

# Defining Causes of Intramammary Infection in Dairy Cattle Using a Novel Sampling Technique



Veterinary Research  
Scholars Program  
University of Missouri

Shannon Ankney, Luis Rivero, Monica Witzke, Allison Naclerio, Pamela Adkins, and John Middleton  
Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, Missouri

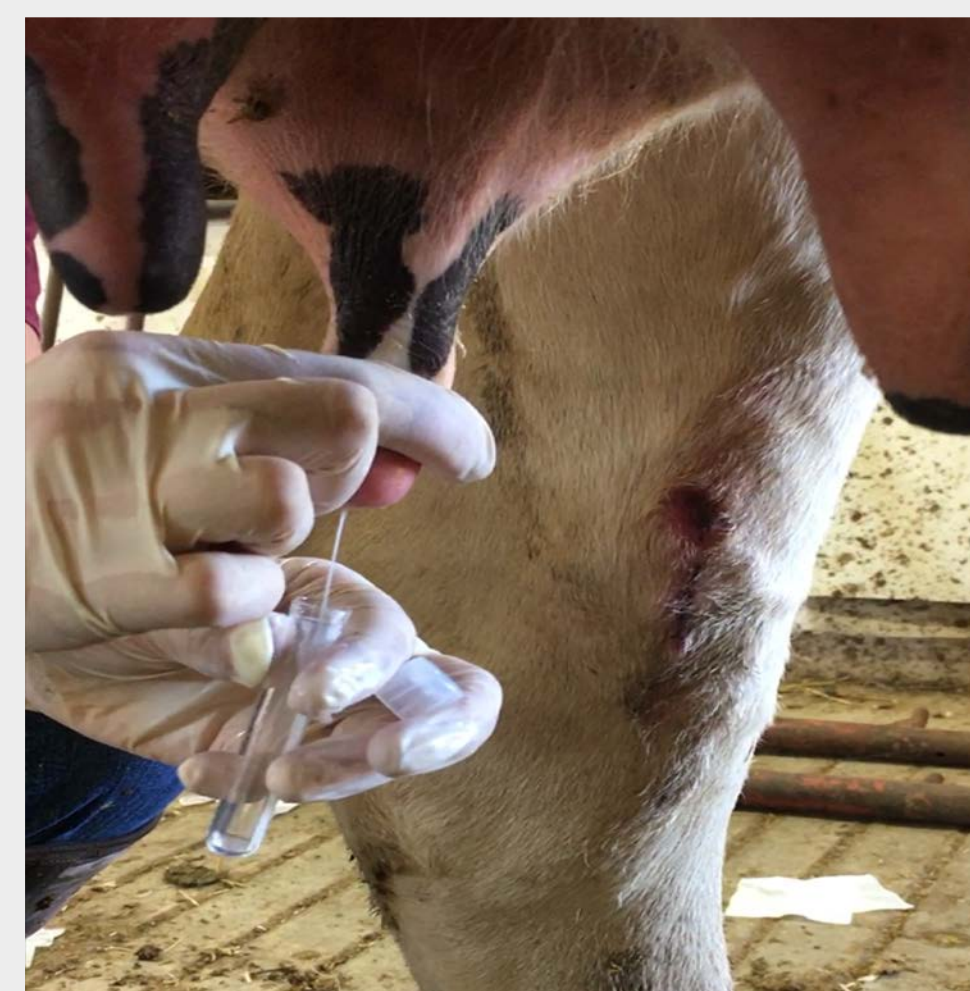
## Background

The most common cause of mastitis in dairy cattle is a bacterial intramammary infection (IMI). The most common method for diagnosing an IMI is aseptic collection of milk via the teat orifice; however, teat canal inhabitants or contaminants could cause false-positive results. By collecting and culturing a milk sample via aspiration from the gland cistern, false-positive results may be eliminated. **The objective of this study was to compare the culture results of milk samples collected directly from the gland cistern to those of milk samples collected via the teat orifice to more accurately determine which bacteria cause IMI.**

