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The respiratory microbiota and its impact on health and disease in dogs and cats: A One Health perspective

Aida I. Vientós-Plotts[1,2,3] | Aaron C. Ericsson[1,4,5] | Carol R. Reinero[1,2,3]

[1]College of Veterinary Medicine, University of Missouri, Columbia, Missouri, USA

[2]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, Missouri, USA

[3]Comparative Internal Medicine Laboratory, University of Missouri, Columbia, Missouri, USA

[4]University of Missouri Metagenomics Center, University of Missouri, Columbia, Missouri, USA

[5]Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, Missouri, USA

Correspondence
Aida I. Vientós-Plotts, 900 E. Campus Dr., Columbia, MO 65203, USA.
Email: vientosplottsai@missouri.edu

Abstract

Healthy lungs were long thought of as sterile, with presence of bacteria identified by culture representing contamination. Recent advances in metagenomics have refuted this belief by detecting rich, diverse, and complex microbial communities in the healthy lower airways of many species, albeit at low concentrations. Although research has only begun to investigate causality and potential mechanisms, alterations in these microbial communities (known as dysbiosis) have been described in association with inflammatory, infectious, and neoplastic respiratory diseases in humans. Similar studies in dogs and cats are scarce. The microbial communities in the respiratory tract are linked to distant microbial communities such as in the gut (ie, the gut-lung axis), allowing interplay of microbes and microbial products in health and disease. This review summarizes considerations for studying local microbial communities, key features of the respiratory microbiota and its role in the gut-lung axis, current understanding of the healthy respiratory microbiota, and examples of dysbiosis in selected respiratory diseases of dogs and cats.

KEYWORDS
asthma, lung, microbiome, nasal, pneumonia

1 | INTRODUCTION

Identification of bacteria from the lower respiratory tract has relied on traditional laboratory-based culture methods. However, it has been reported that only 1% of all bacteria on earth can be cultured in the laboratory, therefore lack of cultivable bacteria does not necessarily indicate a sterile environment.[1,2] In fact, when using culture-independent molecular sequence approaches (targeted metagenomics) on samples from the lower respiratory tract, rich and diverse microbial communities have been identified.[3,4] Although the lower respiratory tract has a low biomass,[1,5] complex microbial communities are present in health and critical for homeostasis. Deviations in microbial community composition (known as dysbiosis) may lead to development, persistence, exacerbation, or progression of respiratory diseases.[6] Understanding which microbial communities are associated with health and how the community composition changes with disease may provide insight into respiratory disease pathogenesis and allow for targeted interventions to modulate the respiratory microbiota back to a healthy state.

Abbreviations: ASV, amplicon sequence variants; BALF, bronchoalveolar lavage fluid; CAP, community-acquired pneumonia; CB, chronic bronchitis; CCR, canine chronic rhinitis; CIPF, canine idiopathic pulmonary fibrosis; CIRDC, canine infectious respiratory disease complex; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; FCR, feline chronic rhinitis; GIT, gastrointestinal tract; IL, interleukin; OP, oropharyngeal; OTU, operational taxonomic unit; PD-L1, programmed death ligand 1; qPCR, quantitative PCR; SBP, secondary bacterial pneumonia; TH, T helper cell; Treg, T regulatory cell; WHWTs, West Highland white terriers.

2 | THE DOGMA OF LUNG STERILITY

The lungs of healthy humans previously were thought of as sterile according to culture-based studies,[7,8] and bacterial growth in bronchoalveolar lavage of healthy individuals was considered to represent contamination.[9] In healthy dogs, one of the first studies describing bacteria in bronchoalveolar lavage fluid (BALF) concluded that BALF was "invariably contaminated with resident flora of the upper airways."[10] In healthy cats, bacteria were also consistently identified, but the number of colony forming units identified in BALF samples was so low that they were thought to be oral contaminants in the sampling instrument, and were not considered consistent with infection.[11] In fact, the idea that healthy lungs are devoid of bacteria was so common and deeply engrained that when the National Institute of Health launched the Human Microbiome Project in 2008 it did not include the lungs as a site to study.[12] Advances in metagenomics and the use of culture-independent methods have proven that this idea is incorrect with healthy lungs containing a rich and diverse ecosystem of bacteria, viruses, fungi, and archaea.[3,13] In contrast to results of laboratory-based cultures, the presence of microorganisms identified using molecular approaches provides a global view of commensals that play key roles in homeostasis in health, with deviations of commensal bacteria in disease. Because research on the respiratory microbiota of dogs and cats has focused largely on bacterial communities, this review will not further discuss viruses, fungi, or archaea.

The first description of the lower airway microbiota in healthy humans was published in 2010.[1] Bronchoalveolar lavage and airway brushing samples of healthy volunteers and humans with asthma were evaluated.[1] Although there were similarities between the upper and lower airway microbial composition, these anatomic regions were distinct from each other.[1] Furthermore, airways of humans with respiratory disease have a higher proportion of bacteria belonging to the phylum *Proteobacteria*.[1] Since then, many studies have confirmed that respiratory dysbiosis is associated with respiratory disease.[14] The term dysbiosis historically has been used in the context of gastrointestinal disease, likely because the intestinal microbiota and its interactions with the host immune system have been the focus of research for far longer and in more depth than the lungs.[12] One of the most common examples of how these studies of the gastrointestinal tract (GIT) have advanced medicine is the use of probiotics to modulate the microbiota in diseases inside and outside of the GIT.[15] These advances have not only made considerable impact in the fields of gastroenterology and immunology, but the application of this knowledge to other sites has the potential to further our understanding of respiratory disease and can provide novel avenues for intervention, such as modulation of the airway microbiota.

