heart rate and respiratory rate were normal. An increase in all palpable lymph nodes was observed. Hemogram and biochemical data were collected for renal and hepatic function, thus analyzing urea, creatinine, aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT), and collected peripheral blood from the ear tip by performing the blood smear for identification of the agent. The treatment of support fluid therapy, vitamin complex based vitamin B12 and fero. When identifying the presence of *Babesia* spp. and *Anaplasma* spp. in the slide confirmed the clinical suspicion of the complex bovine parasitic sadness. The treatment was administered intramuscularly 10 ml (Diminazene diacetate) with Oxytetracycline for four consecutive days, once a day. In addition to vermifugation performed with ivermectin. After five days, a new drug protocol for bovine parasitic sadness was established, using 4 ml (Imido-



carb Dipropionate) intramuscular single dose. Copro-parasitological examination was carried out to evaluate the efficacy of the vermifuge used on the first day (Ivermectin 2%) and to rule out endoparasitosis. A high amount from the Strongyloidea. Was administered 20 ml orally of Albendazole for three days, the dose administered as observed was double, which is associated with twice the dose of levamisole at the dose of 1 ml for every 10 kg of oral weight. Animal presented clinical worsening where again it was carried out support treatment with physiological solution, glucose and vitamins complex b.

**Results:** At the end of the protocol the animal died, it was sent to the necropsy. At the necropsy, oral, nasal, ocular and vulvar mucosa with a whitish appearance were observed, and the gastric and intestinal walls were hemorrhagic. The presence of petechiae and ecchymoses in the pyloric region was noticeable in the abomasum. In the small intestine, in a portion of jejunum, the loops were blackened and hemorrhagic (suffusion). In the abdominal cavity, the presence of yellowish fluid (suggestive of peritonitis), whitish omentum, liver with rounded edges was noticed, with a sandy and spleen appearance and reactive mesenteric lymph nodes. In the thoracic cavity hydropericardium, however, the lung did not present alteration.

**Conclusions:** However, even when dealing with a well-known disease and with treatment protocols already established, one must at all times consider new forms of diagnosis to reduce the economic loss that this pathology generates the great and small producers.

**Key-words:** Babesiosis, Anaplasmosis, vectors.

TD-P02

## Relationship between feeding management for milking cows and dairy farming productivity, especially of nutritious status of cattle and daily milk yield in Mbarara, Uganda

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**Objectives:** A three-year project, Safe Milk Promotion in Mbarara Project has been being conducted sponsored by Japan International Cooperation Agency since September 2016 to improve dairy farming productivity in Mbarara, Uganda. In this study, feeding management for cattle and dairy farming productivity: daily milk yield and nutritious status of cattle were investigated, and their relationships were analyzed for the use of improvement of the productivity.

**Materials and methods:** Thirty farms participated in the project and the farm information of herd size, milk yield and herd management practices were collected. Reproduction and nutri-

tion information was collected by asking owners and/or workers working on the 30 farms in Mbarara district from October 2016 to June 2017. The contents of the information are the following: breed, parity, date of last calving and blood ketone level for cows which calved within three weeks. Feeding management information of contents of feedings and grazing management at the farms was also collected. Descriptive epidemiology was performed for the information collected. Univariable analysis was also performed for the collected data to analyze relations between feedings and blood ketone body level; relations between feedings and individual daily milk yield; and a relation between individual daily milk yield and blood ketone level. For univariable analyses, linear mixed effect models were used for response variables of continuous data.

**Results:** In the participate farms, 25.6% of adult cows (130/507) was local breed, and the other was mixed with dairy breeds. The mean, median and range of the information collected from the 30 farms were the following: age, 5.0, 5, 2-13; parity, 2.8, 3, 0-10; individual daily milk yield (l), 10.1, 9, 1-32; blood ketone body (mmol/l), 0.75, 0.7, 0.2-1.8. Contents of the feedings were pasture, concentrate (brewers waste, maize bran, barley and formula feed) and forage (fresh grass (napier grass), corn silage, grass silage, haylage, hay, lablab, sorghum, banana peel). From univariable analysis, the cows which were fed concentrate at the farms had higher blood ketone body level ( $n$  of observations = 51,  $n$  of groups = 13, difference: 0.26, SE = 0.08,  $p < 0.05$ ) and higher milk yield ( $n$  of observations = 426,  $n$  of groups = 28, difference: 4.14, SE = 1.38,  $p < 0.01$ ) than those which were not. The higher milk producing cows had the higher blood ketone body level ( $n$  of observations = 47,  $n$  of groups = 13, slope: 0.03, SE = 0.01,  $p < 0.05$ ).

**Conclusion:** Dairy cattle breeds were commonly introduced in dairy farms in Mbarara, and many mixed breed cows were there in the farms. Those cows might have higher milk producing ability if they are fed adequate feedings. In this study, the cows which were fed concentrate produced higher milk yield, also had higher blood ketone level. This is maybe because by being fed concentrate, they produce more milk, but the energy balance of cows is negative, which means that the quantity of feeding, in other words, the dry matter intake is not adequate and is below. In order to keep yielding high milk production without nutritious disorders like ketosis, adequate feeding management is important especially in Uganda where dairy industry is developing quickly and milk production and demand is getting bigger and bigger because cows there have so many varieties of productive abilities.



## UH-P01

### Effects of sampling volume of foremilk for the evaluation of somatic cell counts and bacterial counts

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**INTRODUCTION:** Mastitis is a serious issue for the dairy industry in Japan. Monitoring of somatic cell counts (SCCs) and bacterial counts (BCs) in foremilk is important for detection of abnormal milk and to control hygienic milk quality at dairy farms. However, factors affecting the variability of the values of foremilk SCCs and BCs have not been fully clarified. In this study, we focused on the relationship between sampling volume of milk and SCCs and BCs of foremilk samples.

**MATERIALS AND METHODS:** This study used a total of 12 Holstein-Friesian cows, maintained at a Rakuno Gakuen University dairy farm, exhibiting BCs exceeding  $1 \times 10^4$  cfu/mL. Fixed volumes of foremilk samples (5, 10, 15, 20, 25, 50, 75, 100, 125, or 150 mL) were collected from the 1 or 2 quarters of individual animals before each milking. SCCs and BCs of collected foremilk samples were measured using a DeLaval Cell Counter and 3M<sup>TM</sup> Petrifilm<sup>TM</sup> AC plates, respectively. Bacterial isolates were speciated using traditional culture methods, including biochemical screening. Data were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using the Mann-Whitney U test;  $p < 0.05$  was considered significant.

**RESULTS:** Coagulase-negative staphylococci (CNS) (4 quarters), Other streptococci (OS) (4 quarters), CNS+OS (4 quarters), and *Staphylococcus aureus* (1 quarter) were isolated from foremilk samples. Average BCs ( $\times 10^4$  cfu/mL) of continuously collected foremilk samples were as follows: 5 mL,  $9.1 \pm 11.4$ ; 10 mL,  $1.3 \pm 0.9$ ; 15 mL,  $1.0 \pm 0.8$ ; 20 mL,  $0.6 \pm 0.6$ ; 25 mL,  $0.4 \pm 0.6$ ; 50 mL,  $0.2 \pm 0.2$ ; 75 mL,  $0.3 \pm 0.3$ ; 100 mL,  $0.7 \pm 1.2$ ; 125 mL,  $1.2 \pm 2.6$ ; 150 mL,  $0.3 \pm 0.5$ . The BCs with 5-mL foremilk sampling were significantly ( $p < 0.01$ ) higher than those with other sampling volumes. With 10-mL foremilk sampling, BCs were significantly ( $p < 0.01$ ) higher than with 20- and 25-mL sampling. Significant differences were not detected among the BCs for foremilk sampling at 25 to 150 mL.

Average SCCs ( $\times 10^4$  cells/mL) of continuously collected foremilk samples were as follows: 5 mL,  $69.1 \pm 60.0$ ; 10 mL,  $57.1 \pm 45.0$ ; 15 mL,  $65.1 \pm 68.4$ ; 20 mL,  $59.1 \pm 67.3$ ; 25 mL,  $48.3 \pm 61.7$ ; 50 mL,  $49.9 \pm 74.8$ ; 75 mL,  $49.2 \pm 72.9$ ; 100 mL,  $35.2 \pm 28.4$ ; 125 mL,  $35.9 \pm 28.8$ ; 150 mL,  $37.3 \pm 30.7$ . The SCCs with 5-mL foremilk sampling were significantly ( $p < 0.05$ ) higher than those with foremilk sampling at 50 to 150 mL (excluding 75 mL). Significant differences were not detected among the SCCs for animals with foremilk sampling at 50 to 150 mL.

**DISCUSSION and CONCLUSION:** We concluded that, for Holstein-Friesian cows, foremilk sample volumes of at least 50 mL per quarter are necessary for the proper monitoring of SCCs and BCs.

## UH-P02

### Udder disinfection and milking machine cleaning on large Holstein-Friesian farms

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**Objectives:** The milking machine cleaning products and the udder disinfectants are used daily and in large amounts in dairy herds. The milking machine cleaning has an important role in the reduction of bacterial numbers of milk. A good teat disinfectant should (1) have a broad and rapid bactericidal activity, (2) not irritate the teat skin, (3) not have corrosive impact on teat cups, and (4) cause no residue risk in milk. The aim of our study is the conduct a survey on the milking technique, the udder pre- and post-milking teat disinfection and the cleaning of milking machines in the Hungarian large Holstein-Friesian dairies.

**Materials and Methods:** The survey was conducted between September and October 2014 by using a questionnaire. A total of 43 large dairy farms completed the survey and half of the persons filling out the questionnaire were farm managers. The survey covered practically the whole area of Hungary. A total of 31,430 cows were kept on the farms, which corresponded to 17.9% of the 176,000 Hungarian dairy cattle stocks. Furthermore, seven personal interviews were conducted with farm managers representing a total of 6,130 cows.

**Results:** On the farms the cows are most commonly milked twice a day. Almost two-third of the farms use a 5-phase milking machine cleaning system. Milking machines are cleaned twice a day in almost three-quarter of the farms and three times a day in almost one-fifth. The number of acid descaling washes per week ranges from once to 21 times a week. Sodium hypochlorite was the most preferred active ingredient of alkaline detergents, followed by sodium hydroxide and potassium hydroxide. Phosphoric acid was the most commonly used acid, followed by nitric acid, sulphuric acid and organic acids. Price is the most important characteristic when purchasing milking machine cleaning products, followed by the reliability of the distributor and the type of cleaning system used.

Almost two-thirds of the farms use pre-milking teat dipping, almost a quarter still wash the udder with water, and more than 10% use a wash with disinfectant. The udder is wiped with dry paper towels in almost third-quarter of the farms. The forestripping is performed in all herds, in about half of the farms into a cup, the other half onto the floor. Chlorhexidine was the most preferred active ingredient in pre-milking disinfectants, followed by lactic acid and other compounds of chlorine. Iodine was the most common active ingredient in post-milking disinfectants, followed by lactic acid and various compounds of chlorine. The active ingredient is the most important characteristic when purchasing a teat disinfectant, followed by price and ease of use.

**Conclusions:** The efficacy of milking machine cleaning depends on the working solution content, the temperature of water and the application of sanitizer. A quarter of the farms still wash the udder with water, which is not recommended as a routine part of udder preparation. The udder is wiped with dry paper towels in almost third-quarter of the farms, which cannot



play a role in spreading infection. The pre-milking disinfectants containing compounds of chlorine preferred in two-thirds of Hungarian dairy herds, which complies with the international findings. Almost on every farm iodine is considered to be as the optimal active ingredient in post-milking disinfectants, which can largely reduce the incidence of clinical mastitis.

The research was supported by the Hungarian Ministry of Human Capacities [grant number 12190/2017/FEKUTSTRAT]. The project was also supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.1-16-2016-00024, project title: Innovations for Intelligent Specialisation on the University of Veterinary Science and the Faculty of Agricultural and Food Sciences of the Széchenyi István University Cooperation).

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UH-P03

### Evaluating the in vitro susceptibility of mastitis associated catalase-negative, esculin hydrolyzing cocci in Austrian dairy herds

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**Objectives:** Worldwide catalase-negative, gram-positive, esculin hydrolyzing cocci are frequently involved in bovine mastitis. This group of udder pathogens is not only large but also diverse, therefore treatment protocols for best cure rates may vary. In accordance with evidence-based mastitis therapy, decisions on antibiotic usage, especially if prolonged, should be based on proper bacterial identification and proof of the efficacy. Good Veterinary Practice requires susceptibility testing which results in better use of antibiotics and higher treatment success. Aim of this study was to assess the in vitro efficacy of veterinary antimicrobial agents against these organisms in Austria.

**Materials and methods:** Mastitis-causing pathogens (n=111) were obtained from quarter milk samples submitted to the diagnostic laboratory of the University Clinic for Ruminants in Vienna for bacteriological examination. Sequencing of the 16S rRNA gene confirmed isolates as *Streptococcus (Sc.) uberis* (n=77), *Enterococcus* spp. (n=20) and *Lactococcus* spp. (n=14).

Antimicrobial minimal inhibitory concentrations (MIC) were determined using the commercially available MICRONAUT-S Mastitis 3 assay (MERLIN Diagnostika, Germany). A panel of eleven antimicrobials was included in the assay: penicillin G, ampicillin, amoxicillin/clavulanic acid 2:1, oxacillin, cefazoline, cefoperazone, cefquinome, kanamycin/cephalexin 10:1, erythromycin, marbofloxacin and pirlimycin. MIC<sub>90</sub> was calculated and interpretation was performed as far as resistance breakpoints available applying CLSI VET01S Performance Standards.

**Results:** *Sc. uberis* were mostly susceptible to antimicrobials tested and had low MIC<sub>90</sub> for penicillin G, ampicillin, amoxicillin/clavulanic acid, cefazoline, cefoperazone, cefquinome and ka-

namycin/cephalexin but 20.8% and 26.0%, respectively, were resistant to erythromycin and pirlimycin. None of the Enterococci was resistant to penicillin, ampicillin or amoxicillin/clavulanic acid, but all had high MIC<sub>90</sub> values for the cephalosporins, oxacillin, marbofloxacin and pirlimycin. *Lactococcus* spp. showed a deviating in vitro activity profile.

**Conclusions:** Very few antimicrobials used for mastitis treatment have a CLSI breakpoint. Based on our results, penicillins are recommended as first-line agents for treatment of mastitis caused by gram-positive, catalase-negative and esculin hydrolyzing cocci.

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UH-P04

### Antibiotic sensitivity of Clinical and Subclinical Mastitis Pathogens in Turkey

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**OBJECTIVES:** The aim of this study was to estimate the mastitis pathogen incidence and antimicrobial sensitivity in cows affected by clinical or subclinical mastitis from 24 commercial dairy farms, located in different Turkish regions.

**MATERIAL AND METHODS:** A total of 324 milk samples from 24 industrial dairy farms were selected from (sub)clinical mastitis cases, and sent to a central lab (Agrilab) by veterinarians between January 2016 and November 2017.

Milk samples were sent with frozen ice packs at temperature between +2 and +8°C (max. 24-48 hours) to the Agrilab veterinary laboratory, located in the Aegean region.

The Minnesota Easy Culture System was used for bacterial culture, containing 3 different media; Factor (Gram-positive bacteria), MacConkey (Gram-negative bacteria) and Modified TKT (Streptococci). For further species identification, API 20 strep and API 20 E, Baird-Parker Agar (*Staph. aureus*), and PPLO Agar were used. The routine methods were used for other bacterial identification. Antibiotic susceptibility was done with disk diffusion on Mueller-Hinton agar.

Cefquinome (CFQ), Neomycin-Bacitracin-Tetracycline (NBT), and Cefapirine (CEF) sensitivity disks were used because they are frequently used in the field, sensitivity thresholds (mm inhibition zone) were >19 for CEF, >13 for NBT and >22 CFQ.

**RESULTS:** Most frequently isolated pathogens (n=371) of (sub)clinical mastitis was *E. coli* (26.1%), Others (16.7%), No growth (14.2%), Enterococcus (9.7%), *S. aureus* (12.1%), *S. uberis* (7%), and CNS (5.1%).

Resistance of these pathogens to the 3 antibiotics was *E. coli* (CFQ; 12%, NBT; 10%, CEF; 46%) *S. aureus* (CFQ; 13%, NBT; 0%, CEF; 5%), *Strep. uberis* (CFQ; 4.3%, NBT; 0%, CEF; 4.7%), CNS (CFQ; 0%, NBT; 0%, CEF; 0%).

*E. coli* and *Strep. uberis* showed no difference in cefquinome resistance on farms (n=11) where cefquinome was used (10%, n=51; 0%, n=11, resistance respectively) compared to the



farms (n=13) where it was not used (15%, n=46; 7%, n=15, respectively). However, *Staph. aureus* showed higher resistance to cefquinome (n=14, 43%) on farms where it was used, than on farms where it was not used (n=31, 16%).

**CONCLUSION:** Incidence of environmental pathogens was high, suggesting environmental mastitis prevention strategies should be the focus on the farms in the study. Sensitivity of the most frequently isolated pathogens for the 3 antibiotics was generally high, except the sensitivity of *E. coli* to cefapirin. However, antibiotic sensitivity data of both NBT and cefapirin should be interpreted with care because inhibition zone thresholds for sensitivity are based on human pathogens and not on cattle mastitis causing pathogens.

UH-P05

### Antimicrobial susceptibility trends for strains derived from bovine mastitic milk in Japan

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**Objectives:** Bovine mastitis is a diagnosis that comprises one-third of the diseases in dairy cows and results in extensive economic losses to dairy farms. In addition, treatment of mastitis is currently dependent on intramammary infusion of antimicrobial substances and injection into the whole body, and depending on the medicine; however, tolerance has become more widespread and represents a problem. Treatment of mastitis should accurately identify the causative pathogen and treat it according to its characteristics. Treatment based on the accurate diagnosis of the causative bacteria and its drug susceptibility test results enables the most effective treatment, reduces the use of antibiotics and prevents the emergence of resistant bacteria. However, there have been no reports widely investigating the antimicrobial susceptibility of mastitis-derived strains in Japan. The purpose of this study was to investigate antimicrobial susceptibility of strains derived from mastitis milk in Japan and to identify related trends.

**Materials and methods:** A total of 1,349 samples of bovine clinical mastitis milk collected from countrywide in 2015 were applied to 5% sheep blood agar and cultured at 37°C for 24 hours, and separated strains were identified by molecular biology using PCR and sequencing. Minimum inhibitory concentrations (MIC) were measured for the identified 411 strains. MIC analysis was performed by the agar plate dilution method based on the Clinical & Laboratory Standards Institute (CLSI) method.

