Research Focus

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Lactococcus: an emerging mastitis pathogen

If you've ever cultured milk from a cow with mastitis, you may have gotten a result of "Streptococcus species." Many species of Streptococci have been implicated as a cause of bovine mastitis.

This is a general category of infections which can include many gram-positive organisms that are not more specifically identified as are more common pathogens such as *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. Historically, these less common organisms are grouped in this category because identifying each individual isolate would be costly, requiring additional time and expense to achieve a species level diagnosis. "Strep species" may include organisms that are actually in the Enterococci, Lactococci, or Aerococci genus, in addition to other species of *Streptococcus*. From a mastitis management perspective not much is known about this catch-all group of organisms.

Longitudinal characterization of mastitis causing pathogens previously identified as "other Streptococcus species."

In recent years, *Lactococcus* species has emerged on some farms as a pathogen identified in chronic high SCC cows, even after treatment with intramammary antibiotics. These cows were all originally identified with "Strep species" mastitis, but were more specifically identified with *Lactococcus lactis* infections with additional diagnostic testing. This has lead us to wonder exactly which organisms are in this broad category? And, can we identify any differences that may impact management?

Previously, *Lactococcus lactis* and *Lactococcus garvieae* are bacteria that had only rarely been identified with mastitis, and were considered not to be of significant importance until these more recent investigations. The first cases of mastitis were found in water buffalo in Brazil in 1996 and 1997 from subclinical mastitis samples. It was then found in milk samples from dairy cows in 1999, again from subclinical mastitis.

In 2014, Quality Milk Production Services (QMPS) began to investigate mastitis caused non-*agalactiae Streptococcus* species. All samples in this category were cultured by QMPS and Countryside Veterinary Clinic in Lowville, NY, and were further speciated using new technology called Matrix-assisted desorption ionization time of flight (MALDI-ToF). MALDI-ToF is a fast and accurate technology used to identify bacteria and fungi with mass spectrometry. Of the 473 samples that were identified in this category (out of 8,361 samples processed), 155 were *Steptococcus dysgalactiae*, 150 were *Streptococcus uberis*, 112 were *Lactococcus lactis* and 22 were *Enterococcus saccharolyticus*. The remaining 34 isolates belonged to various species in the *Streptococcus*, *Enterococcus*, *Aerococcus* and *Lactococcus* genera. This observational study included samples from 143 farms.

Cows from farms with DHIA records were tracked for two test days after their mastitis event to evaluate the association of various pathogens with subsequent SCC. SCC "resolution" was defined as a SCC less than 200,000 cells/ml on DHIA test 15 to 45 days after the end of treatment (regardless of treatment product). *Streptococcus dysglactiae* and *Streptococcus uberis* both showed a higher rate of SCC resolution (70% of 80 cows and 77% of 13 cows, respectively) than Lactococcus *lactis*, which showed a lower rate of resolution with only 33% of animals showing a SCC resolution (n=57). Cows infected with *Streptococcus dysgalactiae* and *Lactococcus lactis* responded differently to therapy.

This initial study helped illuminate that *Lactococcus lactis* is an important mastitis pathogen, and was even more common on some farms than others. To investigate Lactococcus species further, we focused our 2015 investigation on five farms that had greater than 20 cows with intramammary infections due to *Lactococcus lactis* in the summer of 2014. We were able to resample cows 14 to 28 days after the initial sample to assess bacteriological cure and track the cows through DHIA records for additional information.

229 animals were identified with this "Strep species" type of infection and accurately identified to the genus and species level using MALDI-ToF. Of the organisms identified, 67 were *Streptococcus dysglactiae* (26.5%), 28 were *Streptococcus uberis* (11.1%), 118 were *Lactococcus lactis* (46.6%), and 16 were various species in the *Streptococcus*, *Enterococcus*, *Aerococcus* and *Lactococcus* genera. Only a few animals had each type of infection which prevented further analysis of these infrequent infections.

Table 1: MALDI-TOF identification of isolates of Gram-positive, catalase-negative, non-ß hemolytic cocci.

