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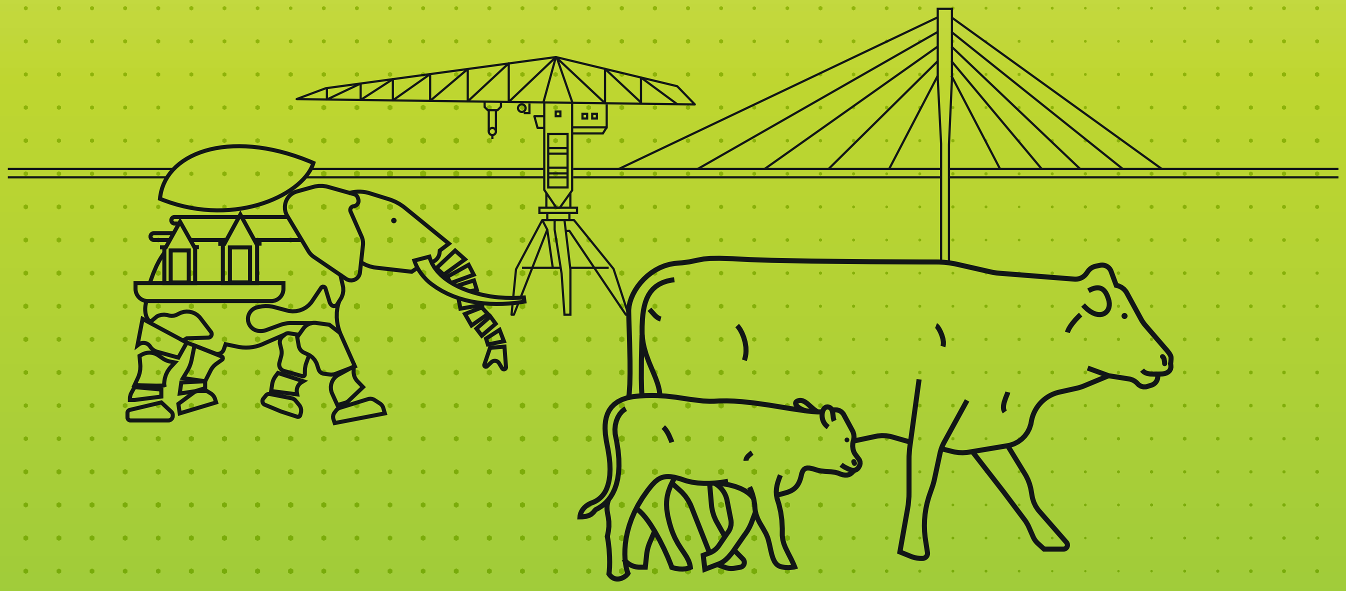

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Session “Welfare, lameness and pain management

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Vetinformatics: Next-generation discoveries of today, bovine diagnostics of tomorrow

M. A. Scott, DVM, PhD
Texas A&M University
College of Veterinary Medicine and Biomedical Sciences
Veterinary Education, Research, and Outreach Program
matthewscott@tamu.edu

Introduction

Infectious diseases in cattle pose significant economic and animal welfare challenges worldwide. Multifactorial infectious disease complexes such as bovine respiratory disease, mastitis, and gastrointestinal infections, including liver abscesses, remain the most prevalent and costly infectious diseases, ultimately culminating in reduced productivity, increased morbidity and mortality, and heightened antimicrobial use. Emerging and re-emerging pathogens, along with evolving antimicrobial resistance patterns, further complicate disease management within production systems. Additionally, shifting incidence rates of endemic and emerging infectious diseases are further associated by consequences of human activity, such as climate change, production systems intensification, and global trade conditions, increasing the risk of outbreaks and transmission across varying regions worldwide. These factors emphasize the need for improved disease surveillance, prevention strategies, and rapid diagnostics to mitigate losses and ensure sustainable livestock production.

Current diagnostic methods for infectious diseases in cattle typically rely on the visual recognition of clinical signs, gross and histopathologic assessment, and serological testing. While advancements in some molecular methods, such as PCR, and biosensor technologies have improved disease detection, significant molecular techniques and relevant knowledge gaps remain. Moreover, current diagnostic approaches rely on diagnostic laboratory-based testing, leading to delays in treatment and veterinary recommendations. Further, distinguishing between subclinical infections, co-morbidities, and asymptomatic or latent disease carriers remains a continuous challenge within livestock production systems. As such, subjectivity and relative delay in precise clinical assessment, along with the lack of rapid, sensitive diagnostic and/or prognostic tools for infectious disease cases, impede our ability to improve the understanding of multifactorial disease pathogenesis, specific antemortem etiological identification, and tailored treatment and prevention strategies specific to a given production system.

The trend toward on-farm, point-of-care diagnostics and integrated multi-omics approaches offers promise in addressing these diagnostic limitations, but further research is needed to enhance accessibility, affordability, and real-time disease monitoring in livestock populations. As such, omics research, the comprehensive evaluation of cellular and molecular systems through high-throughput biochemical assays and computational analysis platforms, has emerged as a leading field for investigating infectious disease in livestock[1]. In recent years, omics-based methodologies have utilized and integrated genomic, transcriptomic, proteomic, and metabolomic approaches to provide a more holistic view of host-pathogen relationships, transmission dynamics, genetic and environmental risk factors, and predictive features associated with disease outcomes. Both ours and other researchers' current research philosophies aim to utilize technological advancements in biochemistry, computing, and statistical modeling to improve knowledge concerning the pathophysiological process, microbial community structure, and immunological response in cattle which either resist or develop infectious disease. As such, the aim of this report and presentation is to explore recent advancements in sequencing and bioinformatic research focused on bovine infectious disease, to identify novel components and potential solutions to infectious disease processes for future diagnostic and prognostic tool development.

Genomics and metagenomics in infectious diseases of cattle

Genomic sequencing focuses on the complete sequencing and analysis of genetic material from a single organism, such as a host or pathogen, or from a collection of organisms within a sample in a metagenomic context[2]. Regarding infectious diseases of cattle, genomic sequencing is used to uncover genetic factors that influence disease susceptibility and severity, identifies traits and virulence factors that contribute to pathogen adaptation and infectivity, and aid in characterizing the composition and dynamics of microbial communities to provide insights into their roles in cattle health and disease.

Of immediate zoonotic and animal health importance, highly pathogenic avian influenza (HPAI) type A (H5N1) has emerged in U.S. dairy cattle, marking a significant shift in the virus's host range. First reported in March 2024, outbreaks in Texas dairy farms were attributed to the B3.13 genotype of clade 2.3.4.4b, a reassortment between a Eurasian H5N1 strain and endemic North American low pathogenicity avian influenza strains[3-6]. Genomic analyses confirmed these strains, with affected cattle exhibiting abrupt declines in milk production in addition to reporting of varying clinical signs. Subsequent studies identified nearly identical genome sequences across infected cattle, wild birds, and other mammals, such as cats, underscoring the virus's potential capacity for cross-species transmission[7]. These developments highlight the evolving epidemiology of H5N1, necessitating enhanced genomic surveillance tactics to track and potentially mitigate further spread among livestock and potential zoonotic transmission.

Metagenomic sequencing is changing the landscape of infectious disease diagnostics in cattle by allowing for unbiased identification of multiple pathogens and/or pathobionts, including novel and unexpected ones, directly from clinical samples. This area of sequencing-based research aims to characterize the composition and functional potential of tissue or system microbiota, identifying shifts in microbial diversity and abundance correlated with treatment outcomes and disease mitigation schemes[8-10]. For example, metagenomic sequencing has been employed to detect uncommon and novel infectious agents in livestock, enhancing our understanding of viral diversity and aiding in the development of more accurate diagnostic strategies[11]. Moreover, metagenomic sequencing has been conducted to investigate the relationship between the bacterial microbiome and resistome (i.e., the composition of antimicrobial resistance genes), disease development and/or mortality, and management tactics, such as metaphylaxis or vaccination[12-14]. Collectively, these metagenomic-based studies offer novel insights into microbial influences in which disease development possesses on the microflora of cattle and provide innovative hypotheses towards the development of advanced diagnostic and control measures against bovine respiratory disease (BRD) in large-scale commercial operations.

Transcriptomics in infectious diseases of cattle

Transcriptomics, the study of RNA from cells or tissues, provides insights into molecular functions, gene regulation, and co-expression patterns[15]. Through transcriptomic approaches, researchers may identify conserved gene expression patterns in cattle under various conditions, leading to the discovery of regulatory pathways and biomarkers for early disease detection[15,16]. Here, work has been led to discover specific host-driven gene expression profiles, such increased type-I interferon production, alternative complement activation, and M1 macrophage activity, alongside reduced lipid metabolism and specialized pro-resolving mediator production, as strong indicators of cattle likely to develop BRD within 28 days of production[17-21]. Additionally, host transcriptomics has been leveraged to improve the understanding of how conventional BRD-control strategies, namely metaphylaxis and vaccination against BRD pathogens, influence long-term immunomodulation and metabolism in beef cattle[22-24]. Further, transcriptome studies of BRD-associated pathobionts, specifically *Mannheimia haemolytica* and *Histophilus somni*, have refined bacterial genome annotations and functional insights[25,26]. By comparing gene expression patterns in both cattle and pathogens, researchers have uncovered pathways regulating inflammation, immune response, and cellular homeostasis that contribute to the development of early diagnostic tools, improved disease monitoring, and targeted therapies to mitigate BRD progression.

Proteomics and metabolomics in infectious diseases of cattle

Proteomics, the extensive evaluation of proteins produced or modified by an organism or system via high-throughput assays, allow for the discovery of functional features into infectious disease mechanisms and identification of novel biomarkers associated with disease outcomes[27]. This approach allows for protein identification, quantification, and post-translational modifications in relation to disease outcomes or interventions. For example, recent proteomic research has detected biomarkers in milk from cows with mastitis, both in general and based on whether the insulting bacteria was Gram-positive, Gram-negative, or culture-negative[28]. Specifically, this research provides host-derived markers that may be leveraged in advancing on-farm diagnostics and reduce the reliance of culture-based assays. Likewise, this technology has been utilized in BRD vaccination trials, leveraging peripheral lymphoid tissue in calves vaccinated and challenged with viral agents; such research improves our perception of vaccine efficacy and supports new concepts concerning BRD vaccine discovery and development research[29].

Metabolomics, the evaluation and quantitative analysis of small molecules produced during metabolism, provides a snapshot of metabolic states and biochemical transformation within a biological system[30,31]. Recent metabolomic research in Holstein calves has demonstrated that increased isobutyrate may detect *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection potentially earlier than current diagnostic methods, with further evidence that MAP infection is related to changes in fat metabolism and energy shortage compared to non-infected healthy cattle[32]. Within BRD-focused research, studies have provided possible diagnostic molecules to differentiate cattle undergoing wildtype viral challenge from those responding to attenuated viral vaccinations[33,34]. These advancements highlight the potential of metabolomics to enhance diagnostic development and insights into common infectious diseases of cattle.

Conclusion

Advancements in high-throughput bioinformatics and data analysis techniques have provided cattle health researchers with valuable tools to evaluate management practices, create innovative diagnostic and therapeutic solutions, and assess commonly used strategies in commercial production. Next-generation sequencing and omics approaches have deepened our understanding of infectious diseases in cattle by revealing the complex interplay of genetic, molecular, and microbial factors. Ongoing interdisciplinary collaboration and technological innovation will be crucial in applying these research insights to develop effective disease prevention and control measures in cattle production systems.

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Metagenomic characterisation of lower respiratory tract virome of pre-weaned dairy calves in Estonia - association with lung consolidations, clinical disease and inflammatory markers

Mõtus K.[1], Kirjonen H.[1]; Kaura R.[1]; Theuns S.[2]; Hettiarachchi A.[2]; Coppens S.[2]; Pardon B.[3]; Orro T.[1]

[1]Estonian University of Life Sciences, Tartu, Estonia [2]PathoSense BV,
Lier, Belgium
[3]Ghent University, Merelbeke, Belgium

Kerli Mõtus – kerli.motus@emu.ee

Objectives

Next-generation sequencing technologies applied in cattle have identified both novel and known viruses in the respiratory tract, though their pathogenicity often remains unclear. This study aimed to characterize the lower respiratory tract virome of pre-weaned dairy calves in relation to lung consolidations, clinical disease and systemic inflammatory response.

Materials and Methods

Between January and April 2024, a case-control study was conducted during which 30 large-scale Estonian dairy herds were visited. Thoracic ultrasonography (TUS) was performed on pre-weaned calves having no prior history of respiratory disease and no exposure to live intranasal vaccines. From ten calves in each farm, tracheobronchial lavage (TBL) and blood samples were collected, along with clinical data. Five calves with lung consolidations of at least 1 cm (classified as diseased based on ultrasonography – TUS+) and five without consolidations (classified as healthy – TUS-) were included. Based on California scoring system (Love et al., 2014), calves with a total score of ≥5 were classified as clinically diseased (CL+); while those with a score below 5 were considered healthy (CL-). Nanopore sequencing was used to analyse the viral metagenomic profile of the TBL samples. From blood samples, fibrinogen (Fib), serum amyloid A (SAA), haptoglobin (Hp), interferon gamma (IFN-γ), interleukin 1 (Il1) and 6 (Il6) concentrations were measured. Random-effects logistic and linear regression models were applied to analyse the association between virus positivity and lung consolidation, clinical disease and inflammatory marker concentrations.

Results

The 300 calves included in the study ranged in age from 3 to 59 days, with a median age of 16 days. The most frequently identified viruses were bovine coronavirus (prevalence 36.0%), bovine rhinitis B virus (13.7%), picobirnavirus (8.3%), bovine rhinitis A virus (6.7%), rotavirus A (6.3%), bovine adenovirus (3.7%), and astrovirus (3.3%). At lower prevalences, the following viruses were identified: bovine respiratory syncytial virus (1.7%), influenza D virus (1.7%), norovirus GIII (1.3%), bovine adeno-associated virus (1.3%), kobuvirus (1.0%), nebovirus (0.7%), rotavirus C (0.3%), sapelovirus (0.3%), bovine picornavirus (0.3%), bovine enterovirus (0.3%), and parainfluenza virus 3 (0.3%).

The most prevalent viral coinfections were rhinitis B and bovine coronavirus (4.3%), picobirnavirus and bovine coronavirus (3.7%), rotavirus A and coronavirus (2.7%), and rhinitis B virus and rotavirus A (2.7%).

Calves infected with bovine rhinitis A virus had, on average, 2.6 times higher odds of being TUS+ (95% CI: 1.0–7.0, p = 0.056). Additionally, bovine rhinitis A virus infection demonstrated a trend toward increased serum Hp concentrations (p = 0.089). Bovine coronavirus was marginally more frequently detected in CL+ calves compared to CL- calves (OR = 1.9, 95% CI: 1.0–3.6, p = 0.064) and was significantly associated with increased serum Hp levels (p = 0.021). Calves infected with bovine respiratory syncytial virus exhibited increased levels of IFN-γ (p = 0.044).

Rotavirus A was less frequently detected in TUS+ calves, with an odds ratio of 0.3 (95% CI: 0.1–0.8, p = 0.021). Additionally, picobirnavirus infection showed a trend toward lower SAA concentrations (p = 0.074).

Conclusions

This study highlights the complexity of the calf lower respiratory tract virome and underscores the importance of expanding routine laboratory diagnostic capabilities to better understand and manage viral infections. Bovine rhinitis A virus, bovine coronavirus, and bovine respiratory syncytial virus emerged as likely important respiratory pathogens. In contrast, infections with rotavirus A, bovine adenovirus, picobirnavirus, and bovine rhinitis B virus appear to have limited or no harmful respiratory effects. Additionally, some of the detected viruses may have an enteric origin.

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Assessment of the impact of paromomycin at 150 mg/kg on gut microbiota in healthy calves

Pascal Butty[1], Ann~~e~~ Trotel[1], Damien Achard[1], Y. Jaquemet[2], L. Hernandez[2], J. Le Guennec[3], P-Y. Moalic[3], F. M'Zali[4]

[1] Ceva Santé Animale, Libourne, France
[2] Pigase, 227 chemin de Sapeins, 01480 Chaleins, France
[3] Bio Chêne Vert Finalab, 4 rue Théodore Botrel, 22600 Loudéac, France

[4] Aquitaine Microbiologie / ADERA, Bâtiment Bordeaux Biologie Santé, 2 rue Dr Hoffmann Martinot, 33000 Bordeaux, France

Objective

This study aimed to evaluate the effect of oral paromomycin administration over five days on the resistance development in the intestinal commensal microbiota of healthy calves.

Materials and Methods

Twenty-five healthy calves aged 12 to 21 days, sourced from seven French farms, were included in the study. They were housed collectively, fed milk replacers twice daily, and randomized into a treatment group [Gabbrovet Multi®, Ceva Santé Animale, 150 mg/kg daily for five days; n=23] or a control group without treatment (n=2). Daily monitoring included fecal consistency, depression scores, and appetite (evaluated on a 0-2 scale). Fecal samples were collected at four time points: before treatment (Day-1), during treatment (Day+4), and after treatment (Day+20, Day+36). Samples were immediately frozen at -80°C and transported to a microbiology lab for the isolation and analysis of commensal *Escherichia coli* strains. For each fecal sample, 20 purified and randomly selected *E. coli* colonies were isolated for microbiological assessments. To manage the high number of strains, related strains were grouped using mass spectrometry (MaldiTof Biotyper Compass Explorer software). Minimum inhibitory concentrations (MICs) for paromomycin were determined using a customized microdilution method (UMIC), along with aminoglycoside antibiograms following CLSI guidelines. Resistance evolution was tracked by comparing strain data at different time points relative to Day-1, using the CA-SFM kanamycin breakpoint for Enterobacteriaceae.

Results

A total of 1,780 *E. coli* strains were isolated and analyzed. On Day-1, commensal *E. coli* populations comprised 69% susceptible and 31% resistant clones. Treatment with paromomycin did not induce resistance in previously susceptible *E. coli* clones. Instead, the treatment eliminated a substantial proportion of susceptible *E. coli*, resulting in a temporary predominance of preexisting resistant and pathogenic clones within the intestinal microbiota. This disruption was short-lived, as susceptible clones began to reappear post-treatment. By Day+36, susceptible *E. coli* accounted for 40% of the population, compared to 7% on Day+20, 10% on Day+4, and 69% on Day-1.

Conclusions

No evidence of clonal resistance acquisition in commensal *E. coli* was observed during this study. MIC values for individual clones remained stable across sampling points. The paromomycin treatment effectively disrupted the microbiota by favoring preexisting resistant clones, but the flora gradually reverted to its initial composition after treatment cessation. The high bactericidal dosage used likely played a role in preventing resistance development. However, caution is advised for lower dosages (e.g., prophylactic use), which are known to increase the likelihood of resistance emergence.

NOTES PERSONNELLES

TRANSCRIPTOMIC OF LAMB´S ABOMASUM INFECTED AND NON-INFECTED WITH *HAEMONCHUS CONTORTUS.*

Luciana Morita Katiki[1], Bruna Gonçalves Santos Costa[2], Rodrigo Giglioti[1], Ana Claudia Alexandre de Albuquerque[3], Carolina Rodrigues Gimenez[2], Alessandro Francisco Talamini do Amarante[4], Helder Louvandini[2]

[1]Instituto de Zootecnia – Nova Odessa/SP – Brazil
[2]Centro de Energia na Agricultura/USP – Piracicaba/SP – Brazil
[3]Universidade Estadual Paulista/UNESP – Jaboticabal/SP – Brazil
[4]Universidade Estadual Paulista/UNESP – Botucatu/SP - Brazil

Email:lmkatiki@sp.gov.br
Tel:55-19-99718-4963

Gastrointestinal nematode infections are considered the most important disease of grazing sheep and due to increasing anthelmintic resistance. Studying the transcriptome from *Haemonchus contortus* infected and non-infected naïve lambs using RNA-sequencing can provide measurements of transcript levels associated to host response to this parasite. Naïve lambs (n=6) were infected with 3,000 larvae weekly from 3 to 8 months of age and other were kept naïve (n=6) without infection. Abomasum transcriptomes of 4 infected sheep was compared to abomasum transcriptomes of 4 non-infected. RNA samples were obtained from fundic abomasal mucosa and RNA-Seq datasets were generated using an Illumina HiSeq 2000 sequencer. The total of 19,093 evaluated genes, analysis revealed 20 significant differentially expressed genes (17 upregulated and 3 downregulated in infected relative to non-infected group (False Discovery Rate (FDR) ≤ 0.05; and Fold-Change (FC) of > ± 2.0). The enrichment analysis KEGG provided specific biological with upregulated gene pathway related to IL-17RB in infected sheep. This pathway plays an essential role in defending against extracellular infections. IL17RB (Interleukin 17 Receptor B) is a receptor for IL-17B and IL-17E (also known as IL-25). When IL-17E binds to IL17RB, it induces NF-κB activation, leading to the production of inflammatory cytokines such as IL-4, IL-5, and IL-13. This signaling is associated with promoting Th2-type immune responses, which are involved in defense against parasites reactions. IL17RB is primarily expressed in tissues associated with mucosal immunity. It is also expressed in certain immune cells, including eosinophils, mast cells, and T-helper cells. Results of this study offer insights into the abomasum transcriptome during *Haemonchus* infection and provide a better understanding of the key regulator genes involved in *H. contortus* infection in sheep.

Biography

Luciana Morita Katiki, DVM, MSc, PhD, researcher at Instituto de Zootecnia (São Paulo State Department of Agriculture) since 2005. Research area: small ruminant parasitology.

Respiratory microbiota description of small ruminants with Q fever antecedents by metabarcoding

Toledo-Perona R.[1], Gomis J.[1], Contreras A.[2], Toquet M.[1], Bailon N.[1], Quereda J.J.[3], Gómez-Martín A.[1*]

[1]Microbiological Agents Associated with Animal Reproduction (ProVaginBIO) Research Group, Department of Animal Production and Health, Veterinary Public Health, and Food Science and Technology, Faculty of Veterinary Medicine, Cardenal Herrera-CEU University, CEU Universities, Valencia, Spain.
[2]Department of Animal Health, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain.
[3]LisBio Research group, Department of Animal Production and Health, Veterinary Public Health, and Food Science and Technology, Faculty of Veterinary Medicine, Cardenal Herrera-CEU University, CEU Universities, Valencia, Spain.

*angel.gomezmartin@uchceu.es

The aim of this study was to investigate the upper respiratory tract microbiota of goats and sheep from two intensive dairy herds and one semi-intensive meat herd with a clinical history of Q fever. A total of 26 females (10 goats and 16 sheep) were sampled during the first week post-partum/abortion. The animals were divided into two groups: G1 (n = 14, females with abortions); and G2 (n = 12, females with normal delivery). Nasopharyngeal swabs were analysed using 16S rRNA gene metabarcoding to describe the respiratory microbiota present during the first week following delivery or abortion.

Overall, the reproductive symptomatology (G1/G2) did not significantly affect the respiratory microbiome diversity. No differences were observed between females regarding the microbial richness and evenness (alpha diversity) or in the bacterial community structure (beta diversity). Proteobacteria was the dominant phylum across both groups (50.23%), followed by Firmicutes (33.15%), Actinobacteriota (8.62%), and Bacteroidota (5.89%). No statistical differences were observed between G1 and G2 for the phylum taxa. The most prevalent genera were *Moraxella* (22.95%), *Mannheimia* (11.11%) and *Lactobacillus* (7.46%). A total of 20 genus from the *Lactobacillales* order, two genera from the *Bifidobacteriales* order and one from the *Brevibacteriaceae* family were observed in nasopharyngeal samples. Specifically, two genera from the lactic acid bacteria (LAB) bacterial group, *Aerococcus* and *Atopostipes*, were significantly higher for G2 compared to G1 ($P < 0.05$). Finally, bacterial species with the highest abundance were *Mesomycoplasma ovipneumoniae* (3.82%), *Moraxella boevrei* (2.14%) and *Lactobacillus brevis* (2.05%). Diverse potential probiotic species LAB genera were found: *Lactobacillus* (n = 18), *Streptococcus* (n = 6), *Weissella* (n = 3), and one specie from the genus *Facklamia*, *Leuconostoc*, *Bifidobacterium* and *Pediococcus*.

To the best of the authors' knowledge, this is the first study to investigate the respiratory microbiota of female small ruminants affected by Q fever. Although no significant diversity differences were observed between individuals, certain bacterial genera with potential probiotic effects exhibited higher abundance in normal delivery females but also globally in the respiratory tract compared to other anatomical sites, such as the reproductive tract in ewes (Swartz et al., 2014). The application of metabarcoding techniques to study the respiratory tract of goats and sheep infected with *C. burnetii* represents a novel approach in advancing the understanding of Q fever impact in this sector.

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DECIDE: Descriptive epidemiology of *Mycoplasmosis bovis* in four European countries

Vandewalle J.[1], Jourquin S.[1], Santman-Berends I.[2], Veldhuis A.[2], Guelbenzu M.[3], Hostens M.[4], Bokma J.[1], Pardon B.[1]

[1]Calf Health Research Group, Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

[2]Royal GD, Arnsbergstraat 7, Deventer 7418 EZ, The Netherlands
[3]Animal Health Ireland, 2-5 The Archways, Carrick on Shannon, Ireland
[4]Department of Animal Science, Cornell University, Ithaca, NY 14853, United States

Ghent University – Jolien.Vandewalle@UGent.be

Objectives: *Mycoplasmopsis bovis* is a leading cause of disease, mortality and antimicrobial use in all cattle industries worldwide. A better understanding of the epidemiological patterns of *M. bovis* over time can contribute to improved control and prevention. Therefore, the primary objective of this study was to describe the epidemiology of *M. bovis* in four European countries, and to determine the baseline prevalence and epidemiologic threshold. Additionally, effects of sample type, laboratory test and production type sampled between countries were described.

Materials and methods: Anonymized diagnostic test results from samples tested for respiratory pathogens in cattle were collected from Belgian (2017-2023), French (2017-2023), Irish (2021-2022) and Dutch (2018-2023) laboratories. In total, 12700 samples were tested for *M. bovis*. An epidemiological threshold was calculated both overall as for each individual country using the 95th percentile of positive sample rates. All periods meeting this threshold were considered as epidemic episodes. Duration of each episode was determined by the number of months the threshold was exceeded. The baseline was determined by the arithmetic mean of all pre-epidemic rates. The effects of sample type, laboratory test and production system were assessed by comparing the different rates between countries.

Results: In total, 23.9% (3040/12700) of the samples were positive for *M. bovis*, ranging between 11.7% and 38.8% in the different countries. The average positive sample rate per month was 24.6%, and varied between 13.3% and 40.8% over the observation period. In general, when calculated over all countries, the overall epidemiological threshold was 36.7%. When determined per country, the epidemiologic thresholds were 39.6%, 37.5%, 62.1% and 60% for Belgium, The Netherlands, Ireland and France, respectively. Considering the overall 36.7% threshold, 29 epidemics were observed over all four countries, each only lasting between one and three months. When using the country specific thresholds, 4, 2, 1 and 2 epidemics were observed in Belgium, The Netherlands, Ireland and France, respectively. In each country, duration of the epidemic using the country specific thresholds also varied between one and three months. Overall, the baseline prevalence of *M. bovis* was 23.7%. In all countries, autopsy was the most common sample type (50.9%), while PCR was the most frequently used diagnostic method (75.8%). For autopsy, the positive sample rate was 23.0%. The positive sample rate was 27.8% for PCR and 11.0% for culture. In France, 39.7% of the samples where from veal calves, whereas veal only represented 0 – 1.2% of the total samples in other countries. In Ireland, only PCR results on autopsy were included in the dataset, and 34.3% of the calves were sucklers, a production type that was not represented in other countries.

Conclusion: Although an epidemiological threshold could be determined, epidemics are limited in occurrence and duration. In addition, even when the threshold is reached, only a limited increase compared to the baseline prevalence was seen. Therefore, *M. bovis* should be considered an endemic disease requiring targeted diagnosis, control and prevention all year round. The prevalence can be substantially higher in production types like the veal calf industry, and the suckler calf industry as seen in Ireland. Higher positive sample rates were observed for PCR, potentially leading to higher thresholds in countries where PCR was dominantly used. Both production type and diagnostic tests used can influence the national surveillance data.

Association between housing, management and environmental factors and respiratory disease prevalence in dairy calves

Donlon J.D.[1], Mee J.F.[2], and McAloon C.G.[3]

[1]Teagasc Animal & Grassland Research Centre, Grange, Dunsany, Ireland
[2]Teagasc Animal & Grassland Research Centre, Moorepark, Fermoy, Ireland
[3]University College Dublin School of Veterinary Medicine, Dublin, Ireland

John.donlon@teagasc.ie

Objectives

Bovine respiratory disease (BRD) is detrimental to the health and welfare of dairy calves. Calf housing environment is commonly referenced as one of the major contributing factors to the prevalence of BRD on a given farm. However, in a recent systematic review by Donlon et al (2023) the evidence base for the relationships between housing environment factors and BRD was found to be lacking. Hence, the objective of this cross sectional study was to assess the association of calf housing design, environment and management with BRD (as diagnosed by lung consolidation and clinical signs).

Materials and Methods

In total, 83 dairy farms were each visited twice in total in autumn and in spring over three years. These farms were recruited via two methods, i). random selection via the Irish Cattle Breeding Federation, ii). private veterinary practitioners who were asked to refer farms with known historic BRD problems. At the autumn visit, the calf housing was surveyed, data loggers (continuous monitoring of temperature and humidity) were installed inside the calf housing and a survey of calf management practices was conducted with the farmer. At the spring visit calves were examined to diagnose the presence of BRD and environmental and calf samples were collected. In total 1,640 calves (20 per farm) between 4 and 6 weeks were examined using thoracic ultrasound (TUS) and the Wisconsin clinical score (WCS). Bacterial air load was quantified [using blood agar (BA) versus VRGB agar (VRGBA)] at three locations within the calf housing (alleyway, feeder, and middle of pen) using an impaction air sampler. Nesting scores (scale of 1 to 3;1 meaning a lying calves legs are fully visible and 3 meaning a lying calves not visible due to bedding) were assigned. Two multivariable generalized linear regression models (GLR) were constructed with prevalence of TUS lesions (score 3 or greater;at least a single patch of lobar consolidation) and positive WCS (aggregate score ≥ 5 or two or more scores ≥ 2) as the outcome variables, respectively.

Results

The descriptive results are presented first. The calf house survey revealed that vented sheeting was the most commonly observed inlet design (17 farms), while central ridge outlet and no outlet present were the joint most common outlet designs (29 farms each).The environmental data loggers indicated that in the week prior to calf examination in the spring the median within-calf house air temperature was 8.8 ^{O}C and relative humidity 76.7%. In total, 173 (10.5%) calves were classified as having complete consolidation of at least one lung lobe by TUS and 155 (9.5%) calves were diagnosed with BRD using the Wisconsin clinical score. The most frequently observed nesting score was 1 (on 33 farms). Results from the GLR models showed the ratio of bacterial air load (BA:VRGBA) in the middle of the calf pen was positively associated with BRD prevalence in both models ($p < 0.05$). Housing temperature exceeding 20 ^{O}C in the week prior to examination was also positively associated with BRD prevalence in both models ($p < 0.05$). In the WCS model, inlet design, feeding method (automatic feeder vs bucket vs teat feeder), milk type (milk replacer vs whole saleable milk vs whole saleable milk & milk replacer vs other), nesting score (score 2 & 3 protective),

mechanical ventilation and calf occupied area (positively associated) were all significant predictor variables for BRD ($p < 0.05$). In the TUS lesion model, the minimum temperature humidity index in the week prior to examination (negatively associated), number of calves in the housing (positively associated), outlet design, colostrum feeding method and colostrum source were significant predictors of BRD ($p < 0.05$).

Conclusions

The modeling carried out in this work highlighted several design and environmental factors in calf housing that play a role in determining the prevalence of BRD in preweaning dairy calves. Some of these may be modified to reduce prevalence of BRD but this will require further investigation.

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Intranasal vaccination of calves in the first week of life: duration of shedding of vaccine virus & effect on specific serum IgG titers

L. Gille, H. Kuijk, P.A.A. Penterman, G. Hoflack, G. Vertenten and B. Sustronck
MSD Animal Health Benelux, Boxmeer/Brussels, The Netherlands/Belgium

Linde.gille@msd.com

Objectives

Intranasal vaccination is possible shortly after birth, allowing for early prevention of viral pathogens such as bovine coronavirus (BCoV), bovine respiratory syncytial virus (BRSV) and bovine para-influenzavirus 3 (PI-3). However, PCR identification of pathogen presence in nasopharyngeal swabs and/or broncho-alveolar lavages shortly after intranasal vaccination with attenuated vaccines often raises the question whether the detected genetic material is originating from the vaccination or from a field infection with a wildtype strain. This study aimed to provide information on the duration of nasal shedding of vaccine virus after intranasal application of two live attenuated viral vaccines, one containing both strains of BRSV and PI-3, and one containing BCoV. Concurrent use of both vaccines was also tested to see the influence on shedding. Furthermore, the effect of nasal vaccination on serum antibody titers was verified.

Materials and Methods

A controlled longitudinal multicenter field trial was set up on three large dairy farms located in the Netherlands & Belgium. Multiple vaccination protocols were evaluated:

- Experimental groups

A total of 58 calves were randomly allocated to four groups and enrolled in the first week of life: Group RSP (n=17): vaccinated intranasally with vaccine A [Bovilis® INtranasal RSP™ Live], Group NASC (n=17): vaccinated intranasally with vaccine B [Bovilis® Nasalgen®-C], Group RSP+NASC: (n=17) vaccinated concurrently with both intranasal vaccines in a different nostril, Group Control (n=7): non vaccinated control animals

- Sampling

On inclusion, and weekly thereafter for 4 consecutive weeks a serum sample and a deep nasopharyngeal swab (DNPS) was taken. At the endpoint of the study (week 4), a non-endoscopic bronchoalveolar lavage (nBAL) was performed on all calves.

- Laboratory testing

Serum:

- Bovine respiratory syncytial virus (BRSV) antibody: ELISA (In-house).
- Bovine corona virus (BCoV) antibody: ELISA (BioX).
- Bovine parainfluenza 3 virus (PI3) antibody: ELISA (IDEXX).

NPS and nBAL:

- RT-qPCR for BRSV, PI3 and BCoV. In house vaccine strain specific RT q-PCR for all three vaccine strains.

Results

Specific serum antibody titers for the three viruses showed a typical decline from birth onwards. No significant difference between the experimental groups at any time point could be detected by repeated measures ANOVA.

BRSV vaccine and wild-type virus: On two farms, BRSV was already detected at the time of intranasal vaccination. On those farms, this wild virus circulation seemed to persist at a low level for two to three weeks. On the third farm, wild-virus BRSV was detected at two, three and four weeks in both non-BRSV vaccinated and BRSV vaccinated calves. In only one out of the 34 BRSV vaccinated calves the presence of vaccine strain RNA was detected in nasal secretions two weeks after the vaccination.

PI3 vaccine and wild-type virus: On one farm, PI3 wild type virus was detected before intranasal vaccination and persisted for three weeks. On all farms, PI3 vaccine virus RNA was detected in calves which received an intranasal vaccination containing PI3 for maximum of three weeks after the vaccination. Two calves of the control group also tested positive for vaccine virus RNA one to two weeks after vaccination. In one calf, wild type PI3 virus was detected in nasopharyngeal secretions and in lung lavage fluid four weeks after vaccination.

BCoV vaccine and wild-type virus: On farm A, wild type BCoV was detected one week after intranasal vaccination and persisted for the complete study period, whereas on farm B wild type BCoV was already detected before vaccination and persisted for three weeks thereafter. On farm C, neither wild type nor vaccine strain BCoV RNA could be detected during the entire trial period.

Conclusions

Intranasal vaccination resulted in PI3 vaccine strain RNA presence in nasopharyngeal secretions for a maximum of 3 weeks, with possibility to transfer to non-vaccinated calves.

Intranasal vaccination resulted in sporadic detection of BRSV vaccine strain RNA in nasopharyngeal secretions for a maximum of 2 weeks.

BCoV vaccine strain RNA was not detected in nasopharyngeal secretions from one week after intranasal vaccination onwards.

One month after intranasal vaccination no vaccine strain RNA was detected in BAL samples.

Intranasal vaccination had no effect on pathogen specific serum antibody titers.

Inflammatory markers as potential indicators for ultrasound-confirmed bronchopneumonia in calves

Kirjonen, H.[1]; Kaura, R.[1]; Pardon, B.[2]; Orro, T.[1]; Mõtus, K.[1]

[1]Estonian University of Life Sciences, Tartu, Estonia
[2]Ghent University, Merelbeke, Belgium

Helen Kirjonen – helen.kirjonen@emu.ee

Objectives

Currently, no reliable inflammatory marker has been established to identify cattle affected by bacterial bronchopneumonia who require antimicrobial treatment. This study aimed to evaluate the potential of three acute phase proteins — fibrinogen (Fib), serum amyloid A (SAA), and haptoglobin (Hp) – along with three cytokines – interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon gamma (IFN-γ) – to differentiate calves with lung consolidations from healthy calves, while also accounting for their clinical disease status.

Materials and Methods

Between January and April 2024, 30 Estonian dairy farms, each housing over 400 cows, were visited. A total of 502 calves with no history of respiratory disease were included in this cross-sectional study. All calves underwent clinical examination, thoracic ultrasonography (TUS), and blood sampling. According to the California scoring system (Love et al., 2014), calves with a total score of ≥5 were classified as clinically diseased (CL+), while those with a score below 5 were categorized as healthy (CL-). Similarly, calves were classified as TUS-positive (TUS+) if a lung consolidation of at least 1 cm in diameter was detected, and as healthy (TUS-) if no such consolidation was observed. For the analysis, the calves were divided into four distinct groups: "healthy" (CL- and TUS-), "upper respiratory tract disease" (CL+ and TUS-), "subclinical pneumonia" (CL- and TUS+), and "clinical pneumonia" (CL+ and TUS+). To assess the differences of inflammatory markers across calf groups, random-effects linear regression models, and a Tobit regression model (for IL-1) were applied. The log-transformed inflammatory marker served as the dependent variable, with calf group as the primary predictor variable. Calf age, presence of diarrhoea, navel inflammation, disbudding time prior to sampling, and administration of NSAIDs prior to sampling were all accounted for in the analysis. After Bonferroni correction, statistically significant associations were determined at 5% significance level. Receiver operating characteristic (ROC) analysis was performed to assess the diagnostic performance of APPs and cytokines in identifying thoracic ultrasound-confirmed bronchopneumonia at two consolidation depth thresholds (≥1 cm and ≥3 cm). The empirical cut-point estimation was conducted using the Youden method.

Results

Of the 502 calves, 49.0% were categorized as "healthy", 14.9% as having "upper respiratory tract disease", 22.3% as having "subclinical pneumonia", and 13.8% as having "clinical pneumonia". Statistically significant increase in Fib and SAA concentrations was observed when comparing the "healthy" group to the three disease groups. Compared to healthy calves, IL-6 concentrations were elevated in those with subclinical pneumonia, and serum Hp concentrations were significantly higher in calves with clinical pneumonia. Fib concentrations were higher in the "clinical respiratory disease" group compared to the "upper respiratory disease" group, but no significant difference was observed between the "subclinical respiratory disease" and "upper respiratory disease" groups. Serum SAA, Hp, and IL-6 concentrations showed no significant differences between calves with upper respiratory disease and those with subclinical or clinical pneumonia. No significant differences in IL-1 and IFN-γ serum concentrations were observed between the groups.

The optimal cut-points for predicting lung consolidation (≥1 cm) were: Fib at 5.6 g/l (AUC = 0.61), IL-6 at 16.7 ng/l (AUC = 0.57), SAA at 65.4 mg/l (AUC = 0.56), Hp at 279.6 mg/l (AUC = 0.55). The optimal thresholds to predict lung consolidation (≥3 cm) were: Fib at 5.6 g/l (AUC = 0.68), SAA at 94.6 mg/l (AUC = 0.58), Hp at 405.0 mg/l (AUC 0.56), IL-6 at 5.1 ng/l (AUC = 0.54).

Conclusions

The study revealed that systemic Fib, SAA, Hp, and IL-6 levels were significantly higher in pneumonic calves compared to healthy ones. However, these inflammatory markers showed limited effectiveness in distinguishing between calves with pneumonia and those with upper respiratory disease. Among the six markers tested, Fib demonstrated the greatest potential as a diagnostic tool for pneumonia detection. Nonetheless, the practical utility of APPs and cytokines for predicting pneumonia in calves is limited due to their inadequate performance. As highlighted in this study, their effectiveness may be constrained by between-calf variability, influenced by factors such as age, concurrent diseases, and variations in the progression and severity of pneumonia, all of which contribute to greater heterogeneity. Furthermore, differences in herd epidemiological conditions and individual responses to diverse pathogens are critical factors to consider.

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Randomized field trial in veal calves on the efficacy of NSAID treatment for pneumonia using ultrasonographic lung reaeration as a cure criterion

Chantillon L.[1], Jourquin S.[1,2], Pas M.L.[1], Debruyne F.[1], Lowie T.[1,2], Boone R.[3], Bokma J.[1,3], Pardon B.[1,2]

Salisburylaan 133 (entrance 7), 9820 Merelbeke, Belgium

[1]*Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

[2]*qTUS, Damme, Belgium*

[3]*Veterinary Practice Venhei, Kasterlee, Belgium*

laurens.chantillon@ugent.be

Objectives: This randomized clinical trial aimed to compare the efficacy of treatment between calves treated only with tulathromycin (NSAID -) and calves treated with both tulathromycin and ketoprofen (NSAID+) in veal calves, using lung reaeration on quick thoracic ultrasound (qTUS) as a cure criterion. Additionally, the effects of maximum consolidation depth on cure and healing time were evaluated.

Materials and methods: The trial involved 293 veal calves, aged 14 to 21 days, randomly assigned into one of two groups: one receiving both tulathromycin and ketoprofen (NSAID+; n = 147)) and the other only receiving tulathromycin (NSAID-; n = 146) on day 1 (7-day metaphylaxis). The NSAID+ group only received one injection with ketoprofen. Clinical scoring, using the California (Davis) score chart, and qTUS were performed on all calves on day 1, 5 and 9 post treatment. For calves with consolidation ≥1cm on day 1, additional follow-up was done on day 3 and 7 (every 48 hours). Regression of maximum consolidation depth <1cm was considered as a cure criterion. On each time point, maximum consolidation depth was used to categorize calves into four qTUS categories: healthy (full aeration, no consolidation), mild pneumonia (consolidation <1cm), moderate pneumonia (consolidation 1–2.5cm) and severe pneumonia (consolidation ≥3cm). Cure and healing time were compared between treatment groups.

Results: On day 1, 34.8% (102/293) of the calves had a consolidation ≥1cm. After 48 hours (day 3), cure was 28.9% (13/45) in the NSAID- group and 35.1% (20/57) in the NSAID+ group ($P = 0.67$). By day 9, final cure of the initial cases was 26.7% (12/45) and 35.1% (20/57) in the NSAID- and NSAID+ group, respectively. In both groups, cure was similar at all observation points ($P > 0.05$). Overall, full aeration at day 9 of calves with mild, moderate and severe pneumonia on day 1 were 54.5% (36/66), 18.3% (13/71) and 12.9% (4/31), respectively ($P < 0.01$). When compared to calves with mild pneumonia on day 1, odds to have consolidation ≥0.5cm by day 9 were increased for both calves with either moderate (odds ratio (OR) = 5.35; 95%CI: 2.47-11.59; $P < 0.01$) or severe pneumonia at that time (OR = 8.10; 95%CI: 2.55-25.74; $P < 0.01$). For the initial cases, median healing time was 6 days (Interquartile range (IQR) = 8; Minimum (Min)=2; Maximum (Max)=10) and was similar in both treatment groups ($P > 0.05$). Treatment duration for calves with moderate pneumonia (Med=6; IQR= 8; Min=2; Max=10) was lower than the median treatment duration of calves with severe pneumonia (Med=10; IQR= 5; Min=2; Max=10) ($P < 0.05$). Davis score was during the whole follow up period not significant different between treatment groups. Slaughter weight on week 13 and at the end of the production cycle, and average daily gain were not significantly different ($P > 0.05$) between the two treatment groups.

Conclusion: In this study, cure was low and ancillary treatment with ketoprofen did not effect ultrasonographic cure of pneumonia. Using ultrasound-based categorization systems are useful to assess treatment effects and can provide insights to reasons for treatment failure, as initial qTUS-scores were associated with both cure and treatment duration.

Use of thoracic ultrasound as a diagnostic tool to identify and evaluate corrective actions during an outbreak of *Mannheimia haemolytica* and bovine Coronavirus in a commercial dairy farm

Bertani Sabrina Elisa[1,2], Sala Giulia[3,4], Boccardo Antonio[1], Pravettoni Davide[1], Moroni Paolo[1], Gentile Arcangelo[2]

[1] Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy

[2] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy

[3] Department of Veterinary Sciences, University of Pisa, San Piero a Grado, Italy

[4] Centro di Ricerche Agro-ambientali "E. Avanzi", University of Pisa, San Piero a Grado , Italy

Bertani.sabrina88@gmail.com

Objective: Thoracic ultrasound (TUS) has been widely studied in recent years for the diagnosis of bovine respiratory disease (BRD) and is currently considered a valuable diagnostic method for field conditions despite some limitations (Buczinski and Pardon, 2020). In addition to its diagnostic applications, TUS is being explored to assess treatment success and monitor the health of replacement heifers (Fiore et al. 2022). This study aimed to evaluate how TUS can be used to detect early outbreaks of BRD in dairy herds.

Material and Methods: The study was conducted in an Italian Holstein herd of approximately 400 lactating cows between August 2022 and December 2024. Calves were housed in individual pens until 30 - 40 days of age, after which they were grouped and fed using automated systems. Colostrum was administered within 4 hours (4 Lt) and 12 hours (2 Lt) of birth. Cows were vaccinated against neonatal diarrhea pathogens, and calves received intranasal BRD vaccination in the first week of life, followed by parenteral boosters at 30 and 51 days. Passive immunity transfer was assessed between 2 and 7 days of life by measuring serum total protein (sTP) and categorized according to Lombard et al. (2020). Before grouping, all calves (25 – 35 days of age) underwent TUS screening, and scores were assigned according to Ollivet and Buczinski (2016). Calves with TUS scores <2 were considered healthy, those with a score of 2 were treated with meloxicam and rescanned only if they manifest clinical sign of BRD, and those with scores ≥3 received antibiotic treatment and were monitored after 3 weeks. In August 2023, the farm experienced a BRD outbreak caused by Mannheimia haemolytica and bovine coronavirus. The clinical outbreak was resolved by December 2023, with subclinical cases disappearing by March 2024. Data were analyzed using SPSS. Continuous variables were expressed as means ± standard deviations, while categorical variables were presented as frequencies and percentages. Changes in TUS scores over time were analyzed using the chi-square test, with statistical significance set at $p < 0.05$.

Results: A total of 599 calves were evaluated during the study. Of these, 458 (76.5%) had TUS scores <2, 106 (17.7%) had scores of 2, and 35 (5.8%) had scores ≥3. The mean sTP value was 5.97 ± 0.7 g/dL. A statistically significant increase in TUS scores was observed from April 2023, with score 2 increasing from 12.5% to 25% by June 2023 and peaking at 56.3% during the clinical phase of the outbreak in August 2023. Similarly, scores ≥3 rose to 23.1% in August 2023. Following the change of the timing of the vaccination and the change of the clinical BRD treatment, scores ≥3 declined by September 2023, while score 2 levels returned to baseline by January 2024 only after the implementation of a coronavirus vaccination protocol.

Conclusion: This study suggests that monthly TUS data analysis can provide early detection of respiratory pathogens in dairy herds, facilitating prompt intervention and management of pulmonary diseases.

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New pulmonary scoring at slaughter for assessment of respiratory disease impact in veal calves

Gardavaud[1] A., Hauray[2] K., and Arcangioli[1,3] M.-A.

1- Pathologie du bétail, VetAgro Sup, Université de Lyon, Marcy l'Etoile, France
2- Le Clair Matin, Bourg-En -Bresse, France
3- UMR Mycoplasmoses Animales, Anses, VetAgro Sup, Université de Lyon, Marcy l'Etoile, France *marie-anne.arcangioli@vetagro-sup.fr*

Veal calves are highly susceptible to respiratory diseases that can impact livestock productivity. Differently from swine health management (Leneveu *et al.*, 2009), only two studies used lung evaluation grid at slaughterhouse in calves (Leruste *et al.*, 2012; Schneider *et al.*, 2009).

Objectives

We created a pulmonary scoring grid, and scoring classes, to be used in slaughterhouse as a new tool to assess the impact of respiratory management (vaccination), in addition to technical data of carcass traits or to therapeutic index.

Material and methods

A grid scoring from 0 to 24 points was designed and three different attempts were made at slaughterhouse before the final design. The simplicity and robustness of the final grid were tested by comparison between two persons on one batch of 93 calves.

Correlation with calf fattening, conformation and color of carcasses was done for 13 different farms/lots and 565 lungs/calves. The lots were of 3 to 104 calves.

Four lung-score classes were defined to obtain sufficient theoretical counts to perform a Pearson khi squared test. These classes were constituted by grouping next grid scores (0-2; 3-5; 6-8 and 9-24) to respect a clinical gravity grading. After control of not normal distribution of results within a same herd/lot, Kruskal-Wallis test was used to analyze the correlations between these data and the grid results by herd/lot. Finally, a Pearson Khi squared test was used to assess the correlation of distribution between individual carcass traits and lung class result. P-values were calculated by simulation of Monte Carlo (five repetitions) in cases where the theoretical minimum size of five required to achieve the classical Pearson khi-squared was not achieved.

Results

Reproducibility was high, with correlation coefficient of 0.96 between the two observers. Only seven lungs on the 93 displayed scoring difference of three points or more.

At the lot level, the median lung score was comprised between 0 and 3.5, whereas individual lung scores ranged from 0 to 22. It was the same for the values of fat cover, muscle conformation or color of carcasses. Nevertheless, each herd/lot displayed different results.

At the individual level, the lung scores were correlated with the technical data except for the color of carcasses. High scores were more likely in degraded carcass classes (lean or classified P/EUROP).

Conclusions

This study confirms the impact of pulmonary diseases on carcass traits (Pardon *et al.*, 2013). By the other hand, the number of 0 class grades within the degraded carcasses' classes also confirms that other elements strongly impact them. Thus, the lung notation grid provides additional information and may confirm the influence of lung disorders on calves' technical results. It would be useful in monitoring a respiratory disease management measures like vaccination.

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Lung ultrasonography combined with liver position assessment for detecting respiratory disorders in limousin bull calves upon feedlot arrival

Stéfanie Bernheim, Alfredo Zanini

ZOETIS France 107 Avenue de la République - 92320 Chatillon, France
SELARL VETALLIER, 96 Grande Rue, 03420 Marcillat-en-Combraille, France
stefanie.bernheim@zoetis.com

Introduction

Respiratory disorders are a leading cause of morbidity and mortality in feedlot cattle, significantly impacting economic profitability and animal welfare. Early detection at feedlot arrival is essential to enable timely and targeted interventions. However, traditional diagnostic methods, such as clinical examination and thoracic auscultation, lack sensitivity and specificity, particularly in larger, more reactive breeds like Limousin bull calves. The logistical challenges posed by these animals, including their size, weight, and vivacity, necessitate screening protocols that are simple, rapid, and minimize the number of manipulations.

Thoracic ultrasonography has emerged as a valuable diagnostic tool, offering real-time visualization of pulmonary abnormalities associated with respiratory disorders. Despite its promise, its diagnostic scope could be enhanced by the inclusion of additional health markers. This study evaluates whether the integration of liver position scoring, used as a quick indicator of rumen fill on the right side, could complement thoracic ultrasonography. This approach offers a faster alternative to the traditional rumen fill scoring grid on the left side, potentially creating an efficient, “all-in-one” solution for assessing health status at feedlot arrival.

Materials and Methods

A cohort of 142 Limousin bull calves, aged 6 to 12 months, was assessed upon arrival at the feedlot between March and November 2024. Thoracic ultrasonography was performed using a Draminski iScan Multi device equipped with a 6 MHz convex probe (depth range: 12–18 cm). The thoracic area was clipped and moistened with 70% alcohol. Abnormal lung findings were scored on a scale from 0 (no abnormalities) to 4 (consolidation >3 cm). Simultaneously, liver position was evaluated on the right side, relative to the 7th rib. Scores of 0 or 1 indicated the liver was posterior to the 7th rib (suggesting reduced rumen fill). A score of 2 indicated the liver was at or anterior to the 7th rib (suggesting adequate rumen fill).

Rectal temperatures were recorded, and calves were classified as sick if their temperature exceeded 39.6°C and their liver score was ≤1. Diagnostic performance was evaluated using Receiver Operating Characteristic (ROC) curves, comparing unilateral and bilateral lung scoring with or without the liver score criterion.

Results

Baseline Comparisons were conducted to build various evaluations of the ultrasonography diagnostic performance. Temperature and Lung Abnormalities: Calves with severe lung abnormalities (scores ≥3) had significantly higher temperatures compared to those with mild abnormalities (scores ≤2) (Mann-Whitney U test, $p < 0.05$).

Temperature and Liver Position: a significant negative correlation ($r = -0.341$, $p < 0.001$) was observed between liver scores and temperatures, with calves having a liver score of 2 exhibiting lower temperatures.

Liver Score and Lung Abnormalities: Liver scores were negatively correlated with lung abnormality scores on both the right ($r = -0.451$) and left sides ($r = -0.271$, $p < 0.001$), suggesting that calves with better rumen fill (liver score = 2) were less likely to exhibit severe lung abnormalities.

Diagnostic Performance: Unilateral Lung Scoring (Right Side Only): using a right lung abnormality score ≥3 achieved an AUC of 0.73, with moderate sensitivity (52.2%) and specificity (84.0%). Adding a temperature threshold (>39.6°C) improved the AUC to 0.75, enhancing specificity (98.3%) but not sensitivity.

Bilateral Lung Scoring (Sum of Right and Left Scores ≥4): this approach improved sensitivity (65.2%) and achieved an AUC of 0.74 ($p < 0.05$, Wilcoxon test). When combined with a temperature threshold, the AUC increased to 0.81, with specificity of 95.8% and a negative predictive value of 93.4%.

Adding liver position assessment refined classification. Calves with a liver score of 2 were less likely to exhibit high temperatures or severe lung abnormalities, highlighting its utility as a rapid indicator of rumen fill and health status. As practical challenges the larger size and increased reactivity of Limousin calves posed logistical challenges for ultrasonography. However, liver position assessment, performed alongside right lung scanning, required no additional equipment or time, proving to be a simple and efficient method.

Conclusion

This study demonstrates that combining lung ultrasonography with liver position assessment significantly improves the accuracy and practicality of health screening in feedlot cattle. The integration of liver position as a rapid rumen fill indicator enhances the diagnostic performance of ultrasonography by providing complementary information about the animal’s health status. This combined approach offers a practical, all-in-one solution for assessing respiratory and general health in larger, more reactive animals, addressing the logistical constraints of handling beef calves. The findings strongly support adopting this protocol into routine feedlot arrival practices to optimize early detection of respiratory disorders and improve management outcomes.

Efficacy of an annual herd vaccination with a live BoHV1 marker vaccine under field conditions

Clara Bourel[1], Geert Vertenten[2]

[1]MSD Santé Animale, Beaucouzé, France

[2]MSD Animal Health, Boxmeer, Netherlands

Clara.conroy@msd.com

Objectives

Many large cattle herds in France have been positive for Bovine Herpes Virus type 1 (BHV-1) for many years. In France, each year, 1 of 5 BHV- 1 positive herds has an active circulation of the virus in the non-vaccinated animals as most infected farms only vaccinate the gE+ cattle. If implemented, total vaccination is often a biannual vaccination because of lack of studies with annual vaccination.

The objective of this study was to evaluate the efficacy in the field of a live marker vaccine against BHV-1 with annual booster, in agreement with the SPC, on 4 BHV-1 positive farms in France.

Material and methods

Farms :

The farms were chosen because of the high level of contamination by BHV-1. Some of them had been infected for years, with unsuccessfully eradication programs.

Farm 1 : Dairy farm with 2 automatic milking systems, infected by BHV-1 virus since 2006. At the inclusion in the study in June 2020, 370 females older than 12 months (heifers and cows) were present on the farm. The seroprevalence for BHV-1 was 70 % in this group.

Farm 2 : Dairy farm with a herringbone milking parlor, infected by BHV-1 virus since 2008. At the inclusion in the study in December 2021, 437 females older than 12 months (heifers and cows) were present on the farm. The seroprevalence for BHV-1 was 92 % in this group.

Farm 3 : Beef farm with genetic breeding (Charolais), infected by BHV-1 few months before the inclusion in the study in June 2020. In June 2020, 155 females older than 12 months (heifers and cows) were present on the farm. The seroprevalence for BHV-1 was 85 % in this group.

Farm 4 : Beef farm (Limousin), infected by BHV-1 since 2010. At the inclusion in the study in September 2020, 183 females older than 12 months (heifers and cows) were present on the farm. The seroprevalence for BHV-1 was 73 % in this group.

Antibody ELISAs

Sera of the animals older than 12 months were frequently analyzed by the Center for Diagnostic Solutions (MSD Animal Health, Boxmeer, The Netherlands). A commercial available ELISA that is specific for antibodies to gE of BHV-1 (HerdChek IBR gE Antibody ELISA; IDEXX) was used to detect animals infected with field virus. This test does not detect animals vaccinated with gE-deleted IBR marker vaccines, whereas cattle vaccinated with a non-marker (gE positive) IBR vaccine test positive.

Vaccine:

In addition of culling of the gE BHV-1 positive animals, the follow vaccination schedule with a live IBR marker vaccine (Bovilis IBR Marker Live; MSD Animal Health) was implemented at the inclusion onwards, with regular veterinarian visits:

- One intranasal vaccination of the calves older than 15 days
- One intramuscular vaccination of the calves older than 3 months, with a booster 6 months later
- Once a year (spring or autumn) intramuscular booster vaccination of the heifers and cows.
- Within 15 days post-arrival: Intramuscular vaccination of the purchased cattle.

Results

During the years after implementation of the control program (culling and total herd vaccination), the incidence of new seroconversions decreased and remained low. As a result, the herd seroprevalences decreased from 70% (farm 1), 92% (farm 2), 85% (farm 3) and 73% (farm 4) to 0%, 29%, 0% and 17% respectively in the group of animals older than 12 months. In December 2024, four years after the vaccination program was installed, two herd were certified free of BHV-1.

Conclusions

Despite the repeated failures in some farms for years, it is possible to eradicate Bovine Herpes Virus type 1 by implementing a clear and structured vaccination program. Annual herd vaccination according to the SPC of a live IBR marker vaccine, including culling of the infected animals, resulted in the eradication of BHV-1 under field conditions in herds with a high seroprevalence.

Seroprevalences of respiratory pathogens in German dairy herds: results of a nationwide survey

~~Thomas Breuer~~[1], Torsten Steppin[1], Andrea Lindner[2], Kathrin Jahn[2], Marcus Klawitter[1], Ulrich Löschner[1], Ansgar Busch[1], Luis Leon[1], Meik Becker[1]

[1]Zoetis Deutschland GmbH, Leipziger Platz 18, 10117 Berlin, Germany
[2] BioCheck GmbH, Mölkauer Straße 88, 04288 Leipzig, Germany

thomas.breuer@zoetis.com

Objective: Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 - Virus (PI3) and the bacteria *Mycoplasma bovis* (*M. bovis*) are central pathogens in the Bovine Respiratory Disease (BRD) complex. Although *M. bovis* frequently appears as a secondary pathogen, it can also act as a primary pathogen and is difficult to treat due to its inherent resistance to certain antibiotics. The goal of this epidemiological study was to assess exposure to these pathogens at individual-animal and herd level. Building on the data collected, evidence-based recommendations for prophylactic measures will be developed to sustainably improve respiratory health in German dairy herds.

Materials and Methods: Between 2015 and 2021, 239 cattle farms across Germany participated in this study. A total of 1,252 blood samples were collected from animals with a recent history of respiratory disease. The sampled animals included 66% calves from dairy farms, 25% adult dairy cows, and 9% animals of other ages. Sampling typically focused on five animals per herd, the exact number of samples per farm varied. 180 farms provided five samples (75.3%), 29 farms provided 1–4 samples (11.8%), 26 farms provided 6–10 samples (10.8%), and 5 farms provided 11–17 samples (2.1%). A total of 1,252 blood samples were analyzed in the laboratory (BioCheck GmbH, Leipzig, Germany) using an ELISA test (Multiscreen Respiratory ELISA Kit for Bovine blood sera and plasma, Bio-X Diagnostics, Rochefort, Belgium) to detect antibodies against BRSV, PI3, and *M. bovis*. A descriptive analysis was performed at both the individual-animal and farm levels. A farm was considered positive for a given pathogen if at least one of its five samples tested positive. Information about size and structure of the facilities, mobility and mortality associated to BRD, vaccination regimes and management programs were not available.

Results: The screen shows that both BRSV and PI3 are highly prevalent at the farm level, with 95% of the 239 farms having at least one positive sample. In contrast, the proportion of farms testing positive for *M. bovis* was lower, at 48.5% (116 farms). Only two farms (1%) were free of all three pathogens, while 45.1% of the farms tested positive for all three pathogens.

At the individual-animal level, 73.7% and 79% of the 1,252 tested cattle showed evidence of contact with BRSV and PI3, respectively, whereas 24.5% tested positive for *M. bovis*. Despite the lower prevalence of *M. bovis*, it was detected in almost half of all farms and in about a quarter of the sampled animals.

In 55.5% of the farms testing positive for PI3, all the sampled animals were positive. Among the BRSV-positive farms, this was true in 43.2% of cases, and among the *M. bovis*-positive farms, in 22.4%.

Conclusions: Overall, the results clearly indicate that BRSV and PI3 are widespread at farms and animals and that *Mycoplasma bovis* has a significant relevance in many herds. Considering that *M. bovis*-infections are also subclinical, the presence of the pathogen can be assumed as higher.

Because *M. bovis* is not immediately identified as a cause of respiratory infection in calves - due to its natural antibiotic resistance and its pathophysiology - initial antibiotic therapy may not be fully effective. This can lead to the development of *M. bovis*–related clinical manifestations (such as lung lesions, arthritis, or otitis media), which often require long-term treatment and thus increase overall antibiotic use. Therefore, based on the data presented, it is recommended to implement prophylactic vaccination strategies against both BRSV and PI3 as primary viral pathogens. Additionally, vaccination against *M. bovis* should be considered to prevent secondary complications.

How many cells should be counted when performing a differential count on bovine non-bronchoscopic bronchoalveolar lavage fluid?

Justine Clinquart[1], Thomas Lowie[1], Stan Jourquin[1], Bart Pardon[1]
[1]Department of Internal Medicine, Reproduction and Population Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke-Melle, Belgium
Ghent University – Justine.clinquart@ugent.be

Objectives: Noninfectious airway inflammation, for example, due to high levels of particulate matter, can predispose to airway infection. The most accessible method to assess airway inflammation in living animals is cytologic analysis of bronchoalveolar lavage fluid. However, this is a labor-intensive method due to the high number of cells ideally included in the differential count. Therefore, the objective of this study was to determine if counting 300 cells would yield comparable results compared to 500 cells.

Materials and methods: Differential counts based on 300 and 500 cells were performed on 134 Hemacolor stained, cytospin preparations. Bland-Altman plots and calculation of beta gamma tolerance intervals were performed. For lymphocytes, bootstrapping was implemented to account for the non-normal distribution. The maximal acceptable difference was set at 5% for macrophages and neutrophils, and 2.5% for lymphocytes.

Results: By the calculation of beta gamma tolerance interval it was predicted with 90% certainty that 95% of future results would lie in the interval of -3.99% to 3.76% for macrophages, -3.39% to 3.74% for neutrophils, -1.78% to 1.62% for lymphocytes. Tolerance intervals were not calculated for eosinophils due to their distribution and a slight proportional bias. The maximum observed difference was 1.13%.

Conclusions: For diagnostic purposes, macrophages, neutrophils and lymphocytes enumeration based on 300 cells seems a reliable and less time-consuming alternative in comparison to the 500 cell differential count.

Comparative analysis of humoral immune response induced by different vaccines against bovine respiratory syncytial virus

Demil A.[1], Martinelle L.[2], Dourcy M.[3], Bayrou C.[4]

1. Anatomy, Department of Morphology-Pathology ULiège, Liège, Belgium
2. CARE-FePex ULiège, Liège, Belgium
3. Immunology-Vaccinology Laboratory, Department of Infectious and Parasitic Disease ULiège, Liège, Belgium
4. Bovine Health service, Clinical Department of production animals ULiège, Liège, Belgium

Corresponding author: calixte.bayrou@uliege.be

Objectives: Bovine respiratory syncytial virus (BRSV) classified within the *Orthopneumovirus* genus of the *Pneumoviridae* family (ICTV 2024). BRSV is a major cause of acute respiratory syndrome in calves of both beef and dairy breeds. It causes intense respiratory disease outbreaks in young animals every year, mainly during winter. BRSV has a global distribution and causes significant economic losses in the cattle industry. Our preliminary phylogenetic study identified circulating BRSV strains from subgroup II, and for the first time in Belgium, subgroup VIII, a recently described phylogenetic subgroup in Italy and Croatia. Vaccines against BRSV have been available since the late 1970s, with six vaccines currently on the market in Belgium: four live attenuated and two inactivated (phylogenetic subgroup II or III). However, the effectiveness of these vaccines in the field is uncertain, particularly with the emergence of this new viral strain. In this study, we assessed the immune response to vaccination.

Material and methods: Five BRSV vaccines were administered to three seronegative heifers. Serum samples were collected prior to vaccination and on days 14, 28, 56, and 84 post-vaccination. Sera were analysed using a commercial enzyme linked immunosorbent assay (SVANOVIR® BRSV-Ab ELISA) to quantify BRSV-specific IgG1 levels and serum neutralization assays (SN) were performed to evaluate the neutralizing capacity of the antibodies produced against one currently circulating BRSV strain (subgroup II). Analysis of data obtained from IgG1 levels was performed using one-way ANOVA and Tukey multiple comparison test. For SN test, the Kruskal-Wallis test and the Dunn's multiple comparison test were used. Values of $p < 0.05$ were considered significant.

Results: IgG1 levels increase from day 14 for all vaccines, adjuvanted vaccines induce higher IgG1 levels than non-adjuvanted vaccines with a significant difference for one multivalent inactivated vaccine ($p<0,05$). However, inactivated vaccines show the lowest serum neutralization values. The modified live adjuvanted vaccine has significantly higher SN values on days 14 and 28 ($p<0,05$)

Conclusion: Live attenuated vaccine strains from subgroups II or III demonstrate effective neutralization of subgroup II BRSV strain. We aim to isolate a field strain from subgroup VIII to perform additional serum neutralization tests using this recently described strain.

Does early vaccination against respiratory pathogens reduce antibiotic usage, compared to non-vaccinated animals under the same conditions?

Driesse M.[1], Bakx A.[2]

[1]Boehringer Ingelheim Animal Health bv, Amsterdam, The Netherlands

[2]Veterinary practice DAP Thewi B.V., Vught, The Netherlands

Objectives

The objective of this case study was to investigate whether early vaccination of veal calves against Bovine Respiratory Disease (BRD) in the week of arrival can reduce antibiotic usage for respiratory disease, compared to non-vaccinated animals under the same conditions. Secondary performance parameters such as weight, feed efficiency and ultrasound lung scores were also evaluated.

Material and methods

Veal calves arriving on the farm on 22 and 23 November 2022 were housed into 2 identical barns (barn 1 and 2). The calves in barn 1 (group 1) were vaccinated on 25 November 2022 with a live intranasal vaccine (Bovalto® Respi Intranasal, Boehringer Ingelheim Animal Health Netherlands bv) containing Bovine Respiratory Syncytial Virus (BRSV) and Para Influenza 3 (PI3) and simultaneously with a systemic inactivated vaccine containing BRSV, PI3, *Mannheimia haemolytica* and Bovine Viral Diarrhea Virus (BVDV) (Bovalto Respi 4, Boehringer Ingelheim Animal Health Netherlands bv) and boosted with the inactivated vaccine 3 weeks later. Lung ultrasound was performed in the week of arrival and 10 weeks later. Samples for serology and nasal swabs were collected at different time points. Antibiotic usage and secondary parameters of calves in barn 1 and 2 were recorded until calves were slaughtered in May to June 2023. The antibiotic usage, secondary parameters and slaughter data of both barns were analyzed and compared with each other. The differences between actual and expected slaughter weight, corrected for weight at arrival, number of days fed, feed intake, breed and gender of calf, was compared between barns.

Results

In total 435 vaccinated calves in group 1 and 438 calves without vaccination in group 2. The origin of the calves was NW Europe, with only German calves in group 2. There were twice as many females in group 1 (68 vs 37) and higher number of beef types in group 2 (38 vs 66). Average weight at arrival was 47,9 kg for group 1 and 45,7 kg group 2. The mortality rate was 1,8% in both groups. The antibiotics usage (defined daily dose) for individual treatment of respiratory disease was 2x higher in group 2 (2,82 vs 5,55). The difference in number of individual antibiotic treatments per group was significant 29% vs 38%, for group 1 and 2 respectively. There was a trend for less repeat treatments and more healthy lungs (ultrasound) at 10 weeks for group 1. Less growth loss in calves with pneumonia at 10 weeks was observed in group 1 (1,91 vs 7,77 kg). The difference in actual versus expected slaughter weight was 3,2 kg in favor of group 1. There was a trend for lower "poor grade" animals and fewer lung condemnations at slaughter and a higher herd index value for group 1. Similar pathogens in both groups were found in nasal swabs. Results of serology show a stronger increase in titer for *Mycoplasma bovis* in group 1. Both groups seroconverted for *Mannheimia haemolytica* at different time points.

Conclusions

This case study has shown that early vaccination of veal calves against Bovine Respiratory Disease (BRD) can reduce antibiotic usage for respiratory disease significantly. And that vaccination contributes to less growth loss due to respiratory disease, a higher corrected slaughter weight and healthier lungs.

Investigating bovine coronavirus and respiratory pathogen prevalence in cattle: findings from southern Belgium

EVRARD Julien[1], DELOOZ Laurent[1], BLOCKX Zoé[1] and GREGOIRE Fabien[1]

[1] Regional Association for Animal Registration and Health (ARSIA) asbl, Ciney, Belgium

Corresponding author: julien.evrard@arsia.be

Objective

Bovine coronavirus (BCoV) is a causative agent of enteric and respiratory diseases in cattle. The role of coronavirus in the Bovine Respiratory Disease (BRD) complex is not clear and remains controversial. Limited data are available on its prevalence in Southern Belgium. The major aim of this study is to determine (1) the virus's prevalence brought by veterinary practitioners: carcasses for necropsy, nasal swabs, and broncho-alveolar lavage fluids, and (2) the association with other major bovine viral and bacterial respiratory pathogens: bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (PI3), *Mannheimia haemolytica* (MHA), *Pasteurella multocida* (PMU), *Histophilus somni* (HSO) and *Mycoplasma bovis* (MBO).

Material and Methods

From September 2023 to December 2024, all samples that arrived at the laboratory with a "BRD" anamnesis were tested using VetMAX™ Ruminant Respiratory Screening Kit. Samples from winter 2025 will be included in this study to provide for a better understanding of the situation. Until the end of December 2024, the laboratory tested the lungs of 577 bovine carcasses, 476 nasal swabs, and 111 broncho-alveolar lavage fluids. The size of the farm, the type of cattle and their age are also recovered, but the total number of current samples
(January 2025) does not allow for a relevant analysis.

Results

Currently, BCoV prevalence was 7.97% for carcasses, 13.51% for broncho-alveolar lavage fluids and 23.11% for nasal swabs.

On carcasses (autopsy), the apparent prevalence of other respiratory pathogens in BCoV-negative and BCoV-positive samples was respectively: 10.17% and 10.87% (BRSV), 4.71% and 4.35% (PI3), 20.53% and 28.26% (MHA), 36.16% and 41.30% (PMU), 19.77% and 28.26% (HSO), 25.42% and 32.61% (MBO).

On broncho-alveolar lavage fluids, the apparent prevalence of other respiratory pathogens in BCoV-negative and BCoV-positive samples was respectively: 7.29% and 0.00% (BRSV), 4.17% and 0.00% (PI3), 23.96% and 33.33% (MHA), 59.38% and 60.00% (PMU), 15.63% and 0.00% (HSO), 29.17% and 26.67% (MBO).

On nasal swabs, the apparent prevalence of other respiratory pathogens in BCoV-negative and BCoV-positive samples was respectively: 10.00% and 8.20% (BRSV), 3.64% and 4.37% (PI3), 47.27% and 35.79% (MHA), 73.64% and 64.21% (PMU), 30.00% and 24.86% (HSO), 31.82% and 16.94% (MBO).

Conclusions

These preliminary results indicate that BCoV is frequently found by PCR in filed samples in Souther Belgium, with the highest prevalence in nasal swabs (23.11%), followed by broncho-alveolar lavage fluids (13.51%) and carcasses (7.97%).

The association of BCoV with other respiratory pathogens revealed mixed results, with certain pathogens showing higher prevalence in BCoV-positive samples. Specifically, *Mannheimia haemolytica* (MHA), *Pasteurella multocida* (PMU), and *Mycoplasma bovis* (MBO) were more prevalent in BCoV-positive samples across various sample types.

These findings underscore the potential significance of BCoV in the BRD complex, although further research is required to establish its role conclusively. The inclusion of samples from winter 2025 will provide additional insights, enhancing the robustness of the analysis. This study highlights the importance of ongoing surveillance and comprehensive diagnostic approaches to better understand the epidemiology of respiratory pathogens in cattle.

Bovine respiratory pathogen associations in Holstein-Friesian dairy calves

H. Kuijk, L. Gille, P.A.A. Penterman, G. Hoflack, G. Vertenten and B. Sustronck
MSD Animal Health Benelux, Boxmeer, The Netherlands

geert.gerard.hoflack@msd.com

Objectives

Bovine respiratory disease (BRD) is a major health problem during calf rearing in many farms. The objective of this study was to obtain further insights into the importance of different pathogens circulating among very young Holstein-Friesian dairy calves. In addition, possible pathogen associations were explored.

Materials and Methods

A longitudinal study was performed from September 2023 until February 2024 and involved three large (≥ 600 lactating cows) Holstein-Friesian herds in Belgium (n=1) and The Netherlands (n=2). Respiratory samples, consisting of deep nasopharyngeal swabs from one week to one month old calves, were collected in those herds and submitted for pathogen detection. Pathogen detection was performed using semiquantitative real-time PCR tests targeting seven bovine respiratory pathogens: bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus type 3 (PI3V), bovine coronavirus (BCoV), *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasmopsis bovis*, and *Histophilus somni* (VetMAX™ Ruminant Respiratory Screening Kit, ThermoFischer Scientific inc.). The results were analyzed using R software (R Core Team, 2024). Mixed multivariable logistic regression models were constructed for each of the seven respiratory pathogens. As predictors, the PCR results of the other six pathogens besides the outcome pathogen were considered for the analysis. Herd was included as random effect in each model. Statistical significance was set at $p < 0.05$.

Results

In total, 286 deep nasopharyngeal swabs were collected during the study period. At least one pathogen was detected in 220 (76.9%) of those samples. In 78 of the positive samples (35.5%) only one pathogen was detected. Fifty-nine samples (26.8%) were positive for two pathogens, whereas 83 samples (37.7%) were positive for three or more pathogens. BCoV was the most frequently detected virus (82 positive/286 samples, 28.7%), and it was nearly always detected in conjunction with other pathogens, as it was the sole pathogen in only 1 sample (0.01%). PI3V was detected in 14.7% of the samples and in most cases multiple pathogens were detected in conjunction with PI3V (73.8%). BRSV was detected in 29 out of 286 samples (10.1%) and was the only pathogen detected in 12 of those 29 samples (41.4%). *Pasteurella multocida*, *Mycoplasmopsis bovis*, *Mannheimia haemolytica*, and *Histophilus somni* were detected in, respectively 51.7, 31.3, 9.44, and 5.6 % of the samples.

In the present study, a PCR positive result for BCoV was associated with an increased detection rate of *Mycoplasmopsis bovis* (OR 3.39, $CI_{95\%}$ 1.60-7.18, p=0.003) and *Mannheimia haemolytica* (OR 6.52, $CI_{95\%}$ 1.90-22.3, p=0.006). PI3V presence was associated with an increased detection rate of BCoV (OR 3.63, $CI_{95\%}$ 1.43-9.20, p=0.02). The detection rate of *Pasteurella multocida* increased in the presence of *Mannheimia haemolytica* (OR 4.36, $CI_{95\%}$ 1.22-15.60, p=0.024) and *Mycoplasmopsis bovis* (OR 3.84, $CI_{95\%}$ 1.12-13.2, p=0.032).

Conclusions

In most cases, multiple pathogens can be detected in deep nasopharyngeal swabs from young dairy calves. In this study in dairy calves under one month of age BCoV was the most frequently detected viral pathogen and in nearly all cases other pathogens were also detected in conjunction with BCoV. Significant associations between the presence of BCoV and the presence of *Mannheimia haemolytica* and *Mycoplasmopsis bovis* in nasopharyngeal secretions of Holstein-Friesian dairy calves were observed. *Pasteurella multocida* was the most frequently detected bacterial pathogen in young Holstein-Friesian dairy calves and its presence was significantly associated with the presence of *Mannheima haemolytica* and *Mycoplasmopsis bovis*. These observations could be useful for the implementation of specific combined preventive measures at the farm level.

Non-inferiority trial in veal calves on the efficacy of oxytetracycline and florfenicol treatment for pneumonia guided by quick thoracic ultrasound

Stan Jourquin[1,2*], Florian Debruyne[1], Laurens Chantillon[1], Thomas Lowie[1,2], Randy Boone[3], Jade Bokma[1,3], Bart Pardon[1,2]

[1]*Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*

[2] *qTUS, De Vage 7, 8340 Damme, Belgium*

[3]*Veterinary Practice Venhei, Geelsebaan 95-97, 2460 Kasterlee, Belgium.*

Objectives: This randomized clinical trial aimed to compare the efficacy of a quick thoracic ultrasonography (qTUS) individualized treatment length between oxytetracycline (OTC) and florfenicol (FF) in veal calves. Additionally, the study assessed the association of consolidation depth at treatment initiation with cure and treatment duration.