**Results:** The breakdown of causative strains of mastitis was as follows: *Staphylococcus aureus*, 54 strains; *Staphylococcus xylosum*, 15 strains; *Staphylococcus saprophyticus*, 11 strains; *Staphylococcus saprophyticus*, 11 strains; *Staphylococcus equorum*, 8 strains; *Staphylococcus equorum*, 8 strains; *Staphylococcus hyicus*, 8 strains; *Staphylococcus haemolyticus*, 6 strains; *Streptococcus uberis*, 64 strains; *Streptococcus dysgalactiae*, 51 strains; *Streptococcus equinus*, 31 strains; *Streptococcus bovis*, 12 strains; *Streptococcus agalactiae*, 5 strains;

*Streptococcus porcinus*, 6 strains; *Enterococcus faecium*, 8 strains; *Enterococcus faecalis*, 6 strains; *Escherichia coli*, 80 strains; *Klebsiella oxytoca*, 13 strains; *Klebsiella pneumoniae*, 13 strains; *Enterobacter cloacae*, 7 strains; *Serratia marcescens*, 3 strains; *Pseudomonas aeruginosa*, 6 strains; and *Pasteurella multocida*, 4 strains.

For gram-positive bacteria, the penicillin MIC for *Staphylococcus* spp. showed high susceptibility ( $\leq 0.125\mu\text{g / ml}$ ), while *Streptococcus* spp. had a high sensitivity to penicillin and pirlirymycin ( $\leq 0.125\mu\text{g / ml}$ ), but resistance to oxytetracycline and kanamycin, indicating the acquisition of natural tolerance. *Enterococcus* spp. had a high resistance to all tested drugs. For gram-negative bacteria, the MIC for enrofloxacin and marbofloxacin against *E. coli* and *Klebsiella* spp. showed high sensitivity ( $\leq 0.125\mu\text{g / ml}$ ), but showed resistance against other antibacterial drugs. *P. aeruginosa* also showed resistance to many antimicrobials. Overall, strains exhibiting resistance to cefazolin were more common.

**Conclusion:** Cefazolin-resistant strains were observed, probably due to disproportional use of cefazolin. As gram-positive bacteria showed higher sensitivity to penicillin than other antimicrobials, it seems necessary to broaden the choice of drugs to include penicillin, in addition to cefazolin. Fluoroquinolone is only permitted as a second-choice drug in Japan. Only if gram-negative bacteria only showed sensitivity to fluoroquinolone, primary treatment with fluoroquinolone within a limited range should be considered when treating peracute coliform mastitis with severe clinical signs.

UH-P06

### Bacterial identification and antimicrobial susceptibility of microorganisms isolated in goat milk

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In small ruminants, no detailed protocols for treatment of mastitis, as have been developed in cows, are available. Effective antimicrobial agents should be used for treatment considering the broad-spectrum product against the major causal agent of disease. The goal of this research was to evaluate the frequency of bacterial groups isolated in goat milk and its antimicrobial susceptibility. For this, Saanen goats in lactation were screened from six properties (A to F). Initially, milk samples (n=270) were cultured in Petri plates containing sheep blood agar (5%), incubated at 37°C for 24 and 48 hours. The identification of the bacterial groups was performed using coagulase and biochemical tests. Subsequently, the positive samples were grouped according to the bacterial type isolated to perform the antibiogram of each dairy farm, using the disc diffusion methodology. Antimicrobials drugs tested were penicillin G (10 IU), ampicillin (10  $\mu\text{g}$ ), oxacillin (01  $\mu\text{g}$ ), clavulanic acid-associated amoxicillin (30  $\mu\text{g}$ ), cephalexin (30  $\mu\text{g}$ ), erythromycin (30  $\mu\text{g}$ ), enrofloxacin (05  $\mu\text{g}$ ), ciprofloxacin (05  $\mu\text{g}$ ), sulfonamide (300  $\mu\text{g}$ ), streptomycin (10  $\mu\text{g}$ ), gentamicin (10  $\mu\text{g}$ ), lincomycin (02  $\mu\text{g}$ ), neomycin (30  $\mu\text{g}$ ), novobiocin (05  $\mu\text{g}$ ) and sulfa associated with trimethoprim (25  $\mu\text{g}$ ). Data from antibiotic sensitive test



were classified as resistant, intermediate and sensitive. Seventy six samples (76/270, 28.1%) of milk were positive in the bacteriological isolation. The main bacterial group isolated from goat milk were coagulase-negative Staphylococci - CNS (69/76, 90.8%). Others bacterial groups isolated in minor proportion were *Corynebacterium* spp (4/76, 5.3%), and coagulase-positive Staphylococci - CPS (3/76, 3.9%). The antibiotics tested for CNS showed 100% (six properties, 6/6) of resistance for ampicillin and 66.7% (4/6) for penicillin. The data from antibiotic sensitive test was considered intermediate for erythromycin (4/6, 66.6%) and streptomycin (4/6, 66.6%) and sensitive for amoxicillin and clavulanic acid (5/6, 83.3%), gentamicin (5/6, 83.3%), lincomycin (5/6, 83.3%), oxacillin (5/6, 83.3%) and sulfonamide (4/6, 66.6%). Antibiotics that showed 100% sensitivity (6/6) among the tested isolates were cephalexin, ciprofloxacin, enrofloxacin, neomycin, novobiocin and trimethoprim-associated sulfamethoxazole. CSP was isolated from three properties (B, C and E), presenting sensitivity for most of the antibiotics tested (11/15, 73.3%). There was 100% of ampicillin resistance and intermediate response to erythromycin, streptomycin and neomycin. Only in one property (A) was isolated *Corynebacterium* spp and it was resistant to amoxicillin and clavulanic acid, ampicillin and penicillin; intermediate reactions were detected for erythromycin, streptomycin and oxacillin, with sensitivity to the other antibiotics tested. Our results can be used to establish specific therapeutic protocols for dairy goats avoiding problems related to bacterial resistance and recurrent cases of mastitis.

UH-P07

### Randomized clinical trial to evaluate the effectiveness of enrofloxacin as a second-line antibiotic for treatment of acute *Escherichia coli* mastitis

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*Escherichia coli* (*E. coli*) is epidemiologically one of the most severe pathogens, and is the major bacterium that causes mastitis on dairy farms. Systemic administration of antibiotics is recommended for the treatment of severe coliform mastitis because of the risk of bacteremia, and several studies have reported on the treatment efficacy of enrofloxacin (ERFX), a bactericidal concentration-dependent fluoroquinolone agent, in treating mastitis. In Japan, the use of fluoroquinolones as a first-line drug is restricted by an antimicrobial resistance risk management measure that is enforced by the Ministry of Agriculture, Forestry, and Fisheries. However, there is a lack of prospective data on the use of ERFX as second-line treatment for acute coliform mastitis caused by *E. coli* in dairy cows. The objective of the present study was to evaluate the effectiveness of enrofloxacin (ERFX) as a second-line antibiotic for treatment of acute *Escherichia coli* (*E. coli*) mastitis. Forty-two cows with naturally occurring acute *E. coli* mastitis were enrolled. On the first day of treatment (day 0), empirically selected antibiotics (oxytetracycline (OTC): n=32, kanamycin (KM): n=10) were ad-

ministered. Although systemic signs improved in 10 cows (first-line group) on the next day, the signs remained unchanged or worsened in 32 cows on day 1, including two cows that were found dead. The 30 surviving but unimproved cows were randomly assigned to second-line groups constituting an ERFX group (n=19) or a control group (n=11) that was treated with other antibiotics (OTC n=6, KM n=4, none n=1). Response to each treatment was evaluated by measuring clinical signs from day 0 to day 3, subsequent quarter milk recovery, and the 60-day survival rate. Although there were no marked differences in the clinical scores of the three groups on day 0, the mean clinical score of the first-line group was significantly lower than that of the second-line groups on day 1. In the second-line groups, the appetite of the ERFX group improved significantly compared to that of the control group (p<0.05) on day 3, but no notable differences were observed in any other clinical signs. The rate of quarter milk production returning to preinfection level was 40.0% (4/10) in the first-line group, 36.4% (4/11) in the ERFX group, and 11.1% (1/9) in the control group. Although the quarter milk production recovery rate of the ERFX group was nominally closer to that of the first-line group than to that of the control group, these differences did not achieve significance. The 60-day survival rate was 80.0% (8/10) in the first-line group, 68.4% (13/19) in the ERFX group, and 54.5% (6/11) in the control group; these values did not differ significantly by statistical analysis. In conclusion, the present study evaluated the use of ERFX treatment for acute *E. coli* mastitis in animals that exhibited poor responses to initial treatment. Notably, administration of ERFX (as opposed to OTC or KM) as a second-line treatment significantly restored appetite and (nominally) improved subsequent milk recovery or the 60-day survival rate. These results show a positive effect of ERFX administration as a second-line treatment for acute clinical *E. coli* mastitis.

UH-P08

### Evaluation of a herbal therapy in bovine subclinical mastitis: A clinical assessment

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The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. In the present scenario, the treatment of subclinical mastitis (SCM) is only antibiotic therapy. But, its overuse and/or indiscriminate use have caused havoc by producing resistance in the pathogens to such antibiotics. A serious consequence of the use of antibiotics in milk is their effect on the manufacture of dairy products and the development of sensitivity syndromes in human beings. For this reason, nowadays, the concept of using non-antibiotic strategies for controlling



mastitis is gaining more attention. One possible approach to control mastitis is through improving the immune cells of the diseased udder during immunosuppressive stages that would greatly impact the ability of the animal to resist pathogenic infection. One such approach is based on enhancement of the animal's natural defense mechanism by use of some non-specific immunomodulator such as herbs which, in turn, minimizes the use of antibiotics. Present study was planned to evaluate the immunotherapeutic potential of a commercial preparation (magic-3 vet) in mastitis in dairy cows. Therapeutic trial of the preparation involved 24 lactating cows with SCM. The cows were divided randomly into 2 groups of 12 each. The cows in group 1 (G1) served as control. The cows in group 2 (G2) were given a commercial preparation (magic-3 vet) @ 100 ml orally twice a day for 7 days. The immunotherapeutic potential of herb in specific SCM was evaluated in terms of elimination of intramammary infections (IMI), reducing udder inflammation, and improvement of milk quality and immunopotential of udder defense up to d 90 post-initiation of treatment. The therapy could eliminate IMI 60.97% and 65.85%, on d15 and d30, respectively. The differences in the elimination of IMI in treatment vs. control were observed to be statistically significant ( $p < 0.05$ ) on d 15 and ( $p < 0.01$ ) on d30. No significant difference in CMT score, pH, EC, SCC, fat, SNF, protein, lactose values was noticed in cows of control group. Therapy with the preparation showed a significant decline in CMT score, pH, EC, SCC and NAGase enzyme activity of milk from d7. There was no significant alteration in fat, SNF, protein and lactose values in the treatment group during the present course of the investigation. The mean phagocytic activity in control group did not differ significantly throughout the course of study. In commercial preparation treated cows, a significant increase ( $P < 0.05$ ) was observed in phagocytic activity at d 7 of treatment, and it remained significantly elevated throughout the course of study as compared to d 0. The finding of the study indicates beneficial effects of herbal therapy against subclinical mastitis of lactating dairy cows. The positive effects of the therapy may be attributed due to their anti-bacterial, anti-inflammatory and immunomodulation potential as substantiated by the elimination of intramammary infections, a decrease of milk SCC and enhanced phagocytosis of milk leukocytes in the present study.

UH-P09

### Application of Novel Biosurfactant, Mannosylerythritol lipid-B, for improving the bovine mastitis.

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**Introduction:** Bovine mastitis is globally known as a disease of major economic effects on the dairy industry. The most common treatment method available against bovine mastitis is the intra-mammary infusion of antibiotics. However, antibiotics causes the problem of antimicrobial resistance. Mannosylerythritol lipids (MELs) are a glycolipid class of biosurfactants produced by a variety yeast and fungal strains that exhibit excellent interfacial and biochemical properties such as antimicrobial

activities and anti-inflammatory action inhibiting the secretion of inflammatory mediators from mast cells. MELs is not an antibiotics. *Pseudozyma tsukubaensis* produces a large amount of a diastereomer type of MEL-B, which is one type of MELs. We previously reported that MEL-B is commercially available as a ceramide-like bio humectant. The present study was undertaken to elucidate the effect of MEL-B ointment on the symptom of sub-clinical mastitis.

**Materials and Methods:** Fifteen teats of 10 dairy cows with subclinical mastitis were used. Udders were coated with 7 ml of 10%(w/w) MEL-B for 7 days. Milk was collected at day 0, 3, 7, 14 and 21 (day 0 was considered as the day of onset of coating) to measure milk yield and somatic cell count (SCC). Teat canal condition score, Hardness of udder and were recorded.

**Results:** Other *streptococci* (20%), *Staphylococcus aureus* (13.3%), *Escherichia coli* (13.3%) were detected in the milk. Milk yield was not changed significantly. On the other hand, SCC was decreased significantly up to about 40% compared to that at day 0. The teat canal condition score was significantly improved with MEL-B until day 21. Moreover, the hardness of udders were softened from significantly until day 21.

**Conclusion:** These results suggest that MEL-B can improve the mastitis symptom such as SCC, teat canal condition and hardness of udder, possibly due to its anti-microbial and moisturizing activities. Therefore, the MEL-B have a strong potential as a new ingredient for improving bovine mastitis without using an antibiotics.

UH-P10

### Effect of combination treatment of refractory chronic mastitis in the dairy cow, using intramammary perfusion with hypertonic saline solution followed by short-term dry-off

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**Objectives:** Bacteria are commonly thought to resist antibiotics and immune cells by producing biofilm in the mammary gland during cases of mastitis caused by pathogens (e.g., *Streptococcus uberis*), this resistance results in chronic and refractory mastitis. Following a report from Chiba et al. that a solution of >1 M NaCl degrades biofilm, we treated refractory, chronic mastitis with a combination of intramammary perfusion, using a hypertonic saline solution, (HSS) and a short-term dry-off (stop milking of the affected udder for 3 days); we then analyzed the results.

**Materials and Methods:** 1) Objects: A total of 42 cows (50 udders) with refractory chronic mastitis, which was uncured by long-term intramammary infusion of antibiotics (>3 rounds of continuous infusion for 3 days each) or short-term dry-off, following causative bacteria identification by culture of mastitic milk. 2) Treatment procedures: We administered an intramammary infusion of 250 mL HSS (hypertonic saline, Kyoritsu Seiyaku, Japan) in the trial group (38 udders) and 250 mL physiological saline (physiological saline V injection for animals,





Zenoaq, Japan) in the control group (12 udders); we drained the fluid after a waiting period of 10 minutes, during the first perfusion. Subsequently, we perfused by immediate infusion and drainage, three times repeatedly. Following perfusion, we conducted short-term dry-off with an intramammary injection of cefazolin (Cefamezin Z®, Zenoaq, Japan). 3) Investigations: Bacterial isolation and identification tests, using mastitic milk, were performed on days 0, 3, 7, 14, and 21 of treatment. We defined specimens without pathogens on day 21, or with Other streptococci isolated at a density of <250 colonies/mL of mastitic milk, as cured. Total protein (TP) and albumin/globulin (A/G) ratio were measured by blood biochemistry on day 0, and the peripheral white blood cell population was analyzed by flow cytometry on days 0, 3, 7, 14, and 21. Smear samples of the first intramammary perfusion fluid were stained with 0.1% crystal violet, and the density of biofilm in the samples was measured by image analysis software, Image J® (Wayne Rasband(NIH)).

**Results:** Cure rate was significantly higher in the trial group than in the control group (71.1 % vs 33.3 %,  $p < 0.05$ ). The density of biofilm in the first intramammary perfusion fluid was significantly higher in the trial group than in the control group ( $p < 0.05$ ). The rate of peripheral CD14-positive cells tended to increase on day 3 in both groups. There was no difference in TP and A/G ratio between cured and uncured individuals in the trial group.

**Conclusion:** In this study, we found that combination treatment of refractory mastitis in the dairy cow, consisting of intramammary perfusion with hypertonic saline, followed by short-term dry-off, was effective. This method may promote degradation of intramammary biofilm and increase the population of monocytes in peripheral blood; thus, this treatment enhances the protective effects of antibiotics and cellular immunity against pathogens, resulting in a high efficacy. We expect that this method can be considered as novel treatment for refractory mastitis with evidence of biofilm formation.

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UH-P11

### The use of Cabergoline as a strategy for the prevention of new intramammary infections during the dry period and clinical mastitis after calving

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In an era of reduced antimicrobial use, drying-off presents a major challenge to the dairy producer, not least because of the increased milk production seen in the modern dairy cow, with its associated increase in risk of new intramammary infection (IMI). The Official Journal of the European Union published guidelines for the prudent use of antimicrobials in veterinary medicine (2015/C 299/04); these guidelines make specific reference to the treatment given to cows at drying-off, stating that one should “avoid the systematic treatment of cows at drying off. In the absence of an IMI at drying off, there is little/no justifi-

cation for antibiotic use and it is necessary to consider and implement alternative strategies and approaches to reduce the risk of new IMI. One such strategy would be to address the increased yield seen in many cows at dry off. Cabergoline (Velactis®, Ceva Sante Animale, France), inhibits prolactin release, reducing milk production and thereby the risk of new IMI during the dry period. This tool may offer an opportunity to support attempts to reduce the use of antibiotics at drying off.

**Objectives:** The objective of this study was to investigate the impact of the use of cabergoline at drying off on udder health at calving and in the early part of the subsequent lactation in UK dairy herds

**Materials and methods:** Over a 12-month period, ‘uninfected’ cows were recruited, at drying off, from 6 herds. ‘Uninfected’ cows were defined as having the last three SCC <200,000 cells/ml and being free from clinical mastitis within the same time frame. Cows were randomly allocated to one of seven different treatment groups. Cows received either cabergoline alone (ie no intramammary therapy), intramammary antibiotic (ADCT) alone, internal teat sealant (ITS) alone, ADCT in combination with ITS or ADCT, ITS or ADCT and ITS in combination with cabergoline. Bacteriology and somatic cell counts were used to assess intramammary infection status at drying off and post-calving. Clinical mastitis and other aspects of production were monitored for the first 100 days of the subsequent lactation. Univariable and multivariable analyses were undertaken to investigate the impact of cabergoline use as well as interactions with other approaches to control of IMI.

