

Bovine mastitis and *S. chromogenes*; concerns, conundrums, and characteristics

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An important cause of mastitis are minor pathogens such as non-aureus staphylococci (NAS), which are the most commonly isolated bacteria from milk samples of dairy cows. The most common NAS species globally and especially in Canada, is *Staphylococcus chromogenes*. To understand why *S. chromogenes* is so common we need to study how it interacts with the cow's immune system. We can do that by analysing its genetic code, which gives it the instructions to make proteins that interact with the cow immune system. By genetic comparisons between *S. chromogenes* strains and close NAS relatives, we can narrow down several *S. chromogenes* specific virulence factors. These factors might explain the more persistent and seemingly adapted propensities of *S. chromogenes* in bovine mammary glands. Several of these virulence factors are related to binding and immune avoidance mechanisms. We will test these virulence factors in experimental assays such as biofilm tests, tissue component binding tests, and blood cell assays. Next, we will remove these virulence factors in deletion mutant and observe how they perform in these experimental assays. Genetic manipulation of *S. chromogenes* is notoriously difficult but we have developed a successful method. Further research in dissecting the underlying mechanisms of *S. chromogenes* virulence factors will be essential to understand the overlying host-pathogen mechanisms of mastitis and recognizing some virulence factors essential to *S. chromogenes*, and others potentially important for all NAS. Such research will focus on the particular interactions virulence factors have with the epithelial cells of the bovine mammary gland, as well as the mechanisms involved in avoiding white blood cells by *S. chromogenes*.

Take home message: This research will be essential to scrutinizing the significant virulence factors involved in the bovine mammary gland adapted *S. chromogenes*. By identifying the virulence factors involved, we can begin to break down the adhesion, persistence, and host avoidance mechanisms involved and come up with a solution to lower the impacts of bovine mastitis.

Evaluation of calves testing strategy to support Johne's disease control programs and early disease detection assays

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The lack of testing strategies in dairy calves can partially explain unsuccessful eradication worldwide of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in dairy herds. The current diagnostic tests to detect MAP infection are less sensitive in the earlier stages of MAP infection. We propose to evaluate the effects of testing dairy calves on within-herd MAP prevalence as part of a new JD control program. First, testing and control measures to develop the inclusion of calves in the testing strategy. The second objective is to evaluate the sensitivity and specificity of fecal qPCR for the gene ISMAP02, ELISA, and interferon-gamma assay (IGRA) in naturally MAP-infected calves. Therefore, the implementation to follow calves naturally infected with MAP over time, considering field conditions, would provide a better evaluation of the applicability of the IGRA. Considering IGRA an indirect marker for MAP exposure, it would be possible to measure the likelihood of latent infection becoming an active infection based on IGRA, which will support early decisions of suspected MAP-positive calves on the farm. Eight dairy farms were selected based on the presence of MAP-positive environmental samples. We will be testing animals <12 mo of age using ELISA and fecal qPCR as reference tests and IGRA every two months, and twice a year whole-herd sampling using fecal qPCR and ELISA on animals >12 mo of age. The results will apply to dairy farms worldwide and provide a better understanding of MAP transmission between calves.

Take home message: IGRA might be a potential indirect marker for MAP exposure to detect latent infection in calves. IGRA testing of blood samples will be at least 10% more sensitive and specific in detecting early immune response in MAP-positive calves than ELISA and direct qPCR from feces.