3 | METHODS FOR MICROBIAL ANALYSIS

Culture-based protocols have long been considered standard procedure for the identification of bacteria from various sites in the body in clinical settings. These protocols were known to have limitations because microscopically the number of bacteria would far outweigh the number of colonies that would grow on media, and success of culture relied on the ability to replicate an organism's environment under laboratory conditions.[16] The development of culture-independent methods highlighted our limited understanding of microbial diversity. For example, in 1987, 11 bacterial phyla were described, and as of 2020 this number has grown to at least 85, the majority of which have no cultured representatives.[17-20]

The most common culture-independent method of microbial identification relies on high-throughput sequencing of amplicons of the 16S rRNA gene, which has both conserved and highly variable features of the bacterial genome.[21] When sequenced, DNA from a single specimen can yield thousands of shorter genome sequences. These sequences can be grouped based on a prespecified degree of similarity (eg, 97%) referred to as operational taxonomic units (OTUs) and compared to a 16S rRNA database[22] (SILVA,[23] GREENGENES,[24] Ribosomal Database Project [RDP][25] and National Center for Biotechnology Information [NCBI][26]) of cultured and uncultured bacteria for identification. Recently, new methods have been developed that resolve amplicon sequence variants (ASVs) from amplicon data without using the similarity indices that define molecular OTUs.[27] These methods can distinguish sequence variants by a single nucleotide and can infer biological sequences in the sample before introducing amplification and sequencing errors. The sensitivity and specificity of ASV methods are as good or better than OTU methods and can better discriminate ecological patterns.[28-31] More importantly, ASVs are not limited by reference databases, allowing for detection of novel taxa, and are reusable across studies, which has essentially led to the replacement of OTUs with ASVs.[27,32,33] This approach is only 1 example of several methods available to sequence and analyze data, all with their own pitfalls and bias.[34-37]

Although capable of providing substantially more information about microbial communities, culture-independent approaches have several limitations. One limitation is that identification of 16S rRNA reflects presence of bacterial DNA fragments, which is not necessarily reflective of viable bacteria.[1,38-41] Without viable isolates, it is impossible to perform antimicrobial susceptibility testing necessary for management of patients with active infections. Additionally, potential contamination associated with sampling methods will have more impact on the composition of microbial communities in samples with lower biomass, as compared to samples with a larger biomass such as feces.[37] For this reason, it is of the utmost importance that reagents and samples taken from bronchoscopes and other instruments are included in the analysis as controls.[42] Other limitations associated with logistics include high cost of sample processing (especially for a small number of samples), limited availability of the technology, variable number of 16S copies per bacteria, and required expertise to analyze and interpret the data. Lastly, clinicians are limited by our still nascent appreciation of these microbial communities. Until a better understanding of the lung microbiota in the context of both health and disease is available, it will be challenging to incorporate sequencing data into clinical decision-making. Some of these challenges may be overcome by the integration of other -omics, such as metabolomics

or proteomics, which allow for the quantification of metabolic end products, by metabolites or proteins of bacteria within a microbial community.[33,43]

4 | PARALLELS BETWEEN THE LUNGS AND THE GIT: ANATOMY, PHYSIOLOGY, IMMUNOLOGY, AND RELEVANCE FOR MICROBIAL POPULATIONS

The internal surface area of the lungs of humans is estimated to be 30 times that of the skin,[44] and larger than the GIT.[45] The microbial biomass of healthy human lungs is estimated to be 10^3 to 10^5 bacteria per gram of tissue, much lower in comparison to the lower GIT (10^{11} to 10^{12} bacteria per gram of tissue).[46] Although the respiratory tract is composed of a series of structures (from the nose to the upper airways to the mucociliary apparatus) that are meant to filter as many particulates, irritants, and microbes as possible, it is in constant contact with the outside world. This situation contrasts with that of the GIT which has sphincters to compartmentalize its parts. Although both the lungs and the GIT are lined by mucosa with a shared embryological origin, they each provide unique environmental features including but not limited to temperature, pH, and oxygen for their respective microbiota. The respiratory tract has a gradient from ambient temperature to core body temperature whereas the GIT has a uniform temperature throughout its entirety.[5,47] Healthy airways in humans have a relatively neutral pH[48] compared to microbes in the GIT that must be able to tolerate changes in pH as they travel from the oral cavity to the large intestine. Additionally, although the lungs can become anaerobic in severe disease, they are usually oxygen rich, compared to the largely anaerobic environment in the GIT.

The respiratory tract has unique physical and mechanical defenses as well as innate and adaptive immune defense mechanisms.[49] Physical defenses include the anatomic barriers the air must travel through such as the nasal turbinates and turbulent airflow, closure of the glottis during swallowing, sneezing, and coughing reflexes that allow for expulsion of irritating particulates.[49] The mucociliary apparatus is composed of epithelial cells that form tight junctions preventing pathogen entry and have cilia protruding into a mucus layer that helps trap and clear particulates and pathogens.[50] Innate immune defenses include chemicals with antimicrobial properties secreted by phagocytic cells and the airway epithelium, as well as macrophages and neutrophils that can recognize and destroy pathogens. Alveolar macrophages, the most abundant cell population in healthy airways, can eliminate debris, apoptotic cells, and surfactant, functioning as one of the first lines of defense.[51,52] Adaptive immune defenses provide for antigen-specific responses and include T-cells involved in cell-mediated immunity and B-cells (with resultant production of antibodies) involved in humoral immunity. Adaptive immune responses are bridged by the innate immunity system allowing for well-orchestrated responses to threats perceived by the host.

