**2011F034R Characterization of coagulase negative staphylococcal species isolated from intramammary infection and extramammary sites on dairy farms**

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Background: Bovine mastitis causes losses of over $300 million annually to the Canadian Dairy Industry. More antibiotics are used in the dairy industry for treatment of mastitis than for all other reasons combined. The coagulase-negative staphylococci (CNS) are a group of more than 50 bacterial species which are the most commonly recovered bacteria from the bovine mammary gland. Previous technologies used to identify CNS species have been demonstrated to be inaccurate therefore little was known about the ecology of different CNS species on dairies therefore control measures could not be implemented.

Objectives:

1. Develop methods to identify different CNS species using high resolution melt analysis (HRMA)
2. Determine species distribution of CNS isolated from the environment, body sites, and IMI’s from dairy facilities
3. Compare species of CNS isolated from milk with those isolated from extramammary sites to determine potential sources of CNS IMI.
4. Use whole-genome sequencing to distinguish between different strains of CNS, to further characterize the relationships between CNS strains isolated from different sites, enabling the accurate determination of the epidemiology most commonly associated with CNS species.

Methods:

1. 5,000 CNS isolates were obtained from the CBMMQRN Mastitis Pathogen Program Collection from 92 Canadian dairy farms and genotyped at the UCVM. Two different speciation techniques were used with either rpoB or cpn60 as the target gene.
2. Ten different dairy farms in Saskatchewan were sampled to determine CNS reservoirs. On each farm 75 – 100 animals were used to collect quarter milk samples as well as body site samples from the teat skin, mizzle and perineum. Environmental samples were also taken from housing areas, milking units, farm equipment, feedstuffs, non-bovine animals and water sources. Bacteria from all samples were cultured and classified.
3. CNS isolates identified during objective 2 were used. A CNS intramammary infection was defined as an udder quarter from which at least 200 cfu/mL of the same CNS species is isolated on 2 consecutive cultures.
4. All CNS isolates were obtained from the National Cohort of Dairy Farms (NCDF) and cultured. Whole genome sequencing was completed from 500 randomized isolates, spanning 26 species, previously identified by HRMA analysis, which were collected from 91 herds and different geographic regions, Atlantic (Nova Scotia), central (Ontario and Quebec), and the prairies (Alberta). The final assembled genomes were are saved into different formats for subsequent analysis.

Outcome:

1. The agreement between the two techniques (87%) indicates that the two speciation methods are comparable for the identification of CNS isolates from IMI.
2. 158 cows were infected with CNS across the 10 dairies with 198 CNS infected quarters among the infected cows. This is slightly lower than the CNS prevalence expected based on the CBMMQRN cohort study (20%). 154 body site samples were positive for CNS and 25 environmental samples were positive (isolation rates of environmental samples may have been decreased due to the sampling protocol). The major species isolated from milk were S. chomogenes (54% of milk samples), S. xylosus (19 %) and S. haemolyticus (11 %). The major species isolated from body sites were S. equorum (47% of body site samples), S. xylosus (21 %) and S. sciuri and S. vitulinus (9%). The major species isolated from the environment was S. equorum (52 % of environmental samples). The effect of different CNS species on mammary glan infection was determined and S. chromogenes and S. xylosus do not have a major impact whereas S. haemolyticus significantly increased SCC.
3. The major CNS species isolated from milk samples was S. chromogens which was not found on body sites or the environment. Therefore this is a contagious pathogen that transmits during milking time. Control programs for contagious pathogens focus on milking tiem hygiene, milking machine maintenance, appropriate treatment of clinical mastitis during lactation, dry cow therapy and culling of chronic animals. S. xylosus was recovered from both milking samples as well as body sites and the environmental suggesting that it is an opportunistic pathogen. Opportunistic pathogens can be transmitted in a contagious or environmental manner. If S. xylus is isolated from milk samples than good environmental mastitis control programs should be implemented (bedding management, housing design and cleanliness of surfaces). S haemolyticus is a contagious pathogen and has the greatest effect on mammary gland inflammation, therefore good contagious mastitis control practices will control the majority of CNS infections and improve udder health and animal productivity.
4. Whole genome sequencing analysis is not yet completed but will generate useful information. We will be able to compare speciation methods to this gold standard. We will be able to compare species and strains of CNS with regard to effects on milk production and microbial resistance. Additionally, we will be able to develop practical and effective treatment and control strategies from the most common CNS species.

Recommendations on farm: The majority of CNS species appear to be contagious pathogens, therefore good contagious mastitis control practices will control the majority of CNS infections and improve udder health and animal productivity.

Benefits to industry: This research has resulted in the characterization of the behaviour of the common CNS species on dairies in Western Canada. This allows for the development of rational control strategies for these emerging mastitis pathogens. This will directly benefit Alberta’s agriculture and food industry by improving in udder health through mastitis control.

Improved udder health will result in increased animal welfare, increased animal productivity, reduced bacterial contamination of milk and reduced antimicrobial use in dairy cattle.

KTT:

* 2 PhD, 1 MSc, 1 undergraduate, 2 research associated trained
* 3 manuscripts prepared for submission to scientific journals
* 4 scientific presentations and 3 industry presentations