Row Labels	Total
Streptococcus dysgalactiae	155
Streptococcus uberis	150
Lactococcus lactis	112
Lactoccus garvieae	16
Streptococcus parauberis	3
Streptococcus suis	1
Streptococcus gallolyticus	6
Streptococcus oralis	1
Enterococcus saccharolyticus	22
Enterococcus faecium	1
Enterococcus casselifl ves	1
Enterococcus facecalis	3
Aerococcus viridans	2
Grand Total	**473**

The difference in bacteriological cure rates in 2015 mirror the difference in SCC reso-

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Influence of cow characteristics and premilking udder preparation on milk flow and teat condition

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could be observed in cows with round teat-end shape when stripping time and lag time were increased, respectively.

Primiparous cows had the lowest risk of bimodal milk let-down while cows towards the end of lactation were more likely to show a bimodal milk flow pattern. Increasing stripping time resulted in decreased risk of bimodal milk let-down.

The risk of short-term changes in teat condition was lower in cows in later stage of lactation. Second lactation cows were least likely to demonstrate short-term changes after milking. While cows with flat teat-end shape had lower risk to develop short-term changes in teat condition, increasing two minute milk or lag time could decrease likelihood of short-term changes in teat condition in all cows.

We were able to identify associations between cow characteristics, milking routine, milk flow parameters, and short-term changes in teat condition. Our data suggests that premilking udder preparation matters and is in accordance with previous research indicating that excellent milk flow rates can be achieved with a stripping time of 8 to 12 seconds and a lag time of 90 seconds. Increasing stripping time resulted in higher two minute milk, less time in low flow, and less bimodal milk let-down (Fig. 2). Expanding lag time could increase two minute milk, decrease time in low flow, and diminish the risk of short-term changes in teat condition postmilking. Moreover, our results indicate that consideration of the interactions between different cow characteristics and premilking udder preparation has the potential to substantially improve parlor efficiency and udder health.

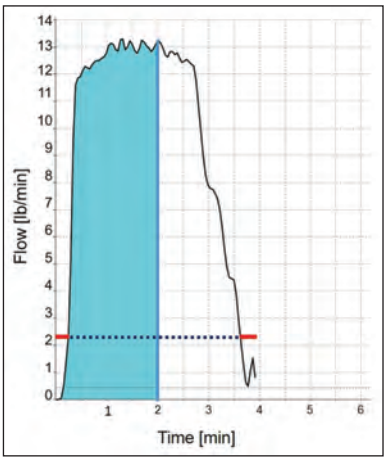

Figure 2: Milk flow curve showing a unimodal milk let-down. The blue area under the curve illustrates the amount of milk harvested within the first two minutes (two minute milk; lb). The red lines indicate time in low flow (seconds below 2.2 lb/min flow rate). Note the difference in two minute milk, time in low flow and unit on time compared to the milk flow curve in Figure 1.

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lution found in 2014. Difference between the bacteriological cure rate for *L. lactis* (59% cures, n=66) was significantly different than *S. dysgalactiae* (92% cures, n=49) and *S. uberis* (89% cures, n=18). There may also be a difference in risk of a repeat mastitis event - at least a second clinical mastitis event was recorded for 26% of cows with *S. dysgalactiae* (n=67), 31% of *L. lactis* (n=118) and 14% of *S. uberis* (n=28). The difference between *S. uberis* and *L. lactis* indicated a potential trend for an increased risk of recurrent mastitis for cows with *L. lactis* infections. Finally, there was a trend towards a difference in number of cows leaving the herd after a *Streptococcus dysgalactiae* infection (19%), compared to those with *Streptococcus uberis* (36%) or *Lactoccocus lactis* (31%).

Identifying differences in intramammary infections due to different bacteria shows why this information on clinical mastitis cases in cows is important. Even within a group of bugs that previously haven't been differentiated, different infection characteristics are observed, which may impact management decisions. *Lactococcus lactis* appears to be more common than originally thought and warrants further investigation so it can be managed to the best of our abilities within the dairy industry. ❐

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