Materials and methods: The trial involved 320 veal calves, randomly assigned into one of two groups: one receiving OTC (n=160) and the other FF (n=160) on day 1 (2-day metaphylaxis). Clinical scoring and qTUS were done on day 1 and every 48 hours for a 10-day period. Regression of maximum consolidation depth to <1cm was used as a criterion for cure and to stop antimicrobial treatment, meaning that after day 1, only calves with consolidations ≥1cm were given further treatment. On each time point, maximum consolidation depth was used to categorize calves into four qTUS categories: healthy (no consolidation), mild pneumonia (consolidation <1cm), moderate pneumonia (consolidation 1–2.5cm) and severe pneumonia (consolidation ≥3cm). Cure, treatment duration and the number of antimicrobial dosages (NAD) were compared between treatment groups.

Results: On day 1, 30.0% (96/320) of the calves had consolidation ≥1cm, which increased to 50.9% (162/318) by day 9. After single metaphylactic treatment, cure was 20.9% (9/43) and 20.9% (9/43) in the OTC and FF group, respectively. Calves with severe pneumonia had lower odds to be cured after first treatment than calves with moderate pneumonia (Odds ratio (OR) = 0.17; 95% Confidence interval (CI): 0.04 - 0.63). By day 9, final cure of the initial cases was 27.9% in both the OTC- and FF-group. In both groups, cure was similar at all observation points ($P > 0.05$). Overall, final cure of all calves with either moderate or severe pneumonia during the trial was 41.2% (52/102) and 19.0% (12/63), respectively ($P = 0.004$). Median treatment duration was 4 days (Interquartile range (IQR) = 2-6; Minimum (Min)=2; Maximum (Max)=8) and was similar in both treatment groups ($P = 0.59$). Treatment duration for calves with moderate pneumonia (Med=6; IQR= 4-6; Min=2; Max=8) was lower than the median treatment duration of calves with severe pneumonia (Med=8; IQR= 4-8; Min=2; Max=8) ($P = 0.004$). When compared to calves with mild pneumonia on day 1, calves with moderate ($P = 0.01$) and severe pneumonia ($P < 0.001$) had significantly longer treatment durations.

Conclusion: In this study, cure was low and not different between both antimicrobials. Categorizing calves based on consolidation depth appears useful as both cure and treatment duration were different for the mild, moderate and severe group.

Evaluation of vaccine efficacy for bovine respiratory disease (BRD) by lung ultrasonography in dairy calves

Anastasia Lisuzzo1, Giorgia Taio 1, Ortensio Bonato 2, Arnaldo Azzolin 2, Nicola Morandi 3, Giacomo Catarin 1, Matteo Gianesella 1, and Enrico Fiore 1

1 Department of Animal Medicine, Production and Health, University of Padua, 35020 Legnaro, Italy - anastasia.lisuzzo@unipd.it; giorgia.taio@phd.unipd.it; giacomo.catarin@unipd.it; matteo.gianesella@unipd.it; enrico.fiore@unipd.it.
2 Veterinarian free practitioner - bonito.ortis@gmail.com; arnaldoazzolin@gmail.com.
3 Boehringer Ingelheim Italia, 20139 Milan (MI), Italy - Nicola.MORANDI@boehringer-ingelheim.com.

Contact person: Prof. Dr. Enrico Fiore, enrico.fiore@unipd.it.
Presenting author: Dr. Anastasia Lisuzzo, anastasia.lisuzzo@unipd.it.

Objectives: Bovine respiratory disease (BRD) can significantly reduce the health and welfare of dairy calves. Vaccination of farm animals remains one of the first and most important lines of defense against this disease. Lung ultrasonography (LUS) represents a new performant tool to detect BRD and to assess the lung lesions' evolution. To better characterize the vaccination efficacy with BRD evolution, a field study was performed using lung ultrasonography as screening and monitoring tool in vaccinated dairy calves.

Materials and methods: Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. A total of 149 Holstein Friesian dairy calves were enrolled from three dairy farms located in Veneto Region (Italy) during the pre-weaned period. Each animal received 10% of the body weight (BW) of colostrum within 4h after the calving. No animals were subjected to antibiotics against BRD pathogens. Animals were randomly divided into: Group CTR (control without vaccination; n=41); Group INT (intranasal vaccination; n=46); and Group VAC (intranasal and parenteral vaccination; n=62). All animals were evaluated with clinical examinations, blood sampling for biochemical analysis and electrophoresis, and LUS in four time points: T0 (10±2d), T1 (17±2d), T2 (31±2d for Group CTR and INT, 38±2d for Group VAC), T3 (45±2d for Group CTR and INT, and 52±2d for Group VAC). Immediately after T0, the groups INT and VAC received the intranasal vaccination (Bovalto®RespiIntranasal; Boehringer Ingelheim Animal Health Italia S.p.A.) against modified-live PI3V and BRSV. After 1 week (T1), the Group VAC received the first parenteral vaccination (Bovalto®Respi3; Boehringer Ingelheim Animal Health Italia S.p.A.) against inactivated PI3V, BRSV, and Mannhemia haemolytica to differentiate the administration time between the two vaccines. Furthermore, the Group VAC received the parenteral vaccination booster after 3 weeks from T1 (T2). Clinical examinations were used to establish Wisconsin (BRD≥4) score. The LUS was used to establish ultrasonography score (US; BRD≥3) and modified lung lesion score (LLS; BRD≥10.5). Only calves classified as healthy based on US at T0 were selected for statistical analysis (CTR, n=17; INT, n=28; VAC, n=31). A mixed model using the fixed effect of Group and Time. Animals were used as a random and repeated effect. A post-hoc pairwise comparison among least square means were performed using Bonferroni correction. The p≤0.05 was accepted as significant.

Results: There were no differences between groups in either clinical assessments or LUS at the beginning of the study. However, the VAC calves appeared with a condition of failure of passive immunity transfer at T0 based on total protein (<55g/L) and γ-globulin (<10g/L) with subsequent biochemical analysis. Despite this difference, the VAC calves improved in immune condition with maintenance of lung health status, while the CTR calves presented a worsening immune condition and lung lesions. Specifically, the CTR calves showed a progressive increase from T0 to T3 in US and LLS (US from 1.7 to 3.0; LLS from 3.1 to 11.1), both indicative of disease at the end of the trial. These data were positively associated with total hepatization area (from 3.6 to 25.8 cm2) and lactate (from 20.4 to 38.9 mg/dL), and negatively associated with total protein (from 58.9 to 49.1 g/L), α1-globulin (from 4.3 to 2.0 g/L), α2-globulin (from 5.9 to 3.9 g/L), β2-globulin (from 8.4 to 6.1 g/L), and γ-globulin (from 11.5 to 5.8 g/L). The INT calves showed an increase in US (T0: 1.4; T1: 1.6; T2: 1.8; T3: 1.6) and LLS (T0: 4.5; T1: 5.7; T2: 6.5; T3: 5.1) at T2 without changes in total hepatization area (~9.7±2.9 cm2) and lactate (~18.1±1.6 mg/dL) during the trial. The α1-globulin (~3.5±0.2 g/L), β2-globulin (~8.2±0.2 g/L), and γ-globulin (~7.5±0.6 g/L) did not showed changes over time despite a progressive increase was observed in total protein (from 53.4 to 57.4 g/L) and a decrease in α2-globulin (from 6.2 to 3.5 g/L). The VAC calves maintained the US (~1.4±0.2), LLS (~4.9±1.1), and total hepatization area (~4.7±3.2 cm2). Instead, a progressive decrease was observed in lactate (from 18.1 to 11.8 mg/dL), α1-globulin (from 3.9 to 3.0 g/L), and α2-globulin (from 5.1 to 3.4 g/L), and a progressive increase in total protein (from 47.7 to 53.1 g/L), β2-globulin (from 6.8 to 7.7 g/L), and γ-globulin (7.9 to 11.1 g/L).

Conclusions: In this field study in dairy calves, intranasal (Bovalto®RespiIntranasal) and parenteral (Bovalto®Respi3) vaccinations was effective in improving immune condition in calves having failure of passive immunity transfer involving maintenance of lung health assessed by lung ultrasonography.

Efficacy of a fixed combination of florfenicol and meloxicam to address lung lesions in veal calves with BRD

Anastasia Lisuzzo [1], Damien Achard [2], Alessio Valenza [3], Luca Cozza [4], Giacomo Catarin [1], Barbara Contiero [1], and Enrico Fiore [1]

1 Department of Animal Medicine, Production and Health, University of Padua, 35020 Legnaro, Italy - anastasia.lisuzzo@unipd.it; giacomo.catarin@unipd.it; barbara.contiero@unipd.it; enrico.fiore@unipd.it.
2 Ceva Santé Animale, 33500 Libourne, France - damien.achard@ceva.com.
3 Ceva Animal Health S.p.A., 20127 Milan, Italy - alessio.valenza@ceva.com.
4 Veterinarian free practitioner - cozzavet@gmail.com.

Contact person: Prof. Dr. Enrico Fiore, enrico.fiore@unipd.it. - *Presenting author*: Dr. Anastasia Lisuzzo, anastasia.lisuzzo@unipd.it.

Objectives: Bovine respiratory disease (BRD) is a syndrome affecting up to 61 % of veal calf herds. The diagnosis in field and treatment decisions are generally based on examination for BRD clinical signs. However, the low sensitivity and specificity can delay diagnosis and caused treatment failure. In contrast, lung ultrasonography is recognized as a more performant tool to detect BRD and to assess the lesions' healing process. To better characterize the BRD prevalence in veal calf herds and the healing process of lung lesions after treatment, a field study was performed using lung ultrasonography as screening tool and a combination of florfenicol and meloxicam for treatment.

Materials and methods: Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. A total of 84 veal calves of 30±9 days and 53±5 Kg were enrolled in the trial. Animal clinical and ultrasonography examinations were performed twice weekly for the first 60d and then weekly until 180d. Lungs were collected at the slaughterhouse to evaluate gross lesion prevalence. Clinical examinations were used to establish Wisconsin (BRD≥4) and California (BRD≥5) scores. The lung ultrasonography was used to establish ultrasonography score (US; 0-5 points score) and modified lung lesion score (LLS; BRD≥10.5). Cranial lesions depth (cm) was measured for each lesion of both lung sides. Animals with the US≥3 or cranial lesion depth ≥3cm and at least 1 clinical sign were treated with one subcutaneous administration of a fixed combination of florfenicol and meloxicam (40mg/Kg +0.5mg/Kg; Zeleris®, Ceva Santé Animale). Treated animals (Flor-Mel group) was monitored at+1, +3, +5, +7, +9, +11, and +14d post-treatment. The non-treated animals during the production cycle were classified as control group. The success (animals that improved after treatment), relapse (animals that required retreatment), chronicity (animals without improvements and with more than three treatments), and mortality (dead animals for BRD) rates of the treatment were evaluated until 45 days after treatment (45-day rates). Differences over time in the Flor-Mel group were assessed by PROC GLIMMIX procedure of S.A.S.-software using the effect of time, sex, breeds, and animal (random and repeated effect). A post-hoc pairwise comparison was performed using Bonferroni correction. Groups comparisons in disease prevalence and lung gross lesion were assessed by chi-square test. The average daily gain (ADG) was evaluated by one-way ANOVA for group effect. A p-value<0.05 was accepted.

Results: Flor-Mel and Control groups enrolled respectively 36 and 48 calves. The BRD prevalence was low and similar between groups at arrival (Control: 2.1% for Wisconsin score, 6.3% for California score, 0% for US, and 0% for cranial lesions depth; Flor-Mel: 0% for Wisconsin score, 2.7% for California score, 0% for US, and 0% for cranial lesions depth). No differences were also observed at the end of the production cycle in disease prevalence or ADG (Control: 0% for Wisconsin score, 0% for California score, 22.9% for US, 10.1% for cranial lesions depth, ADG 1.50Kg/d; Flor-Mel: 0% for Wisconsin score, 5.6% for California score, 22.2% for US, 20.7% for cranial lesions depth, ADG 1.44 Kg/d). Prevalence of lung gross lesion was also similar (Control: 20.8%; Flor-Mel 27.7%; General prevalence: 23.8%). Treatment success 45d-rate was 97.1%, chronicity 45d-rate was 2.9%, mortality 45d-rate was 0%, and relapse 45d-rate was 0%. Both clinical scores increased after treatment with a peak at +5d. Afterwards, both clinical scores decreased within 2 to 4 days (+7 to +9d after treatment). The US score was 4.7±0.3 at treatment and decrease at +3d (US=3.64), +5d (US=2.41), +11d (US=1.68) after treatment. The LLS was 15.6±1.9 at treatment and decrease at +1d (LLS=12.52), +5d (LLS=6.98), +7d (LLS=4.33) after treatment. The right cranial lesion depth was 5.0 cm at treatment and decrease at +3d (3.9 cm; -22%), +5d (2.1 cm; -58%), +11d (0.9 cm; -82%) after treatment. The left cranial lesion depth was 4.5 cm at treatment and decrease at +3d (2.5 cm; -40%), and +5d (1.3 cm; -71%) after treatment.

Conclusions: In this field study in veal calves, the frequent lung scan coupled with clinical examination led to early detection of BRD clinical cases, 5 days before most frequently used clinical scoring tools. Prompt treatment with a fixed combination of florfenicol and meloxicam (Zeleris®) was associated with a high success rate, an absence of relapse, a rapid healing of lung lesions, and a similar growth to control calves. In conclusion, targeted treatments following periodic clinical and ultrasonographic monitoring allowed an early diagnosis of active bronchopneumonia with relevant reduction in the prevalence of lung lesions at slaughter.

Effect of intranasal bovine respiratory vaccination on mortality, average daily weight gain (ADWG), treatment rate and use of antimicrobials in Finnish calf rearing unit

S. Nikunen[a], B. Sustronck[b], K. Mikkelsen[a], G. Vertenten[c], S. Ekström[d], E. Sandell[d]

[a] MSD Animal Health Nordics, Copenhagen, Denmark
[b] MSD Animal Health Benelux, Brussels, Belgium
[c] MSD Animal Health, Global Ruminant Biologicals, The Netherlands
[d] Snellmanin Lihanjalostus Oy, Pietarsaari, Finland

katja.mikkelsen@msd.com

Objectives:

As bovine respiratory disease (BRD) is common problem in calf rearing units in Finland, objective of our study was to investigate, if one intranasal vaccine given at birth farm, before transport to rearing farm, affected mortality and daily weight gain in calf rearing unit as well as treatment rate and use of antimicrobials during calf´s first 6 months.

Materials and methods:

Dairy farm with approximately 540 cows participated in this study as well as a calf rearing unit, which received 7 calves each week only from this same dairy farm. Control group consisted of 198 calves and the vaccination group 191, making total of 389 calves. The control group calves were not vaccinated while in vaccination group calves received vaccination against bovine respiratory syncytial virus and parainfluenza 3 virus [Bovilis RSP Live vet, Intervet International B.V.]. Calves were administered 1 ml of the vaccine into both nostrils within one week after birth.

Calves were fed with colostrum and transitional milk before they were transported to next unit at the age of approximately 3 weeks. They were weighed before transport. At calf rearing unit calves were fed milk replacer until approximately 2 months of age, in addition to concentrates and silage. Calves were moved from calf rearing unit at the age of approximately 6 months to finishing unit. At that moment all calves were weighed second time to obtain average daily weight gain during the rearing period. Mortality and antibiotic usage (defined daily dose, DDD) were recorded into the Finnish medicine recording system Naseva, from which DDDs and number of respiratory disease treatment episodes were collected. All treatments were individually administered and only consisted of oxytetracycline. R (R Core Team 2024) multilevel regression techniques were used to assess the associations between vaccination and ADWG as well as vaccination and used DDD in the calves.

Results:

The average age of calves at arrival to the calf rearing unit for control group was 19.0 (SD ±7.5, median 18.0) days and their average weight was 56.6 (SD ±9.9, median 56,0) kg. Same parameters for vaccination group were 21.6 (SD ±9.3, median 19.0) days and 55.5 (SD ±9.7, median 55.0) kg.

The average age at relocation was for control group 195.5 (SD ± 34.6, median 191.5) days and weight 205.0 (SD ±35.9, median 199.5) kg and for vaccination group 233.8 (SD ±42.1, median 227) days and 253.0 (SD ±46.5, median 242.5) kg respectively.

Average daily weight gain from arrival day until final weighing was in control group 843.8 (SD ±133.7, median 845.2) g/day and in vaccination group 933.8 (SD ±130,7, median 928.3) g/day. The final multivariable regression model with confounding factors age at transport, breed and gender showed that vaccinated calves grew 77.2 (95% CI 50.9-103) g/day more than non-vaccinated control calves ($p<0.001$). Resulting in a final weight, adjusted for age, of 15.9 kg (95% CI 9.61-22.1 kg) more compared to non-vaccinated calves ($p<0.001$).

Defined daily dose for calves in the control group was 4.3 DDD (SD ±5.1, median 3.2 DDD) and in vaccination group 3.9 DDD (SD ±4.3, median 6.5 DDD). The final multivariable linear regression model, with gender, breed, age at transport and age at final weighing as confounding factors, revealed that vaccinated calves received 1.4 DDD

(95% CI -2.5 - -0.37 DDD) less antimicrobials than non-vaccinated control calves (p-value < 0,01). Age at transport affected DDD by decreasing its value with 0.9 units by 1 day increase in age at transport (p<0.001).

No difference in the number of times the calves were treated for respiratory disease was observed between the vaccinated calves and the control calves.

No difference in mortality rate between the two groups, 3 calves died in vaccination group (mortality rate 1.57%) and 4 calves in the control group (mortality rate 1.99%) was demonstrated.

Conclusions:

In this study we found that the vaccination of calves with one dose of intranasal vaccine given within one week after birth at the farm of origin resulted in a significant increase in the average daily weight gain of the calves and in a significant decrease in the use of antibiotics. No effect on mortality or number of respiratory disease treatment episodes was observed.

To the authors knowledge this is the first study to show significant effects of an intranasal vaccination administered before transport to the calf rearing unit on both ADG and DDD in regards to BRD. More studies are needed to provide knowledge on other procedures than intranasal vaccination to improve the quality of the calf before entering the calf rearing unit.

Agreement among deep nasopharyngeal swab and non-endoscopic broncho-alveolar lavage sampling to detect respiratory pathogens in young Holstein-Friesian dairy calves.

H. Kuijk, L. Gille, P.A.A. Penterman, G. Hoflack, G. Vertenten and B. Sustronck
MSD Animal Health Benelux, Boxmeer/Brussels, The Netherlands/Belgium

Pleun.penterman@merck.com

Objectives

Deep nasopharyngeal swab (DNPS) and non-endoscopic broncho-alveolar lavage (nBAL) sampling are the two most frequently used methods in practice to identify airway pathogens in calves with bovine respiratory disease. The agreement between those two sampling methods is not well characterized in case real-time PCR tests are applied for the detection of viral and bacterial pathogens. The objective of this study was to evaluate the agreement between DNPS and nBAL for identification of viral and bacterial pathogens in young Holstein-Friesian dairy calves by means of PCR.

Materials and Methods

A cross-sectional study was performed from September 2023 until February 2024 and involved three large (≥ 600 lactating cows) Holstein-Friesian herds in The Netherlands (farm A and B) and Belgium (farm C). Respiratory samples, consisting of DNPS and simultaneously nBal from one month old calves, were collected in those herds and submitted for pathogen detection. Pathogen detection was performed using semiquantitative real-time PCR tests targeting seven bovine respiratory pathogens: bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus type 3 (PI3V), bovine coronavirus (BCoV), *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasmopsis bovis*, and *Histophilus somni* (VetMAX™ Ruminant Respiratory Screening Kit, ThermoFischer Scientific inc.). The results were analyzed using R software (R Core Team, 2024). Agreement among DNPS and nBAL sampling was evaluated by calculating the Cohen's kappa statistic (κ) and the proportion of positive agreement (popa) for each of the seven respiratory pathogens.

Results

In total, 57 calves were included in the study. Twenty-one calves from farm A, 16 calves from farm B and 20 calves from farm C. Distinct pathogen prevalences were observed on the three farms. On farm A, the only viral pathogen detected was BRSV (9.5% DNPS, 33.3% nBAL). Neither BCoV nor PI3V were detected on farm A. The dominant bacterial pathogen on farm A was *Pasteurella multocida* (52.4% DNPS, 57.1% nBAL). Additionally, *Mannheimia haemolytica* was also detected on farm A (9.5% DNPS, 4.7% nBAL). *Mycoplasmopsis bovis* and *Histophilus somni* were not detected on farm A. On farm B, the predominant viral agent detected was BCoV (75.0% DNPS, 31.2% nBAL). The other viral agent detected was PI3V (6.2% DNPS, 6.2% nBAL). BRSV was not found on farm B. The most prevalent bacterial species detected on farm B were *Pasteurella multocida* (93.7% DNPS, 93.7% nBAL) and *Mycoplasmopsis bovis* (75.0% DNPS, 100% nBAL). *Mannheimia haemolytica* was found in 50.0% and 25.0% of the DNPS and nBAL samples, respectively. All samples were negative for *Histophilus somni*. On farm C, BRSV (0% DNPS, 5.0% nBAL), BCoV (5.0% DNPS, 15.0% nBAL) and PI3V (0% DNPS, 10.0% nBAL) were all present at a low level. As in farm B, the most prevalent bacterial species found on farm C were *Pasteurella multocida* (100.0% DNPS, 90.0% nBAL) and *Mycoplasmopsis bovis* (60.0% DNPS, 85.0% nBAL). *Histophilus somni* was detected on farm C but only in 2 out of 20 nBAL (10.0%) samples. *Mannheimia haemolytica* was not found on farm C.

Good agreement between DNPS and nBAL sampling for the detection of *Mannheimia haemolytica* (κ 0.62, popa 66.7%) and *Mycoplasmopsis bovis* (κ 0.69, popa 84.2%) was found. For *Pasteurella multocida* moderate agreement (κ 0.40, popa 87.9%) between DNPS and nBAL samples was observed. No agreement was found between DNPS and nBAL samples for *Histophilus somni* (κ 0.0, popa 0.0%). For the viral pathogens fair, poor and no agreement was found between the DNPS and nBAL samples for BCoV (κ 0.25, popa 38.1%), BRSV (κ 0.15, popa 20.0%) and PI3V (κ -0.03, popa 0.0%) respectively.

Conclusions

On large Holstein-Friesian herds, distinct circulation patterns of respiratory pathogens can be observed among young dairy calves. Therefore, tailor made preventive approaches on herd level, based on diagnostic sampling are justified. In young Holstein-Friesian calves, consideration on which diagnostic sampling technique to apply is recommended since the agreement between sampling methods is not always evident. The results of our study suggest that DNPS and nBAL sampling methods are similar in their ability to detect *Mannheimia haemolytica*, *Pasteurella multocida* and *Mycoplasmopsis bovis* in young dairy calves. In contrast, the viral agents BRSV and PI3V seem to be more readily detected in nBAL samples, whereas BCoV is more frequently present in DNPS samples in young dairy calves. Inconclusive results were obtained for *Histophilus somni*, probably due to its very low prevalence in the present study. Since in most cases the etiologic agent involved is unclear, a combination of both DNPS and nBAL sampling seems relevant to increase the sensitivity of the diagnostic procedure.

Prevalence of bovine coronavirus on farms with respiratory disease in Northern Germany

K. Stemme

*Kirsten.stemme@msd.de

[1] MSD Tiergesundheit, Intervet Deutschland GmbH, Feldstraße 1a, 85716 Unterschleißheim, Germany

Objective

Bovine coronavirus (BCoV) is known to cause enteric infections such as neonatal diarrhoea in calves and winter dysentery in adult cattle. In recent years, its role in respiratory disease has been increasingly investigated. A high prevalence of BCoV was detected in nasal swabs in European dairy herds (Berge and Vertenten, 2022). However, no data are available for Germany on the prevalence of respiratory BCoV in dairy herds. Therefore, the present study was performed.

Material and methods

The study was carried out on 28 dairy farms with a history of respiratory diseases in the northern part of Germany. Average size of the farms was 250 (50-700) dairy cows. Female calves were mainly reared on the same farms. On each farm, 10 nasal swabs from newborn calves (< 3 weeks), 10 nasal swabs from recently weaned calves and 5 nasal swabs from fresh cows were collected for BCoV detection. For analysis each 5 swabs were pooled into one sample. Samples were sent and analyzed in the same laboratory using semiquantitative real-time PCR (RT-PCR; based on Kishimoto et al. 2017).

Results

The presence of BCoV specific RNA was confirmed in 43 out of 139 (30.9 %) pooled samples. At farm level, detection was possible in 19 out of 28 farms (67.9 %).

On average, 2 out of 5 samples tested were positive for BCoV specific RNA on the farms where the virus was detected. On six farms only one sample was positive, while on one farm the virus specific RNA was found in all 5 samples. Regarding the size of the herd, there was no correlation between the number of cows and positive results.

In terms of age groups, virus specific RNA was mainly detected in calves. In newborn calves under 3 weeks of age 35.7 % (20 out of 56) and in weaned animals 33.9 % (19 out of 56) of the samples were positive for BCoV specific RNA. In cows, only 4 out of 27 pooled samples (14.8 %) were positive.

Conclusion

The results of the study indicate that BCoV specific RNA is commonly present in nasal swabs in dairy herds in Northern Germany with a history of respiratory problems. Therefore, vets should consider BCoV in their diagnosis in respiratory diseases.

Reference

Kishimoto et al. 2017: Development of a one-run realtime PCR detection system for pathogens associated with bovine respiratory disease complex. J. Vet. Med. Sci. 79, 517-523

Case report: bovine coronavirus associated respiratory infections in rearing calves

Egon Thesing[1], Siegfried Marquardt[2], Geert Vertenten[3]

[1]: Intervet Deutschland GmbH, [2]: Veterinary practice Marquardt&Walter, Goch; [3] Global Ruminant Business Unit, MSD Animal Health, Boxmeer, The Netherlands

egon.thesing@msd.de

The respiratory significance of the bovine coronavirus (BCoV) has grown increasingly over the past few years (Vlasova 2021) and has now taken a firm place in the etiology of enzootic bronchopneumonia. A commercial vaccine (Bovilis®Nasalgen-C®) has been available in the European Union since October 2023. The following describes a pneumonia outbreak in calves in a dairy herd due to co-infection of BCoV and *Pasteurella multocida*. The affected animals were vaccinated against other pathogens at an early stage. In addition, this individual case is placed in the context of a prevalence study on the detection frequency of important viral respiratory pathogens under the same geographical and production conditions.

Materials & Methods

Mid-November 2023, 12 rearing calves aged 3-4 months fell ill with respiratory disease in a 300 hundred cow dairy herd on the Lower Rhine. Up to this point, all calves were clinically healthy. The general conditions in terms of barn climate, husbandry and feeding were good. At the age of 3 and 7 weeks, all calves were immunized with an inactivated, trivalent vaccine (Bovilis® Bovipast RSP / MSD Animal Health) covering the following pathogens: BRSV, Parainfluenza 3 Virus and *Mannheimia haemolytica*. Only calves in this age group showed typical signs of respiratory disease (fever > 39.5°C; tachypnoea, partly serous, partly mucosal nasal discharge). During diagnostic examination, a nasal swab (deep nasal swab; DNS) and a pulmonary irrigation sample (non-endoscopic broncho-alveolar lavage; nBAL) were taken of each affected calf for molecular biological investigations of several respiratory-relevant pathogens. Furthermore, blood samples were taken from these calves 3 weeks later. The corresponding serum was tested for different antibody concentrations relevant to the respiratory tract at the Center for Diagnostic Solutions, Boxmeer (Bovine Coronavirus (BCoV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 Virus (PI3), *Mannheimia haemolytica* (Mh), *Mycoplasma bovis* (Mb) and *Pasteurella multocida* (Pm)).

Additionally, to have an impression about the prevalence of important viral pathogens in Germany, data on the prevalence of bovine coronaviruses in similar conditions were collected from 2019 to 2024. Nasal swabs or lung lavage samples were obtained in 54 cases from dairy calves up to an age of 4 month with clinical signs of bovine respiratory disease. Each farm contributed 2 to 5 samples from various regions in northern Germany, which were analysed as pooled samples using viral polymerase chain reaction (PCR) in two different laboratories.

Results

In all calves, the clinical condition improved rapidly after the administration of antibiotic and anti-inflammatory therapy. In the molecular biological investigations for several respiratory-relevant pathogens, only the bovine coronavirus could be detected in a very high pathogen concentration in both compartments. The bacteriological examination of the pulmonary irrigation samples led to the detection of *Pasteurella multocida* in two animals. The findings from the direct pathogen detection could be confirmed by the serological results. All animals had very high BCoV antibody titers, while the other viral pathogens remained normal in this regard. Only the two calves with *Pasteurella multocida* detection in the nBAL also showed very high Pm antibody levels.

The detection rate of important viral pathogens in respiratory samples of dairy calves for BCoV, PIV-3, and BRSV were 63 %, 15% and 15 % respectively.

Conclusion

The results of this case report show that a pronounced clinical respiratory symptomatology can arise from a co-infection of BCoV and *Pasteurella multocida* in calves that are generally exposed to good conditions and are vaccinated against the classic respiratory pathogens.

Sampling of the lower respiratory tract with the non-endoscopic broncho-alveolar lavage method is a good option for the clarification of respiratory problems, especially for the bacteriological pathogen detection.

BCoV was more frequently identified through PCR than other respiratory viral pathogens as it is shown already in other European studies (Vertenten 2024).

Therefore, this underestimated pathogen must be etiologically included in the detection of respiratory diseases.

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Isolation of lactic acid bacteria from the respiratory tract of calves in cattle feedlots

Toquet M.[1], Pedralba A., Gomis J.[1], Bataller E.[1], Jiménez-Trigos E.[1], Toledo-Perona R.[1], Bailon-Larrañaga N.[1], Gómez-Martín Á.[1*]

[1]Microbiological Agents Associated with Animal Reproduction (ProVaginBIO) Research Group, Departamento Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Cardenal Herrera CEU, CEU Universities, Carrer Tirant lo Blanc, 7, 46115 Alfara del Patriarca, Valencia, Spain.

*angel.gomezmartin@uchceu.es

Bovine respiratory disease (BRD) is an infectious multi-factorial disease with a major economic impact on cattle feedlots. Predisposing factors, such as viral infections or environmental factors, are required for the animals to become infected by certain bacterial agents. Given the complex aetiology of the disease, antibiotics are often used inconsistently and can alter commensal bacterial populations of the nasopharynx and disrupt the balance of the microbiota. Moreover, the use of antibiotic therapy increases the risk of antimicrobial resistance (AMR). Probiotic bacteria, such as lactic acid bacteria (LAB), could be part of the commensal microbiota in feedlot calves, but negatively affected by stress during the fattening phase. Additionally, the use of antibiotics also could have a negative impact on LAB populations. Therefore, the aim of this study was to assess the presence of LAB in the respiratory microbiota of feedlot calves, healthy or chronically affected BRD, and their fluctuation during fattening, and to investigate a potential negative impact of antibiotics use on LAB.

For this purpose, a total of 97 samples were collected from 20 artificially-bred calves (15 days old), 20 naturally-reared and pasture-raised calves (7 months old), 7 dead calves, and 3 additional pasture-live fed calves undergoing antibiotic treatment for BRD. For the 40 healthy calves, one nasal swab per animal was taken, from both nostrils, the day after the entry of the calves in the fattening farm (T0) and 53 days later for the artificially-fed calves and 66 days later for the pasture-fed calves (T1). For the calves undergoing antibiotic treatment, swabs were collected before (T0) and two days after the treatment (T1). Regarding the dead calves, which presented a chronic case of BRD unresponsive to antibiotherapy and were euthanised for welfare reasons, an extra two nasal swabs were used for each side of the tracheal bifurcation. The swabs were transported at 4ºC to the laboratory and were introduced in microcentrifuge tubes containing De Man, Rogosa and Sharpe (MRS) broth. The tubes were homogenised using a vortex and 100µL of the solution were plated on MRS agar and incubated 48h in anaerobiosis at 37ºC. The rest of the volume was kept at 37ºC as a preculture and 50µl of this preculture were plated on new MRS plates if no colonies were isolated from a sample after 48h. Isolated colonies with different morphologies and colours were frozen at -80°C in cryotubes with 50% of MRS broth and 50% of glycerol.

Lactic acid bacteria were isolated from 10.5% of the artificially-bred calves with a concentration ranging from 10 to 300 CFU/mL at T0. Nevertheless, at T1, this percentage increased and 31.6% of the samples had a similar LAB concentration. In the pasture-raised calves, 94.4% of the samples showed a concentration of LAB ranging from 10 to 3,000 CFU/mL at T0 and T1. In the euthanised calves, isolation of LAB was observed in 85.7% of the nasal fossae samples and in 100% of the tracheal samples. The concentration varied from 10 to over 3,000 CFU/mL. In the case of pasture-raised calves treated with antibiotics, at T0, only one sample (33.3%) showed LAB on MRS plates. After antibiotic therapy (T1), 100% of the samples were positive for the culture of LAB with a concentration ranging from 10 to 300 CFU/mL. When using the preculture, LAB were isolated in 100% of the samples.

The calf respiratory tract hosts LAB as part of its commensal microbiota although the abundance of this bacterial population remains low, especially at an early age. This abundance, as well as the frequence of isolation, tend to increase with age. The use of antibiotics does not seem to affect the frequence of isolation, on the contrary, but the abundance of LAB appears to be lower in the treated pasture-fed calves, although the small number of antibiotics-treated animals included in this study limits this assessment of the interactions of antibiotics with LAB populations. Euthanised calves that were suffering chronic cases of BRD showed a frequence and concentration of LAB similar to healthy pasture-raised calves. As the number of AMR becomes alarming, bacterial populations with beneficial effects should be studied to develop alternatives to antibiotic treatment. In this sense, the LAB isolated and frozen during this study will later be assessed regarding their probiotic potential and AMR profile.

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D. BLACK[1]

Climate change and sustainability - the role of the practitioner – An opportunity and a responsibility

1- Vetsalus

Our planet is facing a catastrophic environmental crisis: climate change, biodiversity collapse, species extinctions, food and resource scarcity. The Intergovernmental Panel on Climate Change (IPCC) has stated that "the scientific evidence is unequivocal: climate change is a threat to human wellbeing and the health of the planet. Any further delay in concerted global action will miss the brief, rapidly closing window to secure a liveable future" [1]. Antimicrobial resistance may be just as great an issue, threatening the lives of many millions of people. We also know that 75% of new infectious diseases are zoonotic – many originating as a result of human behaviours at the animal-human-environment interface.

Livestock farming is integral to societies worldwide, contributing to food security, economic sustainability, and cultural heritage. However, the environmental impacts of animal agriculture, including greenhouse gas emissions, biodiversity loss, pollution, soil quality and water consumption, now demand urgent attention. Veterinarians, traditionally guardians of animal health, animal welfare and food security, working at the human-animal-environmental interface, are ideally placed to play a pivotal role in continuing the transition to sustainable and regenerative, climate-resilient food systems.

The increasing human population, estimated to reach 9.8 billion by 2050, coupled with rising demand for animal products, underscores the urgency of addressing the environmental footprint of animal agriculture [2]. Farmers, as custodians of our land, and supported by their professional advisers have a great opportunity to take the lead here, championing sustainable and regenerative farming systems, taking steps to protect our natural habitats and ecosystems, reversing the biodiversity crisis and mitigating climate change.

The veterinary professions (vets, nurses, technicians) have a unique One Health perspective regarding the interplay between animal, human and environmental health. We have a position of responsibility and influence and the potential to be a leading force for sustainability.

In a 2024 British Veterinary Association survey [3], 93% of the UK veterinary profession stated that sustainability was important to them while a joint survey by Vet Sustain and VDS [4] indicated that further information and support is sought. From 446 respondent practices, albeit a potentially skewed sample, only 72 (17.5 per cent) said that their practice had a policy regarding environmental sustainability while 240 (54 per cent) felt that a greater knowledge of sustainable solutions for veterinary practices would encourage their practice to create an environmental sustainability policy. Many things we do in clinical practice are designed to optimise patient welfare and outcome, in addition to maintaining health and safety of the veterinary team. Changes made for sustainability do not need to compromise these standards.

The author is a founder Director of Vet Sustain, a not-for-profit organisation based in the UK and created in 2019 by a group of veterinary professionals; the Vet Sustain team are mostly volunteers who dedicate their time to championing the health of humans, animals and ecosystems. The Vet Sustain vision is for the veterinary profession to be enabled as leading forces for sustainability, with a mission to enable and inspire veterinary professionals to continually improve the health and wellbeing of animals, people and the environment. A recent impact review of Vet Sustain undertaken by Human Behaviour Change for Life (personal communication) concluded that "Vet Sustain has become established as a key driver of sustainability within the veterinary sector, empowering professionals with the tools and confidence to integrate sustainable practices into their work. The organization has fostered a growing community of engaged professionals". Furthermore, the report identified that increased knowledge leads to increased confidence and thereby advocacy which drives change with a feedback loop to encourage further engagement (Figure 1).

Vet Sustain initially took inspiration from The United Nations Sustainable Development Goals (SDGs) which are "the blueprint to achieve a better and more sustainable future for all…addressing the global challenges we face, including those related to poverty, inequality, climate change, environmental degradation, peace and justice" [5].

Vet Sustain defines sustainability as meeting the needs of the present without compromising the ability of future generations to meet their own needs, supported by the pillars of environmental stewardship, social and economic equity, and animal health and welfare. And so, Vet Sustain compiled the following six interrelated Veterinary Sustainability Goals (VSGs) to highlight the roles of veterinary professionals in driving sustainability, and to unite our professions around the goals and actions required to address multiple connected challenges facing our society. It is notable that there is a significant overlap between the VSGs and the SDGs because the veterinary professions are uniquely placed at the human-animal-environment interface, and so we can address the SDGs, in our own work including in practice, policy, research, academia, government, non-governmental organisations and industry, and also in the sectors we influence, through the animals under our care.
Since the Veterinary Sustainability Goals were first defined, they have been further distilled into the "6 Ws";

Wildlife; Diverse and Abundant Wildlife
Conserve and enhance natural landscapes, habitats and biological diversity and abundance of wild terrestrial and aquatic plant and animal species.

Warming; Net Zero Warming
Implement and promote decarbonisation through energy efficiency, the generation and use of renewable energy, mitigation of global warming and sequestration of carbon.

Waste; A No-Waste Society
Minimise the usage and disposal of resources and materials, and support a transition to a circular economy.

Welfare; A Good Life for Animals
Safeguard and advocate for the health and welfare, in life and at the point of death, of animals under our care and those that are affected by human activity.

Wellbeing; Health and Wellbeing
Safeguard and enhance the physical and mental wellbeing of people and support a transition to livelihoods and lifestyles that are fit for the future.

Water; Enough Clean Water for All
Uphold best practice in fresh water conservation and protection to mitigate water stress and prevent water pollution.

In light of these challenges, the role of veterinarians must continue evolving to lead the transition towards sustainable, climate-resilient food systems while maintaining the highest standards of animal welfare, food security, and economic stability in the agricultural sector [6].

These are areas in which the veterinary professions have a unique perspective within a holistic approach, often described as 'One Health' [7]. As professions, we have a position of both responsibility and influence and the potential to be a leading force for sustainability. We can drive meaningful change at four key levels (Vet Sustain - Spheres of Influence 2024):

As individuals in the way we live our lives;
the choices we make as individual citizens in households; e.g., personal and household purchasing decisions, food choices, selecting a pension provider, travel choices, financial investments, selecting energy and utility providers, waste and recycling, animal care and welfare, and educating dependents.

In the operations of our veterinary businesses;
the decisions we make or influence within our veterinary workplaces; e.g., corporate social responsibility, workplace policies and processes, ethical supply chains, waste and recycling, sustainable pensions and investments, training and travel, energy utilities and waste, engaging team members and supporting wellbeing, measuring and monitoring impacts, engaging and educating clients on animal welfare and sustainable animal management practices, including biodiversity and land use.

As trusted advocates;
The influence we have on our local, social, and professional communities; e.g., involvement in professional associations and groups, engaging and educating communities on animal welfare, advocating for more sustainable animal management practices including biodiversity and land use, influencing educational institutions, and social media advocacy.

Through Leadership
Platforms we can leverage to influence wider society; e.g., Leadership positions and advocacy within NGOs, investor groups, commercial businesses, political groups and international institutions.

When considering the veterinary business aspects, there are several resources available such as The Greener Veterinary Practice Checklist from Vet Sustain [8] – this checklist highlights simple steps to embed sustainability into clinical practice; when done one step at a time, it is not difficult to do. Some examples of continued change for sustainability in clinical practice include;

Responsible resource use;
Responsible use of resources reduces the environmental impact, preserves resources for the future and can bring cost savings. A simple, and yet key, change for veterinary practices is switching to a green energy provider and promoting a 'switch off' culture.

Optimising waste management is a further key area that can reduce environmental impact. Reducing resource use is the first step in the waste reduction hierarchy [10], followed by re-use and recycling. A great example of a switch to a re-usable system is changing from single-use plastic sharps containers, which once full are incinerated along with their contents, to re-usable sharps containers.

A large proportion of healthcare waste is plastic. The use of plastics in medicine has revolutionised healthcare: their material properties have allowed invaluable equipment to be developed, however plastics have an environmental impact in their synthesis and disposal, or persistence in the environment. Currently, less that 5% of medical plastics are estimated to be recycled in the human field [9], whilst it is suggested that 45-64% are considered to be potentially recyclable; these data are not yet available for the veterinary sector.

Being sustainable in business operations;
Consideration should be given to clinical protocols to ensure they are optimal, lean and efficient. Travel associated with activities of the practice or hospital could be rationalised to reduce carbon emissions; both of supplies delivered and the delivery of veterinary services. This is a key area for the largely ambulatory farm vet – as ranges and availability of fast charging networks improve, a move to hybrid or all-electric vehicles is more attractive – or even hydrogen power. The use of technologies such as trackers to further improve farm visit efficiencies could be considered.

Use and advice on using medicines responsibly;
Appropriate use of antimicrobials, including antiparasitic agents, is essential to mitigate the development of antimicrobial resistance. The use of selective deworming and routine use of faecal egg counts in practice provides a leading example of responsible medicine use within the veterinary sector. We have a vital role as veterinary surgeons to make decisions about the treatment protocols we choose and how we educate the clients about appropriate pharmaceutic use and disposal.

Turning to the role of the livestock vet specifically, much of what we do and have done for a long time contributes to sustainability, but we rarely view our work through that lens. Some of the key ways vets contribute to sustainable and regenerative agriculture:

Healthy Farm Animals Have Less Environmental Impact;
Regenerative agriculture is a mindset (Figure 2) and vets have a role to encourage farmers we work with to adopt and transition to sustainable regenerative principles when appropriate. Regenerative agriculture principles embrace the fact that farmed animals are sentient beings, promoting animal welfare, ensuring that farmed animals have a good life and a humane death and discouraging the prolonged or routine use of unnecessary medicine interventions. A key and integral of regenerative agriculture is around soil health which is absolutely fundamental to increase carbon capture and storage in vegetation and soils. Healthy farm animals enhance productivity, reducing inputs, avoiding unnecessary losses and thus reducing the environmental impact of farming systems. Vets are the gatekeepers of veterinary medicines, and play a key role in minimising the use of therapeutic medicines, while promoting preventative medicines and practices. Selection and breeding of highly efficient animals, appropriate for the environment in which they are being managed will further reduce their impact from emissions. For example, utilising genomics and advanced breeding technologies to select and amplify the genetics of low methane emitting dairy cows [11]. Finally, ensuring the health of domestic animals reduces zoonotic disease risks to people and above all, the potentially catastrophic effects of disease spillover to naïve wildlife populations. [12]

Addressing the Challenges and Offering Solutions;

Farm vets as trusted advisors and advocates are integral to supporting sustainable and regenerative farming practices, and have a responsibility to be well informed of the many challenges faced by livestock agriculture. At the same time, through their wide-reaching influence, farm vets can offer solutions and lead conversations championing continued change. However, it can be difficult to distil out the facts and for vets to navigate the complexity and conflicting information regarding this subject. As advocates, vets can lead non-combative and constructive discussions amongst individuals or groups with opposing views particularly by taking a "challenges and solutions" approach [13].

Vets should avail themselves of evidence-based information and the key considerations when approaching these conversations with farmers and others, and to act as a starting point towards encouraging a more regenerative mindset. To do this well, we need to upskill ourselves beyond our clinical comfort zone to gain better understanding of (for example) grazing practices, farm ecology and leadership of change.

It is critical that vets are informed and understand the science, technology, terminology and relevant local and international legislation in this area. Few topics are simple when addressing sustainability holistically and all aspects are in a balance – for example maximising one area, such as carbon footprint per kg of output can readily impact other areas such as animal welfare or human wellbeing. So, it is important to understand the nuances and avoid "good versus bad" or "myth busting" type approaches

Advocating for Sustainable Food Production;

Veterinarians are in a unique and privileged position to play a leading role in continuing the transition to climate-smart practices and foster innovations in animal health, genetics and breeding, feeding, and husbandry to reduce environmental impact and protect biodiversity. As trusted experts, we are well-placed to engage with farmers to discuss their aspirations regarding sustainability and the challenges they currently face due to a changing climate. Veterinarians can provide education about the science of regenerative farming and climate change, the benefits of promoting biodiversity and offer guidance around legislation and policy changes. Furthermore, we can help guide changes to mitigate environmental impact and adapt to a changing climate including optimised feed and resource management, reduced waste, water conservation, adapted production species, improved soil health and transitioning to regenerative and agroforestry systems.

Raising Awareness and Advocating for a `Less and Better' Philosophy [14];

The detrimental impacts of animal agriculture occur within a backdrop of unsustainable farming practices, but also of overconsumption, particularly in middle and high-income countries. Current interventions, including investment in smaller-scale or regenerative livestock farms, as well as direct measures to mitigate emissions from animal agriculture like feed additives, have not yielded the desired impacts at the necessary scale or speed.

Take Home Message

Sustainable farming systems are essential in tackling the climate and ecological crises, and the vet-led team has an essential role to play. As veterinary professionals, we can have an impact at several levels; as individuals in the way we live our lives, as veterinary businesses within our local communities, and as trusted advocates advising clients on many aspects of sustainability. Veterinary professionals have a unique One Health perspective regarding the interplay between animal, human and environmental health. We have a huge opportunity to make a difference as well as a position of responsibility and influence. Veterinary professionals have the potential to be a leading force for sustainability, thereby safeguarding both animal welfare and environmental integrity for present and future generations alike.

We must all feel empowered to engage with sustainability: for the future of our planet and to ensure ongoing provision of outstanding veterinary care.

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NOTES PERSONNELLES

Risk factors for insufficient silage quality in German dairy farms

Hoedemaker M.1, Litjens I.1, Birnstiel K.1, Campe A.2, Hielscher M.3, Paul P.4, Stoll A.4, Do Duc P.1, Woudstra S.1, Steudtner B.1, and Adler F.2

1Clinic for Cattle, University of Veterinary Medicine
2Institute for Biometry, Epidemiology and Information Processing, University of Veterinary Medicine, Hannover
3Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, Free University of Berlin
4Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig-Maximilians University Munich

Conserved feed stuff such as silages are widely used as major components in modern dairy cow diets. Quite often silages do not meet the quality requirements which negatively affect palatability and feed intake and may cause severe disturbances of ruminal function and even intoxication with far-reaching negative impact on animal health. It was the aim of this study to identify risk factors for insufficient fermentation and microbiological quality of grass (G) and corn (C) silages obtained from dairy farms in three structurally different regions in Germany with intense dairy cow farming.

As part of a large cross-sectional study performed in Germany, data were collected on 666 dairy farms in the region North (N) (n=237), region East (E) (n=227) and region South (n=202), which stored their silages in bunker silos. During an one-time visit, samples of silages from open silos which were actually fed to the dairy cows were collected and the fermentation and microbiological quality was assessed. Furthermore, information of the silage management was obtained via an interview with the farmer. Fermentation quality was evaluated based on the content of butyric acid, acetic acid, dry matter (DM) and pH. With respect to fermentation quality, silages were categorized in acceptable (very good to good fermentation quality; 72-100 points) and deficient (mediocre to poor fermentation quality; 0-71 points). With respect to microbiological quality, silages were categorized in acceptable (score 1), slightly deficient (score 2), severely deficient (score 3) and spoiled (score 4). For statistical analysis, score 1 (acceptable) was compared with the combined scores 2-4 (deficient). Hyopthesis driven and following association and correlation studies, possible risk factors were identified and tested in univariable and multivariable mixed general linear models for their effect on fermentation and microbiological quality.

Overall, 1,475 silage samples were collected (n=843 grass silages (G), n=632 corn silages (C)). Almost all C had good fermentation quality (overall: 98.1%; N 97.8%, E 100.0%, S 96.1%), whereas in G, only 68.1% had good fermentation quality (N 72.5%, E 75.1%, S 55.3%). Acceptable microbiological quality was found in 58.1% of C (N 46.9%, E 73.5%, S 52.8%) and 75.6% of G (N 68.2%, E 79.4%, S 82.3%). For G, a crude ash content $\geq$10% DM was found to be a risk factor for low fermentation quality in N (OR= 2.8) and E (OR=2.6). Other risk factors were the lack of silage additives (N OR=3.5) and, unexpectedly, an even cutting surface (S OR=2.5). For C, no multivariable models could be built due to the lack of variation of the target variable. Univariable analysis did not reveal any risk factors for N and E. For region S, a crude ash content of $\geq$4.5% DM (OR 7.1) and a silo without side walls (OR 15.9) turned out to be risk factors for low fermentation quality. With respect to microbiological quality, an ensiling time of < 6 wks was identified as risk factor for G (N OR=3.9, S OR=5.9). For C, the model did not reveal risk factors in N, whereas in E, a short ensiling time of < 6 wks and the lack of side walls of the silo were risk factors (OR 3.6 and 3.2, respectively). For region S, the year of the visit (2018 vs. 2019) turned out to be a risk factor for deficient microbiological quality of C (OR 2.7).

Silage quality based on fermentation and microbiological characteristics differed between region and was better in region East than in region North or South. Whereas fermentation quality was better in C than in G, the microbiological quality was more often deficient in C than in G. Some of the investigated risk factors repeatedly turned out to effect silage quality negatively: a high crude ash content, lack of silage additives and silo without side walls with respect to fermentation quality, and short ensiling time and silo without side walls with respect to microbiological quality. Our results suggest that an improvement of harvesting techniques, ensiling management and silo construction might help to produce silages of good quality.

Association of AI-predicted milk yield residuals to behavioral patterns and transition success in multiparous dairy ows

C. Kemel[1], M. Salamone[1,2], B. Aernouts[2], I. Adriaens[2], G.Opsomer[1], P. Hut[3], M. Hostens[4,5]

[1]Faculty of Veterinary Medicine, Merelbeke, Belgium

[2]KU Leuven, Geel, Belgium

[3]Faculty of Veterinary Medicine, TD Utrecht, the Netherlands

[4]Cornell University, College of Agriculture and Life Sciences, Ithaca, New York

[5]Faculty of Bioscience Engineering, Ghent, Belgium

Objectives

The transition period between two lactations remains one of the most challenging periods during the productive life of dairy cows and is commonly known to be associated with diminished animal welfare and reduced economic performance in dairy herds. The development of data-driven health monitoring tools based on on-farm available milk yield data has shown potential in identifying health-perturbing events. Using data from the current lactation, comparing expected with realized milk yields makes monitoring the transition period difficult, due to the lack of milk data at the onset of lactation. For multiparous cows, an opportunity lies in the use of data from the previous lactation to predict the expected production of the next one. Recently, a random forest model (nextMILK model) was developed, using milk yield data from the previous lactation to predict the first test day milk yield after calving. The expected milk yield was subtracted from the actual production to calculate the milk yield residuals in the transition period (MRT). Results of previous research suggest that MRT at the onset of a new lactation is associated with the health and metabolic status of transition dairy cows. The main objective of this study was to link these MRT to different behavioral parameters, measured by two types of sensors of high-producing multiparous Holstein-Friesian dairy cows in the transition period.

Material and Methods

Sensor data from 2381 lactations on 8 commercial farms in The Netherlands were used in the study. Lactations of primiparous cows were excluded due to a lack of historical milk yield data. Cows were fitted with two types of sensors, where feeding behavior (eating time and rumination time) was monitored with the commercially available “Nedap Smarttag Neck” sensors (Nedap, Groenlo, The Netherlands) attached to the neck collar of each cow. The “Nedap Smarttag Leg” sensors were attached to one of the front legs of each cow, to monitor walking (standing time) and lying behavior (lying time). The MRT were calculated using the previously mentioned nextMILK model based on test day milk records of the previous lactation, which were registered with an interval of 4-6 weeks as part of the dairy herd improvement program of the Dutch breeding organization CRV (Arnhem, The Netherlands). Three milk residual categories (LOW, MED, HIGH) were subsequently defined, and behavioral differences between the categories were studied.

The data was analyzed using R. Behavioral data and MRT categories were used in linear mixed-effect models for statistical analysis, with behavioral parameters (standing, lying, eating, and rumination) as the dependent variable of interest. Unique herd, animal identifier, parity, DIM, calving season, and MRT categories were used as explanatory variables, with random effects for time relative to calving and lactation identifier respectively. Biologically relevant two-way interactions of DIM with parity, herd, calving season, and MRT category were also evaluated.

Results

Postpartum eating time for cows in the LOW MRT category was consistently lower compared to the HIGH MRT category, except on day 21, where differences were not significant. Significant differences were also observed between the MED MRT and LOW MRT categories during the postpartum period, except for a few days. Rumination time in the 21 days after calving was significantly lower in the category with the most negative milk yield residuals (LOW MRT) compared to the other categories. Lying times in the postpartum period varied between the different MRT categories, with small, but significant differences on dispersed days, where the lying time of the LOW MRT category was higher than both other categories. Standing times were highest in the LOW MRT group compared to both other groups after calving. In contrast to the differences observed between the different MRT categories in the postpartum period, our prepartum models showed no significant differences for any of the analyzed behavioral parameters.

Conclusions

Based on the present study, we found different behavioral patterns for eating, rumination, lying, and standing times for cows with different milk yield residuals in the transition period. Our findings strengthen the idea of using MRT as an indicator of transition success in multiparous dairy cows and to predict the cow’s adaptive capacity throughout the transition period. As such, this indicator might be a solid basis for the development of new health monitoring tools.

Evaluation of 7 critical success factors on 39 Belgian dairy herds by using an overall risk analysis tool as a herd management tool

De Bleecker, K., Van de Wouwer, E., van Mol, W., Bekker, G., De Graef, E.

Objectives

Critical Success Factors(CSF) help to make the dairy herd as a business operation more efficient and increase their profits while also setting goals. CSF are identified as milking, housing, feed and water management, animal welfare, disease-incidence, youngstock rearing and working routines. Key Performance Indicators (KPI)on the other hand help to see how well the management is staying on track with these CSF. Successful execution of CSF should generate a positive outcome on herd management and therefore on economic results of the farm. A Risk Analysis Tool, developed by Animal Health Care Flanders 5DGZ) to evaluate these 7CSF was used by the field veterinarians of DGZ. Results for the 7CSF were obtained by scoring 40KPI on more dan 100Management Control Points(MCP). Study objectives were to evaluate the evolution of these 7CSF which were scored during 5 consecutive farm visits on 39 Belgian dairy farms between 2021 and 2024.

Materials and methods

Results of the scoring of the 40KPI and 7CSF of 5consecutive farm visits, were during each visit in real-time submitted in a central mobile web application called ‘Farmfit’. This was performed on 16Belgian dairy farms in East-Flanders, 15 in Western Flemish Brabant and 8 in Wallonia with an average cattle population of 150 animals in total (range 100-240). Scoring was done on a scale from 1 to 5, with score 1 being labeled as very high-risk and score 5 as very low-risk. Time between visits was 6 months (grazing-stable season). A standard visit included a questionnaire for the farmer, a farm tour and a talk to conclude the scoring and to agree on action points to address high-risk KPI in order to improve the scoring of the CSF during a next visit. Analysis of the data (with Excel-Microsoft®)included an average score per CSF for all 39dairy herds for the 5visits and an evolution in mean scores between visit1 and 5 for all herds. Differences between small and bigger herds was evaluated for each individual CSF as well as differences between the 3regions within Belgium where these herds where located. Finally, an evaluation in score evolution was performed between the dairy herd with the highest risk on visit1 and the herd with the lowest risk on visit1.

Results

We found that average CSF scores for all herds and all visits for housing was 3.6, working routines was 3.5, feed and water management 3.5, milking 4.5, youngstock rearing 3.6, disease incidence 4.0 and animal welfare 4.3. Evolution between visit1 and visit5 showed that mean score for housing went from 3.5 to 3.7, for working routines from 3.2 to 3.7, for feed and water management from 3.0 to 3.6, for milking from 4.1 to 4.7, for youngstock rearing from 3.2 to 3.7, for disease incidence from 3.7 to 4.6 and for animal welfare from 3.9 to 4.8. Evaluation of evolution of mean scores between small(<150 animals) and big herds(>150 animals) showed no differences. Also, the evaluation of evolution in mean scores between the 3 Belgian regions showed no differences. Evaluation of the mean scores between visit1 and visit5 for the herd with the highest risk in visit1 showed an increase in total score for the 7CSF from 22 to 30. The same evaluation between visit1 and 5 for the herd with the lowest risk in visit1 showed and increase for the 7CSF from 28 to 31.

Conclusions

All 39 herds had a considerable low risk in average on the CSF milking, suggesting that the integrated Quality Control System (IKM- Integrale Kwaliteit Melk) within the dairy sector in Belgium, installed since 2000, has led to a better management on Milking. Youngstock rearing, feed and water management and workings routines showed a considerable higher risk than other CSF which suggest room for improvement in most of the 39 herds. Results show that the systematic approach of consecutive visits using the Risk Analysis Tool, coupled with agreed action points, led to higher scoring of CSF and to better performance results during the 5consecutive visits. This indicates that this approach according the Plan Do Check Act (PDCA) quality circle of Deming, led to a continues improvement of quality of the herd management. Finally, this Risk Analysis Tool, as performed on a mobile application on-farm, proved to be a good herd management tool for performance improvement.

How far can fecal material be transported on tires?

Tvistholm B.B. and Budde M. SEGES
Innovation, Aarhus, Denmark

Objectives: The structure in Danish cattle herds is evolving, with herd sizes increasing and more operations with multi-sites. The average Danish dairy herd now consists of 250 dairy cows. With larger herd sizes, there are more transports to and from the herd. Many Danish dairy farms lack efficient logistics and biosecurity, leading to potential cross-contamination between internal and external transport. This study aims to determine how far fecal material can be transported on truck tires, depending on the season and road surface

Material and Methods: The study was conducted on a dairy farm in western Jutland and was done at two different days - in June on a sunny day (24°C) and in December on a misty day (2° C). One front tire of the truck was covered with 8 l of cattle slurry taken from the farm's storage tank where fresh slurry was added daily. An area of 10x10 cm was swabbed immediately after with a sterile cotton gaze swab (10x10 cm) moisturized with sterile buffered saline solution (0.9 %). The swabs were repeated after driving 10 m, 50 m, 100 m, 250m, 500 m and 1000 m on a gravel road. After each swab a mark was placed on the tire to avoid swabbing the same area twice. The procedure was repeated on the other front tire and then driving on asphalt. The swabs were cultured for E-coli using NMKL125 at Eurofins Steins Laboratorium A/S (Ladelundvej 85, 6600 Vejen, Denmark). E-coli was chosen as an indicator bacterium for fecal contamination.

Results: There was a difference in the initial sample taken from the tire immediately after applying slurry between summer and winter. In summer, there were 29,000 and 18,000 cfu/swab on each front tire after applying slurry. On asphalt, there was a rapid reduction in E. coli numbers, reaching the lowest detection limit of less than 225 cfu/swab after 100 m. On gravel roads, the decline was less pronounced, with the lowest detection limit reached at 500 m.
In winter, the slurry contained a much higher number of E. coli. The initial sample contained 211,500 cfu/swab for gravel road testing and 130,500 cfu/swab for asphalt testing. There was a steep decline in E. coli numbers, reducing to 42,750 cfu/swab after 25 m on gravel and 13,500 cfu/swab on asphalt. After 1000 m, there were 225 cfu/swab on both road types. The decline in E. coli count was more pronounced on asphalt. Despite the overall decrease, there were sudden rises in E. coli numbers during the tests on both road types, possibly due to uneven slurry application or new contamination from fecal material on the road or the fender liner.

Conclusion: Fecal material, and therefore potential pathogens, can be transported on truck tires. This study investigates how road surface and season affect the number of E. coli found on tires. The results show that
E. coli numbers decrease faster on asphalt compared to gravel. In summer, the lowest detection point (225 cfu/swab) was reached at 100 meters on asphalt and 500 meters on gravel. In winter, the lowest detection point was reached after 1000 meters on both surfaces. However, the E. coli content in slurry was approximately 10 times higher in December compared to June. This indicates seasonal differences in E. coli content, with higher numbers in winter. Due to the higher initial E. coli levels in winter, it is not possible to directly compare the seasonal effects on the distance fecal material can be transported on tires.

Protein reduction strategies in dairy nutrition: maximizing efficiency and performances through amino acid balance

Knapp Emilie[1,2], Aert Dries[1], Richards Sion[3]

[1] *Agrifirm-quartes nv (booiebos 5, 9031 Drongen, Belgium)*

[2]*Rumexperts (rue des Bada, 4317 Faimes, Belgium)- eknapp@rumexpert.vet*

[3] *Balchem EMEA (Italy/UK units)*

Objectives

This study aimed to evaluate whether maintaining milk production and supporting animal health is feasible by reducing dietary protein levels while supplementing amino acids (AAs) to meet dairy cows' nutritional needs. Additionally, the study assessed if supplementing AAs through concentrate feed in an automated milking (robotic) system could be a cost-effective strategy that maximizes return on investment (ROI). The goal was to enhance protein efficiency, reduce excess protein intake, and promote more sustainable dairy farming with lower environmental emissions while maintaining economic viability.

Material and Methods

The study took place on a commercial dairy farm with 120 Holstein cows managed via a robotic milking system. The cows were divided into two equal groups during 6 months : a control group (C) and a test group (AA). Both groups received the same semi-total mixed ration (STMR) at the bunk, consisting of a grass-based diet (9.4 kg grass cut) at 14% crude protein (CP) per kg dry matter (DM). Nutritional needs were met through two types of pellets dispensed by the robotic system: first, a high-Protein Pellet (HP): 40.5% CP, 272 g PDIE, 3.03 g DV methionine, and 13 g DV lysine, given at 1.5–2.5 kg/day/cow. Second a high-Energy Pellet: 18% CP, provided at 0–4.5 kg/day/cow.

In the AA group, the HP pellet was replaced by a Low-Protein Pellet (LP) containing 35% CP, 205 g PDIE, and supplemented with 6.93 g DV methionine and 21.3 g DV lysine, iso energetic. The robotic system distributed concentrates uniformly across both groups, ensuring consistent feeding conditions. Milk production and quality were recorded daily by the robotic system. Feed intake was monitored to confirm both groups consumed the provided STMR. Health and reproductive parameters were tracked via the robotic system. Additionally, feces, health scores, and blood samples were collected monthly from 10 cows per group to analyze metabolic and excretion parameters. The cows were paired based on lactation stage, days in milk (DIM), and production levels at the beginning of the trial. Data were analyzed using mixed models in JMP 17, with repeated measurements and pairs nested in subject ID as a random effect.

Results

In average, the AA group exhibited a decrease of 0.7% CP/kg DM and consumed 125 g less PDIE per day but received 8 g more DV lysine and 7.2 g more DV methionine. Higher-producing cows in the AA group had even lower CP (-1%/kg DM) and PDIE (-160 g/day) but higher methionine and lysine intake.

Over the 6-month trial, pluriparous cows in the AA group had higher average milk production (35.93 kg vs. 32.71 kg; P=0.06), while primiparous cows showed no production improvement (30.96 kg vs. 30.1 kg). However, primiparous cows in the AA group had higher milk fat content (4.25% vs. 4.03%; P=0.08), which improved profitability. The ROI for pluriparous cows was 1:11.The AA group had a longer average DIM (+13.79 days), so production comparisons at the same DIM revealed a significant increase for the AA group (+2.48 kg/day; P=0.0091), resulting in an ROI of 1:2.5 and a 9% improvement in protein efficiency. Health and reproductive parameters did not differ significantly between groups. Pair analysis throughout lactation showed a significant production increase in the AA group (+4.1 kg; P<0.01) and higher distribution of production pellets (+0.7 kg; P=0.05), yielding a positive ROI of 1:7. Blood analysis indicated a non-significant decrease in urea for both primiparous and pluriparous cows (P=0.11). Primiparous cows in the AA group had significantly lower CPK values (P=0.03). Fecal analysis revealed a reduction in protein percentage/kg DM (-6.6%; P=0.06) and ash content, along with an increase in NDF content (+10.2%; P=0.01).

Conclusion

Supplementing amino acids in a low-protein diet, aligned with AA requirements, can improve protein efficiency without compromising milk production, activity, or herd health. Distributing AAs through robotic milking systems based on production or lactation stage proved effective, yielding a positive ROI despite AA costs. Pluriparous cows showed increased milk production, while primiparous cows did not; instead, they exhibited higher milk fat content and lower CPK values, suggesting improved ruminal efficiency and reduced protein loss during their growth period (average age at first calving: 24.3 months). This strategy supports sustainable dairy farming by maintaining productivity while reducing protein intake and environmental impact.

Post-alert management of dairy cows in sensor-based health monitoring systems: veterinary involvement and antibiotic use

Hendirk Bresser[1], *Jens Tetens*[1], *Joachim Lübbo Kleen*[2]

[1]Georg-August-Universität Göttingen, Department of Animal Science, Albrecht-Thaer-Weg 3, D-37075 Göttingen

[2]Alta Genetics, Zijlsterweg 4, NL-9892 PK Feerwerd

Introduction

The increasing adoption of sensor-based health monitoring systems in dairy farming is a promising tool for early detection of diseases and abnormal behaviours in cows. These systems generate real-time alerts, allowing farmers to react proactively. However, the following steps—especially the role of veterinary intervention and the use of antibiotics—are critical in determining the overall success of these systems. This study investigates how farms respond to sensor-generated alerts, the level of veterinary involvement, and patterns of antibiotic use.

Methods

Seven German dairy farms, with a total of 4,655 cows, have used the Alta Cow Watch system (Nedap N.V., NL Groenlo) to monitor cattle health based on activity, rumination, and feeding behaviours. Herd managers were asked to complete daily surveys to log their responses to health alerts, noting whether clinical examination, veterinary diagnosis, or treatment followed. The data were analysed to assess farm response, with particular focus on veterinary interventions and treatments provided.

Results

Out of 802 fully completed surveys, 54% of the sensor alerts led to some kind of response, while 39% were ignored, and 7% had been addressed already before the alert. Clinical examinations of some kind were conducted in 95% of the cases where action was taken, but veterinary diagnoses were only in 5% of these cases. Treatments followed after 74% of the examinations, with 26% involving antibiotics and 72% involving pain relief or fever reduction. In 59% of cases, another form of treatment (e.g., drenching) was used.

Discussion

The low rate of veterinary involvement, just 5%, raises concerns about the underutilization of professional diagnoses in response to health alerts. This could be because of cost considerations or the belief that minor issues can be solved without intervention by a veterinarian. Research shows that while sensor systems can detect subclinical problems early, farm managers do not always seek veterinary assistance unless clear clinical symptoms are present (Stangaferro et al., 2016). This reliance on internal assessments might limit the benefits of early detection, especially in more complex or serious cases that require veterinary diagnosis. Inappropriate or delayed treatment could also exacerbate health issues, ultimately affecting animal welfare and productivity (Knight, 2020).

The use of antibiotics in 26% of treatments is another point of concern, especially in light of global efforts to reduce antimicrobial resistance. While early detection by sensor systems should ideally reduce the need for antibiotics by enabling more targeted, non-antibiotic treatments (Lovarelli et al., 2020), the data show that antibiotics are still commonly used. A careful balance between timely treatment and antibiotic stewardship is needed, highlighting the rersponsibility of veterinarians in guiding the appropriate use of antibiotics. The relatively high use of pain relief and fever-reducing treatments (72%) suggests a preference for managing symptoms rather than addressing underlying causes, which further emphasizes the need for veterinary input. Finally, it has to be stated that the use of antibiotics without veterinary examination or oversight is legally questionable and may violate animal welfare regulations.

Conclusion

Sensor-based monitoring systems provide valuable tools for early disease detection in dairy cows. However, the low rate of veterinary involvement and the significant use of antibiotics indicate areas for improvement. Enhanced veterinary collaboration and antibiotic stewardship are essential to fully realize the benefits of these systems. Increased training and the integration of sensor data into daily management could improve decision-making and help ensure better animal health outcomes while reducing the overuse of antibiotics.

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J. A. A. Mc. ART

Recent advances in calcium and phosphorus disorders in cattle

DVM, PhD, DABVP (Dairy Practice)
Department of Population Medicine & Diagnostic Sciences
College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
jmcart@cornell.edu

The transition period in dairy cows is marked by significant physical and physiological changes. This presentation focuses on the dynamics of calcium, the relationship of hypocalcemia with inflammation, and the resulting implications of this association with milk production in early lactation cows.

Calcium physiology and dynamics

The onset of lactation dramatically increases the demand for calcium. Prior to calving, cows require approximately 21 g of calcium daily for maintenance, which increases to 44 g at calving as they excrete approximately 23 g of calcium into their colostrum in addition to their maintenance requirements. By 7 days in milk (DIM), a cow making 45 kg of milk per day requires 77 g of calcium for maintenance needs and milk production—this is a tripling, and for very high producing cows a quadrupling, of calcium requirements within a 7 to 10-day period. This demand is met through a combination of dietary intake, bone resorption, and renal conservation, regulated by parathyroid hormone and vitamin D metabolites. Nutritional methods to optimize calcium metabolism are an important aspect of transition cow management but beyond the scope of this presentation.

Subclinical hypocalcemia (SCH) in early lactation dairy cows is characterized by low blood calcium concentrations without obvious clinical signs. Multiple studies have explored the categorization of blood calcium concentrations in early lactation, and there has historically been little consensus on the optimal test day or cut point for classification of hypocalcemia given the different DIM in which cows were tested and different cut points used to define hypocalcemia. (Oetzel et al., 1988; Martinez et al., 2012; Chapinal et al., 2011; Rodriguez et al., 2017; Wilhelm et al., 2017; Neves et al., 2018; Venjakob et al., 2018).

Research within the McArt Dairy Cow Lab has aimed to determine when early lactation hypocalcemia is detrimental and define this timing and cut points for diagnosis based on epidemiologically and economically important outcomes such as disease incidence, milk production, and reproductive efficiency. From several years of studies, we have found that cows can be categorized based on their early lactation calcium dynamics into 4 groups based on their blood calcium concentrations at 1 and 4 DIM: normocalcemic, transient SCH, persistent SCH, and delayed SCH. These classifications are associated with varying risks of adverse health events and differences in milk yield, in that cows with persistent or delayed SCH have higher incidences of metritis, displaced abomasum (DA), and herd removal compared to normocalcemic and transiently hypocalcemia cows (McArt and Neves, 2020). Cows with transient SCH produce more milk than the other calcium dynamic groups (McArt and Neves, 2020; Seely et al., 2021), and cows in both the normocalcemic and transient SCH groups have increase dry matter intakes postpartum than persistent or delayed SCH cows (Seely et al., 2021).

In summary, the dynamics of calcium in the early postpartum period in normocalcemic and transient SCH cows are associated with reduced disease incidence, increased intake, improved reproduction, and for transient SCH cows, increased milk yield. Alternatively, cows with persistent or delayed SCH have increased disease incidence, reduced intake, poorer reproduction, and reduced milk yield—together, we define cows in these calcium dynamic groups as being "dyscalcemic" (McArt and Oetzel, 2023). Dyscalcemia is defined as a blood calcium concentration ≤2.2 mmol/L at 4 DIM.

Inflammation and dyscalcemia

Postpartum inflammation is a normal and expected occurrence after parturition (Bradford et al., 2015). However, when early lactation inflammation is excessive or ongoing, it has been linked with adverse early lactation outcomes such as increased disease and reduced production and reproductive measures (Huzzey et al., 2009; McCarthy et al., 2016; Martins et al., 2021). It is still unknown why some cows experience excessive inflammation, but we do know that mammals exposed to lipopolysaccharide respond with a drop in blood calcium—this happens in mice, pigs, horses, and cows (Carlstedt et al., 2000; Toribio et al., 2005; He et al., 2020; Chandler et al., 2022). Recently, we have asked ourselves if dyscalcemia is a disease or a marker of a disease process. Research from our team has shown there is a strong association between inflammation and dyscalcemia, in that cows with dyscalcemia exhibit higher concentrations of inflammatory markers such as serum amyloid A, haptoglobin, and lipopolysaccharide binding protein (Seminara et al., 2025). Interestingly, these difference in serum amyloid A, haptoglobin, and lipolysaccharide binding protein concentrations appears as early as 2 DIM, which is well before the diagnosis of dyscalcemia at 4 DIM. This suggests that dyscalcemia is a good marker of excessive inflammation; however, we still do not understand if dyscalcemia is caused by excessive inflammation or if some other insult is causing both excessive inflammation and dyscalcemia. More work is needed in this very early lactation period to untwine these complex relationships.

Prepartum low phosphorus diets and postpartum calcium

Although the mechanisms behind phosphorus regulation in dairy cows are still debated, it is clear that phosphorus is not controlled solely in an indirect manner via the regulation of calcium. Grünberg (2023) provides an excellent review of current knowledge on phosphorus regulation, and readers are highly recommended to peruse this article. What is known is that phosphorus and calcium homeostasis are intertwined, and recent research exploring low prepartum dietary phosphorus shows an effect of this feeding strategy on postpartum calcium homeostasis. Research from Wächter et al. (2022) shows that cows fed a prepartum diet low in phosphorus(0.16% dry matter), when compared to cows fed adequate prepartum phosphorus (0.30% dry matter), had elevated blood calcium concentrations in the days leading up to calving and at least through 4 DIM. Recent work by Frizzarini et al. (2024) assessed possible physiological mechanisms for the relationship of low prepartum dietary phosphorus (achieved via feeding of sodium aluminum silicate—commonly referred to as a "binder") and postpartum calcium concentrations compared to the physiological mechanisms we know exist between the feeding of prepartum negative dietary cation anion difference (DCAD) diets and postpartum calcium. Their conclusion was that both diet strategies improved postpartum calcium homeostasis but via different mechanisms.

In relation to postpartum inflammation and calcium dynamics, further research assessing the effects of these dietary strategies on markers of inflammation and dyscalcemia are warranted. For example, is preventing the immediate drop in calcium postpartum good or bad—is this drop in calcium important for regulation of milk production? Although it is perhaps difficult to assess physiological interactions between phosphorus and calcium metabolism, investigation into the impact of feeding low phosphorus prepartum diets, whether formulated or achieved via addition of a binder, on early lactation health and production outcomes is required.

Conclusions

Understanding the dynamics of calcium in early lactation is crucial for managing dairy cow health and productivity. Monitoring calcium concentrations and addressing transition cow nutrition, comfort, and maternity management can help mitigate the risks of adverse health events, improve milk yield, and enhance reproductive performance, whether the source of these issues is excessive inflammation, reduced calcium concentrations, or a different instigating cause. Although much is known about feeding negative DCAD diets prepartum, recent and ongoing research is assessing the physiological mechanisms and health and production outcomes from feeding low phosphorus prepartum diets. The effect of these prepartum nutritional strategies on excessive inflammation and dyscalcemia is unknown, and more work is needed to understand the impact of these management strategies on early lactation cow health.

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NOTES PERSONNELLES

A REVIEW OF INTEGRATED LIVESTOCK SYSTEMS USING MACHINE LEARNING TECHNIQUES: TRENDS AND GAPS FROM ANIMAL WELFARE, SUSTAINABILITY AND 'ONE HEALTH' PERSPECTIVES

Diego Sicuso[1], Annalisa Previti[1], Abrha Bsrat[2], Vito Biondi[1], Michela Pugliese[1], Annamaria Passantino[1]
[1]Department of Veterinary Sciences, University of Messina, Italy
[2]Mekelle University, College of Veterinary Medicine, Ethiopia

Integrated Livestock Systems (ILS) is an environmentally sustainable agricultural practice integrating livestock, aquaculture, crops and forestry to optimize productivity, reduce waste and improve food security. ILS promotes interactions between these components to create a balanced ecosystem while addressing sustainability challenges (Amanullah 2024). Within this framework, integrated systems, particularly those involving cattle, play a critical role in mitigating intensive livestock production's environmental and social problems. Conventional intensive systems emit greenhouse gases, degrade soil and pollute water, and cause biodiversity loss (Ghimire et al 2021). Using land sustainably and recycling nutrients through manure management reduces the environmental impact and improves soil fertility, contributing to sustainable food production. It includes livestock such as poultry, pigs, ducks, cattle, buffalo, sheep, and goats with fish farming activities. This approach is useful in regions with high demand for fish and animal products, like China (Li et al. 2017), South Africa (Akinde et al. 2022), and Vietnam (Thanh Hai et al. 2020).

As ILS has re-emerged due to its potential for maximising production and sustainability, this study

reviews the current state of research on ILS using machine learning techniques, with a focus on identifying trends, gaps, and challenges. It aims to explore the role of ILS in promoting sustainability, enhancing animal welfare, and supporting the One Health framework, while highlighting the potential of advanced Text Mining (TM) and Topic Analysis (TA) methods to uncover insights and inform future research directions.

A systematic literature review was performed using the Scopus® database and the following keywords: “Integrated AND farm AND system AND bovine”, “Integrated AND farm AND system AND pig”, “Integrated AND farm AND system AND hens”, “Integrated AND farm AND system AND fish”, “Integrated AND farm AND system AND animal AND welfare”, and “Integrated AND farm AND system AND One AND health”. The review followed PRISMA guidelines, involving a rigorous screening process to select articles on ILS. A total of 880 records published between 1983 and 2023 were initially retrieved. After applying inclusion criteria such as language (English), publication year (1983-2023), document type and presence of abstract, ILS unrelated articles without animal involvement were excluded. This process yielded 19 eligible articles for detailed analysis.

Descriptive statistics were used to characterize the dataset. This revealed a surge in research interest in 2023, reflecting the growing interest in this field. Of these 19 articles, 36.84% (n=7) were from India, 15.79% (n=3) from Thailand, the 10.53% from China and one article (5.26%) each from Italy, Romania, Australia, Sierra Leone, Nigeria, Malawi and the Philippines. Regarding livestock focus, 47.36% (n=9) addressed poultry, 21.05% (n=4) pigs, 15.78% (n=3) cattle, and 15.78% (n=3) livestock in general.

