

Distinct bacterial metacommunities inhabit the upper and lower respiratory tract of healthy feedlot cattle and those diagnosed with bronchopneumonia

Edouard Timsit,¹ DVM, PhD, DECBHM; Matthew Workentine,¹ PhD; Trevor Alexander,² PhD

¹ Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, T2N 4N1, Canada

² Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, T1J 4B1, Canada

Introduction

Recent evidence suggests that specific nasopharyngeal bacterial communities can provide colonization resistance against respiratory pathogens in cattle. However, the role that bacterial communities of the lower respiratory tract plays in respiratory health remains largely unknown. Therefore, our study aimed to compare the nasopharyngeal and tracheal bacterial communities between healthy feedlot cattle and those diagnosed with bronchopneumonia (BP).

Materials and Methods

Deep nasal swabs (DNS) and trans-tracheal aspiration (TTA) samples were collected from 60 feedlot steers with BP and 60 healthy pen-mates at 4 western Canadian feedlots. DNA was extracted from each sample and the V4 region of the 16S rRNA gene was amplified and sequenced using an Illumina MiSeq. After quality check with FastQC 0.11.5 and MultiQC 1.0, primers and low-quality sequence were trimmed off the raw sequence reads using cutadapt 1.14. The trimmed reads were used to construct amplicon sequence variants (SVs) using dada2 1.4.0 in R 3.4.1. Downstream analyses were done in R 3.4.1. Alpha diversity was assessed using Shannon diversity index and Chao1 species richness estimators. For beta diversity analysis, samples were clustered into bacterial metacommunities using Dirichlet multinomial mixtures (DirichletMultinomial R package, 1.20.0). Cluster association with health status and sampling location was done using a Person's χ^2 test.

Results

A total of 7 phyla were observed across all samples. Among these phyla, Tenericutes (47.4%), Proteobacteria (26.0%), and Firmicutes (20.7%) were the most abundant,

representing 94.1% of the total abundance. A total of 91 genera were identified. The most abundant genera were *Mycoplasma* (46.8%), *Lactococcus* (18.02%), and *Histophilus* (10.1%). Chao1 ($P < 0.001$) and Shannon diversity indexes ($P < 0.001$) revealed a lower bacterial richness and evenness in the nasopharynx and trachea of steers with BP compared to their healthy pen-mates. Using Dirichlet multinomial models, we showed that bacterial communities present within the bovine airways clustered into 4 distinct metacommunities that were significantly associated with sampling location and health status (Chi-squared test; $P < 0.001$). Metacommunity 1, enriched with known bacterial BP pathogens like *Mycoplasma bovis*, *Mannheimia haemolytica* and *Pasteurella multocida*, was dominant in the nasopharynx and trachea of steers with BP. In contrast, metacommunity type 3, enriched with *Mycoplasma dispar*, *Lactococcus lactis*, and *Lactobacillus casei*, was mostly observed in the trachea of healthy cattle. Metacommunity type 4, enriched with *Corynebacterium*, *Jeotgalicoccus*, *Psychrobacter*, and *Planomicrobium*, was present only in the nasopharynx of both healthy and sick steers. Metacommunity type 2, enriched with *Histophilus somni*, *Moraxella*, and *L. lactis*, was present in both healthy and sick steers but was primarily observed in 1 of the 4 feedlots.

Significance

Our study showed that distinct bacterial metacommunities inhabit the nasopharynx and trachea of healthy feedlot cattle and those diagnosed with BP. Bacterial communities in the trachea of healthy cattle were more diverse, had a lower relative abundance of bacterial pathogens, and a higher relative abundance of *L. lactis*, *L. casei* and *M. dispar*. Because *L. lactis* and *L. casei* can inhibit the growth of *M. haemolytica* in vitro, their presence may have provided colonization resistance against respiratory bacterial pathogens.