Respiratory defenses can be altered by resident microbial populations. For example, although airway mucus represents an important protective barrier, mucus hypersecretion, one of the pathologic hallmarks of chronic airway diseases such as asthma,[53] is modulated by the lung microbiota.[54-57] As another example, dendritic cells (DCs) in the lungs are exposed to both commensal and pathogenic bacteria, resulting in expression of pattern recognition receptors to regulate local immune homeostasis.[58] Expression of programmed death ligand 1 (PD-L1) on DCs is necessary for regulatory T (Treg) cell development, with these cells crucial for establishment of tolerance to allergens and regulation of immune responses against self and non-self antigens.[59,60] In mice, absence of microbial colonization or blockade of PD-L1 in the first 2 weeks of life promotes a hyperallergic environment,[59] providing evidence that the respiratory microbiota plays an essential role in development of a tolerogenic environment in the lungs. As a third example, regulation of T helper 17 cells by the microbiota has been demonstrated in mice[61] and asthmatic humans.[62] The T helper 17 cells can either blunt alveolar macrophage response or promote pro-inflammatory cytokines such as interleukin-1β and interleukin-6.[63] As a final example, in addition to promoting immune homeostasis in health, the microbiota provides another line of defense by acting as a gatekeeper, providing resistance to pathogenic organisms on the mucosal surface.[64]

5 | ORIGIN OF THE LOWER AIRWAY MICROBIOTA IN HEALTH

The composition of the lower airway microbiota is thought to be determined by a balance of 3 factors: immigration, elimination, and reproduction of microbes.[3] Immigration occurs primarily by microaspiration and inhalation of microbes in air, with a lesser contribution from direct dispersion along the mucosal surface. Repetitive, non-pathologic microaspiration has been documented to occur in healthy human adults[65] and mice,[66] and has been suggested in dogs[67] (but not to date in cats) making it the most likely source of entry for microbes into healthy lungs. Several studies have confirmed that the lower airway microbiota more closely resembles that of the oropharynx than the nasopharynx, GIT or inhaled air, and that the nasal microbiota contributes minimally to the lower airway communities in healthy humans[3,38,41,68,69] and dogs[70] with no data yet available in cats. Elimination occurs via the mucociliary apparatus, cough, and immune defenses. Reproduction of bacteria in the lower airways is influenced by several variables impacting bacterial growth including pH, relative blood perfusion, oxygen tension, relative alveolar ventilation, temperature, epithelial cell structure, and concentration of inflammatory cells.[64,71] A proposed model of lung biogeography has suggested that in health immigration and elimination exert a more profound influence on the composition of lower airway microbiota, but, in disease, membership appears to be more heavily influenced by regional growth conditions.[71] Other features that influence microbial composition in disease include increased immigration in the form of gastroesophageal dysfunction or reflux (a prominent feature of severe lung disease),[72-74] decreased elimination in the presence of mucociliary apparatus impairment,[75] or increased elimination by exacerbation of cough or influx of

inflammatory cells.[76,77] Additional studies are needed to determine if differences exist in the 3 major factors (immigration, elimination, and reproduction of microbes) or their relative contributions in establishing microbial communities in the respiratory tract of dogs and cats.

6 | GUT-LUNG AXIS

The gut-lung axis has been described as a 2-way communication hub between these 2 mucosal sites wherein crosstalk occurs via chemical signals produced by organisms and the host immune responses they trigger. As we gain better understanding of the interplay between these sites it is not surprising that studies in humans[78-80] and cats[81] have shown that dysbiosis at either site can promote inflammation and dysbiosis at the other site. Additional studies are needed in dogs. The mechanisms by which these interactions occur are still poorly understood. It has been proposed that the GI microbiota plays an important role in the education of the immune system during a critical window early in life[82] and in lung defense against bacterial invasion.[83] Because the respiratory microbiota is heavily influenced by immigration, it seems reasonable that any disease that increases potential for aspiration (eg, gastroesophageal reflux, dysphagia), would impact the microbial community composition of the airways.[84] As our knowledge about the respiratory microbiota increases, additional studies investigating the relationship between the microbiota and these 2 mucosal sites may offer insight into alternative therapeutic strategies to treat, manage, or prevent respiratory diseases.

7 | AIRWAY MICROBIOTA IN HEALTH

Although upper airways substantially contribute microbes to lower airways, these 2 populations are distinct from each other.[70,71,85] Differences in clearance and limited local replication result in regional differences in bacterial concentrations with the more proximal regions of the lung containing a higher number of bacteria than more distal regions.[86] In healthy adult humans, predominating taxa vary in nasal, oropharyngeal (OP), and lower airway sites.[86,87] The nasal microbiota of humans is primarily composed of phylum *Bacillota* (formerly *Firmicutes*; family *Staphylococcaceae*) followed by *Pseudomonadota* (formerly *Proteobacteria*) and Actinomycetota (formerly *Actinobacteria*; family *Corynebacteriaceae*).[87] The OP microbiota of humans harbors primarily *Firmicutes* (*Veillonellaceae*, Streptococcaceae families) and *Bacteroidota* (formerly *Bacteroidetes*; family *Prevotellaceae*).[4] Although the lower airways are composed of similar predominant phyla, the families represented differ from other sites within the respiratory tract including *Bacteroidota* (family *Prevotellaceae*) and *Bacillota* (genera *Streptococcus*, and *Veillonella*).[3,4] For the sake of uniformity, changes in phylum-level taxonomies recently were adopted by the International Committee on Systematics of Prokaryotes (ICSP) and similarly updated at NCBI. To avoid confusion, we will describe previous findings here using the archaic taxonomic designations used in the referenced publications.