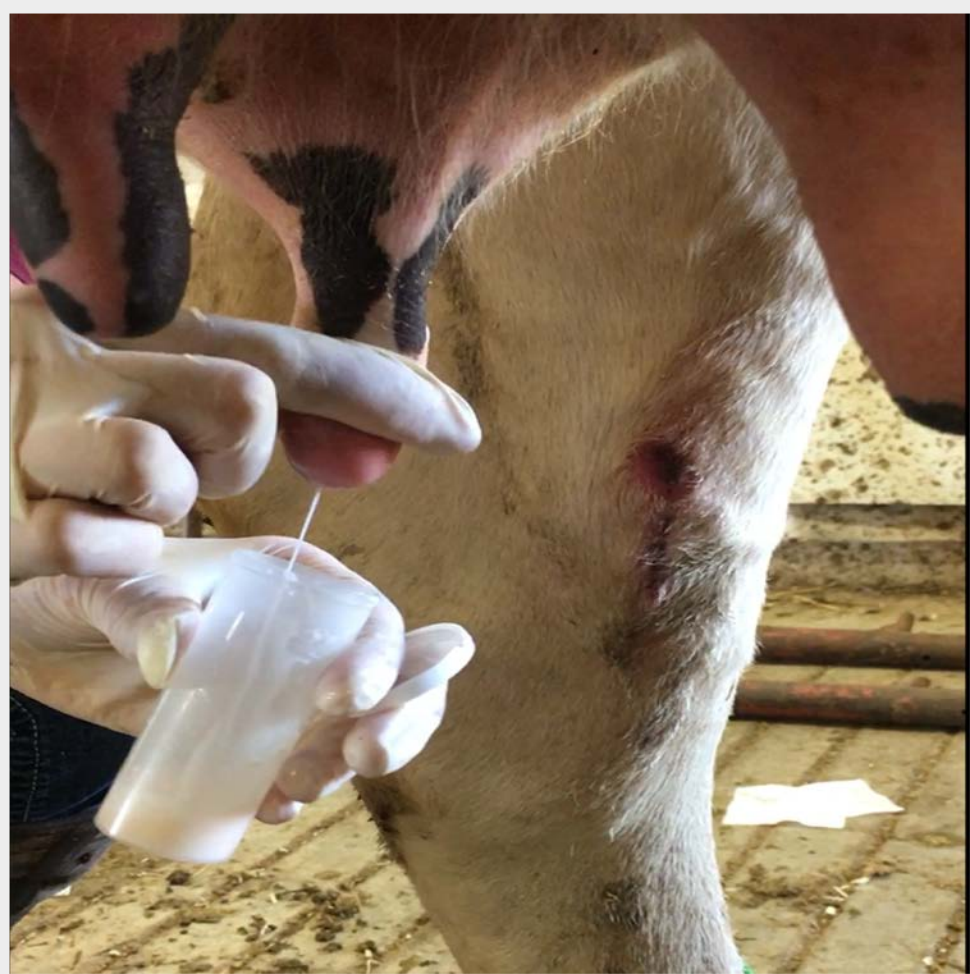
## Sample Collection

- Milk samples were collected from cows at the University of Missouri's Foremost Dairy.
- Two quarters were randomly sampled per cow.

- A milk sample was aseptically collected via the teat orifice using procedures described by the National Mastitis Council.



