

Being Nosy: Characterization of the Upper Respiratory Tract Bacteriome of Healthy Dairy Calves in PEI

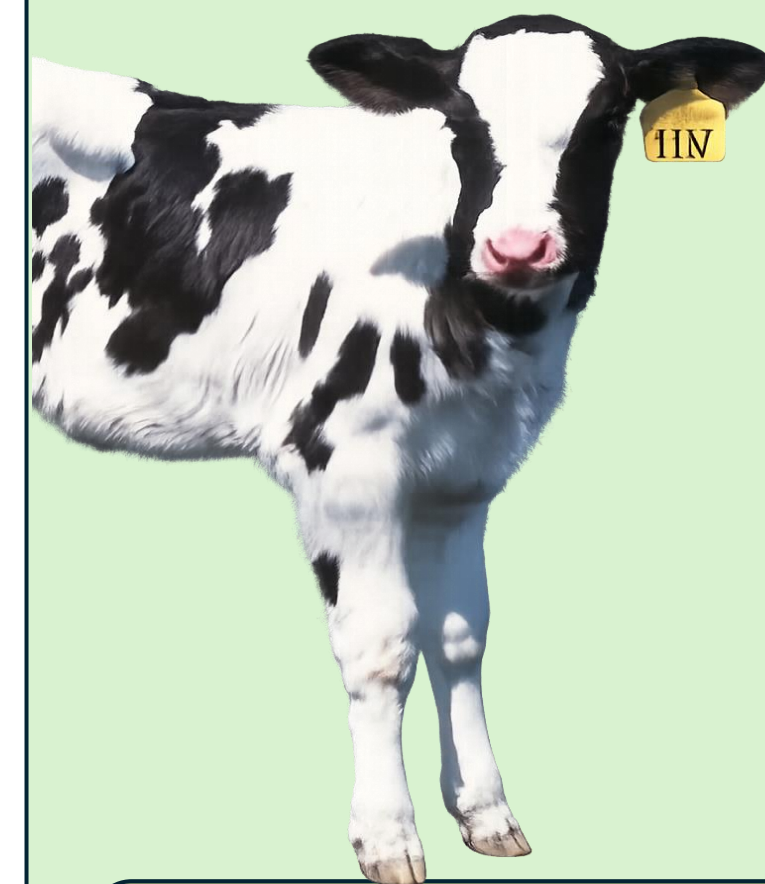
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Introduction

- Bovine Respiratory Disease (BRD) is a major cause of morbidity and economic loss within the cattle industry and results from complex interactions between viral, bacterial, environmental and host factors.
- Recent metagenomic studies have characterized viral and bacterial communities within the respiratory tract of cattle, improving our understanding of bovine respiratory health^{1,3,4}.



- Despite their susceptibility to BRD, the respiratory virome and bacteriome of healthy dairy calves remain poorly characterized
- No studies have characterized the respiratory virome and bacteriome of PEI dairy calves, limiting baseline data in this region.

Objectives

1. Characterize the bacteriome of the upper respiratory tract of healthy PEI dairy calves.

2. Compare the microbial communities based on weaning status and age.

Results

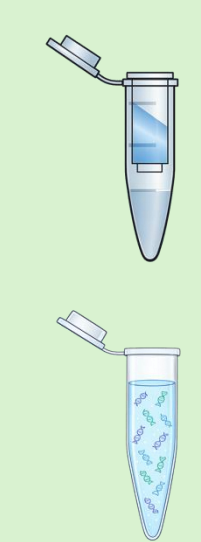
1. Sampling

- Nasopharyngeal swab samples were collected from 20 apparently healthy calves from a PEI dairy farm.



- Swabs were stored in Safe Collect tube and properly stored until processing.

2. Sample Processing



- Bacterial DNA Extraction was performed using ZymoBIOMICS DNA Microprep Kit
- The full 16S rRNA gene sequence library was created using 16S barcoding kit (Oxford Nanopore Technologies)



- Sequenced 16S RNA genes using Oxford Nanopore MinION mk1B device

3. Analysis

- Taxonomic classification was performed using the EPI2ME software based on SILVA_138_1 database
- Statistical analysis and data visualization were completed using R v4.6.1

Results

Alpha Diversity - Significant difference was found only between observed richness among calves aged 1-5 weeks and calves aged 20-23 weeks.