7.1 | Dogs

Proteobacteria, *Bacteroidetes*, and *Firmicutes* are the most abundant bacterial phyla in the nasal cavity of healthy pet dogs.[88-91] In healthy research dogs (n = 16), the nasal microbiota was primarily composed of families *Mycoplasmataceae* and *Moraxellaceae*.[70] The high proportion of *Tenericutes* in research dogs compared to pet dogs may be attributed to their local research environment, genetic background, diet, or geographical location.[90] A study comparing the nasal microbiota of dogs with a variety of facial conformations, age, and environments determined that brachycephalic breeds have a distinct nasal microbiota profile, with higher proportions of *Veillonellaceae*, *Rothia*, *Pasturellaceae*, *Polynucleobacter*, *Staphylococcus cohnii*, and *Mangrovibacter* compared to meso- and dolicho-cephalic dogs, but differences based on age or environment were not detected.[92] Like the nose, the oropharynx and lower airways of healthy dogs were dominated by the phylum *Proteobacteria*. However, within phylum *Proteobacteria*, when resolved to the taxonomic level of family, nasal swabs were dominated by *Moraxellaceae* followed by *Pasteurellaceae* and *Pseudomonadaceae*; OP swabs were dominated by *Pasteurellaceae* followed by *Moraxellaceae* and *Pseudomonadaceae* and the lower airways were dominated by *Pseudomonadaceae* and *Moraxellaceae* with fewer *Pasteurellaceae*. Resolved to the taxonomic level of genus, *Cutibacterium*, *Streptococcus*, *Acinetobacter*, and *Pseudomonas* have been found to be among the most abundant in the lower airways of research[70,93] and pet dogs.[94] Whereas *Bacteroidetes* and *Firmicutes* predominate in the respiratory tract of humans in health,[1] the respiratory microbiota of healthy dogs is dominated by *Proteobacteria* with similar topographical continuity between upper and lower airways.[70] The translational relevance of differences in predominating taxa between humans and dogs needs additional exploration.

A previous study[94] compared the lower airway microbiota of dogs of different breeds (West Highland white terriers [WHWTs], other terriers, shepherds, and brachycephalics) and environments (research, rural, and urban). Research dogs had higher richness (number of different taxa within a community) than dogs in rural and urban conditions, and significant differences were found in microbial community composition between pet and research dogs, but not between pet dogs in urban and rural conditions. Excluding WHWT and research dogs, the lung microbiota was not significantly influenced by breed.[94]

7.2 | Cats

Similar to dogs, the nasal[95] and OP[85] microbiota of healthy cats is dominated by phylum *Proteobacteria* followed by *Bacteroidetes* and *Firmicutes*. Resolved to the family level, the nasal microbiota is primarily composed of *Moraxellaceae*, and *Bradyrhizobiaceae*[95] whereas the oropharynx is dominated by *Pasteurellaceae*, *Moraxellaceae*, *Porphyromonadaceae*, and *Pseudomonadaceae*.[85] The lower airway microbiota of healthy cats is also dominated by *Proteobacteria*.[85] A study of 6 healthy research cats showed BALF samples were primarily composed of *Pseudomonadaceae* (33.90 ± 7.12%), *Sphingobacteriaceae*

TABLE 1 Summary of findings of amplicon sequencing studies of respiratory samples from healthy dogs and cats.

Healthy		Cohort description [Sample type collected]	Predominant taxa Phyla (family)	Platform (sequenced region)
Dog	Nose	Pet dogs (n = 12)[89] [nasal swab]	Proteobacteria (*Moraxellaceae*)	FLX-titanium amplicon pyrosequencing (V1-V3)
		Research dogs (n = 16)[70] [nasal swab]	Proteobacteria (*Moraxellaceae*, *Pasteurellaceae*, *Pseudomonadaceae*) > Tenericutes	Illumina MiSeq (V4)
		Pet dogs (n = 23)[88] [nasal swab]	Proteobacteria (*Moraxellaceae*)	Illumina MiSeq (V4)
		Working (detection) dogs (n = 34)[90] [nasal swab]	Proteobacteria (*Moraxellaceae*) Bacteroidetes	FLX-titanium amplicon pyrosequencing (V4-V6)
		Pet dogs (n = 25)[91] [nasal swab]	Proteobacteria (*Psychrobacter*)	Illumina MiSeq (V4)
		Pet dogs (46)[92] [deep nasal swab]	Proteobacteria (*Moraxellaceae*)	Illumina MiSeq (V1-V3)
	OP	Research dogs (n = 16)[70] [OP swab]	Proteobacteria (*Pasterellaceae*)	Illumina MiSeq (V4)
	Lung	Research dogs (n = 16)[70] [BALF]	Proteobacteria (*Pasterellaceae*)	Illumina MiSeq (V4)
		Pet dogs (n = 4)[93] belonging to the cohort reported in another publication[94] [BALF]	Proteobacteria > Bacteroidetes > Actinobacteria > Firmicutes	Illumina MiSeq (V1-V3)
		Pet dogs: terriers (n = 10), shepherds (n = 11), brachycephalic (n = 9), WHWTs (n = 6) Research dogs: beagles (n = 9)[97] [BALF]		
Cat	Nose	Pet cats (n = 28)[95] [deep nasal swab]	Proteobacteria (*Moraxellaceae* > *Bradyrhizobiaceae*)	Illumina MiSeq (V4)
	OP	Research cats (n = 6)[80,93] [OP swab]	Proteobacteria (*Pasteurellaceae* > *Moraxellaceae* > *Pseudomonadaceae*)	
	Lung	Research cats (n = 6)[85,98] [BALF]	Proteobacteria (*Pseudomonadaceae* > *Bradyrhizobiaceae*)	
		Research cats (n = 11)[81] [BALF]	Proteobacteria (*Pseudomonadaceae* > *Sphingomonadaceae*)	Illumina MiSeq (V4)

Abbreviations: BALF, bronchoalveolar lavage fluid; OP, oropharyngeal swab; WHWT, West Highland white terrier.

