

# Comparison of the nasopharyngeal bacterial microbiota of beef calves raised without the use of antimicrobials between healthy calves and those diagnosed with bovine respiratory disease

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## Introduction

It has been shown that the composition of the nasopharyngeal bacterial microbiota plays a role in respiratory health. However, the role of this microbiota in the development of bovine respiratory disease (BRD) is still not well defined. What we know about the composition of the bovine nasopharyngeal bacterial microbiota mainly comes from beef calves that have received mass medication (metaphylaxis) with an antimicrobial on arrival at a feedlot. Unfortunately, antimicrobials do not just target pathogenic bacteria, and antimicrobial use can have an impact on the entire respiratory microbiota, including commensals. There are currently no data on the composition of the nasopharyngeal microbiota of healthy feedlot cattle and those diagnosed with BRD that are raised without antimicrobials (i.e. natural cattle), limiting our understanding of the disease. As the beef industry begins to move away from the use of antimicrobials, this information will become increasingly valuable. Therefore, the objective of this study was to characterize and compare the nasopharyngeal bacterial microbiota in feedlot cattle raised without antimicrobials that were healthy or diagnosed with BRD.

## Materials and Methods

Newly-received beef-crossed feedlot calves (arrival body-weight  $\pm$  SD = 217  $\pm$  36 kg) diagnosed with BRD (n = 82) and pen-matched controls (CTRL; n = 82) were clinically examined and sampled by deep nasopharyngeal swab. Calves with a rectal temperature  $\geq$  104°F that had two or more visual BRD signs were enrolled in the study to the case group. Calves with a temperature < 104°F that did not have any visual BRD signs were enrolled to the control group. Total DNA was extracted from the swabs and the V4 region of the 16S rRNA gene was sequenced. Sequencing data were processed using DADA2 to infer exact sequence variants, and the resulting variants were used to evaluate the microbiota. In order to take into account the influence of days on feed (DOF), calves were artificially assigned to 1 of 3 different DOF groups after collection of sequencing data (group A = 3-12 DOF; group B = 13-20 DOF; group C = 21-44 DOF). Alpha and beta diversity were compared between health groups and among the 3 DOF groups.

## Results

The most prominent identified genera across both BRD-CTRL groups were *Mycoplasma* (22.2%), *Moraxella* (19.5%), *Histophilus* (19.0%), *Psychrobacter* (9.8%), *Mannheimia* (6.3%), *Pasteurella* (4.4%), *Pseudomonas* (1.8%), and *Alysiaella* (1.0%). However, the order of these genera by abundance was different between BRD-CTRL groups. In the BRD group, the top 3 most abundant genera were *Mycoplasma* (27.0%), *Histophilus* (21.4%), and *Moraxella* (17.6%). Comparatively, the order in the CTRL group was *Moraxella* (21.5%), *Mycoplasma* (17.2%), and *Histophilus* (16.6%). Surprisingly, the relative abundance of the primary BRD pathogens (*Mycoplasma bovis*, *Histophilus somni*, *Mannheimia haemolytica*, and *Pasteurella multocida*) did not significantly differ between these groups. Observed species richness was lower (P = 0.031) in cattle diagnosed with BRD compared to healthy ones. A PERMANOVA showed that both health status (P = 0.007) and DOF group (P < 0.001) were significant sources of variation in microbiota composition. These differences were driven by multiple sequence variants, including *M. bovis*, *H. somni*, and several *Moraxella* spp. Of note was that *M. bovis* was more frequently identified in cattle diagnosed with BRD, as well as in both health groups for those sampled at a later DOF.

## Significance

The bacterial microbiota of the nasopharynx in beef calves raised without the use of antimicrobials in general differed between healthy calves and those diagnosed with BRD. As well, microbiota differed among calves sampled at different timepoints in their feedlot lifecycle. The increased proportion of calves that harbored *M. bovis* in their nasopharynx, specifically calves that were diagnosed with BRD and those sampled at a later DOF, reaffirms a potentially significant role for this bacterium in beef cattle respiratory health.