

Understanding bovine mammary gland niches for staphylococci using different milk sampling techniques

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Introduction

Mastitis is usually caused by a bacterial intramammary infection (IMI) with *Staphylococcus* being the most commonly isolated genus. The standard method for diagnosing an IMI is to aseptically prepare the teat end and collect milk via the teat orifice into a sterile vial for bacterial culture. While this method has been advocated for decades, there has been debate about the importance of some bacteria isolated from such samples when the milk somatic cell count (SCC) in the mammary quarter is < 200,000 cells/ml (threshold for a healthy mammary gland). Recently, Hiitio et al. (2016) showed that milk samples collected directly from the gland cistern were less likely to contain staphylococcal DNA when using a commercial PCR diagnostic test kit than milk samples that were collected via the teat orifice. Unfortunately, the PCR only differentiated staphylococci into *Staphylococcus* spp. and *Staphylococcus aureus*, and so it is unknown which of the non-aureus staphylococcal (NAS) species may be more likely to inhabit the teat canal than those that cause a true IMI. These data provide preliminary evidence that some staphylococci in milk may be teat canal inhabitants and not intramammary pathogens that cause mastitis. The objective of this study was to determine if some species of staphylococci isolated from milk are only isolated when the sample is collected through the teat orifice and absent in cisternal milk collected using the method described by Hiitio et al. (2016).

Materials and Methods

All cows at a MU Foremost Dairy had mammary quarter milk samples collected via the teat orifice to establish herd prevalence of staphylococcal isolates in milk. Cows with at least one mammary quarter culture positive for a *Staphylococcus* spp. and one mammary quarter culture negative were subjected to a second sampling. Milk was aseptically collected via the teat orifice as before, following which a milk sample was aseptically aspirated from the gland cistern of the same

quarter using a sterile needle and vacutainer tube (Hiitio et al., 2016). Milk samples were cultured for bacteria according to National Mastitis Council guidelines. An aliquot of milk collected via the teat orifice was also used for SCC enumeration. Agreement between the culture results from cisternal milk and milk collected via the teat orifice was evaluated using the kappa coefficient.

Results

Mammary quarter-level prevalence of staphylococcal IMI among the 160 cows (640 quarters) sampled during the whole herd culture was 12% (79/641 quarters). To date, 20 mammary quarters on 10 cows have been sampled using both sampling techniques at the same time. Among the samples collected via the teat orifice, 10 quarters yielded no growth and 10 quarters yielded growth of a *Staphylococcus* spp. Among the samples collected by cisternal aspiration, 1 sample could not be obtained, 13 quarters yielded no growth, and 6 samples yielded a *Staphylococcus* spp. Among the 19 quarters that had results for both samples, 84% (16/19) agreed and 16% (3/19) did not agree (kappa = 0.68). Of the 3 quarters that did not agree, the cisternal sample yielded no growth and the teat orifice sample yielded a *Staphylococcus aureus*, a *Staphylococcus chromogenes*, and an undifferentiated *Staphylococcus* spp., respectively.

Significance

Data collection is ongoing and more numbers are needed; however, in general, there was good agreement between cisternal and teat orifice collected samples. Preliminary data corroborate other's data suggesting that some strains of staphylococci may only inhabit the teat canal and not the mammary parenchyma.