TM identified the most frequent and relevant terms from abstracts using the R programming language. The Term Frequency-Inverse Document Frequency (TF-IDF) weighting system was applied to assign importance to each term, considering both its frequency within individual documents and its prevalence across the dataset. Key terms with the highest TF-IDF (≥0.15) included "antimicrobial," "resist," "crop," "dose," "pond," "livestock," "unit-1," "goat," "aquaculture," and "manure".

To explore the thematic structure of the literature, TA was performed using Latent Dirichlet Allocation, a probabilistic topic modelling technique. This method revealed five distinct topics: T1-ILS and antimicrobic resistance: exploring the relationship between integrated systems and the reduction of antimicrobial resistance in livestock; T2-ILS in aquaculture: investing the role of integrated systems in enhancing aquaculture productivity and sustainability; T3-Geographical distribution of ILS: analyzing regional differences in ILS adoption and practices; T4-Economic model for ILS: examining the economic viability and cost-benefit analysis of integrated systems; T5-Sustainability in ILS: addressing the environmental and social dimensions of ILS, particularly resource efficiency and resilience.

The analysis shows ILS’s increasing role in sustainable agriculture, but also identifies critical research gaps. Notably, studies on cattle systems are underrepresented despite their considerable environmental impact, highlighting the need for further investigation. By improving resource efficiency, ILS supports the transition to more resilient food systems, addressing environmental challenges posed by conventional livestock production.

Animal welfare emerges as a key ILS issue, essential for productivity, preventing diseases and public health. Poor animal welfare can result in livestock becoming a reservoir of pathogens, posing risks to food safety and public health. Adopting a “One Health” perspective - a holistic approach linking human, animal and environmental health - is vital for sustainable livestock management.

In conclusion, this study highlights the value of leveraging ML techniques to advance knowledge in ILS. By uncovering trends and gaps, these tools can guide future research and innovation, ensuring that ILS continues to evolve as a cornerstone of sustainable agriculture.

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A novel consolidated blood biomarker index to promote animal welfare and health monitoring

Delhez P.[1], Theron L.[1], Tran M.-N.[2], Christiaens B.[2], Evrard J.[3], Lemal P.[4], Gengler N.[4], Rao A.-S.[1], Bertozzi C.[2]

[1]RumeXperts (rue des Bada 27, 4317 Faimes, Belgium) ;[2]Elevéo by awé groupe (Rue des Champs Elysées 4, 5590 Ciney, Belgium), [3]ARSIA (Allée des Artisans 2, 5590 Ciney, Belgium), [4]Gembloux Agro-Bio Tech, ULiège (Passage des Déportés 2, 5030 Gembloux, Belgium)
Speaker : Pauline Delhez, pdelhez@rumexpert.vet

Introduction and Objectives

Ensuring animal welfare is both an ethical imperative and a critical challenge in the dairy industry, particularly as consumer expectations and regulatory pressures increasingly demand sustainable and responsible farming practices. Early lactation, especially the first 60 days post-calving, is a pivotal period in a cow's production cycle, characterized by profound metabolic and physiological changes. These changes make fresh cows highly vulnerable to metabolic disorders, inflammatory responses, and nutritional imbalances, which not only compromise animal welfare but also lead to economic losses through reduced productivity and increased veterinary costs. Current monitoring approaches often focus on isolated health parameters, limiting their ability to detect early signs of multifactorial or subclinical health issues. To address this gap, this study introduces an innovative, consolidated blood biomarker index that integrates multiple parameters—albumin, urea, cholesterol, non-esterified fatty acids (NEFA), globulins, and the albumin-to- globulin ratio. By providing a holistic view of health status, this index facilitates early detection of health risks, supports proactive interventions, and promotes improved welfare and reduced reliance on antibiotics in fresh cows.

Materials and Methods

The index was calculated using a formula primarily centered on albumin levels, scored on a scale from 1 to 10 (1 point for albumin levels ≤25 g/L and 10 points for levels ≥34 g/L). Adjustments, either penalties or bonuses, were applied to the score based on additional blood biomarker values, including urea, cholesterol, NEFA, globulin, and the albumin-to-globulin ratio. Thresholds were established through veterinary expertise and scientific literature. Data used to develop the index were obtained from a cohort of 260 animals with days in milk ranging from 1 to 400. Descriptive statistical analysis was conducted to evaluate the index's distribution and applicability.

Results

The analysis of index values calculated from 260 animals showed a mean score of 8.34 with a standard deviation of 4.89. Notable differences were identified across farms, corresponding to variations in management practices observed in the field. Expert feedback confirmed that the index is most effective when applied to fresh cows (0–60 days in milk). Fresh cows with an index score of 0 or lower were categorized as being at high risk for health problems, those scoring between 0 and 6 were considered suboptimal, and cows with scores above 6 were classified as low risk.

Conclusion

In summary, this innovative consolidated blood biomarker index, validated through expert consultation, provides a practical and effective tool for evaluating cow health, particularly in fresh cows. Its use can support timely interventions and promote improved welfare in the dairy sector. By fostering sustainable and ethical farming practices, this approach aligns with current industry and societal goals.

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Preliminary evaluation of a phytogenic dry-off tablet on dairy cow transition health and performance

Delhez, P.[1], Hamard H.[1], Theron L.[1], Ackaert G.[2] Rao A.-S[1]

[1]RumeXperts (rue des Bada 27, 4317 Faimes, Belgium)

[2]AHV International B.V. (Schokkerweg 10, 8042 PC Zwolle, Netherlands)

Speaker : ltheron@rumexpert.vet

Introduction and Objectives

The dry-off period (DO) represents an important transition in the lactation cycle of dairy cows, significantly impacting health and productivity. During this phase, cows undergo substantial physiological stress, exerted by metabolic, hormonal, and immunological changes. Mismanagement during this critical stage can result in metabolic disorders, compromised immune function, reduced colostrum quality, calving difficulties, and reduced longevity. To improve animal welfare and health outcomes, effective dry-off strategies are needed. Recent advancements in dry-off strategies emphasize the use of dietary interventions, nutritional adjustments, and targeted treatments to support metabolic adaptation and minimize negative energy balance. This study evaluates the impact of a novel dry-off tablet (StopLac®, AHV; containing a novel Allium-derived compound as its main bioactive ingredient). The test parameters included a wide range of biomarkers related to metabolism, mineral balance, enzyme profiles, and milk production in the early subsequent lactation, aiming to identify key biomarkers of efficacy and measurable benefits.

Materials and Methods

A longitudinal, randomized, double-blinded design was used in this pilot study involving 20 multiparous Holstein cows from a single commercial dairy farm. The animals were divided into two groups: a treatment group (n=10) receiving the StopLac® tablet and a non-treated control group (n=10) receiving standard care. Biomarkers in blood and milk, as well as clinical metrics were recorded at day 1 before treatment with StopLac® (DO-1), day 3 after DO (DO+3), day 40 after DO (DO+40), and day 7 after Calving (C7). Statistical analyses were conducted using R software, and temporal boxplots were generated to visualize trends. Cohen's d effect sizes were calculated to quantify the magnitude of differences between groups at each time point.

Results

- Energy Metabolism (serum): Lower non-esterified fatty acids (NEFA) levels were observed in the treatment group DO+40 (median difference of 50%), indicating reduced fat mobilization prepartum. In contrast, higher urea levels in the treatment group at DO+40 and C7 (median differences of 13% and 15%, respectively) suggested better amino acid utilization. Lower values of serum aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH) in the treatment group at C7 (median differences of 9.5% and 17%) further supported the observed beneficial metabolic effects.

- Mineral Profile (serum): Treated cows showed higher calcium (median difference of 9%) and phosphorus levels (median difference of 24%) at C7, which are important indicators of postpartum mineral balance at the onset of substantial milk production.

- Udder Health and Inflammation (milk): No significant differences in somatic cell count or early biomarkers of host defense and inflammation such as cathelicidin were observed between groups across the study. Significant reduction in lactose, and mild reduction in pH were observed in the treatment group at DO3.

Conclusion

The results of this first field trial with a limited number of dairy cows over a restricted period, focusing on the dry-off and early post-partum periods, showed that the dry-off tablet StopLac® exerts promising beneficial effects on the energy and mineral metabolism, hence reducing the metabolic stress inherent to the transition period. The reduced metabolic stress can support udder health regeneration during the dry-off period, resulting in a better resilience to metabolic and infectious challenges at the onset of lactation.

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The use of hepato-protectors during the transition period to manage hyperketonemia in dairy cows

Anastasia Lisuzzo,1 Alessio Valenza,2 Andrea Biancucci,3 Alex Bach,4 Damien Achard,5 Matteo Gianesella,1 and Enrico Fiore,1

1 Department of Animal Medicine, Production and Health, University of Padua, 35020 Legnaro, Italy; 2 Ceva Animal Health S.p.A., 20127 Milan, Italy;
3 Veterinarian free practitioner;
4 Catalan Institution for Research and Advanced Studies, 08010 Barcelona, Spain;
5 Ceva Santé Animale, 33500 Libourne, France.

Authors' information:
Anastasia, DVM, PhD, anastasia.lisuzzo@unipd.it;
Alessio, DVM, PhD, alessio.valenza@ceva.com;
Andrea, DVM, biancucci.andrea.85@gmail.com;
Alex, DVM, PhD, alex.bach@icrea.cat;
Damien, DVM, PhD, damien.achard@ceva.com;
Matteo, DVM, PhD, matteo.gianesella@unipd.it;
Enrico, DVM, PhD, enrico.fiore@unipd.it.

Objectives: Hyperketonemia (increased blood level of β-hydroxybutyrate or BHB) is a major metabolic disease in dairy cows negatively affecting animals' health. A proper functioning of the energy metabolism (gluconeogenesis and Krebs-cycle) is essential to reduce the risk of hyperketonemia during post-partum period. For these reasons, hepato-protectors composed by precursors of energy metabolism pathways (as propionate, gluconeogenic amino acids (AA), vitamins and co-factors) are usually used in dairy cows during the transition period. A field study was performed to better characterize the efficacy of hepato-protectors to reduce the risk of hyperketonemia in dairy cows.

Materials and methods: Animal care and procedures received ethical approval (n.204359/2023). A total of 445 Holstein-Friesian dairy cows were enrolled at the begging of the dry period, and randomly and equally divided into three groups: CTR (control without treatment); BOG-ERB (treated with Bograss® and Erbacolina PLUS®, CEVA Animal Health); MON (treated with monensin, Kexxtone). The BOG-ERB received 2 ml of Bograss®/10 kg BW, and 70 mL/animal/ treatment of Erbacolina PLUS® at 12d before calving and 6d after calving; while the MON received 35.2 g/animal of monensin at 21d before calving. Blood sampling was performed at -21 and -7d before calving, at the day of calving, and at +7, +14, +28, and +55d after calving. All animals were evaluated for NEFA and BHB levels, while a complete biochemical analysis was performed for 45 animal per group (30 pluriparous, and 15 primiparous). Differences in biochemical parameters were assessed with a linear mixed-effects model. A post hoc pairwise comparison was performed using Bonferroni correction. A logistic regression analysis was performed using blood BHB values (healthy, BHB<1.2 mmol/L; hyperketonemia, BHB≥1.2 mmol/L). The model considered the risk factors of treatment, time, parity, and season. A *p-value*<0.05 was considered significant, whereas a 0.05≤*p-value*≤0.10 was considered a trend.

Results: Hyperketonemia prevalence in BOG-ERB group was between 2.9% to 11.1% during the post-partum period. In contrast, the prevalence was 6.1% to 24.1% in CTR, and was 5.3% to 16% in MON. In fact, the logistic regression identified for BOG-ERB a OR of 0.43 (CI: 0.3 to 0.8) compared to the CTR, and of 0.44 (CI: 0.1 to 0.8) compared to MON. The BOG-ERB cows showed the lower risk of hyperketonemia during the post-partum period considering a lowest level of NEFA from +7d to +28d, and BHB from +14d to +55d. Moreover, the CTR incurred in hypoglycemia at +7d. Regarding the liver, the AST and GGT concentrations were lowest in BOG-ERB from calving until the end of the study. The albumin level was greatest in BOG-ERB from +14d to +55d in association with the lowest level of urea. Serum ALP concentration was greatest in BOG-ERB from -7d before calving to +55d associated with greater level of Ca up to +14d. Furthermore, serum Cl and Na were greater in the same group around calving. Regarding cows' performances, the BOG-ERB had the greatest pregnancy/artificial insemination (BOG-ERB: 55.7; CTR: 49.8; MON: 49.9), and a greater milk production compared to CTR group without differences with MON group (BOG-ERB: 43±0.5 Kg/day; CTR: 42±0.5 Kg/day; MON: 43.5±0.5 Kg/day). Finally, the MON group produced more milk during the first 150 DIM, while the BOG-ERB produced more milk after 210 DIM. Conclusions: Supporting animal metabolism through the use of hepato-protectors decreases lipomobilization, metabolic stress, and protein catabolism. This condition reduces the risk and prevalence of hyperketonemia during the post-partum period with greater reproductive performances and a more constant milk production.

Effects of live yeast supplementation during transition period on gene expression in the rumen epithelia

A Bach 1, F Chaucheyras-Durand 2, M Gauthier 3, E Sulmont 3, F Fàbregas 4, E Garcia-Fruitos 4, A Aris 4

1 Department of Animal Science, University of Lleida, Lleida, Spain
2 Lallemand Animal Nutrition, 31702 Blagnac, France; UMR MEDIS 454, INRA-UCA, 63000 Clermont-Ferrand, France 3 Lallemand Animal Nutrition, 31702 Blagnac, France
4 Department of Ruminant Production, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), 08140 Caldes de Montbui, Spain

E-mail: alex.bach@icrea.cat

This study aimed to investigate (1) the dynamics of gene expression in the rumen epithelia as cows transition from a dry to a lactating diet, and (2) the potential effects of live yeast supplementation on these tissues. Twenty-one Holstein cows were assigned to two treatments: 300 g/d of corn supplemented with 1 × 10^{10} cfu/d of live Saccharomyces cerevisiae CNCM I-1077 (LY; n = 10) or no supplementation (control; n = 11), starting 21 ± 2.6 days before until 21 days after calving. At 14 ± 2.6 days before, and 7 and 21 days post-calving, rumen biopsies samples were collected via endoscopy. Gene expression was assessed using quantitative PCR, while microbiota composition was analyzed using high-throughput sequencing of total DNA. In terms of performance, LY cows showed a higher dry matter intake (18.2 kg/day) and milk production (38.7 kg/day) during the first 21 days in milk (DIM) compared to control cows (15.7 kg/day and 32.7 kg/day, respectively). These results suggest that live yeast supplementation before calving could enhance feed intake after calving, thereby supporting increased milk production during early lactation. In the rumen epithelium, LY supplementation increased expression of TLR4 (toll-like receptor 4, notably activated by endotoxins) before calving and a more consistent immune response throughout lactation. IL10 (an anti-inflammatory cytokine) expression was greatest ($P < 0.05$) at 7 DIM in control cows, whereas it was highest before calving in LY cows. This suggests that live yeast could modulate ruminal immunity by enhancing anti-inflammatory responses before calving, potentially reducing inflammatory responses post-calving. TNFα (tumor necrosis factor α) expression was affected by an interaction between treatment (live yeast vs control) and stage of production. Control cows had the lowest expression of TNFα at 21 DIM, while LY cows maintained steady expression throughout the production stages. OCLN expression, coding for a protein (occludin) involved in tight junctions in the epithelium, was highest during the first week after calving in control cows, whereas it was highest before calving in LY cows. This may suggest that live yeast could strengthen the rumen epithelial barrier, potentially due to the concomitant increase in dry matter intake.

Taken together, these data suggest that strain specific probiotics have potential to improve the rumen epithelial barrier and the rumen mucosal immune response around calving transition.

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The effect of using starch or fat as energy sources on foot health in Holstein and Simmental bull

Aykut ACAR[1], Hilmi Can Acar[1], Göksen Ayalp[2], Ahmet Akkoç[3], Özge Sızmaz[4], Abdülkadir Orman[5], **Hıdır Gençoğlu[1*]**

[1]Bursa Uludag University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Bursa - Türkiye
[2]Balıkesir University, Faculty of Veterinary Medicine, Department of Surgery, Balıkesir - Türkiye
[3]Bursa Uludag University, Faculty of Veterinary Medicine, Department of Pathology, Bursa - Türkiye [4]Ankara University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Ankara ‑Türkiye
[5]Bursa Uludag University, Faculty of Veterinary Medicine, Department of Zootechnics, Bursa – Türkiye

gencoglu@uludag.edu.tr

This trial was aimed to investigate the effects of using starch or fat as energy sources in the Holstein and Simmental bull diets on dry matter intake, feed conversion ratio, fat pad thickness, foot health and fatty acids compositions of digital cushion. The study was conducted between January and July 2020 at Bomonti Dairy Farm (Kırklareli, Türkiye). Fourteen Holstein friesian beef cattle (405 ± 50 kg initial body weight) and fourteen Simmental beef cattle (450 ± 50 kg initial body weight) were randomly divided into four groups and each group consisted of 7 animals. All animals were fed with fat or starch-based total mixed ration (TMR) based on energy sources. The experimental groups of the study were designed as follows: Simmental beef cattle group fed with fat (SF, n:7) or starch (SS, n:7) and Holstein beef cattle fed with fat (HF, n:7) or starch (HS, n:7) based ration as energy source. The rations in the groups were designed as isocaloric and isonitrogenous. Leftover ration in front of the animals from the beginning of the study were collected, and daily dry matter consumption and feed conversion ratios were calculated based on groups. At the end of the study, the animals were sent for slaughtering. After the animals were slaughtered, 4 feet of each animal were collected. The fat pad of each claw was removed from each foot and analyzed for fatty acids compositions of digital cushion The fat pads on the collected from feet were visualized by ultrasonography and the digital cushion fat pad thickness was measured. The fat sample was taken from the cranial part of the footpad and using a microtome device were stained with hematoxylin and eosin dye. The stained samples were examined under a microscope for mononuclear cell infiltration, brown fat tissue, and bleeding. The statistical analyses were performed using SPSS (v28.0) package program and Medcalc (11.5, 2011) program. ONE WAY ANOVA was used to evaluate the effects of fat and starch on performance. Roc Curve Analysis was used to evaluate the relationship between mononuclear cell infiltration and fat pad thickness in collected feet. The dry matter intake of Holstein beef cattle fed based fat (HF, 11.9 kg/d) higher than fed starch based diet for Holstein (HS, 11.2 kg/d) and Simmental (SS, 10.9 kg/d). The feed conversion ratios of SS (0.14 ±0,003) and HS (0.13±0,006) groups were found to be higher than SF (0.10±0,008) and HF (0.11±0,01) groups ($P<0.05$). Fat pad thickness of animals, HF (1.2104) was higher than those fed HS (1.0993) and SS (1.1293) ($P<0.05$). Fat pad thickness in the SF group (1.2289); It was found to be higher than the HS (1.0993) and SS (1.1293) groups ($P<0.05$). There was no difference between the groups in terms of the relationship between mononuclear cell infiltration and bleeding ($P>0.05$). According to the results of this study, it was concluded that feeding with fat based TMR may have a potential positive effect on foot health.

Keywords: Beef cattle, fat, starch, simmental, holstein friesian

Herd-level risk factors associated with indicators of early lactation negative energy balance in Irish seasonal calving pasture-based dairy herds

Marian, R.[1], Kelly, E.[1], McAloon C.A.[1], McAloon, C[1]., Ryan, E.G.[1]

[1] School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, D06W6F6, Republic of Ireland

Objectives

Negative energy balance (NEB) can have significant negative consequences for milk yield, animal welfare, fertility performance and sustainability. The identification of risk factors could yield substantial benefits through a subsequent preventative health approach. The objective of this retrospective observational study was to identify risk factors for NEB in dairy herds through the analysis of test day milk solids from a large Irish seasonal-calving milk recording dataset.

Material and Methods

Study data was obtained from an Irish Cattle Breeding Federation (ICBF, Co. Cork) national milk recording dataset, and included 28,573,326 test day milk records from 2014 to 2023 inclusively. Data was confined to individual cow milk recordings carried out in the months of January to June, and milk recordings with a minimum of 20 cows recorded at each herd test. Bulk tank equivalent (BTE) protein and prevalence of high fat to protein ratio (FPR) were calculated as indicators of negative energy balance. A high herd fat to protein ratio was defined as a test day FPR of >1.4. Four separate models were developed to identify risk factors associated with BTE protein and prevalence of high FPR for the periods 0-30 DIM and 30-60 DIM separately. Herd and year were included as random variables.

Results

The final dataset after cleaning contained 17,312,806 test-day records, with 55,464 herd-years analysed for the 0-30 DIM models and 79,181 herd-years analysed for the 30-60 DIM models. The following variables were significantly associated ($P<0.001$) with an increased prevalence of high FPR in herds between 0 and 30 DIM: a higher percentage of primiparous cows in a herd (OR: 1.286); and mean EBI (economic breeding index – an Irish genetic multi-trait selection index) predicted transmitting ability for fat kg (OR: 1.064). The percentage of primiparous cows in the herd also increased the prevalence of high FPR in herds between 30 and 60 DIM (OR: 1.51). A higher percentage of parity 4+ cows in a herd was associated with a decreased prevalence of high FPR in study herds in the 0-30 DIM time period (OR: 0.85); and in the 30-60 DIM time period (OR: 0.77).

In the BTEP 0-30 DIM model, there was a positive association between BTEP and the percentage of primiparous cows in the herd ($P<0.001$); and mean EBI predicted transmitting ability for fat yield in kg ($P<0.001$). However, BTEP was negatively associated with the percentage of parity 4+ cows in the herd ($P<0.001$); range in yield and mean 305 day yield in a herd ($P<0.001$); and the number of cows recorded on any test day ($P<0.001$). Apart from the percentage of parity 4+ or primiparous cows, the linear associations for the other variables were weak. The same variables remained in the BTEP model for the 30-60 DIM time period, but with a positive association found for all variables including the percentage of parity 4+ cows in the herd.

Conclusions

These findings highlight the importance of herd composition, genetic selection, and regular milk recording in mitigating early lactation NEB, thereby supporting productivity and animal welfare in pasture-based systems. In particular, the finding of an increased odds of a herd having high FPR in association with an increased percentage of primiparous cows in the herd suggests that the nutritional management of primiparous cows in the Irish seasonal calving system warrants closer attention.

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Use of a new generation rumen-protected choline for improved transition health and performance

Pauwelyn B.[1], Passchyn P.[2], and Dubey D.[1]

[1] Kemin Europa, Herentals, Belgium

[2] Milkadvice, Torhout, Belgium

Cows with good transition health achieve higher milk production and better fertility compared to those with metabolic diseases (Carvalho et al. 2019). Many metabolic diseases are linked to the negative energy balance and impaired liver health in the first weeks after calving. Studies show that 9.6 to 63.6% of cows experience subclinical ketosis during the transition phase (Loiklung et al. 2022). However, a recent meta-analysis revealed that using rumen-protected choline (RPC) resulted in an average increase of 1.6 kg of energy-corrected milk (ECM) (Arshad et al., 2020).

CholiGEM™, a new generation RPC, combines high choline concentration with high bioavailability (Kihal et al., 2022). Marques et al. (2024) found that feeding CholiGEM in the transition period improved ECM during the first 150 days after calving with 1.7 kg/d for multiparous cows and 0.7 kg/d ECM for primiparous cows. Additionally, RPC was found to alter the uterine microbiome, reducing the presence of *Fusobacteriae* and implying a lower risk of metritis. The effect of CholiGEM on ECM production was recently reaffirmed, with an increase of 2.3 kg/d. Additionally, positive impacts on fertility and culling rates were observed. At 150 days in milk (DIM), 65.6% of cows in the CholiGEM group were pregnant, compared to 56.8% in the control group ($p = 0.03$). Furthermore, culling rates decreased from 36.5% to 25.5% ($p = 0.008$) (Wollenstarski et al., 2025, under publication).

The current study investigated the effect of CholiGEM on performance and health parameters of dairy cows in two Belgian field trials. Trial 1 involved two groups of 75 multiparous cows (average milk production: 36.5 kg/d) in an OFF-ON trial design. The control group received a close-up pellet for the last 14 days before calving and a starter pellet for the first 40 days after calving. The treatment group received the same pelleted feeds with the addition of 30 g/d CholiGEM. Trial 2 involved 75 cows in total (average milk production: 30 kg/d) that were blocked in two groups based on parity and expected production level. The treatment group received 30 g/d of CholiGEM from calving until 60 days after calving via an additive dispenser on the milking robot. For both trials milk performance was measured, as well as cow health and reproduction data in Trial 1.

In Trial 1, cows fed CholiGEM produced 2.3 kg/day more ECM for the first 120 days post-calving. In Trial 2, CholiGEM increased daily ECM production by 1.4 kg for the first 180 days of lactation. It is clear that the positive effect of RPC on ECM production persisted long after the supplementation stopped.

Regarding ketosis in Trial 1, no clinical cases (BHBA >2 mmol/L) were detected during supplementation with CholiGEM, while 13% of control cows showed clinical ketosis. Cows receiving CholiGEM in Trial 1 took 55 days less to conceive and required 24% fewer services per conception. Conception at first service increased from 31% to 38%.

In conclusion, an RPC strategy during the transition period using CholiGEM increases milk yield persistently and improves overall fertility, making it an effective strategy for enhancing transition health and performance.

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Effects of a yeast, mineral, and antioxidant bolus on transition cow performance under Flemish field conditions

Angelique Rijpert-Duvivier

Van Deventerlaan 31, 3528 AG Utrecht Nederland
Elanco the Netherlands B.V. – *angelique.rijpert_duvivier@elancoah.com*

Introduction and objective: The transition period, particularly the first 30 days in milk (DIM), is a critical time for dairy cows, marked by increased disease susceptibility and reduced performance as a result. Strategies to improve feed intake, rumen environment, and nutrient status during the peripartum period are considered important to help cows adapt to the lactation phase.1 This study investigated the effects of a postpartum bolus containing live yeasts, minerals, and antioxidants on eating and rumination behavior, health events, and fertility in dairy cows under Flemish field conditions.

Materials and methods: Between November 2023 and March 2024, 142 freshly calved cows from a Flemish dairy herd (n > 300) were enrolled in this study. Cows were randomized by parity and calving month to either a treatment group (B; n=50) receiving a single 220g bolus (YMCP Vitall®, TechMix, LLC., Stewart, USA) within 6 hours of calving or a control group (C; n=59) receiving no treatment. Total eating and rumination time per day was monitored for the first 10 DIM using SenseHub™ Dairy (Allflex Merck & Co., Inc., Rahway, NJ, USA). Furthermore, early lactation health events and fertility treats were recorded. Data were analyzed using JMP 17, with significance set at $P < 0.05$ and trends at $P < 0.10$. Excluded were 33 cows due to missing data or pre-calving health issues.

Results: Parity 2 cows in group B exhibited numerically higher total eating time over the first 10 DIM, with significantly longer eating times immediately postpartum (Day 0; 224 and 164 min/day for B and C respectively, $P = 0.006$) and a trend towards longer times at 8 DIM compared to group C (264 and 226 min/day for B and C respectively, $P = 0.06$). Similarly, parity 3+ cows in group B showed numerically higher total eating times, significantly exceeding group C at 1 DIM (199 and 157 min/day for B and C respectively, $P = 0.02$) and 8 DIM (233 and 195 min/day for B and C respectively, $P = 0.03$), and trending higher at 7 DIM (215 and 185 min/day for B and C respectively, $P = 0.099$). Rumination time was significantly higher in group B for parity 2 cows at 1 DIM (473 and 359 min/day for B and C respectively, $P = 0.008$), with no significant differences observed in parity 3+ cows. Both groups showed a marked decrease in eating time on 2 DIM, coinciding with their introduction to the high-production group. Health events within the first 10 DIM were numerically lower in group B (9 cows; 18%) compared to group C (12 cows; 20%). Mortality and culling rates were also lower in group B (1 cow; 2%) compared to group C (4 cows; 7%). Importantly, diseased cows in group B maintained similar eating and rumination times to healthy cows, whereas diseased cows in group C showed significant reductions concerning eating time at DIM 1,2,3,4,5 and 7 and ruminating time at DIM 1,2,3,7 and 8.In parity 3+ cows, group B tended to be inseminated earlier (75 days) than group C (88 days; $P = 0.09$). The interval from calving to conception was numerically shorter in group B (87 days) compared to group C (104 days; $P = 0.11$). The relative risk of successful first insemination was 1.3 times greater in group B.

Discussion and conclusion: This study indicates that a fresh cow bolus containing live yeasts, minerals, and antioxidants may offer benefits to dairy cow health and subsequent performance. The bolus appears to support eating and to a lesser extend rumination time in the crucial days following calving, potentially aiding rumen adaptation and nutrient utilization. While overall health events were similar between groups, the bolus group showed more resilience, with diseased cows maintaining eating and rumination times comparable to healthy cows. These observations align with a previous study where a similar bolus containing live yeasts, minerals, and antioxidants led to higher dry matter intake compared to controls in cows with induced inflammation.2 Furthermore, promising trends towards earlier insemination and improved first service conception rates in older cows suggest potential fertility advantages.

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Tumors in cattle: a retrospective study

Julia Schoiswohl[1,2], Geurten Naila[2], Cassandra Eibl[2], Reinhild Krametter-Froetscher[2]

[1]Institut for organic farming & livestock biodiversity, Species-appropriate animal husbandry, animal welfare and herd management, HBLFA Raumberg-Gumpenstein, Trautenfels 15, Stainach-Puergg, 8951, Austria
[2]Clinical Centre for Ruminant and Camelid Medicine, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, Veterinaerplatz 1, Vienna, 1210, Austria

Julia.schoiswohl@raumberg-gumpenstein.at

Objectives: The incidence of tumors in cattle is increasing, which reduces their productivity (Shruthi et al., 2018). Thoracic and abdominal neoplasia in domestic ruminants is resulting in economic losses of meat, milk, and productivity. The disease has non-specific clinical presentation and abnormalities, often making it challenging to identify, characterize, and stage. In routine veterinary practice, imaging techniques are crucial for diagnosing diseases (Tharwat and Al-Sobayil, 2017). The prognosis depends on various factors, including the disease stage, diagnosis, cow's value, and withdrawal losses caused by therapeutic efforts (Galofaro et al., 2005).

Material and Methods: The aim of this study was to collect and evaluate data from cattle diagnosed with a tumor disease in the period from 2010 to 2021 at the Clinical Centre for Ruminant and Camelid Medicine of the University of Veterinary Medicine Vienna, Austria.

Results: Between 2010 and 2021 in total 27 cattle (24 female, 3 male) with neoplasm were investigated at the Clinical Centre for Ruminant and Camelid Medicine. The average age was 5.1 years (range 13 days-16 years). There are significant more female animals than male animals (p=0.001) referred with neoplasm to the Clinical Centre. Animals showed a duration of illness between one day and one year before they were referred to the Clinical Centre for Ruminant and Camelid Medicine.

Cattle with tumors were reported ill for an average of 44 days. Animals with a benign tumor had a medical history of 52 days, animals with a malign tumor a medical history of 26.6 days and with an unknown dignity 184 days. A statistical association between duration of illness and the malignancy of the tumor was not found (p=0.498). Poor body status was documented in 11 cattle (9 malign, 2 benign), a satisfactory nutritional status in 13 cattle (10 malign, 3 benign). A statistical association between the nutritional status and the malignancy of the tumor was not found (p=0.940).

In 26 cattle a tentative diagnose could be made with the clinical examination and different further diagnostic approaches include blood investigation, imaging techniques (x-Ray, ultrasound, CT), histological investigation of tissue sample, results of fine needle aspiration and diagnostic laparotomy. Post-mortem pathohistological investigations diagnosed a tumor in 1 cattle. Imaging techniques were applied in 11 cattle, an ultrasound investigation was performed in 11 cattle. X-ray was performed in 2 cattle. In 14 cattle fine needle aspiration was performed and this methode got a tentative diagnose tumor in all 14 cases. A laparotomy was performed in 2 cases (mesotheliom, lymphatic leukosis). The cow with the mesotheliom showed a left displacement of the abomasum and the cattle lymphatic leukosis exhibited ascites. In 2 cases blood investigation led to the diagnose lymphatic leukosis (2 cattle). Histogenetically 53.8 % of the tumours were of epithelial, 22.2 %, 38.5 % of mesenchymal and 7.7% pigment- forming cells origin.

Conclusion: Studies according tumors are rarely repoerted in the literature. Generally, this study should be of scientific interest and also useful for veterinarians in terms especially of diagnosis and prognosis.

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Preliminary evaluation of an herbal dry-off tablet on dairy cow transition health and performances

Delhez, P.1, Hamard H.1, , Theron L.1, Ackaert G.² Rao A.-S1
1RumeXperts (rue des Bada 27, 4317 Faimes, Belgium)
²AHV International B.V. (Schokkerweg 10, 8042 PC Zwolle, Netherlands)
Speaker : ltheron@rumexpert.vet

Introduction

The dry-off period (DO) represents a pivotal transition in the lactation cycle of dairy cows, significantly impacting health and productivity. During this phase, cows undergo substantial physiological adjustments, including metabolic, hormonal, and immune changes, which prepare them for the subsequent lactation. Mismanagement during this critical stage can result in metabolic disorders, compromised immune function, and reduced milk yield. The European Commission issued Regulation (EU) 2019/6 which came into force on January 28, 2022. This regulation aims to limit resistance and enhance animal welfare by mandating Selective Dry Cow Therapy. This calls for effective strategies essential to maintaining transition cow health and optimizing productivity. Recent advancements in dry-off strategies emphasize the use of dietary interventions and targeted treatments to support metabolic adaptation and minimize negative energy balance. This study evaluates the impact of a novel dry-off tablet (StopLac®, AHV; Active substance Allicin, $C6H10OS2$) on a wide range of biomarkers related to metabolism, mineral balance, enzyme profiles, and milk production, aiming to establish its potential benefits and identify key biomarkers affected by the intervention.

Materials and Methods

A longitudinal, randomized, double-blinded pilot study was conducted involving 20 multiparous Holstein cows from a single commercial farm. The animals were divided into two groups: a treatment group (n=10) receiving the DO tablet and a control group (n=10) receiving standard care. Biomarkers were assessed across blood, milk, and clinical metrics at day 1 before DO (DO-1), day 3 after DO (DO3), day 40 after DO (DO40), day 7 after Calving (C7). Statistical analyses were conducted using R software, and temporal boxplots were generated to visualize trends. Cohen's d effect sizes were calculated to quantify the magnitude of differences between groups at each time point.

Results and conclusion

- Energy Metabolism (blood): Lower non-esterified fatty acids (NEFA) levels were observed in the treatment group DO40 (median difference of 50%), indicating reduced fat mobilization prepartum. Additionally, higher urea levels in the treatment group at DO40 and C7 (median differences of 13% and 15%, respectively) suggest better amino acid utilization and metabolic preparation for calving. Lower values of aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH) in the treatment group at C7 (median differences of 9.5% and 17%) further support reduced energy reserve mobilization.
- Mineral Profile (blood): Treated cows showed higher calcium (median difference of 9%) and phosphorus levels (median difference of 24%) at C7, which are critical for postpartum recovery and metabolic health.
- Milk Health and Inflammation: No significant differences in somatic cell count or early inflammation biomarkers such as cathelicidin were observed between groups across the study. Significant reduction in milk lactose, and mild reduction in milk pH were observed in the treatment group at DO3.

In conclusion, the dry-off tablet demonstrated promising energy and mineral metabolism benefits, indicating its potential to improve cow adaptation during the dry-off transition. These results highlight their role in reducing metabolic stress and supporting transition period recovery. While the study provides compelling preliminary evidence, further research with larger sample sizes is essential to confirm these findings and optimize dryoff period protocols.

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Dynamics of udder health key performance indicators – a national perspective 2017-2023

Farre M[1]. and Astrup LB[1].
[1]SEGES Innovation, 8200 Aarhus N, Denmark.

Objectives:

The objective of this study was to conduct a comprehensive analysis of Dairy Herd Improvement (DHI) data at the national level over five consecutive years (2017-2023), with a particular emphasis on the development of udder health in dairy cows. The study aimed to differentiate between improve-ments in udder health that arise from adjustments in data parameters—such as selective reporting or methodological manipulation—and those resulting from genuine, measurable enhancements in over-all udder health.

Material and methods:

In Denmark, approximately 91% of all dairy cows are enrolled in DHI-recordings, with mandatory documentation of all udder treatments in the Danish National Cattle Database. This data collection adheres to the International Committee for Animal Recording (ICAR) guidelines and the International Dairy Federation (IDF) definitions for DHI. Consequently, comprehensive datasets are available, en-abling robust national-level comparisons of various udder health parameters. Among the key metrics available for analysis are the national averages for new infection rates, instances of acute mastitis, bulk tank somatic cell count (BTSCC) in shipped milk, and BTSCC recorded on test days. The present study utilized these extensive datasets, spanning five years, to conduct descriptive analyses that ex-amine the dynamic factors influencing udder health. Specifically, the study explored trends associated with changes in milk shipment patterns, longivety, and antibiotic prescription practices, providing valuable insights into the factors impacting udder health over time.

Results:

Over the course of five years, four key parameters consistently and mutually corroborated a trend of improved udder health in Danish dairy cows. First, the new infection rate during lactation decreased from 11.93% to 10.95%. Second, the number of treated clinical cases of acute mastitis declined from 0.25 to 0.23 cows per year. Third, the average BTSCC dropped from 210,640 to 186,820. Fourth, the proportion of chronically infected cows decreased from 15% to 13%. Simultaneously, two additional parameters provided further evidence that the observed improvements were indicative of genuine progress in udder health, rather than reflecting selective data manipulation. First, the difference between the volume of milk produced and the total amount of shipped milk de-creased, suggesting that improvements in udder health were not attributable to an increase in dis-carded waste milk. Second, the reduction in antibiotic treatments was not offset by a redirection of antibiotic consumption into other treatment categories. Notably, the prescription of broad-spectrum antibiotics for mastitis also decreased, and the dosage per treatment remained consistent throughout the five years, reinforcing the notion that the observed changes reflected true advancements in udder health management, rather than adjustments in data or treatment practices.

Conclusion:

The reduction in the new infection rate during lactation has had a cascading effect on the number of clinical mastitis cases requiring treatment, leading to fewer treatments and a decrease in (BTSCC). Furthermore, the proportion of shipped milk relative to the total DIH-recorded milk production has increased, suggesting that improvements in udder health are not the result of altered milk disposal practices. Taken together, these data provide compelling evidence that the observed changes in udder health parameters reflect genuine, sustained improvements rather than biased adjustments due to fac-tors such as increased milk discard, higher culling rates, or shifts in antibiotic prescription practices. Therefore, the observed enhancement in udder health can be attributed to more effective management strategies and the implementation of preventive measures.

Effect of additional prednisolone in the intramammary treatment of clinical mastitis

J. M. Swinkels[1], G. Porcheron[2], O. Roy[2], M. Catalas[2], D. Ledoux[3], A. de Boyer des Roches[3], and F. Leboeuf[4]

swinkels.jantijn@gmail.com, oroy@cebiphar.com, MCatala@cebiphar.com, dorothee.ledoux@vetagro-sup.fr, alice.deboyerdesroches@vetagro-sup.fr, frederic.leboeuf@msd.com

1 MSD Animal Health, Boxmeer, The Netherlands,

2 Cebiphar, Fondettes, France,

3 Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, France.

4 MSD Animal Health, France.

Objective

Intramammary (IMM) antibiotics combined with prednisolone are frequently used to treat clinical mastitis (CM), although field trials confirming the additional effects of prednisolone are scarce. This trial aimed to evaluate the effect of adding prednisolone to cefapirin compared to cefapirin alone on milk parameters and cow behaviour, assessed directly or using cow and milk sensors in cases of naturally occurring CM.

Material and Methods

Nine French dairy farms with Holstein Friesian cows in free stalls, using a Lely A4 or A5 Automated Milking System (AMS), and equipped with milk and cow sensors, were selected for this trial. Milk sensors (Lely) collected data on milk production, color, temperature, Somatic Cell Count (SCC) and conductivity at quarter level, while cow sensors (SenseHub neck monitoring tags, MSD Animal Health) monitored daily cow rumination and activity.

CM was detected using AMS Reports 12 and 23 and/or indicators of high conductivity and/or a high SCC in the T4C or Horizon software. Farmers were instructed to verify alerts at least twice daily, and clinically confirm mastitis before contacting the local veterinarian for inclusion within 2 hours.

On the day of inclusion (D0), the veterinarian performed local and general clinical examinations and collected a milk sample for bacteriology and cortisol measurement. Treatment involved either a commercially available IMM tube (300 mg cefapirin and 20 mg of prednisolone (P), Mastiplan LC, MSD Animal Health), or a IMM tube containing only 300 mg of cefapirin (no prednisolone: NP). The treatment distribution was randomized using statistical software to determine the sequence of treatments (P or NP). Both farmers and veterinarians were blinded to treatments.

The treatment was administered three additional times at the subsequent milking sessions, i.e. at D1 (and possibly D2). Before treatments, the farmer scored cow behaviour, assessed the CM case clinically, and collected a milk sample for cortisol assays.

On D14, the farmer observed cow behaviour using a scoring grid, the veterinarian performed a final clinical examination and collected a milk sample for bacteriology and cortisol measurement. Milk samples were frozen until transferred to the laboratory. Milk and cow sensor data were collected from 14 days before to 14 days after the occurrence of CM.

Statistical analyses were performed using a univariable and multivariable models incorporating biologically plausible fixed factors and random effects, with logistic regression used for behaviour analysis. Fixed factors were removed when not showing significance based on Chi-square tests.

Results

Due to early detection and veterinarians' prescription habits for additional parenteral treatment in more severe (grade 3) CM cases, mainly mild (grade 1 and 2) CM cases were included. In total, 88 CM cases were identified, characterized by changes of milk appearance, increased SCC and conductivity, decreased milk yield, and reduced activity and rumination.

The most frequently isolated pathogens were coagulase-negative staphylococci (CNS) in groups NP (n=44) and P (n=44) at 34% and 25%, respectively, followed by Enterobacteriaceae (3% in NP and 28% in P) and *Streptococcus uberis* (17% in NP and 11% in P). Cultures were negative in 26% and 22% of the CM cases, respectively. The overall bacteriological cure rates were 77.3% in group NP and 82.6% in group P, with no significant difference (Chi-square: $P = 0.6$).

Kaplan-Meier survival estimates indicated a significant difference ($P = 0.03$) in the time to return to normal quarter SCC (< 200,000 cells/ml) in the P group (faster) compared to NP. CM cases in the P group also had a significantly lower milk cortisol level ($P < 0.01$) during treatment. In the multivariable model, no significant difference was found between the two treatment groups in milk conductivity, 24h rolling average of rumination ($P = 0.72$), activity ($P = 0.98$), milk yield ($P = 0.90$) or behaviour ($P > 0.05$).

Conclusion

Adding prednisolone to IMM antibiotic treatments in mild CM cases significantly shortened the time for SCC to return to normal and reduced cortisol levels in milk during treatment, thereby improving milk quality and potentially enhancing animal welfare. The additional prednisolone treatment did not influence milk yield, rumination, activity, or behaviour under the conditions of this study.

Treatment of clinical and subclinical mastitis at calving with cephalexin in cows subject to selective dry cow therapy in Hungary

Peter Kovács*

*University of Veterinary Medicine Department of Animal Hygiene, Herd-Health and Mobile Clinic, Budapest, Hungary - peter.kovacs.dvm@gmail.com

Objectives - In our study we examined the results of pathogen based selective dry cow therapy and post-partum treatment of fresh cows with intramammary infection.

Material and methods - The trial was conducted in an eastern Hungarian dairy farm. On the farm approx. 650 Holstein Friesian dairy cows are milked 3 times a day. The average milk production was 35-37 kg/cow/day during the trial period, with an average DHI SCC of 93 000 – 193 000 SCC/ml during the same time.

The animals eligible for this treatment protocol were pregnant cows without production problems or major health problems. The animals were enrolled one month before the scheduled dry off date. The udder of these animals should not have presented major defects (asymmetry, severe lesions of the skin, blind quarter), and the production must have been normally balanced between the quarters. Palpation of the udder tissue should have been supple, painless and the retro-mammary lymph nodes should not have been inflamed.

Animals' first microbiological examination happened less the 7 days before the expected dry off date. If the results were negative, the animals were dried off with teat sealants only (Ubroseal blue Dry Cow – TS Group). If the microbiological result was positive the cows got an antibiotic treatment and a teat sealant (RILEXINE® 375 DC + Ubroseal blue Dry Cow – R375 + TS Group). After calving during the first 7 days the animals were examined again, and milk samples were collected for microbiological examination. If the second test was negative or no Gram + infection was identified, the animals didn't get any treatment. If there was Gram + infection in one or more quarters, and the cows ID number ended with an even number, she was treated with antibiotics (RILEXINE®200 LC – I+ Group). If there was Gram + infection in one or more quarters, and the cows ID number ended with an odd number, she was not treated with antibiotics (I-Group). In case of CM, the affected quarter was treated regardless of the ID number of the cow. 14 days after the antibiotic treatment the cows were examined again.

Results - Drying off: Altogether 202 cows were dried off and had a result after calving. TS group was made of 99 animals, from which 71 (71.72%) were healthy after calving. The remaining cows had at least one quarter infected with the following pathogens: 1 (1.01%) *Bacillus spp.*, 13 (13.13%) *CNS*, 2 (2.02%) *CNS/Escherichia coli,* 1 (1.01%) *CNS/Streptococcus dysgalactiae*, 7 (7.07%) *Escherichia coli*, 1 (1.01%) *Streptococcus dysgalactiae*, 4 (4.04%) *Streptoccus uberis*, 3 (3.03) *yeasts/moulds*.

R375 + TS Group was made of 103 cows from which 83 (80.58%) were healthy after calving. The remaining cows had at least one quarter infected with the following pathogens: 11 (10,68%) *CNS*, 1 (0.97%) *CNS/Escherichia coli*, 3 (2,91%) *Escherichia coli*, 1 (1.01%) *Serratia marcescens*, 3 (2.91%) *Streptoccus uberis*, 3 (2.91) *yeasts/moulds*.

Treatment during the lactation: On the first week's test after calving, 5 cows showed the signs of clinical mastitis. 3 (60%) cases were caused by *Escherichia coli*, 1 (20%) was caused by *Streptococcus uberis* and 1 case (20%) had a negative microbiological result (possibly also *Escherichia coli* infection). 3 cows (60%) were from the TS Group, and 2 (40%) from the R375 + TS Group.

I+ Group: 16 cows were treated who had a positive microbiological result in one or more quarters, but didn't show any signs of clinical mastitis. Altogether 25 quarters had a positive microbiological result. 14 days after the antibiotic treatment, in 16 quarters (64.00%) the previously identified pathogen was eliminated, while in 9 quarters (36.00%) the same pathogen could have been identified in the control test. In case of *CNS* infection (18 cases) the cure rate was 55.56%. In case of *Streptococcus uberis* infection (5 cases) the cure rate was 80% and in case of *Streptococcus dysgalactiae* infection (2 case) the cure rate was 100%.

Conclusions – The selective dry cow therapy is a safe choice of practice, the lack of antibiotics during the drying off in case of non-infected cows didn’t cause a dramatic decrease in the udder health of the freshly calved animals.

The treatment of infected animals after calving, even though there are no signs of clinical mastitis can be beneficial in case of major mastitis pathogens, with high cure rates. In case of CNS infection, the effectiveness and the necessity of the treatment is questionable.

Evaluation of an on-farm test kit for the selective antimicrobial treatment of clinical mastitis on a large dairy farm in the United Kingdom

Peter Plate

Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire AL9 7TA, United Kingdom

pplate@rvc.ac.uk

Mastitis is a major reason for antimicrobial use on dairy farms, and while reducing the incidence has the highest priority to reduce antimicrobial usage, further progress may be achieved by some farms by selective antimicrobial treatment of clinical mastitis.

Objectives:

The study aimed to evaluate a commercial on farm test kit [MastDecide^{TM}] for the selective antimicrobial treatment of clinical mastitis, assessing sensitivity and specificity against conventional laboratory testing as well as mastitis outcomes in comparison with blanket treated cases.

Material and methods:

A large dairy farm in the South of England was recruited and staff trained in the application of the MastDecide^{TM} kit. The test system differentiates bacteria at the Gram positive, Gram negative and no growth level, based on colour changes of the liquid in two Eppendorf tubes (Leimbach and Krömker, 2018). Staff were asked to treat mastitis cases in even numbered cows with antimicrobials as stated in their farm protocol while collecting a sterile milk sample and applying the trial kit in cases in odd numbered cows, and in those only to treat gram positive cases with antimicrobials, while not applying antimicrobials for mild and moderate gram negative or "no growth" cases. The remainder of the collected milk samples were frozen and sent in batches to a recognised commercial laboratory for routine bacteriological culture and species identification using Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Using on farm records and milk recording data mastitis and production outcomes were compared between blanket and selectively treated cows.

Results:

211 cases were submitted by the farm, of which 187 cases were enrolled with correct allocation and complete records. Of those 79 cases were treated according to on farm culture and 108 were blanket treated.

Of 68 suitable samples submitted to the laboratory the on-farm culture kit led to the correct treatment in 75 % of cases. The sensitivity to detect staphylococci was poor (40 %, 4 out of 10 for Staphylococcus aureus and 43 %, 10 out of 23, for other staphylococci). The sensitivity against streptococci was 100 % (6 out of 6 detected).

Of the 79 cases cultured 29 were not treated with antimicrobials, a saving of 37 % of treatments. Milk from cultured untreated cows entered the bulk tank on average after 3.2 days, milk from treated cows (culture and blanket treated) was withdrawn from the tank on average for 9.7 days.

Cure rates, based on the next somatic cell count in a 14-42 day window after the onset of the case (with a cut off value of 200,000/ml) were identical at 70 % in the blanket treated and cultured cows.

The recurrence rate within the current lactation was not significantly different, 38 % for cultured cows and 42 % for treated cows (p=0.6).

Conclusion :

On-farm culture has the potential to reduce antimicrobial treatments and discarded milk without negatively affecting mastitis outcomes, however, it is important to be aware of the predominant bacteria on the farm, using a specialist laboratory, and of the strengths and weaknesses of individual test kits detecting those bacteria. The selective approach can further be refined by taking the individual mastitis history into account (Ruegg, 2018, Schmenger et al., 2020).

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Bovine mammary excretion of *Salmonella* Dublin: fact or fiction?

Millemann Yves 1, Grisot Lionel 2, Vacelet Rémi 3, Ravary-Plumioën Bérangère 1, Gandoin Christelle 1, Bouillin Corinne 1, Arnaud Florence 4

1 Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France 2 Groupements Techniques Vétérinaires Bourgogne Franche-Comté, Frasne, France 3 Centre Technique des Fromages Comtois, Poligny, France 4 Union Régionale des Fromages d'Appellation d'origine Comtois, Poligny, France.
yves.millemann@vet-alfort.fr

Background:

Salmonella Dublin (*S.* Dublin or SD) is a specific serotype of *Salmonella* that has adapted to cattle (El Sayed et al, 2018) and is known to cause severe and often invasive disease in humans (Kidurkiene et al, 2020; Mohammed et al, 2017). There are regularly reports of food poisoning incidents linked to cheese contaminated with this pathogen (Ung et al, 2019; De Sousa Violante et al, 2022; Ministère de l'Agriculture et de l'Alimentation, 2020). In cattle, SD can be detected during mammary excretion (Spier et al, 1991; Velasquez-Munoz et al, 2023), although the characteristics of this excretion remain poorly understood. To better characterize the nature and modalities of SD mammary excretion, Montbéliarde cows identified as excreting the bacterium during routine raw milk inspections were selected for monitoring over a two-week period, which included milk counts to assess the level of contamination.

Methods:

From ten farms where bulk tank milk was contaminated, ten Montbéliarde cows were selected based on their natural individual excretion of SD in milk. Each cow was hospitalized for two weeks and monitored clinically, with regular samples collected: milk samples were taken twice daily, faecal samples were collected three times during hospitalization, and blood samples, along with samples from various organs (including tonsils, uterus, liver, spleen, mesenteric lymph nodes, and retro-mammary lymph nodes), were obtained following necropsy. *S.* Dublin was detected through enrichment in parallel on two different media with colony counts.

Results and discussion:

Nine out of the ten monitored cows were confirmed to be intermittent mammary excretors of SD, exhibiting relatively low excretion levels (up to 300-350 CFU/mL; **Table 1**). The excretion levels varied over time but generally showed a rapid decline. On average, 12 out of 28-30 milkings tested positive, with a maximum of 13 consecutive positive milkings recorded. These cows were typically in their third or fourth lactation and began to show mammary excretion at various stages of lactation. It is noteworthy that up to 19 consecutive milkings could yield negative results, complicating the identification of excreting cows within the herd. When the investigation was conducted (regarding the last four included cows) at the quarter level, it was observed that each cow tended to have only one excreting quarter consistently over several days. Importantly, these cows did not exhibit any signs of illness and did not show faecal excretion. Among the nine confirmed mammary excretors, two presented SD in the retromammary lymph nodes, and this was observed exclusively in these organs.

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Table 1. Evolution of mammary excretion of *Salmonella* Dublin over time in the 10 hospitalised cows.

Day / cow nb.	Cow nb. 1	Cow nb. 2	Cow nb. 3	Cow nb. 4	Cow nb. 5	Cow nb. 6	Cow nb. 7	Cow nb. 8	Cow nb. 9	Cow nb. 10
D1 AM	ND	ND	ND	+	++	+	+	*NA*	*NA*	ND
D1 PM	++	ND	ND	ND	+++	ND	ND	++	++	++
D2 AM	+	ND	ND	ND	++	ND	+	++	+	+++
D2 PM	+	ND	ND	ND	++	+	+	+	++	++
D3 AM	++	ND	+	ND	++	+	ND	+	+	++
D3 PM	+	ND	+	ND	++	ND	+	ND	++	++
D4 AM	+	ND	+++	ND	+++	ND	ND	ND	+	++
D4 PM	+	ND	++	ND	++	ND	ND	ND	++	++
D5 AM	++	ND	+	ND	++	ND	ND	ND	+	ND
D5 PM	+	ND	+	ND	+	ND	ND	ND	++	ND
D6 AM	+	ND	+	ND	+	ND	+	+	++	ND
D6 PM	+	ND	+	ND	+	+	ND	ND	+	+
D7 AM	+	ND	+	ND	+	ND	ND	ND	++	+
D7 PM	+	ND	+	ND	ND	ND	ND	ND	ND	ND
D8 AM	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
D8 PM	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
D9 AM	+	ND	+	+	ND	ND	ND	ND	ND	ND
D9 PM	ND	ND	ND	ND	ND	ND	+	ND	+	ND
D10 AM	+	ND	+	++	ND	ND	ND	ND	ND	ND
D10 PM	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
D11 AM	ND	ND	ND	+	ND	ND	ND	ND	ND	ND
D11 PM	ND	ND	ND	ND	ND	+	+	ND	ND	ND
D12 AM	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
D12 PM	ND	ND	ND	ND	ND	+	+	ND	ND	ND
D13 AM	ND	ND	ND	ND	+	ND	ND	ND	+	ND
D13 PM	ND	ND	ND	ND	ND	+	++	ND	ND	*NA*
D14 AM	ND	ND	ND	ND	+	+	ND	ND	ND	ND
D14 PM	ND	ND	ND	ND	ND	ND	ND	ND	ND	*NA*
D15 AM	ND	ND	ND	ND	++	ND	ND	ND	ND	*NA*
D15 PM	*NA*	ND	*NA*	*NA*	*NA*	*NA*	*NA*	ND	*NA*	*NA*

+: presence (less than CFU/mL); ++: between and CFU/mL; +++: over CFU/mL; ND : not detected; *NA* : not applicable (no sampling).

NOTES PERSONNELLES

E. MORGAN

Update on antiparasitic resistance in cattle

Professor Eric Morgan
Institute for Global Food Security
School of Biological Sciences
Queen’s University Belfast (Ireland)

Resistance of parasites to antiparasitic drugs can develop through a number of heritable mechanisms. Increasing antiparasitic resistance is therefore an inevitable consequence of using chemical antiparasitics as the mainstay of parasite control in cattle, as in other livestock species. Complacency around the resistance situation in cattle has been shaken by recent reports and the realization that easy alternatives are not in place to support current grazing practices should resistance become further established, especially among gastrointestinal nematodes (GIN).

Anthelmintic resistance in Europe – current status and consequences

Reports of anthelmintic resistance (AR) among ruminants in Europe have been collated in an online database and initial results published in Vineer et al. (2020). The number of publications on cattle is much lower than that for small ruminants, and is biased towards Belgium, France, Italy and the UK. Notably, where AR was not observed at high prevalence, this was associated with low research effort. Resistance has been reported against all major anthelmintic groups, including the benzimidazoles (e.g. albendazole), imidazothiazoles (e.g. levamisole) and macrocyclic lactones (e.g. ivermectin and moxidectin) from nine countries at prevalence up to 100%. Where studies have identified species involved, *Ostertagia ostertagi* is commonly implicated alongside *Cooperia oncophora* (Kelleher et al. 2018), which id concerning as the former is more pathogenic and can affect older animals later in the production cycle. Resistance has also been reported in lungworm, *Dictyocaulus viviparus*, and liver fluke, *Fasciola hepatica*.

The consequences of AR in GIN of cattle could become keenly felt. The pathogenesis of GIN strikes at the heart of protein assimilation by cattle, whether producing meat or milk (Charlier, Höglund et al., 2020), and intensification of grazing systems has only been possible through their effective control, which is now threatened. This could have serious economic consequences (Charlier, Rinaldi et al., 2020) and impede aims to curb greenhouse gas and other emissions from livestock (Kyriazakis et al., 2023). Clinical disease is also likely: recent cases of ostertagiosis in cattle, a disease not seen under effective control by anthelmintics, have been associated with AR (Bartley et al., 2021). Knowledge of anthelmintic effectiveness on individual farms is increasingly essential information to support effective health plans.

Measuring anthelmintic resistance in cattle

Some laboratory tests are available to evaluate AR in GIN in vitro, using the response of eggs or larvae to different concentrations of anthelmintic. These are moderately reliable for benzimidazoles (egg hatch test, or identification of genetic markers using PCR) and a work in progress for macrocyclic lactones (various measures of larval motility) but are mainly research tools currently and not generally available commercially. The only practical way to check effectiveness across all anthelmintic groups is in vivo using the faecal egg count reduction test (FECRT). Faecal egg counts (FEC) in samples from animals on the day of treatment and 7-14 days later, depending on the drug, are used to estimate percentage reduction in FEC (%FECR), which should exceed 95% for acceptable efficacy.

The guidelines for correct implementation and interpretation of the FECRT have been recently updated (Kaplan et al., 2023) and can be obtained in user-friendly format from the COMBAR project (https://www.combar-ca.eu/media). Unfortunately, a number of complications can occur in practice (Morgan et al., 2022). The test considers total FEC and variations in GIN species composition along with differences in species-specific anthelmintic efficacy can mask AR and lead to different test outcomes even on the same farm. Failure to deliver an effective dose, e.g. through poorly calibrated equipment or pharmacokinetic factors such as with pour-on anthelmintics can also affect results. Crucially, in cattle, starting FEC are often low and rapid declines in FEC due to immunity in first season grazers can give the impression of higher %FECR than is caused by treatment alone, again masking AR. It is important to follow test protocols as closely as possible, but this makes the FECRT quite onerous and this means it is rarely conducted in practice. Composite (pooled) FEC can simplify testing requirements, decrease cost and increase accessibility, but at the price of accuracy (Rinaldi et al., 2019).

The majority of cattle farms therefore apply anthelmintics without knowing how effective they are, risking exacerbation of early stage AR. Initially, low grade AR will not lead to overt treatment failure because the majority of the parasite population is still killed, but continuing to use failing anthelmintics in such circumstances will likely accelerate their demise. Waiting for therapeutic failure (i.e., persistence of poor performance or clinical signs after treatment) before considering AR will incur production costs and narrow the options available for remedy.

The way forward for sustainable parasite control?

Ideally, more sustainable approaches should be implemented on all farms to preserve continued anthelmintic efficacy. These tend to focus on building refugia by leaving at least some individuals untreated as a source of susceptible genotypes (Charlier et al., 2014). This strategy does not come without risk, as reticence in preventive treatments could lead to excessive build-up of infective larvae on pasture and impacts on health, growth and other performance outcomes. Models are increasingly used in research to assess this trade-off (e.g. Berk et al., 2016; Filipe et al., 2023) but are not yet widely applied to practical parasite management.

In practice, any approach that decreases reliance on whole-herd interval anthelmintic treatments should improve prospects in terms of AR development, while having co-benefits for the development of immunity in youngstock and for reducing environmental impacts of antiparasitics through off-target effects (Sands and Wall, 2018). Environmental impacts and trade-offs with the production benefits of anthelmintic use are subjects of growing interest in Europe, with opportunities to engage in collaborative networks for research (https://www.cost.eu/actions/CA23154/) and practice (https://wormsparc.com/). Increasingly, anthelmintics should be viewed as a tool to remedy the limits of alternative approaches, not a first line of defence (Vercruysse et al., 2018).

Resistance in other parasites

Apart from helminths, in which the issue is arguably the most widespread and pressing, failure of antiparasitic drugs has also been reported for coccidia and for ectoparasites, especially psoroptic mange (Van Mol et al., 2020). While context-specific factors apply especially in how to prevent and manage resistance, the principles of integrated parasite management with reduced reliance on chemical antiparasitics applies across parasite taxa.

Conclusions

Anthelmintic resistance in cattle is already a major problem but submerged by poor recognition. Better performance monitoring especially in youngstock and wider uptake of tests for AR, especially the FECRT, would help to rectify this knowledge gap and provide actionable intelligence to guide health plans on individual farms. Other parasites are also developing resistance to chemical anthelmintics. Since major advances in cattle production systems on pasture have relied on effective parasite control, these might have to be re-imagined for robustness in a future world in which effective anthelmintics can no longer be relied on as a given. Early adopters of alternative and complementary parasite management tools might secure a lasting competitive advantage, and assistance from well-informed advisors on how to slow AR is valuable.

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NOTES PERSONNELLES

Prevalence and antimicrobial susceptibility of bacterial species associated with interdigital phlegmon (footrot) lesions in French adult cattle using interdigital swab culture

Busnot M.[1], Lutz C.[2], Lequeux G.[3], Guatteo R.[1], Relun A.[1]

[1] Oniris, INRAE, BIOEPAR, Nantes, France
[2] SNGTV, Hochfelden, France
[3] Labocéa, Fougères, France

Oniris VetAgroBio – anne.relun@oniris-nantes.fr

Objectives

Interdigital phlegmon (IP), also known as footrot, is an infectious cause of acute lameness in cattle and is thought to result from the penetration of *Fusobacterium necrophorum* into the interdigital space. Although IP generally responds well to parenteral antibiotic treatment, nearly 50% of rural French veterinary clinics reported at least one case of IP unresponsive to treatment in 2023. One hypothesis is that treatment failures may be linked to the presence of specific bacterial species or the development of antimicrobial resistance among bacteria associated with IP. This study aimed to assess the prevalence of bacterial species isolated from IP lesions in adult cattle in France and to characterize their antimicrobial susceptibility profiles.

Material and Methods

A multicentric cross-sectional study was conducted in France from February 2024 to June 2025, aiming to enrol 70 adult cattle with acute IP across 10 veterinary clinics. Ethical approval was obtained prior to the study. Veterinarians performed a clinical examination to confirm IP, defined as symmetric painful swelling of the foot with sudden onset of lameness, often accompanied by a fetid odour and possible fissure in the interdigital cleft. Inclusion criteria were: cattle > 18 months old, no antibiotic treatment in the previous 15 days, first signs of IP within 24 hours, absence of complication and absence of other cause of lameness or concurrent diseases.

The interdigital space was gently cleaned and disinfected before collecting a sample. Using a sterile swab, veterinarians scraped the moist interdigital area and placed the swab tip containing the collected material into a modified Stuart's Transport Medium. The swabs were stored at 4°C and shipped to the laboratory within 4 days.
In the laboratory, swabs were cultured, and bacterial isolates were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Antimicrobial susceptibility testing

was performed on the isolates using the E-test or the disk-diffusion method following EUCAT guidelines for anaerobes. Samples were classified as polymicrobial if two or more bacterial species were identified. Data from bacteria with intrinsic resistance to the tested antimicrobials were excluded from the analysis. Multidrug resistance was defined as resistance to ≥3 antimicrobial classes.

Results

At this stage, 27 samples have been collected and analysed, 11 of which were classified as polymicrobial. A total of 22 bacterial species were identified, with *Bacteroïdes pyogenes* (8/27), *Fusobacterium necrophorum* (6/27) and *Peptinophilus indolicus* (4/27) being the most frequently isolated. Antimicrobial susceptibility testing showed that 7 out of 24 tested isolates were resistant to at least one antimicrobial, and multidrug resistance was observed in one isolate. This isolate of *B. pyogenes* was resistant to sulfamethazole/trimethoprim, tulathromycine, and ceftiofur. Resistance was particularly common among *B. pyogenes*, with 4 out of 7 isolates displaying resistance to at least one antimicrobial, mostly sulfamethazole/trimethoprim and tulathromycine. Conversely, all 4 isolates of *F. necrophorum* were either fully sensitive or exhibited intermediate sensitivity to all tested antimicrobials. The most frequently observed resistances were to sulfamethazole/trimethoprim (5/24) and tulathromycine (3/24), while resistance to tilmicosine (2/24), oxytétracycline (2/24), tetracycline (1/24) and ceftiofur (1/24) was less common.

Conclusion

These preliminary results indicate that *Bacteroïdes pyogenes*, in addition to *Fusobacterium necrophorum*, may contribute to the etiopathogenesis of IP in cattle and could harbour antimicrobial resistance. However, bacterial isolates from IP in French adult cattle do not appear to have acquired widespread resistance to the antibiotics commonly used for IP treatment. Treatment failures might instead be explained by other factors, such as delayed treatment initiation, improper dosage or misdiagnosis of the foot lesion.

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Botulism in cattle: Assessing risk factors and vaccination interest in the Netherlands

Debora Smits[1], Inge M. van Geijlswijk[2], Menno Holzhauer[1], Yannique M.F. Jacobs[1], Lotte C.A.J. Roos[1], Geert Vertenten[3], Miriam G.J. Koene[4]

Royal GD, Postbus 9, 7400 AA Deventer

[1]Royal GD, Deventer, The Netherlands
[2]UU FVM, Utrecht, The Netherlands
[3]MSD Animal Health, Boxmeer, The Netherlands
[4]Wageningen Bioveterinary Research, Lelystad, The Netherlands

Affiliation d.smits@gddiergezondheid.nl

Objectives

Botulism in cattle is a serious and frequently fatal illness caused by toxins produced by the bacterium *Clostridium botulinum* which can lead to paralysis and death. Botulism typically occurs when cattle ingest feed or water contaminated with the botulinum toxin coming from carcasses, for example poultry or wildlife.

In the case of botulism in cattle, immediate removal of the (potential) source of the toxins is most important, but vaccinating cattle against botulism is also very useful as a preventive measure. Until 2021, a vaccine from South Africa was used for vaccination against botulism in the cascade arrangement.

Since Regulation (EU) 2019/6 came into effect on January 28 2022, the use of vaccines from countries outside the EU is only allowed for list A and B diseases (Commission Implementing Regulation (EU) 2018/1882). This means that vaccination against botulism is no longer possible in the EU because it is not a list A or B disease and there is no vaccine with marketing authorisation in the EU. However, Regulation (EU) 2019/6 is not applicable when it concerns veterinary medicinal products intended for research purposes (Article 2(7)).

A study to investigate the risk factors for botulism outbreaks at cattle farms in the Netherlands has been initiated by Royal GD, which included the possibility for farmers to vaccinate their cows against botulism in the event of an outbreak or when a farm is at increased risk for botulism.

The objective of this study is to identify risk factors, to assess the extent of damage and losses associated with a botulism outbreak and evaluate the demand for vaccination, in order to:

- Convince the European Commission to amend the regulation at this point
- Or to encourage a pharmaceutical company to market a vaccine in the EU

The study affiliates with the 'national animal health surveillance' performed by Royal GD, and is being carried out in collaboration with the Pharmacy department of Faculty of Veterinary Medicine Utrecht University and Wageningen Bioveterinary Research.

Material and Methods

Throughout the project, cattle farmers are given the opportunity to vaccinate their animals against botulism in case of an outbreak, a history of botulism or by an increased risk for botulism. Criteria for inclusion in the study are when vaccination is considered a beneficial measure for the particular farm, and under the provision that farmers agree to share farm details (including herd size, region, close presence of (free range) poultry, the number of affected animals in the event of an outbreak) and allow a farm visit from a veterinarian from Royal GD.

The vaccine used in this study [Botuvax, MSD Animal Health, South-Africa] is approved and widely used in, amongst others, South Africa and Namibia. The vaccine contains *Clostridium botulinum* type C- and D-toxoids. The decision to vaccinate is ultimately the responsibility of the farmer.

Results

Since the start in April until the end of 2024, 28 farms were included in the study; five because of an actual outbreak and the others because of a history with the disease or an elevated risk. The project will continue to run in 2025. Preliminary results show that, although outbreaks are rare, the impact on animal health and welfare, along with financial and emotional strain experienced by the farmer, can be enormous.

Conclusion

The results so far underline the need for a readily available vaccine. This is underscored by the motivation of many farmers who have experienced an outbreak in their cattle to persist with vaccination efforts, given the considerable impact of botulism.

Effect of deltamethrin on intramammary infections, qualitative and quantitative parameters of the milk, stress and fatigue status and antioxidants profile in Holstein cows during peak lactation period under heat stress conditions

Arsenopoulos K.V.[1], Gelasakis A.I.[2], Triantafyllou E.[3] and Papadopoulos E.[1]

[1]School of Veterinary Medicine, Aristotle University of Thessaloniki Greece
[2]School of Animal Biosciences, Agricultural University of Athens Greece Vet-
[3]Analyseis (Veterinary Microbiology Laboratory), Larissa Greece

Intramammary infections represent an important health issue in dairy cows worldwide, which are proven to be largely associated with the presence of flies. The aim of the study was to investigate the possible effects of the fly repellent deltamethrin on intramammary infections, qualitative and quantitative parameters of the milk, stress and fatigue status and antioxidants' profile in Holstein cows during peak lactation period (3 months post-partum) under heat stress conditions.

The study was conducted in one dairy cattle herd of Holstein breed reared under intensive management in Central Macedonia Greece. The herd had a history of subclinical and clinical mastitis (especially during spring and early summer) combined with severe fly infestation. None of the participated cows were treated with antimicrobials during the previous 30 days. A total of 50 multiparous cows (3 to 5 years old), in peak lactation period (1 to 3 months post-partum) were enrolled in the study. The cows were randomly divided in 2 equal groups (25 cows each); Group A cows (n=25) were individually dressed twice on their back with deltamethrin (Deltanil®, Virbac) post morning milking (at Days 0 and 30), while at the same days, placebo treatments were, also, applied on Group B cows (n=25, control group). Numbers of flies landing on the animals in both groups were recorded by direct observation of the animals. Ten fly traps were set in locations inside the pens at the animals' level, but not accessible by them, at equal distances on Days 0 and 30, in order to capture and identify fly species present in the farm. Milk yield was recorded and milk samples were collected from each individual cow, starting from Day 0 and every 15 days for 4 times thereafter (Days 15, 30, 45 and 60), to measure somatic cell counts (SCC) and electrical conductivity in each milk sample were counted and there were performed bacteriological cultures. Also, the milk production of each animal and the electrical conductivity were recorded. Furthermore, blood samples were collected for serum cortisol (SC), creatine kinase (CK), and antioxidants concentration measurements. Results of the studied traits were statistically analysed using mixed linear regression models, incorporating the fixed effects of treatment with deltamethrin, sampling occasion, and their interaction term, as well as the random effect of animal.

The present study recorded that deltamethrin application was associated with significantly higher milk production (by 12.0 kg, $P < 0.001$), decreased somatic cell counts (by ca. 360,000 somatic cells/mL, $P<0.001$), milk electrical conductivity (by 3.3 MilliSiemens per centimetre $P < 0.001$), SC (by 7.3 μg/dL, $P < 0.001$), CK (by 648 μg/dL, $P < 0.001$) concentrations and increased antioxidant capacity (Oxy-adsorbent increased by 72.2 units, $P < 0.001$).

Our findings highlight the significance of flies control when designing biosecurity and udder health management protocols. Moreover, it has been evidenced that deltamethrin (Deltanil®, Virbac) facilitates the effective control of flies and contributes to the formation of a more welfare-friendly environment for intensively reared dairy cows under heat stress conditions.

A novel tool designed to measure and support appropriate reduction of parasiticide use in cattle

Jennifer E. Allan[1], Robert W. Howe[2], Nicholas J. Bell[1]
[1] Herd Health Consultancy, Highwood, Smugglers Lane, Wimborne, UK, BH21 2RY.jenny.herdhealth@gmail.com
[2] Clint Mill, Cornmarket, Penrith, UK, CA11 7HW.

Objectives and introduction

Parasite control measures have traditionally been dominated by the routine use of parasiticides in the cattle sector, particularly in youngstock. However, product residues in the environment (1–3) and development of parasiticide resistance (4) are recognised as negative impacts from the routine use of these products.

Integrated parasite management (IPM) is the method now being used to describe a package of preventative measures such as vaccination, diagnostics and pasture management. IPM aims to optimise the amount of parasiticides used over a grazing season through management changes, diagnostics and reduced exposure to parasite contaminated pasture.

From 2014 to 2020 the UK livestock industry reduced its antibiotic use by 52% (5), one of the key drivers of this was the ability to measure antimicrobial usage.

The aim of this project was to develop a tool to both;

1. Measure parasiticide usage in cattle
2. Assess appropriateness of parasiticide use

The tool's methodology described in this paper allows analysis of not only the amount of parasiticide used, but also facilitates and assesses the implementation of IPM and critically avoids a simplistic focus on treatment reductions, in order to protect animal health. All UK licensed parasiticide products are included. To the authors' knowledge this methodology describes the first attempt to evaluate parasiticide usage**.**

Material and methods

There are two sections to the tool:

1. Parasiticide Use Calculator:

This section describes two metrics; the 'Total doses' (TD) of parasiticide product administered and the 'Cumulative Days of Parasiticide Activity' (CDPA). These metrics are designed to record parasiticide usage and to provide objective, benchmarkable data.
The two metrics described are able to be utilised across variable time periods, animal groups and types of cattle system. The TD metrics calculate the number of doses given across all animal management groups and includes all products given. CDPA is defined as the number of days that a parasiticide is present and acting against a target parasite. It is represented as a percentage of days within a defined timeframe. This metric is proposed to act as a proxy for; time product active exerts selection pressure (SP) at therapeutic levels on target and; time product active and metabolites, are potentially excreted into the environment (urine, dung etc). For illustrative purposes the authors have chosen to focus on products given across the full year and products given during the grazing period to animals that have grazed. The grazing period was focussed on as products given during this time will impact resistance, immunity development and the environment more so, than at housing.

2. Question Based Review:

This section uses flowcharts to facilitate delivery of IPM decision making, including prevention management practices and diagnostics used to drive treatment decisions. The answers given are inputted within the flowcharts by veterinary advisors or suitably qualified persons during an annual veterinary review. They are designed to promote better practice which consequentially drives appropriate reduction in overall usage and assesses IPM decision making. The first three charts focus on diagnostic decision making; whilst animals

are at pasture, once animals are housed for the winter and for liver fluke control.
The second three charts focus on preventing animal exposure to parasite environmental challenge. Management factors (e.g. vaccination, biosecurity, nutrition and nuisance fly control), grazing rotation management and what pastures naive animals are turned out onto are focused on.

Results

Five model farms detailing a variety of scenarios found on UK cattle farms, were developed and trialled through the tool. These were used to illustrate functionality, to demonstrate how the tool can differentiate farms according to usage, and identify key differences in treatment protocols.

All elements of IPM were represented to create a holistic tool. Encouraging reduced parasiticide use needs to be balanced against animal health and welfare considerations. The individual farm context is paramount to responsible use and needs to always be taken into account. It is acknowledged that there will be differing industry opinions on IPM actions and as more evidence is published and knowledge gaps are filled, these flowcharts will be reviewed and updated.

Conclusions

At present, there is no monitoring of parasiticide usage in the UK and to the authors

knowledge there is currently no other tool available to measure parasiticide usage or IPM decision making in the cattle industry. Availability of this data will accelerate the drive for change and reduction where it is appropriate. This tool will provide data to understand and appropriately reduce usage of parasiticides in cattle, as the industry progresses towards full adoption of IPM.

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Q fever awareness among European ruminant farmers

Raphaël Guatteo[1], George Valiakos[2], Vincent Dedet[3] and the members of the European Q fever committee[4]

[1] Oniris, INRAE BIOEPAR, 101 Route de Gachet, F-44300 Nantes, France

[2] Faculty of Veterinary Science, University of Thessaly, 43100 Karditsa, Greece

[3] Auzalide Santé Animale, la Chouannière, 143 – Le Cannée, 35380 Paimpont, France

[4] For more details on the European Q fever committee, visit https://euqfevercommittee.com/

Objectives: In the context of the One Health approach and the management of zoonosis, a European Q Fever Committee was established in 2024, bringing together veterinary and medical experts from various disciplines and countries. As a first preliminary step, an unprecedented and large survey of ruminant (cattle, sheep and goats) farmers was conducted in eight European countries, to assess their degree of awareness and level of knowledge on Q fever, and their related practices in hygiene and medical control of the disease.

Materials and methods: This survey was conducted by an independent company for opinion polling. Ruminant farmers were targeted in each country (Germany, France, Spain, Italy, UK, Belgium, Netherlands and Poland). The questionnaire used for a similar study conducted in France in 2019 was translated into English, and then into the respective local languages. Interviews were conducted using the Computer-Assisted Telephone Interview technology. Each interview was recorded and lasted 20 to 25 minutes. Interviews took place over the summer of 2024. As the data collected were dispersed over several countries and referred to various animal species, only descriptive statistical analysis was performed.

