### **Comparison of Milk and Udder Skin Microbiota of Dairy Heifers** Veterinary Medicine versity of Missour



Travis Cline<sup>1</sup>, Pamela Fry<sup>1</sup>, John Middleton<sup>1</sup>, Aaron Ericsson<sup>1,2</sup>

<sup>2</sup>University of Missouri Metagenomics Center, <sup>1</sup>College of Veterinary Medicine, University of Missouri, Columbia, MO

# BACKGROUND

College of

Dairy product consumption is increasing globally and will be more vital as the world's population continues to grow and demand for animal protein increases. Even with all the improvements in dairy production through improved genetics and animal husbandry, milk production is still greatly impacted by disease. Mastitis, an inflammation of the udder usually caused by bacterial intramammary infection (IMI), is the most economically important disease of dairy cattle. While lactating cattle are most often affected by mastitis, replacement heifers can develop IMI prior to their first lactation which can impact future productivity. The source of prepartum IMI in heifers is not completely understood. Udder skin colonization is a risk factor for some IMI in heifers. Metagenomics provides a broad spectrum approach to analyzing the bacterial flora of the skin and milk of heifers. The objective of this project is to evaluate the microbiota on the teat and udder skin prior to parturition and correlate these data with the microbiota in milk in early lactation.



#### RESULTS

## **METHODS & MATERIALS**

Udder swabs were taken approximately 14 days prior to parturition. Swabs were collected using a sterile electrostatic duster. Mammary quarter milk samples were collected twice during the first 10 days of lactation.

Figure 1: Principal component analysis (PCoA) depicting the relationship of the operational taxonomic units (OTUs) of udder skin swabs and quarter milk samples. Udder swabs are clustered in the lower left and milk samples clustered in the upper right. Quarters with an IMI defined by traditional culture are depicted by a double asterisk (\*\*).



Figure 2: Milk Microbiome: Relative abundance of OTUs detected in at least 6 of 12 quarters (RF, RR, LF, LR). Prominent OTUs are labeled on the right. The \* depicts the quarters with IMI. Heifer #2 was negative in all quarters.



Skin Microbiome: Figure abundance OTUs Relative Of detected in udder skin swabs. Prominent OTUs are labeled on the right. CTL = unused swab.



Milk samples were cultured according to National Mastitis

Council guidelines to detect IMI.



Based on culture results, heifers were selected for metagenomic analysis. To date, 3 heifers have been evaluated in a pilot study: milk culture negative (n=1), streptococcal IMI (n=1), and *Staphylococcus chromogenes* IMI (n=1).

# **CONCLUSION**

Multiple genera of bacteria were detected in each sample type. The PCoA plot shows a clear difference between the types of bacteria present on the exterior of the udder (skin) and within the udder (milk samples). The skin surface appears to have a more diverse bacterial population than the milk. Interestingly, heifer #1 has an obvious dysbiosis in the mammary quarter with the streptococcal IMI. Further, infected mammary quarters, as identified by routine milk culture, also seem to have different metagenomic profiles from non-infected quarters on the same heifer and heifer #2 (uninfected heifer). Larger numbers of heifers will be



#### **ACKNOWLEDGEMENTS**



#### Samples were prepared for MU DNA Core for metagenomic analysis. Next generation DNA sequencing was performed using Illumina.