(22.43 ± 5.10%), and *Bradyrhizobiaceae* (15.83 ± 2.18%).[85] Another cohort of 8 healthy cats housed in the same research facility had mainly *Pseudomonadaceae* (80.4 ± 0.34%) in BALF samples.[81] The differences between these 2 cohorts may be attributed to changes in the microbiota associated with maturation with 1 cohort of cats being between 4 and 6 months of age[85] compared to the other study in which the cats were 3 years old,[81] with 6 months being the age at which cats reach sexual maturity. A third study including healthy pet cats had similar predominance of *Pseudomonadaceae* (78.3 ± 7.6%).[96] In healthy cats, using principal component analysis to assess β-diversity of site-specific bacterial populations, complete separation was found between upper airway (OP) and lower airway (BALF) samples, suggesting distinct microbial populations adapted to their niches.[85] Interestingly, in healthy cats, blood had compositional similarity (ie, with a few dominant taxa) with lower airway communities.[85]

Table 1 and Figure 1 summarize the findings of studies reporting targeted amplicon sequencing of respiratory samples from healthy dogs and cats.

8 | AIRWAY MICROBIOTA IN DISEASE

Dysbiosis has been defined as "compositional and functional alterations in the microbiota driven by a set of environmental and host-related factors that perturb the microbial ecosystem."[99] It can be associated with loss of commensal organisms, overgrowth of pathogens or pathobionts, or overall reduction in diversity, all of which could result from disruption in entry, clearance, or local replication. It is unclear if dysbiosis sets the stage for disease or if inflammation, infection, or neoplasia trigger changes in microbial community

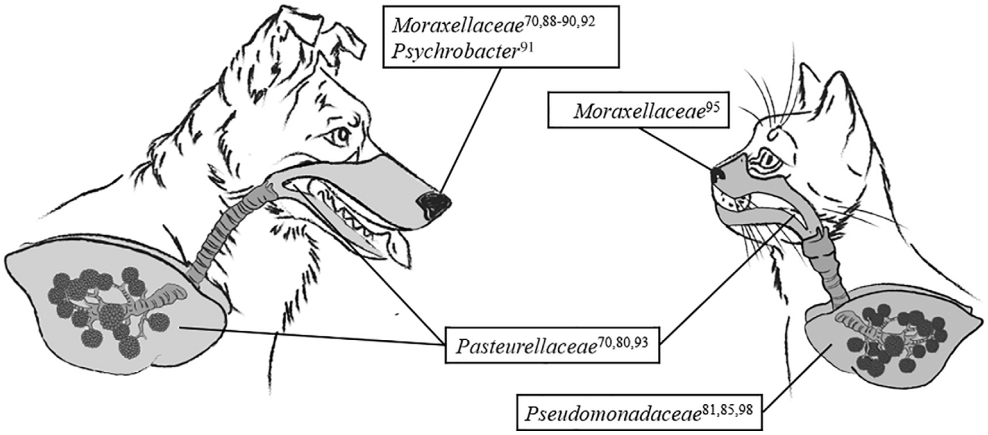

FIGURE 1 The healthy nasal, oropharyngeal, and lower airway microbiota of dogs and cats are populated by different families within the phylum *Pseudomonadota* (formerly *Proteobacteria*).

composition. Although several hypotheses and models to explain the relationship between respiratory dysbiosis and disease have been proposed, most agree that it is likely bidirectional with amplification.[100]

In health, the lungs are considered a nutrient-poor environment, as evidenced by the low protein content of BALF.[101] In disease, protein-rich edema can fill the alveolar space, providing an environment in which bacteria can grow and perpetuate inflammation, endothelial damage, injury, and further edema.[100] This cycle sets the stage for bacteria to thrive, further altering microbial community membership[71,102] (Figure 2). Respiratory dysbiosis has been described in humans with nasal disease,[103,104] asthma,[1,105,106] chronic obstructive pulmonary disease (COPD),[1,106] bronchiectasis,[107,108] cystic fibrosis,[109,110] bacterial pneumonia,[109,110] and interstitial lung diseases.[111] For this review, the disease processes for which the microbiota have been described in dogs and cats will be discussed including inflammatory and neoplastic nasal disease (dogs and cats), chronic bronchitis (CB; dogs), asthma (cats), bacterial pneumonia (dogs), and interstitial lung diseases (dogs) and are summarized in Table 2.

8.1 | Nasal diseases in dogs and cats

8.1.1 | Dogs

Chronic rhinitis and nasal neoplasia are the most common causes of nasal discharge in dogs.[115] Canine chronic rhinitis (CCR) is characterized by lymphoplasmacytic or mild neutrophilic infiltrates in the nasal mucosa causing nasal discharge, sneezing, and coughing.[116] The etiology of CCR is unknown, and although infectious, immune-mediated, and allergic mechanisms have been suggested, dogs may respond poorly to antimicrobials, glucocorticoids, and antihistamines, making these etiologies unlikely.[115] In dogs, carcinoma accounts for up to 66% of nasal neoplasia, with adenocarcinoma being the most common subtype.[115]

To date, a single study has compared the nasal microbiota in CCR (n = 8), nasal neoplasia (n = 16), and healthy (n = 23) dogs.[88] The group of dogs with nasal neoplasia had a variety of tumors (adenocarcinoma [6], osteosarcoma [3], squamous cell carcinoma [2], esthesioneuroblastoma [2], transitional cell carcinoma [1], and lymphoma [1]). A significant difference in nasal microbial community composition was observed between healthy dogs and those with neoplasia, but not between healthy dogs and those with CCR or between dogs with neoplasia and those with CCR. With respect to individual taxa, this study identified that although *Moraxella* spp. decreased, *Pasteurellaceae* significantly increased in dogs with CRR and neoplasia. Collectively, the study suggested that dysbiosis of the nasal microbiota occurred in dogs with nasal disease.