Results: 905 farmers completed the interview, providing exploitable answers. Cattle (n=571), sheep (n=198) and goat (n=177) farmers were represented (some were farming several species). In the Netherlands, only goat farmers (n=20) and in Germany, Belgium and Poland, only cattle farmers (n=80, n=60 and n=80, respectively) were interviewed. Respondents were mostly men (77%); 70% were over 40 years of age, which is comparable to the current situation in Europe. The average number of heads of animals per farm was 103 for cattle, 234 for sheep and 236 for goats. Overall, answers demonstrated a significant lack of farmers' awareness about the disease, regardless of the country: approximately 75% of all farmers were either unaware of the disease or only familiar with its name in all countries. Veterinarians and digital channels were identified as the two main sources of information for animal health, both in comparable importance across countries, and even among the youngest farmers (25-35 years of age). Personal experience with Q fever is very limited (2-5% of respondents) in most countries, but is higher in the Netherlands (12%) and France (13%). In the Netherlands, the largest zoonotic outbreak, linked to goat farming, occurring in 2007-2009, might explain this score. The French results might be ascribed to the awareness campaigns set up by the French Q fever committee over the last decade. Dutch and French farmers were also those who rated the risk of introduction of Q fever on their farm as the highest (> 20 % of respondents consider this risk as high or very high). Less than 10% of other respondents perceived this risk as moderate. Most farmers aware of the disease know that the bacterium can be transported by wind over several kilometers and is transmissible from animals to humans. There was less consensus about the impact of Q fever on the performance of breeding stock: e.g. among cattle farmers, the awareness rate in Spain was nearly twice that of France (57 vs. 31%, respectively). Even though the majority of respondents aware of the disease mention abortion as a manifestation of the disease, other clinical signs were rarely acknowledged. Following Q fever diagnosis on a farm, at least 90% of farmers mention hygiene measures and manure management as priority control measures. Vaccination and treatments are equally considered by about 25% of farmers, except in Italy where vaccination is only mentioned by 6% of farmers and in Germany where it is not mentioned at all. In contrast, 50% of British cattle farmers and 38% of Spanish cattle farmers refer to vaccination over other treatments. In all countries, apart from the Netherlands (100%) and France (27%), vaccination rates against Q fever are generally low (89-95% of respondents do not vaccinate), a puzzling fact since those farmers are aware of its zoonotic trait. However, among farmers with personal experience with Q fever, the vaccination rate is higher (29-50%, and even 71% in France).

Conclusions: Even though in some countries only farmers of a specific ruminant species were interviewed, to the authors' knowledge, this is the largest survey on the perception of Q fever by ruminant farmers in Europe. Although some countries present particular profiles, it should be noted that awareness of the disease is limited among farmers, including infection control and prevention of transmission. The established European Q fever committee intends to use the reported results to tailor specific awareness/information campaigns for each country.

Resistance in veal calves at slaughter in Germany – Changes over time and potential association with antimicrobial use

Bernd-Alois Tenhagen, Mirjam Grobbel, Carolina Plaza Rodriguez, Annemarie Käsbohrer and Matthias Flor
German Federal Institute for Risk Assessment, Max Dohrn Straße 8-10, 10589 Berlin

German Federal Institute for Risk Assessment, Department Biological Safety –
Bernd-Alois.Tenhagen@bfr.bund.de

Objectives: Veal calves are frequently exposed to high antimicrobial use (AMU), which is associated to the development of antimicrobial resistance (AMR). In Germany, calves under 8 months raised for fattening purposes have been included in the national antimicrobial minimization strategy since 2014. There, AMU is measured on farm level as average days under treatment / year and compared with the AMU of other farms in the sector. The included benchmarking system requires farms with highest AMU in the sector to design a management plan with their cooperating veterinarians to reduce AMU. It is the objective of this study to analyse changes in AMR in isolates from veal calves collected at slaughter in Germany and to investigate their association with AMU on the population level.

Material and Methods: Within the framework of the Zoonoses Monitoring Program, caecal content was collected from veal calves at slaughter in uneven years between 2015 and 2023. Samples were tested for *E. coli*, ESBL/AmpC-producing *E. coli* (selective isolation), *Campylobacter* spp. (not in 2017) and *Enterococcus* spp. (not in 2015). AMR was tested using broth microdilution as prescribed in Commission Implementing Decisions 2013/652/EU (2015-2020) and (EU) 2020/1729 (2021-2023). All minimum inhibitory concentrations were evaluated using epidemiological cut-off values as provided by EUCAST, or, if those were not available, values defined by the European Reference Laboratory for Antimicrobial Resistance (EURL-AR) and published by the European Food Safety Authority (EFSA). While AMU was only descriptively analysed, trends in AMR over time were evaluated using logistic regression with resistance to a certain antimicrobial as binary outcome and year as independent variable. Analyses were done per bacterial species and per antimicrobial. Only antimicrobials tested at all instances were included in the trend analyses. These were 14 antimicrobials for *E. coli*, four for *Campylobacter* spp. and ten for *Enterococcus* spp..

Results: AMU decreased between 2014 and 2022 by 27% with the major reduction occurring in the first year. The decrease was more pronounced in antimicrobials from AMEG category B (-59%) than in categories C (-3.5%) and D (-29.9%). Specifically, the strongest reduction was observed in the use of 3rd and 4th generation cephalosporins (-79.5%) and polymyxins (i.e., colistin, -65.7%). In contrast, an increase by 24.6% and 13.4% was observed for phenicols and macrolides, respectively.
Resistance of *E. coli* to azithromycin and sulfamethoxazole decreased between 2015 and 2023. Otherwise, no decrease in resistance in *E. coli* and neither in the *Campylobacter* spp. or *Enterococcus* spp. was observed. Prevalence of ESBL/AmpC-producing *E. coli* did not show a general trend. It increased significantly between 2015 (60.6%) and 2019 (70.8%), and dropped to 65.6% in 2021 where it remained in 2023. The decrease was, however, not significant.

Conclusions: Overall, the benchmarking system has contributed to a massive reduction of antimicrobial use in food producing animals in Germany. Sales of antimicrobials dropped by 63% between 2014 (the year the system was implemented) and 2022. However, in calves under 8 months, the decrease was less pronounced (-27%), partially explaining the failure to reduce AMR in veal calves. However, the reduction in AMU differed between antimicrobial classes and for some antimicrobial classes there was actually an increase indicating a shift in antimicrobial choices.

Bovine endometritis: where are we in practice ?

Sylvie CHASTANT, Reproduction,
Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France
sylvie.chastant@vet-alfort.fr

Over the last 20 years, the approach to endometritis, a frequent condition and the focus of a whole range of reproductive monitoring activities, has been totally reconsidered. Its definition, pathogenesis, diagnosis and treatment have all been reassessed.

Endometritis and metritis, the same battle?

Although both involve inflammation of the uterus after calving, the two diseases are distinct in their clinical expression, pathogenesis and treatment. "Endometritis" is an inflammation restricted to the uterine mucosa, developing beyond 21 days post-partum; its origin lies in a deregulation of the immune system, with an overactivity of pro-inflammatory pathways; it is not associated with any impairment of the general condition, but only with infertility (increased interval between calving and first oestrus, and between calving and fertilization); finally, its treatment relies on local, intra-uterine antibiotic therapy. "Metritis", on the other hand, is an inflammation of the entire uterine thickness, developing during the first three weeks after calving; it is directly linked to viral and/or bacterial growth in the uterine cavity, with an impact on general condition, milk production and, later, through the development of endometritis, on reproductive performance; the treatment of choice is general antibiotic therapy, possibly combined with the administration of anti-inflammatory drugs, or even an infusion.

Forgetting about delayed uterine involution

Since the 1970s, endometritis has traditionally been blamed on "delayed uterine involution", i.e. a delay in the process by which the uterus returns to its non-gestational size after calving. Its diagnosis was therefore based on evidence of abnormally large anatomical dimensions, such as a horn diameter at the base greater than 4 cm. However, a comparison with an objective technique for detecting endometrial inflammation (neutrophil count on an endometrial smear taken from the uterine body) showed that there was no relationship between uterine dimensions and the presence of endometrial inflammation. The only dimension of diagnostic value is the external diameter of the cervix: if this is greater than 7.5 cm (measured by hand transrectal palpation or ultrasound), endometritis is present in 70% of cases. But this criterion is not very sensitive, detecting only 36% of cows with endometritis. If we go back to the diameter of the horns at their base (greater than 4 cm), the positive diagnostic value of this parameter is only 56%: only just over half of cows with horn diameters greater than 4 cm actually have endometrial inflammation. In addition, only a quarter of cows actually affected by endometritis have "abnormally" large horns.

In the end, the technique of choice for diagnosing endometritis is the examination of vaginal secretions: the presence of pus flakes allows to diagnose 60% of affected cows, and 75% of cows showing this sign actually have genital inflammation. Rather than talking about "uterine involution checks" when examining cows around 30 days post-partum, it would therefore be more appropriate to talk about "genital health checks", especially as this involves not only uterine health but also ovarian function. In addition, systematic examination of cows for endometritis can be scheduled as early as 21 days post partum, saving 10 days on the traditional (at least in France) delay of 30 days post partum.

Vaginoscope, speculum or glove ?

Examination of the vaginal content (presumed to be uterine secretions that have passed through the cervix) is therefore the examination of choice for diagnosing endometritis. Vaginal content can either been observed in situ (thanks to a vaginoscope or a speculum) or collected to be examined outside the vagina, with a gloved hand or a Metricheck® device. All methods are considered to have the same diagnostic accuracy. Each practitioner can therefore choose the one that suits him best. Vaginoscope and speculum require rinsing in a bucket containing a disinfectant solution between two animals, the speculum remaining a heavy metal instrument and potentially blunt if the female moves. They also require a light source. The Metricheck® device is sometimes difficult to extract from the vagina, due to the resistance of the vestibular sphincter, and requires a wide movement of the practitioner's shoulder (painful when repeated), but is a suitable tool for examining small cows such as Jersey cows. The gloved hand is an option rapid to implement, allowing secretions to be easily collected and examined; nevertheless, its easiness depends on the diameter of the practitioner's forearm, and some breeders fear that the introduction of the arm is responsible for a bacterial contamination of the uterine lumen: bacteriological examinations allowed to deny this fear, showing no alteration of uterine bacterial content following this practice.

No vaginal pus: is the cow necessarily healthy?

The observation of pus flakes in vaginal secretions leads to the conclusion that the cow suffers from endometritis, in the clinical form of the disease. Notwithstanding, vaginal pus (yellow, white) before 21 days post partum is not pathological, but simply reflects the physiological mobilization of leukocytes in reaction of the uterine bacterial contamination, systematic after calving. Beyond 21 days post partum, the presence of vaginal pus is pathological, in 85% of cases indicating uterine inflammation; in the other cases, the inflammation is indeed vaginal and endometritis is overdiagnosed. However, the sensitivity of the method remains its major limitation: examination of vaginal secretions reveals only 60% of cases of uterine inflammation in the cytological sense of the term. As with mastitis, endometritis can be clinical (with pus in the vagina) or subclinical (the endometrial smear shows uterine inflammation, but no clinical signs are visible). Only an endometrial smear can confirm the absence of inflammation. A cow whose vaginal secretions are perfectly transparent is therefore not necessarily free from endometrial inflammation.

What should/could be done to diagnose more cases of endometritis?

Uterine ultrasound does not significantly increase the detection rate of endometritis. Occasionally, it may reveal the accumulation of an limited amount of pus at the apex of the horns, in the form of hyperechoic clusters, or a larger quantity: this latter situation is qualified of pyometra, the accumulation of pus being associated with a persistent corpus luteum. Endometrial cytology is the reference technique (gold standard) for endometritis diagnosis. Nevertheless, its implementation in the field faces several difficulties: cervical catheterization on a closed cervix, handling glass microscope slides besides cows, time required for smear examination under a microscope, the need to go back to the farm to carry out the treatment. Unfortunately, cervical catheterization using a cytobrush mounted on an insemination gun is indispensable, as the vaginal smear is of no diagnostic interest. An alternative can be the leukocyte esterase test that eliminates the need for slides, staining and reading: the cytobrush is immersed in NaCl, and the presence of leukocytes is revealed in the solution by means of the "Leukocytes" zone of a urine strip (which reacts to the esterase-type enzymatic activity of these cells). Even if the concordance with the smear reading is only partial (kappa concordance coefficient = 0.46, i.e. average), this test effectively identifies cows with impaired fertility who would benefit from intra-uterine antibiotic treatment.

Postpartum, but not only

Endometritis is usually detected in the second month post-partum. As explained above, the diagnosis can be made as early as D21, and all cows in the herd should be examined, whether or not they present a risk factor (metritis, dystocia, twins, placental retention, ketosis..). Cows diagnosed with endometritis and treated at the previous visit should also be systematically checked. But endometritis should also be looked for in circumstances other than the post-partum evaluation of genital health: for example, when a pregnancy diagnosis is negative, or when ovarian function is abnormal (no estrus detected since calving, presence of a follicular cyst, etc.). Indeed, in the first case, endometritis may be the cause of non-fertilization and/or embryonic mortality; in the second, endometritis may be the cause of ovarian dysfunction, since via the production of inflammatory cytokines, inflammation of the endometrium can disrupt the synthesis of GnRH, FSH and LH (anovulatory anestrus) and/or that of prostaglandins F2alpha (persistent corpus luteum or short luteal phases). Not only endometritis being the cause of the ovarian dysfunction, but it would also

be pointless to treat ovarian dysfunction to induce estrus with the presence of endometritis drastically reducing the chances of successful insemination.
indeed, as observed in mares, the presence of endometrial inflammation at insemination has a major impact on the success rate of this insemination, with a reduction in pregnancy rate by a factor of 2 compared to cows free from endometrial inflammation. And it seems that a high proportion of cows (around 50%, at least in the Holstein breed) still suffer from sub-clinical endometrial inflammation at the time of insemination.

It’s the cow’s responsability

The absence of a clinical endometritis around D30 does not fully guarantee that the cow will be non inflamed at the time of insemination. There are several possible explanations: firstly, as explained above, the cow could in fact be suffering from a sub-clinical form of endometritis at D30 (which is the case for around half of all cows with endometrial inflammation); secondly, among cows effectively free of any form of endometritis at D30, some (re)activate endometrial inflammation later post partum, and possibly at the time of insemination. The reasons for these late inflammatory reactivations have not been precisely identified, but are likely associated with the fragile physiological balance of the lactating cow (Figure 1).

While the development of metritis can be attributed to the aggressiveness of bacterial or viral pathogens, the development of endometritis is more closely linked to the cow’s intrinsic inability to control inflammation. It is therefore physiological for a cow to develop endometrial inflammation immediately after calving. In this species, the uterine lumen is subject to ascendant contamination by environmental bacteria immediately after calving. Recognition of the bacteria by receptors present on endometrial cells (PAMP, TLR) triggers the synthesis of inflammatory cytokines (TNFalpha, interleukin 1 and 8) and intense leukocyte diapedesis. Through phagocytosis, leukocytes then suppress bacterial populations. The very intense initial inflammation (during the first week post partum) is therefore beneficial. Thereafter, uterine inflammation diminishes to zero at around 45 days post partum. If the cow fails to extinguish the initial inflammation, the persistence of this inflammation becomes deleterious to her reproductive performance (Figure 2).

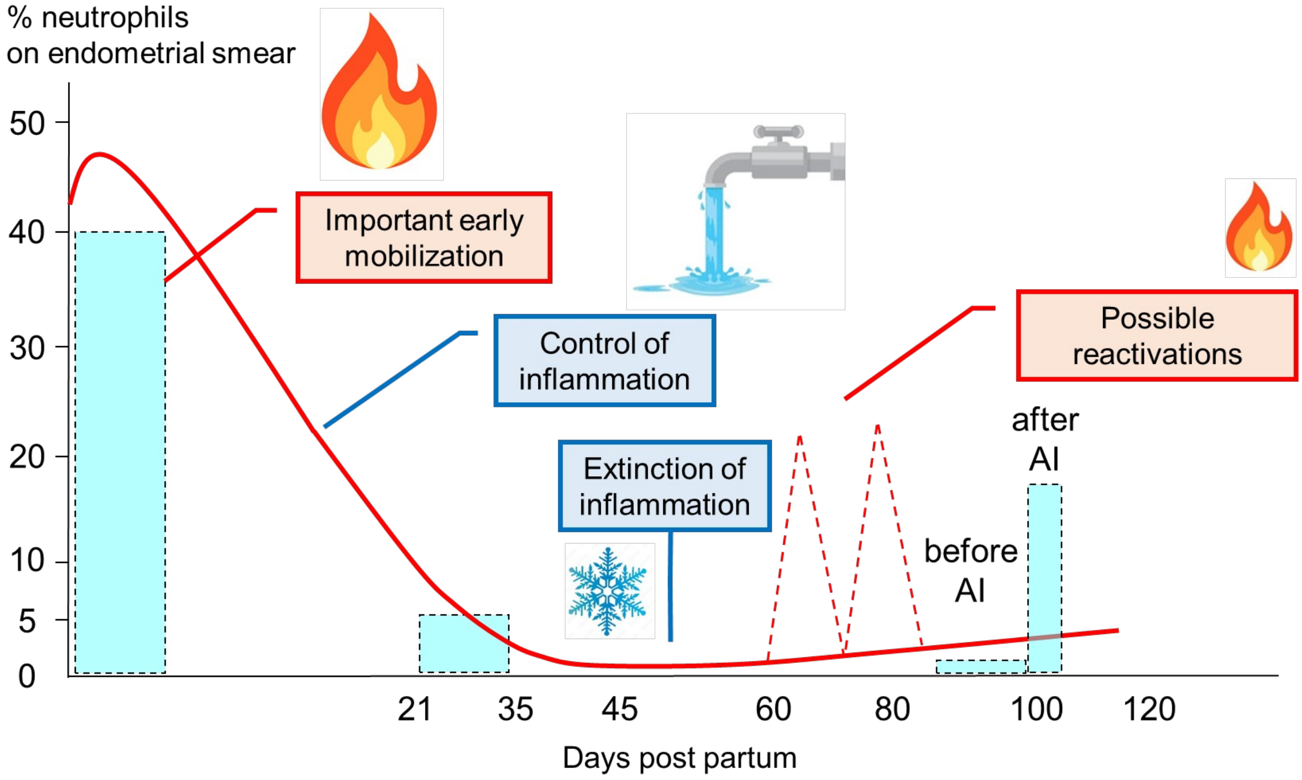


Figure 1: Evolution of intra-uterine inflammation after calving

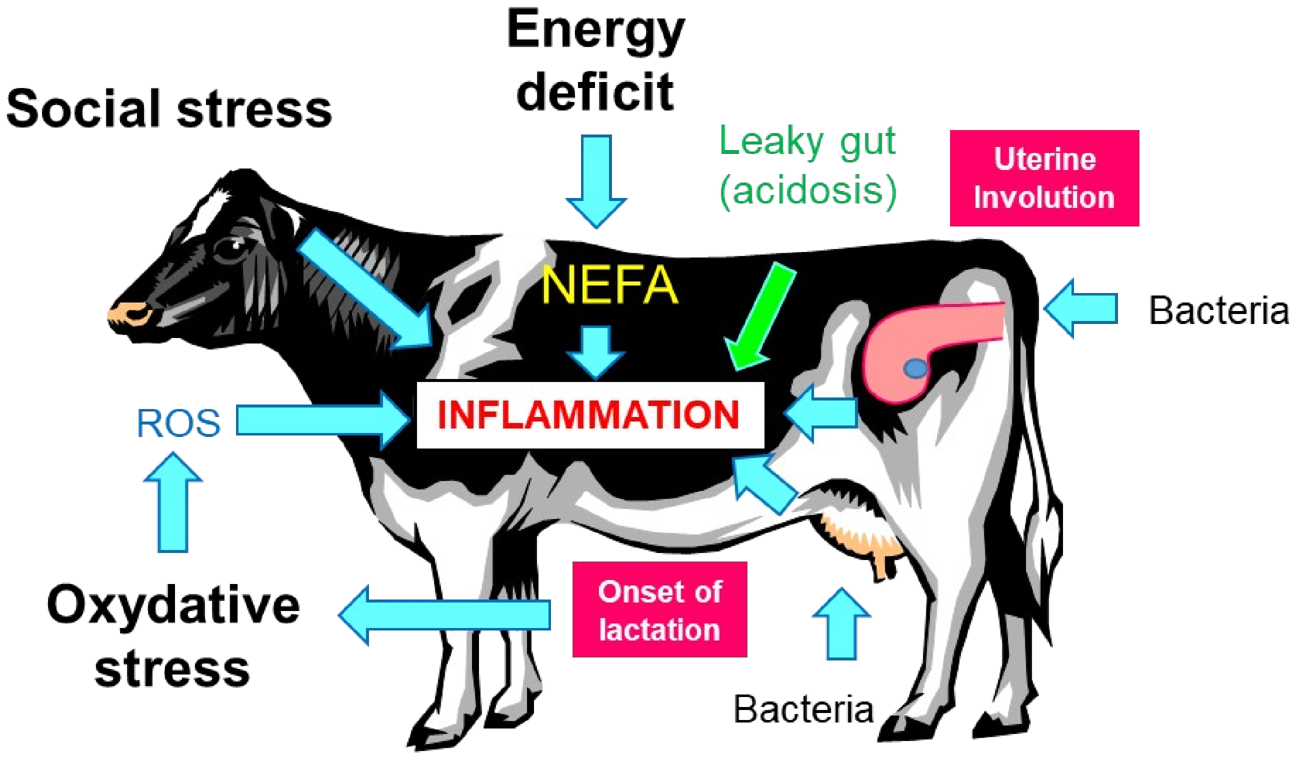


Figure 2. Pro-inflammatory context of post-partum dairy cows

The physiological context of dairy cows disrupts both stages in the evolution of post-partum inflammation. Hypocalcemia, even subclinical, and oxidative stress, associated with the onset of lactation, reduce the phagocytosis capacity of leukocytes: the cow then struggles to effectively control the initial bacterial population in the first post partum weeks; during the second month post partum, energy deficit and still oxidative stress place her in a pro-inflammatory state. It can thus reasonability hypothetized that disruptions in the subtle balance of the dairy cow's metabolism and immunity may lead to reactivations of uterine inflammation during the reproductive period and thus at the time of insemination.

A problem for dairy cows only?

The prevalence of endometritis has mainly been investigated in Holstein cows and around 30 days post-partum: between 30% and 50% of cows are affected by cytological endometritis. However, metabolic and immune challenges are different in many other breeds: the prevalences, both post-partum and around insemination, are little known, if at all, in other breeds, whether dairy or beef. Results in beef females are controversial.

Anti-inflammatory drugs or antibiotics?

Although the inflammatory phenomenon is responsible for fertilization failures and embryonic mortality associated with endometritis, the treatment of this condition is primarily based on intrauterine antibiotic therapy. The administration of various anti-inflammatory molecules has proven ineffective, both systemically and by the intra-uterine route. The only antibiotic for which the literature provides a sufficient body of evidence demonstrating its effect is cefapirin, a first-generation cephalosporin. The effectiveness of antibiotic treatment and the ineffectiveness of anti-inflammatory treatments could be explained by the mechanisms involved in the development of endometritis: an initially insufficient inflammatory reaction would allow a bacterial load to persist in the uterus, and then this bacterial load, even if minimal, would trigger an exaggerated and persistent inflammatory reaction due to the pro-inflammatory context in which the (dairy) cow is set after calving.

Treatment of endometritis should not be judged solely on the apparent clinical cure rate (disappearance of pus in the vaginal content) but more relevantly on cytological examinations and ultimately on the improvement of reproductive performance. After cefapirin treatment, reproductive performances of endometritis-affected treated cows equivalent to those of healthy cows (figure 3). The intrauterine injection can be performed either with the plastic catheter provided with the syringe containing the antibiotic or with a modified embryo transfer gun, more rigid and of smaller diameter (OPTISOIN® device, Embryovet, France).

Many other intrauterine treatments have been proposed, including antiseptics and phyto/aromatherapy products. Antiseptic solutions do not lead to an improvement in reproductive performance and are even associated with a deterioration of performance in the majority of publications. Phyto/aromatherapy products are intended for external use and most often not registered as drugs, with determined Maximal Residues Limits. In the context of the rational use of antibiotics, it would be very interesting to evaluate their effectiveness for the treatment of endometritis, but to date, scientific demonstration is lacking.

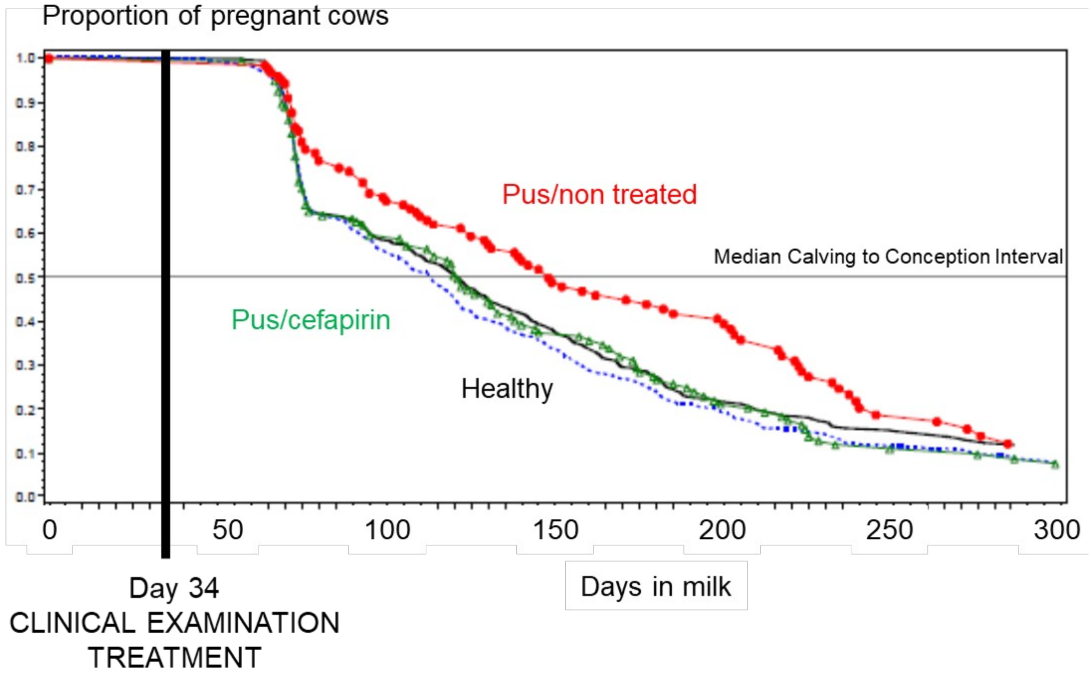
Regarding systemic treatments, whether prostaglandins F2alpha or antibiotics, they prove ineffective for the treatment of endometritis, both clinical and cytological. Only the specific form of endometritis known as pyometra benefit from a treatment with prostaglandins F2alpha.

CONCLUSION

Although endometritis is a common condition with a significant impact on reproductive performance, much work is needed to improve the accuracy and the easiness of diagnostic technique in the field.

Figure 3: Effect of cefapirin intra-uterine treatment on reproductive performance of endometritis-affected cows. From Tison et al 2017.

Holstein cows are examined at Day 34 post partum and diagnosed affected by endometritis from the presence of vaginal pus. Median Calving-to-Conception interval is 158 days in affected non treated cows (red; n=103), vs 120 days in healthy cows (black; n=374), and 120 days in affected treated cows (green; n=121).



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The economic impact of a systematic application of fertility programs in lactating dairy herds, an European perspective

Federico Randi[5], A. Wicaksono,[1,2] F. Edwardes,[1] W. Steeneveld,[3] B. H. P. van den Borne,[1, 4] P. Pinho,[5] H. Hogeveen[1]

1. Business Economics Group, Department of Social Sciences, Wageningen University and Research, Hollandseweg 1, 6706 KN, Wageningen, the Netherlands
2. Veterinary Public Health and Epidemiology Division, School of Veterinary Medicine and Biomedical Sciences, IPB University, Kampus IPB Dramaga, 16680, Bogor, Indonesia
3. Faculty of Veterinary Medicine, Department of Population Health Sciences, Section Farm Animal Health, Utrecht University, Yalelaan 7, 3584 CL, Utrecht, the Netherlands
4. Quantitative Veterinary Epidemiology Group, Department of Animal Sciences, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands
5. CEVA Santé Animale, 10 Av. de la Ballastière, 33500, Libourne, France

federico.randi@ceva.com

Objectives:

Hormone-based reproductive management programs can enhance dairy cows' reproductive performance. This study aimed to compare the economic impact of two types of reproductive management programs: one using systematic hormonal treatments for cows within a specific Days in Milk (DIM) range, and another using cow-specific hormonal treatments based on veterinary diagnoses of ovarian dysfunction during fertility checks.

Materials and Methods:

A dynamic, stochastic bio-economic simulation model, representing a 200-cow herd, was extended to include ovarian dysfunction and fertility inputs. Four hormone-based reproductive management programs were modeled:

1. Baseline Program: Reflecting current Dutch herd practices, cows are inseminated based on estrus detection, and non-cyclic cows receive hormone treatments based on veterinary diagnoses. Treatments include PRID-Synch for anoestrus cows, Ovsynch for cows diagnosed with cystic ovarian disease, and PGF2α administration to subestrous cows.
2. TAI only: Systematic hormonal treatment for timed artificial insemination (TAI) using Double Ovsynch for 1st AI and resynchronization for all the cows found non-pregnant at pregnancy diagnosis 32±3 days after AI according to ovarian status either with or without PRID supplementation.
3. TAI + Estrus Detection (ED): Similar to the second program but includes estrus detection for all the cows not pregnant after TAI.
4. Estrus Detection + TAI: Cows inseminated using ED after the VWP and if not detected in estrus are submitted to a PRID-Synch protocol.

All cows found non-pregnant at ultrasonographic pregnancy check performed at 32±3 after AI were re-synchronized based on the presence or absence of a corpus luteum (CL), cows lacking a CL received PRID-Synch cows with a CL received Ovsynch. The annual mean net economic return (NER) was calculated for each program.

Results:

The highest NER was observed in the FTAI+ED program, with €23,764 higher net revenues compared to the default program, followed by the FTAI and ED+TAI programs with €19,550 and €14,314 higher net revenues, respectively. Although systematic hormone-based programs incurred higher costs due to more hormone use and increased calving and feed costs, the additional revenues from milk and calves outweighed these costs. For example, the FTAI+ED program had €8,953 higher total costs but generated €32,654 higher revenues annually.

Conclusions:

In summary, systematic hormone-based reproductive management programs provided economic advantages over the default program, where hormones are administered based on veterinary diagnoses during fertility checks.

Strategic trace mineral injection to improve reproductive performance in beef cows in Belgium

Theron Leonard[1], Hamard Hélène[1], Brennet Guillaume[1], Gerards Cyprien[1], Rao Anne-Sophie[1], Guadagnini Marcello[2]

1. Rumexperts, Rue des Bada 27, 4317 Faimes, BE
2. Axiota Animal Health, 2809 East Harmony Road #190 Fort Collins, CO, USA

Speaker: ltheron@rumexpert.vet

Introduction and Objectives

Adequate supply of trace minerals is key to maximize cow reproductive outcome. In beef herd raised on pasture, trace mineral deficiencies or suboptimal status can impact ovarian cyclicity and conception.

Previous studies[1,2] have shown how a trace mineral injection before insemination can increase antioxidant enzymes blood concentration and improve conception rates. The aim of this study is to investigate the effectiveness of a trace mineral injection administered prior to the breeding period in enhancing the odds of pregnancy in beef cows in Belgium.

Material and Methods

Between November 2023 and January 2024, 360 Belgian Blue female cattle, belonging to 11 farms in Belgium, were enrolled into the study. Animals on each farm were randomly assigned to either a treatment group (n = 174) or a control group (n = 186) based on their ear tag numbers. Nulliparous heifers (n = 152) were enrolled at an average age of 761 ± 146 days, while cows (n = 208) were enrolled an average of 141 ± 108 days postpartum. All animals were required to be non-pregnant at the time of enrollment. Treatment animals received a subcutaneous injection of a multimineral solution (Multimin™; Axiota Animal Health) at enrollment, containing 60 mg/ml Zn, 15 mg/ml Cu, 10 mg/ml Mn, and 5 mg/ml Se, administered at a dose of 1 ml/100 kg body weight. All animals were artificially inseminated following estrus detection. Vaginal discharge was assessed at each insemination, and differences between the treatment and control groups were evaluated using a *chi-square* test. Reproductive outcomes were evaluated by calculating the risk of pregnancy during the 150 days post-enrollment using a Cox's proportional hazard model. The model accounted for farm, month of enrollment, parity, treatment group and its interactions with farm and parity. Statistical significance was defined as $p < 0.05$, while tendencies were considered at $p < 0.10$.

Results

The first insemination occurred 34±28 days post-enrollment. No significant differences in vaginal discharge at insemination were observed between treatment groups ($p = 0.29$). When analyzing all animals together, no significant differences in pregnancy risk were detected; however, the interaction term for treatment and parity showed a p-value of 0.07. As a result, the model was stratified and analyzed separately for nulliparous heifers and cows. Treatment had no significant effect on pregnancy risk in nulliparous heifers ($p = 0.5$), whereas in cows, treatment approached significance ($p = 0.08$) with a hazard ratio for pregnancy of 1.51 (95% CI: 0.93–2.43).

Conclusion

Despite the relatively small sample size, this is the first study to evaluate the use of an injectable trace mineral combination in Belgian Blue cattle aimed at improving reproductive performance. The findings in multiparous animals align with previous studies by Mundell and Vedovatto, which demonstrated similar outcomes in Angus and Nellore cattle treated with the same trace mineral solution prior to timed artificial insemination. Based on this preliminary data, which should be confirmed by a larger study, the findings support the use of injectable trace minerals in Belgian Blue cows before insemination to improve reproductive performance.

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Risk factors for ultrasound diagnosed endometritis and its impact on fertility in Scottish dairy herds

Ciara McKay[1], Lorenzo Viora[1], Katharine Denholm[1], John Cook[2], Richard Vazquez Belandria[1*]

[1] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, G61 1QH, UK

[2]World Wide Sires, Yew Tree House, Carleton, Carlisle, Cumbria, CA1 3DP, UK

*Corresponding author: Richard.Vazquez@glasgow.ac.uk

Background: The aim of this study was to investigate the risk factors for and the impact of ultrasound-diagnosed endometritis (UDE) on lactating dairy cows' reproductive performance.

Methods: Data were analysed from 1123 Holstein and Holstein-Friesian cows from two Scottish dairy farms. A reproductive ultrasound examination was conducted on two occasions, at 43 ± 3 and 50 ± 3 days in milk (DIM), to screen for hyperechoic fluid in the uterus. Statistical analyses were performed using multivariable logistic regression modelling and Cox proportional hazards models.

Results: The overall incidence of UDE was 8.8% (99/1123). Risk factors for UDE included calving during autumn/winter seasons, increased parity and the presence of two or more diseases in the first 50 ± 3 days postpartum. The presence of UDE was associated with a reduced odds of pregnancy after all artificial inseminations up to 150 DIM.

Limitations: The retrospective design of this study led to some inherent limitations with the quality and quantity of data collected.

Conclusions: The findings of this study indicate which risk factors should be monitored in postpartum dairy cows to limit the impact of UDE on future productive performance.

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TESTICULAR ULTRASONOGRAPHY: A KEY TOOL TO PREVENT REPRODUCTIVE AND HEALTH PROBLEMS IN BUCKS

Bailon-Larrañaga N.[1], Gómez-Martín Á.[1], Contreras A.[2], Toledo-Perona R.[1], Toquet M.[1], Reyes L.E.[3], Quereda J.J.[4], Gomis J.[1]*.

[1] Microbiological Agents Associated with Animal Reproduction (ProVaginBIO) Research Group, Department of Animal Production and Health, Veterinary Public Health, and Food Science and Technology, Faculty of Veterinary Medicine, Cardenal Herrera-CEU University, CEU Universities, Valencia, Spain.

[2] Department of Animal Health, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain.

[3]Clinical Veterinarian for Small Ruminants.

[4] Research Group Intracellular Pathogens: Biology and Infection, Department of Animal Production and Health, Veterinary Public Health, and Food Science and Technology, Faculty of Veterinary Medicine, Cardenal Herrera-CEU University, CEU Universities, Valencia, Spain.

*jesus.gomis1@uchceu.es

The aim of this study was to evaluate the use of testicular ultrasonography as a predictive tool for reproductive health in bucks, investigating whether there is a relationship between testicular alterations, sperm quality, and the infection of important pathogens with reproductive tropism in small ruminants such as *Coxiella (C.) burnetii*, Caprine Arthritis Encephalitis Virus (CAEV), *Mycoplasma (M.) agalactiae*, and *Chlamydia (C.) abortus*.

To carry out this study, a total of 219 bucks (young; 1-3 years, and adults; > 3 years) located on the same dairy farm were used. This farm had a previous clinical history of Q fever, Caprine arthritis encephalitis (CAE), Contagious agalactia, and Enzootic abortion. For this reason, a serological diagnosis for *C. burnetii*, CAE virus, *M. agalactiae*, and *C. abortus* was initially performed in the study population. All ultrasonographic examinations of the testes were performed using a B-mode ultrasonography scanner (Sonosite M-Turbo) with a 7.5 MHz linear array transducer. The ultrasound diagnosis was conducted to assess the severity of lesions in the testicular parenchyma of both testes in each male. Subsequently, the ultrasound images were analyzed by using a specialized computer program (ImageJ) and then classified based on the total affected area into three severity scores (Slight: 1-10mm; Moderate: 10-30mm; Severe: 30mm). Regarding the sperm quality analysis, an ejaculate sample from each male was obtained by electroejaculation using a small ruminant rectal probe (Minitube, Tiefenbach, Germany). Before semen collection, the abdominal area and the external surface of the foreskin were cleaned and disinfected with 1% chlorhexidine. Electrical stimulation was applied to all bucks and collection cup was placed to collect the semen samples. Ejaculates were evaluated immediately after extraction including a subjective assessment of various quality parameters (volume, sperm concentration, mass motility, and morphology).

The results of the serological analysis showed that 71/198 (35.8%) serum samples were positive for C. burnetii and 127/216 (58.8%) were positive for CAE virus. Furthermore, 20.7% of the samples were positive for both etiological agents. On the other hand, 100% of the animals were negative for M. agalactiae and C. abortus. Testicular microlithiasis were the most frequent abnormalities detected by ultrasonography. 18.2% (40/219) and 50.2% (110/219) of males had unilateral and bilateral lesions in testicles, respectively. Regarding the severity of the lesions, bilaterally affected bucks showed 28.2% of slight lesions, 35.4% of moderate lesions, and 36.4% of severe lesions. For the unilaterally affected males, 65% showed slight lesions, 25% moderate lesions, and 10% severe lesions. The presence of males of different ages revealed that age had significant effects ($p < 0.001$) on the presence of bilateral testicular damage, with a higher percentage (65.8%; 50/76) of parenchymal microlithiasis observed in adult males. Moreover, 46% (23/50) of them presented severe lesions. Additionally, age had significant effects ($p < 0.001$) on other variables, such as CAEV serology, showing a higher number of CAEV-seropositive adult bucks. The testicular area affected by microlithiasis was also influenced by age, with higher values in adult animals for both the right ($p = 0.0099$) and left ($p = 0.0233$) testicle areas. In terms of sperm quality results, younger males exhibited significantly ($p = 0.027$) higher sperm concentrations.

In conclusion, testicular ultrasonography proves to be an effective, non-invasive, and reliable tool for assessing the reproductive health of bucks. Through ultrasonography, a significant number of animals with testicular abnormalities can be quickly detected, which may indicate compromised fertility (Stewart & Shipley, 2021). Age increases the risk and severity of testicular lesions and predisposes bucks to CAEV infections, potentially causing structural damage, which can be detected early via ultrasound (Turchetti et al., 2013). However, further research is necessary to determine the potential correlation between testicular damage, the presence of pathogens in the reproductive system, and sperm quality.

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Diagnosing and treating reproductive tract diseases in dairy cows

Jocelyn Dubuc[a], Juan Carlos Arango-Sabogal[a], Véronique Fauteux[a], José Denis-Robichaud[b], and Sébastien Buczinski[a]

[a]Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Canada
[b] Independent researcher, Amqui, Canada – josedr@hotmail.ca

Objectives

This project aimed to identify diagnostic criteria for purulent vaginal discharge (PVD) and endometritis in cows, which are ≥ 100 days in milk (DIM), and to assess the impact of intrauterine cephapirin on pregnancy per artificial insemination (P/AI) in cows diagnosed with these reproductive tract diseases.

Material and Methods

Study 1: Diagnosis

Non-pregnant cows ≥ 100 DIM from 24 commercial Holstein herds were enrolled between September 2020 and December 2021. At enrollment, cows were examined for vaginal discharge using a Metricheck device (Simcro), which was scored as 0 = no discharge, 1 = clear mucus, 2 = mucus with flecks of pus, 3 = mucopurulent discharge, 4 = purulent discharge, and 5 = foul red-brown discharge.[4] Endometrial samples collected with a modified cytobrush technique[3] were used for an esterase test, which was scored as 0 = negative, 0.5 = trace of leukocytes, 1 = small amount of leukocytes, 2 = moderate amount of leukocytes, and 3 = large amount of leukocytes.[2] Cows were then inseminated following a standard Ovsynch56 protocol,[1] and their pregnancy status was determined by the herd veterinarian. Multiple thresholds for PVD and endometritis were modeled from randomly split datasets (0.7:0.3) to identify the combination that could best predict P/AI, determined as the modeled combination yielding the highest area under the receiver operating characteristic curve (AUC).[5]

Study 2: Treatment

Non-pregnant cows ≥ 100 DIM from 31 commercial Holstein herds were enrolled between January 2022 and March 2023. At enrollment, cows were diagnosed for PVD and endometritis using the techniques and thresholds identified in the first part of the study. Cows with reproductive tract diseases were randomly assigned to a treatment with 500 mg of cephapirin benzathine (Metricure, Merck Animal Health) or a negative control, while unaffected cows were considered the healthy controls. Insemination and pregnancy status were done as previously described. The odds ratio (OR) and predicted probability (95% confidence intervals; CI) of P/AI for cows with reproductive diseases compared to unaffected cows were obtained from a multilevel logistic regression model.

Results

Study 1: Diagnosis

A total of 1,064 enrollments were used to identify the optimal thresholds. The AUC for the different threshold combinations varied between 0.566 and 0.898. The combination with the highest AUC was a vaginal discharge score ≥ 2 for PVD and an esterase score ≥ 0.5 for endometritis.

Study 2: Treatment

Of the 1,686 enrollments, 30% were diagnosed with PVD, and 30% with endometritis. Overall, 36% of the inseminations resulted in pregnancy. Cows with reproductive tract diseases had lower odds to become pregnant than unaffected cows, but these odds were lower for untreated cows (PVD: OR = 0.54, 95% CI = 0.43-0.66; endometritis: OR = 0.59, 95% CI = 0.47-0.71) than for cows that were treated with intrauterine cephapirin (PVD: OR = 0.86, 95% CI = 0.74-0.97; endometritis: OR = 0.88, 95% CI = 0.77-0.98). Indeed, the predicted probability of P/AI for untreated cows with PVD and endometritis was 23% and 25%, respectively. It was 37% and 38% for treated cows with PVD and endometritis, respectively, and 43% for unaffected cows.

Conclusions

We identified thresholds for PVD (mucus with flecks of pus or worse) and endometritis (trace of leukocytes or more) that best predicted P/AI in dairy cows ≥ 100 DIM. Cows with reproductive tract diseases that were treated with intrauterine cephapirin had higher odds to become pregnant than untreated cows, almost to the level of unaffected cows.

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Q fever in dairy cattle: comparison of prevalence at herd level according to clinical signs reported

Philippe Gisbert[1], Carla Azevedo[1], Juan Munoz-Bielsa[1], Michaël Treilles[2]

[1] *Ceva Sante Animale, 10, Avenue de la Ballastière,CS30126, 33501 Libourne cedex France*
philippe.gisbert@ceva.com
[2]*Qualyse, Z.A. Montplaisir - 79220 Champdeniers-Saint-Denis, France*

Coxiella burnetii is an intracellular gram-negative bacterium responsible for Q fever in many animal species and humans. The disease occurs worldwide, with the exception of New Zealand, where it has never been described. Among animals, ruminants are the most affected. They are also the source of most human cases. Clinically, in small ruminants, Q fever is dominated by abortions. However, in cattle, in addition to abortions, other clinical signs such as metritis, retained placenta, and fertility problems may occur (Gisbert *et al.*, 2024).

Objective

The aim of this study was to compare the prevalence of Q fever infection at herd level between farms reporting abortions and those reporting only other reproductive disorders.

Materials and methods

PCR testing of bulk tank milk (BTM) is a simple and affordable way of testing for the presence of *Coxiella burnetii* in dairy herds. In 2020, Ceva and Qualyse developed QTest to facilitate the collection and transport of samples to the laboratory. QTest combines an easy sampling medium (FTA® card) that inactivates micro-organisms while preserving nucleic acids, with real-time PCR analysis carried out in a single laboratory in France. The use of QTest increases the sensitivity of BTM analyses (Treilles *et al.*, 2021).
Each QTest is accompanied by a sample identification form. On this form, the breeder or veterinarian sending the sample indicates any reproductive disorders present on the farm. It is possible to tick several boxes:

- Abortions / premature delivery / stillbirth / weak born offspring (APSW complex)
- Metritis / endometritis
- Retained placenta
- Infertility

The objective of QTest is to identify the presence of *Coxiella burnetii* in herds exhibiting reproductive dysfunction. In the present study, the results obtained over a four-year period, from October 2020 to September 2024, were subjected to analysis. The herds were classified into two groups. The first group comprised herds that had reported the APSW complex, while the second group consisted of herds that had reported any of the other reproductive disorders. As multiple clinical signs could be reported, it was decided to assign to the APSW complex group, herds where both APSW complex and at least one other clinical sign were reported.
The results of the two groups were then compared using the chi-square test.

Results

During the study period 2014 samples were collected from farms where at least one reproductive disorder had been reported. The geographical origin is as follows:

- France n=780
- United Kingdom n=563
- Italy n=294
- Germany n=197
- Greece n=35
- Canada n=32
- Republic of Ireland n=31
- Belgium n=22
- Ukraine n=19
- Romania n=15
- Tunisia n=11
- Poland n=6
- The Netherlands n=6
- Turkey n=2
- Bulgaria n=1

APSW complex was reported on 1261 farms (62.6%). The presence of *Coxiella burnetii* in BTM was detected in 44.0% of them (n = 555). 753 farms (37.4%) reported only other reproductive disorders. Of these, 40.4% were positive for *Coxiella burnetii* in BTM (n = 304). The difference in prevalence was not statistically different between the two categories (p=0.11, Chi-square test).

Conclusion

These results suggest that the presence of *Coxiella burnetii* in dairy cattle can be associated without difference with major reproductive disorders (abortions, premature delivery, stillbirth, weak born calves) or other reproductive disorders (metritis/endometritis, retained placenta, infertility). Testing for *Coxiella burnetii* should therefore be considered in herds with reproductive problems, even in the absence of a high abortion rate.

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Fertility performances of lactating dairy cows submitted to timed artificial insemination or timed embryo transfer with fresh or frozen in vitro produced embryos in a seasonal pasture-based system

Alan Crowe[1,3], Jose Maria Sánchez[2], John Browne[3], Stephen G. Moore[1], Michael McDonald[3], Rafaela Rodrigues[4], Maria Fernanda Morales[4], Leandro Orsi De Freitas[4], Maria Belen Rabaglino[3], Pedro Pinho[5], Federico Randi[5], Patrick Lonergan[3], Stephen T. Butler[1]

Affiliations:

- Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland
- Centro Nacional Instituto de Investigación y Tecnología Agraria y Alimentaria (CSIC-INIA), Madrid, Spain
- School of Agriculture and Food Science, University College Dublin, Ireland
- Vytelle LLC, Hermiston, OR, US
- CEVA Santé Animale, Libourne, France

Objectives: This study aimed to compare pregnancy per service (P/S) in lactating dairy cows following timed artificial insemination (TAI) or timed embryo transfer (TET) using either fresh or frozen in vitro produced (IVP) embryos.

Materials and Methods: Oocytes were collected weekly for up to 9 weeks using transvaginal ovum pick-up from elite dairy donors (ET-DAIRY; n = 40; Holstein-Friesian and Jersey) and elite beef donors (ET-ELITE-BEEF; n = 21; Angus). Both heifers and cows were included in these donor groups. Additionally, oocytes were collected from the ovaries of beef heifers of known pedigree following slaughter (ET-COMM-BEEF; n = 119). After in vitro maturation, fertilization, and culture, single Grade 1 blastocysts were transferred either fresh or after freezing and on-farm thawing into lactating Holstein-Friesian dairy cows synchronized with a 10-day PRID-Ovsynch protocol.

On day 0, cows received a 2-mL intramuscular injection of GnRH analogue (Ovarelin, 100 µg of gonadorelin diacetate tetrahydrate; Ceva Sante Animale, Libourne, France) and a progesterone-releasing intravaginal device (PRID Delta; 1.55 g Progesterone, Ceva). On day 7, a 5-mL intramuscular injection of PGF2α (Enzaprost, 25 mg of dinoprost trometamol; Ceva) was administered, followed by a second 5-mL injection of PGF2α on day 8 and removal of the PRID. On day 9.5 (32 hours after PRID removal), a second intramuscular injection of GnRH was given. On day 10, 243 cows received AI (16 hours after the second GnRH), and 863 cows were assigned to receive ET on day 17. Blood samples were collected on day 17 to evaluate serum P4 concentrations.

Pregnancy rates were determined between days 32-35 after synchronized ovulation using transrectal ultrasonography. Pregnancy was confirmed between days 62-65 to determine embryonic loss. Pregnancy data were analyzed using generalized linear mixed models, with service treatment (TAI vs. TET) as a fixed effect.

Results: Mean pregnancy per service at day 32 was similar between AI (48.8%) and ET (48.9%) and did not differ between dairy and beef embryos (50.3% vs. 48.1%, respectively). P/S was lower (P = 0.003) following the transfer of frozen embryos compared to fresh embryos (41.6% vs. 56.1%, respectively). Pregnancy loss between days 32 and 62 was higher (P = 0.003) for ET (15.1%) compared to AI (4.7%), with greater losses observed for frozen beef (18.5%), fresh beef (17.3%), and frozen dairy (19.2%) compared to fresh dairy (6.0%).

Serum P4 concentration on day 7 was associated with P/S at days 32 and 62. Cows in the lowest quartile for serum P4 concentrations (Q1) had a lower probability of being pregnant on day 32 (33.4%) compared to cows in the three upper quartiles (45.7%, 55.6%, and 61.2% for Q2, Q3, and Q4, respectively).

Conclusions: Pregnancy per service event on days 32-35 was similar for TAI and TET, although 9.6% of cows initially synchronized for ET were rejected. Of cows that were pregnant on day 32, pregnancy loss was greater for TET than for TAI. P4 concentrations on day 7 were positively correlated with pregnancy outcomes at days 32 and 65.

The incidence and timing of pregnancy loss derived from timed artificial insemination or timed embryo transfer using fresh or frozen in vitro-produced embryos

F. Randi [5], A.D. Crowe,[1,2] J. M. Sánchez,[2,3] S. G. Moore,[1] M. McDonald,[2] M. S. McCabe,[4] P. Lonergan,[2] and S.T. Butler[1]

1. Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, P61 C996.
2. School of Agriculture and Food Science, University College Dublin, Ireland, D04 V1W8.
3. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctr. de la Coruña km 5.9, 28040, Madrid, Spain.
4. Teagasc, Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Grange, Co. Meath, Ireland, C15 PW93.
5. CEVA Santé Animale, Libourne, 33500, France.
federico.randi@ceva.com

Objectives:
This study aimed to characterize the incidence and timing of pregnancy loss from the service event (timed artificial insemination or timed embryo transfer) to parturition.

Materials and Methods:
Lactating Holstein-Friesian cows were randomly assigned to receive either timely artificially inseminated (TAI, n = 243) or timely embryo transferred (TET, n = 863) with a fresh or frozen in vitro-produced blastocyst following a protocol consisting off on day 0, cows received a 2-mL intramuscular injection of GnRH analogue (Ovarelin, 100 µg of gonadorelin diacetate tetrahydrate; Ceva Sante Animale, Libourne, France) and a progesterone-releasing intravaginal device (PRID Delta; 1.55 g Progesterone, Ceva). On day 7, a 5-mL intramuscular injection of PGF2α (Enzaprost, 25 mg of dinoprost trometamol; Ceva) was administered, followed by a second 5-mL injection of PGF2α on day 8 and removal of the PRID. On day 9.5 (32 hours after PRID removal), a second intramuscular injection of GnRH was give, and cows were either inseminated 32h later (TAI) or received a blastocyst 7,5 days after the last GnRH injection.

The blastocysts were derived from oocytes collected from the ovaries of elite dairy donors (14 Holstein Friesian and 8 Jersey) and elite beef donors (21 Angus) using transvaginal ovum pick-up. Additionally, oocytes were collected from the ovaries of beef heifers of known pedigree following slaughter at a commercial abattoir (n = 119).

Blood samples were collected on day 7 from all cows to determine progesterone concentration, and from subsets of cows on day 18 (n = 524) and day 25 (n = 378) to determine mRNA abundance of interferon-stimulated gene-15 and pregnancy-specific protein B concentration, respectively, for early pregnancy diagnosis. Transrectal ultrasonography was conducted to determine pregnancy status on days 32, 62, and 125 after synchronized ovulation. Parturition dates were recorded for all cows that reached term delivery.

Results:
The predicted probability of pregnancy (%) varied at each time point (days 7, 18, 25, 32, 62, 125, parturition) depending on treatment (AI: 77.0, 60.2, 52.3, 48.8, 47.0, 44.6, 44.0; fresh ET: 100.0, 69.5, 60.3, 56.1, 48.4, 46.8, 45.5; frozen ET: 100.0, 61.7, 52.2, 41.6, 32.9, 31.8, 30.2). Regardless of treatment, the largest proportion of pregnancy loss occurred from the service event (AI on day 0 or ET on day 7) to day 18, with minimal loss occurring between day 62 and parturition (AI: 1.8%, fresh ET: 1.9%, frozen ET: 3.5%).

Treatment differences in the predicted probability of pregnancy per service event were detected between fresh ET vs. frozen ET on day 32, and both AI and fresh ET vs. frozen ET on days 62, 125, and at parturition. There was a greater probability of pregnancy loss between days 32 and 62 following ET (Fresh: 11.3%, Frozen: 18.0%) than AI (4.0%). The percentage of cows that calved following the transfer of a fresh embryo (45.5%) was similar to AI (44.0%), but lower when a frozen embryo was transferred (30.2%).

Conclusions:
In conclusion, AI and fresh ET led to a higher probability of cows becoming pregnant and maintaining the pregnancy to term compared to frozen ET. Cows that were still pregnant on day 62 had a very high likelihood of maintaining the pregnancy to full-term parturition, regardless of treatment. Further work is needed to improve the likelihood of pregnancy establishment and reduce embryonic and fetal mortality following the transfer of a cryopreserved in vitro-produced embryo.

Coxiella Burnetii seroprevalence evolution during the last 14 years in the Northwest of Spain

U Yañez[1], J Álvarez[1], C Pison[1], A Acción[1], R Barrionuevo[1], JJ Becerra[1], A Jiménez[2], P Gisbert[3], D Remmy[3], PG Herradón[1], AI Peña[1], JM Cao[4], A Prieto[4], LA Quintela[1]

[1] Reproduction and Obstetrics Unit, Facultad de Veterinaria, Universidad de Santiago de Compostela,Lugo, Spain. luisangel.quintela@usc.es
[2] CEVA SALUD ANIMAL S.A., Barcelona, Spain.
[3] CEVA SANTE ANIMALE, Libourne, France

[4] INVESAGA, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain.

Objectives

Coxiella burnetii is the etiological agent of Q fever in humans and coxiellosis in animals (Trachsel et al., 2023). Its environmental resistance made *C. burnetii* a highly infectious pathogen presents in every country around the world, with the exception of New Zealand and the Antarctica (Trachsel *et al.,* 2023). Coxiellosis in cattle is often asymptomatic, but a possible relationship with various reproductive problems has been suggested. Due the zoonosis ability and the reproductive disorders associated, *C. burnetii* has been studied for the last decade. Spain is one of the European countries with the highest Q fever incidence. However, there is limited epidemiological data in cattle in Galicia (Northwest of Spain). In fact, there is only one study made about the prevalence of this bacterium in dairy cattle in this region (Yáñez *et al.,* 2024). Therefore, the aim of this study was to determine the seroprevalence in the Northwest of Spain of *C. burnetii* using ELISA bulk milk testing and the evolution of the bacterium seroprevalence in bulk tank milk samples (BTMS) between the years 2010 and 2024.

Material and methods

Thus, 50 BTMS were collected during the year 2010 and 36 BTMS were collected from January to June of 2024. All the BTMS were collected in 50 ml sterile Falcon tubes after mixing the bulk tank milk and were refrigerated (4-8 ºC) after the collection at the farm. At the same time, the BTMS were analysed using ELISAi. ELISAi was carried out with the commercial kit "ID Screen® Q Fever Indirect Multi-species" (IDvet, Grabels, France). The reading of the ELISAi were done with the spectrophotometer "Multiskan EX" (Thermo Scientific®, Waltham, MA, EE. UU.) and a 450 nm wavelength. Absorbances were converted to a optic density ratio between the sample and the control (S/P). S/P= (Sample optic density-Negative control optic density)/ (Positive control optic density-Negative control optic density) *100. The S/P titter was categorized negative (S/P ≤ 30), doubtful (30 < S/P ≤ 40) and positive (S/P > 30).

Results

In total, 97,3 % (35/36) of farms showed antibodies (> 30 S/P) against the bacterium in 2024, in comparison to data obtained in 2010, when 60% (30/50) of the dairies were positive (>30 S/P). Likewise, 62.5% of the regions in 2010 showed antibodies against *C. burnetii* (Lugo = 53.9% and A Coruña = 71.4%). As expected, in the ELISA bulk milk testing done in 2024 the 100.0% of the regions showed antibodies against this bacterium. These results show a high presence, exposure, and geographical distribution of *C. burnetii* in dairy cattle farms in this region, and it also suggests that C. burnetii has increased the prevalence in both provinces studied during the last 14 years. On the other hand, a study carried out in 2018 concluded that in Basque Country the seroprevalence in sheep flocks did not significantly vary from 2005 (40.3%) to 2015 (32.1%), on the other hand the seroprevalence diminished during this period (Álvarez-Alonso et al., 2018).

Conclusion

This study suggests the high exposure and presence of *C. burnetii* in Galician cattle farms and the possibility of its involvement in reproductive disorders in farms with specific epidemiological profiles. Finally, it is important to consider that the high presence of *C. burnetii* in dairy farms could pose a risk to public health, therefore genotyping the strains circulating in herds in Galicia would be interesting to characterize the epidemiology.

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Prevalencesand effects on reproductionof *Coxiella burnetii* infection in dairy cattle farms in Galicia.

U Yañez[1], J Álvarez[1], C Pison[1], A Acción[1], R Barrionuevo[1], JJ Becerra[1], A Jiménez[2], P Gisbert[3], D Remmy[3], PG Herradón[1], AI Peña[1], JM Cao[4], A Prieto[4], LA Quintela[1]

[1] Reproduction and Obstetrics Unit, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain. luisangel.quintela@usc.es
[2] CEVA SALUD ANIMAL S.A., Barcelona, Spain.
[3] CEVA SANTE ANIMALE, Libourne, France
[4] INVESAGA, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain.

Objectives

Coxiella burnetii is the etiological agent of coxiellosis in animals and Q fever in humans. It is a highly infectious pathogen with high environmental resistance (Trachsel *et al.*, 2023). In cattle, coxiellosis is often asymptomatic, but a possible relationship with various reproductive problems has been suggested (Yáñez *et al.*, 2024). Dairy cattle in Galicia (Northwest of Spain) represents the 40% of the dairy cows in Spain (Agricultura & ALIMENTACIÓN Edita, n.d.). However, there is limited coxiellosis epidemiological data in this region (Yáñez *et al.,* 2024). Therefore, the aim of this study was to determine the prevalence of *C. burnetii* and its effects on reproductive parameters in dairy cattle farms in Lugo and A Coruña.

Material and methods

36 bulk tank milk samples (BTMS) were collected and analyzed using real time quantitative polymerase chain reaction (qPCR). Furthermore, reproductive data from the past six months for each farm were obtained to study their relationship with the presence of *C. burnetii*. All the BTMS were collected in 50 ml sterile Falcon and were refrigerated (4-8 ºC) after the collection at the farm. DNA extraction was made with the commercial kit High Pure PCR Template Preparation Kit" (Roche® Mannheim, Alemania). qPCR was performed using the commercial kit EXOone Coxiella burnetii - oneMIX qPCR kit 100 tests" (Exopol S.L., Zaragoza, España) for gene IS1111 detection.

Results

52.3% of farms showed active shedding of the bacterium, with high prevalences in both provinces (Lugo = 53.9% and A Coruña = 71.4%). In contrast, other studies found either lower (Algeria, 26.6%, *Agag et al., 2024) or higher (Lombardy, Italy, 60.0%, Vicari et al.*, 2013) shedding in BTMS. But, our results showed similar prevalence in these provinces than other regions of Spain, as Basque Country (51.7%) (Astobiza *et al.,* 2012). Regarding reproductive parameters, the abortion rate and the incidence of endometritis were significantly higher (*p-value* < 0.05) in farms highly positive for *C. burnetii* (Ct < 32). The abortion rate was also higher in these farms than in negative ones (5.0% *vs* 3.0%) as found in 77.0% of the articles analysed in a systematic review (Gisbert *et al.*, 2024). The percentage of positive farms was higher in those with endometritis (35.0% *vs* 29.0%). This finding aligns with a 2024 study showing higher endometritis in seropositive farms compared to seronegative ones (Yáñez *et al.*, 2024).

Conclusion

This study confirms the high exposure and presence of C. burnetii in Galician cattle farms and suggests the possibility of its involvement in reproductive disorders in farms with specific epidemiological profiles. Finally, it is important to consider that the high presence of C. burnetii in dairy farms could pose a risk to public health.

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Factors influencing reproductive performance in Holstein dairy cows subjected to ovulation synchronization with Double Ovsynch protocol

Zoltán Szelényi[1], Eman Mostafa[1], Ildikó Lipthay[2], Attila Sánta[2], Nikoletta Torák[1], Julie Messel Larssen[1], Ottó Szenci[1]

University of Veterinary Medicine, István u. 2, Budapest, Hungary

[1]Department of Obstetrics and Farm Animal Medicine Clinic – Szelenyi.Zoltan@univet.hu
[2]RougeVet Veterinary Practice, Alsónémedi, Hungary – rougevetkft@gmail.com

The synchronisation of the ovulation before the first insemination is worldwide used in dairy cattle. A total of 2542 lactating Holstein cows (producing 38.7 ± 8.7 kg of milk/d in two Hungarian dairy farms were enrolled in this study to evaluate the outcomes of first artificial inseminations. In this retrospective data evaluation, we analyzed the effects of ovulation synchronisation to herd level reproductive parameters and pregnancy losses.

The conception rates were adversely affected by increasing lactation numbers (P = 0.017). Cows in their first lactation had a conception rate (CR) of 46.5%, while second-lactation cows showed the highest CR of 48.5%, with an odds ratio (OR) of 1.34 (P = 0.023). The CR and OR exhibited a numerical decline for cows in their third lactation, with a CR of 43.9% and an OR = 1.16 (P=0.36). Cows in their fourth or higher lactations, the CR decreased to 37.5%, with an 0R of 0.89 (P = 0.53). Semen type was strongly associated with conception (P <0.001). The application of conventional semen resulted in a CR of 42.2%, while the utilization of sex-sorted semen significantly improved CR to 69%. The OR for sex-sorted semen is 3.06 (P <0.001), emphasizing its substantial advantages over conventional semen. In terms of parity, primiparous cows exhibited a CR of 47.8%, while multiparous cows had a slightly lower CR of 44.1%. Although the resulting differences between both groups approached significance (P = 0.062), there is no statistical difference between the two groups. On the other hand, the initiation day of the Double Ovsynch protocol had no significant impact on CR (P = 0.52) meaning that splitting the starting day of the first GnRH injection did not modify the conception results.

Calving interval in the study animals was 379.4±1.7 days. With different variables examined, calving intervals ranged from 355.3± 7.51 days to 392.2 ± 2.8 days, respectively. The calving interval data were affected by location, year of program, season, dry period length and used semen type. Cows with a dry-off period of > 40 days and those with dry-off periods between 40 and 50 days demonstrated shorter days open with 88.9 and 83.3 days, respectively, and calving intervals of 355.3 and 358.5 days, respectively (P < 0.001). Conversely, extended dry-off periods, particularly those lasting more than 120 days, were associated with increased days open duration to 113.7 ± 2.6 and a prolonged calving interval of 392.0 ± 2.6 days. The type of semen used for insemination significantly influences the reproductive cycle intervals. Specifically, sex-sorted semen was associated with shorter intervals from calving to the first AI (71.2 ± 0.4, P < 0.001), as well as reduced days open and calving intervals (103.8 ± 1.2, and 380.9 ± 1.2, respectively, P = 0.002). On the other hand, conventional semen extended the interval from calving to the first AI, days open, and calving intervals (69.2 ± 0.2, 94.6 ± 2.7, and 371.4 ± 2.8, respectively).

Pregnancy loss was 8,7% in terms of all animals. Parity plays a significant role in pregnancy loss (PL) rates in dairy herds (P < 0.001). In particular, multiparous cows exhibited a PL of 10.3%, significantly higher than the 7.0% observed in primiparous cows. The OR of 1.54 (P = 0.006) indicates that multiparous cows are 54% more susceptible to PL than primiparous cows. Similarly, PL increases with increasing the number of lactations. in the first lactation, serving as the reference group, the PL was 8.2%. The PL declined slightly to 7.1% for cows in their second lactation, with an OR of 0.85, but this difference was not statistically significant (P = 0.41) compared to the first lactation cows. Conversely, cows in their third lactation experienced an elevated PL of 10.6% with an OR of 1.16 (P = 0.19), suggesting an increased but statistically insignificant proportion. Cows in their fourth lactation or more revealed the highest PL at 14.9%, indicating a nearly doubled risk compared to cows in their first lactation (OR = 0.007, P = 0.009). Other factors, like year, season and semen type did not show a significant effect on pregnancy loss rates.

Our results highlight that high producing dairy herds can achieve new standards with the application of synchronized ovulations. In the future we plan to identify further factors which may enhance conception rate results and keep herd level parameters as low as possible.

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Mastitis therapy in Norway: Comparing local to combined systemic and local penicillin treatment of clinical mastitis

Thorell M.[1], Smistad M.[2] and Holmøy I. H.[1]

[1] Faculty of Veterinary Medicine, Norwegian university of life science, Ås, Norway
[2]Tine SA, Ås, Norway

Objectives

Bovine mastitis is one of the most important diseases worldwide in the dairy industry, and antibiotics are frequently used as treatment. Data from Norway's health recording system show that treatment of mastitis constitutes more than 60 percent of antibiotic treatments in the cattle industry. In Norway, treatment is always initiated by a veterinarian, and the standard treatment for clinical mastitis is a combination of systemic and intramammary penicillin. The objective of this randomized clinical trial was to compare the standard mastitis treatment with an alternative treatment protocol using only intramammary treatment, focusing on bacteriological cure as the outcome.

Material and methods

11 veterinary practices across different parts of Norway were recruited for the study. Cows in these practices with clinical mastitis during lactation were eligible if i) the farmer consented to participate, and ii) the farm participated in the milk recording. Exclusion criteria were suspected gram-negative cause of mastitis or teat injuries. Cows were randomized into two treatment groups, both including penicillin as the active substance: group one, the 'combination treatment' (systemic injection day one combined with intramammary treatment in five days), and group two, the 'local treatment' (intramammary treatment in five days). Clinical examination including milk samples for bacteriological culture was performed by the veterinarian prior to initiating treatment. The farmer collected a new milk sample 21 days after treatment. Based on the initial clinical examination, the mastitis was classified as mild (abnormal milk), moderate (abnormal milk and local signs of inflammation) or severe (the cow also had signs of systemic illness). Cow-level data were retrieved from the Norwegian dairy herd recording system at the end of the trial. Treatment success was evaluated based on bacteriological cure rate on day 21. Since penicillin was the active substance of both treatment protocols, only cases with gram positive cause of mastitis were evaluated in the analysis.

Results

Preliminary results from the first 102 teats, collected from 77 cows across 51 farms are presented here, data collection for this study is ongoing and the remaining results will be included in the final presentation at the conference. Reasons for exclusion were culture negative at treatment (n=17), gram negative or yeast infection (n=15), the cow required extra treatment (n=3), teats that could not be treated locally (n=3), the teat was dried off before day 21 (n= 6) or the sample on day 21 was contaminated or missing (n=12), resulting in 46 included cases of clinical mastitis. Of these, 9/46 (19,6 %) were classified as mild, 28/46 (60,9 %) as moderate and 9/46(19,6 %) as severe mastitis. *Staphylococcus aureus* was the most frequently detected udder pathogen 20/46(43,4%). The results show that the bacteriological cure rate (negative sample on day 21) for the combination treatment was 14/24 (58,3 %) and 15/22 (68,2 %) for the local treatment. The cure rate for *Staphylococcus aureus* was 50 % in both groups.

Conclusions

Preliminary descriptive results show no difference in bacterial cure rate in the two treatment protocols. This is promising in terms of the potential to reduce antibiotic consumption for mastitis in Norwegian dairy cows. The number of excluded cases is high in clinical mastitis trials. By increasing the sample size, we will enhance the reliability of the results.

Ketoprofen as the sole initial treatment for mild and moderate bovine mastitis: efficacy and antibiotic reduction

V. Krömker[1], U. Falkenberg[2], N. Wente[1], Y. Zhang[1], S. Leimbach[1], J. Nitz[1], P. Gisbert[3], F. Nankemann[1]

[1] *Microbiology, Department of Bioprocess Engineering, Faculty II, Hannover University of Applied Sciences and Arts, D-30453 Hannover, Germany* *volker.kroemker@hs-hannover.de*

[2] *Cattle Health Service - Animal Disease Fund of Mecklenburg-Vorpommern, Germany*
[3] *CEVA Santé Animale, Libourne, France*

Targeted mastitis treatment concepts are the most scientifically validated approaches to comply with the prudent use of antimicrobials. However, this approach is still a challenge for many farmers who often lack experience with it. Another option for more prudent use of antibiotics in mastitis therapy could be the initial treatment with a non-steroidal anti-inflammatory drug (NSAID) for non-severe mastitis.

Objective

The aim of this study was to compare the treatment of mild and moderate mastitis with ketoprofen to the usual treatment with intra-mammary antibiotics.

Materials and methods

A randomized, multi-herd, non-inferiority study was carried out to compare two treatments of non-severe mastitis cases. The animals in the studied group were treated with systemic NSAID (ketoprofen (KE); KE group; n=104), followed by antibiotic treatment only in cases without clinical improvement, while the animals in the reference group (AB group) received an intramammary antibiotic (n=118). The study included 222 cases of non-severe clinical mastitis on three conventional dairy farms in Northern Germany between November 2022 and November 2023. Study outcomes were clinical cure at day 5, bacteriological cure, CM recurrence in a period of 60 days and new infection risk. Mixed logistic regression was used to analyze the effect of treatment on outcomes. The non-inferiority assumption was checked with the confidence interval of the treatment difference for clinical cure at day 5, calculating using the least square means and their standard deviations.

Results

With regard to the clinical cure on day 5, the treatment in the KE group was inferior. Clinical cure [84.7% (100/118 and 61.5% (64/104)] and bacteriological cure [79.3% (73/92) and 61.2% (41/67)] were significantly higher in the AB group than in the KE group. The risks for recurrent cases [7.3% (8/109) and 15.7% (14/89)] were significantly lower in the AB group than in the KE group. The new infection risk did not differ significantly between the treatment groups [6.8% 50 (8/118) in the AB group and 6.7% (7/104) in the KE group].
In 87% of the cases (n=90) of the KE group, a subsequent antibiotic treatment was not necessary to reach clinical cure on day 5.

Conclusion

Treatment of mild and moderate mastitis with ketoprofen represents an alternative that promoted the deliberate and selective use of antibiotics. Furthermore, as ketoprofen has no withdrawal period for milk, less milk was discarded in the ketoprofen group than in the antibiotic group.
However, some limitations with regard to treatment efficacy in Gram-positive mastitis were observed. A targeted mastitis concept based on the identification of the bacterium involved could help to select cases to be treated with ketoprofen alone and therefore to overcome this limitation.

SmartDCT™: Selective Dry Cow Therapy made simple

Luc Durel[1], Silvia Beschi[2]

1Global technical and medical information manager – VIRBAC S.A., Carros, France – luc.durel@virbac.com

[2]M-team (Mastitis and Milk Quality Research Unit), Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Objectives – The goal of the selective dry cow therapy (SDCT) strategy is to eliminate unnecessary antibiotic (AB) treatments, while treating with AB cows with an intramammary infection (IMI). Veterinarians and farmers face a challenge in selecting dairy cows eligible for AB-based dry-off treatment. Milk culture from every quarter of the udder, shortly before any treatment application, is considered the gold standard diagnostic method. A strong suspicion of IMI can be attributed to the detection of a bacterial species in an aseptically collected milk sample. Despite its benefits, this method is both expensive and time-consuming. IMI can be ruled out by the somatic cell concentration (SCC) in the same milk sample, despite the infection's unknown etiology. Pooled quarter SCCs, when combined with medical history elements, can provide decision-making rules for SDCT. A number of algorithms have already been released. These algorithms remain more or less easy to use and may require the analysis of copious farm records. Additionally, they tend to suggest a single decision rule, or rules for the animal categories (primiparous or multiparous). The aim of this work was to develop a simplified algorithm (SmartDCT™) based on mathematical rules, to test this new method against a set of historical data from dairy farms, and to compare the results obtained with those provided by other algorithms.

Materials and Methods – The objective of SmartDCT™ is to calculate the SCC cut-off in the herd that helps differentiate infected and non-infected cows. As many medical tests, composite SCC predictive values rely on the true prevalence (p) of IMIs in the herd. This prevalence is generally unknown, but it can be estimated as the proportion of cows showing composite SCC>200.000 cells/mL milk over the total population of cows tested at the last test day. Test specificity (*Sp*) and sensitivity (*Se*) depend on the composite SCC level of individuals. *Se* and *Sp* modeling was borrowed from the existing literature (Lipkens et al, 2019). Combining p, *Sp* and *Se* helps calculate how many cows are truly infected (TP), or non-infected (TN), and conversely falsely infected (FP), or non-infected (FN). The art of SmartDCT™ consists in limiting the FP:FN ratio between 1.45 and 1.75, and identifying the corresponding SCC level considered as the proper cut-off for SDCT. SmartDCT™ discourages SDCT in case of estimated IMI prevalence >30%, in case of frequent S. aureus, and S. agalactiae IMIs. All calculations are automated and SmartDCT™ is proposed as an on-line application for free. SmartDCT™ was tested against a dataset of 4- to 6-weekly composite SCC records from 6,935 lactating cows, and 104 farms. Records were available from the lactation of enrolment starting at first test day after calving until the last test day before dry-off. SmartDCT™ results were then compared to the outcomes of algorithms in use in Belgium, The Netherlands, the US, the UK, and New Zealand.

Results – The average percentage of cows that could virtually have been dried off without AB according to the different algorithms varied between 37.0% and 67.8%. The lowest reduction in AB use at dry-off is expected to be achieved via the NZ (i.e. SCC primiparous: <120,000 cells/ml and multiparous cows: <150,000 cells/ml for all test-days in lactation of enrolment) and the NL (i.e. SCC primiparous: < 150,000 cells/ml and multiparous cows: < 50,000 cells/ml at last test-day before dry-off) approach, respectively. The SmartDCT™ and UK algorithm (i.e. SCC <200,000 cells/ml at last three test-days before dry-off) are expected to allow for the highest reduction in AB use at dry-off. For the SmartDCT™ approach, the percentage of cows that are estimated to be dried off without AB increased with decreasing prevalence of high SCC cows. However, caution is needed in the interpretation of the data as the SmartDCT™ app does always recommend BDCT from a herd prevalence of high SCC cows >30%.

Conclusion – The SmartDCT™ algorithm is an elegant tool to support farm vets in determining the most optimal SCC threshold for distinguishing infected from non-infected cows at dry-off as the herd health status is taken into account. The expected reduction in AB use when applying the SmartDCT™ approach will depend on the prevalence of high SCC cows and the herd size.

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Impact of residues in colostrum from first generation cephalosporin treated cows at drying off on antimicrobial resistance in neonatal dairy calves

J.M. Swinkels*[1], D.C. Speksnijder[2, 3], Q. Lu[2], S. Sietsma[3], A. Timmerman[2], A.L. Zomer[2]

swinkels.jantijn@gmail.com, d.c.speksnijder@uu.nl, q.lu@uu.nl, ssietsma@ulp.nu, a.j.timmerman@uu.nl, a.l.zomer@uu.nl

1 MSD Animal Health, Boxmeer, The Netherlands; 2 Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; 3 University Farm Animal Clinic ULP, Harmelen, the Netherlands

Objectives

Antimicrobial resistance poses a threat to both human and animal health. Especially, the transmission of Extended Spectrum Beta-Lactamase producing Enterobacteriaceae (ESBL-pE) from animals to humans is considered a risk. Dairy calves are known to harbor ESBL-pE in their gut, although the source of this bacteria is not yet fully understood. It is possible that transmission occurs from the dam via feces or colostrum. First-generation cephalosporins (1GC) are widely used for dry cow therapy (DCT) but may select for ESBL-producing bacteria, a risk believed to be lower with narrow-spectrum penicillin (NSP). This study compares the effects of a 1GC versus NSP-DCT on the selection and spread of cefotaxime-resistant (CTX-R)-*E. coli* in the gut of neonatal calves.

Material and Methods

On three commercial Dutch dairy farms harboring ESBL-*E. coli* in the herd, cows were screened for CTX-R-*E. coli* in their feces using selective enrichment with 1 mg/ml CTX in LB broth, followed by plating on MacConkey agar before drying off (DO). Cows carrying CTX-R *E. coli* were randomized to receive either standard NSP-DCT (4*600 mg cloxacillin) or 1GC DCT (4*300 mg cefapirin) at DO. Post-calving, calves were fed their dams' colostrum for 2-3 days. Fecal samples from the calves born to these cows were collected on day 2, day 7, and day 14 after birth. Samples were stored at 2-8 °C and sent to the laboratory within 48 hours.