**Results:** A total of 714 uninfected cows with a mean in milk production 24 hours before the drying off of 18.1 litres (Range 2.7 - 42.0) were recruited in the study. Approximately 100 cows were included into each of the treatment groups. The prevalence of infection with major pathogens post calving was lowest in the group receiving cabergoline and ITS (CAB-ITS). Multivariable analysis revealed that the rate of clinical mastitis did not vary between treatment groups, with the exception of the CAB-ITS group in which the rate of clinical mastitis was lower than any of the other groups. Quarters from cows receiving cabergoline in addition to ITS at drying off were at significantly lower risk of developing clinical mastitis compared to quarters in cows receiving an internal teat sealant alone (OR 0.27, 95% CI 0.12-0.62) or antibiotic alone (OR = 0.31; 95%CI: 0.14-0.72). The benefit of the use of cabergoline appeared to be independent of milk yield at drying off.

**Conclusions:** This study has demonstrated a significant decrease in the incidence of clinical mastitis, in the early part of the subsequent lactation following the use of cabergoline at drying off and suggests that the use of antibiotics in such cows is not optimal. Moreover, this study suggests that the optimal treatment for uninfected cows at drying off is the use of an internal teat sealant in combination with cabergoline, offering producers to opportunity to improve udder health in the absence of the use of antibiotics.

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UH-P12

### Cabergoline as a tool to reduce the incidence of milk leakage. Results in Russian farms

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The incidence of milk leakage has been proven to be a risk factor for acquiring new intramammary infections (IMI). Schukken et al. (1993) shown an incidence of milk leaking of 30%, and leakage of milk was reported to be associated with 6.1 times more risk of developing an IMI. In a more recent study Zobel et al. (2013), observed a relationship between the milk production at dry-off and the percentage of milk leakage (ML). Cows dried-off with an average of 10.9 kg of milk at final milking had an incidence of 27% of ML compared to an incidence of 75% in cows dry-off with an average of 14.1 kg. Cabergoline (Velactis®, Ceva Santé Animale, Libourne, France) is a prolactin release inhibitor and reduces milk secretion, the percentage of milk leakage and the new intramammary infections

**Objective:** The objective of this study was to investigate the efficacy of cabergoline (Velactis®, Ceva Santé Animale, Libourne, France), on the incidence of milk leakage 20-24 hours, 30-34 hours and 48-52 hours after dry-off in commercial dairy farms in Russia.

**Materials and methods:** A total of 482 dairy cows from 3 commercial farms (parity 1=250, parity 2= 98 and parity 3+= 134) located in the north of Moscow were enrolled the day of the dry-off and followed up until 48-52 hours after dry-off. All cows were dried off abruptly (without changing in feeding or milking frequency) and treated with a single intramuscular injection of 5.6 mg cabergoline (n = 239) after last milking and the rest (n=243) were considered as a control. Milk leakage was observed at three different time-points after the dry-off: 20-24, 30-34, and 48-52 hours.

The study was multi-centric, randomized and blinded. The drug administrator was a different person to the person who did the milk leakage observations. The individual cow was the experimental unit and the quarter was the data collection. Data were analyzed using mixed effects logistic regression analysis with herd as a random effect and treatment, parity, and their 2-way interaction as fixed effects. The analysis was conducted with STATA® (version 14.0). Number of quarters leaking milk per cow in each group was estimated with corresponding 95% confidence interval

**Results:** Overall, the percentage of cows with milk leakage was lower in cabergoline treated (16.7%) compared with control (50.2%) cows ( $P < 0.001$ ). Cows treated with cabergoline were only 0.12 times as likely to show milk leakage compared with placebo (CI=0.06-0.22). Percentage of milk leakage at different observation times were 27.9%, 38.6% and 21.8% for the first, second and third from untreated cows compared to 5.4%, 7.5% and 9.6% in cabergoline treated cows

When comparing the data by parity, primiparous cabergoline group leaked 14.0% and untreated group leaked 53.7%, whereas multiparous treated group leaked 20.0% vs. 46.7% in the untreated group. Multiparous cows had a slightly lower milk production at dry off ( $18.2 \pm 0.91$  kg/d), than primiparous cows ( $20.5 \pm 0.91$  kg/d). This lower milk production may have contributed to a lower difference in milk leaking 40% in lact 1 vs 27% in lact 2+.

**Conclusion:** It is the first time that milk leakage incidence is evaluated in Russian commercial farms. Our data provide evi-

dence that a single injection of cabergoline reduces risk factors for udder health in primiparous and multiparous thorough reducing the percentage of milk leakage after dry them off. Considering our results, cabergoline can be a useful tool to be used in farms to reduce the milk production and the risk for new intra-mammary infections after the dry-off.

UH-P13

### Cabergoline treatment at dry-off facilitated the remodelling and the lactoferrin immunoprotection of the mammary tissue in dairy cows.

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In ruminants, the early phase of drying-off is a period of intense mammary gland involution that is due, in part, to the dramatic decline of prolactin (PRL) release. The speed at which the bovine mammary gland involutes following the abrupt cessation of lactation is also directly related to the risk of new intramammary infections. Thus, strategies to hasten involution following dry-off could have implications in preventing mastitis and optimizing mammary tissue regenerative processes.

**Objectives:** Our aim was to assess the effect of PRL inhibition by cabergoline on the remodeling and the lactoferrin immunoprotection of the mammary tissue.

**Materials and methods:** Fourteen Holstein dairy cows were injected with a single i.m. administration of 5.6 mg cabergoline (Velactis®, Ceva, Sante Animale, Libourne France) (n=7) or placebo (n=7) at the day of drying-off (D0). Mammary secretion samples were collected using a teat-cannula once during lactation (D-6) and at D1, D2, D3, D4, D8 and D14 after the drying-off. The mammary secretion samples were used for lactoferrin and zymography analyses to detect the activity of enzymes such as MMP, matrix metalloproteinases involved in the remodelling of mammary tissue during involution. Mammary epithelial cells (MEC) were also purified from mammary secretions after centrifugation and immunocytochemical binding in order to evaluate the MEC exfoliation. Mammary biopsy samples were collected one week before drying-off (D-6), at D1 and at D8 and used for lactoferrin immunochimistry and zymography analyses.

**Results:** The activity of MMP9 increased after drying-off in mammary secretions ( $P < 0.001$ ). Cabergoline increased the activity of MMP9 (1.7 fold,  $P < 0.05$ ) in mammary secretions and MMP-2 in mammary tissue after drying-off (1.4 fold,  $P \leq 0.01$ ). MEC concentration progressively increased in mammary secretions after drying-off ( $P < 0.01$ ). Cabergoline induced an increase in MEC concentration ( $P = 0.04$ ). Lactoferrin content progressively increased in mammary secretions during involution. The rise of lactoferrin content in mammary secretions was significant starting at D4 in the cabergoline treated cows ( $P \leq 0.05$ ) whereas it only happened at D8 in controls ( $P < 0.05$ ). Overall, cabergoline treatment increased lactoferrin content of mammary secretions ( $P = 0.10$ ). The total lactoferrin immunos-



taining in the mammary tissue increased after drying-off ( $P < 0.05$ ). Compared with during lactation, this increase was observed at D1 and D8, respectively for cabergoline treated cows and control cows ( $P < 0.05$ ).

**Conclusions:** Our results indicate that cabergoline treated group enhanced the extracellular matrix mammary remodelling, the MEC exfoliation from the mammary epithelium and hastened the udder immunoprotection by lactoferrin compared to placebo group. Therefore, cabergoline treatment could be used as a tool to facilitate the dry-off in dairy cows.

UH-P14

### Cabergoline treatment at dry-off accelerated mammary involution as indicated by mammary secretion composition changes in dairy cows.

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In ruminants, the early phase of drying-off is a period of mammary gland involution that is marked by the cessation of prolactin (PRL) release. The speed at which the bovine mammary gland involutes following the abrupt cessation of lactation is directly related to the risk of new intramammary infections

**Objectives:** Our aim was to assess the effect of PRL inhibition by cabergoline (Velactis®, Ceva Sante Animale) on the speed of the mammary gland involution, through analysis of the changes of mammary secretion composition.

**Materials and methods:** Fourteen Holstein dairy cows were injected with a single i.m. administration of 5.6 mg cabergoline ( $n=7$ ) (Velactis®, Ceva Sante Animale) or placebo ( $n=7$ ) at the moment of dry-off (D0). Mammary secretion samples were collected using a teat-cannula once during lactation (D-6) and at D1, D2, D3, D4, D8 and D14 after the drying-off. The mammary secretion samples were used for milk fat, lactose, true protein, alpha-lactalbumin and SCC analysis. Mammary biopsy samples were collected one week before drying-off (D-6), at D1 and at D8 and used for RNA extraction and RT-PCR analyses.

**Results:** As expected, SCC progressively increased whereas lactose content decreased in mammary secretions after drying-off ( $P < 0.001$ ). The increase in SCC was 2.4 fold higher in cabergoline treated cows than in control cows ( $P < 0.01$ ). The decrease of lactose content in mammary secretions progressively decreased during involution and was associated with paralleled change in GLUT-1 mRNA level coding the main glucose transporter in the udder. These decreases were faster in cabergoline treated cows compared to controls with lower lactose content in cabergoline treated cows already by D1 than in controls ( $P < 0.05$ ) and significant decrease in GLUT-1 mRNA levels at D1 and D8 respectively for cabergoline and control treatments compared to D-6 ( $P \leq 0.05$ ). Cabergoline treatment tended to increase fat content at D3 after drying-off ( $P < 0.10$ ). No significant effects of cabergoline treatment were observed both in true protein and in alpha-lactalbumin contents in mam-

mary secretions or in alpha-lactalbumin and kappa-casein mRNA levels in mammary tissues.

**Conclusions:** The changes in lactose, SCC and fat in mammary secretion and GLUT-1 mRNA level in the udder, indicate that cabergoline treated group accelerated mammary involution without affecting milk protein synthesis in the mammary tissue compared to placebo group. Cabergoline treatment could be used as a tool to facilitate the dry-off in dairy cows

UH-P15

### Presence of milk leakage on days following dry-off increased the risk of clinical mastitis and new intramammary infections at calving in commercial dairy herds around Europe

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Between lactations, a non-lactating period, the dry period (DP), is necessary for optimal milk production in the subsequent lactation. The DP starts with an abrupt cessation of milking which involves an udder engorgement due to milk accumulation, leading to possible milk leakage (ML). Presence of ML in the week after the last milking is known to be a risk factor for new intramammary infections (IMIs) during the early DP which may impact udder health in the following lactation. Schukken et al (1993) demonstrated that cows leaking milk after dry-off (DO) were 4 times more likely to develop clinical mastitis (CM) and had 6.1 times more risk of developing an IMI with a major pathogen during the dry period than cows that did not leak.

**Objective:** The objective of this study was to investigate the relationship between the presence of ML after DO and new IMI during the DP and at calving based on Somatic Cells Count (SCC) and CM from dry-off up to 30 days after calving.

**Materials and methods:** The presence of ML was assessed at quarter level in 1175 dairy cows at different points after DO (i.e. 20-24, 30-34, and 48-52 hours). The cows belonged to 41 herds from 8 European countries. For all the quarters included in the study the teat end score was evaluated at the moment of dry-off. An IMI at cow level was defined as either showing CM from DO up to 30 days after calving or a rise in individual somatic cell count (ISCC) (e.g. from below 200,000 cells/ml at the last test day before dry-off, to above 200,000 cells/ml at the first test day after calving). Statistical analysis was performed, using STATA® (version 13.0) software

**Results:** A total of 24.5% (95% CI: 22.0-27.0) of the cows were diagnosed with ML between 20-52 hours after DO. Quarters that experienced ML had 2.0 times as many odds ( $P=0.08$ ) on developing CM than quarters without ML. Cows that experienced ML after dry-off had 1.5 times as many odds ( $P=0.08$ ) on new IMI in the first 30 days after calving than cows that did not experienced ML.

**Conclusion:** Our results showed that the presence of ML im-



plies a risk for developing CM and acquiring a new IMI during the dry period and in the first 30 days after calving. Farmers and veterinarians need to address this problem through specific strategies in order to reduce the number of cows leaking milk after DO.

UH-P16

### The efficacy of Tylosin injection for treating subclinical SA mastitis in dry cow therapy

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**Objectives:** *Staphylococcus aureus* (SA) becomes a problem as the pathogen of gram-positive refractory mastitis. SA shows poor response to treatment and also extensively exists as subclinical mastitis causing increased somatic cell counts without manifesting any definitive sign. Dry period is recognized important for treatment of chronic mastitis, and currently employed treatment is systemic administration of tylosin formulation during the dry period especially targeting SA-infected udders. The results of the investigation on treatment efficacy of 5- and 3-day consecutive administration of tylosin formulation during the dry period for subclinical mastitis are summarized below.

**Materials and Methods:** Starting from March 2014 to September 2017, composite milk was sampled from 293 cows in 11 farms (Tahara-shi, Aichi, Japan) for bacterial culture. Cows with SA detected as the pathogen while not showing any clinical sign were included in the study. Test cows received intramuscular injection of 30 mL tylosin formulation (Tylan200 Injection for Animals) from the first day of the dry period as dry cow therapy, in addition to intramammary injection of ointment for dry cow (KP Dry or Cepravin). Cows were assigned to 3 groups and received Tylan200 for 5, 3, 0 (treated with ointment for dry cow only) days (T5, T3 and T0 groups, respectively). Teat sealant was not used. Milk was sampled on Day 7, 14 and 28 post calving for bacteria test. Occurrence of clinical mastitis was monitored until Day 30 post calving, and bacteria test was performed for affected udders in the same manner.

**Results:** SA detection rate in test cows before the dry period was 10.2% (30/293 cows). An udder in which SA having detected before the dry period was not detected in bacteria tests of milk performed 3 times post calving and no clinical mastitis occurred until Day 30 post calving was determined to be cured. Udders from which scheduled milk sampling could not be performed were removed from the study. Cure rates were 50.0% (4/8), 42.9% (3/7), and 16.7% (1/6) in T5, T3 and T0 groups, respectively.

**Conclusions:** Previously reported cure rates with Tylan200 treated during the dry period were 80% and 87%. These reports have not identified bacterial species and revealed the combined cure rates of subclinical mastitis caused by Gram-positive bacteria. The cure rate in the present investigation, limited to subclinical mastitis by caused SA was 50.0% in T5 and 42.9% in T3 group, which was higher than that in the T0 group. These results suggest that systemic administration of Tylan200 would have high significance for treatment of refractory subclinical mastitis caused by SA during the dry period.

Due to small sample size of this investigation, further investigation will be required.

UH-P17

### Determination of the presence of *Salmonella* spp. in milk samples from two dairy herds located in the Sabana de Bogota-Colombia

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**Objectives:** The objective of this study was to determine the presence and prevalence of *Salmonella* spp. shedding in bovine milk samples in two dairy herds in the Sabana de Bogotá – Colombia.

**Materials and methods:** Milk samples were collected from 80 Normand and Holstein milking cows that comprised the whole milking herds. The milk samples were obtained from the 4 quarters and pooled by each animal. Each quarter was disinfected and the first milk streams were discarded before sample collection. Each sample was collected in 50-ml falcon tubes, which were identified with all the precise information pertaining to the animal.

Samples were processed according to the Colombian technical norm (NTC) 4574: food microbiology and animal food microbiology, and the *Salmonella* Oxoid PreciSTM methodology, which has been validated and approved by ANFOR according to the ISO regulation 16140. All the milk samples were pre-enriched in non-selective broth, enriched in selective broth and afterward they were cultured in selective and differentiating agars; for the Oxoid PreciSTM methodology, the protocol provided by the manufacturing company was followed and the *Salmonella* spp. compatible colonies were isolated in a chromogenic agar (Agar Brilliance) using the presence of the caprylate esterase enzyme for confirmation. The biochemical identification of the isolates was done using the identification system RapidOne® for enterobacteria. The confirmation of the suspected *Salmonella* spp. colonies was done using the commercial kit PCR-Salmonella (CorpoGen® BM-00007) to detect the 284bp genic fragment that corresponds to gen *invA*, which is detected in agarose gel. The extracted DNA samples were sent to MacroGen for sequencing and identification of the serovars present in the samples.

**Results:** From all the cultured milk samples, 21.25% (n=17) were *Salmonella* spp. positive. The results in each herd showed that only 1.25% (n=1) of the samples were positive in herd 1 and 20% (n=16) of the samples in herd 2. All the isolates were confirmed as *Salmonella* spp. by using the visualization of the 284bp fragment specific for the *invA* gen of this microorganism.

The sequencing results indicated that 58.8% (n=10) of the isolates were *S. Newport*, 11.76% (n= 2) of the isolates were *S. Typhimurium* and *S. Bredeney*, *S. Anatum* and *S. Virchow* were determined in 5.88% (n=1) of the isolates each. However, in



the 11.76% (n=2) of the isolates, species identification could not be achieved.

**Conclusions:** *Salmonella* spp. was isolated in 21.25% of the milk samples in these two herds and *S. Newport* was the most prevalent isolated serovar. There were significant differences in the prevalence between the two herds.

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UH-P18

### Evaluation of the distribution of drug residues in different dairy products during the cheese production process

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**OBJECTIVES:** Antibiotics are widely used to treat bacterial infections in lactating cows. In case of insufficient withdrawal periods or increased or incorrect dosage, antibiotic residues will occur in milk. The European Union has regulated the maximum residue limits (MRLs) allowed in milk (Council Regulation 37/2010/EC) and regulatory authorities have established withdrawal periods that producers have to observe before sending milk from medicated cows to the market. However, this does not guarantee the absence of antibiotic residues in milk, but only that their concentration remains below the MRLs. Antibiotic residues in raw cheese milk are a public health concern. Moreover, even if below the MRLs, they may potentially interfere with the cheese making process, i.e. by inhibiting starter cultures' growth or acidic production with costly economic losses to the dairy industry. To date, very little is known about antibiotic persistence during cheese production and its potential effects on the cheese making process.

This study had the following objectives:

to evaluate the persistence of veterinary drug residues in pasteurized milk;

to determine the distribution of drug residues between polar and non-polar constituents of milk resulting from skimming;

to evaluate the distribution of drug residues in milk products (whey cheese, curd, cream) in a dairy product (hard cheese made from raw milk and soft cheese made from pasteurized milk);

to define a proper scientific evaluation regarding the role of MRLs of veterinary drugs in dairy products as safety factor and consequently to give indications on the relationship between MRLs in milk according to the type of cheese-making process.

**MATERIALS AND METHODS:** Analyses were performed with the LC-MS/MS equipment (6470 Triple quad LC/MS equipped with a 1290 Infinity UHPLC Agilent Technologies and TSQ Quantum triple quadrupole equipped with a Finnigan SurveyorTM HPLC system Thermo Scientific).

**RESULTS:** The pasteurization step did not cause a significant decrease in the concentration of antibiotic residues in milk, with degradation percentages <20% for most of the analysed antibiotics at all concentrations tested. Cephalosporins ( $\beta$ -lactam family) presented a greater instability to heating, probably due

to the nature of substituents at C-3 and C-7 that play an important role in determining the facility with which the  $\beta$ -lactam bond is hydrolysed or broken.