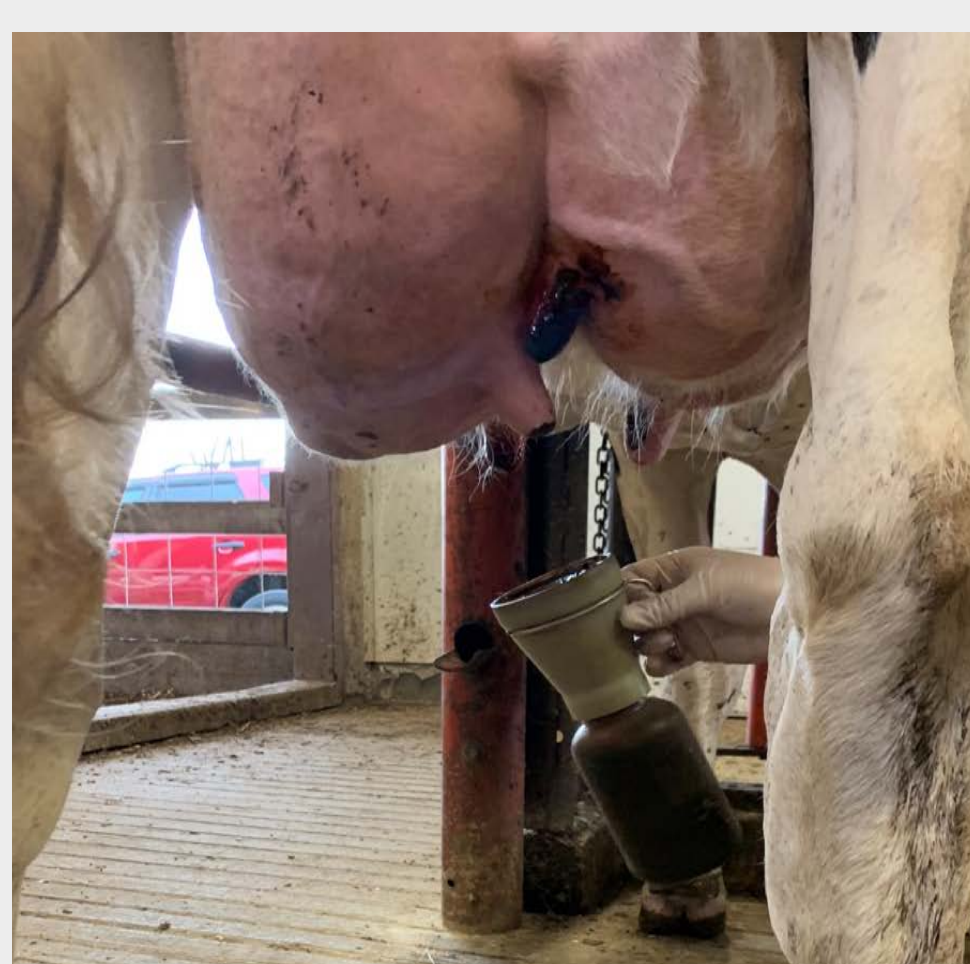
- Next, milk for somatic cell count (SCC) enumeration was similarly collected.



