Airway Microbiota in Healthy and Bronchitic Dogs

Veterinary Research Scholars Program
University of Missouri

Alexa R. Personett[1], Aaron C. Ericsson[2,3], Megan Grobman[1], Hansjörg Rindt[1], and Carol R. Reinero[1]
Department of Veterinary Medicine and Surgery[1], Department of Veterinary Pathobiology[2], MU Mutant Mouse Resource and Research Center[3], College of Veterinary Medicine, University of Missouri, Columbia, MO

Introduction

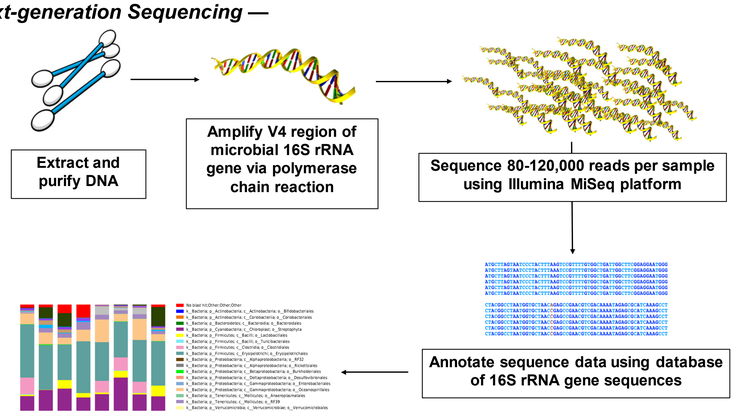
- Chronic bronchitis (CB) is a common and irreversible inflammatory disorder of the lower airways of dogs, usually of unknown cause. The typical syndrome, defined by a cough >2 months duration with airway inflammation, is associated with non-degenerate neutrophils. Another poorly characterized syndrome is chronic eosinophilic bronchitis, which has similar clinicopathologic features and was also included in this study.
- Based on culturing methods, the lower airways have been considered to be sterile in disease-free states.
- In healthy humans, culture-independent techniques have recently demonstrated the existence of a core airway microbiota that differs in diseased states. Dysbiosis of the microbiota may contribute to chronic respiratory diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease (COPD).
- Dysbiosis of the canine airway may similarly contribute to respiratory disease like CB, making the airway microbiota a potential new target for treatment.
- **The objective of this study is to compare the upper and lower airway microbiota in healthy versus bronchitic dogs and determine if bacterial richness and diversity, or overall community structure, differs in bronchitic airways.**

Methods

Animals —

- Healthy research dogs (n=16) and client-owned dogs diagnosed with CB at the University of Missouri Veterinary Medical Teaching Hospital (MU VMTH) (n=14) were studied.
- Research dogs had unremarkable physical exams and nasal, oropharyngeal, and bronchoalveolar lavage fluid (BALF) samples were collected.
- Clinicopathologic features of bronchitic dogs were gathered from the medical record system of the MU VMTH. BALF was collected as part of the diagnostic work-up of pet dogs with CB and prospectively banked for the study.

Next-generation Sequencing —



Study Participants

- ***Signalment of research dogs –*** All healthy research dogs were intact female beagles. The group mean age was 3.8 years (range: 2 to 8 years) and group mean body weight ± SD was 11 ± 1.4 kg.
- ***Signalment of pet dogs with CB –*** Eleven different purebreds were represented. Seven dogs were males (4 neutered, 3 intact) and seven were females (all spayed). The group mean age was 7.7 years (range: 1 to 13 years) and group mean body weight ± SD was 18.1 ± 16.3 kg.
- ***Clinicopathologic features of pet dogs with CB –*** Clinical signs included chronic cough (n=14), exercise intolerance (4), and increased respiratory effort (3). Thoracic radiographs taken in seven dogs all showed a diffuse bronchial or bronchointerstitial pattern. Bronchoscopy performed in ten dogs revealed hyperemia of the airways (n=7), narrowing or collapse of bronchi and bronchioles (7), bronchiectasis (4), and mucus accumulation (3). Bacterial culture showed aerobic growth in five of twelve dogs, and Mycoplasma PCR was positive for one of three dogs.