The skimming process did not seem to cause a substantial decrease of antibiotic residues in milk.  $\beta$ -lactams and sulphonamides were mainly retained in the aqueous phase of milk while quinolones and tetracyclines showed a higher affinity for the cream layer.

Antibiotic recovery in skimmed milk was higher than 85% for most  $\beta$ -lactams, macrolides, and sulphonamides (with the exception of spiramycin-I, tylosin A and sulfaquinoxaline).

Tetracyclines and quinolones (especially flumequine) were more significantly lost by skimming, but were still recovered with efficiencies ranging from 55% to 80%.

About antibiotic effect on new whey starter, evaluating three parameters (pH, SH e logarithmic survival of lactic bacteria), we can conclude that Enrofloxacin, Sulfadimetoxin and Monensin don't have any effects on lactic bacteria at maximum residue limit concentration. Amoxicillin and Oxytetracycline don't have a significant effect. Penicillin G, Cefquinome, Ceftiofur and his metabolite DesfuroylCeftiofur, have an important effect on lactic bacteria grow and on curves of pH e SH.

We have shown that Desfuroylceftiofur residues clearly persist in new whey starter and curd after the cheese making process. Approximately half of the original concentration was detected in the aqueous new whey starter after overnight incubation. Remarkably, the antibiotic was found in the proteic curd at concentrations exceeding the initial amount added to milk. It will be interesting to evaluate the analyte concentration also in whey obtained at the end of the cheese production process, before overnight incubation.

Persistence of antibiotic residues in raw cheese milk, and especially their potential accumulation in curd, may have important implications for the establishment of MRLs, and therefore need to be addressed in further studies. Antibiotic residues in milk are a well-known public health concern, but even concentrations at or below the MRLs might interfere with the cheese making process, with potentially significant implications for the dairy industry. We have tested that the presence of Desfuroylceftiofur in n.w.starter and in the curd inhibits its microbial charge or alter its related chemical-physical properties

**CONCLUSION:** Further work is required to describe this issue for different antibiotics, to compare different protocols for cheese production, and to evaluate the persistency in cheese over time.

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UH-P19

### An Observational Cohort Study on Persistency of an Internal Teat Sealant Residue in Milk after Calving in Dairy Cows

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**Introduction:** Internal teat sealants (ITS) can be used alone, or in combination with antimicrobials, to prevent occurrence of new intramammary infections (NIMI) during the dry period of dairy cows. An ITS forms a physical barrier to the entry of bacteria responsible for mastitis and remains in the teat cistern over the dry period until it is physically removed manually at first milking, or by suckling by the calf. Study objectives were to evaluate prevalence of quarters with an observable ITS plug at first milking following calving, investigate persistency of ITS residue in milk after calving, and investigate whether quarters without a sealant plug at the first milking after calving were equally protected from NIMI acquisition during the dry period compared to those with a sealant plug.

**Materials and Methods:** An observational cohort study was carried out on 557 quarters of 156 cows treated with ITS in 6 farms in Quebec, Canada. The presence of an ITS plug at first milking and ITS residue in milk at each milking were observed by producers. Single quarter milk samples of all enrolled cows were collected on the day prior to dry off (S1), and after calving at 3 to 4 days in milk (S2) and 5 to 18 days in milk (S3) for bacterial identification to detect intramammary infections (IMI) using bacteriological culture followed by MALDI-ToF mass spectrometry identification. The effects of various factors on persistency of ITS residue in milk were studied using a mixed negative binomial regression model. A generalized mixed model with a logit link was used for modelling the effect of the presence or not of an observable ITS plug at the first milking on odds of NIMI.

**Results and Discussion:** We observed an average persistency of residue of 4.0 (SD: 2.3 days). When an ITS plug was still present at first milking (83% of quarters), the elimination of ITS residue in milk after calving was significantly longer (4.5 days, on average, compared to 1.2 days when ITS plug was absent). Among cows with an ITS plug at calving, we observed a higher number of days of residue excretion in older cows. Predicted number of days with residue were 3.9, 3.7, and 4.9 days, for 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> or higher parity cows, respectively. Quarter position did not influence number of days of excretion. When a plug was not observed; quarter position, cows' parity, and dry period duration were all significantly associated with number of days of ITS residue. Quarter position, however, had a relatively small impact on number of days of ITS residue excretion (0.5 vs. 0.7 days for front and rear quarters, respectively). Predicted number of days of residue excretion were 0.4, 0.3, and 2.6 days for 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> or higher parity cows, respectively. Dry period duration also had a relatively modest impact; number of days of residue were 0.1, 0.2, and 0.6 days, for quarters with dry period of 6, 8, and 10 weeks, respectively.

The absence of an association between presence or not of an observable ITS plug and odds of NIMI at calving, suggests that cows were possibly still protected against NIMI during most of the dry period. Therefore, we can hypothesize that loss of the plug occurred around calving, because of suckling by the calf, or for other reasons. In fact, our producers confirmed that suckling by the calf could occur on some occasions, especially for calving during night time. Although we were able to highlight some statistically significant risk factors explaining persistency of residue following calving, observed differences were often relatively small. Therefore, clinical relevance of these risk factors may be low.

**Conclusion:** Results from our study revealed that an ITS plug was present until first milking after calving for 83% quarters and ITS residue could be observed in milk up to 12 days in milk.

There was no evidence that quarters without ITS plug at first milking after calving were at higher risk of NIMI during the dry period.

UH-P20

### Effect of gamma irradiation treatment of freeze-drying colostrum on colostrum bacteriology and immunoglobulin G concentrations

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**Objectives:** Colostrum provides calves with immunoglobulins and nutrients, but may also expose calves to pathogens. Therefore, for the use as a colostrum replacer, colostrum should be pasteurized without losing IgG concentrations. Gamma-rays disinfection system is an established technology of well-documented safety and efficacy for inactivation of microorganisms and insects. Therefore, the aim of this study was to evaluate the effects of gamma irradiation treatments of bovine colostrum on colostrum bacteriology and immunoglobulin G concentrations.

**Materials and methods:** Colostrum was collected from Holstein cattle in 3 farms. Refrigerated colostrum was pooled and freeze-dried over a period of 41 hours. Freeze-drying colostrum was exposed to gamma-rays (5, 10, and 15 kGy). Pre- and post-irradiated colostrum samples were analyzed for bacterial contamination through standard plate count, and tested for immunoglobulin G concentrations. Statistical significance was defined as  $p < 0.05$ .

**Results:** Total bacterial count of pre-irradiated colostrum was  $3.1 \times 10^5$  colony forming units/ml. There were no bacteria in the post-irradiated colostrum samples regardless of irradiation dose. IgG measure in pre-irradiated colostrum was 46.5 mg/ml. After gamma irradiation, IgG was 43.7, 41.7, and 39.1 mg/ml at the dose of 5, 10, and 15 kGy, respectively. Though freeze-drying colostrum did experience loss of IgG as irradiation dose increased, the colostrum IgG concentrations remained high overall, and there were no significant differences in IgG concentrations.

**Conclusions:** This study demonstrated that gamma irradiation of colostrum could be successfully conducted to decrease colostrum microbial counts while maintaining colostrum IgG concentrations, and 5 kGy dose seemed to be the most suitable choice to irradiate freeze-drying colostrum.



UH-P21

## Effects of dietary clinoptilolite supplementation on udder health and chemical composition of milk in dairy cows over two consecutive years

### In-feed clinoptilolite supplementation and udder health in dairy cows

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**Objectives:** The aim of the present study was to investigate whether the supplementation of clinoptilolite (Vibrosorb®, Croatia) in the concentrate feed of dairy cows has an effect on udder health and chemical composition of milk in dairy cows over two consecutive years.

**Materials and methods:** Forty-six clinically healthy Holstein-Frisian cows, aged between 3-5 years, were randomly assigned to one of two groups: control (n=23) and CPL-fed group (n=23). The CPL group received 50 g zeolite in the ratio for dairy cows, twice a day. Initial milk sampling was performed prior to adding CPL to feed. Additional sampling sessions were carried out on a monthly basis up to the 7<sup>th</sup> month of pregnancy, and next lactation starting from the first month of lactation and over the next 6 months. Milk samples were analysed for chemical composition (milk fat, proteins, lactose, solids non-fat dry and urea), somatic cell counts (SCC) and by bacteriological examination. The data were analysed using the program Stata 13.1 (Stata Corp., USA). The average values of milk components were compared by the Student t-test between two groups for each sampling point. In repeated samplings within the same group of cows, the values for a milk component were compared using the repeated ANOVA. Post hoc analysis of values was performed by the paired t-test.

**Results:** The results showed that clinoptilolite supplementation had no adverse effects on the tested chemical composition of milk at any of sampling points. However, the chemical composition of milk was found to be more stable in CPL-fed cows, especially at next lactation. A statistically significant difference between single samplings in CPL-fed cows was found for milk fat and urea. The SCC in milk did not differ significantly between groups but was higher in the control group (412,260 vs. 339,980, respectively). Average milk fat, proteins, lactose, solids non-fat dry and urea were slightly higher in the control than in CLP-fed cows (4.38±0.77 vs. 4.02±0.56, 3.85±0.37 vs. 3.64±0.32, 4.38±0.16 vs. 4.37±0.14, 9.21±0.36 vs. 8.94±0.29, 21.66±7.00 vs. 20.90±5.11, respectively). The values recorded in the frequency of isolation of the mastitis causative agent differed significantly between groups (P=0.01). Cows from the control group had a four-fold higher and at next lactation had a seven-fold higher incidence of intramammary infections than CPL-fed cows.

**Conclusions:** The observed differences in the content of a particular milk component and SCC between the groups of cows did not differ significantly at any of sampling points. The beneficial outcome of the study may be attributed to the antibacterial, immunostimulating, detoxifying and antioxidative effects of CPL on udder health.

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UH-P22

## Regional Attempts to Enhance Milk Quality Through Introducing Management of HACCP in Milk Production in Shibeche Town, Hokkaido

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Accidents related to food safety such as antibiotic contamination in milk tanks results in huge losses not only for the milk plants but it also degrades the costumers' in foods. Shibeche town consists of 250 dairy farms with 160,000 tons of milk produced annually, there were 30 accidents from 2009 to 2013, with 342 tons of milk wasted in which 87 percent of the cases (5.2 cases per year) were caused by contamination by antibiotics. To minimize human error, which may cause accidents related to antibiotic contamination, we have introduced and constructed a regional HACCP management system for milk production. In 2014 to 2016, during the trial to diffuse the HACCP management system, accidents decreased to 3.6 cases per year. Furthermore, after starting the normal operation of the HACCP management system from April 2017, there were no noteworthy accidents as of November 2017.

To make these attempts and movements possible, the associated organizations, which were organized by the town office, farmers' cooperatives, the mutual aid associations, the extension centres, the milk record associations, the milk equipment companys, and the milk processors, learned of and obtained licenses associated with HACCP and then worked to share the meanings and importance of the HACCP management system with farmers. During this trial, associated organizations and farmers worked to diffuse the new management system and were able to construct "P-D-C-A" cycle together under the process and accumulation of "work together and learn together" which eventually minimized food accidents.

Milk which produced in Shibeche town in supplied to milk processors and much of it is processed into premium butter. Our products, in any shape, whether fluid milk or premium butter, are consumed with confidence in terms of both safety and reliance by consumers.

UH-P23

## Case Report: Field experience with a polyvalent mastitis vaccine in a commercial dairy farm infected with *Klebsiella*

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**Objective:** Even though mastitis control programs are frequently used in dairy production, mastitis is still one of the most frequently occurring and costly disease in modern dairy farms (Halasa et al., 2007) The goal of this study was to evaluate the efficacy of a polyvalent vaccine on a farm with *Klebsiella* mastitis problems.

**Materials and Methods:** A farm with 40 cows in free-stall, located in Hayato-cho, Kirisima-shi, Japan, with river sand bedding. *Klebsiella* has been isolated from rice husks that this farm has been using. Even though the farm has been using calcium hydroxide, *Klebsiella* mastitis has been frequently seen since 2013. For this reason, a polyvalent mastitis vaccine based on *E. coli* J5 and *S. aureus* (STARTVAC<sup>®</sup>) has been used for the first time in October 2016 with the blanket/rolling protocol i.e. all animals received two shots of the vaccine with 4 weeks interval followed by vaccinations every 3 months. The heifers were vaccinated 1st dose with 45 days before calving, 2<sup>nd</sup> dose with 10 days before expected parturition data (EPD), and then every three months (rolling protocol) after 2<sup>nd</sup> dose. The observation parameters were quarterly investigation of the number of mastitis cases, deaths and culls. As a result of efficacy of vaccination milk production data was measured (Bradley et al., 2015). To prove the cost benefit of the vaccine in the farm, all costs related to treatments/vaccination have been also calculated. The results were statistically analyzed by Dr. SPSSII X<sup>2</sup> test.

**Results:** Mastitis incidence rates were 85.7% in 2014, 86.3% in 2015, 59.1% in 2016, and for 2017, 27.7%. There were 5 death/culls from October 2015 to September 2016 and 1 death/culls after vaccination (from October 2016 to September 2017). As a result of decrease in total mastitis cases and lower severity grade of mastitis cases daily milk production has increased from the point of vaccination. From October 2015 to September 2016, the average milk production per cow per day was 36.5 liters whereas from October 2016 to September 2017 the average milk production per cow per day was 37.3 liters. Therefore, 0.8 liters were produced more each day per cow after implementing vaccination. Similar results were observed in study made by Bradley et al., 2015. Regarding cost benefit of vaccination, cost of mastitis treatment was 416,280 yen in 2013, 837,700 yen in 2014, 846,020 yen in 2015, 551,820 yen in 2016 and 215,040 (9 months total) in 2017. The vaccination cost was 120,000 yen in 2016 and estimated 301,500 yen in 2017. Therefore, total cost of treatments and vaccination is lower than treatments alone.

**Conclusion:** Along with other management practices to control *Klebsiella* mastitis, vaccination with a polyvalent mastitis vaccine based on *E. coli* J5 and *S. aureus* (STARTVAC<sup>®</sup>) resulted in less culls/ deaths, less severe mastitis cases and increased daily milk production. Thus, vaccinating with rolling protocol (vaccinating the whole herd every 3 months) on farm suffering problems due to environmental mastitis has been proved to be efficacious and profitable.

UH-P24

### The effect of vaccination on bacterial count, somatic cell score, milk yield and vaginal temperature following experimental *Escherichia coli* mastitis

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**Objectives:** Vaccination against coliform mastitis has become part of mastitis control programs in the past three decades as a means of reducing severity of clinical mastitis. The study objective was to evaluate the effect of vaccination on clinical response following *Escherichia coli* intramammary challenge.

**Materials and Methods:** Cows (n = 36) were randomly assigned in late lactation to one of three treatment groups (n = 12 per group); ENV (Enviracor J-5, Zoetis Inc., Kalamazoo, MI), BOV (J-5 Bovilis, Intervet Inc., MSD Animal Health, Madison, NJ) and unvaccinated controls (CTL). Vaccines were administered subcutaneously in three doses; approximately 60 and 21 d prior to expected calving and 14 d postpartum. At a mean of 102 days in milk (range 72 to 137 d), all cows were challenged in one rear quarter with approximately 100 CFU/mL of *E. coli* 727. Requirements for the challenge quarter were a SCC of <100,000 cells/mL and clear of intramammary infection in three consecutive samples collected in the week preceding challenge. Milk was aseptically collected from challenged quarters at 0, 6, 12, 15, 18, 21 and 24 h, and at 2, 3, 4, 5, 6, 7, 30 and 60 d post-challenge for bacteriology and SCC. Milk yield was measured by an inline milk meter (Afimilk Ltd., Kibbutz Afikim, Israel). Vaginal temperature was recorded at 15 min intervals using a temperature data logger (Star ODDI, Iceland). Temperatures were averaged every 3 h in the first 24 h post-challenge, and daily for 7 d post-challenge. For analyses, bacterial counts and quarter SCC were transformed into log<sub>10</sub> CFU/mL and somatic cell score (SCS), respectively. Post-challenge milk yield was expressed as a percent of the 7 d mean milk yield from the week pre-challenge. The outcomes of interest included bacterial count, SCS, vaginal temperature and milk yield % and were analyzed using the GLIMMIX procedure in SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA). Variables offered into the models included the main effects of treatment, parity, breed and time, and associated two-way interactions. Significance was declared at  $P < 0.05$ .

**Results:** Seven cows were removed from the study during the 7 d post-challenge for antibiotic treatment; three each from ENV and CTL groups, and one BOV. Of these, two CTL cows were euthanized. The need for veterinary attention was determined without regard to vaccination status. Mean vaginal temperatures were elevated (>39.4°C) at 12 h post-challenge for both ENV (39.4 °C ± 0.1) and BOV (39.7 °C ± 0.1) cows, whereas CTL cows had lower temperatures at this time (38.9 °C ± 0.1;  $P < 0.01$ ). No differences in body temperature were observed between groups from 15 h post-challenge, and for the remainder of the 7 d post-challenge period. Bacterial counts and SCS did not differ between treatment groups. On d 2 post-challenge, vaccinated cows produced less milk, expressed as a percentage of average pre-challenge milk yield (BOV 44.0% ± 4.5 and ENV 44.4% ± 4.8) than CTL cows (66.9% ± 5.2;  $P < 0.01$ ), and BOV cows milk yield remained lower than the other groups on d 3 ( $P < 0.05$ ), but there were no treatment differences from d 4 to d 60. All groups returning to 85% of





pre-challenge milk yield by 7 d post-challenge.

**Conclusions:** Overall, the clinical responses to *E. coli* intramammary challenge were not different between BOV and ENV treated cows. Fever was observed earlier for vaccinated cows, as indicated by vaginal temperature  $>39.4^{\circ}\text{C}$  at 12 h post-challenge. The larger decline in milk yield observed for vaccinated animals was short-lived, with no differences between treatments from 4 d post-challenge. While not statistically significant, the mortality rate was higher for unvaccinated controls, and the number of cows requiring treatment during the 7 d post-challenge was higher for CTL and ENV groups, compared with BOV. Further analyses of antibody concentration in blood and milk are underway and may shed light on the clinical responses observed.

