The nasopharyngeal microbiota of preweaned dairy calves with and without ultrasonographic lung consolidation

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Introduction

Preweaning is a high-risk time period for dairy calves; pneumonia is a predominant cause of mortality and use of antibiotics on farms. Given the concern of antibiotic resistance, there is demand to investigate alternative therapies. Administering probiotics to alter the respiratory microbiome and reduce the risk of pneumonia has shown promising results in mice. Although the bacterial community dynamics of the upper airway have been investigated in dairy calves, they have not been evaluated in calves diagnosed with pneumonia using lung ultrasound (US). By allowing visualization of lung consolidation, US increases the sensitivity of pneumonia diagnosis. Elucidating phenotypes of the commensal community in healthy and pneumonic dairy calves is crucial to investigating probiotics as a preventative for pneumonia. The primary objective of this prospective case-control study was to describe bacterial communities in the nasopharynx (NP) of preweaned dairy calves with and without lung consolidation. Secondary objectives included evaluating the effects of previous antibiotic therapy and age on the composition of NP microbiota.

Materials and Methods

A total of 257 Holstein heifer calves were enrolled into a separate study investigating the genomics of resistance to bovine respiratory disease (BRD) over a 4-week follow up period. Calves were examined twice using US and clinical respiratory score (early exam: 4 weeks old; late exam: 7 weeks old). From this population, case and age-matched controls were selected to undergo deep NP swabbing for the current study. Cases were defined by the presence of lobar pneumonia (1 or more consolidated lung lobes). Controls were not affected by lobar pneumonia. The NP swabs were taken at the time of exam and calf information was collected from farm management software. Swabs were placed in phosphate buffer solution, and stored at -80°C. Following DNA extraction, the V4 region of the 16S rRNA gene was amplified using PCR. Libraries were sequenced using the MiSeq platform. Sequences were processed through Mothur and output was analyzed in RStudio. Diversity data was analyzed using t-tests and general linear models. Comparisons of relative abundance (RA) of genera between groups were performed using Kruskal Wallis tests. Multiple linear regression was used to investigate the impact of time point of exam and antibiotic treatment, in the month prior to examination, on RA of genera.

Results

In total, 50 swabs were collected. Two swabs were lost during transport, therefore, 48 swabs (cases = 23, controls = 25) obtained from 44 calves, were used for analysis. Thirty-five (73%) swabs were collected during the late exam. The most common genera were *Acinetobacter* sp, *Escherichia* sp, *Mycoplasma* sp, *Pasteurella* sp, and *Psychrobacter* sp. Alpha diversity was not different between cases and controls (P=0.78), nor was it different between early and late time points (P=0.37). The RA of *Mycoplasma* sp was higher in controls (P=0.03) and the RA of *Pasteurella* sp tended to be higher in controls (P=0.08). The RA of both *Mycoplasma* sp and *Pasteurella* sp was not affected by exam time point or antibiotic treatment ($P > 0.75$ and $P > 0.12$, respectively).

Significance

To the authors' knowledge, this is the first study to evaluate the community dynamics of the NP microbiota from calves with and without lung consolidation. We did not expect to identify a higher RA of *Mycoplasma* sp in the control calves, as a previous study showed a higher RA of *Mycoplasma* sp in calves with BRD. Future studies are needed to understand the discrepancy, including metagenomic studies to infer speciation and determine pathogenicity of the identified genera. It would also be important to know if the duration of consolidation affected the RA of bacteria. This study demonstrates the complexities of identifying how the NP microbiota is affected by disease. This may impact future attempts to utilize NP microbiota characteristics in diagnosis and prevention of BRD.