8.1.2 | Feline chronic rhinitis

Feline chronic rhinitis (FCR) has been reported to account for approximately 35% of cases of nasal discharge in cats, second only to neoplasia (38%),[117,118] with lymphoma being the most common type of neoplasia.[119] The exact etiology of FCR is unknown. It has been proposed that primary viral infections such as feline herpes virus 1 can damage the epithelium and predispose to secondary bacterial infections with pathogens such as *Mycoplasma*, *Pasteurella*, *Actinomyces*, and *Bordetella bronchiseptica*.[120] To date, a single study has described, but not compared, the nasal microbiota in FCR (n = 15), nasal neoplasia (n = 16), and healthy (n = 28) cats.[95] *Moraxella*, *Bradyrhizobiaceae*, *Staphylococcus*, *Pasteurella*, *Chlamydia*, and *Streptococcus* were the most frequently observed families in cats with FCR.[95] The nasal microbiota of cats with FCR and confirmed *Chlamydia felis* or feline herpes virus 1 infections was not significantly different from cats with FCR alone.[95] Cats with nasal neoplasia were diagnosed with lymphoma (7), squamous cell carcinoma (5), adenocarcinoma (2), fibrosarcoma (1), and carcinoma (1). *Moraxellaceae* and *Bradyrhizobiaceae* were the most abundant families in cats with nasal neoplasia. No significant difference was found in microbial community composition between cats with nasal neoplasia and a history of antibiotic administration and those without. However, within cats having nasal neoplasia, *Prevotella copri* and *Staphylococcus sciuri* were significantly more abundant in cats that had received antibiotics within 8 weeks of sampling.

The studies to date in dogs and cats with inflammatory and neoplastic nasal disease document differences (ie, dysbiosis) compared to healthy dogs and cats. The heterogeneity of the groups in the