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UH-P25

### Immune response during onset of coliform mastitis in vaccinated dairy cows

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**Objectives:** Coliform mastitis in dairy cows can cause serious problems in dairy farm management, including milk yield reduction and death, sometime necessitating culling. Bacterial lipopolysaccharide (LPS) is reported to delay the migration of granulocytes (e.g., neutrophils) into the udder, resulting in an enhanced mastitis disease state. There are many reports on LPS and the disease state in coliform mastitis, but limited information is available regarding the host immune response at the onset of coliform mastitis. The purpose of the present study was to investigate the host immune response, including the dynamics of lactoferrin (LF), interleukin 8 (IL-8), and IL-1 $\beta$  concentrations, as well as somatic cell counts (SCC) in milk, at the onset of coliform mastitis.

**Materials and methods:** The relationship between the presence or absence of mastitis vaccination (STARTVAC<sup>®</sup>, HIPRA) inoculation, and the disease state, the bacterial species, LF concentration, IL-8 concentration, IL-1 $\beta$  concentration, and SCC in milk was investigated in 101 cows with coliform mastitis. Animals exhibiting only local symptoms were assigned to an acute coliform mastitis (ACM) group; those showing general symptoms such as anorexia or decreased cutaneous temperature were assigned to a peracute coliform mastitis (PCM) group. Statistical analysis used the Mann-Whitney U test and chi-square test;  $P < 0.05$  was considered significant.

**Results:** Among unvaccinated animals, LF, IL-1 $\beta$ , and SCC were significantly ( $P < 0.01$ ) lower, and IL-8 was significantly ( $P < 0.05$ ) higher, in the PCM group ( $n=49$ ) compared to the ACM group ( $n=13$ ). In the PCM group itself, LF, IL-1 $\beta$ , and SCC were significantly ( $P < 0.01$ ) higher, and IL-8 was significantly ( $P < 0.05$ ) lower, in vaccinated animals ( $n=24$ ) than in unvaccinated animals ( $n=49$ ). There were no significant differences in the distribution of bacterial species, and among bacterial species in terms of LF, IL-1 $\beta$ , IL-8 and SCC when comparing between the vaccinated and unvaccinated animals.

**Conclusion:** Unvaccinated PCM animals exhibited lower val-

ues for LF, IL-1 $\beta$ , and SCC, and an elevated value for IL-8, compared to unvaccinated ACM animals; this difference presumably reflected the fact that the coliform growth rate exceeded the immune response in the unvaccinated PCM group. In addition, the lower IL-1 $\beta$  concentration presumably results from delayed expression and secretion of this cytokine by the migrating neutrophils. On the other hand, the elevated IL-8 concentration in the unvaccinated PCM group presumably reflected release of this factor by monocytes and macrophages present in the udder as part of the initial immune response. Vaccinated PCM animals exhibited elevated values for LF, IL-1 $\beta$ , and SCC, and a lower value for IL-8, compared to unvaccinated PCM animals. We hypothesized that this pattern reflected enhanced activation of the anti-coliform immune response at the time of infection. Considered together, these data indicate that the vaccinated cows (compared to the unvaccinated animals) possessed improved initial immune responses at the onset of coliform mastitis, providing enhanced defense at the early stage of infection.

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UH-P26

### Influence of Bovine Leukemia Virus infection on clinical mastitis

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**Objectives:** Bovine mastitis, a common infectious disease in the mammary glands of dairy cows, causes huge economic losses in the dairy industry. The causes of mastitis vary widely, and one of the host factors involved in mastitis causation is suggested to be in the innate immunity system of the udder. Bovine Leukemia virus (BLV) is the causative agent of Enzootic Bovine Leukosis (EBL), a disease that leads to death. Its occurrence report in Japan has been increasing steadily, and economic loss due to BLV infection is also enormous. Although there have been several reports on the relationship between BLV infection and clinical mastitis, the clinical effect of BLV infection on severity of clinical mastitis during lactation, including the relationship to provirus load (PVL) and severity of clinical mastitis has not been reported. The purpose of this study was to clarify the effect of BLV infection on innate immunity and the resulting relationship to clinical severity of mastitis.

**Materials and Methods:** A total of 125 quarters milk sample were collected from 106 lactating Holstein-Friesian dairy cows with clinical mastitis from 14 dairy farms. Cows were classified into BLV positive cows ( $n=106$ ) and BLV negative cows ( $n=19$ ). Using the polymerase chain reaction method, BLV positive cows were further divided into 2 groups; a group of cows with number of blood BLV provirus copies higher than or equal to 1000 copies / 10 ng DNA (H-PVL group), and a group of cows with less than 1000 copies / 10 ng DNA (L-PVL group). The severity of clinical mastitis was classified as mild, moderate and severe. Somatic Cell Count (SCC), Lingual antimicrobial peptide (LAP) concentration and Lactoferrin (LF) concentration in milk were measured.



**Results:** The clinical severity of mastitis in BLV positive cows was greater compared to BLV negative cows, and BLV positive cows had significantly higher LAP compared to BLV negative cows. SCC tended to increase in cows with both H-PVL and L-PVL as the severity of mastitis worsened. LAP tended to increase with increasing severity in cows with L-PVL, but this tendency was not observed in cows with H-PVL. In mild and severe mastitis, LAP was significantly lower in cows with H-PVL than in the cows with L-PVL. In cows with H-PVL, LF decreased with increasing severity and was significantly lower compared to cows with L-PVL in severe mastitis.

**Conclusions:** These results suggest that neutrophil function and the immune function of epithelial cells in the mammary gland was reduced in cows with H-PVL compared to BLV negative cows. Therefore, the severity of clinical mastitis increased in cows with H-PVL compared to BLV negative cows.

UH-P27

### Improving mastitis diagnosis of presumptive *Streptococcus uberis* isolates applying 16S rRNA gene sequencing

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**Objectives:** As part of antimicrobial resistance avoidance strategies and to ensure a prudent use of antibiotics, bacteriological examination of milk samples is a prerequisite to enable pathogen-specific therapy. *Streptococcus (Sc.) uberis* is a common mastitis pathogen for which an extended duration of adequate intramammary treatment is associated with higher bacteriological cure rates. Decisions on an extended use of antibiotics require accurate identification of the pathogen but not all *Sc. uberis* isolates follow the normal pattern of phenotypic and biochemical criteria used in routine diagnostic procedures. Additionally, *Enterococcus* spp. and some species of the genus *Lactococcus* and *Aerococcus* may show similar biochemical profiles (Wald et al., 2017). The aims of this study were to establish a standardized scheme for reliable *Sc. uberis* identification in routine diagnosis and to evaluate the accuracy of conventional tests and growing patterns of *Sc. uberis* on a selective medium (indicating  $\beta$ -d-galactosidase production) using 16S rRNA gene sequence analysis as a reference method.

**Materials and methods:** Isolates of presumptive *Sc. uberis* (n=336) were obtained from quarter milk samples of Austrian dairy cows with intramammary infections. Isolates were identified biochemically as presumptive *Sc. uberis* if they were growing in catalase-negative, whitish and non-haemolytic colonies, hydrolysing esculin, carrying no Lancefield antigen or Christie Atkins Munch-Petersen factor and were inhibited in growth on a selective differential medium for enterococci. For differentiation between *Sc. uberis* and other esculin-positive streptococci, growth and colony staining on modified Rambach agar medium (MRAM) were evaluated (Watts et al., 1993). Organisms showing blue colony growth and thus  $\beta$ -d-galactosidase production on MRAM were considered as *Sc. uberis*.

Sequencing of the 16S rRNA gene was defined as a reference method to confirm the identity of all bacterial isolates. The accordance of strain identification between 16S rRNA gene sequencing and bacteriological examination respectively MRAM was compared using McNemar's chi-square test.

**Results:** Biochemical characterization classified the isolates into two groups: Isolates (n = 280) clustered in the typical biochemical profile-group (cluster 1) exhibited the same characteristics as the reference quality control strain *Sc. uberis* ATCC 700407. Production of  $\beta$ -d-galactosidase on MRAM was shown by 257 of these isolates (91.8%). 16S rRNA gene sequencing verified 271 (96.8%) isolates as *Sc. uberis*. MRAM agreed with sequencing results in cluster 1 in 264 isolates (94.3%). In contrast, 56 isolates differed in at least one biochemical reaction from the reference strain and were assigned to cluster 2. Thirty-seven isolates (66.1%) were  $\beta$ -d-galactosidase-positive. Based on 16S sequencing results, 36 isolates (64.3%) were identified correctly as *Sc. uberis* with biochemical methods. MRAM agreed with sequencing results in cluster 2 in 47 isolates (83.9%). Identification success in this group differed significantly between routine diagnosis and MRAM application.

Cocci misidentified as *Sc. uberis* were mainly confirmed as *Lactococcus* spp., *Enterococcus* spp., *Sc. parauberis* or *Weissella* spp.

**Conclusions:** The majority of *Sc. uberis* isolates show a typical biochemical profile. Based on our results, the complete set of parameters (catalase reaction, haemolysis, esculin hydrolysis and growth on enterococci agar) are needed for identification of *Sc. uberis*. A modified Rambach agar medium as subsequent secondary test helps to achieve a higher accuracy in isolates with diverging patterns.

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UH-P28

### A diagnostic tool for the detection and quantification of pathogenic bacteria in bovine milk samples from dairy farms in South Korea

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**Objectives:** The aim of this study was to check for prevalent pathogens all over South Korean dairy farms using PCR diagnostic tool (STARTCHECK<sup>®</sup>) which was provided by HIPRA and also to show that STARTCHECK<sup>®</sup> 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 132 Bulk Tank Milk (BTM) and Mastitis Pool samples were collected from different dairy farms in South Korea, between November 2015 to August 2017. The samples were taken 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 samples were then sent to DIAGNOS (HIPRA) in Amer, Girona, Spain. The samples were processed and tested using the Real-Time Multiplex PCR assay as previously described, 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 (100%) were positive for at least one bacteria. *Staphylococcus aureus*, *Escherichia coli*, *Coagulase Negative Staphylococci* (CNS) and coliforms were detected in 20%, 52%, 95% and 25% of samples respectively.

**Conclusion:** 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. It should be noted that the trend is shifting towards environmental pathogens in South Korea.

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UH-P29

### Usefulness of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of non-aureus staphylococci isolated from bovine intramammary gland infections in Poland

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**Objectives:** Non-aureus staphylococci (NAS) have been isolated more and more frequently as the causative agents of bovine intramammary infection (IMI). At present, at least 30 NAS species have been isolated from bovine milk. Although these bacteria are often considered as minor pathogens of the bovine udder, there is an increasing evidence that they may vary greatly in pathogenicity, host-pathogen relationships, epidemiologic significance, ability to produce enterotoxins and resistance to antimicrobials. For these reasons, the accurate species identification of the pathogen causing IMI is often necessary. The aim of the present study was to evaluate MALDI-TOF MS for the identification of NAS, including some rarely encountered species, isolated from IMI in cattle.

**Materials and methods:** The study was carried out on 136 NAS isolates recovered in years 2013-2017 from milk of cows

from three unrelated dairy farms in Lower Silesia, Poland. The bacteria were isolated on Tryptone Soya Agar (Oxoid, UK) supplemented with 5% defibrinated sheep blood. After preliminary examination (colony and cell morphology, Gram reaction, catalase activity) isolates were identified by means of sequence analysis of the 16S rRNA-, *dnaJ*- and *rpoB* genes (encoding the small subunit of the ribosomal RNA, heat-shock protein 40 and the beta subunit of RNA polymerase, respectively). MALDI-TOF MS analysis was performed using a Bruker Daltonics UltrafleXtreme spectrometer with the database version 3.1. According to the manufacturer, identification scores were interpreted as follows: less than 1.700 – no reliable identification, 1.700 – 1.999 – acceptable identification to the genus level and equal to or more than 2.000 – acceptable species-level identification. Each isolate was run at least twice and those isolates which failed to achieve a score equal to or more than 2.000 were retested with a maximum 4 repetitions.

**Results:** One hundred and thirty six NAS isolates were assigned to 18 species. The most frequently detected species were *S. haemolyticus* (n=33, 24%), *S. chromogenes* (n=28, 21%), *S. epidermidis* (n=23, 17%), *S. microti* (n=12, 9%) and *S. rostri* (n=9, 7%). In total, MALDI-TOF MS correctly identified 113 isolates (83%); however, the secure identification (scores equal to or more than 2.000) was obtained for 91 isolates (67%) only. For the three most frequently recovered species – *S. haemolyticus*, *S. chromogenes* and *S. epidermidis* – scores equal to or more than 2.000 were obtained in 79%, 75% and 87% of isolates, respectively. MALDI-TOF MS failed to identify 23 NAS isolates (12 isolates of *S. microti*, 9 of *S. rostri* and 2 of *S. agnetis*).

**Conclusions:** MALDI-TOF MS was shown to be a reliable tool for the identification of most NAS species isolated from IMI in cattle. However, the method requires an expanding of the commercial database, especially for unusual or rarely isolated species.

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UH-P30

### Validation of prediction algorithms for early detection of clinical mastitis caused by Gram-positive and Gram-negative pathogens

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**Objectives:** The use of precision technologies on dairy farms presents an opportunity for automated animal health monitoring. Changes in milk composition and cow activity occur prior to clinical mastitis (CM). This time-series data could be used to identify cows with mastitis and potentially indicate the pathogen type to assist in treatment decisions. The study objective was to validate prediction algorithms using daily milk component data or a combination of milk and activity measures, for their ability to identify CM caused by Gram-negative (GN) and Gram-positive (GP) pathogens.



**Materials and Methods:** Milk yield, conductivity, somatic cell count (SCC), lactose %, protein % and fat % were measured using an in-line milk meter (AfiMilk MPC, Afimilk Ltd., Kibbutz Afikim, Israel) or analyzer (AfiLab) at Virginia Tech (VT) and University of Florida (UF) research dairies between March 2011 and March 2012. Activity measures included daily steps on both farms (AfiPedometer) and total resting time, rest bouts and rest bout duration for VT cows (Afi PedometerPlus). Body weight was measured daily (AfiWeigh). A quarter milk sample was collected at CM detection for bacteriological analysis. Data were extracted from the AfiFarm system for the 14 d before and 14 d after CM detection (n = 166) and for control animals (n = 166) matched for breed, parity and days in milk (DIM).

Algorithms were derived using the change in parameters over a 7 d period (R v. 3.4). Slopes of the 10 explanatory variables of interest (milk yield, conductivity, SCC, lactose %, protein %, fat %, steps, rest time, rest duration, rest bouts) were estimated using linear regression and calculated between d 7 and 5, 4, 3, 2 or 1 before CM. All slope ranges were entered into the models for GN and GP infection, along with breed, parity, body weight and DIM. Backward stepwise elimination mixed effect regression was used to derive models. Infection was treated as a binomial response and farm was included as a random effect. Two models for each pathogen type were developed; the first was offered all milk parameters, and the second was offered both milk and activity measures. Final models included variables that remained significant ( $P < 0.05$ ) or tended to be significant ( $P < 0.1$ ).

An independent dataset was created for external model evaluation using data collected from VT dairy between August 2015 and April 2017. Milk and activity data were collected as described earlier, and were combined with cow data including parity, breed, DIM, body weight, and CM history (diagnosis date and bacteria isolated). Models were evaluated by comparing observed and predicted infections and calculating sensitivity (Se) and specificity (Sp) for the original dataset (internal evaluation) and the independent dataset (external evaluation).

**Results:** When only milk parameters were offered into the model, the significant indicators of GN CM included the change in fat % (d -7 to -2) and lactose % (d -7 to -3 and d -7 to -2). Estimated Se and Sp were 68.8% and 76.6%, respectively, when the model derivation dataset was used for evaluation. Both Se (90.9%) and Sp (85.1%) improved when milk and activity parameters were included in the model. External evaluation of the models yielded similar Se (62.5%) but lower Sp (36.9%) for the milk model, and lower Se (37.5%) and Sp (66.7%) for the combined milk and activity model.

For GP CM, the milk model included parity, breed, change in lactose % (d -7 to -1), milk yield (d -7 to -5 and d -7 to -3) and SCC (d -7 to -1). Internal evaluation yielded Se and Sp estimates of 75.0% and 72.5%, respectively. Combining milk and activity parameters increased Se to 90.9%, and Sp remained similar (70.7%). For both models, external evaluation reduced Se (milk model Se = 38.5% and combination model Se = 53.8%), but increased Sp (milk model Sp = 82.8% and combination model Sp = 83.3%).

**Conclusions:** Estimated Se and Sp improved when a combination of milk and activity measures were used to identify CM caused by GN and GP pathogens. External validation yielded a lower Se and Sp for both models, with the exception of Sp for detecting GP CM. The test dataset reflected a natural CM incidence, whereas the models were derived from a dataset con-

taining a 50:50 ratio of cases to controls. Data collected on farm provides a good resource for animal health monitoring; however, management tools constructed from this data need to be based on the distribution of infections seen in practice.

UH-P31

### Ability of milk electrical conductivity and the sodium, potassium, and calcium concentrations measured by hand-held instruments to diagnose subclinical mastitis at dry-off in dairy cattle

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**Objective:** To evaluate the ability of milk electrical conductivity (EC) and the concentrations of sodium ( $[Na^+]$ ), potassium ( $[K^+]$ ), and calcium ( $[Ca^{2+}]$ ) to diagnose subclinical mastitis (SCM) in quarter milk samples at dry-off.

**Materials and Methods:** Quarter foremilk samples were collected from 115 dairy cows at dry-off. Quarter somatic cell count (SCC) was measured using a Delaval<sup>®</sup> cell counter with  $SCC \geq 200,000$  cells/mL as the reference method for diagnosing SCM. Milk EC, and  $[Na^+]$ ,  $[K^+]$ , and  $[Ca^{2+}]$  were measured at 37°C using Horiba ion-selective hand-held meters (LAQUAtwin). The clinical performance of the tests for diagnosing SCM was evaluated using logistic regression to determine the area under the receiver operating curve (AUC) and the sensitivity (Se) and specificity (Sp) at the optimal cut point. The positive likelihood ratio (+LR) and kappa coefficient ( $\kappa$ ) were also calculated;  $P < 0.05$  was considered significant.

**Results:** Compared to the reference method, milk  $[Na^+]$  had the highest correlation ( $r_s = 0.72$ ), and at an optimal cut-point ( $>39.0$  mEq/L),  $AUC = 0.86$ ,  $Se = 0.77$ ,  $Sp = 0.84$ ,  $+LR = 4.8$ , and  $\kappa = 0.56$ . Milk EC was the second best performing test ( $r_s = 0.67$ ), and at the optimal cut-point ( $>5.2$  mS/cm),  $AUC = 0.85$ ,  $Se = 0.71$ ,  $Sp = 0.86$ ,  $+LR = 5.1$ , and  $\kappa = 0.47$ . Milk  $[K^+]$  was the third best performing test ( $r_s = -0.65$ ), and at the optimal cut-point ( $<28.0$  mEq/L),  $AUC = 0.84$ ,  $Se = 0.68$ ,  $Sp = 0.82$ ,  $+LR = 3.8$ , and  $\kappa = 0.40$ . Milk  $[Ca^{2+}]$  had the lowest correlation ( $r_s = -0.04$ ), and at the optimal cut-point ( $<7.8$  mEq/L),  $AUC = 0.52$ ,  $Se = 0.88$ ,  $Sp = 0.27$ ,  $+LR = 1.2$ , and  $\kappa = 0.18$ .