Feces were enriched in LB broth with 1 mg/ml CTX, incubated, and plated on MacConkey agar for the screening of CTX-R-*E. coli*. Confirmation of *E. coli* was performed using MALDI-TOF mass spectrometry. If a sample tested positive, quantitative analyses were conducted to calculate the CTX-R-*E. coli* colony-forming units (CFU) per gram of feces. Fresh feces were mixed with saline, and ten-fold dilutions were plated on square MacConkey plates with CTX and incubated overnight for quantitative counts. Whole Genome Sequencing was performed on the CTX-R-*E. coli* isolates.

A one-way ANCOVA test was used to assess whether there was a difference in counts between the two groups, with the time points of sampling included as covariates and farms treated as a random factor.

Results

A total of 172 cows were screened for the presence of fecal CTX-R-*E. coli* before DO. Of these, 22 cows (13%) tested positive for CTX-R-*E. coli* and were included in the study, with half receiving 1GC DCT and the other half receiving NSP-DCT. A total of 24 calves were born alive, and 20 calves (10 in each study arm) could be followed until day 14 after birth.

In the calves, mean fecal counts of CTX-R-*E. coli* were recorded as follows: $3.73*10^{6}$ (SD $6.52*10^{6}$) and $2.20*10^{6}$ (SD $4.14*10^{6}$) CFU/gram of feces on day 2; $8.36*10^{6}$ (SD $2.53*10^{7}$) and $3.44*10^{5}$ (SD $8.47*10^{5}$) CFU/gram on day 7; and $1.73*10^{7}$ (SD $3.75*10^{7}$) and $4.91*10^{6}$ (SD $1.00*10^{7}$) CFU/gram on day 14 in the 1GC and NSP groups, respectively. On day 2, all calves harbored CTX-R-*E. coli*; by day 7, CTX-R-*E. coli* was not detected in one calf in the 1GC group and in three calves in the NSP group; by day 14, CTX-R-*E. coli* could not be found in one calf in the 1GC group.

At each sampling point, average CTX-R-*E. coli* counts were higher in calves from dams that received 1GC treatment, but no significant differences in CFU counts per gram of feces were observed compared to calves born from dams receiving NSP-DCT during the study period (F=2.401; p=0.20), when adjusted for farm and sampling time point. We observed acquired resistance genes and point mutations that cause resistance. Transmission of resistant clones was independent of the treatment group and occurred throughout the farm, with multiple clones observed in animals at different time points. CTX-R-*E. coli* was detected in both mother and calf on all farms and in colostrum from one farm.

Results from a worst-case scenario study involving a limited number of animals indicate that fecal counts of CTX-R-*E. coli* in neonatal calves born to dams harboring CTX-R-*E. coli* in their gastrointestinal tract at the time of DO did not significantly differ between cows receiving cloxacillin or cefapirin DCT. However, there were substantial variations in fecal CTX-R-*E. coli* counts among calves within both study groups, suggesting that other cow- or farm-related factors may have a greater influence on the selection and transmission of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-pE) via calves. The presence and spread of CTX-R clones appear to be independent of the type of DCT received, whether cloxacillin or cefapirin.

Sensitivity of isolated strains of bovine mastitis to trimethoprim (TMP), sulfadimidine and the TMP-Sulfadimidine combination

Guillaume Lequeux[1], Mathieu Pichaut[2]

1 LABOCEA, BioAgroPolis, 10 rue Bourgelat, 35306 FOUGERES - *guillaume.lequeux@labocea*.fr

[2]VIRBAC S.A., 13ème rue L.I.D, 06510 CARROS, France

Objective - To determine the Minimum Inhibitory Concentrations (MIC) and the sensitivity of a selection of recently isolated strains of bovine mastitis in the West of France:

- to trimethoprim (TMP) alone
- to sulfadimidine alone
- to the TMP-sulfadimidine combination at the ratio of the veterinary medicinal product AMPHOPRIM® (VIRBAC France) that is 1/5.

Material and methods - Selected strains: *Staphylococcus aureus* (n=15), Coagulase-negative *Staphylococcus* (non-aureus) (15), *Streptococcus uberis* (14), *Streptococcus dysgalactiae* (12), *Pseudomonas aeruginosa* (5), *Klebsiella* spp. (7), *Serratia marcescens* (12), *Enterobacter* spp. (5) and *Escherichia coli* (15) isolated at LABOCEA between 2020 and 2024, identified by MALDI-TOF mass spectrometry (VITEK MS) and stored at a temperature ≤ - 75°C

MIC determination by microdilution in liquid medium according to CLSI VET01-A5 and VET08-S4 standards. Quality control using strains ATCC 25922 and 29213.

Categorization thresholds selected: those of the CA-SFM veterinary 2023 and, when not available, thresholds recommended by Guiguère et al., 2013 (1)

Results – All tested strains were resistance to sulfadimidine alone. By contrast, many strains were sensitive to TMP with the exception of *P. aeruginosa* (100% resistant), and to a lesser extent *Str. uberis* (71%), *Str. dysgalactiae* (33%) and *E. coli* (13%). With the notable exception of *P. aeruginosa* (100% resistant) and to lesser extent *E. coli* (13%), all strains were sensitive to the 1:5 TMP-Sulfadimidine ratio.

Conclusion - The natural resistance of *P. aeruginosa* to TMP and sulfonamides as well as to their combination has been documented. These results are comparable to those published in Switzerland for Gram-positive bacteria (2). The proportion of *E. coli* resistant to TMP alone or the combination is comparable to those of the RESAPATH (a French organization entitled, RESeau de surveillance de l'Antibiorésistance des bactéries PATHogènes animales) 2023 survey (3) indicating respectively 11 and 14% resistance to the combination TMP- sulfamethoxazole (ratio 1/19). The low proportion (<5%) of resistant staphylococci and other Gram negative strains compare to those of RESAPATH. Finally, by contrast to the RESAPATH survey that mention a proportion of 15% streptococci species resistant to the combination TMP-sulfamethoxazole (ratio 1/19), our results were less concerning. From a pharmacodynamics point of view, the TMP-Sulfadimidine combination (ratio 1:5) remains a reliable option for the treatment of clinical mastitis.

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Confirmed identification of *Mycolicibacterium smegmatis*, *Staphylococcus borealis* and *Paenibacillus xylanexedens* in Danish cows with high total bacterial count from the same farm

Astrup LB[1]. and Kløve DC[2].

[1]SEGES Innovation, 8200 Aarhus N, Denmark.
[2]Danish Technical University, Kgs. 2800 Lyngby, Denmark.

Objectives:
The objectives were to confirm MALDI-TOF MS identification of *Mycolicibacterium smegmatis*, *Staphylococcus borealis* and *Paenibacillus amylolyticus*, respectively, and to evaluate antibiotic resistance profile of taxonomically confirmed *M. smegmatis*.

Material and methods:
A Danish farm with high-level management and generally high-level udder-health had suffered abrupt high total bacterial counts (TBC) on Bulk tank milk for a period of approximately 3 months (January through March 2024). Several cows had gone through individual TBC-analysis on composite samples (four quarters pooled) through the DIH-testing and the 10 cows with the highest TBC had also gone through PCR (DNA-diagnostics TBC4-kit, EUROFINS, Dk) and four of the same animals had additionally gone through a broader PCR-test (DNA-diagnostics TBC16-kit, EUROFINS, Dk). One of the cows was slaughtered while the remaining 9 cows had a total of 22 aseptically obtained quarter milk samples shipped for bacteriological analysis by MALDI-TOF MS. These 22 quarters were selected as they were culture-positive at receival at the herd veterinarian, while the remaining quarter samples were culture-negative. The samples were kept and shipped frozen from the veterinary practitioner's lab to MALDI-TOF MS analysis. Before MALDI-TOF MS-analysis each milk sample was cultured on blood agar (5% calf blood, SSI Diagnostica A/S, Hillerød, Dk). All plates were inspected at both 24 and 48 h of incubation. All bacterial colonies were sub-cultured on blood-agar and incubated for 24 h to obtain pure-cultures of all culturable bacteria present in the milk samples irrespective of definitions on contamination. All resulting pure-cultures were analyzed with MALDI-TOF MS. (NB: Additional culture-protocols were carried out and other bacterial species identified, but these results are not included as the present abstract focuses on the identification of rare mastitis-pathogens and not on the full study-aspects related to high TBC). All unusual mastitis-causing bacteria with a MALDI-score of ≥ 2.0 was whole genome sequenced (WGS) (Novogene (UK), Co., Ltd., Cambridge, UK) to confirm their MALDI-TOF MS taxonomy. The WGS-based taxonomy identification was performed using KmerFinder (Center for Genomic Epidemiology). For the *S. borealis* genomes, an Average Nucleotide Identity (ANI) analysis was further performed to verify species taxonomy including the currently available 21 *S. borealis* reference genomes in the NCBI genome database. The species boundary cut-off > 95% was applied. Finally, ResFinder (Center for Genomic Epidemiology) was used to investigate the presence of acquired antibiotic resistance genes (ARGs) of the two M. smegmatis genomes.

Results:
One of the nine cows had two glands that were culture-positive for *M. smegmatis*. One gland was a pure-culture and the other gland had a mixed culture (2 bacterial species) with *S. borealis*. WGS-analysis showed that the isolates were genetically confirmed as *M. smegmatis* and both isolates were suggested multi-drug resistant as each were harboring seven ARGs encoding resistance to a series of different antibiotics, e.g. penicillin, erythromycin, tetracycline and fosfomycin. Notably, both isolates also harbored a *mecA* gene associated with methicillin resistance. Three of the nine cows had one or two quarters each that were culture-positive for *S. borealis*. According to NMC-guidelines two of these quarter-samples were pure-cultures while the third quarter-sample displayed *S. borealis* in mixed culture with *M. smegmatis* as described earlier. The fourth quarter sample was positive for *S. borealis* but at CFU below NMC-definitions of culture-positive samples and was found together with another predominant bacterium (*Enterococcus* sp.). All four *S. borealis*-isolates had their taxonomy genetically confirmed in ANI analysis, with percentage identities ranging from 97-99%. One of the nine cows had a gland that was culture-positive for a pure-culture, but at CFU below NMC-definitions of culture-positive samples. This culture was identified as Paenibacillus amylolyticus by MALDI-TOF MS, but WGS-based taxonomy identification using KmerFinder suggested reclassification as Paenibacillus xylanexedens.

Conclusion:

M. smegmatis is a rare mastitis-pathogen that has also been associated with pyrogranolomatous mastitis.
A recent retrospective study suggested that *S. borealis* is a more common non-*aureus* staphylococci than hitherto acknowledged. But to the best of our knowledge, *Paenibacillus xylanexedens* has never been associated with the bovine udder before. Also, the bacterium was not classified until 2009, which might explain the flawed species allocated by MALDI-TOF MS.
Possibly, our finding of *P. xylanexedens* reflects low-grade contamination of a milk sample. Yet, the finding of all three rare pathogens in pure or mixed culture from cows with high total bacterial counting, and from the same farm, calls for more attention towards the possible complex nature behind the TBC relative to classical udder-health microbiology.

Antimicrobial resistance patterns of mastitis pathogens in a region of Switzerland with raw milk cheese production

Martin Sommer, Brian Gerber, Michèle Bodmer
University of Bern, Vetsuisse Faculty, Clinic for Ruminants, Bremgartenstrasse 109a, 3012 Bern
Affiliation – *michele.bodmer.unibe.ch*

The work presented here was conducted within a larger study dealing with reduction of antimicrobial resistance in the fields of udder health, calf health and uterine health in the canton of Freiburg in western Switzerland by initiative of dairy producers. The producers were provided with a variety of prevention strategies of which they could choose to implement in their herds. For this study the bacterial culture results and the resistance testing of a subset of herds, that had chosen an udder health strategy were described.
Milk samples were submitted from cows with subclinical mastitis during lactation, clinical mastitis and subclinical mastitis before dry-off. Samples were cultured on blood agar and aesculin agar followed by a species identification in the case of aesculin positive streptococci and staphylococci. The antimicrobial resistance testing was performed by the disc diffusion method using EUCAST breakpoints. The resistance testing was performed systematically for all staphylococci, enterococci and coliforms.

A total of 2441 milk samples were collected and submitted by 123 dairy producers for bacterial culture. In total 3028 pathogens were identified. The majority of samples was submitted from cows with subclinical mastitis during lactation defined as cows with somatic cell count of >150000 cells /ml (n=1524), 463 samples were submitted form cows with subclinical mastitis before dry-off and only 123 samples were submitted from cows with clinical mastitis.
The results of bacterial culture revealed 37% of non-aureus staphylococci (NAS), 21% of Aesculin-positive streptococci, 10% contaminated samples (>3 different colonies), 10% coliforms, 7% Corynebacterium spp., 3% *Staphylococcus aureus*, 2% Aesculin-negative streptococci and 12% other pathogens. A total of 67% of the Aesculin-positive streptococci were identified as *Streptococcus uberis*, 12% as *Entercoccus sacharolyticus*, 6% as *Lactococcus garviae* and 15% as other streptococci, enterococci and lactoccocci and *Kokuria* spp.. Among the aesculin-negative streptococci 12 out of 13 results were identified as *Stretpococcus daysgalactiae*.

Looking at the resitance testing 73% of the NAS isolates showed a simple penicillin resistance. Ninety percent of the coliform isolates were resistant against Ampicilline ans 21% against Amoxycilline-clvulanic acid, A total of 12% of the *Staphylococcus aureus* isolates were resistant against penicilline but none showed a resistance against methicillin. Looking at the entercocci, 100% of the isolates were resistant against cephalosprins 3rd and 4th generation but only 23% against penicillin.

Due to sample collection by farmers the percentage of contaminated samples was rather high although the farmers were educated in aseptically collecting milk samples. Unfortunately, in a high percentage of samples the farmers did not indicate if the sample was collected from a cow with clinical and subclinical mastitis respectively, therefore no separate anaysis for clinical and subclinical mastitis respectively was possible. Out of financial reasons resistance testing for streptococci was not performed since in the literature only a very small percentage of pnicillin-resitance was reproted. The situation of antimicrobial resistance in NAS is alarming when comparing to other European studies where 17 to 52% of penicillin-resistance were reported. This may be due to overtreatment of minor mastitis pathogens in a context of rather small herds and high demands on low somatic cell count in bulk tank milk delivered for raw milk cheese production. Looking at the pattern of mastitis pathogens found it is comparable with older Swiss studies and also comparable with international literature.

In conclusion this work shows, that there is a lot of room for improvement in terms of reducing antimicrobial usage in Switzerland by simply prevent overtreatment of subclinical mastitis caused by minor pathogens during lactation. This can easyly be achieved by systematic culturing of cows with subclinical and clinical mastitis. Hopefully these pracitces will lead to an reduced penicillin-resistance in NAS in the future.

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Diagnostic validation and comparative performance of electrical conductivity and california mastitis test for udder health assessment in mediterranean buffaloes (*Bubalus bubalis*)

Maria Chiara Alterisio[a], Paolo Ciaramella[a], Sergio Esposito[a], Anastasia Lisuzzo[b], Enrico Fiore[b], Massimo Pascale[c], and Jacopo Guccione[1*a]

[a]*Department of Veterinary Medicine and Animal Production – University of Study of Napoli Federico II – Via Delpino 1, 80137, Napoli, Italy*

[b]*Department of Animal Medicine, Production and Health, University of Padua, Viale dell'Università 16, Legnaro 35020, Padua, Italy*

[c]*Veterinary practitioner, 81100, Caserta District.*
Department of Veterinary Medicine and Animal Production – University of Study of Napoli Federico II – Via Delpino 1, 80137, Napoli, Italy

Objectives. This study aimed to validate the use of electrical conductivity (EC) and the California Mastitis Test (CMT) as diagnostic tools for assessing udder health in Mediterranean buffaloes (MBs), for the first time. Moreover, their diagnostic performance was compared to a gold standard (GS) combining somatic cell count (SCC) and bacteriological milk culture (BMC).

Materials and Methods. A total of 600 quarters milk samples were aseptically collected from randomly selected, multiparous MBs, between December 2023 and September 2024. All samples were submitted for SCC and BMC to the reference laboratory, while EC and CMT were recorded and performed on-farm after milking. No specific eligibility criteria were applied regarding udder health status at the time of sampling. Based on the GS results (using 200'000 cells/mL as SCC cut-off and presence/ absence of mastitogens bacteria), the samples were categorized as mastitis cases [including both clinical mastitis (CM) and subclinical mastitis (SCM)], or non-mastitis cases [including both intramammary infection (IMI) and healthy (H)] categories. For the validation both EC and CMT were compared with SCC as the benchmark test. Thereafter, EC and CMT were compared to the GS to assess their ability to correctly identify udder health status both independently and associated with BC as potential alternatives to the use of SCC in field.

Results. Overall, 134 mastitis cases (57 CM and 77 SCM) and 466 non-mastitis cases (335 IMI and 131 H) were detected. For the diagnostic validation (EC vs. SCC, and CMT vs. SCC) the Cohen's Kappa revealed an agreement of 0.27 ($P<0.0001$) and 0.51 ($P<0.0001$) for EC and CMT, respectively. An area under the ROC curve (AUC) of 0.75 ($P<0.0001$) for EC and 0.97 ($P<0.0001$) for CMT was calculated, with accuracy values of 0.73 for EC and 0.76 for CMT. Sensitivity (Se) was 0.45 for EC and 0.97 for CMT, while specificity (Sp) was 0.82 for EC and 0.69 for CMT. The positive likelihood ratio (LR+) was 2.45 for EC and 3.15 for CMT, and the negative one (LR-) was 0.68 for EC and 0.04 for CMT. Positive post-test probability was 0.50 for EC and 0.56 for CMT, while the negative post-test probability was 0.63 for EC and 0.09 for CMT.

When both the test was compared to the GS (EC vs. GS, and CMT vs. GS), an AUC of 0.66 ($P<0.0001$) for EC and 0.82 ($P<0.0001$) for CMT, with accuracies of 0.73 for EC and 0.74 for CMT were detected. The Se was 0.46 for EC and 0.97 for CMT, while the Sp was 0.81 for EC and 0.67 for CMT. The LR+ values were 2.41 for EC and 2.92 for CMT, with LR- values of 0.67 for EC and 0.04 for CMT. Positive post-test probability was 0.49 for EC and 0.54 for CMT, and negative post-test probability was 0.62 for EC and 0.10 for CMT.

Finally, when both the tests were combined with BC (EC+BC vs. GS, and CMT+BC vs. GS), an AUC of 0.67 ($P<0.0001$) for EC and 0.86 ($P<0.0001$) for CMT, with an accuracy of 0.79 for both tests were observed. Sensitivity was 0.46 for EC and 0.97 for CMT, while specificity was 0.89 for EC and 0.74 for CMT. The LR+ was 4.00 for EC and 3.77 for CMT, and LR- values were 0.61 for EC and 0.04 for CMT. The positive post-test probability was 0.62 for EC and 0.60 for CMT, while the negative post-test probability was 0.60 for EC and 0.09 for CMT.

Conclusions. The CMT demonstrated better performance as diagnostic tools for mastitis cases, with a higher AUC and sensitivity, as compared with EC. In contrast, the latter seems to be useful for confirming the absence of mastitis due to its higher specificity. Neither test alone should be relied upon for definitive diagnosis; a combination with BC seems to improve their diagnostic performance, but CMT consistently outperformed EC in terms of sensitivity and overall accuracy.

New rapid test for selective treatment of clinical mastitis: An innovative, fast, and easy-to-use solution to reduce antimicrobial usage in cattle.

Sabine Reinhold[1], Marcus Klawitter[2], Antonia Hentzsch[1], Luis Leon[2]

[1] Vetxperts am Weinberg, Weinberge 39A, 06917 Jessen (Elster), Deutschland
[2] Zoetis Deutschland GmbH, Leipziger Platz 18, 10117 Berlin, Germany

marcus.klawitter@zoetis.com

Introduction and Objectives: The treatment of clinical mastitis with antimicrobials is common. In Europe, guidelines for the prudent use of antimicrobials in veterinary medicine promote the selective treatment of clinical mastitis (STCM). This will allow to identify pathogens responsible for mastitis and helping to minimize the use of intramammary and injectable antimicrobials in dairy cows (European Commission, 2020). Implementing STCM prior to treatment offers economic benefits for farmers (Rico et al., 2024) and can lead to a reduction in antibiotic use of up to 60% (Schmenger et al., 2022). Research indicates that STCM does not negatively impact healing, somatic cell count, milk yield, recurrence of mastitis, or culling rates (de Jong et al., 2023). German farmers express interest in and readiness to adopt STCM, recognizing the need for changes in management practices and the time available to make decisions before treatment (Klawitter et al., 2024).
The new VetScan Rapid Mastigram+™ test (Zoetis Inc., USA) is a novel flow test that detects Gram-positive bacteria in milk after 7 to 7.5 hours of incubation at 37°C. According to Busch et al. (2024), veterinarians report several advantages of this new test: 83% describe it as very fast, 58% find it easy for farmers to use and interpret, and 58% believe it contributes to reducing antibiotic use in livestock. Further analysis of the new test in field conditions is necessary, particularly focusing on dairy farms with a high incidence of mastitis caused by *S. uberis*, the most significant pathogen responsible for clinical mastitis in Germany.

Materials and Methods: Thirty quarter foremilk samples (QFS) from fresh cases of clinical mastitis were collected from a commercial dairy farm in eastern Germany. The rapid test (RT) Mastigram+™ and a bacteriological test at the veterinary clinic's milk laboratory (VC) were conducted according to established protocols and the product insert. The enrichment process for the QFS should begin immediately after collection. Due to a slight delay in enrichment and testing, the QFS were maintained at refrigerated temperatures during transportation to the veterinary clinic. Sample enrichment and testing were completed on the same day as collection. Additionally, the QFS were analyzed using MALDI-TOF mass spectrometry at the milk laboratory of the University of Hannover.

Results: Eighteen out of thirty QFS (60%) yielded a positive result for the rapid test, indicating the presence of Gram-positive bacteria. All these samples were confirmed by MALDI-TOF, with 17 identified as *S. uberis* and one as *S. aureus*. At the veterinary clinic's laboratory, all results were corroborated; however, one sample presented *Klebsiella* spp., which was confirmed by both MALDI-TOF and the VC. Twelve samples (40%) returned a negative result on the rapid test, indicating no presence of Gram-positive bacteria. Four of these samples showed “no growth in 0.01 ml” with MALDI-TOF, while the laboratory identified 2 as *coagulase-negative staphylococci* (CNS), 1 as *Klebsiella spp.*, and 1 as having no growth. One sample with a negative RT result was identified as *Klebsiella spp.* by both MALDI-TOF and VC. Five samples that were RT negative for Gram-positive bacteria showed mixed bacterial growth at low concentrations (including *Streptococcus uberis, Staphylococcus capitis, Staphylococcus chromogenes, Sphingobacterium mizutaii*, and *Staphylococcus sciuri*) on MALDI-TOF. Notably, only two *S. uberis* samples in single culture and high concentration (>300 CFU/10 µl) were not detected by the RT. The specificity of the RT (true no growth, Gram-negative rate) was 100%, while the sensitivity (true positive rate) was 89.4% overall and 88.8% specifically for S. uberis (>180 CFU/10 µl).

Conclusions: The new udder health Gram-positive test provides veterinarians and farmers with a highly accurate tool to reduce the use of antibiotics in livestock in accordance with European and local regulations. Furthermore, enhancing time-to-treatment efficiency through a faster diagnostic test contributes to a favorable net cash impact of selective treatment of clinical mastitis (STCM).

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Udder health status of dairy cows at dry-off: Cytobacteriological analysis at quarter level and *gn t itro* susceptibility of relevant intramammary antibiotics

Marcus Klawitter[1], Luis Leon[1], Christian Fidelak[2], Torsten Steppin[1], Ulrich Löschner[1], Ansgar Busch[1], Thomas Breuer[1], Maria Vergara Hernandez[3]

[1] Zoetis Deutschland GmbH, Leipziger Platz 18, 10117 Berlin, Germany
[2] Bovicare, Bernauer Allee 10, 16321 Bernau, Germany
[3] Hochschule Osnabrück, Am Krümpel 3, 49090 Osnabrück

marcus.klawitter@zoetis.com

Objectives: The analysis of udder health status at dry-off is relevant for establishing and continuously controlling management decisions on dairy farms. Information about the pathogens to treat at dry-off and the resistance situation of dry-cow intramammary antimicrobial products is crucial for farm veterinarians. This epidemiological study examines the udder health of dairy cows at dry-off in Germany by analyzing quarter foremilk samples (QFS) to identify bacterial pathogens and antimicrobial resistance, providing guidance for medication prescribers.

Materials and Methods: QFS were collected by 51 farm veterinarians from 115 dairies (mainly Holstein Friesian and Simmental herds) across Germany. The QFS were collected aseptically from the farmers between day seven and one day before dry-off. All QFS were processed at the milk quality laboratory bovicare GmbH (Bernau, Germany) for bacteriology and SCC (Fossomatic, FOSS Analytical, DK) following international guidelines with slight modifications (NMC, DVG - German Society of Veterinary Medicine). The susceptibility was determined using the agar diffusion method, and the evaluation of the inhibition zone results (S = susceptible, I = intermediate, R = resistant) was done according to the literature (e.g., NLSI). The susceptibility was tested for pathogens against the most frequently used intramammary antibiotics/antibiotic fixed combinations: cloxacillin (CLOX), cephapirin (CEPA) as a first-generation cephalosporin representative, cefquinome (CFQ), and the combination penethamate + penicillin + framycetin (PPF). For the statistical analysis, the susceptibility situation was compared using the Friedman Test (within-subjects ranks) (SPSS Version 28, IBM USA). Differences were considered statistically significant when $p<0.05$.

Results: A total of 2,488 QFS from 622 dairy cows were analyzed between June 2021 and June 2024. The mean number of cows per veterinarian was 12 ± 25 (min 1, max 148, geometric mean 6). Of the 2,488 QFS, 1,840 (74.0%) were bacteriologically negative, 61 were contaminated (2.5%), and 65 were not sampled (2.6%). Intramammary infections (IMI) associated with subclinical mastitis were identified in 522 quarter foremilk samples (QFS), representing 20.9% of the total samples. The pathogen distribution was as follows: 198 QFS *KNS* (37.9%), *Corynebacterium bovis* 125 (23.9%), *S. uberis* 72 (13.8%), *S. aureus* 38 (7.3%), *Enterococcus spp.* 32 (6.1%), *S. dysgalactiae* 23 (4.4%), *E. coli / coliforms / Klebsiella spp.* 17 (3.3%), and 17 (3.3%) others. At the cow level and excluding QFS with *C. bovis*, 63.4% of the cows did not present any IMI, 22.8% (142) had one quarter with an IMI, 46 (7.4%) had two quarters with IMI, and 3.5% (22) had three quarters with IMI. Eighteen (2.9%) animals were detected with all four quarters affected. The overall pathogen susceptibility situation was as follows: CEPA (susceptible 84.8%), CLOX (susceptibility 79.4%), CFQ (77.9%), and PPF (67.6%). The results showed a significant difference in in vitro susceptibility between the antibiotics tested. For all pathogens, the susceptibility was found in this range: CEPA > CLOX > CFQ > PPF. For KNS, the range was CLOX > CFQ > CEPA > PPF. For *S. uberis*, this range was determined as CLOX > CEPA > CFQ > PPF. *S. aureus* showed the range CLOX > CEPA > CFQ > PPF. For *Enterococcus spp.:* CEPA > PPF > CFQ > CLOX.

Conclusions: The substantial number of cows without IMI at dry-off allows for selective dry-cow therapy. Most of the cows presented with less than two quarters with IMI, making quarter selective dry-cow therapy advantageous. Most of the major pathogens found at dry-off showed in vitro susceptibility to CLOX. Cloxacillin, classified by the EMA as a group D antibiotic recommended for first-line treatments whenever possible, should be preferred over first- and fourth generation cephalosporins or fixed combinations, as these offer no additional advantage.

Molecular epidemiology of *Streptococcus uberis* intramammary infection in high prevalence mastitis herds

Anyaphat Srithanasuwan[1,2], Yang Zou[1], Witaya Suriyasathaporn[2,3,4], and Ynte H. Schukken[1,5,6*]

[1]Department of Animal Sciences, Wageningen University, 6708 PB Wageningen, the Netherlands [2]Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand, [3]Research Center of Producing and Development of Products and Innovations for Animal Health and Production, Chiang Mai University, Chiang Mai, Thailand, [4]Cambodia Campus, Asian Satellite Campuses Institute, Nagoya Universities, Nagoya 464-8601, Japan, [5]GD Animal Health, PO Box 9, 7400 AA Deventer, the Netherlands, [6]Department of Population Health Sciences, Utrecht University, 3584 CL, Utrecht, the Netherlands

**Corresponding Author – ynte.schukken@wur.nl*

Streptococcus uberis is one of the most common mastitis-causing pathogens, exhibiting a wide range of intramammary infection (IMI) durations. This study aimed to investigate the diversity of *Streptococcus uberis* isolates from Thai dairy cows with subclinical mastitis, focusing on antimicrobial resistance and virulence genes. A total of 138 *S. uberis* strains were isolated from three dairy herds (Farm A – C) with a high prevalence of *S. uberis* mastitis. Data on farm management practices and mastitis control policies were collected during the longitudinal sampling of milk, revealing variations in policies among farms. Poor husbandry and inadequate mastitis control policies were noted across all farms. All available *S. uberis* isolates were selected based on the characteristics of infection episodes, with a maximum of two isolates per episode. Selected isolates were confirmed using MALDI-TOF and subsequently subjected to whole-genome sequencing. Pangenome analysis revealed significant variation in *S. uberis* diversity between farms. It was found that *S. uberis* isolates from certain farms exhibited novel multi-locus sequence types, indicating a highly diverse population. A total of 21 out of 38 virulence genes were identified in all isolates, including *biofilm putative glycosyltransferase, hasC, lmb, sclB, sua, fbpS, srtA, fbp54, surface-anchored protein, mtsB, scaR, mtuA, oppF, pauA, mga, scpA, lbp, cyG, cylA, cps4A,* and *emm*. The proportion of isolates carrying at least one antimicrobial resistance gene varied across herds, ranging from 10.7% to 98.1%. In herds with high genetic diversity, most *S. uberis* infections were transient, lasting approximately 1–2 weeks, with a high prevalence of antimicrobial resistance genes. In contrast, on farms experiencing chronic mastitis lasting up to 10 months, *S. uberis* isolates were predominantly from a contagious strain with low genetic diversity and a limited distribution of antimicrobial resistance genes. These differences were likely influenced by farm management practices and bacterial traits. Additionally, antimicrobial resistance was linked to farm antibiotic policies, while greater virulence diversity and genetic variation suggested a potential connection between virulence profiles and resistance. These findings underscore the diversity and characteristics of *S. uberis* mastitis, which appear to be influenced by farm management practices and bacterial traits. This highlights the need for further studies tailored to specific mastitis scenarios to develop effective control strategies aimed at reducing the impact of *S. uberis* mastitis.

Keywords: *Streptococcus uberis,* mastitis, Whole genome sequence, Intramammary infection, Genetic diversity, Antimicrobial resistance, Virulence factor

Development of a rapid on-farm test for early detection of subclinical mastitis in dairy cattle

Pauline Delhez[1], Léonard Theron[1], Anne-Sophie Rao[1], Béatrice Blanchard[2], Marie-Astrid Vernet[2], Aurélie Fondaire[2], Philippe Coppe[2], Philippe Hivorel[2]

[1]RumeXperts, Faimes, Belgium

[2]Bio-X Diagnostics, Rochefort, Belgium

Speaker : Léonard Theron, ltheron@rumexpert.vet

Introduction and Objectives

Subclinical mastitis is a major concern in dairy farming, causing substantial economic losses and impacting animal welfare. Its absence of visible symptoms often results in delayed detection, highlighting the need for efficient and accessible diagnostic tools to facilitate timely intervention. The current gold standard for assessing mammary health is somatic cell count (SCC), but its reliance on laboratory processing requires the shipment of samples and waiting for results, which can delay decision-making. This study aims to address these limitations by developing and validating a rapid, immunochromatographic test that provides a cost-effective and on-farm solution for early detection of subclinical mastitis directly on farms.

Materials and Methods

The study began by exploring potential biomarkers linked to subclinical mastitis, with a particular emphasis on understanding the inflammatory processes occurring within the mammary gland. A cathelicidin-family antimicrobial peptide (CAT) was identified as a promising alternative to SCC. Given the initial uncertainty regarding CAT's identity, a monoclonal antibody was developed, and antigen purification followed by mass spectrometry confirmed its nature.

The test employs lateral flow immunochromatography, analyzing milk samples with a strip housed in a cassette containing a QR code. This design enables smartphone-based interpretation, offering semi-quantitative classification into categories (<100k, 100k-400k, and >400k cells/mL). The prototype, initially tested on 220 milk samples, underwent iterative optimization using several hundred samples with varying SCC levels. Parameters such as dilution factors, reagent formulations, and gold conjugate concentration were adjusted to enhance performance.

Finally, on-farm validation was conducted with 270 tests to evaluate reproducibility under field conditions. Key performance metrics, such as sensitivity (Se) and specificity (Sp), were evaluated to determine the test's reliability and accuracy.

Results

The CAT biomarker demonstrated high diagnostic accuracy (AUC = 0.93 at a 100k SCC cut-off). The test achieved sensitivity exceeding 80% and specificity above 90% at an SCC cut-off of 100k cells/mL, effectively identifying cows at risk of subclinical mastitis and aligning closely with infection status. These semi-quantitative results highlight its significant advantages over subjective on-farm methods like the California Mastitis Test.

Conclusion

In conclusion, this rapid, cost-effective, and user-friendly test provides a practical solution for early subclinical mastitis detection on farms. By enabling targeted interventions such as selective dry cow therapy, it has the potential to reduce antibiotic usage while improving dairy herd health and productivity.

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Recent advances in the management of the first days of a calf's life

I. Lorenz
Bavarian Animal Health Service, Poing, Germany

RESUME

Research on calf health and welfare has intensified in the past decades. However, calf losses are still a major problem on cattle farms worldwide.Good colostrum management is still recognized as the single most important factor to preventing calf morbidity and mortality, however, it is now known that immunoglobulins are only one of many components of colostrum that are vital for the calf's development. Other non-nutrient factors like leucocytes, hormones and growth factors, oligosaccharides as well as microRNAs have significant effects on the development and maturation of the intestinal and systemic immune functions. They also promote the maturation and function of the intestine, thus enabling the calf to digest and absorb the nutrients provided with colostrum and milk. The improved energetic status of colostrum-fed neonates is reflected by an accelerated maturation of the somatotropic axis, which stimulates body growth and organ development. Colostrum oligosaccharides play a major role in the development of a healthy intestinal flora.Early separation of the dairy calf from its mother is a contentious practice on dairy farms with potential to damage the public image of the industry. Two review articles on the topic found only reduced acute distress as a benefit of early separation. In the area of calf and cow health and calf behavior studies found mostly benefits or no effect for longer cow-calf contact.

Despite immense knowledge and scientific advances, calf losses are still a major problem in dairy farming worldwide. A representative study of over 750 German dairy farms found that on average one in ten calves on the farms does not reach the fourth month of life. This figure does not include the losses of male calves on the farms to which they are given for calf or bull fattening. The main causes of death in calves that died after being born alive were diarrhea and respiratory diseases. On average, a quarter of the calves were treated for diarrhea and about 15% for respiratory diseases (PraeRi, 2020; https://ibei.tiho-hannover.de/praeri/pages/69#_AB). Both problems are classic multifactorial diseases, i.e. in addition to pathogens, unfavorable environmental or management factors contribute to the outbreak of the disease. These risk factors can be roughly differentiated according to whether they weaken the calves' immunity or increase the infectious pressure. Calf nutrition in the first weeks of life is generally recognized as the most important criterion by which calves can be kept healthy due to a good immune status. The underlying scientific findings are presented and discussed in this review.

Components of colostrum

Traditionally, the importance of colostrum supply to calves is primarily based on the absorption of immunoglobulins (Ig) from colostrum into the blood, since, unlike in other species, the bovine placenta epitheliochorialis does not allow intrauterine passive transfer of immunoglobulins to the fetus. In recent decades, however, the importance of other non-nutritional ingredients of colostrum has become the focus of scientific interest (Lorenz, 2021).

Bovine colostrum contains high concentrations of viable maternal leukocytes similar to those in peripheral blood, but with relatively more macrophages/monocytes and fewer lymphocytes. The uptake of colostrum leukocytes through the intestinal barrier is possible, with the preferred uptake route being via the follicle-associated epithelium of Peyer's patches. Viable maternal leukocytes are destroyed by freezing and significantly reduced by heat treatment. Studies comparing the effect of cell-free colostrum with whole colostrum in calves found significant differences in the number and composition of blood monocytes and an altered cellular response to vaccination in calves. However, the influence of maternal leukocytes on common calf diseases has so far been rarely investigated. However, given the current state of knowledge, the routine feeding of previously frozen colostrum to newborn calves should not be encouraged.

In addition, colostrum contains bioactive proteins in greater quantities than mature milk that notably influence metabolic processes. In particular, colostrum contains a variety of growth factors, including insulin-like growth factors (IGFs) and insulin in high concentrations. These substances exert their effects mainly locally, since receptors for IGF-I, IGF-II and insulin are found throughout the intestine of newborn calves, but absorption is negligible. While IGF alone does not affect the development of the intestinal epithelium, an extract from first-milk bovine colostrum increases the circumference and height of the villi in the small intestine as well as the proliferation rate of epithelial cells in the intestine. Feeding colostrum as opposed to a milk replacer with comparable nutrient content stimulates mucosal growth and increases the absorption capacity in the small intestine. Increased nutrient and glucose uptake thus enables increased glucose supply and hepatic glycogen storage, which improves glucose status. The improved energetic status of colostrum-fed newborns is reflected in accelerated maturation of the somatotropic axis, which in particular leads to increased production of IGF-I in the newborn.

Another group of substances found in high concentrations in bovine colostrum are oligosaccharides, the effects of which are not yet fully understood. However, they most likely prevent the adhesion of pathogens to the intestinal epithelium, can improve the uptake of IgG, and especially serve as a carbon source for beneficial bacteria to promote their growth and establishment in the intestine.

Colostrum also contains large amounts of microRNAs. MicroRNAs are short, non-coding RNA molecules that can regulate gene expression at the post-transcriptional level and are thought to provide a method of signaling from the mother to the newborn. They can reach the intestine and be absorbed into the bloodstream because they are protected from digestion in extracellular vesicles. MicroRNAs may play an important role in the development of the intestinal epithelium as well as in the maturation of the calf's immune system.

Factors of successful colostrum delivery

Nevertheless, the passive transfer of immunoglobulins from the mother to the calf via colostrum naturally plays an important role in providing immediate systemic immunity after the calf is born. Colostrum quality is defined by Ig concentration and remains a critical factor in successful passive transfer. Factors affecting colostrum quality and yield have been recently thoroughly reviewed (Westhoff et al., 2024). In brief, there is high individual and herd-level variability in colostrum yield and quality which, however, is only partly understood so far.

Passive transfer of immunity category	Serum IgG category (g/L)	Equivalent serum total protein (g/L)	Equivalent serum Brix %	Recommended percentage of calves on farm in category
Excellent	≥ 25.0	≥ 62	≥ 9.4	> 40
Good	18.0 – 24.9	58 - 61	8.9 – 9.3	~ 30
Fair	10.0 – 17.9	51 – 57	8.1 – 8.8	~ 20
Poor	< 10.0	< 51	< 8.1	< 10

Table 1: Consensus serum IgG concentrations and equivalent total protein (TP) and Brix measurements, and percentage of calves recommended in each transfer of passive immunity category (Godden et al., 2019)

Especially regarding the influence of prepartum nutrition additional research is needed. Important factors that the farmer can influence are heat stress, which should be avoided, and timing of colostrum harvest. IgG concentration drops by about 20 % if colostrum harvest is delayed by six hours. Bacterial contamination of colostrum should be below industry standards (total plate count <100,000 cfu/mL and total coliform count <10,000 cfu/mL).

Because direct laboratory methods for determining Ig in colostrum are laborious and expensive, the use of cow-side testing of colostrum quality has been extensively investigated. Evaluation of studies using a Brix refractometer showed that a cut-off value of 22% Brix can be used to identify high quality colostrum (IgG ≥ 50 g/L), but with a Brix value < 18% the probability of good quality is very low. Therefore, it is suggested that the two cut-off values be used to select good quality colostrum (sample with Brix ≥22%) or to reject poor quality colostrum (sample with Brix <18%). If sample results are between these two values, colostrum supplementation should be considered. To achieve an adequate supply, calves should ingest 3 to 4 liters of first colostrum as early as possible after birth. Prolonged feeding of colostrum or a mixture of colostrum and milk for three days not only increases serum Ig concentrations but also promotes small intestinal growth and maturation (Lorenz, 2021).

For many decades, an individual calf threshold of 10 g/L serum Ig has been used to define failure of passive transfer of immunity. A serum protein concentration of 52 g/L corresponded to 10 g/L serum IgG and was proposed as a test threshold for healthy calves up to 8 days of age. Recent studies have questioned this simplified approach of monitoring passive immunity status. For example, a reduced risk of respiratory disease was found in calves with serum total protein levels ≥ 57 g/L, and serum total protein levels between 58 and 63 g/L were considered optimal to indicate sufficient passive transfer of immunity in dairy calves. In addition, the risk of calf morbidity and mortality was shown to decrease significantly with increased serum Ig concentrations. In light of these findings, a panel of calf specialists revised the passive immunity transfer standards for the U.S. dairy industry in accordance with the latest scientific evidence. The proposed standard includes 4 serum IgG categories: excellent, good, fair, and poor as can be seen in Table 1 (Godden et al. 2019).

Early separation from the dam

Under natural conditions, cows separate from the herd before giving birth and subsequently keep their calf away from the herd for some days. After that, calves join the herd to form groups with their peers engaging in social relationships.

On the vast majority of commercial dairy farms, however, calves are separated from their dams within hours or at least within very few days after they are born. However, public interest in the welfare of farm animals is rising, increasing the potential for contentious practices to damage the public image of the industry. In general citizens without involvement in the dairy industry are mostly unaware of early separation of dairy calves from their mothers, but if they are informed about it they object to it (Lorenz, 2021).

In light of these findings the available scientific literature on the effect of early cow-calf separation on cow and calf health was systematically reviewed (Beaver et al., 2019). The authors concluded that the evidence extracted from the included journal articles does not support a recommendation of early dairy cow-calf separation on the basis of calf or cow health. The articles addressing calf diarrhea pointed to beneficial or no effects of suckling. Mixed results were found for cryptosporidiosis, other common pathogenic causes for diarrhoea have not been evaluated in detail. Furthermore, no consistent evidence of increased risk of pneumonia in calves reared with the cow was found. For measures of calf immunity and mortality, no consistent pattern was observed, with studies split between those showing benefits versus risks associated with suckling. However, in studies detailing the risks of leaving the calf with the dam, colostrum intake was not systematically monitored. The articles addressing calf scours pointed to beneficial or no effects of suckling. With respect to cow health, the review indicates that suckling is protective against mastitis and that cow-calf contact is not a significant risk factor for Johne's disease. Early separation (within 24 h postpartum) was found to reduce acute distress responses of cows and calves in a review on the effects of prolonged cow-calf contact (Meagher et al, 2019). However, longer cow–calf contact typically had positive longer-term effects on calves, promoting more normal social behavior, reducing abnormal behavior, and sometimes reducing responses to stressors. Calf weight gains were increased during the milk-feeding period, when cows were allowed to nurse their calves, without consistent evidence of reduced milk production over a longer period.

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Field evaluation of immune transfer in suckler calves using a brix scale refractometer

Jérôme Chantreau
SNGTV, Saint Désiré, France
chantreauj@gmail.com

Objectives

Passive immunity is essential for the health of calves, which depend on maternal antibodies, particularly immunoglobulin G (IgG), acquired from colostrum to protect against early disease. Over the last 15 years, it has been proposed to use the Brix refractometer to measure IgG indirectly in serum. The scientific literature provides relatively variable measurement thresholds, making it difficult to assess the failure of passive immune transfer (FPT) under field conditions. Establishing effective Brix thresholds can help veterinarians and farmers to more accurately assess passive immunity status and take steps to reduce morbidity and improve calf survival. The aim of this study was to determine a practical threshold for diagnosing FPT in calves using a Brix refractometer.

Materials and Methods

This field study included 193 calves from 23 French cow-calf farms, with a final analysis of 141 calves after exclusions for methodological consistency. Blood samples were taken from calves aged 2-6 days in good apparent health. Collected serum samples were sent to a laboratory for IgG concentration assessment using radial immunodiffusion. Percentage Brix values were then obtained using electronic and optical refractometers on another decanted and/or centrifuged serum sample. However, only data from the electronic refractometer on decanted samples were included in the statistical analysis to control for variability associated with centrifugation. Firstly, ROC curve analysis was used to determine the Brix threshold for FPT detection across various IgG concentration target levels, specifically 10, 16, 18, and 25 g/L, range associated with different degrees of passive immunity transfer. Then, two generalized Youden indices (YG), a measure that takes into account both the prevalence and relative consequences of false positives and negatives, were applied to identify the most clinically relevant threshold. Sensitivity, specificity, predictive values and likelihood ratios were calculated for Brix thresholds across each IgG concentration target level.

Results

A strong correlation was observed between Brix values and IgG concentrations in serum ($r = 0.76$, $p < 0.01$), confirming the Brix refractometer's utility as an indirect tool for assessing IgG levels and diagnosing FPT. For each target level, the thresholds calculated, respectively, from ROC curve analysis, YG with relative consequences of 2, and YG with relative consequences of 1, are as follows: for a target of 10 g/L, the thresholds are 7.9%, 7.4%, and 7.2%; for a target of 16 g/L, thresholds of 8.2%, 8.2%, and 7.7%; for a target of 18 g/L, thresholds of 8.3%, 8.3%, and 7.9%; and for a target of 25 g/L, thresholds of 8.6%, 9.5%, and 9.5%. These results highlight the variability of the threshold for each target value, depending on the calculation method chosen and whether the lowest proportion or the lowest number of misclassified sera is to be preferred. For example, for an IgG concentration threshold of 18 g/L, we can choose the Brix threshold of 8.3% identified from ROC curve analysis (sensitivity 71.2%, specificity 79.3%). This threshold suggests that calves with Brix values below 8.3% are likely to experience FPT. Depending on the immunity transfer objectives and the corresponding Brix threshold chosen, the test can be an excellent or poor predictor: likelihood ratios range from 59.7 to 1.89 for the positive ratio and from 0.06 to 0.55 for the negative ratio. In addition, some variability was observed from year to year, indicating that factors such as environmental conditions, maternal diet and sampling protocols may influence IgG concentrations, or indirect measurements, and have a potential impact on the threshold reliability.

Conclusions

The Brix refractometer serves as a valuable tool for FPT detection in calves under field conditions. However, the reliability of Brix thresholds may be influenced by sample processing protocols and temporal variability in IgG concentrations, underscoring the importance of cautious interpretation. Practitioners should apply Brix thresholds with awareness of these limitations, recognizing that threshold values may need adjustment based on herd characteristics, environmental factors, and annual variations in IgG readings. Finally, at the individual level, in our study, a serum at 7.9% or less may indicate FPT, while one at 8.7% or more is probably good; in between, uncertainty persists. At herd level, FTP can be considered if more than a quarter of calves have a brix lower than 8.4%. Further studies are recommended to refine Brix thresholds and validate their application across diverse farming conditions.

Cost assessment of cryptosporidiosis in dairy cattle

Bas Burger[1], Geert Vertenten[2], Henk Hogeveen[1]

[1] Wageningen University & Research, Department of Business Economics, The Netherlands
[2] MSD Animal Health, Boxmeer, The Netherlands

bas.burger@wur.nl

Objectives:

Cryptosporidium is a widespread zoonotic pathogen. *Cryptosporidium* can infect mammals, birds, reptiles, amphibians and fish. In cattle, cryptosporidiosis is considered a global pandemic. cryptosporidiosis is one of the most important causes of gastroenteritis in newborn calves. When newborn calves are affected, the disease does not only have immediate cost related consequences such as treatment and extra labor, but there are also long-term consequences of the disease such as growth retardation and eventually, reduction of milk yield in the first lactation. There are currently no studies that estimate the economic impact of cryptosporidiosis while taking into account the long-term effects. Therefore, the aim of this research was to estimate the total costs associated with a *Cryptosporidium* outbreak.

Material and methods:

First, an overview of the factors regarding cryptosporidiosis that can incur costs was made. These factors were either included in a partial budget or in a stochastic young stock rearing simulation model. The stochastic model was developed to gain insight in the costs of rearing heifers and was adapted to fit the situation at hand. The parameters of the model that were specifically related to effects of cryptosporidiosis were parameterized through literature or expert knowledge for a typical Dutch dairy farm. One partial budget was developed to estimate the net costs of mortality due to *Cryptosporidium* in the first two weeks after calving. A second partial budget was developed to estimate the costs that are associated with reduced performance in the first lactation after an early life cryptosporidium infection.

Results:

The average costs of rearing a heifer were €2508 in a situation with an incidence of cryptosporidiosis of 32.5%. In a situation with 0% incidence of cryptosporidiosis these costs were €2367. Hence, cryptosporidiosis leads to, on average, €141 higher rearing costs per heifer. In addition, the net costs of mortality due to cryptosporidiosis in young calves was estimated to be €7 per successfully raised heifer. The net costs of reduced milk production in the first lactation were estimated to be €47 per heifer.

Conclusion:

Under Dutch farming circumstances, the economic impact of cryptosporidiosis in young calves added up to €195 (€7 +€141+€47) per successfully raised heifer including the first lactation. Although this is the best possible estimation, given our current knowledge, due to uncertainty of the occurrence and effect of certain effects of cryptosporidiosis, these results should be interpreted with caution. Nevertheless, it is clear that cryptosporidiosis is costly which may have implications for management practices and prevention.

Managing neonatal calf diarrhoea besides antibiotics: a European perspective

Cassandra Eibl 1, Alexandra Hund 2, Lorenzo Viora 3, Hugues Guyot 4, Johanna Wilms 5, José Felix 3, Alexander Tichy 6, Ricardo Bexiga 7

[1]Clinical Centre for Ruminant and Camelid Medicine, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, Veterinaerplatz 1, Vienna, 1210, Austria

[2]Agricultural Center for Cattle, Grassland, Dairy, Game and Fisheries of Baden-Württemberg (LAZBW), 88326 Aulendorf, Germany

[3]School of Veterinary Medicine, University of Glasgow, Glasgow, UK

[4]Clinic for Ruminants, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

[5]Tierarztpraxis Geisenhausen, 84144 Geisenhausen, Germany

[6]Platform Bioinformatics and Biostatistics, Department for Biomedical Sciences, University of Veterinary Medicine, 1210 Vienna, Austria

[7]Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Av. da Universidade Técnica, Lisbon, Portugal

Corresponding author: Cassandra.Eibl@vetmeduni.ac.at

Objectives: Neonatal calf diarrhea (NCD) is a common disease in many dairy and beef herds worldwide with huge economic impact and is associated with poor animal welfare. Irrespective of the cause and severity, animals dealing with NCD can suffer from a wide range of alterations leading to intestinal epithelial inflammation, hypovolemia, metabolic acidosis, sepsis, and associated altered demeanor. Although NCD has been widely studied, little is known in Europe about individual practices adopted in the field concerning the ancillary treatment of NCD including treatment protocols, veterinary and farmer attitudes and decision-making. The aim of this study was to investigate on-farm management practices of farmers and veterinarians in different European countries regarding the ancillary treatment of NCD and the factors influencing them.

Material and methods: An online-based questionnaire was distributed to farmers and veterinarians in Portugal, Belgium, Scotland, and Austria from February 2016 to January 2019. The complete questionnaire included 35 questions, 14 of which were multiple-choice and open-ended questions specifically addressing the management and treatment options for NCD. These included practices such as discontinuing milk feeding, using non-steroidal anti-inflammatory drugs (NSAIDs), parasympatholytics, intravenous fluids, oral fluids, vitamins, and probiotics. Data on antimicrobial usage has already been published and is not included in this abstract. The data were analyzed using logistic regression analysis and regression tree (CART) analyses.

Results: A total of 874 questionnaires (Austria: 546, Belgium 94, Portugal: 163, Scotland: 71) were finally analyzed. All 14 questions were answered by 597 farmers and 277 veterinarians, whereas 221 participants were female and 650 male. About one third of all participants routinely use oral fluids (59%, n=517), intravenous fluids (35%, n=306) and non-steroidal anti-inflammatory (NSAID) (30%, n=287) for NCD. In terms of intravenous fluids, 212 participants used it in recumbent calves, 208 in calves with sunken eyes and 172 in calves with a weak or absent suckling reflex Regarding the use of NSAID, 162 (18.5%) participants indicated that they use them in 50-100% of the NCD cases, especially when calves are unable to stand or have a weak or absent suckling reflex. One third of the participants used flunixin meglumine or meloxicam. Half of the participants (n=474) find dry bedding important for the sick calves. A total of 206 (23%) participants reduced the quantity of milk and 61 participants stopped milk feeding for 24 h in calves showing signs of NCD.

Discussion: Many practices used in the field are not in agreement with best practices. For example, many participants stated that they do not regularly use oral fluids as a treatment in calves suffering NCD, although it is reported as “gold standard” in all calves with at least a weak suckling reflex. The use of non-COX-selective NSAIDs in dehydrated calves can impact the risk of nephrotoxicity. Regarding bedding, many studies have reported the importance of dry bedding in a sufficient quantity to ensure calf health and recovery, yet only half of the participants stated a dry bedding as an important practice in NCD.

Conclusion: These findings show that there are discrepancies between on-farm practices and scientific best practices and there is a need for specific training or guidelines. The introduction of algorithms or decision trees, which have already been implemented for the use of antibiotics in diarrhetic calves, for the selection of ancillary treatment in calves suffering NCD may act as an aid for farmers and veterinarians and may reduce animal welfare issues in diarrhetic calves.

Evaluation of existing and novel sepsis scores in sick calves

Mathilde L. Pas[1], Jade Bokma[1,2], Filip Boyen[1], Laurens Chantillon[1], Stan Jourquin[1], Donatienne Castelain[1], Justine Clinquart[1] and Bart Pardon[1]

[1] Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[2] Veterinary Practice Venhei, Kasterlee, Belgium.

Objectives: Early recognition and timely administration of appropriate antimicrobial treatment are critical for the survival of calves with sepsis. Currently, it remains unclear which sepsis scoring system is most effective for early diagnosis in calves. Therefore, the objective of this study was to evaluate two existing sepsis scores (Fecteau (1998) and Trefz score (2017)), three novel calf sepsis screening scores (CSS, CSSA and CSSB) and blood culture as diagnostic test for sepsis, using a Bayesian latent class approach.

Material and Methods: A retrospective diagnostic test study was conducted to asses sepsis scores in comparison to blood culture for diagnosing sepsis in calves, using Bayesian evaluation. The study included 131 sick calves, that were all ≤ 3 months old, sampled for blood culture and underwent clinical and laboratory examinations.
The Fecteau score included the presence of a focal infection (hypopyon, septic arthritis, soft tissue abscess or mucopurulent nasal discharge), age, hydration status, scleral vessel injections, mental state, umbilicus, and fecal consistency, which were scored according to the model proposed by Fecteau et al. 1997 [1]. The Trefz score, considered calves to have sepsis when ≥2 signs of systemic inflammatory response syndrome (SIRS) were present (i.e. abnormal leucocyte count, abnormal rectal temperature, tachycardia and tachypnea), in combination with hyperemia of mucous mucosae, mucosal or subscleral bleedings or the presence of hypopyon [2]. The calves sepsis screening (CSS) is a currently proposed new scoring system, and consists of the combination of the previously mentioned SIRS-criteria and the presence of an abnormal mental state. Two alternative models for CSS in practice were proposed and tested as well. Calf sepsis screening A (CSSA), in which the leukocyte count was eliminated, thus only including three SIRS criteria, of which at least 2/3 SIRS criteria and abnormal mental state had to be present for the calves to be sepsis-suspected. In calf sepsis screening B (CSSB) the leukocyte count was substituted for abnormal mucosal color.

Results: Sepsis prevalence was 45%, 27%, 56%, 47%, 55%, when using the Trefz score, Fecteau score, CSS, CSSA, CSSB, respectively, while 22% had a relevant positive blood culture. New established models CSS, CSSB and CSSA appeared to have the highest sensitivity of 86%, 84%, 80% respectively, and could be interesting screening tests for sepsis. Sensitivity of the existing Trefz score and Fecteau score was 70% and 39%, respectively. Specificity was highest for blood culture (83%), followed by the Fecteau score (77%) and CSSA (67%). Specificity was similar for the Trefz score (63%), CSS (61%) and CSSB (60%).

Conclusions: The newly developed sepsis scoring systems show promise as screening tools to identify calves at risk of sepsis, after which a calf-side diagnostic test is recommended to confirm the diagnosis. Its use might aid in the rationalization of antimicrobial use in sepsis-suspected calves.

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Systemic pasteurellosis in calves: Investigating *Mannheimia haemolytica* serotype A2 as a potential contributor to fatal systemic infections

J. Reinmold[1,a], C. Helmer[1,b]
[1] SanGroup Biotech Germany GmbH, Mühlenstraße 13, 49685 Emstek, Germany
[a] jennifer.reinmold@san-group.com, [b] carina.helmer@san-group.com

Objectives

Systemic pasteurellosis is a major cause of morbidity and mortality in livestock, particularly in young calves. While *Mannheimia haemolytica (M. haemolytica)* serotypes A1 and A6 has long been identified as a key pathogen in systemic pasteurellosis, recent cases have raised concerns about the role of previously considered less virulent serotypes in severe clinical courses.

Material and methods

This study presents a series of 4 cases involving calves euthanized due to severe respiratory and other clinical symptoms, submitted to veterinary laboratory [SAN Group Biotech Germany GmbH] for investigation. Post-mortem examinations were performed and samples were systematically collected from the pleura, pericardium, lungs, and, in one case, the central nervous system (CNS). These samples were subjected to microbiological analysis. Additionally, Polymerase chain reaction (PCR) serotyping and Next-Generation Sequencing were performed to further characterize the isolates found.

Results

Post-mortem examinations revealed characteristic adhesions and fibrinous deposits in the thoracic and abdominal cavities, indicative of systemic pasteurellosis. One case also showed significant opacity of the meninges, indicating CNS involvement. Microbiological analysis exclusively identified M. haemolytica as the causative agent, with no evidence of other pathogens. Notably, M. haemolytica was successfully isolated from the CNS in one case.
PCR serotyping initially classified the isolates as serotypes A2 or A5. Subsequent next-generation sequencing confirmed that all isolates were of serotype A2.

Conclusions

M. haemolytica serotype A2, typically considered less virulent and often classified as a commensal in cattle, was consistently associated with severe systemic infections, including those with CNS involvement. This serotype is not included in commercial cattle vaccines and has been rarely explored in relation to systemic infections. Interestingly, in small ruminants the serotype A2 is recognized as a pathogenic serotype, for which commercial vaccines are available. Our findings underscore the importance of pathogen typing to assess the clinical significance of different serotypes. The detection of *M. haemolytica* serotype A2 in severe systemic pasteurellosis cases, particularly those with neurological symptoms, suggests that this serotype may be more virulent in cattle than previously recognized. These results align with recent studies and call for further investigation, along with potential reassessment of current vaccination strategies.

Regenerative medicine for dairy calves' health management: a clinical trial assessing the efficacy of Leukocyte-Platelet Rich Fibrin for tissue regeneration after disbudding.

Maria Chiara Alterisio[a], Sergio Esposito[a], Giovanni Della Valle[a], Federica Aragosa[a], Chiara Caterino[a], Gianmarco Ferrara[a], Davide De Biase[a], Gerardo Fatone[a], Paolo Ciaramella[a], Jacopo Guccione[a1]*.

[a]*Department of Veterinary Medicine and Animal Production – University of Study of Napoli Federico II – Via Delpino 1, 80137, Napoli, Italy. 1 Presenting author, *Contact author: jacopo.guccione@unina.it*

Objectives.

Disbudding is a common procedure practiced in the dairy industry and is known to cause pain and discomfort with long healing times in treated calves. Considering all welfare implications related to the hot-iron disbudding, speeding up the healing process becomes a priority; therefore, the use of the Leukocite-Platelets Rich Fibrin (L-PRF) could meet this need. The L-PRF is a platelet-based autologous, hemostatic, biological scaffold used in wound healing in other species, including humans, whose extraction protocol has been recently validated in dairy. Based on the previous statement, this randomized controlled clinical trial aimed to assess the efficacy of L-PRF on the healing of wounds due to disbudding in dairy calves.

Materials and methods.

Seventeen Holstein-Friesian calves were randomly enrolled and underwent a hot-iron disbudding procedure within three weeks of life. A whole blood sample of 20 ml was taken by jugular venipuncture from each calf and employed for L-PRF. Briefly, each sample was centrifuged for different time intervals (total time of 30 minutes), and the fibrin clot was extracted and pressed by a dedicated compression box for 15 minutes. During the extraction procedure, the calves even received the bilateral cornual nerves block procaine-based (5 ml/side of Procamidor duo® 20 mg/ml, IZO S.r.l., Brescia, IT), followed by the disbudding 15 minutes later. At the end of the extraction protocol, each calf received an L-PRF membrane on the right wound (treated side_TS) while the left one was left untreated (control side_CS). The healing process was weekly monitored comparing the results observed between the two groups, up to the complete re-epithelialization of the wounds, both through a direct clinical evaluation and through a digital analysis of the healing area.

Results.

Overall, thirty-four sites of horn-producing cells were disbudded. The clinical procedure of extraction and application of the L-PRF membrane has proved to be well tolerated by the animals and easy to perform despite the fixed times required by the method. On average, the TS healed within the 6-We ± 1 (±standard deviation), while the CS within the 8-We ± 1 ($P<0.05$). The wound on the TS that healed faster required 5-We, while the one that took longer 9-We. Instead for the CS, the time interval for the same parameter ranged from 6-We to 10-We. The TS showed the following healing times: 2/17 wounds at 5°-We, 11/15 at 6°-We, 1/4 at 7°-We, and 3/3 at 9°-We. Instead on the opposite side, 5/17 wounds healed at 6°-We, 1/12 at 7°-We, 7/11 at 8°-We, 2/4 at 9°-We, and 2/2 at 10°-We. A statistically significant difference was found for the comparison between the two groups at 6°-We ($\chi^2= 6.149$, $P<0.05$). The digital analysis revealed that wound on the TS, which healed faster, required 5 weeks and showed a percentage decrease in wound area of 80%, while the wound that took longer to heal required 9 weeks with a percentage decrease in wound area of 80%. In contrast, for the CS, the time interval for the same parameter ranged from 6 weeks to 10 weeks with a percentage decrease of 80%.

Discussion and Conclusions.

Preliminary data shows the overall potential beneficial effects of leukocyte-platelet rich fibrin on the regeneration of wounds due to hot-iron disbudding in dairy calves. Its use might be hypothesized as support for a complete clinical calf management program to reduce disinfectants and antibiotics while maintaining a high level of animal welfare. Nevertheless, further studies are necessary to confirm the encouraging outcomes observed.

Determining the nutritional status of Belgian blue calves hospitalized using urea, albumin and cholesterol: toward a "Calf-CONUT" ratio

Justine Eppe[1], Corine van Leeuw[1], Elena Borelli[1], Hélène Casalta[1],
Salem Djebala[1], Anne-Sophie Rao[2], Léonard Théron[2], and Calixte Bayrou[1]

[1] University of Liège, Liege, Belgium
[2] RumeXpert, Faimes, Belgium

Introduction: Malnutrition or undernourishment is a significant factor contributing to the failure of disease management. This observation led to the development of the CONUT (COntrolling NUTritional status) score in humans [1], which is utilized to monitor patients' nutritional status. The score is based on the concentrations of albumin, cholesterol, and lymphocytes in the blood, demonstrating a sensitivity (Se) of 92.30% and a specificity (Sp) of 85% in predicting fatal outcomes.

In the context of rural medicine, there have been few studies focusing on the nutritional status of calves. The most commonly used prognostic parameters include blood lactate levels, blood pressure, and the shock index. Other studies have indicated significant differences in parameters such as urea, albumin, and hypoproteinemia concerning survival rates. However, none of these studies have approached these parameters from a nutritional perspective.

Objective: The aim of this study was to compare serum urea, cholesterol, and albumin levels in Belgian Blue calves at the time of hospital admission and at the time of discharge or death. Based on the observations made, a ratio will be proposed to interpret the prognosis of the animals.

Material and methods: The calves included in this study had to be of the Belgian Blue breed, aged between 0 and 3 months, and admitted to the clinic for any disease requiring hospitalization, excluding urinary cases (to avoid hyperuremia due to renal failure). Cases of congenital joint stiffness and fractures were also excluded from the study.

Upon arrival at the clinic, a complete physical examination was conducted on the calves. A blood sample (dry tube, Vacutainer® with a 22G needle) was taken at the time of admission. Serum concentrations of urea (immunochemistry, Alinity C, Abbott®), cholesterol (immunochemistry, Alinity C, Abbott®), and albumin (immunochemistry, Alinity C, Abbott®) were systematically measured during the initial examination. When possible, a second blood sample was taken just before discharge or euthanasia to measure the same parameters. The calves were classified based on the outcome of their hospitalization as either "survivors" (group S) or "non-survivors" (group NS).

Results: The two groups were established: S ("survivors"; n=47) and NS ("non-survivors"; n=35). At admission, there were no significant differences in serum cholesterol concentrations between groups S and NS ($p > 0.1$). However, serum albumin concentration was significantly higher in group S compared to group NS ($p = 0.02$). Conversely, serum urea concentration was significantly lower in group S than in group NS ($p = 0.04$). When comparing values at admission and discharge, group S exhibited a significant increase in cholesterol ($p < 0.05$) and a significant decrease in both urea ($p < 0.01$) and albumin ($p < 0.05$).

A urea/albumin ratio, referred to as the Calf-CONUT ratio, to compare results between groups S and NS, was established. An animal with a ratio exceeding 2.69 is associated with a poor prognosis (Se 71%; Sp 76%). Furthermore, when comparing calves in the highest quartile of the Calf-CONUT ratio (indicative of undernutrition) with those in the lowest quartile (indicative of optimal nutrition), the risk of mortality is three times higher for the former group. Furthermore, when focusing on calves with enteritis (n=30), it appears that the threshold can be adjusted to 3.09 for this specific group, accompanied by a significantly improved ROC curve (sensitivity 83%; specificity 78%). In this cohort, the comparison between the highest and lowest quartiles of the Calf-CONUT ratio reveals a similar range of mortality risk as observed in the overall population, with a relative risk (RR) of 4 and an odds ratio (OR) of 9.1.

Conclusions: This study offers initial insights into the nutritional assessment of hospitalized calves, paving the way for innovative approaches to the nutritional and medical management of these animals. Given the unique blood characteristics of Belgian Blue cattle, it is essential to validate these thresholds across other breeds before implementation. Additionally, further studies focusing on specific diseases should be conducted to refine these thresholds.

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Dutch BVDV control program – Evaluation 2018-2023

Strous E.[1], Bisschop I.[1], van Schaik G.[1,2], Mars J.[1], Waldeck F.[1], Scherpenzeel C.[1], de Roo B.[1], Wever P.[1], and Santman-Berends I.[1]

[1]Royal GD, Deventer, The Netherlands
[2]Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

PO Box 9, 7400 AA Deventer, The Netherlands

Royal GD, Deventer, The Netherlands

e.strous@gdanimalhealth.com

Objectives

Bovine viral diarrhea virus (BVDV) is a globally prevalent pathogen affecting cattle. Since 2018, Dutch dairy farmers have been obliged to opt for one of four routes to achieve BVDV freedom in the national BVDV eradication program. These routes include: (1) virus testing the entire herd and ear notch testing newborn calves for ten months, followed by biannual spot testing of youngstock for surveillance; (2) bi-annual spot testing of five youngstock; (3) quarterly bulk milk testing for BVDV antibodies; (4) ear notch testing for both obtaining BVDV freedom and surveillance. The objective of this observational study was to evaluate the efficacy of the overall BVDV program in the Netherlands from 2018 to 2023.

Material and Methods

The efficacy of the BVDV program was evaluated by monitoring a set of indicators over time. These included the number of persistent infected cattle (PI's), percentage of dairy herds with a BVDV-free status, percentage of BVDV-free dairy herds with evidence of introduction of BVDV. Moreover a cost calculation per route was performed.

Results

The Dutch BVDV program appeared to be successful as the percentage of BVDV-free dairy herds increased from 59 percent at the start of the program to 89 percent by the end of 2023. The number of PI's detected each quarter, declined from 500 PI's in the third quarter of 2019 to 83 PI's in the last quarter of 2023. The percentage BVDV-free dairy herds with evidence of (re)introduction of BVDV decreased from 1.29 percent per quarter in the first year of the mandatory program to 0.25 percent per quarter by the end of 2023. The total cumulative costs over the first four years were the highest in Route "ear notch testing" and the lowest in the Route "bulk milk".

Conclusions

In Europe, BVDV control program designs are often tailored to the country's specific situation e.g. prevalence at the start of eradication, risk profile of a country or herd, desired speed of eradication and available funds. These results show the efficacy, advantages and disadvantages of multiple routes towards BVDV freedom.

Monitoring IDV in northern Italy: Virological and serological surveillance in dairy farms (Emilia-Romagna and lombardy regions, 2018-2024)

Prosperi A., Soliani L., Moreno A., Zanni I., Faccini S., Rosignoli C., Torreggiani C., Pupillo G., Mescoli A, Garbarino C. A., Alborali G. L., Luppi A., Chiapponi C.

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy
alice.prosperi@izsler.it

Objectives

Identified for the first time in 2011, Influenza D virus (IDV) has been detected in a broad range of animal species, with cattle regarded as the major viral reservoir. The virus replicates and transmits efficiently in ferrets, which are used as animal model for Influenza A virus transmission to humans.

The IDV genus was initially divided into 3 main genetic and antigenic clusters based on the Hemagglutinin-esterase-HEF gene phylogeny: D/OK (worldwide distributed), D/660 (circulating in the USA), and D/Japan (in Japan). However, this nomenclature no longer adequately reflects the growing viral diversity.

Since 2014, both D/OK and D/660 has been reported to co-circulate in the USA, with reassortment events observed between the two viral lineages.

This study aimed to investigate IDV virological and serological prevalence in Northern Italy dairy farms; additionally, the viral genetic and antigenic diversity was investigated.

Materials and Methods

The samples analysed were collected between January 2018 and November 2024, during Bovine Respiratory Disease Complex-BRDC outbreaks in Emilia-Romagna and Lombardy Regions (Northern Italy). For the virological investigations, 3466 samples (2272 nasal swabs, 1064 lung tissues and 130 bronchoalveolar fluids-BAL), were tested for IDV via a real-time RT-PCR previously described (Faccini el al. 2017, Henritzi et al. 2019). Viral isolation was attempted on real-time RT-PCR positive samples. Viral isolates or PCR positive clinical samples were NGS sequenced using Illumina technology and phylogenetic analyses were performed.

Seroprevalence for IDV antibodies was assessed on 525 serum samples using the haemagglutination inhibition-HI test. Considering the cross-reaction within different lineages, serum samples were HI-screened using a D/OK strain.

Results

Eleven lung tissues, 12 BAL and 172 nasal swabs were IDV positive (total 195 samples), and full genome sequencing was performed on 122 samples. Considering the HEF gene, 76 of them were identified within the previously reported European lineage (D/OK) and 46 strains clustered with the American D/660 lineage, which was firstly reported in Europe in 2018 in Italy (Chiapponi et al. 2019).

Moreover, since 2019, reassortant viral strains have been detected, and after a period with a higher prevalence of D/660 strains (years 2019-2022), an increased circulation of D/OK was detected in 2023 but not confirmed during 2024, in which co-circulation of both strains was detected.

Of the 525 sera tested, 420 were positive with HI titres between 1:20 and 1:640.

Conclusions

BRDC is one of the most expensive diseases affecting cattle farming worldwide. It results from a complex interaction between viruses, bacteria, environmental, and host factors. The risk posed by IDV to animal and human health, as well as IDV's role in BRDC, remains unclear. However, Lion and colleagues (Lion et al., 2021) recently demonstrated that IDV facilitated and promoted lower respiratory tract colonization by Mycoplasma bovis during coinfections.

In March 2018, D/660 lineage was first reported in Europe in Italy, but D/660 strains are now circulating in France, Denmark, and the Netherlands. Our data suggest the rapid spread of D/660 strains, co-circulating with D/OK in the bovine population, with evidence of reassortant events, as already occurred in the USA. According to our surveillance, in Northern Italy most of the IDV strains in 2018 belonged to the D/OK lineage, meanwhile between 2019-2022 an increasing of D/660 strains was observed, but in 2023 D/OK strains were the most prevalent. Finally, the two lineages were co-circulating in 2024. In this six-years surveillance the co-circulation of both clades was observed, sometimes identified in a single farm, which promoted the emergence of reassortant strains.

The HI test results revealed a high seroprevalence (80% within the analysed sera), highlighting the significant circulation of IDV in cattle farms and confirming cattle as the primary viral reservoir.

These data update the IDV serological, genetic, and antigenic diversity surveillance in Europe.

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Efficacy of an oral solution of paromomycin for the treatment of newborn calves with cryptosporidiosis in European farms

Damien Achard[1], Béatrice Besche-Barbazanges[1], Anne Trotel[1], Gaëlle Pagny[1], Emmanuelle Carrié[1], Charlotte Billy[1], Dejan Cvejić[2]

[1] Ceva Santé Animale, Libourne, France
[2] Klifovet AG, Munich, Germany

Objectives

Cryptosporidiosis is a frequent parasitological infection of mammals including calves. The objective of this work is to provide insights from two large field studies conducted in Europe about the efficacy and safety of paromomycin used orally at a dosage of 150 mg/kg BW per day for 5 consecutive days against cryptosporidiosis (*C. parvum*) in naturally infected pre-ruminant calves.

Material and methods

Two independent multicenter European field studies involving 44 dairy and cow-calf farms were carried out following a parallel, controlled, randomized, blinded design. Farms were selected based on recent history of *C. parvum* infection in calves, absence of concomitant preventive treatments against cryptosporidiosis, absence of recent or current BVD infections, and no recent history of diarrhoea due to coronavirus, *E. coli* and rotavirus, or alternatively dedicated vaccination. To be enrolled, newborn calves were required to meet the following criteria: age between 3-14 days on first treatment, faecal score ≥ 2 (loose to watery), negative rapid test for *E. coli*, coronavirus, rotavirus, and positive rapid test for *C. parvum*. Any calf that presented with diarrhoea for > 24 hours or that has been previously exposed to antibiotics, parasiticides or probiotics was excluded. Animals were randomly allocated to two treatment groups. Calves in group 1 received 150 mg paromomycin sulfate/kg b.w. [Gabbrovet Multi®, Ceva], once daily, for 5 days by oral route while calves in group 2 were orally administered 100 μg halofuginone/kg b.w. [Halocur®, MSD], once daily, for 7 days. Several clinical and parasitological parameters were monitored at fixed times during the 21 days of the study: faecal score (0-3), general health observation (0-3), hydration score (0-3), and oocyst counts (number of oocysts per gram of dry faeces). Percentages of calves clinically cured on day 8 was considered a pivotal outcome to evaluate the efficacy of the interventions. Calves were considered cured if for a minimum of two consecutive observations all their clinical scores were equal to zero, to the exception of faecal score that could be equal to zero or 1. Other criteria such as time to clinical cure, number of days when calves were asymptomatic or considered in relapse from Day 0 to Day 8 and the parasitological cure at day 7 (oocyst count = 0) were also assessed. Safety (adverse events, serious adverse events) was evaluated at any time during the studies. The statistical unit was the calf.

Results

Five hundred twenty-five calves, with a mean age of 9 days and a mean body weight of 44 kg were enrolled. Sixty percent were from Holstein-Friesians breed, with remaining calves from various beef or crossbred breeds (Aubrac, Blonde, Limousin, Charolais, Fleckvieh). Sixty-nine percent of calves were female. 260 calves were allocated to treatment group 1, 265 to the treatment group 2. At inclusion, the treatment groups were found comparable for clinical parameters and oocysts count. 93.4% (242/260) of calves in group 1 and 80.6% (212/265) in group 2 were considered clinically cured by day 8. Clinical cure rate in group 1 was found superior to those observed in group 2 ($p<0.0001$). In addition, calves in group 1 were found to be cured faster in comparison to calves in group 2. Hazard ratio (group 1 *vs.* group 2) was 1.40, indicating a higher likelihood (+40%) of being cured in group 1 at any time (best model). Relapses from day 2 to day 8 were low in calves from group 1 and 2 (2.7 and 2.9% respectively). The parasitological cure was similar between the 2 groups, with a value of 77.4% (199/257) in group 1 and 78.8% (205/260) in group 2. The oocyst count decreased steadily after treatment in calves of group 1 from an initial mean log of 11.8 to a mean log of 2.9 on day 7. At least one adverse event was reported in 9.6 and 13.6% of calves in group 1 and 2 respectively. While none of these adverse events were considered possibly related to paromomycin, it was possibly related to halofuginone in two calves.

Conclusions

In these European field studies, daily oral treatment with 150 mg/kg of paromomycin [Gabbrovet Multi®] for 5 days was found safe and highly effective to cure sick dairy and beef calves with cryptosporidiosis and to control their oocyst burden. In addition, this treatment regimen was found superior to treatment based on halofuginone.