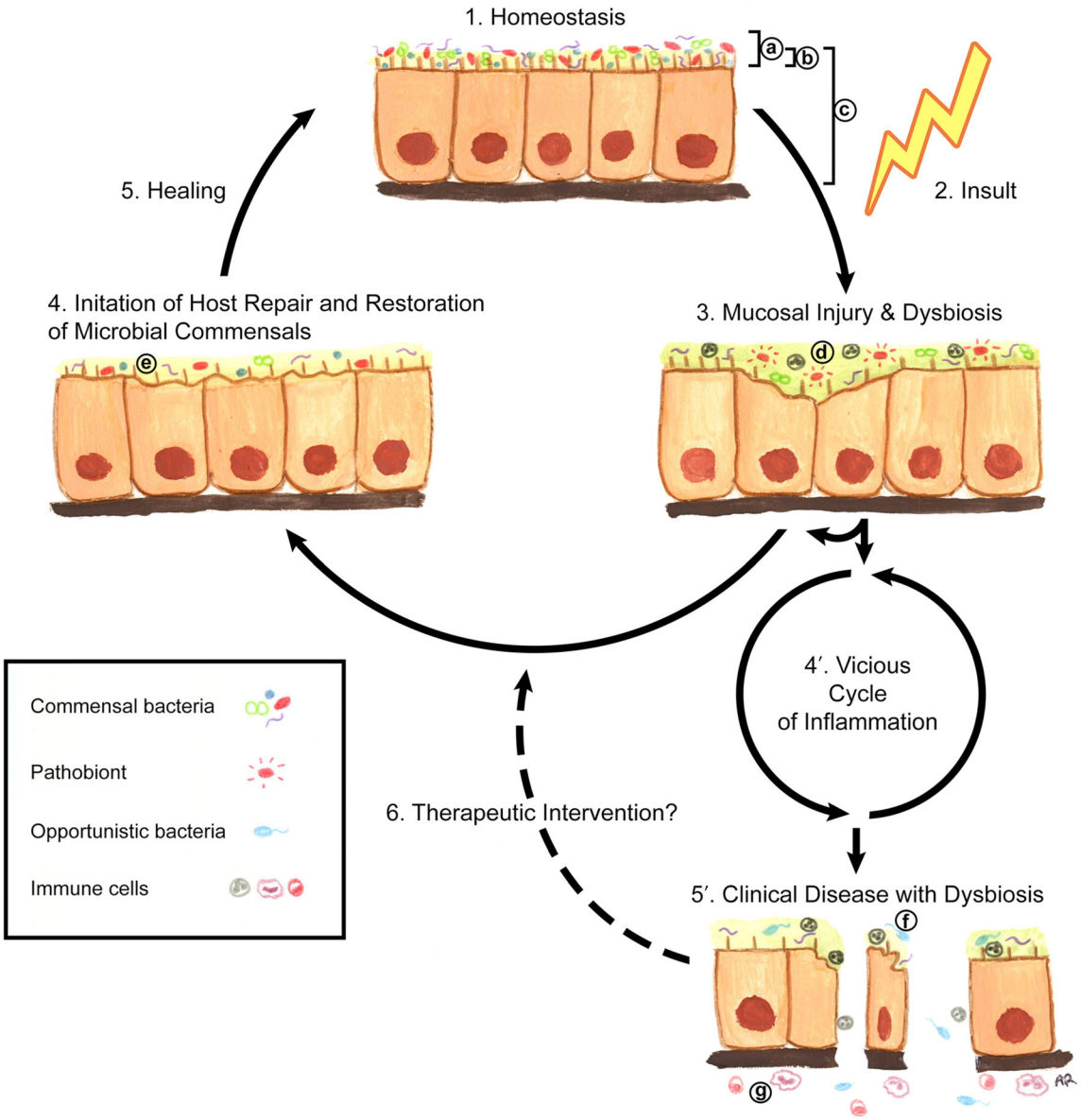

FIGURE 2 The symbiotic relationship between the host respiratory mucosal tissues and microbial communities in health and disease. (1) Homeostasis is achieved with a mutually beneficial symbiotic relationship between the host respiratory mucosal surface and commensal microbial communities in health. Although low in biomass, the microbiota is rich and diverse. Pathobionts, potentially pathogenic organisms which in states of health live as nonharmful symbionts, may be included in the microbial population. The mucosal surface is free of inflammation, with effective physical and chemical barriers and functional mucosal immunity. Commensal bacteria are represented by (A), the mucociliary apparatus by (B), and ciliated epithelial cells by (C). (2) An internal or external insult leads to mucosal injury. (3) With damage to the epithelial cells, mucociliary function is negatively impacted, and microbial communities are altered (generally resulting in decreased diversity). Respiratory dysbiosis is believed to play a key role in the disruption of the homeostatic state. Low-grade inflammation and decreased microbial diversity are represented in (D). Subsequent events determine the fate of the host-microbe relationship with 2 possible outcomes: a pathway of repair and healing or a vicious cycle of inflammation. (4) With removal and recovery from the insult, host repair mechanisms are initiated. Loss of danger signals, termination of inflammation, epithelial barrier repair, increased diversity of commensals, and decreased pathogen colonization are represented by (E). In time and without additional insults, (5) healing can occur, returning the environment to homeostasis. If repair and healing fail to occur and the respiratory mucosal tissues enter a vicious cycle of inflammation (4′), there is a transition to pathogenic bacteria such as pathobionts or opportunists (F), with recruitment of immune cells (G) and perpetuated mucosal injury. (5′) This leads to clinically evident respiratory disease with more profound dysbiosis. (6) With knowledge of the impact of loss of homeostasis on respiratory mucosal tissues, the challenge to the scientific community is to develop therapeutic interventions that target inflammation and restore beneficial commensal bacterial communities.

aforementioned studies in dogs[88] and cats,[95] in combination with small sample size, environmental and genetic contributing factors (such as diet or recent antibiotic or corticosteroid administration) may account for failure to identify more robust differences between groups when compared. Larger studies with more homogenous populations and including breed- and age-matched controls are warranted to determine if specific taxa are associated with CCR, FCR, or a specific type of nasal neoplasia.

TABLE 2 Summary studies referencing disease processes for which the airway microbiota has been described in dogs and cats.

Disease	Cohort description	Findings	Sample type—Platform (sequenced region)
Canine chronic rhinitis and nasal neoplasia	Dogs with malignant nasal neoplasia (n = 16), and CCR (n = 8)[88]	Significant decrease in *Moraxellaceae* and overall decreased diversity in both conditions. Dogs with nasal neoplasia also had significant increases in *Pasteurellaceae* and *Neisseriaceae*.	Deep nasal swab—Illumina MiSeq (V4)
Feline chronic rhinitis and nasal neoplasia	Cats with nasal neoplasia (n = 16), and FCR (n = 15)[95]	Most frequently observed taxa in neoplasia *Moraxella*, *Bradyrhizobiaceae*, *Chitinophagaceae*, *Phyllobacterium*, *Pasteurella*. *Mycoplasma* spp. was not the most abundant but was the most common bacterium in cats with FCR. Most frequently observed taxa in FCR—*Moraxella*, *Bradyrhizobiaceae Staphylococcus*, *Chlamydia*, *Pasteurella*.	Deep nasal swab—Illumina MiSeq (V4)
Canine chronic bronchitis	Pet dogs with CB (n = 53) vs pet dogs with upper airway diseases (n = 11) vs healthy research dogs (n = 16)[112]	Decreased relative abundance of *Pseudomonadaceae* and overall richness in dogs with CB compared to healthy. Suggested that changes in environmental conditions may be associated with changes in the respiratory microbiota.	BALF—Illumina MiSeq (V4)
Feline asthma	Research cats with experimentally induced asthma (n = 8)[81]	*Pseudomonadaceae* predominated before asthma induction. *Sphingobacteriaceae* and *Xanthobacteraceae* predominant taxa after asthma induction. Dysbiosis observed in lower airways as well as GIT.	BALF—Illumina MiSeq (V4)
	Pet cats with spontaneous asthma (n = 26) vs healthy research cats (n = 11)[96]	Higher α-diversity in asthmatic vs healthy cats. Most asthmatic cats had a predominance of either *Filobacterium* spp. (family *Chitinophagaceae*) or *Acinetobacter* spp. (family *Moraxellaceae*). Family *Mycoplasmataceae* only sequenced in asthmatic cats.	BALF—Illumina MiSeq (V4)
Bacterial pneumonia	Dogs with bacterial pneumonia (n = 15) (20 samples)[113]	Airway dysbiosis documented in dogs with bacterial pneumonia. Dogs with CAP had greater loss of β-diversity than dogs with SBP.	BALF, OP—Illumina MiSeq (V4)
	Dogs with *Bordetella bronchiseptica* infection (n = 20) vs healthy research dogs (n = 4)[97]	Decreased richness and α-diversity, increased bacterial load, and frequent coinfection with *Mycoplasma cynos* in dogs with *B. bronchiseptica* infection.	BALF—Illumina MiSeq (V1-V3)
	Dogs with bacterial pneumonia (n = 9)[114]	Blood cultures are insensitive but specific for cultured BALF bacteria in dogs with pneumonia. Dogs with CAP had greater loss of diversity than dogs with SBP.	BALF—Illumina MiSeq (V4)
Fibrotic lung disease	WHWT with CIPF (n = 11)[94]	Lower α-diversity in diseased dogs compared to healthy dogs. Dogs with CIPF had greater abundance of *Brochothrix*, *Curvibacter*, *Pseudarcicella*, and *Flavobacteriaceae*.	BALF—Illumina MiSeq (V1-V3)

Abbreviations: BALF, bronchoalveolar lavage fluid; CAP, community-acquired pneumonia; CB, chronic bronchitis; CCR, canine chronic rhinitis; CIPF, canine idiopathic pulmonary fibrosis; FCR, feline chronic rhinitis; GIT, gastrointestinal tract; OP, oropharyngeal swab; SBP, secondary bacterial pneumonia; WHWT, West Highland white terrier.