**Conclusions:** Milk  $[Na^+]$  and EC provide clinically useful cow-side tests for diagnosing SCM in lactating dairy cattle at dry-off.

UH-P32

### Predicting the infection status of the bovine mammary gland at the end of lactation using RMT and electrical conductivity

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Cognosco, Anexa FVC

**Objectives:** Not all dairy herds undertake DHIA testing. Hence for these herds, somatic cell count data are not available to classify cows as likely infected or not infected prior to assigning



cows to antibiotic dry cow therapy or internal teat sealant (ITS) infusion at the end of lactation. The objective of this study was to evaluate the Rapid (or California) Mastitis Test (RMT) and electrical conductivity (EC) at dry off when used alone, in combination together.

**Material and methods:** Quarter-level milk samples were collected at the time of drying off from 153 clinically healthy cows in 3 herds for RMT, EC, and microbiological culture. The sensitivity (Se), specificity (Sp), and positive and negative predictive values for each test were calculated, using the isolation of any bacteria as the gold standard for infection. The same diagnostic parameters were calculated following assessment of the RMT and EC used in parallel, or in series.

**Results:** The prevalence of any infection was 40% and 16% at the cow and gland levels, respectively, whereas the prevalence of major infections (i.e. *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas* pp, *Klebsiella* spp.) was 4.6% at cow level and 1.3% at gland level. At the cow level and when infection was defined a presence of any isolate, Se and Sp of an RMT score of greater than or equal to trace were 71% and 57% respectively, while at an inter-quarter ratio (IQR; i.e. highest EC/lowest EC within cow) greater than or equal to 1.37, the Se and Sp were 39% and 0.85%, respectively. At the quarter level, Se and Sp of an RMT score of greater than or equal to trace were 46% and 78% respectively, while at an IQR of greater than or equal to 1.14, the Se and Sp were 48% and 66%, respectively. When RMT greater than or equal to trace and IQR greater than or equal to 1.37 were interpreted in parallel, the Se and Sp were 76% and 51%, respectively. When both tests were interpreted in series the Se and Sp were 34% and 91%, respectively. When presence of a major infection was used as the gold standard, Se increased to 100% and Sp was 49%, for RMT greater than or equal to trace in any gland. Sensitivity and Sp were 71% and 86%, respectively, for an IQR greater than or equal to 1.47.

**Conclusions:** When evaluated singly, RMT and EC had only low to moderate diagnostic abilities. When 2 tests were combined and interpreted in parallel, Se increased but only moderately. For herds that do not herd test, RMT has greater utility than EC, and is recommended for making decisions at dry off about cows receiving ITS alone. The gains in sensitivity and specificity achieved by combining 2 diagnostic tests are small, and the additional costs of undertaking two tests do not appear to be justified.

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UH-P33

### Exfoliation rate of mammary epithelial cells into milk on bovine mastitis caused by *Staphylococcus aureus* is associated with bacterial load

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**Objectives:** The exfoliation rate of mammary epithelial cells (MECs) in milk is affected by physiological, breeding and environmental factors. Little is known about the relationship between the MEC exfoliation into milk and mammary-infected *Staphylococcus aureus* (*S. aureus*) load on the bovine mastitis caused by *S. aureus*. The aim of this study was to investigate

the relationship between the *S. aureus* load and the proportion of MEC exfoliation in milk using 5 substantial bovine mastitis models.

**Materials and Methods:** Two udders from each cow were randomly selected after the evening milking for infusion with *S. aureus*. The teats were allowed to air-dried and the *S. aureus* suspension was infused into the gland cistern. The remaining two udders were challenged with the same volume of PBS as a control. Clinical signs such as rectal temperature, udder uniformity, milk abnormalities, appetite, and milk yield on each cow were recorded at the morning and evening milking, and milk samples from individual udders were collected every morning. After infusing *S. aureus* into the udder, 64 randomly chosen milk samples were measured for *S. aureus* counts, SCC, and MEC exfoliation rate.

**Results:** In 64 randomly extracted milk samples from udders at 3 to 21 days after *S. aureus* infusion, it was included that various samples with different numbers of *S. aureus* counts and somatic cell counts. Milk yields from day 3 to 21 days were significantly decreased to less than the pre-infusion level. No significant correlations were found between the *S. aureus* counts and somatic cell count ( $r = 0.338$ ). In contrast, a significant correlation was noted between *S. aureus* counts and the proportion of cytokeratin-positive cells in the milk from the infused udders ( $r = 0.734$ ,  $P < 0.01$ ).

**Conclusions:** In conclusion, the increasing MEC exfoliation rate in milk from mastitis udders caused by *S. aureus* may contribute to reduced milk yield. Further development of this method may prevent dry off of individual udders and culling of cows that retain milk production capacity.

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UH-P34

### Risk factors associated to Capsular Type II *Streptococcus agalactiae* isolated from bulk tank milk in Western Colombia

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**Objectives:** To establish the risk factors associated to *Streptococcus agalactiae* (GBS) with capsular type (CT) II isolated from bulk tank milk in three provinces of western Colombia.

**Materials and methods:** A cross-sectional study was designed. A total of 136 randomly selected cryopreserved GBS isolates previously obtained in a former research were used in the present study. Milk samples were collected from 152 farms located in Caldas ( $n = 77$ ), Quindío ( $n = 29$ ) and Risaralda (46) in Western Colombia during 2013 and 2014.

First, the cryopreserved GBS was molecularly identified by PCR, then those confirmed as GBS underwent to a multiple PCR to identify those genes that codify for the capsular polysaccharide types Ia, Ib, or II to V (Poyart et al., 2007).

The CT II GBS and the information recorded about the farm (i.e. location, type and milking practices, biosecurity, antimicrobial use, and condition of the paddocks and roads towards the



parlor) was used to establish the potential risk factors for CT II GBS. An analysis of principal factors for quantitative variables was done, and then a multivariate logistic regression model with backward elimination was conducted. The quantitative variables that were not included in the principal factors analysis, were further included in the logistic model. Region, type of milking and altitude were also included in the initial logistic regression model and were kept only if they showed a P-value < 0.05.

**Results:** Out of the 136 samples, only 114 (84%) were molecularly confirmed as GBS. CT II prevalence was 58.8% (n = 67), the non-typeable GBS isolates were 9.7% (n = 11), and 31.5% (n = 36) were classified as other CT.

The risk factors associated with TC II GBS were the number of milkers in the parlor (P < 0.01), also, not keeping records of mastitis treatments, not having antimicrobial treatment protocols (i.e. when, how and in what cases to use them), doing treatments by unqualified personnel, and the poor storage of antimicrobials (e.g. exposed to sunlight and high environmental moisture). Each one of the last four risk factors showed an increase in the odds of being positive to CT II GBS about OR = 1.77. Related to the milkers, our findings showed that as the number of milkers increased, the probability of being positive to this CT of GBS increased; parlors having four or more milkers, had seven times more probability of being CT II GBS positive when compared to parlors with a single milker (OR = 7.03). No effects of the geographical region, altitude or type of milking (e.g. by hand or machine milking) were observed (P > 0.1).

**Conclusions:** The main CT GBS observed in dairy herds located in western Colombia was CT II, also the highest report to the date of this CT, this finding suggests that the distribution to the CTs could vary according to the geographical region. It is important to mention that the CT II, although is not one of the most prevalent CT, has also been isolated in humans.

The adequate use of antibiotics and having treatment protocols for mastitis is recommended. It is important to have people trained for treating cows with mastitis to reduce the risk of infections caused by CT II GBS.

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UH-P35

### Difference in transmission between *Escherichia coli* and *Klebsiella pneumoniae* causing bovine mastitis by molecular epidemiological analyses

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*Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) are categorized into environmental pathogens on bovine mastitis. The aim of this study is to consider the difference in transmission between these pathogens causing mastitis by molecular epidemiological analyses.

In June to November, 2014, we isolated and identified causative agent of coliform mastitis on 27 dairy farms in a certain town of Hokkaido. Isolated EC or KP band patterns by pulsed-field gel electrophoresis were compared each the farm. The

strains showing the similarity more than 80 % were classified as a same genotype.

Eighty nine strains of coliforms were isolated from 27 farms. The isolation frequency of EC is 55.1 % (49 strains) and KP is 23.6 % (21 strains). EC were isolated from 23 farms and showed a wide distribution. KP were differentially distributed in two farms (85.7%, 18 strain). The three of same genotype group of EC were found in three farms. Only one of them was different in cows. The other two were different in udders (same cow) or sampling date (same udder). In one of two farms KP mastitis was endemic, three strains from three udders in different cows were a same genotype and two strains were another same genotype by the same token. Additionally in another farm, six strains from five udders in three cows were a same genotype.

The genotype concordance of strains isolated from individual cows and udders indicate the strain have spread between individual cows and udders. In the past, EC and KP causing mastitis were infected from environment to udder. However, our findings suggest that EC as well as KP are not only infected from environment to udder but may also from udder to udder. And the frequency of transmission from udder to udder in KP causing mastitis is higher than EC.

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UH-P36

### Coagulase negative staphylococci may act as a protection factor against *Streptococcus agalactiae* udder infections

Funded by Sistema General de Regalías de Risaralda

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Coagulase-negative staphylococci (CNS) are the most commonly isolated bacteria from cow milk. Recent studies have suggested that CNS may act as a protective factor against udder infections caused by major mastitis pathogens.

**Objective:** The goal of this study was to establish the association between the presence of CNS and the occurrence of pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* in bovine udder.

**Methodology:** The study involved 275 milk composite samples collected in 11 Colombian dairy herds, which were analyzed for mastitis pathogens. Bacteriological status of milk samples was determined by diagnostic procedures recommended by the National Mastitis Council (NMC), further isolated pathogens were confirmed by PCR.

**Results:** Several samples (n=37) were positive to *Streptococcus agalactiae*, 109 to *Staphylococcus aureus*, and 155 to CNS. A logistic regression model was used to establish the effect of being CNS positive against infections caused by *Streptococcus agalactiae* (OR=0,21; P<0,05). The association between CNS and udder infections caused by *Staphylococcus aureus* was not statistically significant.



**Conclusions:** According to these results, it is suggested CNS may protect against udder infections caused by major pathogens, such as *Streptococcus agalactiae*. Therefore this group of bacteria had become an important target for looking for molecules that potentially inhibit the growth of this important pathogen.

UH-P37

### Genetic and phenotypic diversity of *Staphylococcus haemolyticus* isolated from intramammary gland infections in cows

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**Objectives:** In recent years, non-aureus staphylococci (NAS) have become important pathogens causing intramammary infections (IMI) in cattle. *Staphylococcus* (*S.*) *haemolyticus* belongs to the top five most frequently isolated species, accounting for 4.6 – 40.7% of udder infections caused by NAS. Owing to a variability of clinical presentations of IMI as well as for epidemiologic reasons, species-level identification of the pathogen is often necessary. The aim of the present study was to investigate the intra-species diversity of *S. haemolyticus* by various identification methods.

**Material and methods:** The study was carried out on 33 *S. haemolyticus* isolates, recovered from milk of cows with sub-clinical mastitis on three dairy farms in Poland. The bacteria were identified by means of sequence analysis of the 16S rRNA- and *dnaJ* genes, MALDI-TOF-MS analysis as well as the ID 32 STAPH system (BioMérieux, France).

**Results:** Based on phenotypic properties on ID 32 STAPH, 28 different biochemical patterns of *S. haemolyticus* were determined. However, only 10 (30.3%) of the isolates could be identified by this method. Sequence analysis of the 16S rRNA gene proved to have a very low discriminatory power. In contrast, *dnaJ* gene-based analysis revealed a very high level of diversity of bovine *S. haemolyticus* isolates, with two main subpopulations displaying also some phenotypic differences. Heterogeneity of *S. haemolyticus* was confirmed by MALDI-TOF-MS analysis. Only 20 of the bovine isolates could be identified unambiguously and the identification of the remaining isolates needed an extension of the existing database.

**Conclusions:** (1) *S. haemolyticus* is a highly heterogeneous species, thus requiring the improvement of databases for identification. (2) IMI in cattle may be caused by two distinct subpopulations of *S. haemolyticus*, differing clearly by genotypic and some phenotypic properties.

UH-P38

### Physiological and pathological alterations of the mammary gland of Holstein cows in the transition period

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The processes of colostrogenesis and lactogenesis during the transition period (TP) promote changes in the bovine mammary gland, influenced by hormonal action and increased blood flow, culminating in accumulation of lymphatic fluid in the interstitial space, generating physiological edema after parturition. Such physical changes allied to the low viability and functional activity of the neutrophils and low opsonizing activity of IgG increase the susceptibility of the mammary gland to the infections in this period. Therefore, the objective of this work was to characterize the physiological and pathological changes that occurred in mammary gland during the transitional period, using the physical examination and evaluation of the mammary secretion. Thirteen Holstein cows, from 2nd to 4th lactation, raised at a farm located in the city of Nova Odessa, Brazil, were monitored weekly during the TP (week -3 at week +3) and submitted to a specific examination of the mammary gland (n=356 mammary quarters), strip cup test (n=208), California Mastitis Test - CMT (n=152), microscopic somatic cell count - MSCC (n=204) and bacteriological examination of mammary secretion (n=355). The Statistical Social Package Science (SPSS) program was used with the Generalized Estimation Equations test, adopting groups and times as fixed factors, suitable for qualitative variables. On the other hand, the Odds Ratio allowed to evaluate the risk of the mammary infection on the parameters. MSCC was included in the model with the range distribution type for quantitative variables. The mammary quarters were divided into two groups, according to the bacteriological examination: BAC- (n=151) - did not present bacteriological growth throughout the TP; and BAC+ (n=151) - showed bacteriological growth at least two moments during TP. A general rate of mammary infection of 22% (78/355) was observed in the studied period, with 69.2% (54/78) of coagulase-negative *Staphylococcus* (CNS) infections; 10.3% (8/78) caused by *Corynebacterium* spp.; 6.4% (5/78) by *Streptococcus* spp; 3.8% (3/78) by *Serratia marcescens*, and 10.3% (8/78) were associations between different microorganisms. The peak of infection in this study occurred at parturition (33.3%, 16/48) and may be related to the current cortisol peak, leading to a decrease in the functional activity of the immune cells and a greater susceptibility to the mammary infections, besides the physiological alterations occurring in the mammary gland itself such as increased volume, increased skin tension and pendulous udder. In relation to the physical parameters, the BAC+ group presented a lower risk of increased volume (Odds Ratio = 0.245, 95% Confidence Interval - CI = 0.134-0.447), decreased skin elasticity of mammary gland (Odds Ratio = 0.474, 95% CI = 0.259-0.868) and increase of temperature (Odds Ratio = 0.349, 95% CI = 0.173-0.703). These changes may be associated with functional loss of the mammary parenchyma and persistent infectious process. In relation to CMT, differences between groups (P=0.021) were observed in weeks +1, +2 and +3, showing a higher frequency



of positivity in the BAC+ group (36.5%, 23/63) in relation to BAC- group (18%, 12/65). At the time of delivery (colostrum) occurred the highest median value of MSCC -  $1.5 \times 10^6$  cells/mL, different from the weeks +1 ( $0.35 \times 10^6$  cells/mL), +2 ( $0.24 \times 10^6$  cells/mL) and +3 ( $0.08 \times 10^6$  cells/mL). The MSCC was also different between the groups ( $P=0.000$ ), BAC+ had a median value of  $5.0 \times 10^5$  cells/mL, whereas in BAC- the value was  $2.87 \times 10^5$  cells/mL, results compatible with the leukocyte response in the presence of mammary infection, accumulation of leukocytes and scaling of epithelial cells during the colostrum-gene-esis process. Based on the results it was possible to conclude that the transition period of cows evaluated in this study was characterized by the predominance of CNS bacteria and that the physiological changes associated with edema are more evident in mammary glands free of bacterial infection.

YS-P01

### **Preliminary investigation on the effect of milk replacer reconstitution rate and crude protein content on abomasal emptying rate**

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Recent years have seen a trend for increased feeding rates of higher protein milk replacers in pursuit of a younger age at first calving and higher milk yield. The objective of this experiment was to investigate the effect of milk replacer (MR) reconstitution rate (RR) and MR crude protein (CP) concentration on the abomasal emptying rate (AER) of pre-ruminant calves. At 19 ( $\pm 2.8$ ) days of age calves were randomly assigned to one of five treatments in a latin square design based on date of birth and birth bodyweight from 21 March to 2 April, 2017. The treatments were (1) 20% CP MR mixed at 12.5% RR, (2) 20% CP MR mixed at 20% RR, (3) 26% CP MR mixed at 12.5% RR, (4) 26% CP MR mixed at 20% RR and (5) 26% CP MR mixed at 15% RR (control). Every calf was assigned to each feeding treatment for a period of 3-days. Calves were offered 2L of MR containing acetaminophen (50 mg/kg bodyweight) at time 0; acetaminophen absorption was previously validated as a measure of AER. Jugular venous samples for determination of plasma acetaminophen concentrations were collected at 0, 10, 30, 45, 60, 90, 120, 240 and 360 min after the morning feed of MR on day 3 of each period.

The mean time ( $T_{max}$ ) (SEM) of maximum plasma acetaminophen concentration was recorded at 207 (33), 333 (18), 219 (37), 276 (26) and 253 (31) minutes, for treatments one to five, respectively. A preliminary analysis of variance indicated a significant difference between treatment one and two ( $p = 0.049$ ), while the time difference between treatments two and three approached significance ( $p = 0.101$ ). Further analysis will be required to evaluate the relevance of these findings.

YS-P02

### **A welfare assurance programme for dairy calves in New Zealand**

#### **Developing a monitoring and benchmarking programme for dairy calves with clear animal health and welfare outcomes**

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**Objectives:** In 2015, the New Zealand dairy industry came under significant public pressure due to a couple of notable cases of poor calf animal welfare. This threw the spotlight on the whole dairy calf industry, leading to regulatory change and greater oversight.

A veterinarian- led calf welfare assurance scheme was developed to help guide dairy producers and allow them to better





benchmark their practices and identify areas for improvement.

This scheme was piloted in 2016 and refined in 2017, and this paper presents some early data from this scheme.

