Testing a computer-based method to analyze and identify different *Mycoplasma bovis* strains using targeted DNA enrichment

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Mycoplasma bovis infections in dairy herds can lead to clinical symptoms such as mastitis, arthritis, and pneumonia. Additionally, certain cows become subclinical intermittent shedders. Moreover, recent research revealed considerable variation in transmissibility among 20 clinically infected dairy herds. This variability is not well understood, but it is hypothesized that strain differences could explain most of this variation, rather than external farm management factors. However, identifying strains after culture is subjective to numerous biases, and becomes especially challenging without culture. Until now, an efficient and accurate method to identify *M. bovis* strains in clinical samples is lacking. Therefore, the goal of this research was to determine how well a computer-based method, focused on enriching specific genetic material, identifies the quantity and number of milk-isolated *M. bovis* strains. At a 90% enrichment, the approach consistently allowed for the accurate inference of both the correct number and identity of strains in all tested scenarios mimicking mixed infections. Accuracy in distinguishing both the number and identity of strains diminished when the enrichment percentage was set at a lower value, such as 30%, particularly when there were six or more strains present. Strain differentiation of *M. bovis* is extremely important when it comes to controlling and eradicating infections in dairy herds, as it facilitates the tailoring of farm-specific control measures for efficiently reducing the spread and number of affected animals in a herd.
Take home message: This targeted enriched metagenomic approach, which enriches *M. bovis* DNA pieces, has proven to be successful at identification of strains based on variances in DNA nucleotides. Next steps involve applying this approach to field samples taken from animals with various disease presentations and outbreak farms to determine the strains associated with distinct clinical outcomes and transmission characteristics.

Longitudinal analysis of digital dermatitis in cattle: Identifying the bacteria initiating lesion development

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Digital dermatitis (DD), a leading cause of lameness in Canadian dairy cows, presents challenges due to its unclear causation and pathogenesis. Considered a polymicrobial disease, it involves *Treponema* spp. and other anaerobes whose specific roles are not well understood. While previous studies have identified the core bacterial species involved in DD, the specific species initiating the lesion are still unknown. Therefore, this longitudinal study aimed to identify the bacterial species most crucial for initiating lesions and identify the opportunistic species by monitoring changes in their abundance over time. Over 12 weeks, weekly swabs were taken from both hind feet of 53 Holstein dairy cows without DD (M0 stage) in a DD-affected herd. Bacterial DNA was extracted and subjected to qPCR targeting *Treponema phagedenis*, *Treponema medium*, *Treponema pedis*, *Porphyromonas levii*, *Bacteroides pyogenes*, *Fusobacterium necrophorum*, and *Fusobacterium mortiferum*. Out of 53 cows, 8 developed DD, while 31 remained healthy, and 14 developed heel erosion. Analyses of the results have started, and we will be completed by the 2024 conference. Samples will be compared at key time points (Day 0 [DD case emerged], -1, -2, -3 and +1 weeks) between cows developing DD and those remaining healthy. We expect certain targeted bacterial species to increase in numbers before and during lesion onset, indicating their primary role in initiating DD. Conversely, secondary opportunistic pathogens are expected to increase in numbers only after the lesion has been established.
Take home message: This study is expected to yield impactful findings in DD microbiology, including the roles of DD-associated bacteria in lesion initiation, with the goal of disease prevention and targeted early interventions. The findings on lesion initiation will be useful to induce DD in experimental models for evaluating new treatments and vaccines.