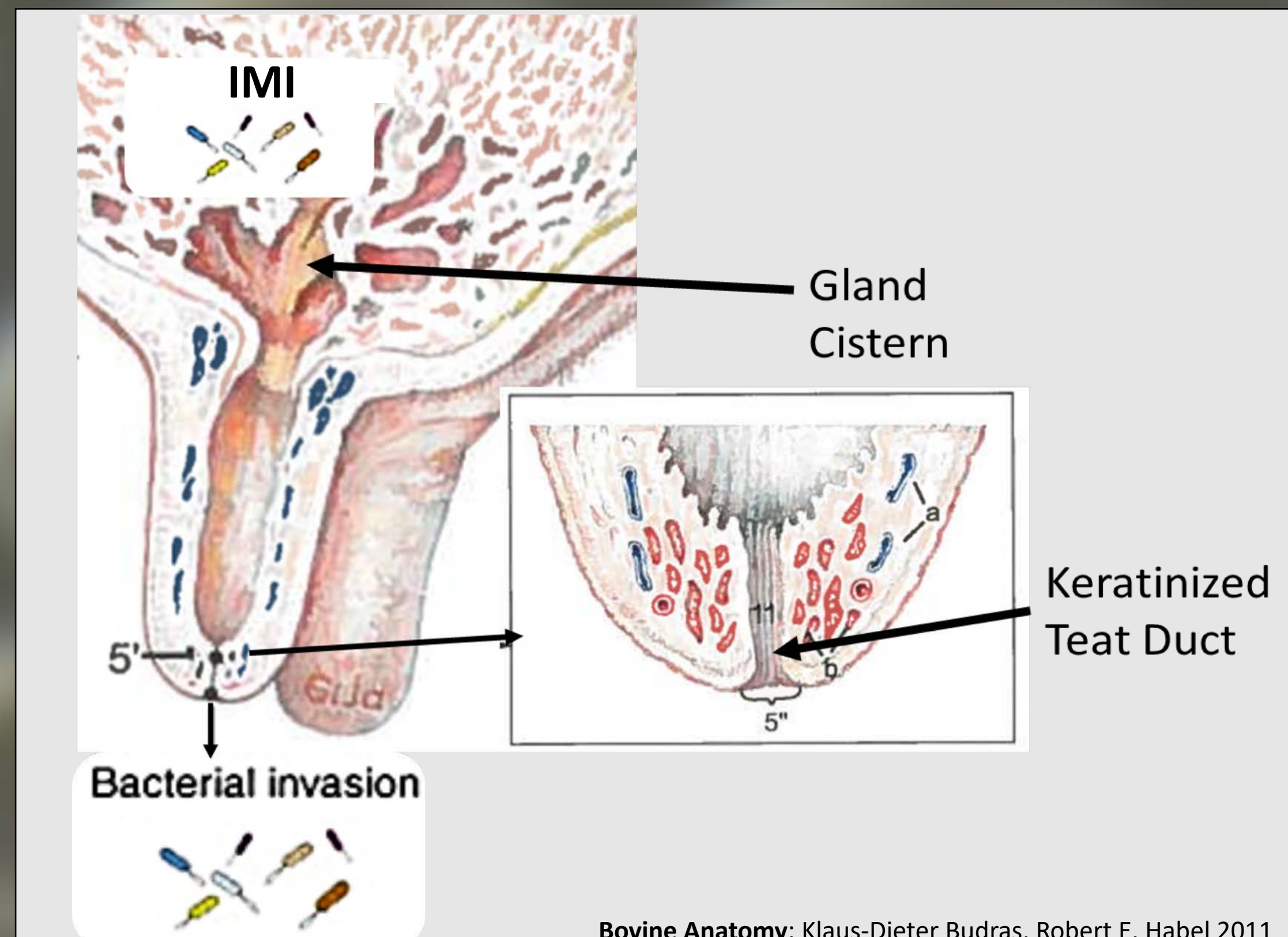
- Finally, a milk sample was aspirated from the gland cistern using a sterile vacutainer needle and tube.