**Materials and Methods:** 23 dairy farms in the Southland region of New Zealand were enrolled in this programme. These farms were all seasonally calving, pastorally-based dairy farms. All farms calved over three months in the spring of 2017.

Measurements were grouped into four areas for benchmarking: colostrum management, biosecurity, preventive health, and calf welfare. Veterinarians and trained veterinary technicians visited the enrolled farms on at least 4 occasions during the 2017 spring season. All farms were given a Brix refractometer to measure and monitor colostrum quality.

The data were collected and uploaded to a dedicated database, for reporting back to farmers at an individual level; for the provision of an online dashboard for live monitoring; and for analysis and final reporting at the end of spring.

Samples and data gathered were both quantitative and qualitative. Each farm also completed a comprehensive questionnaire describing management practices on the farm. The quantitative measurements included pen sizes, colostrum quality, colostrum transfer, and disease monitoring; and the qualitative measures included assessments of housing and shelter such as quality of bedding, quality of feeding management, and environmental conditions.

Calf blood samples were collected from up to ten, 1-7 day old calves per farm on two occasions and tested for total protein (TP) levels to assess colostrum transfer. These were presented against a best practice target TP of 5.5g/dl. Colostrum quality was measured daily using a Brix refractometer.

Data were benchmarked and presented back to farmers in the form of individual farm reports, an online individualized dashboard, a final farm summary report, and a generic project report. In addition, where specific immediate issues were identified during the programme farmers were notified immediately and provided feedback and management support.

**Results:** Of the 23 farms enrolled, 45% of all Brix colostrum readings were above the threshold for colostrum quality of 22. The maximum Brix score was 30 and the minimum was 9.

430 individual calves were tested for TP. 29% of all calves tested had failure of passive transfer (FPT, TP < 5.5g/dl). The within-farm proportion of calves that had FPT varied from 0-100%. 15/21 farms deceased or maintained their proportion of calves with FPT from the first visit to the second visit (2 only had a single visit).

On biosecurity measures, 62% of all farms had a footbath into the main calf shed; 60% had a separate pen or shed for sick calves; 33% had a footbath for this area; 57% used different equipment for the sick calves; and only 38% employed a different person to feed these calves and/or fed them last.

For preventive health, 81% of farms routinely navel sprayed all calves at least once; 33%, 86% and 90% vaccinated against rotavirus, clostridial disease and leptospirosis respectively; 39% BVD tested all calves; and 0% used pasteurization on the milk fed to calves.

From an animal welfare perspective, 95% of farms met minimum pen space requirements per calf for stocking density (>1.5m<sup>2</sup>), and 95% had adequate loading facilities. However, only 68% had adequate shelter and 55% had *ad lib* access to

water. 69% of farms used local anaesthetic when disbudding (this is not yet a mandatory requirement in New Zealand).

**Conclusion:** This project demonstrated the value of monitoring and benchmarking key areas of animal health and welfare for dairy calves for farmers. The improvements seen in the proportion of calves with FPT between the first and second monitoring visit highlight the positive impact of this project, and demonstrate the value of similar approaches.

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YS-P03

### Study on Formation and Utilisation of FMR (Fermented Milk Replacer) for Calf Feeding

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Fermented raw milk, manufactured by the process of fermentation is being utilized to prevent and minimize enteritis and symptoms of diarrhea in calves. However, due to the duration of fermentation, the fluctuation of quality, originating from the operator who processed the milk, and lack of material, these factors disturb the utilization and diffusion of FMR.

We have tried to make FMR, made of commercial milk replacer. FMR has 4 times the density of Commercial Milk Replacer fermented with forms of Lactic Acid Bacteria 10<sup>9</sup>/ml and Coliform 10<sup>3</sup>/ml within 24 hours and its process was faster than the fermentation of ordinal liquid fermented milk. Five days after manufacturing, we observed a number of decreases of both Lactic Acid Bacteria and pH, furthermore the calves preference to intake FMR was lessened. In fact, treating enteritis caused by rotavirus or cryptosporidium by feeding either FMR or ordinal liquid fermented milk supplementary to their nurturing feeds decreased significantly both the number of calves treated as well as the mortality rate due to enteritis.

FMR is an equivalent feed to conventional fermented milk originally made from colostrum or non-saleable milk. We observed that the application of FMR is acceptable in calf-rearing for two reasons; the first is, it is easy to make even when colostrum or non-saleable milk is not available, the second is the process of manufacturing it is relatively faster. Furthermore, we found feeding FMR prevents occurrences of enteritis and makes symptoms milder even after the etiology of enteritis.

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YS-P04

### Capsular antigen type determination of *P. multocida* isolated from calves in Serbia

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**Objectives:** Due to ever changing epidemiology and influence of *Pasteurella multocida* in bovine respiratory disease (BRD), we wanted to check out what *Pasteurella multocida* type can be found on some farms in Serbia. We isolated *Pasteurella multocida* from calves with diagnosed bronchopneumonia on 2 farms in Serbia during 2012-2014 and we have determined type of isolates using multiplex capsular typing system.

**Methods:** A total of 147 diseased Holstein-Friesian calves 3 to 4 months of age, from 2 farms (farm A, n= 72 calves, farm B, n=75calves) were sampled in winter time when was outbreak of respiratory infection. All animals were maintained within the same airspace in naturally ventilated environment buildings. Animals on the both farms were not vaccinated (neither against viral nor bacterial diseases). Deep nasal swabs were taken from all animals included in the study. Conventional bacteriological methods were used for the isolation and the identification of *Pasteurella multocida* was confirmed using the BBL Crystal E/N, ID Kit (Becton Dickinson, USA). For molecular typing of *P. multocida* isolates, previously described multiplex PCR (Multiplex PCR kit, Qiagen GmbH, Germany) was applied (Townsend et al., 2001).

**Results:** Prevalence of *P. multocida* was 56,9% (41 of 72 calves) on farm A, and 26,6% (20 of 75 calves) on farm B. All *Pasteurella multocida* isolates belonged to type A. A higher prevalence of *P. multocida* type A infections was detected in calves from the farm A, a farm with a history of high morbidity and mortality from respiratory diseases in dairy calves.

**Conclusion:** Our results indicate that *P. multocida* type A could be an important pathogen involved in the pathogenesis of dairy calves bronchopneumonia in Serbia.

**Reference:** KIRSTY M. TOWNSEND, JOHN D. BOYCE, JING Y. CHUNG, ALAN J. FROST and BEN ADLER (2001) Genetic Organization of *Pasteurellamultocida cap* Loci and Development of a Multiplex Capsular PCR Typing System. JOURNAL OF CLINICAL MICROBIOLOGY. Vol. 39, No. 3, p. 924–929. DOI: 10.1128/JCM.39.3.924–929.2001.

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YS-P05

### Risk factors, assessment and outcome of failure of passive transfer of immunity in crossbred cattle calves

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GAD Vet. and Anim. Sci. Univ.

Failure of passive transfer of immunity (FPT) due to several maternal or calf factors is one of the most important problems affecting calf welfare. The present study was aimed to investigate FPT with respect to risk factors, assessment and outcome in crossbred cattle calves (Holstein Friesian and Sahiwal). Twenty unassisted calving events culminating into birth of live calves were monitored from the first appearance of labor to the expulsion of fetus. The newborn calves were assessed for vitality before colostrum feeding and a vigour score was calculated.

Observations on dam parity, calf birth weight and colostrum feeding were recorded. Blood samples were collected once between 24 to 48 hours and again between 21 to 28 days of life. Serum immunoglobulinG (IgG), total proteins (TSP), albumin and gamma glutamyl transferase activity were estimated. On the basis of serum IgG concentrations at 24 to 48 hours, the calves were categorized as normal (NC, IgG more than 10 g per L) or having failure of passive transfer (FPTC, IgG less than 10 g per L). The health and growth of calves were monitored for the first 3 months of age. The first colostrum samples from the dams were analyzed for IgG concentrations and somatic cell counts (SCC). The mean serum IgG concentrations at 24 to 48 hours were  $10.85 \pm 0.29$  g per L and 5 out of the 20 calves (25.0 per cent) had failure of passive transfer. At 24 to 48 hours, the FPTC had significantly ( $p < 0.05$ ) lower serum IgG ( $9.39 \pm 0.28$  g per L) and TSP ( $50.20 \pm 3.62$  g per L) concentrations than the NC (IgG  $11.34 \pm 0.27$  g per L, TSP  $65.33 \pm 3.02$  g per L). Significantly ( $p < 0.05$ ) lower volume of colostrum ingested at the first feed ( $0.68 \pm 0.12$  L in FPTC vs  $0.96 \pm 0.07$  L in NC), significantly ( $p < 0.05$ ) higher first feed interval ( $198.00 \pm 116.65$  minutes in FPTC vs  $52.67 \pm 11.66$  minutes in NC) and significantly ( $p < 0.05$ ) higher colostrum SCC ( $4887.02 \pm 412.77 \times 10^3$  cells per ml in FPTC vs  $3294.69 \pm 401.52 \times 10^3$  cells per ml in NC) were found to be the risk factors for causing FPT in calves. Vigour score at birth, colostrum intake in the first 24 hours after birth, dam parity and calf body weight at birth did not influence the passive transfer in calves. The Ig index (ratio of serum IgG at 21-28 days to serum IgG at 24-48 hours) was significantly ( $p < 0.05$ ) higher in FPTC ( $1.13 \pm 0.04$ ) as compared to the NC ( $0.91 \pm 0.03$ ) indicating poor health status of the earlier. In the first 90 days of life, the incidence and severity of diarrhea, and days in treatment were significantly ( $p < 0.05$ ) higher while the age at disease onset and growth rate were significantly ( $p < 0.05$ ) lower in the FPTC. It is concluded that failure of passive transfer of immunity is prevalent in crossbred cattle calves that adversely affects their health and growth. Feeding of poor quality colostrum with high somatic cell count, and quantity and duration of the first colostrum feeding are risk factors for the occurrence of FPT in crossbred cattle calves.

YS-P06

### Influence of metaphylactic use of antimicrobials on systemic inflammatory profile in Holstein calves during the first month of life

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This study assessed the effect of immediate use tulatromycin on the development of systemic inflammatory response in Holstein calves over the first month of life. To this end, 26 healthy Holstein heifer calves were divided in two groups with one given prophylactic antibiotics (ATB+ tulatromycin at 2.5 mg per Kg) and the other no antibiotics (ATB-). The drug was delivered



subcutaneously within 12 hours after birth. Blood samples were collected within twelve hours after birth, and in the windows D3-5, D7-9, D13-15, D20-23 and D27-30 after birth. Absolute total leukocyte numbers were determined with an automatic analyzer (ABC Vet, ABX<sup>®</sup>). Leukocyte differential was performed based on microscopic cell morphology of stained cells at 1000x magnification counting at least 200 cells per sample. The phenotypes of polymorphonuclear leukocytes (PMN) and mononuclear cells were evaluated by staining with the bovine specific monoclonal antibodies recognizing CH138 (PMN) and CD62L. The cells were assayed for the production of intracellular Reactive Oxygen Species (ROS) by chemiluminescence. Electrophoresis on cellulose-acetate membranes provided a cellular protein profile. Haptoglobin was detected using a turbidometric method, and serum iron with a commercial kit (Iron UIBC, Randox<sup>®</sup>). Lower values of hemoglobin ( $P = 0.071$ ), CHCM ( $P = 0.057$ ) and iron ( $P = 0.051$ ) were measured in ATB- than ATB+ calves on D3-5. ATB- calves had a significantly higher frequency of anemia (61.6%) than ATB+ calves (15.4%) ( $P = 0.016$ ) on D3-5. The anemia that was observed in ATB-calves was classified as a normochromic normocytic (38.5%) and hypochromic normocytic anemia (23.1%). The anemia observed in ATB + calves was primarily normochromic normocytic (15.4%) ( $P = 0.038$ ). These findings are compatible with the anemia observed during acute inflammatory processes. Anemia was observed subsequent to navel inflammation more frequently in ATB- calves. While the absolute number of neutrophils ( $P = 0.009$ ) was higher in the ATB- calves on D7-9, the number of lymphocytes was higher in the ATB + calves at this time ( $P = 0.018$ ). The production of endogenous ROS by total leukocytes was higher in ATB- calves (470.46 AFU) than ATB+ calves (387.27 AFU) on D7-9 ( $P = 0.038$ ). We observed a tendency toward a higher percentage of mononuclear cells expressing CD62L+ in ATB+ calves on D3-5 ( $P = 0.098$ ) and D7-9 ( $P = 0.083$ ), in conjunction with navel inflammation. Following peak diarrhea episodes, the mean fluorescence intensity for CD62L+ on granulocytes ( $P = 0.043$ ) and mononuclear cells ( $P = 0.089$ ) showed a tendency toward high values in ATB+ than ATB-calves on D20-23. At the D13-15 sampling, we observed a tendency toward higher numbers of lymphocytes in ATB+ than ATB- calves ( $P = 0.061$ ). The ATB+ calves also tended to have higher levels of beta-globulin ( $P = 0.057$ ), gamma-globulin ( $P = 0.069$ ) and significantly higher haptoglobin levels ( $P = 0.032$ ) on D13-15. ATB+ and ATB- calves had similar prevalence of diarrhea (~ 90%) primarily caused by *Cryptosporidium* on D13-15. In composite, these results lead us to conclude that early metaphylactic use of tulatromycin is associated with higher systemic inflammatory responses in early development that are associated with natural episodes of diarrhea.

YS-P07

## The Influence of the lunar cycle on haemorrhagic complications in castration of young bulls

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**Objective:** We can not deny the influence of the lunar cycle in nature. It is logical to assume that somehow the gravitational

pull affects all fluids. There is no doubt about the Moon's influence on ocean tides.

In its 29,5-day cycle, the Moon passes through its phases. It waxes after the new moon and wanes after the full moon. There are traditional guidelines involving crop planting, slaughtering animals, logging, etc., which follow the lunar cycle and choose their timing for certain tasks according to it.

There is a common fear that the waxing phase of the moon leads to a greater risk in surgical complications. For the purpose of finding the truth, we analysed 63 cases of castration, checking each patient's state after surgery.

**Materials and methods:** Castrating calves and young bulls is traditional for my country. In the past, steers (oxen) were used as working animals but now the procedure is used for the purpose of calming them down, getting better meat prices and avoiding unwanted sexual activity in herds.

From August 2016 until November 2017 we have monitored each castration we performed. Male calves were of different breeds, aging from one week to six months. The method of surgical approach was very uniform: light sedation, retaining the animals in a standing position, cutting off the top of the scrotum, twisting each spermatic cord separately with forceps until the testicle comes off, no suturing (Bohor's method). Therefore, the goal of the report was presenting the data collected during this one year period, examining the post operational bleeding correlated with the moon phases.

**Results:** The results are based on the most concerning post operational complication for the owners, that is the amount of haemorrhage, which has the greatest effect on their satisfaction with the surgical outcome. The question and the essential problem was: the degree of bleeding after the procedure.

Outcomes were put into three categories:

N - no bleeding (no bleeding, occasional drops of blood ceasing in less than an hour after the procedure)

M - mild bleeding (moderate amount of blood, the bleeding ending in up to one hour postprocedure)

C - considerable bleeding (needs to be taken seriously, the patient needs to be reexamined and properly treated)

In total there were 63 cases, 67,6% had been performed in the waxing phase, 52,4% in the waning moon. That shows equal distribution between the "favorable" and "unfavorable" days. In the waxing time 90% of the cases had no bleeding (N), 10% had mild (M).

In the waning time, 94% had no bleeding (N), 6% had mild (M) consequences.

Not one patient needed additional treatment (C), regardless of the Moon's phase. This is the most important fact!

The classification/categorisation of each case depended on our personal, as well as the owner's opinion and observation. Taking this subjective evaluation into consideration, we think that that is still the most relevant assessment for the surgeon.

**Conclusions:** There is in fact no significant correlation between the phases of the Moon and the amount of postsurgical bleeding from the skin and spermatic cords in calf castration. This study shows that there is no real fundament in the public belief (and fear) that performing castration on the wrong part of the lunar cycle could compromise the surgical outcome.



The advantage of this study is that it was performed by one surgeon, so any individual skill differences were excluded. All procedures were performed during everyday practice.

These results may help veterinarians in convincing and changing the minds of owners who are somewhat afraid of wrongly timed castrations because of the moon cycle. Regardless of the results, clients should always be taken seriously, therefore try to synchronize the timing of the operation with their expectations as much possible.

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YS-P08

### Neonatal calves form abomasal curds after first ingestion of first-milking colostrum for efficient absorption of immunoglobulin G

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**Objectives:** Abomasal curd formation is a unique digestive system that occur in milk-fed pre-ruminant calves. In this process, milk is coagulated under the action of chymosin (EC 3.4.23.4) and casein, and the lipids are trapped in the curd, whereas other milk proteins, lactose and minerals are separated in the whey. It has been thought that curd formation is important for digestion and absorption of milk nutrients and immune substances in calves. However, there is no study observing abomasal contents in neonatal calves after first ingestion of first milking colostrum. The objective was to evaluate curd formation in neonatal calves and its physiological function with a focus on immunoglobulin G (IgG) absorption.

**Materials and Methods:** First milking colostrum was collected from 37 Holstein cows at 5 private farms, pooled, checked its coagulation by *in vitro* rennet coagulation test, divided into plastic bags (aliquot 2 L), and stored at -20°C until feeding. In the primary experiment, abomasal contents were temporally observed by ultrasonography in three neonatal calves fed the pooled colostrum once at a volume of 4% of body weight at 38°C using a rubber nipple attached to a bucket within 4.2 h after birth. In the secondary experiment, serum IgG concentrations were compared between 10 calves fed 2 L of the pooled colostrum and 7 calves fed 2 L of the whey solution, which is separated from the pooled colostrum by adding rennet, at 38°C within 3 h after birth. Blood samples were collected from a jugular vein 6 to 7 h after the first feeding and before the second feeding.

**Results:** *In vitro* assay confirmed that the pooled colostrum was coagulated by incubation with rennet while the whey solution was not. All calves suckled whole amount of the pooled colostrum or whey solution within 10 min without assistance. Between 0.5 and 8 h after colostrum ingestion, a curd was visualized as a large echogenic image with a clear outline, which was surrounded by an anechoic image corresponding to whey. Serum from one calf in the pooled colostrum sample set was excluded due to incomplete curd formation in that the whole colostrum did not coagulate into a large mass of curd and a portion of the colostrum remained as its residue caseins detectable from the abomasal fluid. Serum IgG concentrations were significantly higher ( $p=0.0126$ ) in the 9 calves fed the colostrum ( $3.8\pm 1.6$  g/L) than the 7 calves fed the whey solution

( $2.1\pm 0.6$  g/L). One calf exhibiting incomplete curd formation showed low level of serum IgG (2.7 g/L) after the ingestion. Considering similar IgG concentration between the pooled colostrum (54.5 g/L) and the whey solution (59.4 g/L), we strongly suggest that the variations of serum IgG levels between calves are mainly caused by the presence and absence of curd in their abomasum.