Results

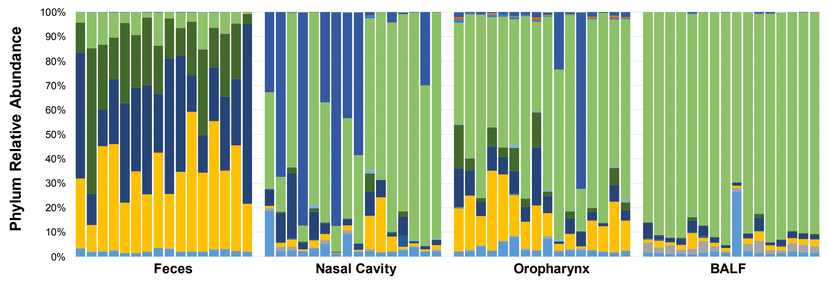


Figure 1. Healthy dog microbiota at the phylum level from fecal, nasal, oropharyngeal, and BALF samples.

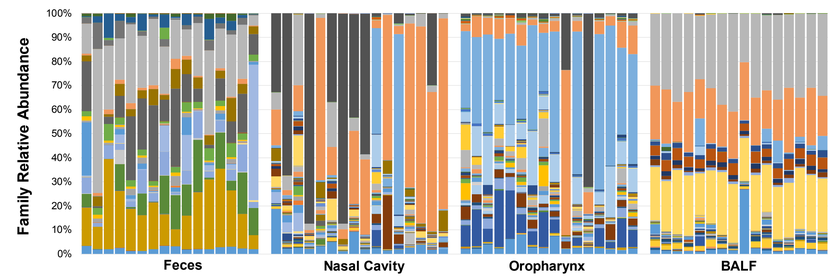


Figure 2. Healthy dog microbiota at the family level from fecal, nasal, oropharyngeal, and BALF samples.

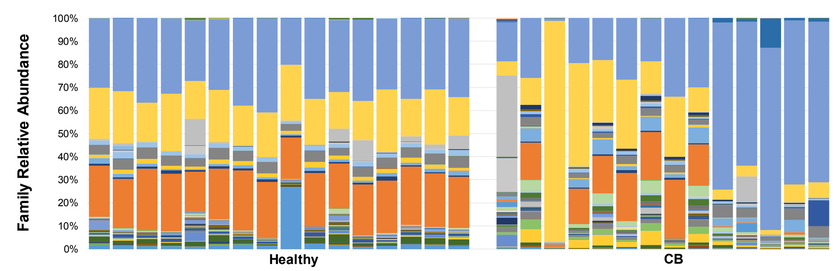


Figure 4. Microbiota at the family level from BALF samples of healthy and bronchitic dogs.

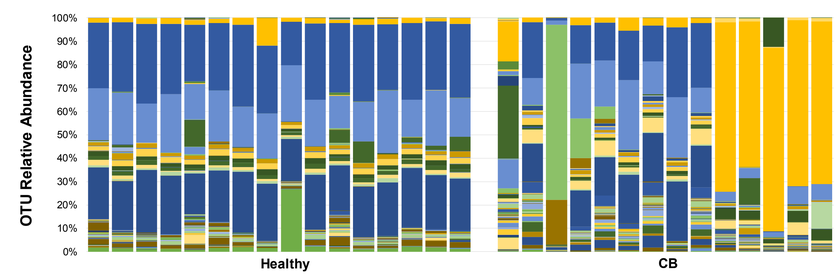


Figure 5. Microbiota at the OTU level from BALF samples of healthy and bronchitic dogs.

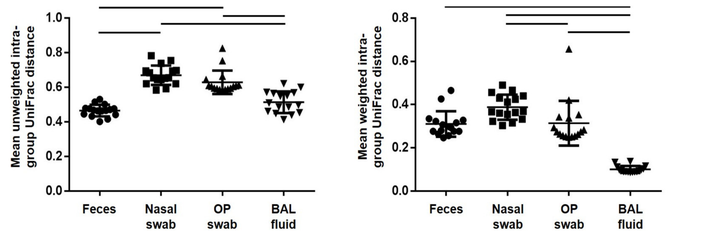


Figure 3. Mean ± SEM unweighted and weighted intra-group UniFrac distance between each sample and all other samples collected from the same sample site. Bars indicate significant differences between groups (P<0.05, Kruskal-Wallis one way ANOVA on ranks with multiple pairwise comparisons via Tukey test).