- After all samples were collected, sampled teat ends were dipped in a post-milking iodine-based teat disinfectant.



- All milk samples were chilled on ice and transported to the laboratory. Samples for culture were stored at -20 °C until plated. Milk samples for SCC enumeration had a bromopol preservative added and were shipped overnight to a commercial laboratory (Mid-South Dairy Records, Springfield, MO). SCC were performed on an automated counter.

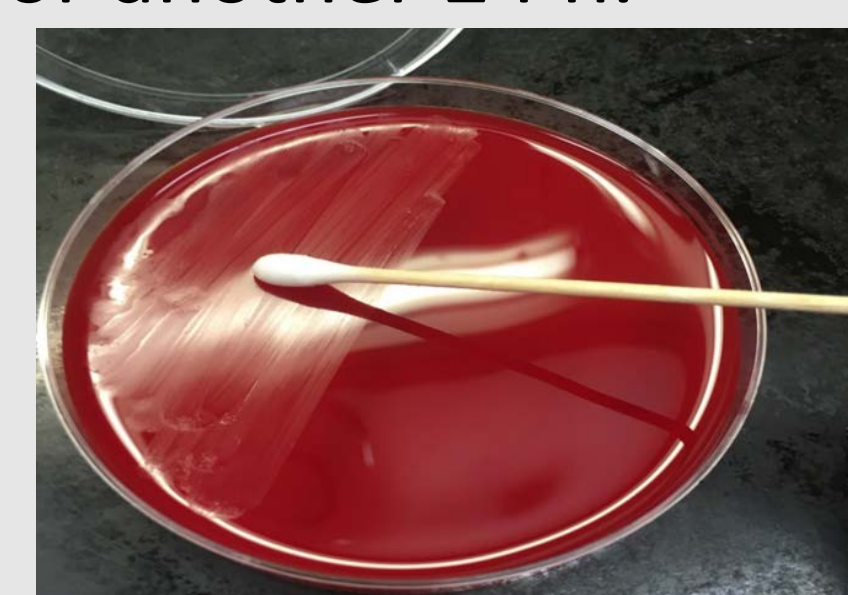


## Bacterial Culture

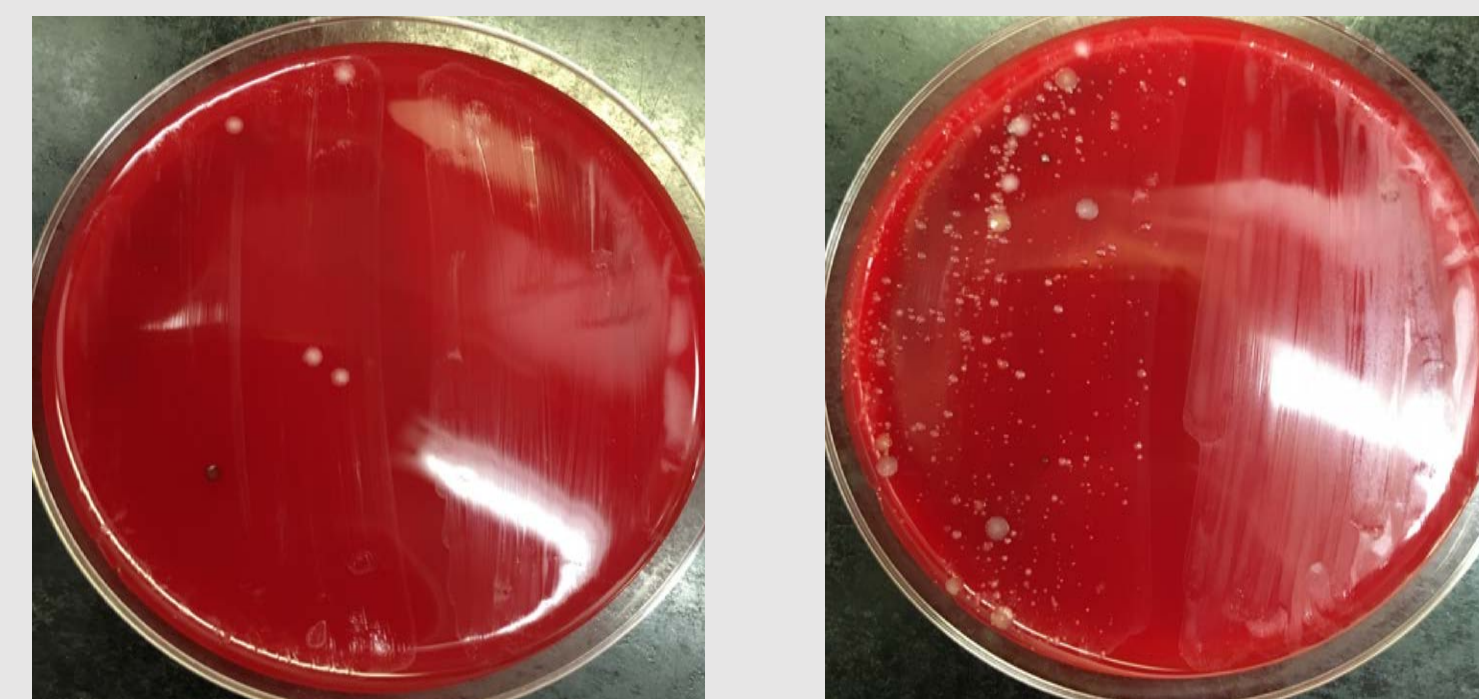
- Milk samples were thawed at room temperature (~22°C).



- Milk was plated on Columbia blood agar and incubated at 37 °C for 24 h and then at room temperature for another 24 h.



- At 48 h the plates were read and the number of morphologically different bacteria colonies were enumerated.