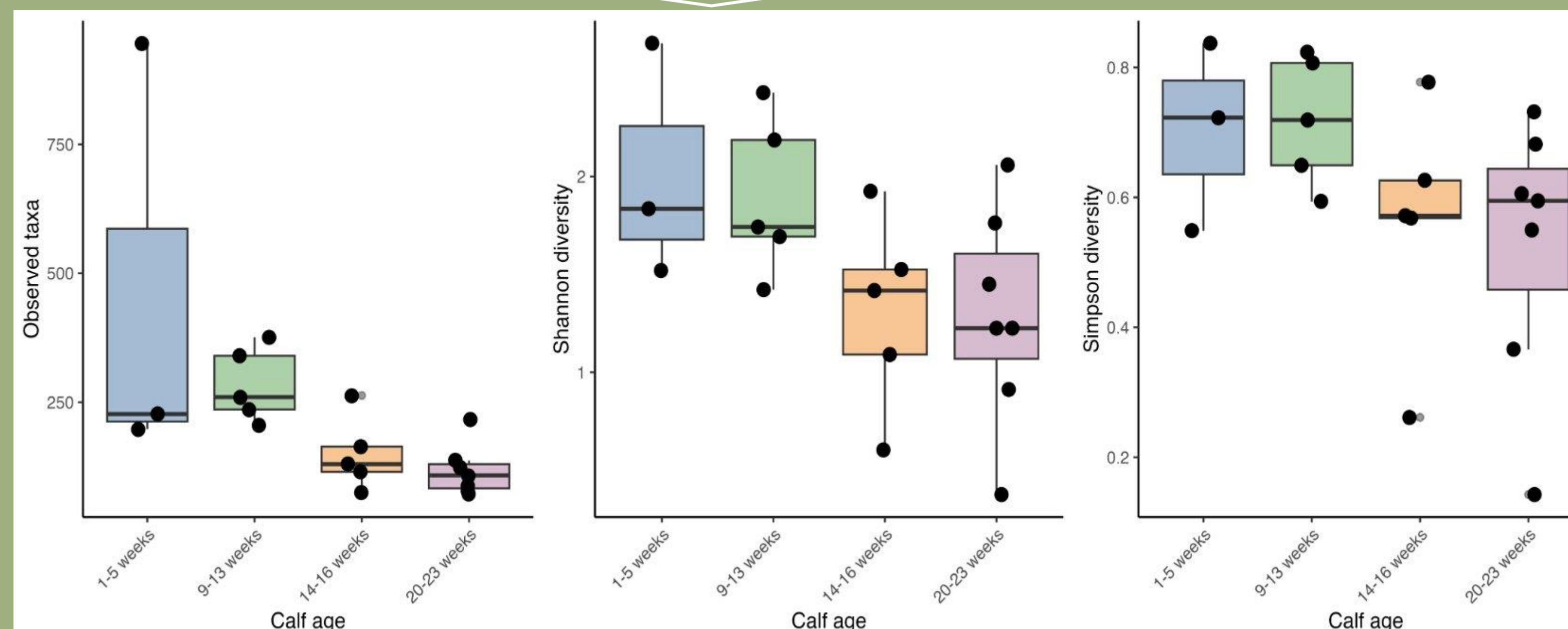


Figure 1. Boxplots showing alpha diversity distributions. Boxes represent the interquartile range with the median shown as a horizontal line. Points represent individual calves. Statistical differences were assessed using Kruskal–Wallis tests followed by multiple-comparison corrected pairwise tests. A significant difference was found in the Observed richness between 9-13 weeks and 20-23 weeks of age (adjusted p-Value = 0.0234).