Assessment of Hydrolyzed Yeast as an alternative Protein Source in Milk Replacers: Effects on Calf Health, Growth and Carcass Quality

Emilie Arcier[a], Didier Bastien, Magdéléna Chanteperdrix[b], Erik Sulmont[a], Marine Gauthier[a], Clothilde Villot[a],

[a] Lallemand Animal Nutrition
[b] Institut de l'Elevage, Qualité des carcasses et des viandes, 8, route de Monvoisin, 35650 Le Rheu, France

Presenting author: Emilie Arcier. Email: earcier@lallemand.com

Corresponding author: Clothilde Villot. Email: cvillot@lallemand.com

Protein is a crucial nutrient in milk replacers (MR), and selecting appropriate protein sources is essential for optimizing animal health, performance, and production costs. Due to the rising cost of milk protein and the increasing human consumption of whey proteins (Thornsberry et al., 2016), research has shifted towards alternative protein sources in MR. This experiment aimed to assess the impact of an alternative protein source on calf growth, development, and health. A total of 44 Holstein male calves were enrolled in the study, divided into two groups. One group was fed a control MR consisting of 50% skimmed milk powder and 18% whey protein, while the other group received a modified MR with 3% of the skimmed milk powder replaced by a specific hydrolyzed yeast (Yelaprosecure; YelaP). This feeding regimen was maintained for the first 63 days. Calves were bucket-fed MR twice daily and had ad-libitum access to solid feed and water. Individual milk refusal and solid feed intake were monitored using automatic feeders throughout the experimental, growing, and finishing phases, which lasted a total of 162 days. Individual body weights, feed consumption, health records, and carcass characteristics were documented. Palatability of the MR was comparable between groups, as there were no significant differences in liquid intake during the experimental phase. Total solid intake was also similar across both groups throughout the entire growing period. The overall health status was consistent, with no significant differences in white blood cell counts, and a low overall morbidity rate of 5%. Average daily gain (ADG) was numerically higher for calves receiving YelaP after the experimental phase (949 vs. 914 g/day, p = 0.29), with a more pronounced difference by the end of the rearing period (1406 g/day for YelaP vs. 1335 g/day for Control, p = 0.16). At slaughter, the YelaP group exhibited higher carcass weights (+5 kg; 151 kg for YelaP vs. 145 kg for Control, p = 0.84) without compromising meat color and displaying more consistent conformation scores, indicating improved overall meat quality .

Incorporating Yelaprosecure in milk replacer at the beginning of the rearing phase for young calves can effectively substitute a high-quality milk protein source, achieving similar performance outcomes without compromising animal health and carcass quality.

Relationship between herd seroprevalence of *Mycoplasma bovis* in bulk tank milk and clinical symptoms in dairy calves.

Marcus Klawitter[1], Torsten Steppin[1], Ingrid Lorenz[2], Ulrich Löschner[1], Ansgar Busch[1], Thomas Breuer[1], Maria Vergara Hernandez[3], Luis Leon[1]

[1] Zoetis Deutschland GmbH, Leipziger Platz 18, 10117 Berlin, Germany
[2] Tiergesundheitsdienst Bayern e.V., Senator-Gerauer-Str. 23, 85586 Poing
[3] Hochschule Osnabrück, Am Krümpel 3, 49090 Osnabrück

marcus.klawitter@zoetis.com

Objectives: Mycoplasma bovis (M. bovis) is a common pathogen in cattle, known to induce pneumonia, otitis, and arthritis in calves (Maunsell and Donovan, 2009). Farm veterinarians often rely on clinical signs to suspect the presence of this pathogen. This study investigates the relationship between major clinical symptoms (respiratory diseases and otitis) in calves and the herd seroprevalence of M. bovis, aiming to assess the diagnostic value of these clinical observations.

Materials and Methods: Bulk tank milk (BTM) samples were randomly collected from veterinarians at dairies (Holstein Friesian and Simmental herds) across relevant dairy regions in Germany. The samples were analyzed for M. bovis antibodies using the ID Screen® Mycoplasma bovis Indirect ELISA (ID vet, France) at the Bavarian Animal Health Services laboratory. BTM samples with a sample-to-positive percentage of ≥30% were considered positive. Additionally, veterinarians provided observational clinical information (OCI) regarding the prevalence of respiratory diseases (yes/no) and otitis (yes/no) at the farm level. Statistical analysis was performed using SPSS (IBM Version 28), with the Chi-squared test and odds ratios (OR) calculated.

Results: BTM samples and OCI from 306 dairy herds were collected and analyzed. Of these samples, 44.8% tested positive for M. bovis antibodies. Respiratory diseases were reported by veterinarians in 86.8% of the herds. Among the ELISA-positive herds for M. bovis, 91.9% exhibited respiratory diseases, compared to 82.7% of the ELISA-negative herds ($p<0.001$), resulting in an odds ratio of 2.371 ($p<0.001$). Otitis was recognized as a clinical sign in 27% of farms, with a prevalence of 42.7% in BTM ELISA-positive farms versus 14.3% in ELISA-negative farms ($p<0.001$; OR 4.462, $p<0.001$).

Conclusions: Clinical signs such as respiratory diseases and otitis at the farm level can be effectively utilized to identify herd BTM seropositivity to M. bovis.

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Evaluation of caudal vena cava point of care ultrasound and its association with shock parameters and prognosis in calves referred as emergencies

H. Casalta (Liège university, Belgium)

Hypovolemia is a major cause of shock in calves, but the clinical examination lacks specificity in evaluating volume status, and other tools available in human and small animal medicine were not developed in large animal practice. Being able to develop a non-invasive tool, technically feasible for non-specialist practitioners, with low investment, to help evaluating calves' volume status in combination with clinical parameters would be particularly interesting in this context. The objective of this study was to investigate the correlation between ultrasound measurements of the caudal vena cava (CVC) and aorta (Ao), and clinical parameters related to hypoperfusion in emergency-referred calves. Forty-five calves were recruited for this study, 28 calves were clinically categorized "in shock" based on clinical perfusion parameters. Point of care ultrasound (POCUS) of the CVC was performed at 2 anatomical sites, the xyphoid process and the right sub-lumbar site. The results showed a positive correlation between CVC and Ao measurements and calf age, consistent with observations in the pilot study. There was no significant difference in the ages of calves between the two groups, with or without clinically defined shock. The diameter of the CVC in a longitudinal view taken at the sub-lumbar level was correlated with LAC levels and was lower in calves with hyperlactatemia ($p=0.019$). The maximal diameter of the CVC measured at the xyphoid process was correlated with non-invasive systolic blood pressure (SBP) measured using a cuff positioned at the tail and decreased with SBP ($p=0.043$). However, there was no correlation between CVC and Ao measurements and clinical shock parameters (heart rate, capillary refill time, pulse). The CVC/Ao diameter ratio measured in a transverse view at the sub-lumbar level was positively correlated with survival ($p=0.036$), but this parameter was not sufficiently discriminative to be used as a prognostic factor (AUC=0.683, 95% CI 0.524-0.841, sensitivity and specificity respectively of 0.62 and 0.81 for a CVC/Ao ratio of 0.58). The CVC diameter and CVC/Ao diameter and area ratios at the sub-lumbar site were lower in calves with enteritis than in those with obstructive digestive diseases ($p=0.042$ and 0.022 respectively), and the maximal diameter of the CVC measured at the xyphoid process was significantly lower in calves with enteritis than in those with non-digestive disease ($p=0.032$). The measurements of the CVC and Ao in this study are static parameters that appear to be related to the calf's volume status. However, calf survival will depend in part on the management of this hypovolemia, so it would be interesting to evaluate the normalization of these parameters during fluid resuscitation, especially to aid in determining prognosis.

Effects of colostrum supplementation on the prevalence and severity of diarrhea in neonatal calves

Miriam Deppe[1], Alexandra Schlagheck[1], Hanna Strodthoff-Scheider[2]

[1]Biochem Zusatzstoffe Handels- und Produktionsgesellschaft mbH, Lohne, Germany
[2] agro prax Gesellschaft für Tiermedizin und Betriebsbegleitung mbH, Ankum, Germany
deppe@biochem.net

Objectives

Diarrhea is a leading cause of calf mortality. While countermeasures often focus on optimizing hygiene or daily feeding routines, the colostrum supply immediately after birth is rarely considered. Apart from contributing to passive immunity, previous research has shown that colostrum may not only influence the prevalence but also the severity of diarrheal diseases. The aim of this study is to investigate the effects of supplementing maternal colostrum with natural bovine colostrum powder on the prevalence of diarrhea and fecal consistency in neonatal calves.

Material And Methods

The study was conducted from 2022 to 2023 on two dairy farms (A and B) in Germany and included a total of 165 Holstein-Friesian calves (Farm A n=80; Farm B n=85). On each farm calves were divided into four groups. Two groups received maternal colostrum only (Control Group (CG) 1 and 2), and two groups received maternal colostrum mixed with a dietary feed supplement containing natural bovine colostrum powder [Colo-Ig, Biochem Zusatzstoffe] (Trial Group (TG) 1 and 2). Groups were divided by sex and maternal colostrum quality, using Brix refractometry. CG1 included female calves receiving colostrum with a Brix value above 22 (Farm A n=21; Farm B n=26). TG1 comprised female calves receiving a quality below 20 Brix (Farm A n=21, Farm B n=21). CG2 (Farm A n=19; Farm B n=15) and TG2 (Farm A n=19; Farm B n=23) were composed of male calves receiving colostrum below 20 Brix. All calves were drenched four liters of colostrum within six hours after birth. The supplementation of colostrum fed to TG1 and 2 followed a dosage table, ensuring a calculated minimum intake of 200g Immunoglobulin G (IgG) (sum of maternal IgG and IgG from powder). As a basis for this calculation, it was assumed that one liter of maternal colostrum with a Brix value of 15 contains approximately 25g of IgG. For each single higher Brix value, an increase of 5g IgG/L maternal colostrum was estimated, resulting in 200g IgG being covered without supplementation from Brix 20 onwards. The colostrum powder used was standardized to an IgG content of 200g/kg. Accordingly, a calf receiving colostrum with a Brix value of 15 was mathematically supplied with 100g IgG via maternal colostrum and supplemented with 100g IgG using 500g Powder.
To evaluate the prevalence and consistency of feces, a fecal scoring was conducted for each calf during the first 14 days of life (0=physiologically normal, 1=pasty, 2=thin feces, remaining on litter, 3=very thin feces, running through litter). The success of passive immunization was evaluated as part of the veterinary stock monitoring but will not be discussed further in this abstract. Throughout the trial, housing and colostrum management did not differ between groups. Due to individual management practices and subjective evaluation procedures, data were analyzed separately by using SPSS with a non-parametric test (Kruskal-Wallis-Test, conf. level=0.95).

Results

The average Brix values of maternal colostrum fed per group were as follows: CG1: Farm A: 25.7, Farm B: 24.02; TG1: Farm A: 18.4, Farm B: 16.9; CG2: Farm A: 18.7, Farm B: 15.5; TG 2: Farm A: 18.3, Farm B: 15.8. During the trial period, none of the groups, neither on Farm A nor Farm B, remained completely free of diarrhea. On farm A all calves in the non-supplemented groups CG1 and CG2 were affected by diarrhea. Diarrhea-free calves only occurred in the supplemented groups TG1 and TG2. In addition, the severity of diarrhea was lower in these groups. Comparing the prevalence of diarrhea between CG1 and TG2, a statistically significant lower incidence of diarrhea was found in TG2 (p=0.042). On farm B, no significant differences in prevalence of diarrhea were measured between TG1 or TG2 compared to CG1. However, comparing CG1 with CG2, the supply of low-quality colostrum without supplementation caused significantly higher incidence of diarrhea (p=0.01) and an increased severity in CG2.

Conclusion

The results indicate that supplementation with dried bovine colostrum powder may contribute to a reduction in diarrhea prevalence and an improvement in fecal consistency. On farm A, the lower number of calves with diarrhea as well as the higher proportion of milder cases in the supplemented groups both point to improved intestinal health. The results from farm B confirm this assumption and show that this is particularly the case when maternal colostrum quality is low. One possible hypothesis for the lower prevalence of diarrhea is that the supplementation of bovine colostrum powder, collected from many farms, supported the calves' resistance to diarrhea by providing a broader spectrum of antibodies against various pathogens present on farm.

DAY 1: the largest database on colostrum quality worldwide

Thibault Devambez[1], Elsa Rimet[1], Audrey Brunet[2]

[1]VIRBAC France, Espace Mercantour, 3ème rue, 06510 CARROS, France - *thibault.devambez@virbac.com*
[2]VIRBAC S.A., 13ème rue L.I.D, 06510 CARROS, France

Objective - Colostrum is the first milk produced by a cow after calving. Colostrum is rich in antibodies that are essential for strengthening the calf's immune system and protecting it against diseases. A high-quality colostrum is crucial for the calf's survival and growth. Colostrum is also an excellent reflection of the cow's health and, when measured on a significant portion of the herd, serves as an indicator of good calving preparation. DAY 1 is a digital tool developed by VIRBAC. DAY 1 is specifically designed to help farmers optimize the quality of colostrum given to calves immediately after birth. This communication reports on the implementation of DAY 1 in France.

Data collection – DAY 1 is a two-layer digital tool:

- DAY 1 Monitoring. The *My Monitoring* (*Mon suivi*, in French) option allows individual registered cattle producers to,
    - Record data related to each calving: colostrum quality, cow breed, vaccinations, feed, etc.
    - Monitor the evolution of colostrum quality within their herd.
    - Generate customized reports to analyze results and identify rooms for improvement.
    - Receive advice on implementing strategies to improve colostrum quality.
- DAY 1 Observatory. The *Observatory* (*Observatoire*, in native language) option gathers data from numerous farms, this observatory creates a national database. This allows farmers to:
    - Benchmark against the peers: By comparing their results to the average, they can identify areas for improvement.
    - Benefit from best practices: The observatory highlights the most effective practices for obtaining high-quality colostrum.
    - Better understand risk factors: By analyzing the data, it is possible to identify factors that can negatively impact colostrum quality.

Results - Over the past … years, DAY 1 has enabled the collection of a significant body of data on colostrum quality:

- Over 11,500 colostrum data points, meticulously linked to 13 animal husbandry variables
- 330 cattlemen and women now registered in the program, and convinced of the support provided by DAY 1
- 103 Veterinary Clinics committed in promoting DAY 1, and that benefit from the program
- A wide range of 13 cattle breeds represented
- An equal volume of colostrum data from dairy and beef cattle origin

Conclusion - In summary, Day 1 offers both a personalized and collective approach, allowing farmers to better understand the importance of colostrum, improve calf health, and optimize production. Day 1 is a real revolution in the field of cattle farming, placing the health of the calf at the heart of concerns from its first hours of life.

How control and eradication of BVDV at farm level influences the occurrence of calf diseases and antimicrobial usage during the first six months of calf rearing

Attila Dobos[1*], Vilmos Dobos[2], István Kiss[3]

[1] Large Animal Clinical Laboratory, University of Veterinary Sciences, Faculty of Veterinary Medicine, Brno, Czech Republic

[2] University of Veterinary Medicine, István u. 2, Budapest H-1078, Hungary

[3] Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Szállás u. 5, Budapest H-1107, Hungary

Attila Dobos: attivet@gmail.com

Objectives

Bovine viral diarrhoea viruses (BVDVs) cause significant economic losses in dairy cattle farms worldwide. Acute BVD outbreaks are rare across Europe nowadays due to successful control programs, while other manifestations of the virus infection are becoming increasingly common (6,7). In calves BVDV infections have mainly been associated with pneumonia and enteritis (1,5). A significant relationship was found between the BVDV infection status of herds and the incidence of calf mortality and respiratory disorders (3,4). Studies also indicated that BVDV plays an important role in enteric diseases when occurring in conjunction with other enteric pathogens. Nowadays the disease manifests mainly as virus-induced immunosuppression, compare with acute BVD outbreak impacting overall herd performance and contributing to increased antibiotic usage in calf rearing. In our study we investigated the effect of rapid BVDV control measures on calf diseases and antimicrobial usage after weaning on a large industrial dairy farm.

Material and Methods

A large industrial dairy farm implemented a BVDV control program in January 2023. Total herd size was 1571 Holstein Friesian cattle. This herd had never been vaccinated against BVDV before the investigation period and no animals are introduced to the farm, they use their own stock for replacement. 1541(cows and heifers) and all 542 newborn calf of blood samples (total 2083) were submitted for RT-qPCR and ab-ELISA tests. Ten animals were investigated by virus neutralization (VN) test. A commercially available qPCR kit was used for screening to identify PI animals as described earlier. The nucleotide sequences of the partial Npro coding genomic region was used for genotyping of the detected viruses as described by Booth et al. (2). Serological investigations were carried out by using the IDEXX BVDV Total Ab ELISA kit (IDEXX, USA) and by virus neutralization (VN) test, the latter to assess vaccine efficacy against the prevailing virus. Herd parameters were collected from the herd-management program between January 2019 and December 2023. Occurrence of calf diseases and cases and antibiotic usage in calves were investigated between calf birth and the age of 6 months.

Results

The rapid BVDV eradication programme began in January 2023 with identifying and eliminating PI animals from the farm. Twenty-one PI animals were found by using RT-qPCR testing of blood sera out of the 1571 animals tested (1.33%). Subsequent testing (between January and December 2023) identified further 28 PI animals amongst the 542 (5.1%) calves tested shortly after birth, and all were instantly removed from the farm. The detected 1b strain confirms previous recent findings on the prevalence of BVDV subgenotypes in the country, i.e. 1b, 1d, and 1f (4).

Before detecting BVDV on the farm (2019) the calf mortality was 5.68%. During the next three years when BVDV infection spread among the herd (2020, 2021 and 2022) annual calf mortality rapidly increased to 7.17%, 7.62% and 7.45%, respectively. Before detecting the BVD virus, 21 out of 43 animals (48%) were recorded with respiratory problems and 12 out of 43 calves (28%) with diarrhoea. During the next three years, respiratory cases tripled compared to the previous year. The total number of antibiotic treatments included 1012 cases in 2019 before the first detection of BVDV in the dairy farm. Over the next three years, antimicrobial usage increased dramatically. Antimicrobial treatments totalled at 1194, 1407 and 1422 during these years, respectively. During the BVDV eradication program, antimicrobial usage decreased rapidly. Less than 600 AB treatments were performed, more than fifty percent (57.9%) less compared to the previous year. Antimicrobial usage decreased for all active ingredients but mostly for the antimicrobials which were associated with calf respiratory diseases (tulathromycin, florfenicol)

Conclusion

Our study clearly demonstrated the positive effects of BVDV eradication: beyond eliminating the virus and reducing its direct impacts, it has evidently improved calf health and more importantly, contributed to the reduction of AB usage, a cornerstone of the One Health perspective of farm animal production.

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Enteropathogenic rotavirus and bovine coronavirus in neonatal calf diarrhoea: anamnestic analysis, clinical presentation, diagnostic, and therapeutic evaluations

1C. Eibl, 2J. Schoiswohl, 1C. Sohm, 1R. Krametter-Frötscher

1Clinical Centre for Ruminant and Camelid Medicine, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, Veterinaerplatz 1, Vienna, 1210, Austria

2Institut for organic farming & livestock biodiversity, Species-appropriate animal husbandry, animal welfare and herd management, HBLFA Raumberg-Gumpenstein, Trautenfels 15, Stainach-Puergg, 8951, Austria

Cassandra.Eibl@vetmeduni.ac.at

Objectives

Neonatal calf diarrhoea (NCD) remains one of the most commonly treated diseases in cattle. In addition to diet or feeding management, failure of passive transport, various pathogens including bacteria, parasites or viruses can play a leading role. Despite this, the widespread use of antibiotics in diarrhetic calves has been reported worldwide, regardless the origin of disease. The aim of this study was to retrospectively evaluate the anamnestic details at referral, pre-treatment, diagnostic management, treatment, the length of clinic stays and therapeutic outcome, in calves tested positive for rotavirus or coronavirus.

Materials and methods

In this retrospective study the clinical records of 156 calves, maximum 35 days old, referred to the Clinical Centre for Ruminant and Camelid Medicine and diagnosed positive for rotavirus or coronavirus, were reviewed. Information comprising anamnesis, clinical presentation, diagnostics, and therapy was collected, and described and correlations were analysed by chi-square test.

Results

A total of 23 calves died or were euthanized during hospitalisation. These calves were younger, had a lower base excess and a lower body temperature than the surviving calves at initial examination. Approximately one third of the animals were treated with antibiotics prior to arrival. These calves had a longer duration of hospitalisation than untreated calves. Besides rota- and coronavirus, the most common pathogen was *Cryptosporidium parvum*, with co-infection of rotavirus and *Cryptosporidium parvum* being the most detected (n=17 faecal samples). The risk of mortality in calves with a co-infection was 3 times higher than in calves with a mono-infection.

Discussion

As viral and parasitic pathogens are more likely to be involved as the primary cause of NCD than bacteria, the majority of the antibiotic treatments are not justified, except in calves with clinical signs of systemic inflammatory response syndrome (e.g. hypothermia, inappetence, recumbency). Recent studies have shown that the use of antibiotics in diarrhetic calves of non-bacterial origin may result in reduced microbiome diversity and prolonged time to normal faecal consistency. This may be another reason that these pre-treated animals had a longer stay in the clinic.

Conclusion

Calves with a non-bacterial diarrhoea should not be treated with antibiotics unless indicated by signs of septicaemia, as antibiotic therapy has been shown to be associated with a prolonged duration of cure. As the mortality in calves with a co-infection is severely increased, pathogen diagnostics are important to rule out further pathogens and to start early treatment according to the findings.

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Alternative strategies for disease prevention: assessment of the effects on colostrum quality and dairy calves' health of a dietary mineral complementary feed administered in dams at dry-off.

Maria Chiara Alterisio[a], Sergio Esposito, Mercaldo Beatrice[a], Sebastiano Tinelli[b], Giovanni D'Onghia[b],
Paolo Ciaramella[a], Jacopo Guccione[a1*]

[a] *Department of Veterinary Medicine and Animal Productions, University of Napoli Federico II, Via Delpino 1, 80137 Napoli, Italy.*

[b] *Veterinary Practitioner, Bari District, Puglia Region, Italy.1 Presenting author, * Contact author: jacopo.guccione@unina.it*

Objectives. The study aimed to evaluate the effects on colostrum quality and calves' health of a dietary mineral complementary feed contained in slow-release intraruminal bolus (Dalmaglass®, Fatro S.p.a., IT) administered in dams at drying-off.

Materials and methods. Fifty-four healthy pluriparous Holstein-Friesian cows were enrolled as the treated group (TG, receiving two boluses as labelled and containing Mg<1%, Na=19%, P=27%, Ca<1% at dry-off) and 53 as the control group (CG, left untreated). Colostrum quality was defined by Brix Refractometer (0-32.0%) for these animals. Colostrometer results were arbitrally divided into three categories (Cat): Cat1=Brix<18%, Cat2=18%<Brix<23%, and Cat3=Brix>23%. Serum total protein (sTP) levels were evaluated 96h postpartum by handheld refractometer to confirm correct liquid feed management. Cases of diarrhoea (within 4 weeks after birth) and respiratory disorders (within 24 weeks after birth) were recorded to assess the effects on the calves' health. Upon the appearance of at least one of the two problems, the animals were at the endpoint (excluded by the study). Variables were analysed by appropriate non-parametric tests. Probabilities<0.05 were considered significant.

Results. The correlation between Brix and sTP values was classified as very strong (r^2 = 0.817; P < 0.001). In the treatment group (TG), significant differences were observed in the distribution of calves across Brix categories: Cat1 (2/54) vs. Cat2 (11/54) (P < 0.01), Cat1 (2/54) vs. Cat3 (41/54) (P < 0.0001), and Cat2 (11/54) vs. Cat3 (41/54) (P < 0.0001). Similarly, in the control group (CG), significant differences were found between Cat1 (12/53) vs. Cat2 (36/53) (P < 0.0001) and Cat2 (36/53) vs. Cat3 (5/53) (P < 0.01). Inter-group comparisons revealed significant differences for the same parameters: TG-Cat1 (2/54) vs. CG-Cat1 (12/53) (P < 0.01), TG-Cat2 (11/54) vs. CG-Cat2 (36/53) (P < 0.0001), and TG-Cat3 (41/54) vs. CG-Cat3 (5/53) (P < 0.0001). Relative risk (RR) analysis demonstrated higher risk in CG-Cat1 (RR = 6.226) and CG-Cat2 (RR = 3.396) compared to TG values (TG-Cat1, RR = 0.161; TG-Cat2, RR = 0.294). Regarding calf pathologies, a significant inter-group difference was observed in the prevalence of diarrhea, with fewer cases in TG (29/54) compared to CG (8/53) (P < 0.0001). The associated RR was markedly lower in TG (RR = 0.199) than in CG (RR = 5.016). No significant difference was observed in the incidence of respiratory disorders between the two groups.

Discussions and conclusions. Preliminary data shows overall potential beneficial effects in improving colostrum quality and reducing the occurrence of diarrhoea in calves. Its use in dams might be hypothesized as support for a complete clinical herd management program. Nevertheless, further studies are necessary to confirm the encouraging outcomes observed.

Prevalence and predictors of bacteriemia in diarrheic sick beef calves in field conditions in France : a prospective study

V. Herry[1], N. Masset[1], M. Treilles[2], M. Ricordel[1], N. Herman[3], F. Perrot[4], G. Lemaire[4], A. Barral[4], R. Guindrey[1], C. Marget[1], C. Trumel[5], A. Geffre[5] , F. Schelcher[6,7], G. Foucras[6,7,] , F Corbiere[6,7]

[1]SELAS EVA, Réseau Cristal, 16 avenue du Général De Gaulle, 79150 Argentonnay, France
[2]Laboratoire Qualyse, ZAE Montplaisir, 79220 Champdeniers-St. Denis, France
[3]Clinique Vétérinaire Des Mazets, Riom-ès-Montagnes, France
[4]Clinique vétérinaire de la Haute Auvergne, Saint-Flour, France

[5] Central Medical Biology Laboratory, Université de Toulouse, ENVT, Toulouse, France
[6]Clinic for Ruminants, ENVT, 31076 Toulouse, France
[7]IHAP, Université de Toulouse, INRAE, ENVT, 31076 Toulouse, France *Corresponding author:*
v.herry@reseaucristal.fr

Objectives

The objectives of the study were to determine the prevalence of bacteremia in neonatal beef calves with diarrhea and altered mental state (= sick beef calves - SCD) and to determinate clinical factors and laboratory parameters associated with bacteriemia in those calves.

Material and methods

A prospective cohort study was conducted in 4 French veterinary clinics from 01/01/2020 to 31/08/2022. Criteria for inclusion were sick beef calves, <30 days old, no previous antibiotic treatment and to be hospitalized in one of the 4 clinics. This presentation focuses on a subgroup of 117 calves with diarrhea (decreased faecal consistency from creamy to liquid) belonging to a larger study group including non-diarrheic and diarrheic calves that was presented at EBF 2023, Berlin. At inclusion, usual clinical features were evaluated and venous blood was aseptically sampled for i) hemoculture in aerobic and anaerobic conditions, ii) hematology , iii) blood gas analysis (including L-lactates, glucose and Ca2+ concentration) and iv) other biochemical analysis (serum total protein, procalcitonin, haptoglobin and fibrinogen). Faeces of diarrheic calves were tested antigenically by immuno-chromatography to detect most frequent enteric pathogens in calves (rotavirus, coronavirus, *E coli* K99, *Cryptosporidium parvum*, *E coli* CS31a+). Calves meeting systemic inflammatory response syndrome (SIRS) criteria were given intravenously a combination of Sulfamimidine and Trimethoprim. Fluidotherapy was implemented according to current recommendation to correct dehydration, acid-base and/or electrolytic imbalances. Briefly, for univariate analysis, qualitative data were compaired using wilcoxon test, quantative date were comparaired using exact fusher test. ROC curves were performed for significant quantitative data and we determined the most discriminating theshold maximizing the Youden index. Then The likelyhood ratio and the odds ratio were determined using Wilson-Brown and Baptista-pike method, respectively. Finally we performed the multivariate analysis using generalized linear model analysis and we trained some classification algorithms (simple CART) to define decision rules allowing for the best discrimination between bacteremic and non-bacteremic calves.

Results

Of the 117 SCD included, 39.3% were bacteremic (46/117), and experienced a higher mortality rate 15 days after inclusion (36% ; 16/45) compared to other calves (7.4% ; 5/68). A high diversity of bacterial isolates was found and *Escherichia coli* (n=21) was by far the most frequently isolated bacteria, followed by *Trueperella pyogenes* (n=6), *Streptococcus uberis* (n=4), *Klebsiella spp.,* (n=2), and *Streptococcus spp.* (n=2). Univariate analysis revealed that age (≤4 days; OR=3.25), rectal temperature (≤38.7°C; OR=2.27), the degree of dehydration (≥10%; OR=3.6), signs of bacterial dissemination (OR=∞), L-lactatemia (≥1.12mmol/L; OR=9.78), glycemia (≤72mg/dL; OR=1,5), total serum protein concentration (≤43mg/dL; OR=19.01), procalcitonin (≥60 pg/mL; OR=3.33), base excess (≥-12 mmol/L; OR=3.11), pCO2 (≥44.9 mmHg; OR=5.76), anion gap (≤19mmol/L; OR=3.76), ionized calcium (≤1.19 meq/L; OR=2.95), hematocrit (≥49.7; OR=4.14), platelet count(≤519.10^{6}/L; OR=4.5) and lymphocyte count (≤$2.2.10^{9}$/L; OR=2.84) differed significantly between bacteremic and non-bacteremic calves. Cryptosporidium parvum was the most frequently isolated pathogens in faeces, followed by *E coli* K99 and rotavirus. There were no association between pathogens isolated from feces of diarrheic calves and the risk of bacteriemia despite a numerically higher proportion of bacteremic calves when *E coli* F5 or *E coli* CS31a+ were isolated. Multivariate model showed that L-lactate, procalcitonin and total protein were associated with bacteremia (accuracy: 0.81 ; sensibility: 0.78; specificity: 0.83). Decision tree models based on clinical features only poorly performed, while models including all available data yielded the best trade-off between sensitivity and specificity (Se: 84% ; Sp: 75%; Accuracy: 80%). The most discriminatory variables used in decision trees were pCO2 (≥39), total serum protein (≤45 g/L), procalcitonin (≥30 pg/mL), heart rate (≥128).

Conclusions

To our knowledge, this is the first prospective study dealing with bacteremia in diarrheic beef calves with altered mental state not previously treated by antibiotics. Overall the rate of bacteremia in SCD remained high and mortality rate was higher in bacteremic than non-bacteriemic calves. This study helps to characterize diarrheic calves with bacteremia, requiring immediate antimicrobial treatment. Particularly, younger calves with higher degree of dehydration, milder metabolic acidosis with lower anion gap and higher respiratory acidosis should worth particular attention. Moreover, total protein, procalcitonin and L-lactate proved to be very useful additional markers to discriminate diarrheic calves with or without bacteriemia. We did confirm previous factors associated with septicemia and point out some new ones ameliorating our predictive models.

Validation of long term continuous interstitial glucose monitoring by sensors in healthy calves

Ch. Lausberg (Veterinary practitionner, Sprimon, Belgium)

Objectives

In newborn calves, glucose serves as the primary energy source, gradually replaced by volatile fatty acids produced by the developing rumen microbiota. Blood glucose levels can be influenced by multiple factors, making regular monitoring crucial. However, there is no solid consensus on glucose monitoring in calves. Currently, blood glucose is measured using portable glucometers or laboratory analysis, which require frequent stressful blood sampling that can affect glucose levels. Continuous interstitial glucose monitoring via sensors offers a non-invasive solution to track glucose fluctuations over several days without disturbing the animals. One recent study investigated the accuracy of a continuous glucose monitoring system (CGMS) placed on the neck in 7 healthy goats and 7 dairy calves by comparing it to a chemistry analyzer under different glucose conditions (normoglycemic, hyperglycemic and hypoglycemic animals) (Brobst et al., 2024). Our study aimed to evaluate the feasibility of using interstitial glucose sensors in healthy untranquilized calves, and to select the best sensor location in terms of accuracy, precision and lifetime.

Materials and methods

The study involved 6 male dairy calves, aged 15 to 38 days. Five sensors were placed on each calf at the following locations: base of the ear, neck, thorax, rump near the base of the tail, and medial side of the thigh. An external jugular catheter was inserted to collect blood samples 8 times daily as long as the last sensor was still functional. Blood samples were analyzed by the hexokinase method. Glucose values obtained from the lab and the sensors were compared by the Bland Altman analysis method.

Results

For each location, the sensor lifetime varied considerably (0 to 13.95 days), influenced by different factors such as sensor defects and accidental detachment due to friction (calf petulant demeanor). Sensors placed on the rump and thorax showed the best average lifetime (respectively 6,7 ± 4,2 and 6,6 ± 4,6 days). According to the Bland and Altman analysis method, the average bias was positive for each location, indicating an overestimation of glucose levels by the sensors. In terms of accuracy, the wide limits of agreement reflected suboptimal analytical accuracy. Sensors on the rump and thorax offered the best balance between accuracy and lifetime. As observed by Brobst et al. (2024), despite suboptimal analytical accuracy, this tool demonstrates potential for clinical use. Further statistical analysis is ongoing to investigate this.

Conclusions

Despite the relatively suboptimal analytical accuracy, these sensors could be useful to investigate different milk feeding practices and to monitor the transition of calves from monogastric to ruminant status.

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NUTRITIONAL SUPPLEMENTATION OF A MULTI-STRAINS YEAST FRACTION HELPS TO SUPPORT OVERALL HEALTH STATUS AND INCREASES BENEFICIAL GUT MICROBIOTA OF PRE-WEANED DAIRY CALVES

Gauthier M.1, Sulmont E.1, Kowalczuk-Vasilev E.2,
Duniere L.1, Chevaux E.1

1 Lallemand SAS, Animal Nutrition, France,
2 University of Lublin, Poland

mgauthier@lallemand.com

High morbidity and mortality rates of calves during pre-weaning period will dramatically impact the sustainability of the farm. The reduction of the prophylactic treatments in calves production has contributed to the development of new microbial-based solutions to improve gut heath and consequently production. YANG is a feed ingredient including yeast fractions of *Saccharomyces cerevisiae* and *Cyberlindnera jadinii*. The objective was to determine the effect on health of YANG when introduced to the calves' diet during the rearing period (5-65 day of life).

Thirty Holstein calves (male and female) born from late June to mid-November 2019, in a medium-size (500 milking cows) dairy farm in Poland were enrolled in the study. All calves received the colostrum from the dam within 2 h and 2.5 L of transition milk, 2 times a day until 5 days of age. At enrollment, 6 days-old calves, were individually housed and were offered daily 8 L of MR, divided in 2 equal meals, in buckets, at a dilution rate of 0.125kg /L, providing 4.6 Mcal metabolizable energy (ME) /kg of dry matter (DM) (21.9% crude protein and 18% fat). They had *ad-libitum* access to water and pelleted starter (3.55 Mcal ME/kg DM). Calves were randomly assigned in the two experimental groups, designated as supplemented (YANG) and Control (C), stratified by dam's parity, gender and initial BW. YANG was supplemented at dosage of 800g/ton of MR for the whole experiment. Fecal consistency (scores) was scored daily by the research team during the morning feeding using a 1 (firm) to 4 (watery) scale. Two fecal samples were collected at time = 10 days and time = 60 days of the trial for each calf. Incidence of health disorders was recorded daily for each animal. Every abnormal health condition such as fever or bronchitis as well as every treatment (antibiotic, electrolyte therapy) was recorded.

A linear mixed model was performed to assess the impact of the supplementation on the different parameters measured. Calf was used as random effect, sex, group, and sex*group interaction were used as fixed effects and initial BW as a covariate. Statistical analysis was performed using IBM SPSS v.25 and RStudio.

No calves of YANG group experienced respiratory diseases during the trial whereas 50% of the non-supplemented calves had respiratory problems (*P* = 0.034). In addition, the number of diarrhea cases was lower for YANG compared to Control group (2.2 vs. 0.9/calf respectively, *P* = 0.007) as well as the duration of the diarrhea case (5.6 vs. 2.9 days/calf respectively, *P* = 0.005). Consequently, a lower fecal score was reported all along the trial for YANG compared to Control calves (1.6 vs 1.9 respectively, *P* = 0.002). Along with those observations, a lower number of calves received a therapeutic treatment in YANG group compared to Control group (18.8 and 62.5% respectively, *P* = 0.034), and the average number of therapeutic treatment used for YANG calves was lower compared to Control calves (0.3 vs 2.7/calf respectively, *P* = 0.005). Finally, less YANG calves were supplemented with electrolytes compared to Control calves (37.5 vs. 81.3% respectively, P =0.035). From a fecal microbiota standpoint, YANG supplementation in neonate calves increased positive bacteria such as *Bifidobacterium* (lactic acid bacteria), Akkermansia (stimulation of mucine production) or *Succiniclasticum* (propionate producers, linked with gut maturation) whereas decreasing *Fusobacterium*, a potential pathogen.

In addition, average daily gain of calves was significantly increased during the first 30 days (+46g/d, P<0.05), with a numerical improvement of final BW at 60 days (+1.6kg) and a tendency for better overall FCR (P<0.10).

The calves supplemented with YANG showed lower morbidity and mortality, both at digestive and respiratory levels, with a consequent decreased number of therapeutic treatments and costs. It confirms that YANG can help maintain health under some challenging conditions. These phenotypic observations can be linked to fecal microbiota modifications in YANG calves: significantly higher abundance of taxa linked to health benefits particularly at early age (*Akkermansia, Bifidobacterium, Succiniclasticum),* and less opportunistic pathogens (*Fusobacterium*) were observed. Positive effects on intestinal health and growth, and the reduction of antibiotic treatments support farm profitability and sustainability.

Study on the impact of antibiotics on the microbiota, feed intake, and growth of calves

Soler J. [1], Lopes E. R. [2], Heittiarachchi A. [3]

[1] Irodservet SL, Barcelona, Spain – irodservet@gmail.com;
[2] Huvepharma NV, Antwerp, Belgium – rui.lopes@huvepharma.com;
[3] Pathosense, Ghent, Belgium - amanda.hettiarachchi@pathosense.com

Objectives

The use of antibiotics to treat diarrhea in calves, while necessary, can have significant effects on the intestinal microbiota. This delicate ecosystem plays a vital role in digestion, immune development, and protection against pathogens. Altering this balance with antibiotics may cause short- and long-term issues, affecting both the health and productive performance of calves.

The main objective of the study was to analyze the influence of antibiotics on the intestinal microbiota, feed intake, and weight gain in calves.

Materials and methods

The study lasted 42 days and involved a total of 24 calves distributed into four homogeneous groups, each consisting of six individuals. The impact of three different oral antibiotic treatments (paromomycin [Parofor®], apramycin [Apravet®] and neomycin) for diarrhea cases was evaluated, and compared to a control group that received no oral antibiotics.

The intestinal microbiota was assessed using two key metrics: alpha diversity, which measures the variety of microbial species within each sample, and beta diversity, which evaluates differences in bacterial composition between groups.

Feed intake data were tracked throughout the study period, and growth performance was evaluated through regular measurements of weight gain.

Results

Impact of antibiotics on the intestinal microbiota

- The control group and the group treated with paromomycin maintained high levels of alpha diversity, especially at the end of the study (day 42), indicating a healthy microbiota. This suggests that paromomycin has a limited impact on microbial balance, preserving bacterial diversity.
- The bacterial compositions of the control group and the paromomycin group were very similar, reinforcing the hypothesis that paromomycin has a minimal effect on the microbiota. Conversely, the group treated with neomycin exhibited a significantly different bacterial composition, indicating a greater disruption of the microbiota.
- The control group and the paromomycin group displayed a higher abundance of beneficial bacteria such as *Lactobacillus johnsoni* and *Megasphaera elsdenii*, particularly after treatment (day 7). These bacteria are essential for intestinal health, contributing to carbohydrate fermentation and the production of short-chain fatty acids, a key energy source for intestinal cells 1,2.

Evolution of feed intake is a crucial indicator for evaluating the general condition of calves:

- Paromomycin group showed a total feed intake of 239.50 kg, with a stable and consistent pattern throughout the trial. This indicates sustained recovery and good digestive efficiency, with the highest feed efficiency.
- Control group achieved the highest total feed intake (244.60 kg). However, despite the high feed intake, this group exhibited the lowest feed efficiency.
- Neomycin group recorded the lowest total feed intake (158.80 kg).

- Apramycin group with a total feed intake of 212.70 kg, this group showed intermediate performance, though it was less consistent than the paromomycin and control groups.

Weight gain is another key indicator of the performance and health of calves. The results aligned with observations on the microbiota and feed intake:

- Paromomycin group achieved the highest average weight gain (27 kg), with low variability among individuals. Additionally, only one calf experienced diarrhea, highlighting the effectiveness of the treatment.
- Although the control group calves experienced diarrhea in the first week, they achieved an average weight gain of 23.25 kg. Variability among individuals was high, possibly due to individual factors.
- Neomycin group with an average weight gain of only 16.5 kg, this group showed the worst performance.
- Calves treated with Apramycin demonstrated intermediate performance, with an average weight gain of 23.25 kg but greater variability in results.

Conclusions

- Treatment with Paramomycin demonstrated effectiveness by preserving intestinal microbiota diversity, supporting beneficial bacteria and promoting efficient digestion. Calves in this group achieved the highest weight gain (27 kg) with minimal variability. This treatment has shown both short-term health and long-term productivity.
- The recovery of calves in the control group without antibiotics allowed the natural evolution of the microbiota to be observed, serving as a reference for evaluating the effects of the treatments.
- Treatments should be selected not only for their clinical efficacy but also for their ability to preserve the balance of the microbiota, which is essential for short- and long-term productive performance. Positive results during the suckling phase will also have a beneficial impact on the subsequent fattening phase.

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Are German calves on a better way since 2004/05? – Actual data about the colostrum supply in German dairy herds

K. Stemme*[1], M. Erhard[2], D. Klaus-Halla[1], S. Reese[3], E. Rauch[2]

*Kirsten.stemme@msd.de

[1] MSD Tiergesundheit, Intervet Deutschland GmbH, Feldstraße 1a, 85716 Unterschleißheim

[2] Lehrstuhl für Tierschutz, Verhaltenskunde, Tierhygiene und Tierhaltung, Tierärztliche Fakultät der LMU München, Veterinärstraße 13/R, 80539 München

[3] Lehrstuhl für Anatomie, Histologie und Embryologie, Tierärztliche Fakultät der LMU München, Veterinärstraße 13, 80539 München

Objectives

Calf mortality is a huge problem in dairy farms and more than 80 % of losses are due to farm-specific management problems. Adequate supply of colostrum is an essential prerequisite for reducing calf losses. In two studies, conducted in Germany in 2004/05 and 2015, it was shown that a large proportion of calves do not receive an adequate supply of colostrum. Therefore, the present study was intended to get more information about the actual situation of calves in Germany.

Material and methods

Two questionnaires analogous to the studies in 2004/05 and 2015 were provided to get information about the farm (herd size, management) and the calves from which a blood sample was taken (time, quantity and type of colostrum administration, time of blood sampling, …).

A total 463 blood samples from calves (taken 24-72 hours after birth) from 69 farms (herd sizes 35 - 2600 cows) were examined in the period from January 2021 to February 2022. For this purpose, a sandwich ELISA testing for IgG according to Erhard et al. (1995) was used.

Results

73% of the calves (n=338) were adequately supplied with colostrum (≥ 10 mg IgG/ml). The IgG-status of the examined calves improved significantly ($p<0.001$) compared to the studies in 2004/05 (61.2%) and 2015 (40.9%). Nevertheless, 8.4% of the calves (n=39) had a failure of passive transfer (< 5 mg IgG/ml serum) and 18.6% (n=86) a partial failure of passive transfer (5–10 mg IgG/ml serum).

More recent studies by Lombard et al. (2020) recommend other threshold values for assessing colostrum supply. According to their studies, a good supply is guaranteed from a serum IgG content of 18 mg/ml, a very good supply from a serum IgG content of 25 mg/ml. If these threshold values are applied to the calves examined in the present study, 22.7% of the calves were well supplied with colostrum (18-24.9 mg IgG/ml).14.9% of the calves were even very well supplied (≥ 25 mg IgG/ml).

There was no influence of breed and sex on colostrum supply, but quantity ($p<0.001$) and type of colostrum administration ($p<0.01$) had significant effects on IgG content in the serum of the calves.

Conclusion

The results show that colostrum supply of calves in Germany has improved significantly compared to 2004/05 and 2015. Even though this development can be considered very positive, a considerable proportion of calves are still not well supplied. The responsible factors still need to be evaluated.

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Cryptosporidium spp. oocyst viability submitted to disinfectants under different light conditions.

Vázquez-Flores, S[1], Barrera-Almanza, S. [1], Sánchez, F. [2], Esteves-Lopes, R. [2], Nenov, V.[2]

[1]Tecnológico de Monterrey, México *svazquef@tec.mx*
[2]Huvepharma, Belgium.

Biosecurity is one of the areas of opportunity to control cryptosporidiosis. The environment where a calf is born in the maternity area is the main contamination source as peripartum cows can shed 2.4×10^9 per 100 g of feces. A calf starts shedding oocysts since the first meconium, and in longitudinal studies the prevalence is from 96.6 to 100% in the dairies if sampled continuously. The pathogen stays viable under different conditions of cold, humidity, and even direct sunlight. Removing manure and cleaning is not enough, classical disinfectants like chlorine, quaternary ammonium compounds, potassium monopersulfate are not useful to destroy oocyst.

Materials and methods. The study was performed *in vitro*, with a sterilized substrate of sand, spiked with 3.3×10^5 oocysts obtained from calves with cryptosporidiosis, set under three different conditions: sunlight, partial sunlight, and shade. Five treatments were used: 1. H_2O_2 vol 1:50; 2. H_2O_2 vol 1:100; 3. Chlorocresol 1:50, 4. Chlorocresol 1:100 and 5. Control (H_2OD_1). The treatments were distributed in blocks in triplicate. The samples were cleaned, concentrated, quantitated, and submitted to *in vitro* culture by Atwill et al., 1997. Oocysts were classified due to viability and non-viability status.

Analysis of oocysts post-disinfectant treatments.

H_2O_2 35% vol 1:100, showed the higher number of complete oocysts, damaged oocysts in partial sunlight.

Analysis of oocysts post-viability treatment submitted to direct sunlight, indirect sunlight and under the shade.

Direct sunlight: In the case of chlorocresol 1:50 the treatment generated damage to the oocysts, and detritus, the quantitation indicated larger numbers of destroyed oocysts, complete oocysts, and complete non-viable oocysts than the rest of the treatments.

Indirect sunlight: In the case of chlorocresol 1:100 the number of non-viable oocyst and detritus was larger than the other treatments.

Shade: The H_2O_2 35% vol 1:100 treatment had the highest number of complete oocysts, and the highest number of non-viable oocysts in treatments under the shade. The highest destruction of oocysts was with chlorocresol 1:100.

The H_2OD_1 control treatment showed the larger number of destroyed oocysts during all the environmental conditions when submitted to viability test. When analyzed with a correlation test, the damage indicated that the viability test was successful due to the amount of viable oocysts.

As conclusions, the study aimed to assess the effectiveness of different disinfectants on cryptosporidiosis oocysts under various lighting conditions. Hydrogen peroxide (H_2O_2) and chlorocresol were tested at different concentrations and compared to a control. The results indicated that chlorocresol, especially at concentrations of 1:50 and 1:100, was effective in damaging oocysts across various light conditions. H_2O_2 at a concentration of 1:100 also showed significant damage to oocysts, particularly under shade conditions. The control treatment consistently showed the highest number of viable oocysts during the cell culturing tests across all environmental conditions.

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Paromomycin sulfate as a metaphylactic treatment for cryptosporidiosis control in newborn dairy calves

Vázquez-Flores, S[1]., Barrera-Almanza, S.[1], Sánchez, F.[2], Esteves-Lopes, R.[2], Nenov, V.[2]

[1]Tecnológico de Monterrey, México
[2]Huvepharma, Belgium.

The objective of the field trials was to mitigate cryptosporidiosis in newborn female Holstein calves before its clinical presentation during the second week of life, aiming to reduce weight loss consequences under various raising systems.

The study involved 120 newborn female calves from three different dairy facilities in Mexico: Study1 (S1) in northern Mexico, Study2 (S2) in central Mexico, and Study3 (S3) in western Mexico. Calves were raised in individual crates and randomly assigned to one of the following treatments: Control (antibiotic-treatment for scours), and commercial prophylactic treatments against *Cryptosporidium* spp. encompassed Halofuginone-lactate (HL), Nitazoxanide (N), and Paromomycin-sulfate (PS).

S1 included 20 calves in elevated crates with Control and 20 with PS. S2: 21 calves in Control, 14 with N, and 23 with N. S3: 30 calves with HL and 28 with PS. Treatment HL was administered from day 2 of life for seven days, while N was given on days 2 and 5 as per label. Personnel involved in the trials were trained to detect early signs of loss of appetite, indicating the need for paromomycin sulphate treatment.

Passive immunity was determined using Brix refractometers at 48h of age. A daily scoring system was used to record diarrhoea for the first 60 days of life. Faecal were collected at 7 and 14 days of age, analysed morphologically with acid-fast staining. Faecal samples from S2 were quantified for *Cryptosporidium* oocysts using a discontinuous flotation test. Calves were weighed at birth, 60(S2/S3), 90(S1/S3), and 120 days (S2). All calves received pasteurized colostrum and milk until 55d of age. Feeding schemes varied: S1 calves were fed five times daily at 20% of their body weight (BW), S2 calves twice daily at 20% of their BW, and S3 calves twice daily at 18% of their BW.

Statistical analysis was conducted using mixed procedures with JMP17.2 and JASP0.18.1, considering statistical significance at $P<0.05$. Fixed effects included treatments, site, and housing, while random effects were scours and appetite, with weight as a covariate within the treatment.

Results indicated no significant differences in passive immunity among study groups at all sites. Morphological analysis of faecal stains showed a seven-day threshold for the clinical appearance of cryptosporidiosis. At the end of treatment, the prevalence of cryptosporidiosis and oocyst shedding on 14d of age showed that PS decreased prevalence in S3(14%), N reduced prevalence in S2(28.1%), while HL increased prevalence in S3(33%).

In the first week of treatment with PS showed a decrease in scours across all sites, the effect continued into the consecutive week in S1($P<0.05$) and S2($P<0.0001$), no follow-up was recorded for S3. During the second week of age, quantification of *Cryptosporidium* oocysts post-treatment showed a lower oocyst count in the PS group compared to the Control and N groups in the S2 cohort ($P<0.05$). S2 showed less pneumonia cases with PS($P<0.05$).

There were consistent increments in weight and height for S1 and S2 when treated with PS. Regarding S2, calves treated timely for diarrhoea showed no significant difference compared to PS treated calves, while calves treated with N prophylactically did not prevent weight loss and had less height at 120 days of age ($P<0.05$).

Comparing different milk feeding schemes and treatments, S1 calves fed 20% BW with PS showed a 13.6 kg weight difference with the control group. In S2, the weight difference was 16.8 kg when comparing the control and PS groups to the N treatment. The smallest weight difference was in S3 (6.9 kg). Passive immunity was a probable contributing factor to the weight increment, with S1 and S2 registering Brix of 9.9 and 9.5 respectively, while S3 had a lower Brix of 7.3.

In conclusion, paromomycin-sulfate (PS) was effective during the peak of cryptosporidiosis, resulting in 34-50% less oocyst shedding, thereby allowing fewer clinical signs, no appetite loss, and fewer pneumonia cases. Weight gain was consistently higher in the PS study group at 60, 90, and 120 days of age, compared to other prophylactic treatments for controlling *Cryptosporidium* spp. The combination of passive immunity, higher milk feeding planes, close clinical follow-up, along with biosecurity measures and timely treatment, collectively contributed to the effective control of cryptosporidiosis in newborn calves.

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Updates on diagnostics for respiratory disease in calves

S. Depenbrock DVM, MS, DACVIM (LAIM)
Davis, CA, USA

Background

Despite decades of research, advances in vaccinology and antimicrobial therapies, and a growing body of research documenting risk factors, the bovine respiratory disease (BRD) complex remains a leading cause of morbidity, mortality, and reason for antimicrobial drug use in calves [1,2]. Modern calf management systems contain numerous risk factors for BRD, and as management practices change, new risk factors for BRD are created. The BRD complex is an excellent example of how disease can manifest due to interactions between the host, environment, and pathogen [3]. Many veterinarians agree that the BRD complex is a multifactorial disease that culminates in bacterial infection of the lungs. However, there is still debate over specific test result cutoffs or exam findings that define normal vs diseased, as well as how to use test findings to make decisions about which animals to treat or not treat. A diagnosis of BRD has been made using combinations of: exam findings, exam scoring systems [4–6], auscultation [7,8], airway sampling [9], thoracic imaging [10,11], biomarkers [12] and necropsy. Each methodology has advantages and limitations. Respiratory imaging, airway sampling and biomarkers are active areas of research that have developed significantly over recent years and are thus reviewed in this session. Diagnosing disease and identifying pathogens are small pieces of the BRD investigation. Finding the underlying causes of diminished host immunity and increased pathogen opportunity depends on finding which aspects of animal management set up an environment in which host immune defenses are compromised and infectious pathogen(s) can invade. An approach to diagnosing management factors associated with BRD is thus likewise briefly discussed.

Diagnosis

Imaging

Clinical signs of BRD are variable, and subclinical disease common [13–16]. Developments in airway imaging have significantly improved the livestock practitioner's ability to diagnose BRD. Early descriptions of diagnostic imaging specifically used to describe lung lesions in BRD cases used ultrasound and radiographs and were published about 25 years ago [17–19]. Since then, clinicians have used radiographs, ultrasound [20,21], endoscopy [22,23], and computed tomography [11] to aid in diagnosis of respiratory disease in cattle. In recent years, thoracic ultrasound (TUS) has gained significant popularity, allowed practitioners to image bovine airways in the field, and arguably improved the fidelity of antemortem BRD diagnosis on the largest scale in both research and clinical settings compared to other imaging modalities and is thus discussed in this session.

Advantages of TUS for diagnosis of BRD include: excellent access to superficial lung fields in calves, relatively fast and technically straight forward to perform by trained individuals, can be performed in-hospital or in the field, and uses ultrasound equipment commonly carried by livestock practitioners. Disadvantages and limitations of TUS include: inability to image lesions deep to aerated pleura and thus some lesions can be missed, requires some technical training, as animals grow the cranial thorax becomes more difficult/impossible to image particularly in heavily muscled or over-conditioned animals, and ultrasound machines and probes are expensive equipment. When compared to post mortem exam, TUS had 94% and 100% sensitivity and specificity for detecting lung lesions in dairy calves [20]. Compared to scoring systems based on clinical signs or auscultation, TUS appears to be more sensitive and specific for the diagnosis of BRD and allows for detection of subclinical disease. For comparison, the sensitivity and specificity for the commonly used Wisconsin and California BRD scoring systems, which are based on clinical signs, were reported as 71.1%, 72.6%, and 91.2%, 87.4% respectively for detection of cases of BRD when ultrasound plus auscultation was used as the standard for BRD diagnosis [24]. Auscultation alone has poor sensitivity for detection of BRD, with a sensitivity of 5.9% when compared to TUS lesions [7]. Some investigations, particularly in beef calves, have found poor diagnostic performance for TUS. For example, one study found no association between TUS and a clinical diagnosis of BRD based on exam findings; cytology obtained from TTW was more closely associated with clinical signs of BRD in this study [8]. However, as noted above, as cattle get larger and gain condition it becomes more difficult to examine the cranial thorax with TUS; the afore mentioned study used a more caudal approach than is typically used in dairy calves. The size and body condition of weaned calves entering beef production systems are much larger than pre weaned dairy calves, and are often restrained in chutes that do not have ideal access for TUS of the cranial thorax; these factors limit the ability of investigators to image much of the right cranial lung lobe. These differences are likely a significant factor in how well TUS performs in reports from dairy calves compared to feedlot cattle.

Multiple different TUS techniques and scoring systems that categorize results have been described. The technique the clinician or researcher uses depends on their goals, what the age/size of the calf is, and what restraint is available or necessary. Practitioners presented with individual cases may choose to scan the entire accessible lung field and document the location, depth, and distribution of each lesion in order to document detailed findings and track patient progress. The researcher or practitioner examining groups of animals may prefer to use a scoring system to categorize TUS findings and create a more uniform way to enter and analyze TUS data. The lungs of young calves can be reliably scanned from the 10th intercostal space (ICS) to the 1st ICS on the right and to the 2nd ICS on the left [25]. This is notably more cranial than most adult ruminants and horses; clinicians should be aware of how cranially the lungs extend and can be imaged with ultrasound to avoid missing significant lesions or entire lung lobes in calves. More limited techniques have also been described; in some settings, the practitioner may find a limited exam more efficient for their specific investigation. One technique describes starting at the 8th to 9th ICS and scanning cranially until internal thoracic artery and vein are visualized; this technique had fair inter rater agreement when multiple people were trained to scan using this technique [26,27]. An exam focused only on the cranial and middle lung lobes describes scanning 1st to 5th intercostal space on the right and 2nd to 5th on the left, and reports 93% sensitivity compared to complete TUS extending to the 10th ICS in veal calves [28]. Even more focused TUS exams for limited applications have been described. One technique examines only the right and left 4th and 5th ICS and reports a sensitivity of 83% when compared to complete TUS extending to the 10th ICS in dairy calves [29]. Another describes scanning only the right 8th-11th ICS, with the specific goal of diagnosing interstitial pneumonia in feedlot cattle, and reports that in animals with >5 B-line counts the probability of interstitial pneumonia was 0.86+0.11 [30]. When considering the use of a limited TUS exam, the practitioner should be aware that lung pathology can be missed, particularly when the cranial aspect of the right cranial lung lobe is not imaged, as this is the most common location for pulmonary lesions in BRD in calves. A study investigating the sensitivity of specific lung lobe lesions for diagnosing BRD reported that identifying TUS lesions in the cranial aspect (sensitivity of 84.7%) and caudal aspect (sensitivity 40.3%) of the right cranial lung lobe were the most sensitive locations for the TUS exam [31]. The reader is referred to a review article on TUS for descriptions and images of ultrasound lung lesions in calves [25].

Scoring systems for TUS findings allow practitioners and researchers to categorize results for data analysis. There are multiple TUS scoring systems and different cutoffs used to define BRD cases published currently; one commonly referenced system uses a 5 point scale ranging in severity from normal to severe with discrete lesion parameters described for each score [20]. There is no broad consensus on how much lung consolidation or extent of pleural lesions define a BRD case, whether clinical or subclinical. However, a commonly used cutoff for cases is consolidation of > 1cm. There is also no consensus on the minimum lesions that warrant treatment for BRD. Arguably, any consolidation is abnormal, however there is no consensus on how best to use this information to make treatment decisions, particularly in subclinical cases. There is evidence that early identification and treatment of lesions found when using TUS for BRD screening improves short term health outcomes [15]. However, there is still great value in identifying abnormal or subclinical animals early in disease to inform veterinarians when/where animal management problems are occurring that favor the development of BRD, even if we do not yet have consensus on treatment of subclinical animals.

Airway sampling

Current techniques used for airway sampling in calves include: nasal swabs (NS), deep nasopharyngeal swabs (DNPS), transtracheal washes (TTW), bronchoalveolar lavages (BAL), and occasionally thoracocentesis or lung tissue sampling (typically post mortem). Samples are used clinically to identify pathogens, determine pathogen antimicrobial susceptibility profiles, gain information on host response to disease, and in a variety of research settings investigating BRD pathophysiology and microbiology.

Nasal swabs and DNPS are typically used to sample upper airway bacteria, viruses and other microbiota associated with BRD or to study the upper airway microbiome. Nasal swabs (which sample more rostrally than DNPS) have been demonstrated to be comparable to DNPS for culture and qPCR of *Mannheimia haemoltyica* in feedlot calves [32] and culturing *Pasteurella multocida* in dairy calves [33]. Upper airway swabs can also be used to sample viruses affecting the upper respiratory tract such as bovine herpes virus, bovine respiratory syncytial virus and bovine coronavirus [9,34]. Bacterial overgrowth is a potential disadvantage of using the NS technique for bacterial culture of the upper respiratory tract, although this is reported as a problem by some investigators [33,35] and not others using this sampling method [32].

The DNPS is likewise used to sample bacteria, viruses, and other microbiota from the upper airway. This technique typically uses a guarded swab (although unguarded, single guarded and double guarded swabs have all been used for DNPS [9,32]) advanced from the nares to the pharyngeal region (usually approximately the level of the medial canthus of the eye), to sample the nasopharyngeal region. This technique is intended to get a sample deeper in the upper airway associated with the pharyngeal region and limit contamination from the rostral nasal cavity [33]. Recent research has identified some other potentially clinically useful data regarding DNPS in cattle. One study demonstrated that bacterial enrichment of samples obtained from DNPS improves detection for BRD pathogens and AMR genes with metagenomic sequencing in feedlot cattle [36]. Two studies suggest that DNPS is the preferred method to maximize recovery of *Mycoplasma bovis*, although it can also be isolated on NS [37,38]. A study of *M. haemolytica* isolates from DNPS demonstrated that when multiple isolates are cultured from a single swab, the AMR genotype was consistent among isolates from the same swab, and that the AMR phenotype likewise only showed minimal differences in MIC [39].

Few studies specifically examine the effects of swab materials used for NS or DNPS in cattle. Studies from other species suggest that the preferred material for swab tips used for PCR is rayon with plastic shafts rather than cotton tips, calcium alginate containing tips, or wood shafts due to PCR inhibitors in these materials [40,41]. However, both rayon and cotton tipped swabs have been used for molecular diagnostics from NS/DNPS in calves [32,42]. There insufficient reports specifically comparing PCR results or reporting PCR inhibition associated with swab type in airway swabs from calves.

Sampling the upper respiratory tract by either NS or DNPS to make inferences about lower airway disease has potential limitations. The upper and lower airways have unique microbial communities [43], and the upper respiratory tract contributes to the microbiome of the lower airways [44,45]. Studies comparing pathogen identification from the upper respiratory samples (NS or DNPS) to samples lower in the airway (TTW or BAL) have shown mixed results depending on the samples being compared, in what calf population, and which isolates are being compared. Some describe acceptable agreement for isolation of *M. haemolytica*, *P. multocida* and *M. bovis* [9], or mixed results where factors like age, BRD status, or geographic location influence agreement in culture or genomic testing [42,44], while others describe clinically unacceptable agreement among culture results [35]. Interestingly, a recent microbiome study investigating multiple respiratory sampling sites reported that the nasopharyngeal and tracheal microbiota were not statistically different from that of the lung [45]. When using respiratory sampling to determine antimicrobial susceptibility of common bacteria associated with BRD (*M. haemolytica, P. multocida, H. somni*), a recent study found that the agreement among susceptibility results between samples obtained from NS, DNPS, TTW and BAL was fair to excellent, depending on the drug and pathogen being tested [46]. However major discrepancies were noted when comparing florfenicol and tulathromycin susceptibility across sampling methods in *P. multocida* isolates and spectinomycin in *M. haemoltyica* isolates.

Ante mortem sampling techniques aimed at getting a sample more representative of the lower airway with less contamination from the upper airway most commonly include TTW and BAL. Some contamination from the upper respiratory tract is still possible for samples obtained from both a TTW or BAL due to tube passage (BAL), calf micro-aspiration, and impaired airway clearance mechanisms (BAL or TTW). Multiple techniques for tracheal sampling have been described in calves including tracheal washes, aspirates and TTW, with the most common in recent publications being similar versions of the TTW [9]. A BAL can be performed with or without an endoscope. A non-endoscopic technique for BAL in calves has been described [47] and a study in calves demonstrates

that cultures from this type of sample are more likely to yield a pure culture than samples from DNPS [35]. Additionally, this same study notes that the BAL was more likely to isolate *H. somni* than DNPS [35].
In addition to pathogen isolation, TTW and BAL are commonly used for cytology. When using TTW for cytology, a cutoff of > 20% neutrophils was associated with a clinical diagnosis of BRD [8]. When using a BAL for evaluation of cytology, a reference interval of 2.3-47.4% neutrophils has been suggested [48]. However, a cutoff of >4% neutrophils was associated with subclinical BRD with 81% sensitivity and 75% specificity in a different study [20].

Much can be learned from airway sampling regarding pathogens associated with BRD to aid in diagnosis, plan antimicrobial therapy, and generally increase our knowledge of BRD pathogenesis. However, no broad consensus exists for the use of airway sampling alone to diagnose BRD. To diagnose lower respiratory disease, it is still prudent to examine the lower airways and concurrent clinical signs.

Biomarkers

Effective biomarkers inform clinicians about physiology and disease processes occurring in their patients. The use of biomarkers to aid in the diagnosis for BRD is an attractive idea because: sampling blood (or other bodily fluids or gasses) may be technically simpler and involve less labor than a complete physical exam and could provide quantitative results for a disease that can be subjective to diagnose on exam, and in which subclinical disease is common. A variety of different types of biomarkers have been investigated for BRD including: blood cell counts, acute phase proteins (ex: fibrinogen, albumin, serum amyloid A, haptoglobin, c reactive protein, α-1-acid glycoprotein), cytokines (ex: IL-1β, IL-4, IL-6, IL-8, TNF-α, IFN-γ), peptides involved in the immune response (ex: substance P, neopterin), hormones or prohormones (ex: cortisol, procalcitonin), enzymes (ex: metalloproteinase), serum metabolites (ex: L-lactate), iron metabolism (iron, ferritin, transferrin, total iron binding capacity), globulins associated with compliment (ex: conglutinin, immunoglutinin), antioxidant measures (NO, total antioxidant capacity), exhaled gasses (ex: CO, N_2O, CO_2), as well a variety of omics (genomics, transcriptomics, proteomics and metabolomics) investigations into biomarkers of BRD [12,49].

Traditional biomarker studies focus on a single, or a small number of biomarkers in combination to assess the ability of the marker(s) to detect BRD. Serum haptoglobin is among the most commonly used biomarkers for BRD in the literature. Multiple cutoff values for haptoglobin to define BRD cases have been suggested; 0.15mg/mL had a sensitivity and specificity of 0.64 and 0.71 respectively [50], whereas a cutoff of >0.81mg/mL reported sensitivity and specificity of 92.9% and 85.7% respectively [51]. Serum amyloid A has also been investigated repeatedly as a biomarker for BRD; similarly, variable sensitivity and specificity have been reported and the use of SAA alone to diagnose BRD has not been supported [52]. A variety of studies combine information from multiple biomarkers to increase the ability of biomarkers to detect BRD, however there is currently no consensus on what combination of biomarkers is ideal for diagnosing BRD.

Some of the newest biomarker research comes from the omics fields. These methodologies are promising for use as biomarkers due to their ability to take vast amounts of information from multiple simultaneously occurring processes in the host and/or pathogen and identify specific aspects of genetics, gene expression, proteins or metabolism that are associated with disease. This technology also has the potential to determine changes very early in disease, before clinical evidence of BRD develops. Though these techniques are not typically used in clinical practice yet, technology and research in these fields are rapidly evolving and may be available for clinical use in the future. A recent study demonstrated that metabolomics could be used to identify changes in blood metabolites associated with experimental infections with *M. haemolytica* and BRSV in calves, and the method provided a 95% sensitivity and 100% specificity for determining infected vs uninfected calves [53]. A study using transcriptomics found that differentially expressed genes could be used to identify calves subclinically infected with BRSV [54]. A multi-omics study used genomics, transcriptomics and metabolomics to assess BRD cases and controls, and was able to make inferences about genetic susceptibility to BRD, determine differentially expressed genes during BRD, and find associations with multiple metabolites in BRD [55].

Biomarkers inform clinicians and researchers on the pathophysiology of BRD and are important to our understanding of host-pathogen and host-environment interactions in BRD. However, there is no consensus on the use of any single or combination of biomarkers to diagnose BRD in clinical practice. It is also difficult to find manuscripts that compare biomarkers between calves with BRD to calves with other infections or inflammatory processes. In clinical practice, the use of biomarkers to aid in the diagnosis of BRD may be most useful in cattle populations where lung imaging is not possible or is not highly effective (such as in some beef cattle operations). As technology, particularly in the omics fields, improves these methods may become clinically useful as standalone diagnostics for BRD. As it stands currently, it seems reasonable to retain some degree of physical exam and imaging in the diagnosis of BRD and use biomarkers as an additional tool in the diagnostic investigation of BRD.

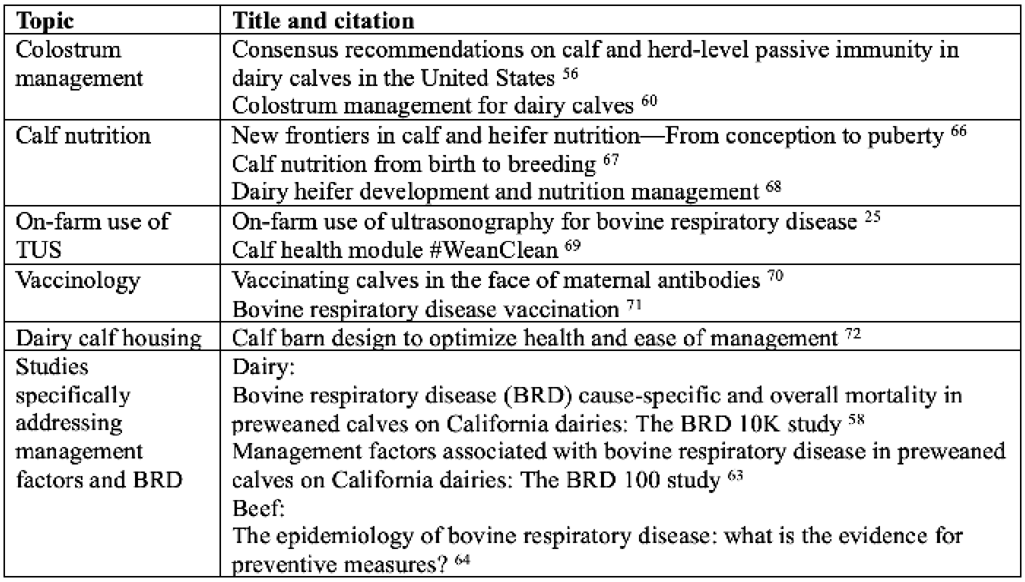
Diagnostics summary

Many tools are available to aid in the diagnosis of BRD in calves. A thorough physical examination is a valuable tool however this method often fails to diagnose subclinical disease and some aspects remain subjective. Lung imaging and diagnostic sampling are common ways to increase the veterinarian's ability to diagnose, categorize, and plan treatment for BRD. In calf populations where lung imaging can be readily used to examine the entire lung field (such as in pre-weaned dairy calves), TUS appears to generally outperform biomarkers and airway sampling for identifying current lung lesions antemortem; TUS allows direct visualization of diseased lungs providing a very high specificity of diagnosis [20], provides immediate results, can readily be used to identify subclinical BRD [15], can confirm and aid in the prognosis of clinical BRD [13,21], and is relatively fast and simple to perform for trained individuals [26]. However, in calf populations where TUS is not possible or cannot successfully image the cranial lung lobes, (such as in larger calves in feedlot settings), airway sampling and biomarkers appear to be particularly useful to aid in making a diagnosis of BRD. Some biomarkers may also have an advantage over TUS for early disease detection, before lung consolidation occurs [12]. As the study of biomarkers improves and accessibility to rapid testing platforms improves, particularly in the fields of genomics, transcriptomics and metabolomics, biomarkers may become more useful in a variety of clinical practice settings for the diagnosis of BRD. Airway sampling and biomarker analysis are important parts of the BRD diagnostic toolkit outside of their ability to define BRD cases. These methods add clinically important information such as pathogen identification, antimicrobial susceptibility profiles, and extensive information on the host's response to disease to aid in prognostication and general understanding of the pathophysiology of BRD in our clients' herds.

Management

Although diagnosing and treating animals with BRD are important parts of BRD management, prevention of BRD is far more impactful for animal health and welfare. A complete discussion on all factors known to be associated with BRD is beyond the scope of this session. Some clinicians may find it helpful to break down the investigation into all three parts of the epidemiologic triad (host, pathogen, and environment) and look for aspects of calf management in each category that may put calf health at risk. The management practices investigated will depend on the age of animals affected by BRD, the type of production system, and farm-specific practices. Broadly speaking, it may be helpful to investigate management practices related to the following non-exhaustive list of examples:

Calf (host): Passive immunity [56,57] (including all practices related to close up cow management [58] to monitoring passive immunity in calves[59]; in dairy calves this will likely also include practices related to colostrum collection, quality checking, storage and



Topic	Title and citation
Colostrum management	Consensus recommendations on calf and herd-level passive immunity in dairy calves in the United States [56] Colostrum management for dairy calves [60]
Calf nutrition	New frontiers in calf and heifer nutrition—From conception to puberty [66] Calf nutrition from birth to breeding [67] Dairy heifer development and nutrition management [68]
On-farm use of TUS	On-farm use of ultrasonography for bovine respiratory disease [25] Calf health module #WeanClean [69]
Vaccinology	Vaccinating calves in the face of maternal antibodies [70] Bovine respiratory disease vaccination [71]
Dairy calf housing	Calf barn design to optimize health and ease of management [72]
Studies specifically addressing management factors and BRD	Dairy: Bovine respiratory disease (BRD) cause-specific and overall mortality in preweaned calves on California dairies: The BRD 10K study [58] Management factors associated with bovine respiratory disease in preweaned calves on California dairies: The BRD 100 study [63] Beef: The epidemiology of bovine respiratory disease: what is the evidence for preventive measures? [64]

Table 1: Additional resources for calf health management and BRD prevention

administration practices [60]); vaccine practices and comorbidities.

Environment: Housing and air quality in calf housing and at calf level such as ammonia levels [61], dust/particulate matter, and drafts [61,62]; temperature extremes and adequacy of protection from heat/cold stress [63]; hygiene in and around calf housing; bedding type/amount/hygiene; stressors such as transportation [64,65], social stresses from group changes, weaning, or handling/processing/human interaction; nutrition and feeding practices [58,63] (volume, quality, access, time to adjust to feed changes, age-appropriate feeds, leaky nipples for bottle fed calves, etc.).

Pathogen: antimicrobial susceptibility, virulence, and consideration if vaccination is a useful practice for pathogen(s) involved.

A risk assessment tool has been created specifically for clinicians to help clients assess their risk factors for BRD in dairy calves, with the goal of working towards decreasing the risks by implementing incremental change and continually reassessing [59]. This tool is available within a BRD scoring app available at the app store called, 'UC Davis BRD'; the risk assessment tool can be found in the app under 'Menu' then 'Risk Assessment'. Additional resources that the reader may find helpful for calf health management related to BRD prevention are listed in Table 1.

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NOTES PERSONNELLES

Development of a classification system for lung ultrasonographic findings in calves based on the association with cure and production outcomes

Stan Jourquin[1,2*], Thomas Lowie[2], Florian Debruyne[1], Laurens Chantillon[1], Mathilde L Pas[1], Justine Clinquart[1], Randy Boone[3], Jade Bokma[1,3], Bart Pardon[1,2]

[1]Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

[2] qTUS, De Vage 7, 8340 Damme, Belgium

[2]Veterinary Practice Venhei, Geelsebaan 95-97, 2460 Kasterlee, Belgium.

Objectives: The primary objective of this study was to determine associations between ultrasound based classification systems (using maximum consolidations depth, number of quadrants with consolidation and location of consolidation) and clinical indicators (factors) and ultrasonographic cure, average daily gain (ADG) and cold carcass weight (CCW) in veal calves (outcomes).

Materials and methods: A retrospective cohort study was conducted on 1090 white veal calves originating from three Belgian veal calf herds. Clinical scoring (Wisconsin score) and quick thoracic ultrasound (qTUS) follow-up were done at four points during production, namely, arrival (week (wk) 0), at the start of the first metaphylactic treatment with doxycycline (outbreak, wk1), at the end of this metaphylactic treatment (short-term, wk3) and on a long-term evaluation point (wk10-12). Cure was defined as complete reaeration of previously consolidated lung tissue. At each time point, calves were assigned to a qTUS category based on maximum consolidation depth: ‘healthy’ (no consolidation), ‘mild pneumonia‘ (consolidation <1 cm), ‘moderate pneumonia’ (consolidation 1-2.5cm), and ‘severe pneumonia’ (consolidation ≥3cm). Additionally, the number of affected quadrants (1–4), craniocaudal (cranial, caudal or both) and bilateral location of consolidations (left, right or both) were registered.

Results: At the outbreak, 46.9% of the calves were healthy, while 12.4%, 21.4% and 19.4% had mild, moderate or severe pneumonia, respectively. After metaphylaxis (wk3), short-term cure was 59.3% for calves with mild pneumonia, 50.2% for calves with moderate pneumonia, and 32.7% for calves with severe pneumonia, resulting in a total short-term cure of 45.9%. At the long-term evaluation point (week 10-12), final cure was 59.3%, 55.8% and 40.3% for calves with mild, moderate and severe pneumonia at the outbreak, respectively. Both increasing qTUS category and number of affected quadrants resulted in significantly decreased short- and long-term cure. Multivariable regression models indicated that different interactions between qTUS categories and number of affected quadrants were associated with short- and long-term odds of cure, resulting in three ultrasound based classification systems. Calves with clinical pneumonia (Wisconsin score ≥5 and lung consolidation ≥1cm) had decreased short-term odds to cure upon treatment compared to calves with subclinical pneumonia (Wisconsin score < 5 and lung consolidation ≥1cm) (Odds ratio (OR)=0.43; 95% Confidence interval (CI): 0.27- 0.67). For calves with severe pneumonia in wk10-12, ADG and CCW were reduced by 104 g/day (Standard deviation (SD)= 13; 95%CI: .078- .130) and 7.1kg (SD=1.9; 95%CI: 3.4-10.9), respectively.

Conclusion: Categorization based on maximum consolidation depth, the number of affected quadrants or an integrated system of both have the potential to be included in an ultrasound scoring system. However, based on the practicality for recording, reporting and follow-up, the authors recommend categorizing calves into mild, moderate and severe pneumonia based on maximal consolidation depth measurement, referred to as the qTUS score, as an accessible classification system for ultrasonographic lung assessment in calves.

Clinical, laboratory and ultrasonographic findings in 21 adult cattle with acute infectious bronchopneumonia

Nicolas Masset[1], Sébastien Assié[2], Lisa Courtois[1], Romane Gindrey[1], Claire Marget[1], Vincent Herry[1]

[1]SELAS EVA, Réseau Cristal, Argentonnay, France
[2]Oniris, INRAE, BIOEPAR, Nantes, France

Objectives

Bronchopneumonia due to airborne pulmonary infections by viruses and bacteria remains a major health concern primarily affecting young cattle. However, in recent years, veterinarians have reported an increase in cases among adult cattle. These observations are now confirmed by several necropsy studies in the literature, which show a significant proportion and/or an increase in cases of bacterial bronchopneumonia in adults, particularly due to *Mannheimia haemolytica*. However, the clinical diagnosis of pneumonia in adult cattle remains challenging due to the lack of well-defined clinical descriptions specific to adult cattle. Many diagnostic criteria have been extrapolated from knowledge of bronchopneumonia in young cattle. The objectives of our study were threefold: to describe the signalment, clinical presentation, laboratory tests, thoracic ultrasonography (TUS) and follow-up of cases of cows diagnosed with acute bronchopneumonia, to identify causative pathogens, and to identify potential factors associated with outcome.