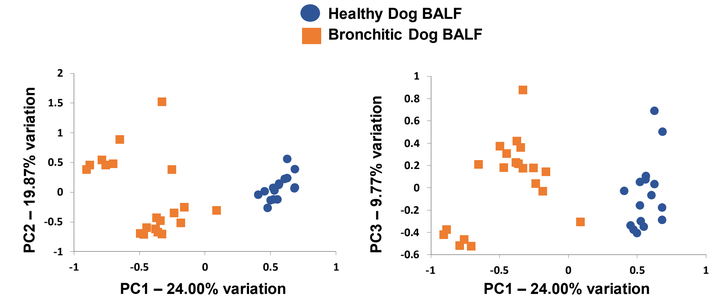


Figure 6. Principal component analysis of airway microbiota from BALF samples of healthy and bronchitic dogs. PC1, PC2, and PC3 account for 24.00%, 19.87%, and 9.77% respectively of the total variance observed.

Phylum	P-value	Abundance in CB vs. Healthy
Actinobacteria	<0.001[a]	Decreased
Bacteroidetes	0.036[b]	Increased
Cyanobacteria	<0.001[b]	Decreased
Fusobacteria	0.004[b]	Increased
Family	**P-value**	**Abundance in CB vs. Healthy**
Alcaligenaceae	<0.001[b]	Increased
Bacteroidaceae	<0.001[b]	Increased
Bradyrhizobiaceae	0.011[b]	Increased
Caulobacteriaceae	<0.001[b]	Decreased
Comamonadaceae	<0.001[a]	Decreased
Flavobacteriaceae	<0.001[b]	Decreased
Fusobacteriaceae	0.005[b]	Increased
Lactobacillaceae	0.009[b]	Increased
Listeriaceae	0.004[b]	Decreased
Methylobacteriaceae	<0.001[a]	Decreased
Micrococcaceae	<0.001[b]	Decreased
Paraprevotellaceae	<0.001[b]	Increased
Prevotellaceae	<0.001[b]	Increased
Propionibacteriaceae	<0.001[b]	Decreased
Order *Rhizobiales*	0.001[b]	Decreased
Ruminococcaceae	<0.001[b]	Increased
Sphingomonadaceae	0.021[b]	Decreased
Streptococcaceae	0.003[b]	Decreased
Order *Streptophyta*	<0.001[b]	Decreased

Figure 7. Table illustrating the significant differences in relative abundance of bacterial phyla and families in healthy and bronchitic dogs (P<0.05, t-test[a] and Mann-Whitney Rank Sum test[b]). P-values ≤0.001 are highlighted.

Conclusions

- Next-generation sequencing demonstrated the existence of a core airway microbiota, similar among dogs in the operational taxonomic units (OTU) present and the abundance of shared OTUs. *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria, Firmicutes*, and *Proteobacteria* were the most abundant phyla present.
- No significant difference was found in richness, or the number of distinct OTUs, between healthy and bronchitic dogs (P=0.126, t-test). Lack of clustering on the PCA suggests that the microbial communities are dissimilar among healthy and bronchitic dogs.
- Compared to healthy dogs, bronchitic dogs showed a significant increase in the abundance of phyla *Bacteroidetes* and *Fusobacteria* and a significant decrease in the abundance of *Actinobacteria* and *Cyanobacteria*. Significant differences were not seen in the more prominent microbial families (e.g. *Moraxellaceae*, *Pseudomonadaceae*), but in other well-represented families. In bronchitic dogs, there was an increase in the abundance of fecal microbial families and a decrease in the abundance of environmental microbial families that are a part of the core airway microbiota.

Acknowledgements

Student support was provided by the Morris Animal Foundation Veterinary Student Scholars Program.

Morris Animal FOUNDATION