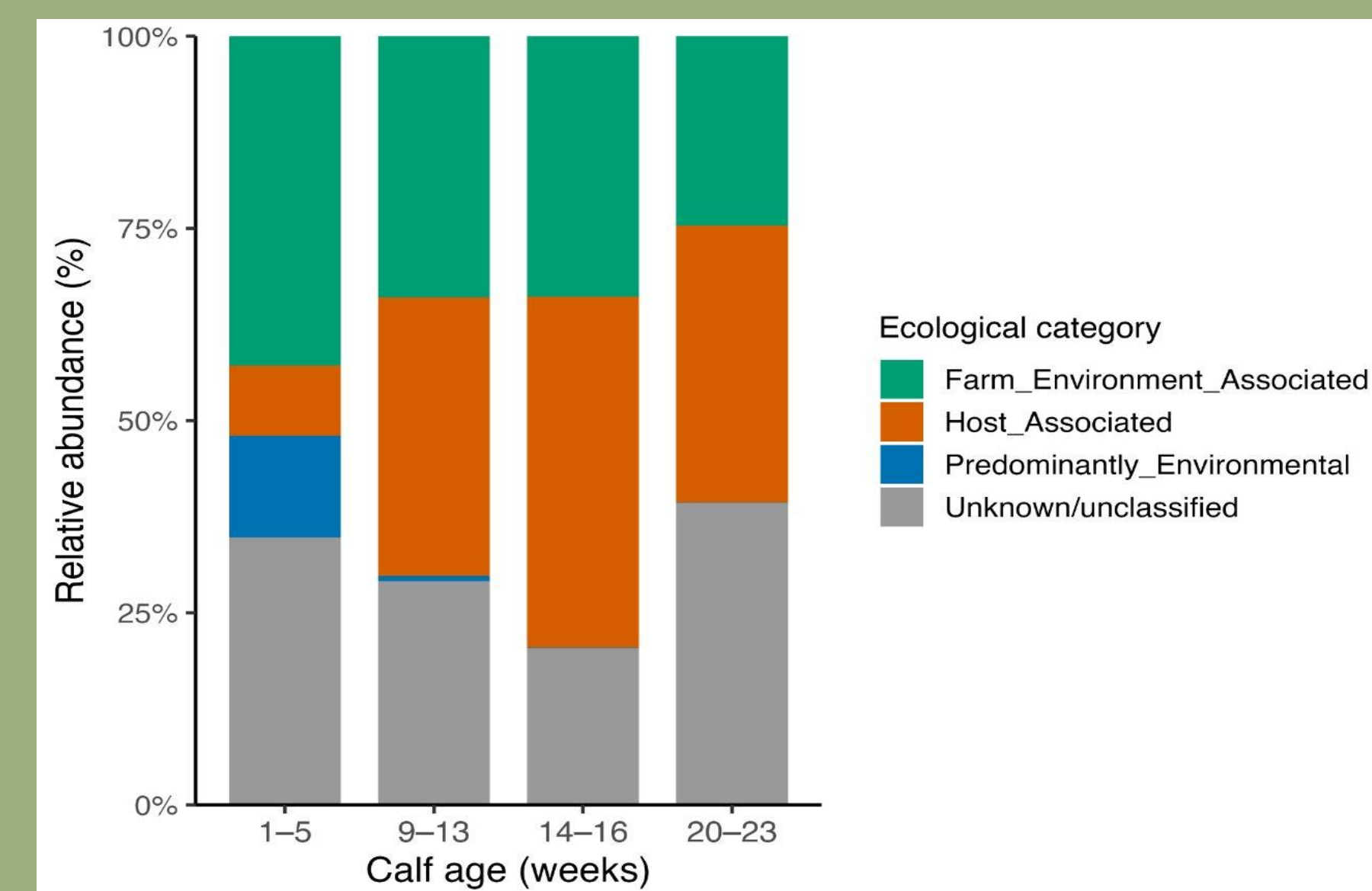


Figure 2. Comparison of the relative abundance of bacterial species by ecological category across age groups.

Relative Abundance of Host Bacteria by Age Group

Younger calves aged 1-5 weeks had a lower relative abundance of host associated bacterial species compared to older calves. Younger calves also showed higher numbers of environmental bacteria whereas older calves present little to no environmental-associated bacteria.

Top 10 Most Abundant Host-Associated Bacteria Among Calves

- Histophilus somni*
- Pasteurella multocida*
- Staphylococcus equorum*
- Psychrobacter maritimus*
- Mycoplasma bovis*
- Mesomycoplasma dispar*
- Streptococcus pluranimalium*
- Metamycoplasma alkalescens*
- Psychrobacter alimentarius*
- Streptococcus ruminantium*