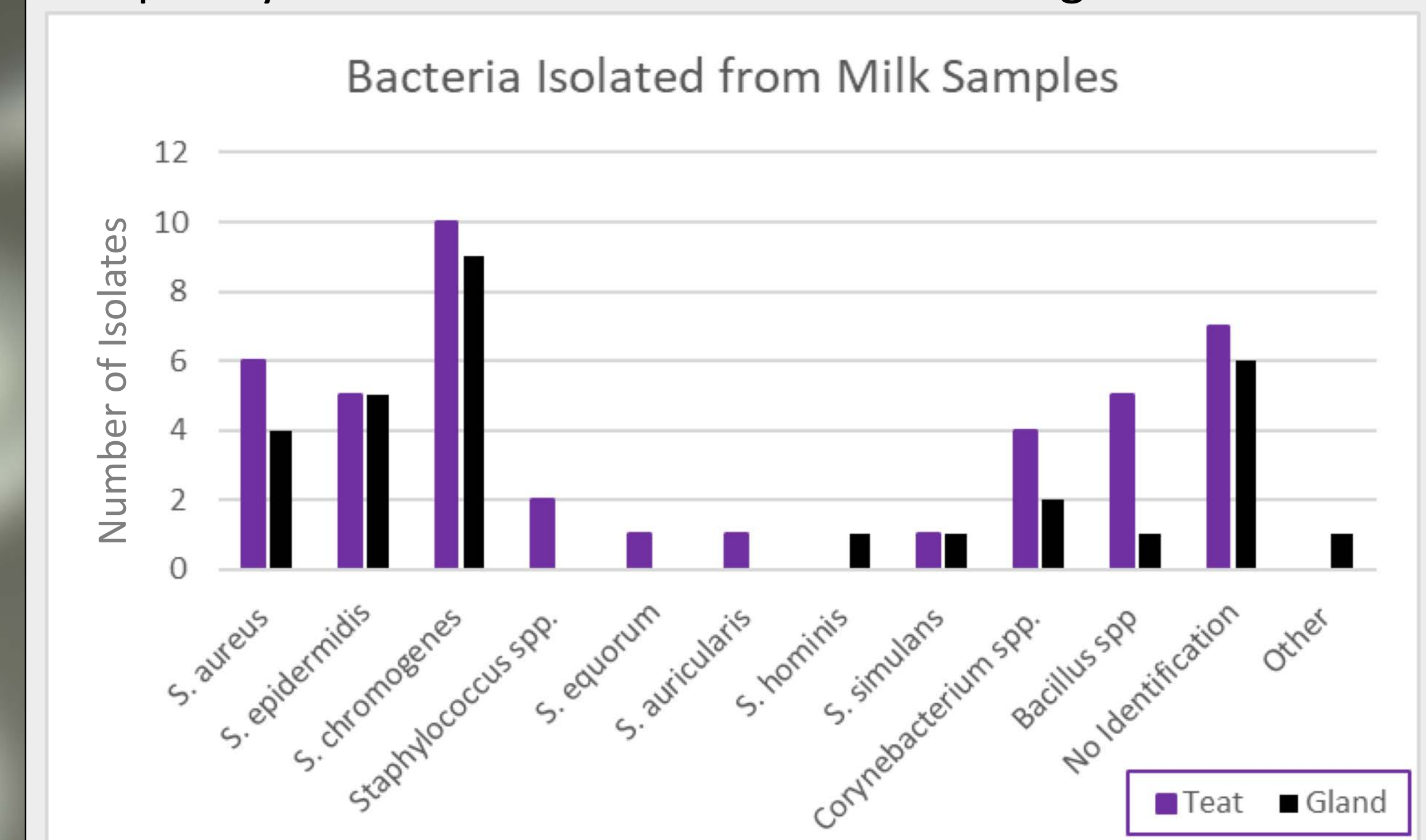
- Bacterial isolates were speciated using Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry.



- Isolates were stored at -80°C for future use.

## Results

To date, 114 quarters on 57 cows have been sampled. Fourteen quarters (n = 14 cows) had incomplete data due to missing or contaminated samples, and 2 quarters had bacterial growth that differed between the two sample sites. Of the samples collected via the teat orifice, 63 yielded no growth, 25 yielded a *Staphylococcus* spp., and 16 yielded another bacterial species. Of the samples collected from the gland cistern, 80 quarters had no growth, 20 yielded a *Staphylococcus* spp. and 10 yielded another bacterial species. Frequency of bacterial isolation is shown in Figure 1.



**Figure 1** - Number of bacterial isolates found in milk samples that were taken via the teat orifice compared to those taken from the gland cistern.

		Gland Cistern		
		Positive Growth	No Growth	
Teat Orifice	Positive Growth	21	16	37
	No Growth	4	57	61
		25	73	98

**Table 1** – Distribution of mammary quarters by culture result. When concordant results are noted between the two samples, the same genus and species were isolated from both.

Among the 98 mammary quarters available for data analysis, 80% (78/98) had concordant culture results and 20% (20/98) were discordant (Kappa= 0.53; moderate agreement). Of the 21 culture positive concordant samples, 81% (17/21) yielded a *Staphylococcus* spp. (Table 1).

Four mammary quarter cisternal milk samples grew a bacterium but the sample collected via the teat orifice was culture negative, whereas 16 samples collected via the teat orifice were culture positive for a bacterium but the cisternal sample was culture negative.

## Conclusion

Overall, 26% (25/98) of mammary quarters were classified as having an intramammary infection (IMI) based on bacteria being isolated from cisternal milk. Among these mammary quarters staphylococcal IMI predominated (68%; 17/25). If the cisternal milk sample was considered the “gold standard” for diagnosing IMI, then samples collected via the teat orifice made a false positive diagnosis 22% of the time and a false negative diagnosis 16% of the time. Most staphylococcal species were associated with intramammary infections with the exception of 1 undifferentiated *Staphylococcus* sp., 1 *S. equorum*, and 1 *S. auricularis* that were each only isolated from samples collected via the teat orifice. Somatic cell count data analyses are still pending.

**Acknowledgements:** Stipend and research support provided by USDA National Institute of Food and Agriculture (Project No. 1017880) and the Veterinary Research Scholars Program.