Material and methods

An observational prospective cohort study was conducted in a private practice in France between 2022 and 2023. Inclusion criteria were: cows ≥ 2 years old, hyperthermia (≥ 39.0°C), tachypnea (≥ 36 mpm), and/or abnormalities at TUS. Signalment and history of cows were collected. At Day 0, a complete clinical and TUS examinations (bilateral, from 4th to 10th intercostal space (ICS)) were performed. The depth of consolidation lesions, depth of effusion and number of B-lines according to ICS and dorsoventral localization were recorded. Blood samples were collected for complete blood count analysis. Nonendoscopic unguarded bronchoalveolar lavage (BAL) samples were performed for pathogen identification (bacterial culture and PCR). Clinical and TUS follow-ups were performed on Day 5 and Day 10. A descriptive statistical analysis was performed. Regardless of their distributions, quantitative variables were characterized by means, SDs, medians, and ranges, and qualitative variables were characterized by frequency of occurrence. Cases were grouped, and comparisons between unfavorable outcomes (death, poor milk yield, early dry-off, early culling) and favorable outcomes (recovery with milk yield recovery, no anticipated culling) were conducted. Univariate statistical analyses were performed to identify variables (clinical, laboratory and ultrasonographic) associated with outcome. The Fisher's exact test was used to evaluate the association between the categorial variables of the 2 groups. The t-test or the Wilcoxon test was used to evaluate the statistical association between the continuous variables of the 2 groups depending on whether normal distribution was present or not, respectively. Statistical significance was set at $P \leq 0.05$.

Results

Twenty-one cows (17 dairy; 4 beef) were included in the study. The median parity was 2 (range, 0-5) and the median age was 3.8 years old (range, 2-8.6). The reasons for consultation were: respiratory diseases (8), milk drop (7), depression (6), anorexia (3) and hyperthermia (3). Ten cases occurred within the 60 days after calving. On inclusion, the most common clinical signs were: poor ruminal fill score (20), modified breathing sound intensity (19), nasal discharge (16), tachycardia (15), and expiratory dyspnea (14). Cough, wheezes, and crackling sounds were only audible in 2, 4 and 2 cows respectively. A left shift of the neutrophils was observed in 17/21 cows. At Day 0, 17/21 cows had consolidation lesions (3 with maximum lesion depth between [1; 3 cm[, 9 between [5; 10 cm[and 5 ≥ 10 cm) and 13/21 had thoracic effusion (4 with maximum lesion depth between [1; 3 cm[, 4 between [3; 5 cm[and 5 ≥ 5 cm).

Bacteria isolated by culture from BAL samples were *Mannheimia haemolytica* (7), contaminated (5), negative (5), *Pasteurella multocida* (2), *Bibersteinia trehalosi* (1), *Bacillus spp* (1) and *Proteus mirabilis* (1) whereas bacteria detected by PCR were *Pasteurella multocida* (18), *Mannheimia haemolytica* (13), *Mycoplasma bovis* (3) and *Histophulis somni* (3). Bovine respiratory syncytial virus was detected in only one sample, and neither bovine parainfluenza 3 virus nor bovine coronavirus was detected.

Based on univariate statistical analysis, cases exhibiting nasal discharge or having abnormal ruminal contraction frequency were more likely to have an unfavorable outcome. Cases with favorable outcomes had significantly less deep consolidation lesions at Day 0 and at Day 5, and a lower respiratory rate at Day 0 than those with unfavorable outcomes.

Conclusions

These findings may help practitioners to diagnose acute infectious bronchopneumonia in adult cows more accurately. Pasteurella species are the main bacteria responsible for acute cases and *Mannheimia haemolytica* is indeed a major primary pathogen associated with BRD in adult cattle. TUS proves to be a valuable cow-side diagnostic tool, allowing visualization of specific lung abnormalities, to confirm the diagnosis of bronchopneumonia. Moreover, TUS provides critical prognostic information by measuring the depth of consolidation lesions.

Procalcitonin as a diagnostic biomarker in bovine respiratory disease: Associations with lung lesions and etiological agents in preweaned dairy calves

Sala Giulia[1,2], Boccardo Antonio[3], Irene Ciabattini[1], Ferrulli Vincenzo[3], Meucci Valentina[1], De Marchi Lucia[1], Sgorbini Micaela[1,2], Pravettoni Davide[3*], Bonelli Francesca[1,2]

[1]Department of Veterinary Sciences, University of Pisa, via Livornese s.n.c., San Piero a Grado, 56122, Italy
[2]Centro di Ricerche Agro-ambientali "E. Avanzi", University of Pisa, San Piero a Grado (PI), 56122, Italy
[3]Department of Veterinary Medicine and Animal Sciences, University of Milan, via dell'Università 6, 26900, Lodi, Italy
Corresponding author: Giulia Sala, giulia.sala@unipi.it

Objectives:

Bovine respiratory disease (BRD) is a multifactorial condition with significant economic and animal welfare implications in dairy farming (Buczinski and Pardon, 2020). Accurate ante-mortem diagnosis remains challenging due to non-specific clinical signs and difficulties in differentiating between upper and lower respiratory tract disease (Buczinski and Pardon, 2020). Procalcitonin (PCT) has been suggested as a potential biomarker for BRD, though studies on this are limited (El-Deeb et al., 2020; Sala et al., 2024). This study evaluates the association between PCT, thoracic ultrasound (TUS) findings and the isolation of etiological agents through transtracheal wash (TTW).

Materials and Methods:

A total of 240 Italian Holstein calves (1-3 months of age) from 10 dairy farms in Lombardy, Italy, were evaluated using TUS (ethical approval number 104/2020, January 15, 2020). n each of the 10 farms enrolled for BRD diagnostics, 6 animals underwent TTW, for a total of 60 animals included in this study, as described by Pravettoni et al. (2020). Bacteriological analysis was conducted using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), while viral pathogens and *Mycoplasma bovis* were identified using Polymerase Chain Reaction (PCR). Additionally, blood samples were collected in lithium heparin tubes, and plasma was obtained by centrifugation and frozen for subsequent analysis. PCT analysis was performed on both plasma and TTW samples using a commercial bovine ELISA kit previously validated for cattle. The animals were classified into different categories based on TUS results and etiological findings. For ultrasound assessment, a 1 cm lesion cut-off was used to differentiate animals with and without BRD (Buczinski et al., 2015). Regarding etiology, animals were categorized as healthy, bacterial infection, viral infection, or mixed infection. A combined classification was also performed, grouping animals into healthy (negative TUS and negative etiology), ultrasound lesions (positive TUS and negative etiology), infection without ultrasound lesions (negative TUS and positive etiology), and BRD animals (positive TUS and positive etiology). Descriptive statistics were performed, with categorical variables expressed as frequencies and percentages, and continuous variables as median and interquartile range (IQR). Non-parametric tests (Mann-Whitney U, Kruskal-Wallis, and Bonferroni post hoc correction) were used to assess differences between groups. Statistical significance was set at $p < 0.05$.

Results:

TUS identified 80% (48/60) of calves as positive using a ≥1 cm lung consolidation threshold. TTW revealed bacterial isolation in 51.7% of cases, with*Pasteurella multocida* (37%) and*Mycoplasma bovis* (13%) being the most prevalent. Viral isolation was limited to bovine Coronavirus spp. (15%) and bovine respiratory syncytial virus (7%). Plasma PCT showed a significant difference between TUS-negative calves (56.0 pg/ml, IQR 68.5 pg/ml) and TUS-positive calves (84.7 pg/ml, IQR 60.1 pg/ml; p-value 0.016). Furthermore, plasma PCT differed between calves with viral (121.2 pg/ml, IQR 170.7 pg/ml) and bacterial (80.8 pg/ml, IQR 45.9 pg/ml) infections compared to calves without pathogen isolation (50.3 pg/ml, IQR 97.3 pg/ml; p-value 0.012). Considering TUS and etiological analysis, plasma PCT differed between healthy calves (40.0 pg/ml, IQR 37.0 pg/ml) and BRD-affected animals (87.0 pg/ml, IQR 59.4 pg/ml; p-value 0.012). However, PCT levels in TTW fluid showed variability and no statistical significance, limiting its diagnostic consistency.

Conclusions:

PCT demonstrated potential as a biomarker for assessing BRD, correlating with TUS findings and the detection of etiological agents. Blood PCT levels were particularly valuable in differentiating healthy from diseased calves, while methodological challenges persisted in the quantification of PCT in TTW samples. Further studies are needed to validate PCT cut-off values for clinical application in BRD diagnosis and prognosis.

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Identifying Asymptomatic Salmonella Dublin Carriers: Can Lymph Node Sampling Improve Detection?

BLOCKX Zoé[1], EVRARD Julien[1], SMEETS Frederic[1], GREGOIRE Fabien[1] and HOUTAIN Jean-Yves[1]

[1] Regional Association for Animal Registration and Health (ARSIA) asbl, Ciney, Belgium Corresponding author: zoe.blockx@arsia.be

Objective

Salmonella enterica subspecies *enterica* Dublin (SD) is the primary serotype affecting cattle in Walloon herds, posing significant risks to both animal and public health. Asymptomatic carrier animals are key to the persistence and spread of SD within herds, making their detection critical for effective control measures. However, current faecal culture methods have limited sensitivity, particularly in adult cattle. A preliminary study conducted at ARSIA indicated that bacteriological culture of superficial lymph nodes on septicemic calves may provide higher sensitivity than faecal culture, offering a potential solution to improve carrier detection.

The objectives of this study are to evaluate lymph node culture in identifying asymptomatic SD carriers in live cattle and to assess the feasibility of integrating this method into pre- purchase testing and herd sanitation protocols. Additionally, the study aims to identify SD carriers among seropositive animals to enhance eradication efforts and mitigate economic losses in affected herds.

Material and Methods

The study involves two types of sampling: blood collection for serology (dry tube) and 16G- needle aspiration of the pre-scapular lymph node following site disinfection with alcohol.

Serological analysis will be conducted using the PrioCHECK® Salmonella Ac bovine ELISA [Thermo Fisher Scientific], applicable to serum, milk, or bulk tank milk. The lymph node aspirate is transferred upon collection into a pre-enrichment medium, following bacteriological culture for *Salmonella* according to the ISO 6579-1 standard.

The study is divided into three parts: (1) longitudinal follow-up of seropositive purchases, (2) herd assessment and detection of asymptomatic carriers, and (3) cohort monitoring for chronic carrier detection. In part one, pre-scapular lymph node aspiration will be proposed for animals with high seropositivity ($S/P \geq 80$) during pre-purchase testing, followed by quarterly serological monitoring over one year. Part two involves three herd screenings at six-month intervals, with lymph node cultures performed on seropositive animals to assess targeted culling strategies. In part three, cohorts of 20 calves from herds with recent salmonellosis diagnoses will undergo monthly serology and lymph node aspirations for three months.

Results

Initial validation was performed on calves that died from *Salmonella Dublin* septicemia to compare culture results from lymph node imprints with needle aspiration of the same nodes.

This validation involved palpation and aspiration of the pre-scapular lymph nodes through the skin to simulate field conditions. A perfect correlation was observed between the needle aspiration and lymph node imprints, with all 10 pre-scapular lymph nodes testing positive for both methods.

During the field phase, a total of 250 lymph node aspirations were conducted across the different parts of the study. Despite targeting animals with high seropositivity and herds with recent salmonellosis diagnoses, no positive cultures were obtained. These findings suggest that either the sensitivity of lymph node aspiration is lower than anticipated in live animals, or the prevalence of asymptomatic carriers within the sampled populations is lower than expected.

Conclusions

Although promising results were obtained in the preliminary study on septicemic calves, the absence of positive cultures in the field phase, despite targeting seropositive animals, suggests that the sensitivity of this method in live animals may be lower than anticipated or that asymptomatic carrier prevalence is limited in the sampled population.

These findings highlight the challenges of detecting asymptomatic *Salmonella Dublin* carriers in cattle and suggest that lymph node aspiration alone may not be sufficient for routine surveillance. Further investigation is needed to refine sampling techniques, identify high-risk subpopulations, and explore complementary diagnostic approaches to enhance detection sensitivity in live animals.

The comparison of production parameters in BVDV endemically infected beef suckler herds before and after eradication including use of a live double-deleted BVDV vaccine

M. Yarnall [a], E. Schmitt- van de Leemput [b], M. Cerviño[c], R. Prieto[c], A. Bolon[d]

[a] Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany
[b] Bovilogique, Villaines la Juhel, France
[c] Boehringer Ingelheim Animal Health España, Barcelona, Spain
[d] Boehringer Ingelheim Animal Health France, Lyon, France

Objectives: BVDV impacts health and performance of bovine herds. In the present study, the impact of BVDV eradication on production performance of suckler herds was tested, using an eradication protocol including vaccination of all female cows of the herd on the same day (DV). Method : performances of cows of two commercial suckler herds was analysed during 12 months before DV (PREVAC, n = 497 cows) and during 9 to 21 months after vaccination (POSTVAC, n = 531 cows).

Results : the proportion of calves weaned compared to the initial number of cows subjected to mating did not differ ($P = 0.2928$) between PREVAC (71%) and POSTVAC (74%). Neither did the proportion of calves born compared to the initial number of cows subjected to mating (PREVAC and POSTVAC, 87% and 84% respectively, $P = 0.3584$). However, the proportion of calves weaned compared to the initial number of calves born was higher POSTVAC (87%) when compared to PREVAC (81%), ($P = 0.0173$), *i.e.* a difference of
6% of weaned calves. Conclusion : data demonstrate that a BVDV eradication protocol using vaccination in suckler herds, improves calf survival and the number of weaned calves at herd level. This study contributes to the knowledge of the benefits of eradication of BVDV in suckler beef herds. The implementation of a BVDV eradication strategy, including vaccination with a live double-deleted vaccine, rapidly improves productivity on endemically infected farms, by improving the number of calves weaned. It would be interesting to include this finding in an overall economical model for the benefits of BVDV eradication schemes in suckler herds.

Six years of *Mycoplasma bovis* serological surveillance in Northern Italy (Emilia-Romagna region, 2019-2024)

Prosperi A., Torreggiani C., Garbarino C.A., Gelmini L., Pupillo G., Zanni I., Chiapponi C., Luppi A.

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy – alice.prosperi@izsler.it

Objectives:

Mycoplasma bovis is the etiological agent of several diseases affecting cattle herds worldwide, such as bronchopneumonia, mastitis, otitis, arthritis and more. Although it is a non-zoonotic pathogen, *Mycoplasma bovis* affects animal health and welfare in all sectors of cattle farming (beef, milk, or rearing) and causes serious economic effects. Economic losses due to *Mycoplasma bovis* respiratory disease in the USA have been estimated to reach up to 108 million US dollars per year (considering a 70% infection rate of a heard).

Bovine Respiratory Disease Complex (BRDC) is one of the costliest diseases affecting cattle farms worldwide, with several species within the *Mycoplasmataceae* family involved in BRDC development. The major representative of these species is *Mycoplasma mycoides* subsp. *mycoides* (largely eradicated from the developed world), followed by *Mycoplasma bovis*. However other mycoplasmas have been isolated from pneumotic cattle, including *Mycoplasma dispar*, *Ureaplasma diversum*, *Mycoplasma bovirhinis* and *Mycoplasma canis*.

Although the pathogenetic role of *Mycoplasma bovis* is recognized, its prevalence during BRDC is certainly underestimated due to the presence of other pathogens (such as the members of the *Pasteurellaceae* family) that are much easier to isolate during calf pneumonia outbreaks. Finally, to the best of the authors' knowledge, this is the first report on *Mycoplasma bovis* seroprevalence in North Italian dairy farms.

Materials and Methods:

The survey was conducted on herds randomly selected in four Provinces (Piacenza, Parma, Reggio-Emilia, and Modena), which are have the highest abundance of dairy farms within the Emilia-Romagna region (Northern Italy), between years 2019 and 2024.

The seroprevalence of *Mycoplasma bovis* antibodies was assessed using 3293 serum samples, collected from 233 dairy farms. Samples were sent to the Parma diagnostic laboratory of the Istituto Zooprofilattico Sperimentale of Lombardia and Emilia-Romagna for diagnostic purposes; the sera were analyzed using a commercial indirect ELISA test, following the manufacturer instruction.

Results:

The seroprevalence of *Mycoplasma bovis* antibodies evaluated during the study appears to be high, with an overall seroprevalence of 46.7% among the analyzed samples. Considering the results aggregated by Provinces, the highest seroprevalence was observed in sera collected in Piacenza province (66.7%), followed by 49.2% in Parma, 47.5% in Reggio-Emilia, and 26.8% in Modena Province. Analyzing the data by year, the relative seropositivity observed was as follows: 70.2% in 2019; 28.3% in 2020; 38.6% in 2021; 50.2% in 2022; 35.1% in 2023; 36.9% in 2024.

Conclusions:

In enzootically infected areas, *Mycoplasma bovis* is widely distributed, although it is not considered an ubiquitous pathogen. The introduction of this etiological agent into a naïve herd usually occurs via clinically healthy calves or young animals shedding the pathogen. Once established on multi-age sites, eradication becomes extremely challenging. In farms already affected by low-grade respiratory disease, the presence of *Mycoplasma bovis* can significantly increase both morbidity and mortality. Infected animals act as reservoirs of infection, shedding the pathogen via the respiratory tract for months or even years.

During the study, the comparatively higher seroprevalence observed in 2019 (70.2%) was significantly reduced in subsequent years. This decrease may be attributed to improved farm-level surveillance plans. However, the convenience sampling method employed in the study might not provide a fully representative picture of *Mycoplasma bovis* circulation in the monitored dairy farms.

Given the challenges in isolating *Mycoplasma bovis* from treated animals or chronical affected cattle, serological detection often proves to be a more reliable diagnostic tool. This is particularly true because antibody levels detected via ELISA tests can persist for several months. Moreover, in Italy, until November 2024, no registered vaccines against *Mycoplasma bovis* were commercially available. Consequently, the seropositivity detected during the study period provides a realistic picture of the prevalence of this pathogen in Northern Italian dairy farm.

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Evaluation of *Mycoplasma bovis* diagnostic tools in relation to management in endemic farms in Wallonia

Seys C.[1], Gille L.[2], Evrard J.[3], Bayrou C.[1]

[1] Bovine Health Service, Clinical Departement of Production Animals, ULiege, Liege, Belgium

[2] MSD, Animal Health, Watermael-Boitsfort, Belgium

[3] Regional Association for Animal Registration and Health (ARSIA) asbl, Ciney, Belgium
Corresponding author: calixte.bayrou@uliege.be

Objectives: Currently, *Mycoplasma bovis* is widely present in European farms, causing significant economic losses. In the absence of effective treatments, prevention remains the main approach to limit the impact associated with this bacterium. In Wallonia, the Regional Association for Animal Health and Identification (ARSIA) is actively involved in preventing this disease through two key measures: (i) detecting carrier animals using bacterial detection methods (culture or PCR) and combined serology (based on IgG2 detection) that can lead to the cancellation of a sale when a bovine is identified as seropositive (>80, MilA ELISA, Bio-X Diagnostics) during purchase blood sampling; (ii) offering an autovaccine developed from *M. bovis* strains isolated from the farm. Although the diseases associated with *M. bovis* presence in farms are well-documented, few studies describe the parameters of farms where *M. bovis* is endemic. The objective of this study is to characterize farms with a known endemic status using the diagnostic tools used in the control of *M. bovis*. An assessment of key management elements in these farms, including autovaccination, was conducted to evaluate their relationship with diagnostic results.

Material and methods: Twenty-three farms with endemic *M. bovis* (according to ARSIA data) were evaluated using questionnaires on their overall management practices (clinical signs, environmental and animal management, feeding, supplementation, vaccination, biosecurity, preventive measures) as well as through culture, PCR, and serology (IgG2) on randomly collected samples across different age groups.

Results: Despite the endemic presence of *M. bovis* in these farms, the serological behavior of calves under 120 days varies between farms. In nearly half of the farms (11/23), calves remain seronegative (<80), whereas in the other farms (12/23), seropositivity appears early in the calves' lives. The factors significantly differentiating the two groups ($p<0.05$) are the association of positive culture and PCR results, as well as better body condition scores among adults in farms where calves are seropositive. No other management elements, including autovaccination, are significantly associated with calves exceeding the seropositivity threshold before 120 days.

Conclusion: This study demonstrates that serology (evaluated by the MilA ELISA at a threshold of 80) is not a sufficient tool to detect the endemic status of a farm in calves under 120 days old, as half of the farms are seronegative. The positive association between seropositivity before 120 days and positive PCR results suggests that animals are more likely to be seropositive when
M. bovis is actively circulating within these age groups. The absence of a link between autovaccination and seropositivity is noteworthy and requires further investigation. One hypothesis is that vaccination results in only a modest increase in IgG2 compared to IgG1, making it less detectable by an IgG2-specific ELISA.

Clinical manifestation of Bluetongue serotype 3 in ruminants in Flanders, Belgium

van Mol, W. [1], Van Praet, W. [1], Van Mael, E. [1], Debouvere, J. [1], De Bleecker, K. [1], Van de Wouwer, E. [1], Ribbens, S. [1]

[1]**Animal Health Service Flanders, Industrielaan 29, Torhout (Belgium)**

Objectives

Following the 2023 outbreak of Bluetongue serotype 3 (BT3) in the Netherlands, Belgium endured a similar outbreak in the summer of 2024. Within weeks, all regions reported confirmed cases of BT3. Whereas Belgium had regained its bluetongue free status in 2023 and had no history of contact with this serotype, no immune animals were present. Consequently, there was limited knowledge about the associated clinical signs and impacts. The objective of this paper was to estimate the herd-level impact of BT3 in Northern Belgium, i.e. Flanders.

Material and methods

A survey was sent to all registered owners of cattle (n=9.142), sheep (n=15.945), goats (n=4.295) and camelids (n=495) with a known e-mail address in Flanders. The number of present animals within each herd was questioned, as well as the observed clinical signs, vaccination history against BT3, and prevention attempts. Clinical signs were reported as ordinal estimates of the observed prevalence: ‘not observed’, ‘0-5%’, ‘5-20%’, ’20-40%’, ’40-60%’, ’60-80%’ and ’80-100%’. The survey addressed general clinical signs, abnormalities of the head, gastrointestinal, respiratory, and locomotory system problems, reproductive performance, and udder health.

Descriptive statistics were performed with Excel (Microsoft, USA). Multiple correspondence analysis (MCA) was performed with the FactoMineR (Le et al., 2008) package in Rstudio (R Core Team, 2024) in order to identify possible clusters within dairy and beef cattle.

Results

In total, 3.650 (12,4%) surveys were completed. Dairy cattle was reported on 906 farms and beef cattle on 774. Among the respondents, 2.179 had sheep, 637 had goats, and 139 had camelids. Confirmed or suspected BT3 was reported by 63.3% of the participants.

The majority of dairy cattle owners (30,9%) reported clinical diseases in 0-20% of their herd. Additionally, 20,1% reported clinical disease in 20-40% of their animals, 11,8% in 40-60%, 6,5% in 60-80%, and only 1,8% in 80-100%. The most commonly reported clinical signs were milk drop, lameness, premature calving, hypersalivation, clinical and subclinical mastitis and abortions. Thus, production was directly affected by a lower production and increased somatic cell count, as well as indirectly by increased prevalence of clinical disease.

Beef cattle showed less variation in disease prevalence, with 42,4% of owners reporting clinical disease in 0-20% of their animals and 45,6% reporting no observed diseases. The most observed clinical signs were similar to those in dairy cattle: lameness, premature calving, abortion and hypersalivation.

Sheep owners reported a high variation in sick animals: 23,2% noted that 0-20% of their herd was sick, followed by 12,8% (20-40%), 10,4% (40-60%), 7,3% (60-80%) and 12,5% (80-100%). The increase in the last group is likely due to the high number of flocks with only a few animals. The most reported problems in sheep were lameness, dyspnea, hypersalivation and death with precedent disease.

MCA results showed that clustering of low prevalences of clinical signs (0-5%) explained the highest fraction of variability. The clinical signs contributing the most were lesions of the head and locomotory system. The subsequent cluster contained high prevalence (80-100%) of the same observed clinical signs. Highlighting that most often relatively few numbers of animals showed any typical sign, although some herds had highly prevalent clinical disease. The variability in disease prevalence between herds could not be explained. No significant clustering of clinical signs was observed.
Almost half of the respondents (47,5%) stated to have vaccinated their flocks. Among those wo did not vaccinate, the most common reasons were late availability of the vaccine, lack of awareness about the option or distrust in the vaccine. In cases where herds were vaccinated but still showed clinical disease, this occurred in in 68,4% of instances within 3-4 weeks after basic vaccination. The number of cases were immune herds still had clinical disease was too small to have any conclusions about the vaccine's efficacy from this survey.

Conclusions

Dairy cattle and sheep herds have reported high variability in clinical manifestation of BT3, as well as in observed clinical signs as in numbers of affected animals. In dairy cattle, production was directly affected by milk drop and decreased udder health and indirectly due to clinical disease. In sheep, mortality was reported as a frequent observation next to clinical disease. Within beef cattle, lower variation in disease prevalence was reported. MCA showed clustering in disease prevalence, supporting the variability in disease prevalence between herds.

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Herd- and animal-level bluetongue antibody prevalence and risk factors in Dutch dairy cattle herds after the 2023 bluetongue serotype 3 epidemic

Katrien M.J.A. van den Brink[1], Jet Mars[1], Nannet D. Fabri[1], Anouk M.B. Veldhuis[1], René van den Brom[1], Carlijn ter Bogt-Kappert[1], Inge M.G.A. Santman-Berends[1]

[1] Royal GD, Deventer, The Netherlands

Introduction

In September 2023, bluetongue virus serotype 3 (BTV-3) emerged in the Netherlands for the first time. Within a few months more than 3,000 cattle herds became infected and farmers reported severe clinical signs, including reduced milk production and increased mortality. After the 2023 epidemic, it became crucial to assess the prevalence of BTV-3 and to identify risk factors associated with BTV-3 infections to support farmers in preparation for the 2024 vector season, as the possibility of BTV-3 overwintering was considered realistic.

Material and methods

Prior to this study, a commercially available ELISA from IDvet used for bulk tank milk (BTM) screening for BTV antibodies was adapted and validated at Royal GD to enable estimation of the within-herd animal level prevalence based on one BTM sample. Subsequently, in this study, a BTM sample was collected from all ~13,500 Dutch dairy herds between January and April 2024 and tested for BTV antibodies. Additionally, participating farmers were asked to fill in a survey about their herd management in 2023 and whether they experienced BTV-3 cases (confirmed and/or clinical). Data from herds that were vaccinated against BTV in the past five years were excluded as vaccine induced antibodies would have interfered with the test results. During the study period a vaccine against BTV-3 was not yet available. Based on the BTM results, an indication of the herd-, within-herd- and animal-level prevalence on region and country level after the 2023 epidemic was estimated. ELISA results were combined with the survey results. Logistic regression models were used to identify management factors associated with I) presence or absence of antibodies in BTM and II) if antibodies were found, with a within-herd antibody prevalence below or above 50%.

Results

Antibodies were found in BTM samples of 64% (95% CI: 63-65%) of 12,756 non-vaccinating Dutch dairy herds. In these antibody positive herds, an average within-herd prevalence of 36% (95% CI: 35-37%) was estimated. In the region where the BTV outbreak started in September 2023, nearly all herds tested BTV antibody positive. On national level, we estimated that the animal prevalence was 23% (95% CI: 22-24%), with a large variation between herds and regions.

For 5,253 dairy herds both ELISA results and data from the survey were available. Factors associated with a lower odds for the presence of antibodies included keeping cattle indoors, large ventilation openings in the barns and mechanical ventilation during housing. Factors associated with higher odds for the presence of antibodies after 2023 included purchase of cattle from BTV-3 high-risk areas, being located in a high risk area, medium or high intensive grazing, and presence of sheep in the farm with clinical signs related to BTV-3. In antibody positive herds, keeping cattle indoors, large ventilation openings in the barn combined with windbreak curtain, location of the farm in a low-risk area and larger herd sizes were associated with lower odds for a high within-herd prevalence. The use of insecticides was not associated with presence nor the level of antibodies against BTV in dairy herds.

Conclusion

This study assessed the antibody prevalence of BTV in all Dutch dairy herds in the first months of 2024 and identified management practices that protect against BTV-3 infection. While most dairy herds have been exposed to BTV-3 in 2023, the prevalence of infection at the individual animal level remained relatively low, suggesting that many animals were still susceptible to BTV-3 in 2024. Several management practices were identified as being associated with a reduced risk and spread of BTV infection, offering farmers additional strategies to mitigate the impact of BTV-3 in their herds beyond vaccination.

High efficacy in Sheep of Bultavo 3 vaccine against BlueTongue virus serotype 3

Jiří Nezval[1], Milan Huňady[1], Lucie Střelcová[1], Martin Tkáč[1], Juraj Kučerák[1], Edmond Jolivet[2], Laure Mouton[2], **Mathieu Chevalier**[2], Hélène Gaude[2]

[1] Bioveta, Czech Republic
[2] Boehringer Ingelheim Animal Health, France

The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary diseases affecting ruminants. The recent BTV serotype 3 (BTV3) outbreak, rapidly spreading through Europe, has a significant impact on animal health and is causing severe losses for farmers. In the absence of cross-protection by existing vaccines, a BTV3 vaccine accelerated development was triggered to respond to this emergency. This new vaccine has been industrialized for mass production and tested to confirm efficacy against the currently circulating BTV3 strain.

BTV3 inactivated vaccines were administered to 3 groups of 6 lambs (26 to 31 days old). The vaccines contained 3 different antigen payloads and were adjuvanted with aluminum hydroxide and saponin. One group of 6 non-vaccinated animals served as control. Vaccinated animals were monitored for possible local and systemic reactions. All animals were challenged 21 days after vaccination with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. The rectal temperature and clinical signs were monitored daily for 16 days in all animals and viraemia was measured by a BTV3 specific qRT-PCR.

No vaccinated animal displayed local or systemic reactions after the vaccination in any of the 3 vaccinated group during the observation period. Following challenge, clinical signs of the BTV disease were observed in all control group animals. One animal of the control group had to be euthanized on ethical grounds. All vaccinated animals were protected from development of clinical signs of BTV3 disease, regardless of the administered dose. Presence of BTV3 RNA was detected in blood samples from all control animals and in two animals from the low-dose vaccinated group and in one animal from the mid-dose vaccinated group. In the group vaccinated with the highest antigen content, no viremia was detected in any animal.

In conclusion, the safety and efficacy of this new vaccine against BTV3 was successfully demonstrated after challenge in lambs. Bultavo 3, with a 1-shot regimen for sheep, is suitable for active immunization of target species older than 21 days of age and resulted in the prevention of clinical signs and mortality and the reduction of circulating virus in the blood of vaccinated animals. Vaccination with Bultavo 3 significantly reduces the risk of disease transmission through midge bites. With this new vaccine, BTV3 outbreaks can be prevented, and farmers can protect not only their herds, but also their livelihoods.

Abortion and congenital abnormalities due to bluetongue virus serotype 3 in southern Belgium

DELOOZ Laurent[1,2], EVRARD Julien[1], PETITJEAN Thierry[1], SMEETS Frederic[1], GREGOIRE Fabien[1], SAEGERMAN Claude[2]

[1] Regional Association for Animal Registration and Health (ARSIA) asbl, 5590 Ciney, Belgium

[2] Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULiège), Fundamental and Applied Research for Animals & Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium

Corresponding author: laurent.delooz@arsia.be

OBJECTIVE

Bluetongue virus serotype 3 (BTV-3) has emerged in the Netherlands in September 2023 and caused severe clinical signs in ruminants including abortions. Some weeks later, the virus was also detected in 6 different herds in the province of Antwerp, which borders the Netherlands to the north of Belgium. Thanks to the local winter conditions, which are not favorable to vectors, the virus had not directly spread to the rest of the Belgian territory. Winter 2023-2024 was quiet and no new outbreak was detected in Belgium. Nevertheless, the 9th July 2024, the first outbreak of the year was detected in the south-east part of Belgium. This was the beginning of an historic bluetongue disease epizootic. BTV-3 was confirmed by PCR in different species including cattle, sheep, goats and camelids. A significant increase in mortality rate was observed in the bovine population and also, an outbreak of abortions with a high rate of congenital anomalies in fetuses which will be discussed in this work.

MATERIAL AND METHODS

In Belgium, a surveillance program based on the compulsory reporting of abortions and subsequent analyses on their products reached several objectives including official surveillance but also the monitoring of other bovine abortive diseases. Some emerging pathogens could be identified, including Bluetongue virus serotype 3. Thanks to this monitoring, 19,639 bovine abortion cases collected from January 2021 to December 2024 were included in this study. Information issued from the anamnesis, such as sampling date, herd identification number, cattle breed, month of pregnancy were encoded in the laboratory database. The geographical localization of each case of abortion was possible using the Lambert coordinates and the Belgian cattle identification and movement traceability system. A standardized panel of analyses was applied to perform the laboratory diagnosis of bovine abortion on submitted fetuses, sera and placenta. Furthermore, a necropsy is systematically performed by veterinary pathologists. A BTV-3 PCR was performed on fetuses with congenital abnormalities or on suspicious abortions according to the veterinary practitioners.

RESULTS

A significant increase in mortality rate was observed in the bovine population and more specifically in the first month of life as well as in the adult population of animals over 24 months. In addition, an unprecedented abortion epidemic hit Belgium, while this had barely been identified in cattle in the Netherlands in 2023. Compared to the 3 previous years, the number of abortions has been multiplied by a factor 3 from August to October in 2024. The virus was detected in the blood of the aborted dam but also in the bovine aborted fetus. Since September, congenital lesions of the central nervous system such as anencephaly, hydranencephaly or hydrocephaly were detected in aborted fetuses. BTV-3 was detected in the spleen of these malformed fetuses and no other known pathogens responsible for congenital anomalies have been identified. The rate of malformations has increased significantly since the 36th week. While the number of abortions tended to decrease slightly from December, the rate of malformed fetuses was significantly higher in 2024 from September to November with an average of 28,85% (IC 95%: 26,8-30,9) and an OR of 24,46 (IC 95%: 18,33-32,66) in comparison with the same period in 2021-2023.

CONCLUSIONS

As the virus has been highlighted in the blood of the aborted dams and also on fetal organs where congenital malformations have been identified, this demonstrates the transplacental passage of the virus from the mother to the calf. This work shows also the virus's ability to cause outbreaks of abortions as well as congenital malformations in bovine aborted fetuses. According to publications relating to this family of viruses, this type of malformation can appear during infections occurring between the 60th and 100th day of gestation. Given the stage of gestation of the malformed and PCR-positive fetuses, it would be possible to approximately date the time of infection and therefore the start of the viral spread. With these data, it seems that the first infections could have occurred as early as March or April, while the first outbreak of 2024 was not identified until July. This means that, despite a situation requiring increased surveillance, it took more than 3 months before the identification of the first case. This study seems to demonstrate that the surveillance of abortions does not allow early detection of BTV-3 but that surveillance should be focused more on the clinical detection of more sensitive animals. Earlier identification of the first cases would have allowed for better awareness in the sector and probably would have encouraged more farmers to vaccinate earlier.

Clinical observations in a randomized trial with two different treatments for bluetongue in sheep

Jorritsma R., Bijkerk H.J.C., Martina R., van Geijlswijk IM.

Faculty of Veterinary Medicine, Utrecht University, The Netherlands

r.jorritsma@uu.nl

750 words Maximum

Objectives

On September 19th 2024, signs of bluetongue were noted in sheep on the farm of the faculty of Veterinary Medicine of Utrecht University, followed by many other clinical cases. It was advised to provide the animals with supportive care, use antibiotics to treat secondary infections, rehydrate them when necessary and provide anti-inflammatory products such as NSAID's. To our knowledge, there is no data on the effect of the treatment with NSAID's on the survival of infected sheep and effects of paracetamol. We therefore decided to evaluate the use of a protocol with NSAID and paracetamol. In addition, we evaluated the added value of antihistamine on the survival of the affected sheep.

Materials and Methods

All included sheep were treated with a single subcutaneous injection of Meloxicam (1 mg/kg) based on the authorized dose in Australia for sheep, at the day of inclusion (day 0). In addition, all sheep were thrice a day orally treated with paracetamol tablets 500 mg resulting in a dose of 10-20 mg/kg starting at day 1 until death or recovery. In addition, animals in one group (AH) received a single treatment with chlorphenamine maleate 1 mg/kg at day 1, 2 and 3 while animals in another group (NAH) received no additional treatment. When the clinical condition of a sheep was worse compared to the previous day, the animal was euthanized. All sheep were clinically scored daily starting at the day of inclusion until recovery, natural death or euthanasia. Clinical scoring was done on scale from 0 (not observed), 1 (mild), 2 (severe), and 3 (very severe) Also, the temperature was measured and presence of dyspnea was scored (yes/no).

Clinical scores were summarized as the most severe score of each symptom on day 0 (baseline) and day 1 (post baseline). We performed logistic regressions to study the association between the severity of al individual symptoms at baseline and the incidence of survival. In a survival analyses, we tested the association between the severity of the clinical symptoms and the probability of survival using the log-rank test. Additionally, it was investigated whether the survival differed between animals that were actively euthanized and those that died due to the disease. We calculated hazard ratios for the survival model with the significant symptoms. Results were considered significant if $P<0.05$.

Results

Most frequently severe clinical symptoms at baseline were salivation, dyspnea, depression and lameness. For the post-baseline period, clinical symptoms that were most frequently scored as severely affected were dyspnea, salivation, nasal discharge, lameness, and depression.

We found a case fatality rate of the combined groups of 57% as only 36 out of 83 sheep in the study survived. Of the 47 subjects that died, 34 (72.3%) were euthanized and 13 (27.7%) died a natural death, which was not significantly different between AH and the NAH. The severity of salivation during baseline was the only significant clinical observation that was associated with the survival of the sheep. We found no significant treatment effect on the survival of the included sheep.

As we could not calculate the median survival for group NAH, we calculated the mean survival in both groups, which was 2.5 days for group NAH and 0.8 day for group AH. This difference in survival was not significant. The hazard of death was approximately 8 times higher for animals with salivation in severity class 3 compared to those in severity class 0 at baseline. The 95% confidence interval for the hazard ratio ranged from 1.8 to 35.4.

Discussion

We confirmed that BTV infections may in general result in a very high case fatality rate despite a protocol with supportive measures and treatments and found an average survival of one or a few days. As the survival of chlorphenamine treated sheep (AH group) on the survival tended to be lower, we suggest that this treatment should not be introduced. It appeared that it was difficult to use the clinical observations for the prediction of the outcome of the BTV-3 infection.

MONITORING OF THE SEROLOGICAL RESPONSE FOLLOWING BTV-3 VACCINATION IN CATTLE, UNDER FIELD CONDITIONS.

Martínez R.[1], Dominguez B.[1], Delgado L.[1]de Smit H.[1]
[1] Laboratorios SYVA S.A., León. Spain.
Parque Tecnológico de León
Avda. Portugal s/n Parc. M20 · 24009 León (España)

raul.martinez@syva.es

Objectives.

The objective of this study was to assess the humoral immune response promoted by vaccination with two doses of a serotype 3 of the bluetongue virus (BTV-3) vaccine [Syvazul BTV3], in cattle. Specifically, how commercial vaccine after administration induces the development of VP7 antibodies against BTV, and neutralizing antibodies against BTV-3, in field conditions. Additionally, clinical observation of the animals was also performed.

Material and methods.

50 healthy dairy cows, from 2 to 11 years of age were selected in a closed commercial dairy farm located in the Netherlands, with no recent history of BTV disease. Absence of VP7 antibodies was previously confirmed by ELISA in bulk tank milk samples. On study day (D) 0 all the animals received a prime vaccination, followed by a booster vaccination on D21, according to the dosage and route recommended for this vaccine. The serological response after vaccination as well as the potential emergence of a BTV outbreak were monitored. From D0 until the end of the study (D64), cows were daily observed by the farmer for any clinical signs related to BTV infection. On D0 (prior to vaccination), D35, D42 and D64, blood samples were collected to be tested by VP7 BTV ELISA and BTV-3 neutralisation assay. EDTA blood samples were also collected at several occasions to assess presence of BTV genome by reverse transcriptase quantitative PCR (RT-qPCR).

Results.

All cows were negative to BTV VP7 ELISA and BTV-3 neutralising antibodies prior to vaccination (D0). On D35, one week after the second dose of [Syvazul BTV3], all animals had seroconverted and showed high titers of BTV3 neutralizing antibodies, as a result of vaccination.

During the study, a BTV outbreak occurred, resulting in detection of viral genome in 50% of the animals starting from D35 to the end of the study (D64). 13 out of the 25 positive cows were infected before the onset of immunity (D42) since they were found positive on D35 or D42. The remaining 12 cows were found positive on D64, thus after the onset of immunity. Up to the end of the study, 50% of the vaccinated cows were not infected, which was demonstrated by RT-qPCR as well as by the absence of a BTV VP7 antibody booster due to infection that was observed in case of the RT-qPCR positive animals.

Neutralising antibodies were detected at D35, with titres ranging between 2.5 and 40. From D42 onwards, a decrease in the titres was observed for the animals infected before the onset of immunity of the vaccine (geometric mean titre (GMT) 8.5 at D35, 27.4 at D42 and 14.8 at D64), whereas in the non-infected animals or those infected after D42, neutralising antibody titres increased further (GMT 8.8 at D35, 9.4 at D42 and 26.6 at D64 and GMT 9.1 at D35, 10 at D42 and 37.6 at D64, respectively).

Clinical signs related to BTV-3 were first detected from D33 onwards. In the overall period, 54% (7 out of 13) of the animals infected before the onset of immunity exhibited specific clinical signs, whereas only 25%(3 out of 12) of the animals infected after D42 displayed BTV specific clinical signs.

Conclusions.

Vaccination with the inactivated BTV3 vaccine [Syvazul BTV3] is a suitable tool to control BTV-3 disease in cattle.

After two doses 3 weeks apart, vaccinated animals showed a clear response of BTV-3 neutralising antibodies, as well as VP7 BTV antibodies.

The occurrence of a natural outbreak of disease evidenced the ability of the vaccine to provide protection to the animals, since either they did not become infected or when infected after the onset of immunity, the proportion of animals developing disease was lower.

Congenital bluetongue virus infection in neonatal calves: A case report

J. Reinmold[1,a], M. Beumer[1,b], C. Helmer[1,c], A. Dierkes[2,d]
[1] SanGroup Biotech Germany GmbH, Mühlenstraße 13, 49685 Emstek, Germany
[2] Tieraerztliche Gemeinschaftspraxis Dr. A. Dierkes und Dr. K. Woeste, Bockholter Str. 53, 49757 Werlte, Germany
[a] jennifer.reinmold@san-group.com, [b] marius.beumer@san-group.com, [c] carina.helmer@san-group.com
[d] dierkes@tierarztpraxis-werlte.de

Objectives

A severe increase in neonatal calf morbidity and mortality was reported on a dairy cattle farm in North-Western Germany during the final quarter of 2024. Despite adequate colostrum intake and intensive supportive therapy, 11 of 12 calves born between October and December died between days 6 and 10 post-birth. Clinical signs included fluctuating milk intake, diarrhea, and neurological symptoms, beginning on days 3–4 of life. A thorough diagnostic investigation was carried out to identify the primary pathogens involved. A key finding was the detection of Bluetongue virus (BTV), highlighting the potential for congenital BTV infections. Notably, adult cows on the farm had been vaccinated against BTV in July and August 2024. The herd showed no clinical symptoms of a BTV infection and maintained normal fertility and milk production throughout the year.This report aims to underscore the role of subclinical infections in adult cattle and their implications for congenital BTV infections, which may predispose the calves to secondary infections and subsequent health complications.

Material and methods

Two calves (born on 27 November 2024 from a cow inseminated on 14 February 2024 and born on 28 November 2024 from a cow inseminated on 23 February 2024) were euthanized at 7 and 8 days of age, respectively, after presenting with terminal opisthotonos, tonic-clonic seizures as well as profuse diarrhea despite intensive treatment. The carcasses were submitted to a veterinary laboratory [SAN Group Biotech Germany GmbH] for comprehensive investigation. Post-mortem examinations were performed and samples were collected from the pleura, pericardium, lungs, liver, intestine and central nervous system (CNS). These samples were subjected to microbiological analysis including MIC (Minimal inhibitory concentration) testing, histopathological examination and Polymerase chain reaction (PCR) testing for various pathogens.

Results

Patho-morphological findings revealed dehydration, reddened intestinal mucosa with yellow, liquid content, enlarged mesenteric lymph nodes and cloudy leptomeninges. Histo-pathological findings of the small intestine suggested a bacterial infection consistent with Escherichia coli. A viral coinfection was considered as likely. No patho-histological alterations were found on the brain samples submitted. Microbiological analysis revealed no bacterial growth in samples taken from the CNS, pericardium, pleura, or lungs. However, E. coli was isolated from the intestinal samples, characterized as F17-positive by PCR and multidrug-resistant in MIC testing. PCR testing of the intestine identified Bovine Coronavirus (Ct 19.2) and Cryptosporidium parvum (Ct 28.9), while Rotavirus A was not detected. Subsequent PCR testing for Bluetongue Virus (BTV) returned a positive result (Ct 30.1).

Conclusions

Initial findings suggested diarrhea due to E. coli, Bovine Coronavirus and Cryptosporidia as major findings to cause the clinical disease pattern as increased mortality rates on the farm. However, the unusually severe, rapid progression, high case numbers and the presence of central-nervous symptoms before diarrhea onset prompted BTV testing, which confirmed infection. Given the winter season and the likely decrease of the biting midges population coupled with the fact that the calves were only 7-8 days old when BTV was detected via PCR, it was concluded that the infection occurred in utero. Likely, the cows contracted BTV during the vulnerable period of pregnancy in summer/autumn 2024, coinciding with high BTV detection rates in north-western Germany. Hence, the virus was transmitted congenitally to the calves, which were born viremic. No patho-morphological abnormalities or congenital malformations were evident in the 2 calves examined. However, the immunosuppressive effects of BTV likely predisposed the calves to secondary infections, as evidenced by the presence of multiple pathogens causing therapy-resistant diarrhea. This underscores the importance of recognizing BTV as a potential cause of immune suppression in neonatal calves, as it can contribute to secondary infections and complicate the clinical picture and treatment outcomes.This case highlights the long-term effects of the ongoing BTV-3 outbreak and the need to consider congenital BTV infections even months after cows appear to recover. With BTV still circulating in 2024 and likely continuing in 2025, awareness of congenital infections in calves is crucial. The offspring likely died from secondary infections triggered by BTV's immunosuppressive effects, rather than the virus itself. Vaccination against BTV should be urgently promoted to prevent serious long-term consequences in cattle and small ruminants.

N. GLADDEN

Pain detection and management in cattle with a special focus on calving

N. Gladden, Dip. ECBHM PhD PGCHE AFHEA MRCVS, Clinical Assistant Professor in Farm Animal Practice, EBVS® European Veterinary Specialist in Bovine Health Management, RCVS Specialist in Cattle Health & Production
Farm Animal Department, University of Nottingham School of Veterinary Sciences (United Kingdom)

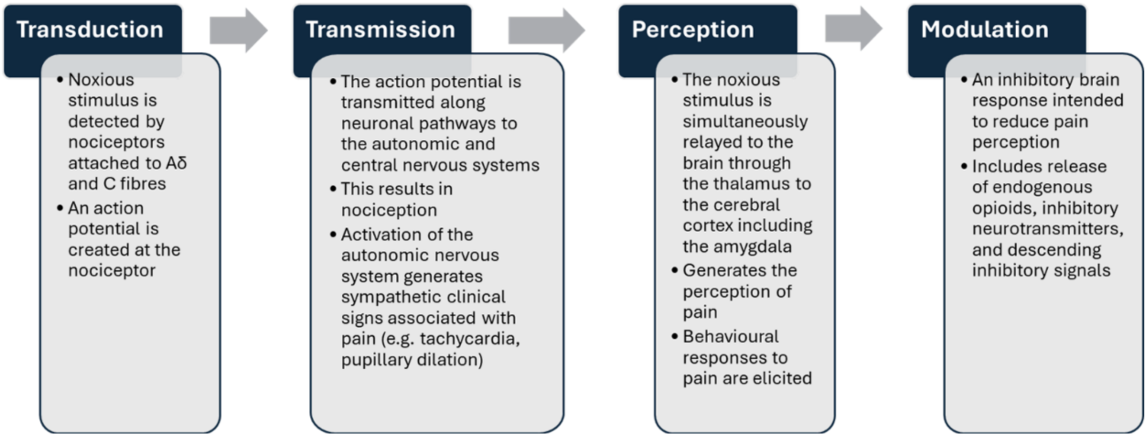
What is pain?

The International Association for the Study of Pain (IASP) currently defines pain as: *"an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"* (Raja et al., 2020). This definition was proposed five years ago and acknowledges that pain is an affective state (*"emotional experience"*), and that nociception and pain are not one and the same. Additionally, this updated definition of pain no longer includes reference to verbal description of pain, recognising that an inability to verbally communicate does not mean pain is not experienced.

Pain is a subjective experience usually associated with a nociceptive event, although it is possible for pain to be experienced in the absence of nociception. Pain perception is a complex phenomenon that can be thought of as a pathway comprising of four steps: transduction, transmission, perception, and modulation [*Figure 1*], although it is important to realise that this is not always a sequential process and some steps may occur simultaneously (Zoltick et al., 2024).

How can we detect pain in cattle?

Accurate detection of bovine pain can be difficult: cattle are prey species believed to mask pain as an evolutionary survival strategy, and the inherently subjective nature of pain means that extrapolation of findings from one species to another is not always appropriate. Nevertheless, whilst challenging, it is crucial to be able to identify bovine pain so that effective mitigation strategies can be implemented. Measures used to detect pain in cattle typically fall into one of three categories: behavioural, physiological, and production indicators (Wagner et al., 2021).



Transduction
- Noxious stimulus is detected by nociceptors attached to Aδ and C fibres
- An action potential is created at the nociceptor

Transmission
- The action potential is transmitted along neuronal pathways to the autonomic and central nervous systems
- This results in nociception
- Activation of the autonomic nervous system generates sympathetic clinical signs associated with pain (e.g. tachycardia, pupillary dilation)

Perception
- The noxious stimulus is simultaneously relayed to the brain through the thalamus to the cerebral cortex including the amygdala
- Generates the perception of pain
- Behavioural responses to pain are elicited

Modulation
- An inhibitory brain response intended to reduce pain perception
- Includes release of endogenous opioids, inhibitory neurotransmitters, and descending inhibitory signals

Figure 1: Four steps of pain perception pathway

Behavioural indicators

Examples of behaviours studied as indicators of pain include posture, pain-directed behaviours (e.g. grooming the painful site), pain-avoidant behaviours (e.g. preferentially lying on the opposite side to a surgical wound), social behaviours, play behaviours, facial expression (e.g. grimace scales), and daily activity. Behaviour is a more sensitive indicator of pain than physiological or production indicators and continuous visual observations are considered to be the optimal approach for measuring behaviour. However, whilst visual analysis of short-term behaviours of individual animals is usually straightforward, assessment of multiple animals or performing visual observations over long durations is time consuming and labour intensive. Moreover, interpretation of behaviours can be subjective and dependent on the observer, further limiting the routine use of behavioural analysis as an indicator of pain. Precision livestock technology is emerging as a valuable tool in behavioural assessment as technology can often mitigate some of the limitations of visual observations. Animal-worn accelerometers have been used to measure activity patterns in animal studies since the late 1990s, with there being a significant growth in interest in the past 10 to 20 years (Brown et al., 2013). Several studies have compared accelerometer-generated data to visual observations of bovine behaviour across a range of production types and life stages, finding that accelerometer-generated data offer an accurate (albeit less detailed) alternative to visual observations of behaviour. More recently, there has been increasing interest in the use of machine vision systems to automate the visual detection and monitoring of animal behaviour. This type of technology offers the benefits of visual observation but in a less time consuming and subjective manner. In the dairy industry, machine vision systems have gained attention for their potential for use in the management of lameness with commercial systems that can identify lame cows with an accuracy comparable to well-trained human observers (Siachos et al., 2025 [In Press]) now being available to farmers.

Physiological indicators

Physiological indicators of pain are commonly used in bovine research as they are straightforward to obtain and analysis is often easier and less labour intensive than assessment of behavioural indicators, especially when sample sizes are large. Indicators of stress attributed to the sympathetic response to a nociceptive stimulus include body temperature, heart rate, heart rate variability, respiratory rate, and biomarkers of stress such as cortisol. Additionally, biomarkers of tissue injury such as creatine kinase are sometimes used where relevant. Whilst physiological indicators are typically good indicators of stress, there can be a wide variation in responses between individuals and it needs to be considered that these indicators are not specific to pain. In order to address this, the use of study designs that compare the physiological responses of animals subject to painful and non-painful experiences that receive analgesia to controls not receiving analgesia [Figure 2] has been suggested as an optimal approach when studying animal pain (Weary et al., 2006).

Production indicators

Many studies have found that measures of production such as weight gain, milk production, reproductive performance and survival are negatively associated with painful experiences such as painful management procedures (e.g. disbudding, castration) or diseases known to be painful (e.g. lameness, mastitis). Although these negative effects have been widely demonstrated, production parameters are not specific to pain; furthermore, the specific contribution of pain to these findings is not fully understood. As such, measures of production are limited as indicators of pain, and are best assessed

	Subject to painful experience (P)	**Not subject to painful experience (p)** • Typically, either this group is subject to no procedure or a non-painful sham procedure
Analgesia administered (A)	PA	pA
No analgesia administered (a) • This group will either be administered a non-analgesic placebo drug or will not receive any treatment	Pa	pa

Figure 2: Optimal design for studies of animal pain (adapted from Weary et al. 2006)

alongside other indicators. Nevertheless, in clinical practice knowledge of the effects of painful procedures or conditions on measures of production is valuable as this information can aid veterinary surgeons and farmers in determining potential effects of painful events and encourage implementation of effective pain mitigation strategies.

How can we manage pain in cattle?

The ultimate goal for effective pain management is an approach targeted at each of the steps in the pain perception pathway [Figure 1]. In practice, this can be achieved by using a multimodal treatment plan, for example a protocol for management of pain associated with caesarean-section might comprise a paravertebral nerve block using a local anaesthetic agent, light sedation using a modified ketamine 'stun' technique (an alpha-2 agonist + ketamine), plus a non-steroidal anti-inflammatory drug (NSAID) to provide longer-lasting post-procedure analgesia [Figure 3].

In much of Europe, it is possible to achieve multimodal analgesia using products licensed for use in food producing species; however, if licensed analgesic options for farmed species are more limited, off licence use of products may be needed to achieve a multimodal approach. If this is the case, veterinary surgeons need to consider the implications of off licence use when formulating treatment plans and ensure compliance with local regulations regarding the administration of medication to food producing animals.

Pain around calving

Maternal pain

Historically parturition has been perceived to be painless for non-human animals, but in recent decades this hypothesis has been challenged and the notion of parturition pain in animals is now widely accepted. Maternal pain during parturition is initially of visceral origin and arises from cervical dilation and uterine relaxation; as parturition progresses, pelvic muscle and perineal distension occur, causing somatic pain to become predominant (Mainau & Manteca, 2011). Postpartum pain associated with uterine involution is well documented in women, and may last for several months, but it is currently unclear whether this is the case in cattle.

Several studies have reported welfare and productivity benefits of NSAID analgesia at calving, with carprofen, flunixin, ketoprofen, meloxicam, and acetylsalicylic acid all having been studied. Differences in study design, NSAID used, and parameters measured make direct comparisons between studies difficult but collectively the results of these studies indicate that provision of analgesia to cows at parturition is recommended. However, it needs to be considered that flunixin has been associated with adverse effects when used in the peri-partum period; therefore use of alternative NSAIDs is advisable.

Paravertebral nerve block	• Local anaesthetic agents block nerve sodium channels • Prevents initiation and propagation of action potential • **Targets transduction and transmission stages**
Modified ketamine 'stun' (xylazine + ketamine)	• Alpha-2 agonists provide sedation and analgesia and can heighten the pain-relieving effects of other analgesic drugs • Alpha-2 agonists inhibit nociceptors, reduce the release of the neurotransmitter Substance P, and have a central effect on pain perception • Ketamine blocks NMDA receptors that mediate nociception • Ketamine is also believed to have anti-inflammatory and pain modulation effects • **Targets all four stages**
NSAIDs	• Inhibition of cyclo-oxygenase results in a reduction in the conversion of arachidonic acid into inflammatory mediators such as prostaglandins • Inflammation heightens the sensitivity of nociceptors; therefore, inhibition of inflammatory mediators reduces this sensitivity • **Targets transduction stage**

Figure 3: An example of a possible multimodal analgesic approach suitable for bovine caesarean-section targeting each stage in the pain perception pathway. Note: this is a hypothetical example, it is recognised that there are many approaches to surgical analgesia/anaesthesia and veterinary surgeons should adopt protocols most appropriate for their individual circumstances.

Neonatal pain

Few studies have investigated pain experienced by neonatal calves at birth and analysis of a smaller range of NSAIDs has been reported, with the predominant NSAID used in neonatal studies being meloxicam. Data from individual studies are inconsistent (likely due to differences in study design) but collectively, studies indicate that birth is likely to be painful for calves and administration of pain relief leads to improved welfare, irrespective of birth experience. A single study has assessed the effect of meloxicam on calves of differing vitality at birth, finding that calves with low vitality experienced heightened benefits of analgesia (Kovács et al., 2022). This is an interesting finding that suggests the effects of pain relief may be influenced by calf birth experience, but further research is warranted to explore this hypothesis further. Clinically, it is important to recognise that neonates may metabolise NSAIDs differently to adult cattle and that NSAIDs are not licensed for use in calves at birth. However, studies have not reported any adverse effects thus far; therefore, judicious administration of NSAIDs at birth can be recommended.

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Spastic paresis in Romagnola cattle: a genetic investigation

Marilena Bolcato[1], Joana G. P. Jacinto[2,3], Irene M. Häfliger[3], Anna Letko[3], Cord Drögemüller[3], Arcangelo Gentile[1]

[1]*Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (Bologna), Italy*;
marilena.bolcato2@unibo.it; arcangelo.gentile@unibo.it

[2]*Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, 3012, Switzerland*;
joana.jacinto@unibe.ch

[3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, 3012 Bern, Switzerland;*
joana.jacinto@unibe.ch; irene.haefliger@vetsuisse.unibe.ch; anna.letko@unibe.ch;
cord.droegemueller@unibe.ch

Objectives:

Bovine spastic paresis (BSP) is a mono- or bilateral tonic spastic state of the hind limbs characterized by overextension of the gastrocnemius muscle causing a "straight hock" with an increase of the tibiotarsal angle. It is described in more than 20 cattle breeds, in almost all European countries but also in the USA, Canada and Australia. Clinical sings usually appear at the age of 3 to 8 months, more unusual are the cases of later onset, as late as 3 years. In the advanced stage of the disease, due to the difficulty in maintaining stance, the general condition is also affected. Although the hereditary contribution appears unquestionable, to date, it has not been possible to definitively determine the pathogenesis and etiology. Since Italian Romagnola cattle show a concerning prevalence for BSP- estimated of 0.6% in 2002 - we performed DNA-based molecular genetic analyses to identify the genetics behind Romagnola BSP.

Materials and methods:

35 affected animals, ranging from 1 to 21month-old were clinically diagnosed with BSP with severity grades ranging from 2 to 4 (median 3.5). From all the 35 cases pedigree and EDTA blood samples were collected. A genome-wide association study (GWAS) was performed using high-density 777k SNP array genotyping data of the 35 BSP-affected and 32 controls. In addition, whole-genome sequencing (WGS) using the Illumina NovaSeq6000 was performed using DNA extracted of 6 BSP-affected Romagnola cattle. The obtained sequence reads were mapped to the ARS-UCD1.2 bovine genome assembly.

Results:

The pedigree data did not indicate a simple Mendelian inheritance. Preliminary GWAS results indicate an absence of genome-wide significant association signals, although some regions exhibiting suggestive hits have been identified. No shared single-nucleotide variants (SNVs) predicted to impact the coding sequence could be detected in the six sequenced cases when compared with more than 500 control genomes from other unrelated breeds.

Conclusions:

These results suggest a more complex inheritance, most likely due to regulatory mutations affecting multiple genes across different regions of the genome. Beyond considerations of animal welfare and the economic impact in the context of cattle production, it is notable to highlight that the BSP seemed to be comparable to the human hyperekplexia - that also has a supposed genetic etiology- and maybe it could become a model for comparative and translational medicine.

Prevalence of foot lesions and their association with carcass characteristics in French young bulls

Sarah Ishak [1,2], Raphaël Guatteo [2], Anne-Raphaëlle Entraygues [1], Marie Gall [1], Aurore Duvauchelle Waché [1], Anne Relun [2].

[1] Institut de l'Elevage, 149 Rue de Bercy, 75012 Paris

[2] Oniris, INRAE, BIOEPAR, 101 Route de Gachet, 44300 Nantes, France

Introduction and objectives

Lameness in young bulls is increasingly reported by various stakeholders, particularly towards the end of the fattening period. This painful condition compromises animal welfare and causes economic losses. While it may be attributed to digital dermatitis (DD), as reported in other countries, it could also be caused by other foot lesions. However, the lack of large-scale studies on countries, it could also be caused by other foot lesions. However, the lack of large-scale studies on foot lesions in French fattening farms limits efforts to raise awareness among farmers and provide effective management recommendations. Identifying foot lesions present on both the front and rear feet is essential for proper management. Additionally, studies have highlighted the economic impact of foot lesions on the young bulls' performance, including reduced average daily gain (ADG), extended fattening period or early slaughtering. This raises the hypothesis of earlier slaughtering for young bulls affected by certain lesions, potentially due to compromised growth or increased management costs. This study aimed to address these gaps by determining the prevalence of foot lesions in French beef and dairy young bulls at the end of the fattening period and examining the association between these lesions and slaughterhouse performance metrics, using a slaughterhouse-based approach.

Materials and methods

This cross-sectional study was conducted in 3 regions of France, which have the highest number of young bull slaughters. Data collection took place twice, once during the spring of 2023 and again during the autumn of the same year. All collected feet were identified, trimmed, and examined post-mortem by three trained professionals, with foot lesions categorized by type, severity, and location based on the French national scoring methods, which are similar to the ICAR Claw Health Atlas. All slaughterhouses had previously agreed to provide the following slaughter data: identification number, breed, date of birth, age, slaughter performances (carcass weight, carcass conformation, and carcass fattening state). Linear regressions were performed to examine the association between the presence of lesions and weight, estimated ADG, and age at slaughter. Logistic regressions were used to evaluate the relationship between the presence of lesions and both carcass conformation and fattening state. All analyses were conducted using the R® statistical software.

Results

In total, 2102 animals were included in the study, with 953 animals examined during spring and 1149 animals during autumn. 97.1% of animals had at least one foot lesion. Most animals (79.9%) had at least one infectious lesion, and 34.4% of young bulls had a lesion severe enough to cause lameness. Sole hemorrhage diffused form (SHD) and heel horn erosion (HHE) were the most common lesions, present in 77.6% and 73.6% of animals. White line hemorrhage (WLH), sole hemorrhage circumscribed form (SHC), and white line fissure (WLF) were detected in 50.9%, 44.2%, and 42.6% of animals. DD affected 34.4% of animals. Additionally, 51.5% of batches were affected by DD. The prevalences of sole ulcers and interdigital phlegmon (IP) were below 1%, at 0.6% and 0.4%, respectively.
Regarding slaughterhouse performance, preliminary results indicate that the presence of certain lesions is associated with changes in carcass weight. For example, the presence of an IP reduces the carcass weight of a Charolais young bull by nearly 30 kg. In contrast, other lesions, such as HHE or hemorrhages, are linked to an increase in carcass weight. Additionally, carcass weight tends to rise with the severity of the lesions. For instance, in a Charolais young bull weighing 411 kg, the presence of HHE severity level 1 increases the carcass weight by approximately 9 kg, level 2 by 24.5 kg, and level 3 by 31.3 kg. A similar trend is observed for WLH, SHD, and SHC. This may be due to extended farm stays for animals with severe lesions, potentially leading to increased carcass weight. However, it is also possible that higher carcass weight acts as a risk factor for certain lesions, making it challenging to determine whether weight is a cause or a consequence. Further investigation is needed to clarify this relationship. The association between lesions and age at slaughter, fattening state, and conformation is still being analyzed.

Conclusion

Infectious and non-infectious foot lesions are highly prevalent in young bulls raised indoors in France at the time of slaughter, affecting both front and rear feet and potentially leading to France at the time of slaughter, affecting both front and rear feet and potentially leading to

Lumbosacral subarachnoid injections and anesthesia in calves : impact of the needle choice on injection success rate and central neural trauma

Gwenola Touzot-Jourde[1], Marie Meurdra[2], Paul David[2], Raphaël Guatteo[2]

[1] EnvA – CHUV des Equidés – Campus Equin Normand
La Fromagerie, 14430 Goustranville France

[2] Oniris VetAgroBio, La Chantrerie, Route de Gachet, 44300 Nantes France

Affiliation – gwenola.touzot-jourde@vet-alfort.fr

Lumbosacral subarachnoid anaesthesia has been reported to provide abdominal antinociception of good quality in calves (1). In humans, injection techniques and needle tip designs have been shown to impact injection success and dural trauma (2, 3). Data collected on calves submitted to lumbosacral subarachnoid injections were gathered to document injection success rate and post-mortem findings on meninges and spinal cord.

Data on needle characteristics and injection success rate were collected from 2 studies (4, 5), one comparing subarachnoid injection volume spread and occurrence of inadvertent epidural injection via myelography under general anesthesia and dissection after *post-mortem* dye injection on ten 36-50 kg calves of 21 to 51 days of age, and the other evaluating anesthesia adequacy during umbilical resection on fourteen 28-42 day-old, 31-60 kg calves followed by a necropsic evaluation the same day of the lumbosacral spinal canal. Ethical approval was obtained for both studies. Needle choice was randomized between a hypodermic (18Gx40 mm) or a spinal (20Gx88mm) needle. The injections were undertaken by a single 6th year veterinary student on 10 calves in lateral recumbency in the first study and by different 5th year students on 14 calves under sedation either standing or positioned in lateral or sternal recumbency during surgical laboratories. A standardized protocol was used to perform the necropsic examination of the spinal canal. In the first study, total and partial epidural injection were identified on the myelogram or after dissection upon methylene blue identification around the *dura mater*. In the second study, trauma of meninges and spinal cord were macroscopically identified and photographed with x10 magnifying lens for later determination of puncture hole areas using GIMP software. Data were compared with non-parametric tests and expressed in median (min-max) with a p value set at 0.05.

In the first study, 10 injections (4 during myelography and 6 *post-mortem*) were undertaken with the hypodermic needle resulting in 70% of successful subarachnoid injections and 30% with a partial injection into the epidural space, 7 injections (3 during myelography and 4 *post-mortem*) were performed using the spinal needle with 43% of successful subarachnoid injections, 14% of partial epidural injection and 43% of total peridural injection. Only a trend to better success rate with the hypodermic needle was evidenced. In the second study, 70% of the 14 calves were injected in sternal recumbency. Based on the animal reactions during surgery and the need for rescue analgesia, It was suspected that 14% of the injections were misplaced in the epidural space (one with each needle) and 21% were deposited only partially into the subarachnoid space. During necropsic examination, no dural puncture could be identified in the one calf that had a suspicion of peridural injection with a spinal needle. Needle placement and readjustment resulted in 83% versus 37% of single puncture hole respectively with hypodermic and spinal needles and a maximum of 3 punctures per animal. Dural puncture surface areas but not shapes were significantly different between hypodermic and spinal needles, 0.5231mm2 (0.2150-0.6815) versus 0.2581mm2 (0.2150-0.3698) respectively. No difference was found in the prevalence of dural hematoma (25 versus 18%) but a tendency to larger hematomas was identified with the hypodermic needle (≥ 18 versus 2mm2, p=0,15). Prevalence of *Pia mater* and spinal cord hematomas and puncture lesions were not different between needles (respectively 25-35% and 27-37% for *pia mater* defects and hematomas, 10-20% for spinal cord defect and hematomas). However, hematoma and lesion sizes showed a trend to be larger with the hypodermic needle.

In the context of performing lumbosacral dural puncture in 3 to 6 week old calves of mainly dairy breed, students showed a preference for the use of hypodermic needle easier compared to spinal needle for which greater length and smaller bore were deemed to impinge easily identified cerebrospinal fluid free flowing upon crossing the *dura mater*. Partial to total inadvertent peridural injections were found using both needles. Lesions of the dura and spinal cord were seen with both needles but the sizes with the hypodermic needle trended to be larger. Quality of calf immobilization during dural puncture, operator's experience and clinical significance of these lesions warrant further practice evaluations.

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A novel recessively inherited syndromic form of bovine spastic paresis in Brown Swiss cattle

Joana Jacinto[1,2,*], Bettina A. Weber[2], Anna Oevermann[3], Dragos Scarlet[4,5], Heinrich Bollwein[5, 6], Jan Franzen[7], Lea A. Hiller[7], Gaby Hirsbrunner[1], Barbara Drews[8], Karl R. Klisch[8], Franz R. Seefried[9], Cord Drögemüller[2]

[1] Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, 3012, Switzerland
[2] Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, 3012, Switzerland
[3] Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bern, 3012, Switzerland
[4] Institute of Veterinary Anatomy, Vetsuisse Faculty Zurich, Zurich, 8057, Switzerland
[5] Clinic of Reproductive Medicine, Vetsuisse Faculty Zurich, Zurich, 8057, Switzerland
[6] Center for Clinical Studies (ZKS), Vetsuisse Faculty Zurich, Zurich, 8057, Switzerland
[7] Institute of Animal Pathology, Vetsuisse-Faculty, University of Bern, Bern, 3012, Switzerland
[8] Division of Veterinary Anatomy, Vetsuisse Faculty, University of Bern, Bern, 3012, Switzerland
[9] Qualitas AG, Zug, Switzerland

*Corresponding author: joana.jacinto@unibe.ch

Objectives:

Bovine spastic paresis (BSP) is defined as a sporadic progressive neuromuscular disease, characterized by unilateral or bilateral hyperextension of the hindlimbs. First described in 1922, the hypothesis that BSP is heritable has been maintained over time. Monogenic and polygenic inheritance have both been discussed as potential causes. However, to date, no genetic variant has been identified as the cause of this disorder. Unpublished whole-genome sequencing (WGS) data of a single infertile BSP-affected Brown Swiss heifer (index case) identified a private homozygous missense variant in *ASCC2* affecting a conserved residue (p.Pro42Leu). The variant allele was absent in 5577 cattle genomes of different breeds sequenced as part of the ongoing Swiss Comparative Bovine Resequencing project and the completed international 1000 Bull Genomes Project. Subsequent microarray genotyping under the umbrella of the Swiss routine genomic system revealed the presence of additional homozygous animals only in Brown Swiss cattle. The allelic frequency in 43.262 genotyped Brown Swiss cattle was 5.7%. The objective of this study was to describe the clinicopathological phenotype of a series of Brown Swiss heifers carrying the homozygous *ASCC2* variant.

Material and methods:

This study included twenty Brown Swiss heifers suspected of being affected by BSP, who underwent a general clinical examination, neurological examination and gynecological examination. The mean age of the cases at the time of examination was 19.6 months (S.D. ± 3.3). In addition, a complete post-mortem examination was performed on the index case. Histology on the neuromuscular system was performed (e.g. muscle semimembranosus, muscle quadriceps femoris, spinal cord, tibial nerve, brainstem). Genomic DNA was obtained from the affected animals (EDTA blood samples) and the homozygous genotype for the p.Pro42Leu missense variant in *ASCC2* was also confirmed by PCR and Sanger sequencing in the 20 studied heifers.

Results:

All animals demonstrated an increased tarsal angle, exhibiting heterogeneity in severity, with 80% of the animals showing this condition bilaterally. The animals exhibited a stiff gait with hyperextension of one or both tarsi and muscle contractions of the feet extensor muscles. The hindlimbs were either placed in the median plane of the body during walking or completely crossed this plane resembling a form of spinal ataxia. Furthermore, proprioception was found to be reduced in three heifers. The neurological examination was compatible with BSP grade 2. The mean number of artificial inseminations per heifer, considering heifers that were inseminated at least once, was 4.4. However, none of the animals examined, although they had received at least one artificial insemination, were confirmed to be pregnant. A gynaecological examination revealed that all animals were cycling, and no morphological changes were observed by rectal ultrasound. Post-mortem collected oocytes (n=3) completely lacked cumulus cells and failed to mature *in vitro*. Histological analysis of the nervous system revealed low-grade multifocal axonal degeneration in the spinal cord and brainstem as well as astrocytic hypertrophy of the brainstem and midbrain. This neuropathological diagnosis is consistent with axonal degeneration.

Conclusions:

The *ACSS2* encodes an enzyme that generates acetyl-CoA, that is central to histone acetylation and gene regulation, particularly in neurons, due to their unique metabolic demands and postmitotic state. This gene is also known to physically interact with *ACSS3*, which has been associated with neuromuscular syndrome in humans, and estrogen genes 1 and 2, which have been associated with female subfertility in humans. Considering the rarity of the identified variant, the predicted effect on the encoded protein, gene function and physical interactions, the identified variant was considered likely to be pathogenic and thus the most likely cause of the observed phenotype. This study represents the first successful genetic analysis of a novel form of BSP associated with ovary dysfunction in Brown Swiss cattle. Our results provide robust evidence for a harmful recessive breed-specific allele, in accordance with monogenic inheritance, most likely explaining a mild form of BSP associated with subfertility. These insights not only enhance our understanding of BSP genetics but also highlight the potential implications for breeding strategies in Brown Swiss cattle.

Inter-observer agreement between veterinarians and hoof-trimmers in recognising and grading bovine hoof lesions

Relun A.[1], Daviet M.[1,2], Berodier M.[3], Carrière A.[4], Delacroix M.[5], Delaunay I.[6], Fayolle G.[3], Geboers P.[6], Lutz C.[7], Peudenier J-M.[8], Prétot MS.[9], Saillard Y.[10], Schelcher F.[11], Thomas G.[2,12], Guatteo R.[1], Duvauchelle-Waché A.[2]

[1] Oniris, INRAE, BIOEPAR, Nantes, France
[2] Institut de l'Elevage, Beaucouzé, France
[3] UMOTEST, Jouy-en-Josas, France
[4] FODSA-GDS Aveyron, Rodez, France
[5] MD Formation, Marcoux, France
[6] CFPPA, Le Rheu, France
[7] SNGTV, Hochfelden, France
[8] OS Normande, Domfront en Poiraie, France
[9] ANPB, Mollans, France
[10] Inoval, Noyal-sur-Vilaine, France
[11] IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France
[12] UMT eBis, Jouy-en-Josas, France

Oniris VetAgroBio – *anne.relun@oniris-nantes.fr*

Objectives

Veterinarians and hoof-trimmers play crucial roles in assessing and managing hoof lesions in cattle. However, differences in their backgrounds can lead to scoring discrepancies, affecting farm monitoring, benchmarking, or even genetic selection. In France, hoof-trimmers traditionally follow a national scoring grid with severity scores, whereas veterinarians may use varied systems, like the ICAR Claw Health Atlas. To standardize scoring, France established a national committee (CTNBB) in 2018, developing a novel system compatible with ICAR but with defined severity scores. This study aimed to assess inter-observer agreement between veterinarians and hoof-trimers in recognising and grading bovine hoof lesions. It also aimed to assess whether a guide based on photos and definitions for each lesion and severity score could enhance agreement among less experienced observers.

Material and Methods

A collection of 563 digital colour photographs of bovine hooves was sourced from CTNBB members. Of these, 277 photographs depicting 13 lesions and 10 lesion-free images were included in an online survey. The photographs were selected by the 2nd author to encompass at least 10 photographs of each scores. Fifteen hoof trimmers, 17 veterinarians and 11 final-year veterinary students completed the survey. They received no prior training to complete the survey. Six students received the novel national guide, while five were given the ICAR atlas. Participants were asked to name and grade up to 2 lesions in each photograph. Inter-observer agreement on lesion recognition was assessed using Fleiss' Kappa (κ), for all lesions and all observers, as well as for the different lesions and the different observers, with and without the guide. Agreement on lesion severity was assessed using the intra-class correlation coefficient (ICC).

Results

Inter-observer agreement for lesion recognition was moderate overall ($\kappa = 0.52$). Stronger agreement (> 0.6) was observed between hoof-trimmers and veterinarians for the main lesions causing lameness (digital dermatitis, sole ulcer, toe necrosis, white line disease, axial horn fissure) or for lesions that were easier to recognise (interdigital hyperplasia, scissor claw). Agreement was moderate for lesions less likely to cause lameness (circumscribed and diffuse sole haemorrhage, heel horn erosion) or less familiar (bulb ulcer). Agreement was weak (< 0.4) for those challenging to identify in photographs (white line haemorrhage, double sole) and for no lesion.

Confidence intervals were too wide to draw firm conclusion on agreement regarding lesion severity, but it tended to range from weak (ICC < 0.5) to moderate (0-5-0.75) for most lesions.

The national guide tended to improve scoring agreement but not lesion recognition.

The lower inter-observer agreement for recognition of hoof lesions in this study compared with previous studies may be attributed to the lack of prior training among observers and the option to score up to 2 lesions in each photograph. The inter-observer agreement for lesion grading should be assessed using larger sets of pictures, examining each lesion separately.

Conclusion

Without prior training, experienced hoof trimmers and veterinarians agreed on recognising the main lesions causing lameness but differed for the others and for lesion grading on photographs. While a comprehensive guide may enhance agreement, continuous training of both professionals appear to be necessary, especially if the scoring is to be used in regional or national programs.

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Effects of individual compared to pair housing of dairy calves on production until the end of first lactation in the UK.

Mahendran, S.A[1]., Wathes, D.C[1]., Booth, R.E[1]., Blackie, N[1].
[1]Royal Veterinary College, UK

Objectives: The aim of this study was to establish the impacts of individual compared to pair housing of pre-weaning calves on health, growth and behaviour until the end of first lactation.