**Conclusions:** Neonatal calves produce a large mass of curd by first ingestion of first-milking colostrum, but about 10% (95% CI 2-40%) of neonatal calves may exhibit incomplete curd formation. Curd formation would enable neonatal calves to efficiently absorb IgG from the colostrum.

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YS-P09

### Longitudinal study on the effects of calf BRD on growth and productivity in Holstein-Friesian cattle

\*Sophie Anne Mahendran John Robert Baggs

FeverTags

**Objectives:** Bovine respiratory Disease (BRD) is the one of the most important growth-limiting diseases of cattle, with both short-term economic effects due to initial treatment costs as well as long-term effects on animal productivity such as growth rates. The aim of this study was to assess the effects of BRD events on the long-term growth rates of dairy cattle, and on the age of first service and initial milk yield of heifers.

**Materials and methods:** A longitudinal study was conducted on one UK dairy farm over a three-year period. Four hundred Holstein-Friesian dairy cattle of both sexes were recruited at birth, with bull calves followed until 12 months of age, and heifers followed until they calved for the first time. Cases of BRD were detected using FeverTags, and treatment using combinations of gamithromycin and flunixin meglumine. Each case of BRD was recorded in the individual animal records. The animals were weighed at birth, weaning (10 weeks of age) and approximately 12 months of age using mechanical weigh scales. The daily milk yield of the heifers was recorded between 4-6 weeks after calving. A generalised linear model was used to assess the association between the growth rates and occurrence of BRD as a calf.

**Results:** Analysis of the average daily live weight gain for pre-weaning calves indicates that both sex ( $p=0.006$ ) and occurrence of repeated episodes of BRD ( $p=0.046$ ) were significantly associated with the average daily live weight gain of pre-weaning calves. Being male was associated with a 0.039kg/day increase in daily weight gain, and having repeated episodes of BRD was associated with a 0.037kg decrease in daily weight gain. Analysis of the daily live weight gain for pre-weaning calves up to 12 months indicated that having repeated episodes of BRD as a calf was associated with a 0.044kg/day reduction in daily live weight gain ( $p=0.05$ ). Data collection for the age of first service and heifer milk yield is still ongoing.

**Conclusions:** The study indicates the long-term reduction in growth rates that repeated episodes of BRD as a pre-weaning calf can have. This highlights the importance of BRD prevention, as well as development of accurate detection methods



and efficacious treatment protocols.

YS-P10

## Development of a novel carcass autolysis scale (CAS) for use in cases of bovine perinatal mortality.

### Gross pathology scale.

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**Introduction:** Once death occurs autolysis commences at a variable rate in different organs. The extent of autolysis is primarily dependent upon the death-to-necropsy interval (DNI). However, there are no studies detailing the extent of autolysis in different organs in calves with a known DNI. Knowing this would allow pathologists to interpret necropsy findings adjusted for the DNI effect.

**Objective:** The aim of this study was to assess the extent of autolysis in different organs and to use these assessments to develop and evaluate a novel carcass autolysis scale (CAS) using macroscopic changes in calves with a known DNI.

### Materials and methods:

#### Calves

This study was carried out on 30 cases of perinatal mortality born at observed calvings on 10 Polish dairy farms between November 2013 and June 2015. The cases were fullterm (260 or more days), singleton, Holstein-Friesian, calves (17 female and 13 male) which were alive at birth (breathing observed or 33% and more of lung area inflated at necropsy) and died, in most cases, within 5 mins of birth (mean 12 min, SD 33 min).

#### Development of carcass autolysis scale (CAS)

Carcasses were examined using the same necropsy protocol by the same pathologists. Necropsy examinations were carried out, on average, 7.43h (SD 2.11h; range 3.30-12.35h) after death. Each of six carcass elements (fluids in thoracic and abdominal cavities and aorta, left kidney, spleen, liver and brain) was individually assessed visually or by palpation and assigned an autolysis score from 1 to 3; (1) no signs of autolysis, (2) mild to moderate autolysis and (3) marked autolysis. Body cavity fluid score codes were (1) serous, (2) serosanguineous or (3) sanguineous/bloody. Aortic tunica intima score codes were (1) pale, (2) pink or (3) red. Two aspects of the kidney were assessed visually and by palpation: kidney cortex consistency and the extent of capsulo-cortical and cortico-medullary separation: (1) firm without separation, (2) soft with focal separation or (3) liquefied and generalized separation with serosanguineous fluid accumulation. Two aspects of the spleen were assessed: splenic capsule colour and the consistency of the cross-section parenchyma surface: (1) blue and firm with visible structure of parenchyma, (2) pink and soft/semi-fluid parenchyma or (3) marked haemoglobin-stained imbibition with liquefied parenchyma. The external colour of the liver was assessed as (1) reddish brown/yellow/orange (2) brown or (3) pink (generalised haemoglobin imbibition). Brain cortex consistency

was assessed visually and by palpation: (1) firm with uniform structure of sulci and gyri, (2) soft with gyri swollen and sulci narrowed or (3) liquefied cerebral and cerebellar cortex.

When the scores were combined from these 6 carcass elements, the minimal and maximal possible carcass autolysis scores (CAS) were 6 and 18, respectively.

**Results:** Of the 30 calves, 21 (70%) had a CAS of 6 points, indicating no autolysis and the remaining 9 calves (30%) had a CAS of 7 points. In the latter cases, mild to moderate autolysis was only detectable in a single parameter/carcass. The most common sites of mild to moderate autolysis (score 2) were the brain (3 calves) and the aorta (3 calves) and less frequently, the spleen, kidney and body cavity fluids (1 calf in each case). Autolysis was not detected in the liver and none of the carcass elements had marked autolysis. The average DNI of carcasses with no autolysis (7.07h, SD 1.46h; range 3.30-10.20h) was shorter than that of carcasses with mild-moderate autolysis (9.06h, SD 2.26h), ( $P < 0.05$ ). Of the 9 calves with mild to moderate autolysis, 7 (78%) had a DNI  $> 8$ h (8-12.35h) and the others (22%)  $< 8$ h (5.30, 6.05h).

**Conclusions:** Gross autolysis is not detectable in the majority of calves which die after birth if the DNI is less than 8 hours. Where present, autolysis is mild to moderate and is first detectable in the brain and the aorta. These are novel practical findings which can be the basis of improved DNI-adjusted necropsy reporting.

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MT-P01

## Determination of reproduction and lactation parameters in the first production year of Brown Swiss and Simmental cattle

### Reproduction Parameters

### Milk Yield Parameters

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This study aimed at determining the adaptation levels of pregnant Brown Swiss and Simmental heifers, imported from Austria. This study focuses on the cows' reproduction and milk yield performances. Insemination, pregnancy and birth parameters for 70 Brown Swiss and 282 Simmental cows were found to be similar. The first insemination interval and gestation length of Simmental cows were shorter than Brown Swiss. For Brown Swiss and Simmental cows, real milk yield was 9205,61 lt and 8351,05 lt; milk yield for 305-days was 8115,71 lt and 7693,44 lt; the lactation period was 356,0 days and 337,7 days. The differences between breeds according to real and 305-days milk yield were statistically significant. The pregnancy rate was 76,92% for Brown Swiss and 85,66% for Simmental cows. 40,00% and 41,21% of pregnant cows conceived in the first insemination. For Brown Swiss, 90,00% were live birth, 4% were stillbirth and 6,00% were abort; for Simmental, 93,56% were live birth, 1,72% were stillbirth and 4,72% were abort. Unassisted, assisted and difficult birth rates were 37,78%, 46,67% and 15,56% for Brown Swiss and 43,58%, 47,25% and 9,17% for Simmental. The milk yields of the cows that calved in November and December were high, while the milk yields of cows that calved in June, July and August were low. Considering that the mean milk yields of Brown Swiss and Simmental cows in first lactation were over eight thousand liters, it can be said that the cows imported from Austria genetically have dairy potential, that they can show this potential from the first yield-year onwards, provided that attentive and rational caring and nutrition programs are implemented for the herd, and that it is necessary to carry out more attentive insemination and pregnancy follow-ups in order to increase reproduction performance.

MT-P02

## Alterations in molecular status of fibronectin associated with perinatal mortality in dairy cattle

### Fibronectin in stillbirth

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**Objective:** Fibronectin (FN) is a large, multifunctional glycoprotein present in significant amounts in biological fluids, extracellular matrix and cell surface of mammalian tissues. FN levels are deter-

mined both in human and veterinary medicine. Increase of FN concentration was shown in rabbits' plasma during sepsis and in rats after turpentine injections. Fetal FN level in cervicovaginal fluid is reported to be a useful predictive parameter of spontaneous preterm birth in humans. Appearance of FN degradation products in amniotic fluid in women was found to be associated with intra-uterine infection. Little is known about FN structure, presence of its molecular variants and concentration changes in dairy cattle. The objective of this study was to determine FN molecular status (i.e. plasma FN concentration, presence of FN degradation products and FN-fibrin complexes) in stillbirth cases in dairy cattle.

**Materials and Methods:** The experimental design was approved by II Local Ethics Commission in Wrocław (permission numbers 23/2012, 58/2014, 60/2014). The study was carried out on 110 dams of stillborn calves that gave birth to 121 calves (11 cows gave birth to two calves each) and 21 cows that gave birth to healthy calves. Six control calves were euthanized and examined according the same protocol as stillborn calves. Sixteen cows in later lactation stage (120-638 days in milk) were enrolled to the study. Sample were collected over a 20-month period between November 2013 and June 2015 in 30 Polish Holstein-Friesian herds. Blood samples from the dams (after calving), lactating cows, abomasal samples from 44 stillborn calves and six euthanized calves and amniotic fluid samples from all control calvings and six stillborn calvings were collected. FN concentration was evaluated by ELISA in all collected plasma and amniotic fluid samples, as well as in six abomasal fluid samples from both control and stillborn calves. The electrophoretic patterns of FN in plasma, amniotic and abomasal fluid were evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting. Supramolecular forms of bovine plasma FN-fibrin complexes were detected by sodium dodecyl sulfate-agarose gel electrophoresis (SDS-AGE) followed by immunoblotting. Additionally in all plasma samples fibrinogen concentrations were evaluated by heat precipitation method.

**Results:** The FN concentration in plasma of stillborn dams was significantly lower than in other cows ( $P < 0.001$ ). The FN concentration in plasma of dams of live born calves was significantly lower than in lactating cows ( $P < 0.05$ ). No significant differences were noted in the concentration of FN in amniotic and abomasal samples. In all plasma samples two major bands of FN were detected. In 9-20% cows after parturition additional two bands with lower molecular weight were detected, contrary to cows during lactation where only two major bands were detected. Lower number of amniotic fluid samples showed degradation of FN compared to abomasal fluid. More FN-Fibrin complexes with higher molecular weight were observed in plasma samples after calving compared to cows in lactation. The highest and the lowest concentrations of fibrinogen were in dams of stillborn calves and in cows in lactation, respectively ( $P < 0.02$ ).

**Conclusions:** The results of our study showed changes in plasma FN concentration, electrophoretic pattern of plasma FN, as well as occurrence of high molecular weight FN-fibrin complexes during the periparturient period compared to lactation. These observation suggest activation of coagulation process and/or inflammatory response in cows after calving. High degradation level of FN in abomasal samples limits its usefulness for determination of FN status.

**Comments:** This work was supported by project NCBR no. PBS2/A8/20/2013.



MT-P03

### **Bilateral convergent strabismus with exophthalmus (BCSE) in a cross breed Iraqi cow**

#### **Cases report**

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A Bilateral convergent strabismus with exophthalmus were reported in adult cow (5 years-old) and calf (3 months-old) cross breed German Brown Holstein. Both animals were presented to the hospital of veterinary medicine in Al Muthanna governorate/ Iraq and revealed a severe visual disorientation that impaired gait and handling. The owner claim that the Bilateral convergent strabismus with exophthalmus (BCSE) was found since birth and gradually progressed to the present condition. The clinical examination of the animals showed a bilateral symmetric protrusion of the eyeballs associated with an anterior-medial rotation and epiphora of both eyes. Disorganized pigmentation also was very obvious on the sclera of both eyes. However, the cow was able to move the eyelids over the cornea. The progressive of this defect might lead to blindness that will be change the behavior and production of the affected animals such as inability to reach the food or drink then reduction in its productivity. The inappropriate breeding with genetically affected cows led to distribution of allele for BCSE gene, which is an autosomal dominant major gene in bovine inherited eye diseases. In conclusion, the present article reported BCSE defect for the first time in a cross breed cow in Iraq. Moreover, this condition considered as a congenital bilateral convergent strabismus with exophthalmus and perhaps inherited in nature according to previous published literatures. The author recommends to do molecular-epidemiological study regarding this defect and advises should be given to the owner regarding the cross breeding and excluding plan of the affected or carrier BCSE defect gene cows to prevent its distribution in the herd of cattle.

MT-P04

### **Lesion profilings of cell lines persistent with BSE in transgenic mice**

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**Objectives:** Bovine spongiform encephalopathy(BSE) is one of the groups of transmissible spongiform encephalopathies(TSEs) that cause fatal infectious neurodegenerative diseases in animals. TSEs strains can be defined by differences in the conformation, glycosylation ratio, protease resistance, and aggregation state of the core of abnormal isoform of prion protein(PrPSc). The brain lesion profiling is the gold standard to identify biological properties of TSEs strains. In this study, we characterized histopathological findings of cell lines persistent with BSE in bovine prion protein overexpressing transgenic mice.

**Materials and Methods:** All transgenic mice were assessed by polymerase chain reaction using bovine prion protein primers.

cell lines persistent with BSE and BSE infected cattle brain homogenates were inoculated in 10 mice each. When a mouse showed above three symptoms over a week, euthanasia and necropsy were performed. Mouse brains were fixed in 10% neutral buffered formalin, immersed in 88% formic acid for inactivation of PrPSc. After tissue processing and paraffin-embedding, serial sections were stained with H&E. The pattern of vacuolar changes was scored in 12 areas. In addition, immunostaining was generated with polyclonal anti-prion protein antibody.

**Results:** Both groups showed moderate to severe vacuolar degeneration in dorsal medullar nuclei, central thalamus and septal nuclei. In addition, relatively severe vacuolar degeneration was observed in mesencephalic tegmentum compared with other white matter lesion. The patterns of PrPSc depositions of transgenic mice inoculated with cell lines were analogous to those of inoculated with BSE infected cattle brain homogenates in immunohistochemistry. Accumulation of PrPSc was observed multifocally in dorsal medullar nuclei, central thalamus and partially in posterior cerebral cortex, hippocampus and hypothalamus. Both groups showed some multifocal plaque-like deposition of PrPSc in central thalamus.

**Conclusions:** The present study revealed histopathological similarities of cell lines persistent with BSE and BSE infected cattle brain homogenates in transgenic mouse. The present data demonstrates the suitability of cell lines as a tool to investigate prion diseases.

MT-P05

### **The Effects of Ractopamine Hydrochloride on Finishing Performance, Carcass Characteristics, and Economic Returns of Feedlot Heifers in the United States**

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Zoetis Inc.

The supplementation of feedlot finishing rations with a beta-agonist repartitioning agent has become standard industry practice in the United States because it helps animals convert feed to lean tissue. Ractopamine hydrochloride was the first beta-agonist approved for use in cattle in the U.S., and has been safely and reliably used for more than 10 years to help finishing cattle efficiently generate more pounds of lean beef. Ractopamine hydrochloride is classified as a beta-1 agonist, known to increase red meat yield with little impact on fat deposition and beef quality/tenderness. Though beta-agonist supplementation is widely practiced, some feedlots have not adopted this cost-effective, beneficial technology. Ongoing research demonstrates how beta-agonist feeding can improve efficiency and boost economic returns without compromising nutrition and health.

This study was conducted at a commercial feedyard in Kansas, USA to evaluate the effects of Actogain™ (ractopamine hydrochloride) on performance, carcass characteristics, and economic returns of feedlot heifers.

Eight hundred fifty crossbred beef heifers were blocked by source and randomly assigned to 24 pens (approximately 35 head/pen). Heifers were on feed an average of 136 days be-



fore the start of the treatment period. Treatments consisted of adding 0 (control) or 300 mg/head/day of ractopamine hydrochloride (Actogain) to the finishing diet for the last 33 or 34 days before harvest. Body weights, feed intake, and carcass characteristics data were collected.

**Results:** A partial economic analysis was performed utilizing carcass value (as reported by the harvest plant), feed cost, and product cost (\$145.50/kg of Actogain™ 45). Body weights at the start of the treatment period were not different between treatments (568 kg;  $P=0.95$ ). Actogain supplementation significantly ( $P<0.01$ ) increased average daily gain (1.22 vs 0.91 kg/d) and final body weight (609 vs 599 kg) compared to non-supplemented heifers. Dry matter intake was not affected by treatment ( $P=0.75$ ). As a result, feed efficiency was increased by 26% for the Actogain treated heifers ( $P<0.01$ ). From a carcass perspective, hot carcass weight (395.4 vs 387.0 kg;  $P<0.01$ ), dressing percentage (64.94 vs 64.66;  $P=0.05$ ), and longissimus muscle area (94.1 vs 91.5 cm<sup>2</sup>;  $P<0.01$ ) were all increased in heifers supplemented with Actogain compared to control heifers. Conversely, 12<sup>th</sup>-rib fat ( $P=0.62$ ), quality grade distribution ( $P>0.25$ ), and yield grade distribution ( $P>0.09$ ) were not significantly affected by treatment. After removing the product cost, carcasses from Actogain supplemented heifers returned more than \$15 per head over their control counterparts (\$1,609.06 vs \$1,593.35;  $P=0.05$ ). During the last 33 or 34 days on feed, Actogain supplementation helped significantly improve growth, feed efficiency, hot carcass weight, longissimus muscle area, and financial return. Use of Actogain in finishing rations offers an exceptional opportunity to increase performance, optimize production efficiency, and maximize profit potential.

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### ***Congenital diplomyelia and hydromyelia in two calves***

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We describe 2 clinical cases of diplomyelia with hydromyelia in newborn calves. Both animals presented clinical signs of a general proprioceptive ataxia affecting the pelvic limbs. The most clear finding during clinical examination of the animals was an unilateral (case 1) or bilateral (case 2) absent patellar reflex. Only the second case was serologically tested positive for Schmallenberg virus which is not a very likely etiology. The cause of the spinal cord lesions in both calves remains unknown.



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