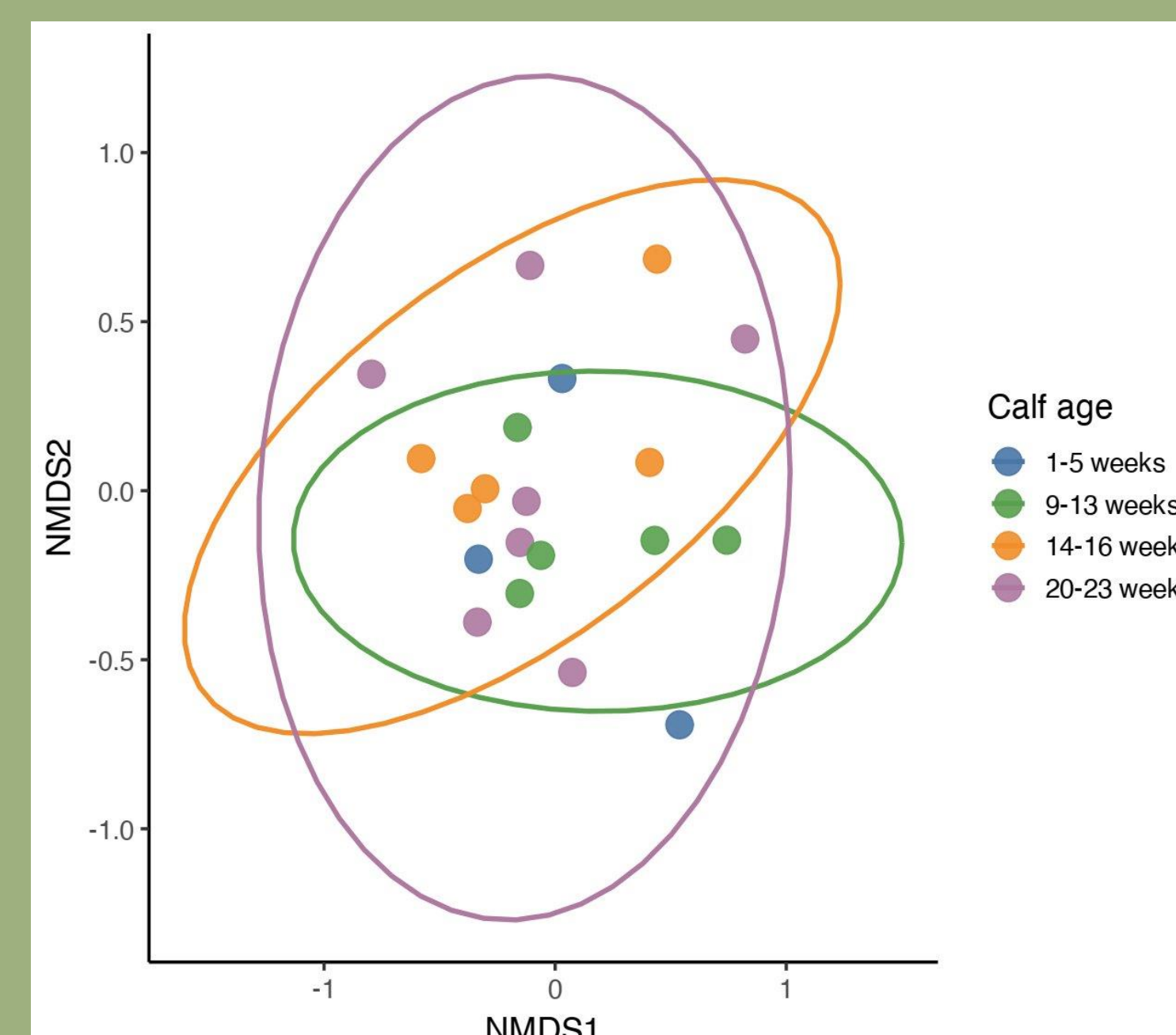


Figure 3. Beta diversity. NMDS ordination plot based on Bray–Curtis dissimilarity of genus-level host-associated bacterial abundance profiles. Beta diversity was not statistically significant ($p > 0.05$). However, 16% of the community variation was attributable to calf age.

Conclusions

- Younger calves (1–5 weeks) presented a lower relative abundance of host-associated healthy bacteria in their URT than older calves, which may increase their susceptibility to opportunistic pathogens.

- Histophilus somni* was the most abundant host-associated bacterium detected, followed by *Pasteurella multocida* and *Staphylococcus equorum*. This contrasts with previous work, which has indicated other host-associated taxa as more abundant members of the healthy bovine upper respiratory microbiome².

The predominance of *H. somni* in apparently healthy calves warrants further investigation through longitudinal sampling and continuous health monitoring.

- More comprehensive farm records of previous health status and treatment history would have enabled the differentiation of pre-existing conditions from age-related effects and provided greater context for the observed microbial profiles.



Future Directions

- Explore strategies to improve the early establishment of the healthy respiratory microbiome in neonatal calves, including prebiotic or probiotic interventions.
- Expand characterization of the respiratory microbiome by sequencing both bacterial and viral communities.
- Future studies should increase sampling across multiple farms, breeds, sexes, age groups, and include both healthy calves and calves with BRD to better characterize the regional respiratory microbiome.

References

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