Evaluation of aseptic techniques used to collect bovine milk samples

Veterinary Research Scholars Program
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Background

Intramammary infections are one of the leading causes of mastitis (Guarin, Paixao and Ruegg, 2017). Bacterial culture of milk is one of the primary methods used to diagnose intramammary infections (Hiitio et al. 2018). Aseptic technique is thought to be vital when collecting milk samples for culture to minimize false positive results caused by bacteria on the teat end entering the sample during sample collection (Hiitio et al. 2016). Recommended practices for aseptically collecting milk include: gloved hands, pre-milking teat disinfection with a teat dip followed by drying with a single-use towel, scrubbing the teat end with gauze soaked in 70% alcohol, discarding a few more streams of milk, and collecting the milk sample (Middleton et al. 2017). While this approach is recommended, it has never been critically evaluated in a peer-reviewed study. **Hence, the objective of this study was to determine if there were differences among teat end preparation techniques with regard to potential contamination of milk samples for culture.**

Photos: Dr. Tessa Marshall

Materials and Methods

Mammary quarter foremilk samples were collected from 168 dairy cows. All 168 cattle were housed on the same bedding, shavings atop mattresses, and were milked twice daily.

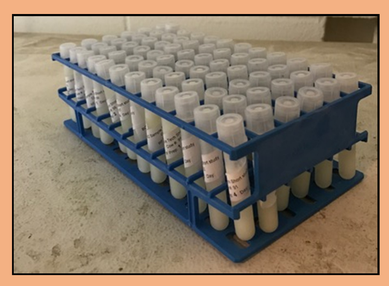
Four different teat preparation methods were used to compare contamination rates in milk samples. Teat preparations were randomized at the quarter level within each cow. Milk samples were taken during two milking shifts.

After samples were collected, they were frozen at -20^{o}C until further processing.

Table 1. Description of the four teat preparation techniques used prior to obtaining foremilk samples.

Control Group (1)	**No preparation before collecting milk**
Treatment Group 2	**Pre-dip and dry teat only before collecting milk**
Treatment Group 3	**Scrub teat end with alcohol only before collecting milk**
Treatment Group 4	**Pre-dip, dry teat, and scrub teat end with alcohol before collecting milk**

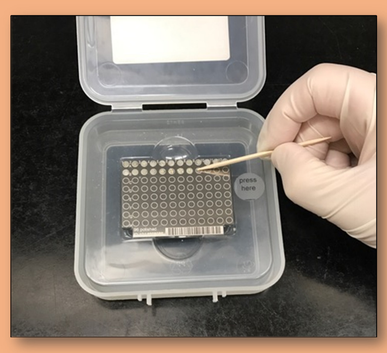
- Milk was thawed at room temperature (~22°C) the day of culture.



- Milk samples were plated on blood agar. The student plating the samples and reading the culture results was blinded to treatment group.

- Milk cultures were read 48 hours after plating, with the number of morphologically different bacterial colony types quantified and isolated.

- Each isolate was then speciated using MALDI-TOF Mass Spectrometry.



Data were analyzed using a Kruskal-Wallis one-way analysis of variance on ranks to compare the median number of colony types isolated from milk samples among the groups with post-hoc pairwise comparisons made using the Dunn's method ($P < 0.05$).

Preliminary Results

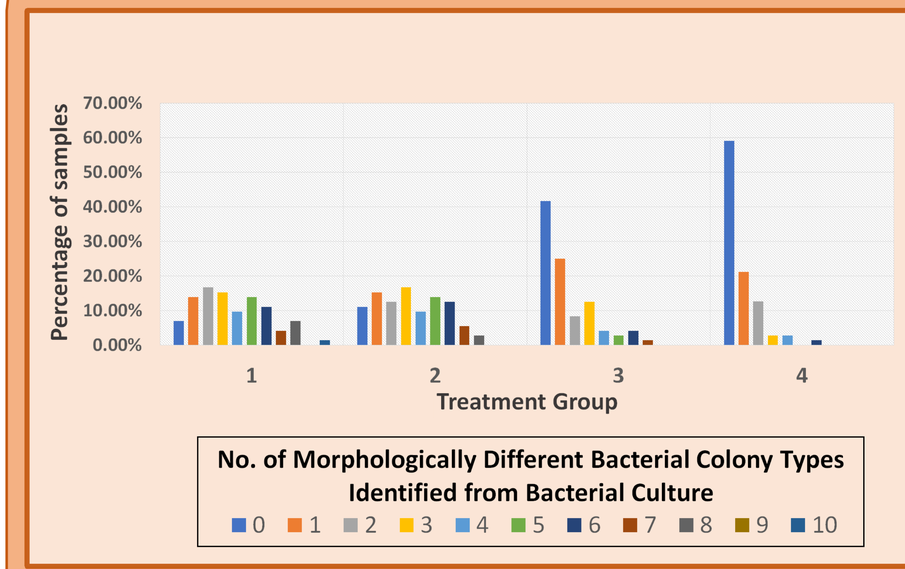


Figure 1. The percentage of samples with morphologically different bacterial colony counts within teat preparation group (1-4). Samples with ≥ 3 colony types were considered contaminated.

To date, 287 of 672 milk samples have been processed (Table 2).

Table 2. Overview of total samples and progress made to date.

Total cows sampled:	**168**
Total milk samples collected:	**672**
Milk Samples Plated as of 7/30/18:	**287**
Morphologically different bacterial colony types isolated and enumerated as of 7/30/18:	**662**
Milk samples left to plate:	**385**

Median numbers of colony types for groups 1 and 2 were significantly higher than groups 3 and 4 ($P<0.001$), but groups 1 and 2 ($P = 1.000$) and 3 and 4 ($P = 0.210$) did not differ from each other (Table 3).

Table 3. The median (range) number of colony types for groups 1-4 ($P < 0.001$).

Group	Median	Range
1	3	0-10
2	3	0-8
3	1	0-7
4	0	0-6

Conclusions

Overall, these data confirm that scrubbing the teat end with alcohol minimizes contamination of the milk sample, but application of pre-dip prior to scrubbing with alcohol may not be necessary.

Acknowledgements

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References

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