Materials and Methods: A total of 457 heifer calves were recruited from a UK dairy, and were systematically allocated into either pair or individual pens at birth, where they stayed until weaning at 8 weeks. Here, weekly visits were conducted with weight and standardized calf scoring carried out. A subset of calves (n=90) had accelerometers attached to monitor activity, with CCTV placed above a further 16 pens to allow behavioural assessments to be made via continuous focal sampling at 1 and 5 weeks of age. Following weaning, all heifers were similarly managed through group housing, feeding with total mixed rations, the use of automatic heat detection for artificial insemination and weighing every two months until conception. Farm staff identified disease occurrences, which were treated following standard operating procedures. First-lactation monthly milk recording was used to measure milk yields and somatic cell counts.

Results: Overall mortality until the end of first lactation was decreased if they were pair housed as a calf (73.8% compared to 63.7% survival, $p=0.015$), but with no impact of housing group on growth (0.72 kg/day). Disease assessment pre-weaning found individually housed calves had increased odds of developing disease and overall lower activity levels ($p<0.01$). Accelerometer data showed that housing group had no effect on lying times, with a mean of 18 h 11 min per day (SD 39 min) spent lying down. The motion index was significantly higher in pair-housed calves ($p < 0.01$), potentially due to more social play behaviour. The total time engaged in cross sucking was not impacted by housing group ($p = 0.72$). Pair-housed calves split their time conducting cross sucking equally between inanimate objects and on their pen mates' body. Individually housed calves spent significantly more time with their head out of the front of the pen ($p = 0.006$), and also engaged in more self-grooming than pair-housed calves ($p = 0.017$), possibly due to a lack of socialization.
In first lactation, heifers that were pair housed as calves had significantly increased odds of developing udder health issues (OR=1.93, $p=0.022$). Despite this, there was no difference in first lactation 305 day milk yields (mean 9,996kg), which was not associated with the housing group. However, the total milk produced per calf recruited into the original study was greater for pair-housed compared with individually housed calves (8088kg vs. 7115kg), which is likely due to the significantly higher hazard of individually housed calves exiting the herd prematurely.

Conclusions: The overall findings of this study indicate that within a UK commercial dairy management system, pair-housed calves were healthier and more active than individually housed calves, while housing group did not influence growth rates or the occurrence of cross sucking. Pair housed calves had improved survivability in the herd, but did have increased udder health issues which did not negatively impact overall milk production.

Long-distance transport of beef cattle to the fattening unit: impact of live yeast and selenised-yeast on blood metabolites and reticulo-rumen pH in newly received Charolais bulls

Emilie Arcier[c], Erik Sulmont[c], Bruno Martin[c], Clothilde Villot[c], Matteo Santinello[a], Isabella Lora[b], , Giulio Cozzi[b*], Mauro Penasa[a], Alberto Guerra[a], Massimo De Marchi[a]

[a] *Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

[b] *Department of Animal Medicine, Production and Health (MAPS), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

[c] *Lallemand Animal Nutrition*

Presenting author: Emilie Arcier, Email: earcier@lallemand.com

Corresponding author: Clothilde Villot. Email: cvillot@lallemand.com

Long-distance transport, comingling and receiving phase at the fattening unit are sources of stress for young beef cattle. This randomised controlled study involved 80 Charolais young bulls that underwent 12 hours of transport from France to Italy and aimed at testing whether the animals have some benefits from the supplementation of live yeast and selenium through slow-release nutritional boluses. The bulls were randomly allocated into two supplementation groups of 40 animals each, named Yeast and Control groups. Bulls of the Yeast group received two slow-release ruminal boluses before leaving France and live yeast was also supplemented in the receiving diet once arrived in Italy. The supplementation Yeast consisted of live yeast *Saccharomyces cerevisiae* CNCM I-1077 and selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R397. Individual BW and blood metabolic profile were assessed before leaving the French commingling centre (d-1), at the arrival to the Italian fattening unit (day 0) and after 7 d (day 7). The rumen environment was continuously monitored through reticulo-rumen sensors (SmaXtec) that measured pH (n=26), temperature (n=34) and rumination (n=34) in a subsample of bulls, equally distributed between Yeast and Control groups. Supplementation did not affect BW loss during transportation (-27 kg) or BW recovery (ADG_{d0-7}=2.32 kg/d) after 7 days. Bulls fed Yeast tended to increase blood glucose (5.06 vs 4.92 mmol/L for Control group; $p=0.07$) and significantly increased blood selenium content (73.4 vs 57.5 µg/L; $p<0.01$), which can potentially benefit animal for stress recovery. Reticulo-rumen data shows that supplementation stabilised the rumen environment by minimizing the daily pH amplitude ($p<0.05$) and the lowering inter-animal variability ($p<0.01$). The Yeast group tended to increase the time spent ruminating (+32 minutes/day; $p=0.09$) at day 7 compared with Control group. More stable ruminal conditions represent an important support for beef cattle health during the receiving period at the fattening unit, when animals face the delicate transition to the fattening unit.

Evaluation of the water intake of pre-weaned calves with a focus on heat stress using on-farm digital technologies

Malina Flessner, Michael Iwersen, and Daniela Klein-Jöbstl

University of Veterinary Medicine Vienna
Veterinärplatz 1, 1210 Vienna, Austria

malina.flessner@vetmeduni.ac.at

Objectives: As more digital technologies are installed in calf barns the evaluation of available tools and data for different applications is important. Drinking water is essential for the calves' health and development. There are limited studies available that assess the water intake of pre-weaned calves. Water intake is influenced by several factors like age, milk and feed intake, activity, and environmental factors.
While global temperatures continue to rise, the effects of higher temperature-humidity-indexes (THI) on calves need to be further investigated. Calves that are under heat stress experience distress, immune system weakness, slower growth, and increased water loss. The goal of this study was to evaluate the water intake in pre-weaned calves in general and in relation to increased THI levels.

Material and methods: This study was conducted on an Austrian dairy farm from October 2023 to September 2024. The calves were housed together in two groups in a semi-open calf barn from the age of five days to twelve weeks. Calves were fed with an automatic milk feeder and a concentrate feeder. They had ad libitum access to water and hay. Individual milk intake and starter intake were recorded by the automatic feeding system. Calves were fed milk replacer ad libitum for the first 42 days and were weaned slowly for the next 43 days. Weights were recorded automatically with a forefront weight scale in the milk station. Calves wore collars with accelerometers to track their activity. For the climate monitoring a temperature-humidity-logger was installed in the animal area of the calf barn. The water bowl maintained a constant water level and the water intake was measured with a water flow sensor. Each calf was uniquely identified by its ear tag using RFID technology in the water station.
For the data analysis an automatic linear model was generated for the water intake to see which factors influenced it most. A partial correlation was carried out to examine the relationship between water intake and THI, when corrected for age, sex, milk intake, starter intake, activity, and individual calves. A Mann-Whitney-U-Test was done to compare between heat-stressed and not heat-stressed calves based on a THI threshold of 72, that is commonly used for heat stress classification in dairy cows.

Results: The calves that were fed ad libitum milk replacer drank on average 0.2 L of water per day. The minimum for the ad libitum group was 0 L and the maximum was 15.7 L. The restrictively fed calves drank 2.9 L on average. In this group the minimum was 0 L and the maximum 21.2 L per day. The mean age for the first water intake was 29 days. The earliest age for the first water intake was 6 days and the latest 62 days. The automatic linear model showed an accuracy of 55.6 % with the most important predictor being concentrate intake followed by age and milk intake. The partial correlation demonstrated a statistically significant positive correlation (0.29) between the water intake and the THI in the calf barn. The mean of the daily water intake of the ad libitum no heat-stress group was 0.1 L in contrast to 0.5 L in the heat stress group. For the restrictively fed calves the water intake was 2.7 L for no heat stress compared to 4.8 L during heat stress. The Mann-Whitney-U-Test showed significant differences for both groups.

Conclusions: Calves that were fed ad libitum milk replacer started drinking water at around four weeks of age. Water intake was mostly influenced by feed intake and age. The water intake of calves increased with a higher THI in both ad libitum and restrictively fed calves.

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Comparative analysis of climatic indices using generalized additive models: Assessing the impact of the most suitable indices on circadian behaviors of grazing cattle in summer conditions

Czaplicki S.[1] , Hornick J.-L.[1], and Dufrasne I.[1]

1 Department of veterinary management of Animal Resources, FARAH, ULiège, Belgium

Affiliation – Czaplicki.Sebastien@Uliege.be

The primary aim of this study was to evaluate several climatic indices using Generalized Additive Models (GAMs), identify the most suitable predictor for cattle behavior, and determine critical temperature thresholds impacting grazing, rumination, standing, and inactivity, with hourly resolution.

Behavioral data were collected from 69 dairy cattle over two summer seasons (2021–2022) using accelerometers recording activities at 5-minute intervals. The cows grazed rotationally across eight shaded plots and had unrestricted access outside milking and feeding times. Milking occurred twice daily at 6:30 AM and 4:30 PM, with a prepared ration provided in the barn following morning milking. Hourly climatic data—including temperature, relative humidity, solar radiation, and wind speed—were recorded at a weather station 6 km from the farm. Several climatic indices—such as Black Globe Temperature (TBG), Temperature Humidity Index, Heat Load Index, Comprehensive Climate Index, Wet Bulb Globe Temperature, Equivalent Temperature Index of Cattle, adjusted Temperature Humidity Index, and Respiration Rate Index—were evaluated using the Akaike Information Criterion. GAMs analyzed non-linear relationships and interactions between time of day, climatic indices, and cattle behaviors. Splines incorporated into the models, along with accounting for individual variability, enabled robust modeling of circadian patterns. From 2,463,528 behavioral observations, harmonization with hourly meteorological data yielded 205,294 observations.

TBG emerged as the most suitable index, achieving the lowest Akaike Information Criterion values across behavioral categories. It outperformed alternatives, with Wet Bulb Globe Temperature and Comprehensive Climate Index ranking second and third, respectively. The hourly analysis revealed circadian patterns aligned with milking and feeding routines. During these periods, behavioral variability was low, driven by human interventions, whereas between these times, behavioral cycles reflected individual differences in responses to environmental and physiological conditions. Critical TBG thresholds marking transitions in animal behaviors were identified, with grazing and rumination showing more consistent patterns than standing and inactivity, which exhibited higher inter-individual variability.

This study identifies TBG as a reliable predictor of cattle behavior under diverse climatic conditions. Using GAMs, the research provides insights into circadian rhythms and temperature-induced behavioral adaptations, informing strategies to optimize grazing schedules and enhance shade availability, thereby alleviating heat stress and promoting cattle welfare in extensive farming systems.

Antibiosis – regional intravenous antibiotic therapy for the treatment of foot infections in cattle – 32 patients

CESTARI H[1], ALONSO JM[2], DUARTE HOG[1], RIBEIRO MG[1], RODRIGUES CA[1], WATANABE MJ[1], ALVES ALG[1], HUSSNI CA [1]

1 UNESP, School of Veterinary Medicine and Animal Science, Botucatu, Brazil.
2 Universidade Federal de Santa Catarina, Curitibanos, Brazil

UNESP - São Paulo State University, School of Veterinary Medicine and Animal Science. Department of Veterinary Surgery and Animal Reproduction, Botucatu, São Paulo, Brazil. Zip 18618-681.

UNESP, School of Veterinary Medicine and Animal Science, Botucatu, Brazil - h.cestari@unesp.br

The objective of this report is to describe the use of antibiosis as an adjuvant in the treatment of foot infections in cattle.

Antibiosis combined with other procedures was performed on 32 cattle of both sexes of different breeds, including dairy and beef cattle. These animals had different foot diseases, such as digital dermatitis, interdigital dermatitis, septic osteitis, septic arthritis, interdigital hyperplasia with infection, heel erosion, foot phlegmon, septic pododermatitis, exungulation and toe necrosis. All treated animals received antiseptics and protective bandages when dressings were applied. Antibiosis was carried out using ceftiofur, ceftriaxone, amikacin, gentamicin, and levofloxacin, which were chosen for each clinical case. The antibiotic was combined with 2% lidocaine without a vasoconstrictor, which reduced pain during manipulation and allowed surgery to be performed concomitantly in cases where this occurred. Antibiosis was repeated every 48 hours.

Sixteen animals received a single application, six animals received two applications, another 10 animals received up to seven applications, and a single animal required 10 antibiotic applications via regional perfusion. Twenty-seven animals were treated with antibiotics associated with surgery, four of which also received systemic antibiotic therapy. Five of the animals had their digits amputated, one with an ascending infection, which required ten applications. Another fifteen patients underwent surgical debridement and removal of necrotic tissue. Of the thirty-two animals, two were euthanized due to the severity of necrosis and gangrene of the digits in one case and diffuse phlegmon of the affected limb in the other, in addition to the high costs of treatment, which became unfeasible. Patients with osteomyelitis and septic arthritis required the greatest number of repetitions of antibiotics. A single application of antibiotics was sufficient to control infection in patients with digital dermatitis or interdigital dermatitis.

It is possible to conclude that antibiosis can be used as a therapeutic complement in cattle foot infections; it is efficient in treating foot infections, and however, attention must be given to the evolution of each case. However, the use of antibiotics raises several questions, particularly regarding environmental, economic and production issues. Compared with the systemic use of these drugs, these drugs are less impactful and more viable, with less time of use, smaller quantity and thus less environmental and economic impact and greater control of the use of antibiotics.

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Therapeutic and prophylactic procedures associated with the elimination of foot infection in a batch of beef cattle – A case report

HUSSNI CA[1], CESTARI H[1], ALONSO JM[2], DUARTE HOG[1], RIBEIRO MG[1], RODRIGUES CA[1], WATANABE MJ[1], ALVES ALG[1]

1 UNESP, School of Veterinary Medicine and Animal Science, Botucatu, Brazil.
2 Universidade Federal de Santa Catarina, Curitibanos, Brazil

UNESP - São Paulo State University, School of Veterinary Medicine and Animal Science. Department of Veterinary Surgery and Animal Reproduction, Botucatu, São Paulo, Brazil. Zip 18618-681.

UNESP, School of Veterinary Medicine and Animal Science, Botucatu, Brazil - carlos.hussni@unesp.br

The objective of this report is to describe the procedures and corrective measures for controlling and eliminating severe and highly occurrent foot infections on beef cattle farms.

Approximately 1500 and 2000 animals per year are produced on a beef cattle rearing and finishing farm, divided into batches of 80 to 150 male Nelore and Angus cattle. Rearing takes place for one month and finishing for three months, when the animals reach slaughter weight. With no previous history of foot disease, a batch of 80 Angus cattle were bred, some of which were lame. Attended by a veterinarian, he trimmed some of the animals and applied dressings, recommending the maintenance of this local treatment. The disease characteristics of foot phlegmon affected other animals in this group.

When the visit was requested, lameness was evident in 21 of the 80 animals, but lameness did not affect the other batches. These animals had septic pododermatitis with the involvement of deep structures and grade 4 or 5 lameness in 12 of the animals and of lesser intensity in the remaining nine. The twelve animals whose foot injuries were considered more serious were sent to the Veterinary Hospital for treatment. Bacteriological culture revealed *Helcococcus spp* and *Staphylococcus spp*. All animals returned to the farm after 15 days, with a significant reduction in the process, after treatments involving surgeries (amputation and debridement), antibiotics (regional intravenous antibiotic therapy), bandages and bandages. The remaining nine animals were treated on the farm by washing the affected digits with soap and water, followed by the application of Hoofcare® (a compound based on citric acid, tea tree oil, copper, and zinc) to the lesions with a brush every two days. At the same time, hydrated lime - $Ca(OH)2$ - was placed in the soil close to the troughs where the animals were fed prophylactically in all batches of rearing and finishing animals. Upon reaching slaughter weight (30 arrobas), the animals were replaced by new batches that, upon arriving at the farm, were inspected, confirming the absence of lameness and foot injuries; after these batches, they were subjected to pediluvium with 5% copper sulfate. The batch affected by foot infection remained on the farm until it reached slaughter weight, which occurred on average in three months during the finishing phase, without any losses. None of the animals from the other batches presented with foot infection. Seven months after the occurrence of the cases, there were no new episodes, with the liming of the soil next to the trough similar to a dry pediluvium maintained for all renewed batches. When new batches of animals arrived at the farm, the initial placement of the footbath with copper sulfate was maintained.

The therapeutic and concomitant prophylactic procedures adopted promoted the recovery of lame animals, controlled the infection without cases arising in other batches and inhibited the recurrence or new emergence of the disease in the herd. Acknowledgments: CNPq/PQ Proc. 309024/2021-8. The Ethics Committee on the Use of Animals at FMVZ – UNESP – Botucatu approved this study.

Detection of *Dichelobacter nodosus* and *Fusobacterium necrophorum* on dairy farms in Slovakia

Pavol Mudroň
University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

Objectives

Foot rot (interdigital phlegmon) is a significant health disorder of dairy cattle caused by bacteria *Dichelobacter nodosus* (*D. nodosus*) and *Fusobacterium necrophorum* (*F. necrophorum*). The herd incidence per lactation is generally below 5%, but also prevalence rates of up to 25% have been reported. The aim of this study was to determine the prevalence of both bacteria in non-lame dairy cows on eight Slovak dairy herds.

Materials and methods

A total of 1,631 Holstein Friesian dairy cows were included in the study. Amies agar gel medium transport swabs (Sarstedt) were used for sampling. The swabs were taken from healthy dairy cows from the plantar interdigital space of one of the pelvic limbs during milking in the milking parlour, without cleaning or other treatment of the feet prior to sampling. For the diagnosis of *D. nodosus*, specific primers have been designed for the detection of the 16S rRNA gene, which is specific to the bacterial species by its sequence. Diagnosis of *F. necrophorum* was performed with primers specifically designed for the leucotoxin A (lktA) gene region. All statistical analyses were performed using R software version 3.6.3 (R Core Team). The outcome of interest was bacterial status (negative or positive). The Chi-squared test was used to assess differences between the prevalence of *D. nodosus* and *F. necrophorum* in dairy cows and between farms and regions. Differences were considered significant if $P < 0.05$. The association of bacteria was also examined in a multinomial logistic regression model. The outcome of the model was bacterial contamination with *D. nodosus* and *F. necrophorum* and the variables were farm, region, number of lactations, annual milk yield, DIM and bacterial contamination with both bacteria. Yearly milk yield and DIM were transformed by taking the natural logarithm to obtain normally distributed data.

Results

The total final sample size for all eight farms was 1,631 sampled dairy cows. The PCR method detected *D. nodosus* and *F. necrophorum* in 1,394 (85.5%) and 373 (22.1%) animals, respectively ($p<0.05$). *D. nodosus* was significantly associated with annual milk yield and *F. necrophorum* detection (OR = 0.69, $p < 0.05$; OR = 2.37, $p < 0.05$). Similarly, *F. necrophorum* was negatively associated with annual milk yield and *D. nodosus* detection (OR = 0.32, $p < 0.05$; OR = 4.55, $p < 0.05$). Effect plots were generated for the interaction terms: *F. necrophorum* positivity (FN) x milk yield (lnkg) and *D. nodosus* positivity (DN) x lnkg, showing the association between milk yield and *D. nodosus* positivity for clear positivity for *F. necrophorum*. If a cow is FN positive, the risk of being DN positive increases with decreasing milk yield. A similar effect was observed for *D. nodosus* positivity (FN) x lnkg. A decrease in milk yield was only associated with an increased risk of being FN positive in cows that were DN negative.

Conclusions

The results of this study show that the foot rot pathogens D. nodosus and F. necrophorum are common in dairy herds throughout Slovakia. Despite the very high prevalence of D. nodosus, there is no evidence of a high number of cases of foot rot in dairy cows in the country. Our data show that D. nodosus is present on the feet of almost every dairy cow. Therefore, the potential risk of cattle developing foot rot is present and farmers must always be aware of the potential for future outbreaks.

Welfare in Swiss dairy heifers: Comparative assessment of skin lesions in 2 housing systems

Clinic for Ruminants

Bremgartenstrasse 109a

CH-3012 Bern

R.M. Schmid[1], A. Loup[1], E. Studer[1], J. Becker[1]

1 Clinic for Ruminants, Vetsuisse Faculty, University of Bern, 3012 Bern, Switzerland – robin.schmid@unibe.ch

During summer, dairy heifers are often managed extensively in Switzerland. This applies to two different types of husbandry systems across the country (i.e., to heifers on alpine transhumance pastures and to heifers on pastures belonging to a year-round lowland farm). In contrast to heifers, dairy cows are more intensively farmed and spend more time in confinement. The prevalence of skin lesions (e.g., hock injuries) in dairy cows is high. The aim of this study was to record the prevalence of skin lesions in heifers and to possibly determine the time of occurrence of these lesions. To this end, heifers from both husbandry systems (alpine pastures vs. year-round lowland farm pastures) were inspected both visually and through palpation, and findings were compared. Inspection took place in the fall (i.e., at the end of the alpine pasturing season before descent to the lowlands). Of the 480 heifers included on a total of 28 farms, skin lesions were found in 13.1% of individuals. Skin lesions commonly associated with barn design (e.g., injuries on the dewlap or hock injuries) and skin lesions on the pelvis were most frequently detected, equally distributed among husbandry systems. In conclusion, the prevalence of skin lesions among Swiss dairy heifers appears to be lower compared with dairy cows, at least as measured in the fall before heifers transition to the main dairy herd. Accordingly, deploying measures to reduce skin lesions is more efficient on dairy farms than in heifer raising systems.

Paid workforce in Hungarian dairy farms: situation and perception of 24 farm managers and veterinarians in relation to herds health status

Emma Sanne[1], Khalil Sellami[2], Audrey Brunet[3]

[1]*ES - Elevage et Stratégie, Allex, France – esanne0@gmail.com*

[2]*ISEAM management school, Lognes, France*

[3]*VIRBAC S.A., GM&MD, Carros, France*

Objectives - In Hungarian dairy farms, the workforce is composed for more than three quarters of paid labor in opposition to family labor model[1]. In this specific work pattern, detecting animal signs, respecting hygiene and applying the right actions are tasks that fall to workers and which have a strong impact on the health and performance of a herd. To master health status, involvement of the labor force is therefore key. Multilevel approaches merging medical and social sciences and customized standard operational procedures appear to have a positive impact on herds health status[2,3]. But a deeper understanding of employees' profile and perception of managers is requested for practical implementation in farms of Hungary.

Materials and Methods - A survey was conducted in May 2024 i) to describe the labor force in Hungarian large scale farms, ii) to understand the paid labor factors of motivation and iii) to identify needs regarding training. 24 farm managers and veterinarians participated. They were selected randomly based on their willingness to participate. It took about 10 minutes to cover the 20 questions form that were mostly multiple choice questions. The questionnaire was divided into 4 sections. Section 1 was covering general information about respondents. Section 2 was addressing labor management. Section 3 was dealing with challenges and opportunities regarding the workforce. And section 4 was kept for additional comments. The data was collected in a Google Sheet and processed to obtain a basic statistical description.

Results - Although the job is kept for more than 3 years in 79% of the cases, 62.5% of the interviewed consider workers to have basic knowledge and experience. The majority of workers are declared permanent (71%) and specialised in their activities (79%): feeding, cleaning, newborn care being the first. The language preferred for training is for 78% Hungarian, 14% Indian and 8% English. For 4 respondents out of 5, the lack of skilled workers is a challenge. Labor shortage and workers motivations are quoted as an issue in 50%. For the latter, managers express that supervisors' attitudes towards workers (83%) and competitive wages (83%) are key factors. In the end, 96% consider the skills of workers have a high impact on animal performances. The methods foreseen to improve workers performances are i) implementation of incentives (43%), ii) improving communication channels (43%), and iii) providing training opportunities (14%).

Conclusion - The above result differs from a previous research which has shown that the first factor motivating calf care workers is calf health[4], in front of economical parameters. But the study of Viidu *et al.* reviews the perception of workers themselves and not managers. This gap highlights a difference of perception between stakeholders. This need for communication is confirmed by the work of Farre *et al.* in which only 47% of farm managers give feedback to their employees[3]. Our research has also shown that not only the local language is spoken by workers but also foreign languages. To be effective, training and any type of communication with employees must anticipate and address this.

In the end, further investigations are required to fasten interactions among managers, veterinarians and workers and improve health status in large scale farms. Comparative studies in other European countries with a paid labor model would also be of interest.

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Serological study of zoonotic diseases in veterinarians, in Portugal – Q Fever

Martins M [a,b], Esteves F[c], Bica I[d],Cardoso J[e], Duarte S.[a,b,f], Anastácio S.[a,b,g], Mesquita JR[h,i], Cruz R[c,i]

[a] *Departamento de Ciências Veterinárias, Escola Universitária Vasco da Gama, Campus Universitário, Lordemão, 3020-210, Coimbra, Portugal (miqueljcmartins@gmail.com; sofia.duarte@euvg.pt; sofia.anastacio@euvg.pt;)*

[b] *Centro de Investigação Vasco da Gama, Escola Universitária Vasco da Gama, Campus Universitário, Lordemão, 3020-210, Coimbra, Portugal*

[c] *Escola superior Agrária de Viseu, Instituto Politécnico de Viseu, Quinta da Alagoa, Estrada Nelas, 3500-606 Viseu (rcruz@esav.ipv.pt; festeves@esav.ipv.pt)*

[d] *UNICISA:E, CINTESIS@RISE, Escola Superior de Saúde de Viseu, Instituto Politécnico de Viseu, R. Dom João Crisóstomo Gomes de Almeida 102, 3500-843 Viseu (isabelbica@gmail.com)*

[e] *CEVA Portugal, Rua Dr. António Loureiro Borges, 9/9ª-9ºA, 1495-131 Algés (jose.cardoso@ceva.com).*

[f] *LAQV, REQUIMTE, Laboratório de Bromatologia e Farmacognosia, Faculdade de Farmácia, Universidade de Coimbra, Polo III, Azinhaga de Stª Comba, 3000-548 Coimbra, Portugal*

[g] *CIBB - Centro de Inovação em Biomedicina e Biotecnologia (CIBB), Universidade de Coimbra, Coimbra, Portugal*

[h] *Laboratórios de Doenças Infeciosas, Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto (jrmesquita@icbas.up.pt)*

[i] *Epidemiology Research Unit (EPIUnit), Instituto de Saúde Pública da Universidade do Porto, Porto 4050-091, Portugal;*

The "One Health" concept is gaining increasing relevance and remains a subject of continuous study due to its role in promoting ecosystem balance and integrating human, animal, and environmental health. Emerging and re-emerging zoonotic diseases strongly exemplify the interconnectedness of the "One Health" pillars, representing significant threats to both human and animal health. The resurgence of certain diseases reflects the impact of environmental changes, highlighting the need for collective, interdisciplinary strategies for their prevention and control, alongside the use of basic, straightforward measures, such as the active implementation of simple biosecurity practices.

Q Fever is a globally distributed disease caused by a bacterium known as *Coxiella burnetii*. Ruminants are recognized as the primary reservoirs, shedding the agent into the surrounding environment through secretions and/or excretions.

The main objectives of this study were to identify risk factors associated with *Coxiella burnetii* infection and to assess the implementation of biosecurity measures among veterinarians working in livestock species. Additionally, it aimed to determine the seroprevalence of Q Fever infection in this population.

In this research, the anti-C. burnetii antibody titers were quantified in serum samples from two groups: the test group, consisting of veterinarians (n=92), and the control group, composed of blood donors (n=184). The analysis was conducted using a commercial ELISA test.
The results showed that 33.7% of the samples in the test group tested positive for IgG anti-Coxiella burnetii, corresponding to 31 out of the 92 veterinarians tested, suggesting exposure to the agent. In comparison, the control group had a seropositivity rate of 17.4%.
Statistical analysis revealed a significant difference between the two groups (P=0.0023), suggesting a significantly higher exposure risk in the test group. This study demonstrated that veterinarians in the test group had a greater likelihood of exposure to the agent (OR=2.41) compared to the control group.
The study of occupational diseases among veterinarians involved in livestock production is a relatively underexplored topic, considering the risks they face during their professional activities.
This is one of the first studies conducted in Portugal to demonstrate the exposure risk of veterinarians to Coxiella burnetii – a risk that could be mitigated through biosecurity measures, which are often overlooked in the daily practice of field veterinarians.

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Surgical correction and post-operative care of a non-congenital complicated entropion caused by an injury of the upper eyelid in a 9-month-old Charolais heifer

Djebala S [1]. Marian R [1]. Leitao MR [1]. Kelly E [1]. Ryan EG [1]

[1] UCD Veterinary Hospital, School of Veterinary Medicine, Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, D04W6F6, Republic of Ireland.

salem.djebala@ucdconnect.ie

Objectives

This case aims to describe the surgical treatment and post-operative care provided for a non-congenital entropion with severe corneal ulceration caused by an injury of the upper eyelid in a 9-month-old Charolais heifer.

Material and methods

A 9-month-old Charolais heifer suffered an injury to her right eyelid four weeks prior to referral to University College Dublin Veterinary Hospital (UCDVH). Initially, the farmer monitored the case and called his private veterinary practitioner two weeks later due to obvious pain, blepharospasm and epiphora.

The referring veterinarian diagnosed entropion of the upper eyelid, which he tried to correct using two long simple interrupted everting sutures. Additionally, a corneal ulcer was identified and treated with topical cephalexin cream (RILEXINE®, Virbac, France). After two weeks of treatment without improvement, he referred the case to UCDVH. Clinical examination revealed significant inversion of the upper eyelid approximately 1 cm from the lateral canthus, resulting in contact between eyelashes and the corneal surface. Additionally, there was swelling and traumatic damage to the upper eyelid which was predisposing to secondary spastic entropion. Staining of the cornea using fluorescein dye

(FLUORESCEINE FAURE®, Vidal, United Kingdom) confirmed the presence of a severe corneal ulcer. Surgical treatment was performed on the 400kg heifer in under standing sedation with the head tied to a head-rest attached to a squeeze crush. Standing sedation was accomplished using a modified ketamine stun using equal quantities (1ml = 0.05 mg/kg) of xylazine 2% (XYLAPAN 2%®, Vetoquinol, United Kingdom) and (1ml = 0.25 mg/kg) ketamine (Ketabel 100 mg/ml®, Bela-pharm, Germany) given intramuscularly and repeated 30 minutes later. Regional anaesthesia using auriculopalpebral and Peterson nerve blocks were achieved using 20 ml and 40 ml of procaïne 40 mg/ml (Pronestesic®, Fatro, Italy), respectively.

A Hotz-Celsus procedure was performed by excising an elliptical flap of palpebral skin from the upper eyelid. The lower incision, 7 cm in length, was made parallel to the lash line, starting 1 cm above the lateral canthus and extending to 1 cm above the medial canthus. The upper conjoined incision was elliptical, with the widest portion measuring 2 cm in width. The incision was closed with a series of appositional inverse cruciate sutures using 6 metric polyglycolic acid (SURGICRYL®, SMI, Belgium). Additionally, a narrow triangular dissection (0.8 cm x 0.8 cm x 0.5 cm) was performed beneath the eyelid, where the sutures placed by the referring vet had traumatised the palpebral mucosa. This incision was sutured with simple continuous sutures using 3-0 polydioxanone material (ETHICON®, SurgiNatal, Rajasthan). Post-operative care involved cleaning serous discharge around the eye and surgical site using swabs soaked in saline solution, followed by the application of cephalexin cream (RILEXINE®, Virbac, France) to the cornea. This treatment was performed twice daily for the first five days after surgery and once daily for the subsequent five days. Additionally, subcutaneous meloxicam 5 mg/kg (LOXICAM 50 mg/ml®, Norbrook, United Kingdom) and intramuscular oxytetracycline (10 mg/kg; Engemycin 10%®, United Kingdom) were given every other day for three days, starting immediately prior to surgery.

Results

In the first four days following surgery, the eye was swollen, painful, and nearly closed, with abundant discharge. The cornea appeared significantly cloudy indicating corneal oedema, likely as a reaction to the surgical manipulations. By the fifth day post-surgery, the eye was open, the cornea was nearly clear, and the amount of discharge had significantly decreased. As a result, the frequency of cleaning and topical treatment was reduced to once daily and systemic treatments were discontinued. The sutures in the mucosa were removed on the fifth day post-surgery, while those in the eyelid skin were removed on the 10th day with an end to all treatments. However, a 2 mm white scar remained visible on the corneal ulcers site when the heifer was discharged 15 days post-surgery.

Conclusions

Treatment of entropion in ruminants, especially small ruminants, is mainly performed by palpebral injections, everting sutures or staples. In the current case, due to trauma and swelling of the upper palpebral skin, secondary spastic entropion and severe corneal ulceration, a Hotz-Celsus procedure combined with palpebral mucosal repair was indicated. Appropriate sedation and regional anaesthesia are critical to a successful surgical outcome, in addition to significant post-operative care.

Bacterial contamination of the surgical site at the time of elective caesarean section in Belgian blue cows: Identified by 16Sr DNA amplicon sequencing.

Salem Djebala [1, 2], Elise Coria [2], Florian Munaut [2], Linde Gille [3], Justine Eppe [2], Nassim Moula [4, 5].

[1] UCD Veterinary Hospital, School of Veterinary Medicine University College Dublin Belfield, Dublin 4, Ireland – salem.djebala@ucdconnect.ie
[2] Liège, Quartier Vallée 2, Avenue de Cureghem 7A-7D, Liège 4000, Belgium - Salem.djebala@ucdconnect.ie; elise.coria@hotmail.com ; fmu-naut@hotmail.com ; justine.eppe@uliege.be
[3] MSD animal health Benelux, 1170 Watermael-Boitsfort, Belgium - linde.gille@msd.com
[4] Department of animal production, University of liege, Quartier Vallée 2, Avenue de Cureghem 6, Liège 4000, Belgium. nassim.moula@uliege.be
[5] GIGA—Animal Facilities—ULiège—B 34, 4000 Liège, Belgium. nassim.moula@uliege.be

Objectives

This study used amplicon sequencing to characterize the microbiome at the surgical site of Belgian Blue (BB) cows undergoing caesarean section (CS). It also compared microbiota composition between samples with positive and negative bacterial cultures and evaluated whether cultured bacteria matched with the bacterial DNA identified by amplicon sequencing.

Material and methods

The study involved 13 BB cows of a previous dataset of 76 animals. Bacteriology was performed on swabs sampled from visceral and parietal peritoneum during the realisation of CS. 16Sr DNA Amplicon sequencing was performed in six samples chosen randomly among the positive swabs to the bacterial culture and seven among the negative swabs.

Results

A total, nine isolates belonging to seven bacterial species were identified by bacterial culture in the six positive samples, these species are: *Aerococcus viridans, Psychrobacter sp*. *Acinetobacter sp*. *Pantoea agglomerans Staphylococcus lentus Clostridium perfringens Pseudomonas sp*.

The study of the surgical site microbiota during the realisation of elective CS showed 2542 distinct bacterial operational taxonomic units belonging to 567 genera derived from 28 phyla. A total of 121,083 reads were identified from the 13 samples. The most abundant bacterial phyla were *Tenericutes* (45%), *Firmicutes* (26%), *Actinobacteria* (15%), *Proteobacteria* (10%) and *Bacteroidetes* (4%), and the others were found at very low rates. The most abundant genera were *Mycoplasma* (44%), *Corynebacterium_1* (9%), *Bacillus* (8%), *Acinetobacter* (6%), *Ruminococcaceae_UCG-010* (2%) and *Ruminococcaceae_UCG-005* (2%), and the others represented 1% or less of the identified genera. Alpha diversity analysis revealed no significant differences for the following indices: Simpson reciprocal biodiversity index, Chao1 richness estimator for bacterial genera and the population regularity index. The alpha diversities of the samples that were positive and negative to the bacteriology were similar. The NMDS analysis showed no difference between the compositions of the surgical site microbiota of the positive and negative cross-sections in the bacteriology. In fact, the analysis of molecular variance (AMOVA) showed no significant difference between the samples positive and the negative to the bacteriology (F score: 0.764; p-value: 0.562). Moreover, the analysis of the homogeneity of molecular variance (HOMOVA) also showed no significant difference between these groups (BV0.059; p-value 0.657). The genera of bacteria species identified by culture represented 13% (8462/64,646) of the reads identified by amplicons in negative samples to bacteriology, and 2.98% (1687/56,437) of the reads in the positive cultures. Overall, five out of nine genera were identified by amplicon sequencing in the same sample in which bacteria species belonging to these genera were identified by culture. In fact, the genera *Psychrobacter* and *Pantoea* were never identified by amplicon sequencing in the same samples in which *Psychrobacter sp. and Pantoea agglomerans* were cultured.

Moreover, the genera *Aerococcus* was not found by amplicon sequencing in two of three samples in which *Aerococcus viridans* was cultured. We also observed that the bacterial species identified by culture were not frequently identified by amplicon sequencing. Indeed, only *Acinetobacter sp*. and *Pseudomonas sp*. were identified by amplicon sequencing in the same sample in which they were cultured. The other species (seven of nine isolates) were not identified by amplicon sequencing in the positive samples to bacteriology. In contrast, the most frequent genera (*Mycoplasma*, *Corynebacterium_1*, *Bacillus*, *Acinetobacter*, *Ruminococcaceae_UCG-010*, *Ruminococcaceae_UCG-005* and the others) and bacterial species found by amplicon sequencing (*Mycoplasma_wenyonii*, *Bacillus_liqueniformis*, *Mycoplasma_AY837724.1.1457* and others) were never found in the culture.

Conclusion

Regardless of culture results, all sampled swabs revealed a high diversity of bacterial DNA (via amplicon sequencing) at the surgical site of CS. Unlike the culture findings, most bacteria identified through amplicon sequencing belonged to the *Mycoplasma* genus, likely introduced to the site via a haematogenous route. Interestingly, discrepancies between culture and amplicon sequencing results suggest that cultured bacteria are not the dominant species in the samples where they were isolated. This study highlights the need for further research to explore the role of these bacteria in post-operativecomplications.

Paratuberculosis in Italy: where are we?

Garbarino Chiara[a], Cerioli Monica[a], Nassuato Claudia[c], Boldini Massimo[a], Gradassi Matteo[a], Ventura Giordano[a], Rosignoli Carlo[a], Tonni Matteo[a], Maisano Marco Antonio[a], Ricchi Matteo[a], Bellini Silvia[a], Santi Annalisa[a], Ruocco Luigi[b], Lomolino Roberto[b], Luppi Andrea[a], Diana Palma[d], Tamba Marco[a]

[a] Istituto Zooprofilattico Sperimentale Lombardia and Emilia-Romagna, National Reference Centre for paratuberculosis, Italy
[b] Ministry of Health, Italy
[c] Veterinary Regional Service, Lombardy Region, Italy
[d] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"

Objectives

We report here the classification of the sanitary status of Italian cattle and buffalo herds for paratuberculosis (PTB) according to the national guidelines. In more detail, we also report data on dairy farms in the Lombardy region, which produce almost the 40% of the Italian milk. The final goal of this report is to encourage the improvement of the health status of the herd thought the collaboration between veterinarians and farmers.

Material and methods

The “National guidelines for the control of bovine paratuberculosis and for assigning the health ranking of herds” were issued by the Italian Ministry of Health in 2013. These guidelines were updated in early 2023 (3) by considering the critical issues that emerged during their application and the inclusion of paratuberculosis in the list of category e) diseases under Regulation (EU) 2016/429 and its Implementing Regulation 2018/1882. Notably, buffalo, sheep and goat species were included within the scope of the plan. The 2013 version was endorsed by all the 21 Italian regions through regional plans, while the most recent revision was applied only by 8 regions. Since 2014 the classification of herds is based on seven risk levels for paratuberculosis infection. The first two levels (PTC, PT0) are assigned by the local veterinary services based on presence (PTC) or absence (PT0) of clinical cases; clinical cases of PTB are subject to notification. The accreditation for the other levels (PT1 to PT5) is obtained upon specific request of the farmers. The health status of the herd is based on results of standardized serological testing schemes which has to be repeated annually. The qualifications for cows and buffalos, are reported in the National Data Bank (BDN), at the moment not for sheep and goats. Here we report data for the Lombardy region, the Italian region with the highest density of dairy cows, with around 5000 dairy herds and accounting for around the 40% of the total national milk production.

Results

At the date of 30/09/2024, the overall percentage of Italian commercial cattle herds with a qualification recorded in BDN was 38.5%; while for buffalo herds it was 7.9%. Regarding the situation of dairy cows in the Lombardy region, the herds joining the plan and the percentage of farms tested has increased over the years: 79.3 % of the total dairy herds joined the plan and 33.9% of the farms were tested. In the last years (2014-2023) we observed a reduction in positive farms from 74.2% to 53.5% and a reduction in the number of positive animals within the infected herds from 4.2% to 1.5%, with considerable stability in recent years. These results have been made possible by all stakeholders involved in the management of the disease. In particular, over the years (1), in the Lombardy region, working groups have been set up between veterinarians, laboratories and other stakeholders, aimed at communication and continuous training of farmers. The epidemiology and the economic impact of the disease were the most useful topics to explain the benefits of applying such a farm management plan, by emphasising that the measures to control paratuberculosis are also functional for the control of other diseases, for instance calf diseases.

Conclusions

The results of the paratuberculosis control and certification plan in Italy and specifically in Lombardy region confirm the possibility of improving the health status of herds.

Due to the chronic nature of the infection, it is pivotal to keep high the motivation of farmers over time. For the achievement of this goal, the collaboration among the Veterinary Services, laboratories, operators and farmers proved to be fundamental.

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Benefits of a ruminant ambulatory service in local farms to improve clinical exposure of BVM students.

Eloi R.G. Guarnieri[1], Suzanne A.C. Clergue[2]

[1]Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong S.A.R.

[2]Centre Hospitalier Universitaire Vétérinaire de Oniris VetAgroBio Nantes, National Veterinary School of Nantes, France

Small ruminant curriculum-based teaching activities (CBA) within the Bachelor of Veterinary Medicine (BVM) program at the City University of Hong Kong have primarily been conducted with the partnership of two local goat farms of over 200 heads. In August 2023, a ruminant ambulatory service (RAS) was established to provide additional clinical exposure of BVM students to small ruminant medicine in Hong Kong. RAS visits were open to BVM student volunteers.

Objectives: To describe and compare the clinical exposure of BVM students under the current CBAs and volunteer-based activities (VBAs).

Materials and methods: Retrospective observational study over 12 months. Reports of veterinary visits to local farms from August 2023 to July 2024 were reviewed. The description and number of farms, animals, students, and procedures performed were recorded. CBAs other than clinical extramural studies and rotations were excluded.

Results: Over one year, the median number of visits per student was 2 [1;2] (25 students) and 4 [1;13] (21 students) for CBAs and VBAs, respectively. The VBAs provided additional exposure to other species than goats, such as sheep, alpacas, and cattle. Students were able to observe and participate in several procedures categorized as Day 1 competencies, such as 'goat handling' (CBA: 2 [3;23]; VBA: 14 [3;174]), 'goat subcutaneous injection' (CBA: 4 [2;23]; VBA: 13 [2;84]), and 'goat drench' (CBA: 1 [0;10]; VBA: 5 [0;66]).

Conclusions: These results demonstrate the importance of the RAS in improving the clinical exposure of BVM students to small ruminant medicine.

Estimation of minimum centrifugation time of microhematocrit tubes to obtain accurate values of packed cell volume and total solids in ruminants

Lardé H.[1], Oliva D.[2], Bouillon J.[3], Whiston R.[4], Peda A.[4], Dowling P.[5], Chapuis R.[3]

[1]Université de Montréal, Faculté de médecine vétérinaire, Rimouski, QC, Canada
[2]Garden State Veterinary Specialists, Eatontown, NJ, USA
[3]Institut Polytechnique UniLaSalle, Université d'Artois, IDEALISS, URL 7519, Mont-Saint-Aignan, France
[4]Ross University School of Veterinary Medicine, Basseterre, St. Kitts
[5]University of Saskatchewan, Western College of Veterinary Medicine, Saskatoon, SK, Canada

Objectives

The packed cell volume (PCV) and total protein concentration in serum or plasma are important parameters to assess in critical cases such as anemic or dehydrated patients. The PCV can be read from microhematocrit tubes after centrifugation. Total solids (TS) are routinely used to estimate the total proteins and are measured in plasma using a refractometer. The minimum centrifugation time to obtain accurate results of PCV and TS has not been determined in most veterinary species. Red blood cell packing time during centrifugation of the blood is species-dependent, and this time is reported to be longer in ruminants compared to dogs, cats and horses (Cornell University, 2025). Guidelines recommend spinning microhematocrit tubes of ruminants for 10 minutes before PCV reading. Our objective was to determine the minimum centrifugation time of microhematocrit tubes to obtain accurate values of PCV and TS in cattle and sheep. We hypothesized that the minimum centrifugation time would be less than 10 minutes in both species.

Material and methods

The American Society for Veterinary Clinical Pathology recommends using at least 40 independent patient specimens to compare results obtained from two different methods (Arnold et al., 2019). We used the results of PCV and TS obtained after 15 minutes of centrifugation as reference values for a given animal, and we compared the results of PCV and TS obtained after 1.5, 3, 5 and 10 minutes to those reference values. Blood samples were collected from 61 castrated or intact crossbred adult male sheep and 40 non-pregnant crossbred cows from the teaching herd of Ross University School of Veterinary Medicine (RUSVM). Animals were deemed healthy based on physical examination at the time of blood collection. Blood samples were obtained (approved by the Institutional Animal Care and Use Committee at RUSVM), from the jugular vein in sheep and from the coccygeal vein in cattle, using a Vacutainer® blood collection system fitted with a 20-gauge needle, in heparinized tubes. For each centrifugation time, PCV and TS values were assessed by two independent investigators. For both parameters, we calculated the difference between the values at each centrifugation time and the reference value at 15 minutes. We evaluated the agreement between values at each centrifugation time and reference values for PCV and TS, using the intra-class coefficient of correlation (ICC), linear regression, and distribution of differences between values obtained at each time of centrifugation and the reference value. To provide recommendations on the minimum acceptable centrifugation time to report PCV and TS in ruminants, we elected to use the best combination of ICC value, linear regression equation, and visual symmetrical distribution on difference plots, for the paired results of PVC and TS by species.

Results

The reference intervals (RI) from the RUSVM diagnostic laboratory are, for sheep PCV 0.27-0.45 L/L and TS 6.0-7.5 g/dL, for cattle PCV 0.24-0.46 L/L and TS 6.0-8.0 g/dL. Results for sheep: 52% (32/61) sheep had a PCV value below the RI and 15% (9/61) had TS above the RI. For the PCV results, the agreement was excellent at centrifugation time of 10 minutes (ICC 0.984, linear regression with a slope of 1 and an intercept of 0). For the TS results, the ICC was also excellent at 10 minutes (0.950), with a slope of 1.0 but an intercept of 0.2 g/L on the linear regression. The distribution on difference plots was symmetrical. Results for cattle: all PCV values were within the RI and 7.5% (3/40) cows had TS above the RI. For the PCV results, the agreement was excellent at centrifugation time of 5 minutes (ICC 0.982, linear regression with a slope of 1 and intercept of 0). For the TS results, the ICC was also excellent at 5 minutes (0.971), however the regression line had a slope of 0.9 and an intercept of 0.5 g/dL. The distribution on difference plots was symmetrical.

Conclusions

The best combination of statistical parameters was met at 10 minutes for sheep and at 5 minutes for cattle. This centrifugation time provides an almost perfect agreement for the PCV (compared to 15 minutes) but is slightly less ideal for the TS (results of the linear regression). However, the total error for TS would not be of clinical significance. Because PCV values of all cattle were within reference intervals and no polycythemic animals were included, validation of these times may be warranted in a broader population.

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Inspiring the next generation of production animal veterinarians

Sarah Wood

University of Bristol, Langford house, Bristol, UK

Bristol Vet School – sarah.wood@bristol.ac.uk

As educators in veterinary institutions and practitioners in clinical practice we are involved in supporting veterinary students towards choosing, embarking on, and succeeding in a career in production animal practice. To ensure we can meet the needs of the industry it is imperative that we are attracting veterinarians of the future to consider this career path, but we must also ensure they have a good understanding of the role, it fits with their personal requirements, and they are adequately prepared to undertake the role.

In this presentation we will discuss what challenges we are facing in terms of recruitment into production animal practice, and what practitioners and those working in educational establishments may be able to do practically to better prepare students for practice.

Key challenges for us to tackle to recruit graduates into production animal roles include addressing equality and diversity issues in the industry. We know that females, those from urban backgrounds and those from ethic minorities don't see themselves in production animal careers (Payne et al., 2022). We must also ensure that the industry can align with the values held by this generation of graduates. Ethical values include concerns around animal welfare of food producing animals, sustainability and food security. Values also of maintaining personal wellbeing and a healthy worklife balance, including opportunities to work flexibly or part-time. "Out of hours" working is key to offering a service to clients but may be an unattractive feature of production animal practice for those considering their career options.

In training students, we must ensure our curriculum aligns with the current workload for production animal practitioners. Our curriculum must meet accreditation standards but also draw on an evidence base where available for what we should be teaching. Graduates must be equipped with the necessary practical skills, underpinning knowledge and the ability to problem solve. They must also be aware of the characteristics that make for a successful production animal veterinarian, and be encouraged to reflect upon and develop these in readiness to work as part of the team in veterinary practice and on farm.

Our teaching methods are key to ensuring veterinary students are well prepared for practices. Innovative teaching technologies and the use of clinical skills laboratories, simulations and models for the acquisition of clinical skills has changed veterinary education in recent years. Continuing to improve and apply our understanding of how adults learn, and how learning in higher education takes place allows us to further develop our veterinary curriculum.

Production animal veterinarians, whether working in educational establishments or in practice serve as role models to veterinary students whether they like it or not, and we play a key role in inspiring the next generation.

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A. FOURNIER

Anaplasmose ovine : maladie émergente dans nos clientèles et mise en relation avec les observations post-mortem à l'abattoir

Antoine Fournier,
Commission Ovine SNGTV
Capveto Tandem, site de Bellac, 16 Rue des Rochettes 87300 Bellac
antoine.fournier46@yahoo.fr

RÉSUMÉ

L'Anaplasmose ovine est une maladie à transmission vectorielle de plus en plus souvent mise en évidence sur le terrain en élevage ovin. Il s'agit d'une maladie due à une bactérie intra-érythrocytaire, parfois peu débilitante et pouvant passer inaperçu cliniquement. Les constatations cliniques de terrain sont peu fréquentes et peu spécifiques : l'anaplasmose se manifeste par des signes cliniques de type amaigrissement, hyperthermie, anémie ou encore ictère. Il en résulte quelquefois des saisies totales pour ictère, le plus souvent chez des agneaux cliniquement sains. Ces saisies génèrent des pertes économiques très importantes. Les outils diagnostiques sont variés : frottis sanguins, sérologie et PCR. Cette dernière se révèle être la plus spécifique et utilisée en routine, réalisable sur sang EDTA mais également sur la rate.

De plus, le dérèglement climatique favorise la propagation de nouvelles espèces de tiques dans nos territoires. Il a été mis en évidence le rôle prépondérant de ces vecteurs dans la propagation de l'anaplasmose ovine.

Compte tenu de l'augmentation des cas retrouvés sur le terrain et des données récoltées à l'abattoir de Bellac en Haute-Vienne pendant 4 ans, nous avons étudié les relations possibles entre les saisies totales pour ictère, l'activité vectorielle des tiques présentes et la mise en évidence d'A.ovis. Il en ressort en 2024, une augmentation significative des saisies totales pour ictère, qui est non corrélée à l'augmentation de la quantité d'animaux abattus. Les analyses mensuelles des saisies pour ictère montrent une évolution similaire depuis 4 ans, avec une augmentation marquée entre avril-mai et les mois de juillet et août. Ces dernières peuvent être mises en lien direct avec l'activité des tiques (les genres Ixodes, Dermacentor et Rhipicephalus majoritairement) présentes dans la zone et pouvant transmettre l'anaplasmose ovine. Compte tenu des pertes économiques engendrées par la saisie totale d'une carcasse ainsi que celles engendrées sub-cliniquement dans les troupeaux, il serait intéressant de poursuivre les recherches en ferme. Pour cela plusieurs outils s'offrent à nous : l'envoi des rates des carcasses saisies pour analyse PCR et des prises de sang sur tube EDTA dans les exploitations pour recherche spécifique PCR A.ovis et la réalisation de frottis sanguin.

Mots clés : anaplamose, ovins, tique, amaigrissement, anémie, ictère, saisie totale, abattoir.

L'anaplasmose ovine : une maladie émergente et sous-diagnostiquée

Agent infectieux et épidémiologie

L'anaplasmose ovine est due à une bactérie intra-érythrocytaire stricte à Gram négatif de l'ordre des Rickettsiales appelée *Anaplasma ovis*. Les caprins peuvent également être infectés par cette bactérie et déclarer des formes cliniques plus sévères du fait de par leur sensibilité supérieure aux ovins. L'infection des ovins par *A.ovis* est souvent subclinique et se manifeste par des symptômes seulement en présence de facteurs favorisants tels qu'une baisse immunitaire à la suite d'un stress, l'introduction d'animaux naïfs dans une zone touchée ou un stress. Un animal infecté deviendra ensuite immunisé mais restera porteur et deviendra donc une source potentielle d'infection pour les autres animaux. Les animaux adultes restent néanmoins plus sensibles que les jeunes (2 et 11).

Initialement, les zones tempérées telles que celles retrouvées en Europe occidentale ou légèrement plus au sud ne permettent l'apparition que de cas sporadiques d'anaplasmose. Les climats tropicaux favorisent le développement des vecteurs incriminés dans la dissémination de cette maladie et donc son incidence. Cependant, depuis quelques années, le changement climatique a facilité la diffusion plus large de l'anaplasmose ovine en Europe et plus au Nord, avec des cas en France, en Allemagne, en Grèce, en Espagne, ou encore en Italie (4 et 7).

Transmission et pathogénie

La source d'infection est exclusivement le sang d'un animal porteur. Les modes de transmissions sont multiples : par des vecteurs comme les tiques (les genres *Ixodes*, *Rhipicephalus*, *Dermacentor*), des tabanidés, certains moustiques ou encore par la transmission iatrogène lors de la vaccination, de la pose de boucle ou de la tonte (1, 2 et 12).

Chez les tiques, la transmission transtadiale (entre différents stades) est décrite mais pas la transmission trans-ovarienne. Le plus souvent, la transmission entre 2 animaux se fait lorsque la tique change d'hôte lors de son engorgement sanguin. La transmission trans-utérine de la mère gravide n'est possible qu'à partir du second tiers de gestation. Le fœtus pourra mourir in-utéro d'anémie (forme d'avortement) ou devenir à son tour une source d'infection pour le vecteur.

Toutes les 24-48h, la charge bactérienne est multipliée par 2 et donc le nombre d'érythrocytes infectés aussi. Il faut ainsi attendre au moins 30 voire 45 jours avant de voir des symptômes de la maladie suite à l'infection. Dans ce cas, il faut que le taux de globules rouges infectés soit supérieur à 15%, ce qui est rarement le cas, la maladie reste alors silencieuse. La multiplication des bactéries en intra-érythrocytaire n'est pas hémolytique, c'est la réaction immunitaire de l'hôte qui provoque l'hémolyse (2, 4, 7 et 11).

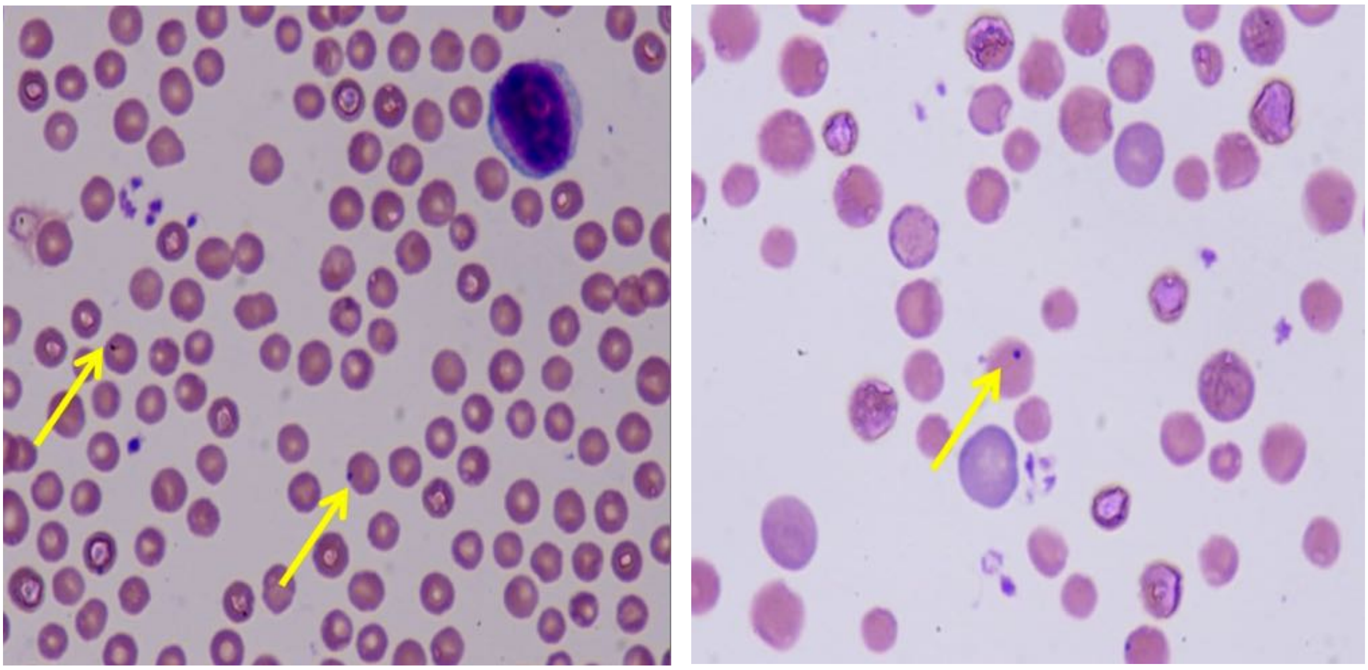

Figure 1 : images de frottis sanguin avec inclusion intra-érythrocytaire : *Anaplasma ovis* ***(8)***

Signes cliniques et diagnostic différentiel de l'ictère

Dans la majorité des cas, l'anaplasmose ovine reste une atteinte subclinique, et entrainer une baisse de GMQ. Lorsque des facteurs favorisants comme un stress immunitaire ou une maladie concomitante sont présents, un épisode clinique peut se déclarer. On observe ainsi, durant 1 à 2 semaines, une phase d'hyperthermie autour de 40°C, de l'anorexie, une baisse d'état général, des matières fécales modifiées (fèces diarrhéiques ou sèches), des fréquences cardiaques et respiratoires augmentées. De l'anémie et parfois même de l'ictère (pré-hépatique) surviennent avant la mort. Chez les agneaux, c'est le plus souvent à l'abattoir que la maladie est décelée, par la saisie totale de la carcasse pour ictère. Les pertes économiques associées sont très importantes, pour des animaux cliniquement sains (4 et 11).

Les béliers peuvent voir leur capacité de reproduction débilitée par des troubles de la spermatogénèse 7 à 8 semaines après l'infection, à cause de la phase d'hyperthermie. Il faudra, après traitement, près de 5 mois pour retrouver la qualité initiale du sperme avant infection. La reproduction des brebis quant à elle n'est pas touchée, les avortements ne sont également pas décrits sauf avec des cas de mort in-utero.

Lorsque l'on parle d'ictère dans les populations ovines et qui plus est sur des agneaux, il faut évoquer les causes suivantes :

- pour les ictères pré-hépatique : l'intoxication au cuivre, les hémoparasitoses (*Anaplasma ovis*, *Mycoplasma ovis* et *Babesia spp*), la leptospirose, *Clostridium perfringens* type A, et les intoxications végétales (mercuriale notamment)
- pour les ictères hépatiques : mycotoxines, lipidose hépatique ou abcès hépatiques à la suite d'une infection
- pour les ictères post-hépatiques : principalement les infestations par la grande douve (*Fasciole hepatica*) (9 et 10 et 13)

Diagnostic clinique : que faire en pratique

Identification morphologique sur étalement de sang : frottis

Cette méthode était initialement celle la plus couramment utilisée avant la mise en place quasiment systématique de recherche d'ADN par PCR. Il faut prélever un échantillon de sang sur un tube EDTA, principalement dans des vaisseaux de taille importante afin de récolter les globules rouges de taille et de morphologie modifiées par la présence de la bactérie en intra-érythrocytaire. Le moment idéal du prélèvement reste la phase clinique mais cette dernière reste souvent très discrète. Une coloration au RAl 555 est rapide et permet l'analyse correcte du frottis en cas de suspicion d'anaplasmose. Les anaplasmes apparaissent comme des inclusions intra-érythrocytaires rouges foncé, le plus souvent uniques, de taille 0,3 à 1m et en position périphérique du cytoplasme (voir Figure 1) (7 et 11).

Sérologie

C'est également une méthode de choix pour le diagnostic des hémoparasites mais la sensibilité peut-être relativement faible. De plus, en fonction des cas, la persistance plus ou moins longue des anticorps dans l'organisme ne permet pas de dater précisément l'infection par rapport à la date du prélèvement. Il est possible d'utiliser la technique de la sérologie couplée : une analyse avant la possible infection par l'agent pathogène et une autre quelques semaines après l'exposition. On cherche ainsi à obtenir une séroconversion avec cette technique, qui reste néanmoins peu utilisée.

Pour pallier ces aléas, la technique de PCR détaillée ensuite sera la plus à même de détecter les cas d'anaplasmose (7 et 11).

Identification d'ADN par PCR

Actuellement et compte tenu des avancées en termes de PCR et de détection d'une faible quantité d'ADN, c'est la méthode de choix utilisée en routine en laboratoire. C'est une méthode très sensible pour détecter l'ADN bactérien sur sang EDTA, permettant de distinguer notamment de la sérologie, les espèces du genre Anaplasma. De plus, elle permet rapidement de mettre en évidence une infection récente mais également le portage persistant d'animaux chroniques (7 et 11).

Étude des données de l'abattoir de Bellac : évolution des saisies pour ictère des ovins depuis 4 ans et leur interprétation.

Ictère ou adipoxanthose

Les saisies à l'abattoir et notamment les saisies totales ont des origines très diverses.

L'adipoxanthose, qui n'entraine pas de saisie, est une coloration jaune de la graisse uniquement. Elle est le plus souvent la conséquence d'une l'alimentation riche en caroténoïdes, molécules liposolubles qui se fixent facilement aux graisses. Cette coloration est rarement retrouvée chez les ovins, car les caroténoïdes sont rapidement éliminés chez cette espèce. Il s'avère qu'un déficit d'enzyme d'oxydation et de transformation des caroténoïdes, d'origine génétique, peut favoriser l'apparition de l'adipoxanthose.

L'ictère correspond à une coloration jaune, anormale, de la peau et des muqueuses par la présence de bilirubine (Figure 2). Cette dernière est issue de la dégradation de l'hémoglobine dans le sang.

Figure 2 : Carcasses ictériques d'ovins saisies à l'abattoir de Bellac. Crédit photo : Dr P.Autef.

Les colorations en cas d'ictère sont hétérogènes sur l'ensemble de la carcasse, allant de l'orange, jaune jusqu'au vert lorsque la bilirubine s'oxyde au contact de l'air en biliverdine.

Trois types d'ictère sont considérés en fonction de leur origine :

- pré-hépatique ou hémolytique à la suite d'une hémolyse massive intravasculaire et donc une libération de bilirubine dans l'organisme,
- hépatique due à une insuffisance hépatique fonctionnelle et majeure,
- post-hépatique ou cholestatique due à une rétention de bilirubine par obstruction des voies biliaires.

La différenciation entre l'adipoxanthose et l'ictère peut se réaliser en observant : les valvules cardiaques, l'endartère des artères moyennes, et de manière plus spécifique, la muqueuse du bassinet rénal. Ces tissus sont jaunes seulement en cas d'ictère.

En cas d'ictère, la carcasse est systématiquement saisie. En présence d'un doute concernant la coloration, la carcasse est mise en consigne pendant 24h. Si de la biliverdine se forme (produit de dégradation de la bilirubine au contact de l'air), l'ictère est avéré et la carcasse est saisie (6).

Évolution annuelle des saisies à Bellac

L'étude se concentre sur la période de janvier 2021 à décembre 2024 inclus. L'abattoir de Bellac, situé en Haute-Vienne (climat océanique avec été tempéré) dans un bassin ovin allaitant important du Limousin, est quasiment exclusivement dédié à l'abattage des ovins. Il y est tué près de 120 000 ovins chaque année et ces dernières années ont montré une légère augmentation depuis deux ans en réponse à une réorientation de clients vers l'abattoir de Bellac. Nous avons sélectionné les saisies totales pour ictère uniquement et non pour coloration anormale afin de quantifier l'importance de ces dernières. Comme nous l'avons abordé au 1.3, le diagnostic différentiel de l'ictère chez les ovins est large, mais la fréquence des saisies peut orienter, sans même l'affirmer, les hypothèses logiques de terrain rencontrées. Des cas d'*A.ovis* ont été décrits en Espagne et au Pays basque à la suite de plusieurs épizooties et l'adaptation des vecteurs à notre climat tempéré orientent également les suspicions en France et dans la région concernée. (4)

La figure 3 met en évidence une évolution ascendante assez récente, surtout en 2024 des saisies pour ictère à l'abattoir de Bellac.
On constate à l'aide de la courbe des pourcentages, qu'il y a une augmentation marquée des saisies pour ictère en 2024 à Bellac et qu'elle n'est pas due à une augmentation des abattages d'ovins sur la chaine. La situation entre 2021 et 2023 était assez stable.

Incidence et évolution saisonnière des saisies pour ictère à Bellac

En se basant sur la même période que précédemment, nous nous sommes intéressés maintenant à l'évolution mensuelle des saisies pour ictère et ainsi rechercher une incidence saisonnière de ces dernières.
Nous constatons des évolutions proches sur les 4 dernières années pleines pour lesquelles nous disposons des données. Une augmentation importante a lieu chaque année dès le mois d'avril-mai pour arriver au pic vers le mois de juin ou juillet. Les saisies diminuent ensuite plus lentement jusqu'en septembre ou octobre. On constate pour l'année 2024 un rebond au mois d'octobre qui peut coïncider avec la hausse ponctuelle des températures ressenties à ce moment-là.

Comme vu dans le 1.2, les tiques responsables du portage et de la dissémination d'*A.ovis* sont maintenant habituées au climat plus tempéré, doux et humide, comme dans le Limousin. Les variations mensuelles constatées pour les saisies à Bellac, peuvent se corréler à l'activité majoritaire printemps-été des stades adultes de *Rhipicephalus spp*, majoritairement impliqué comme vecteur d'*A.ovis*. Les tiques du genre *Ixodes*, également vecteur d'*A.ovis*, sont fortement représentées en région tempérée et ont une activité adulte démarrant mi-avril jusqu'au mois de septembre, comme le montre la Figure 5 en Europe centrale et au Royaume-Uni. Ces régions et notamment le Royaume-Uni possèdent des caractéristiques climatiques océaniques similaires à la région du Limousin concernée ici (12).

On constate également que la moyenne d'abattage mensuelle est la plus haute en mars-avril chaque année, correspondant à la période de production d'agneaux pour Pâques. Elle est ensuite assez stable sur le reste de l'année, notamment par la production d'agneau de contre-saison, et n'est pas en lien avec l'augmentation saisonnière de saisie pour ictère mentionnée ci-dessus.

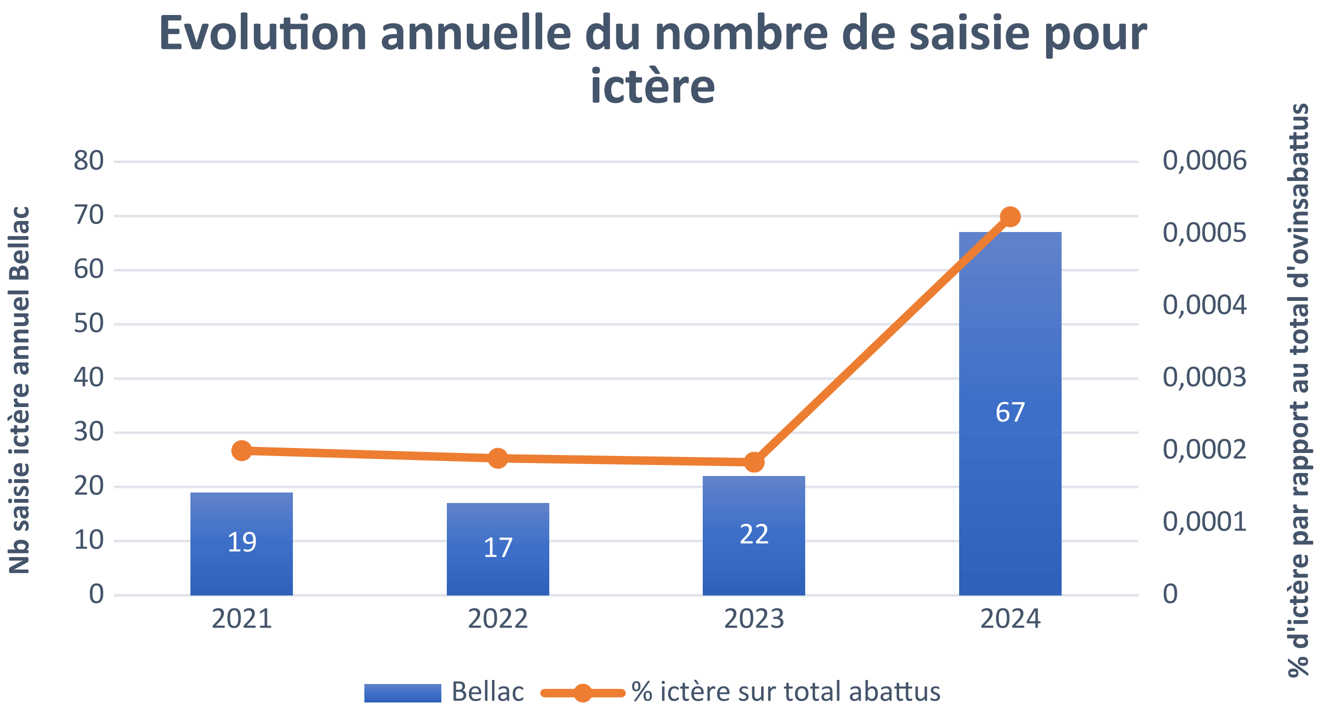

Figure 3 : Nombre de saisie pour ictère par année à l'abattoir de Bellac et pourcentage d'ictère sur le total d'ovins abattus annuellement

Discussion

L'anaplasmose ovine est une maladie infectieuse émergente, cela veut dire qu'elle apparaît ou son incidence augmente dans un lieu donné, ici en France et en Europe, et qui se propage rapidement et majoritairement par les morsures de tique. Lorsqu'elle arrive dans une zone où les animaux sont naïfs, elle est capable de provoquer une épizootie et des pertes économiques importantes. En effet, il s'agit d'une maladie infectieuse qui se présente très discrètement par de l'ictère, le plus souvent observé lors de la saisie d'ovins à l'abattoir. Les animaux arrivant à l'abattoir et tués sur la chaine sont cliniquement sains, cela permet donc d'écarter les causes d'ictère avec une pathologie aiguë : intoxication au cuivre et au mercuriale, entérotoxémie de type A ou encore la leptospirose (7).

Les données de l'abattoir de Bellac montrent que les saisies pour ictère sont majoritaires entre les mois d'avril-mai et de septembre, avec possiblement une augmentation en octobre. Nous avons montré qu'après infection par une tique adulte, il faut attendre entre 30 et 45j, dans le cas d'*A.ovis*, pour que les symptômes débutent. Or, les activités annuelles des différentes espèces de tiques mentionnées auparavant, *Ixodes* spp mais également *Rhipicephalus spp*, montrent un pic dès le mois de mars et jusqu'au début de l'été. Ainsi, les attaques de tiques adultes dans la région de Bellac, seraient corrélées temporellement aux saisies pour ictère à la suite en abattoir. Il y a donc une suspicion d'hémoparasitoses de type *A.ovis* qu'il faut vérifier.

En pratique, après saisie en abattoir, il serait intéressant de remonter aux fermes d'origine de ces ovins infectés. Des PCR pourraient être réalisées sur des lots d'agneaux avant la vente, afin de confirmer la présence ou non d'*A.ovis* et ainsi mettre des mesures en place. La prévention des infestations par les tiques est une perspective, tout comme le traitement curatif à base d'oxytétracycline pour les cas positifs. De plus, lors de ces saisies, l'exploration de la rate pour son poids ainsi que sa composition cellulaire, permettrait de poursuivre le diagnostic d'infection par *A.ovis*. En effet, de nombreux cas de splénomégalie ainsi que de modification du parenchyme splénique en réaction aux globules rouges infectés ont été constatés (7).

On pourrait également effectuer des recherches PCR d'*A.ovis* directement sur les rates prélevées des animaux saisies. En effet, la rate comme organe de stockage, a également un rôle pour phagocyter et éliminer les érythrocytes infectés par les anaplasmes, et permettrait donc de confirmer en post-mortem l'infection à *A.ovis*. Cette technique a déjà été réalisé en Espagne sur de la faune sauvage, néanmoins le frein resterait le cout de sa réalisation (5).

Enfin, le cas de l'espèce *Rhipichephalus bursa* est mentionné dans le Pays basque comme la tique porteuse d'*A.ovis* à 50% et c'était la seule espèce présente. Malgré le climat plus chaud dans cette région qu'à Bellac, il faudrait vérifier l'évolution et la possible présence de cette espèce dans notre biotope sur des ovins parasités (4).

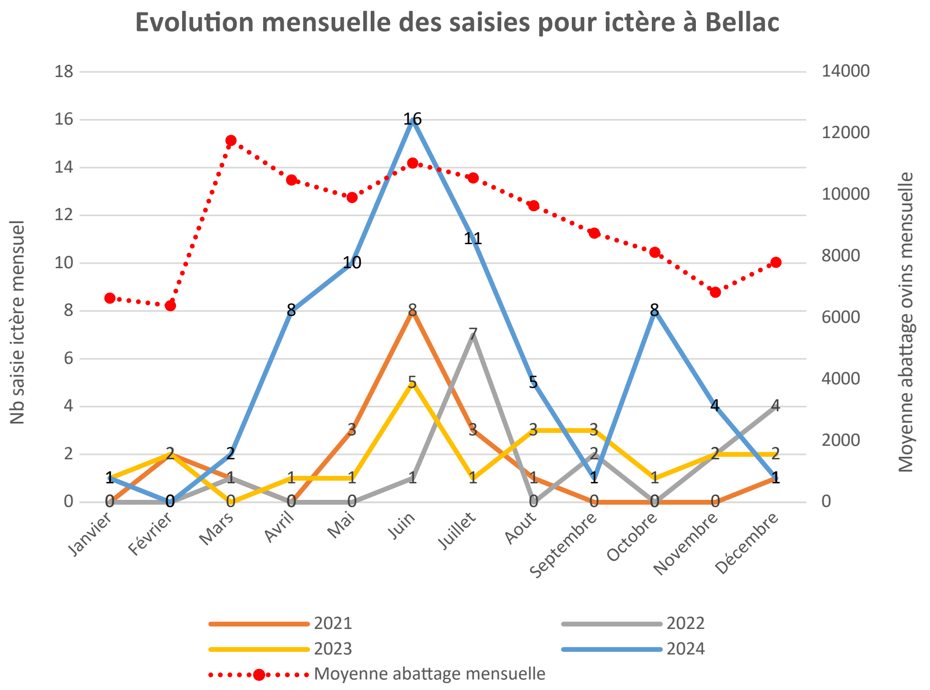

Figure 4 : Nombre de saisie pour ictère avec évolution mensuelle à l'abattoir de Bellac et moyenne d'abattage d'ovins tout confondus mensuellement depuis 4 ans.

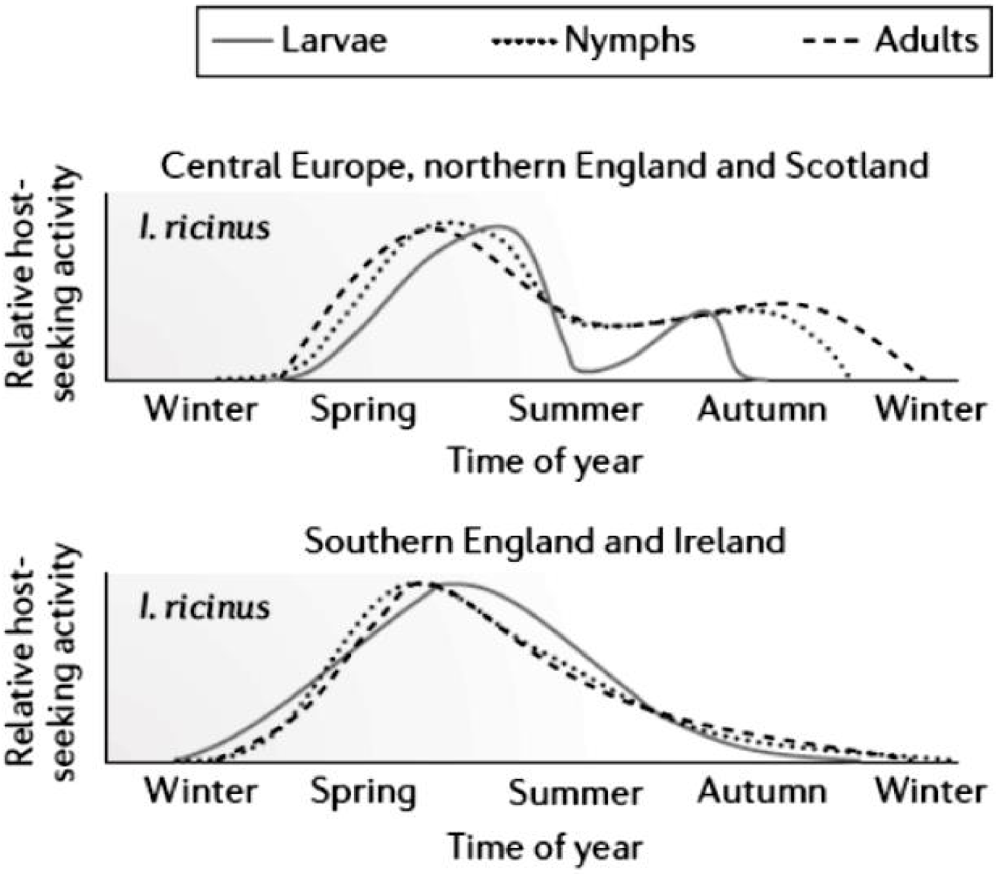

Figure 5 : Activité saisonnière des différents stades d'I.ricinus sur une année en Europe centrale et au Royaume-Uni (3)

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