8.2 | Airway diseases in dogs and cats

8.2.1 | Canine chronic bronchitis

Canine CB is a noninfectious, inflammatory lower airway disease characterized by cough for >2 months, mucus hypersecretion, and nondegenerate neutrophilic airway cytology.[121,122] Affected dogs require chronic corticosteroid administration to decrease airway inflammation and manage clinical signs.[121] Although the pathophysiology of CB in dogs and COPD in humans differs, chronic inflammation leading to irreversible architectural changes such as airway wall remodeling and bronchiectasis, and subsequent impaired mucociliary clearance, can be observed in both diseases.[123] Airway dysbiosis has been documented in humans with COPD[124,125] and in dogs with CB.[112]

The lower airway microbiota was compared among pet dogs with CB (n = 53), pet dogs with respiratory signs in the absence of inflammatory BALF (non-CB; n = 11), and healthy research dogs (n = 16).[112] Dogs with CB and non-CB had decreased richness and alpha diversity compared to healthy research dogs. Additionally, dogs with CB had an increased relative abundance of *Pseudomonas* with increasing absolute

biomass. A similar observation has been made in human COPD patients with high proportions of *Pseudomonas* having higher bacterial loads and higher rates of disease exacerbation.[126] Large intragroup variability was noted in dogs with respiratory disease (CB and non-CB) compared to healthy research dogs, with some dogs harboring high proportions of *Agrobacterium*, *Stenotrophomonas*, and *Bradyrhizobium*. Hierarchical clustering analysis comparing CB and non-CB dogs to healthy dogs showed that Gram-negative *Proteobacteria* (including potential pathogens such as *Enterobacter hormaechei*, *Roseomonas*, and *Stenotrophomonas* spp.) contributed the largest amount of between-group variability. Furthermore, principal component analysis showed that samples clustered according to the 6-month period in which they were collected. These data suggest that in addition to variability that could be expected secondary to different breeds, diets, household environments, and severity of disease, that time of collection (season) also influenced microbial community composition.[112] Although this study had some limitations, such as the lack of healthy pet dogs as a control group, it highlighted the potential influence of the environment on the lower airway microbiota of pet dogs and demonstrated that dysbiosis occurs in dogs with CB. Future studies should enroll larger numbers of affected dogs and comprehensively investigate environmental variables and clinical data to better determine if a correlation exists between a specific microbial profile and environmental factors or disease severity.

8.2.2 | Feline asthma

Feline allergic asthma is a common bronchopulmonary disorder estimated to affect between 1% and 5% of the pet cat population.[127] It is characterized by airway eosinophilia, airway hyperresponsiveness, mucus hypersecretion, and airway remodeling resulting in airway obstruction. Similar to childhood-onset asthma in humans,[53] feline allergic asthma is orchestrated by allergen-specific T helper 2 (Th2) cells that produce cytokines to trigger and amplify a local inflammatory response. Based on these similarities, cats have been proposed as a non-rodent model of allergic asthma with relevance to One Health.[128] Alterations in the lower airway microbiota have been documented in asthmatic patients.[1,129-131] Similarly, airway dysbiosis in cats with experimental and spontaneous asthma also has been described.[81,96]

Changes in the upper and lower airway and GIT microbiota were assessed longitudinally in research cats after experimental asthma induction, in the acute and chronic stages of disease.[81] As cats transitioned from health to chronic asthma, a significant decrease in microbial richness was observed in the lower airways and GIT. In health, the most abundant families in rectal samples were *Lachnospiraceae* and *Veillonellaceae*, *Bacteroidaceae*, *Helicobacteraceae*, and *Fusobacteriaceae*. The most significant changes in relative abundance were observed in chronic asthma with a significant decrease in *Firmicutes* and an increase in *Proteobacteria*. *Pasteurellaceae* and *Porphyromonadaceae* were the most abundant families in the upper airways. No significant change was observed in relative abundance of the aforementioned taxa over time, but *Pseudomonadaceae*, the most abundant taxon in the lower airways of healthy cats,[85] was significantly decreased during the transition from health to chronic asthma. After asthma induction, the most marked changes in microbial community composition were observed in the lower airways.

Respiratory dysbiosis in cats was characterized by a significant change in relative abundance of bacterial phyla, with Proteobacteria decreasing from 94.4% to 65.2% and 36.1%, and *Bacteroidetes* increasing from 1.3% to 25.5% and 62.9% as cats transitioned from health to acute and chronic asthma, respectively.

Although challenging to prove in spontaneous asthma, this study with longitudinal evaluation showed that induction of eosinophilic inflammation in previously healthy cats led to respiratory dysbiosis. Furthermore, the dysbiosis was not confined to the respiratory tract (ie, the primary site of inflammation), but also occurred at the distant site of the GIT. Thus, results provided evidence to support the existence of a gut-lung axis in cats with experimental asthma.

A recent study investigated the lower airway microbiota of pet cats with spontaneous asthma.[96] Because the lower airway microbiota of healthy pet cats has not been characterized for ethical reasons associated with the risk of anesthesia and collection of BALF, comparisons were made to a cohort of healthy research cats. Although cats with experimental and spontaneous asthma were not directly compared and considering that factors such as diet, environment, and genetics can influence the composition of the lower airway microbiota, it was not surprising that cats with spontaneous asthma exhibited more intragroup heterogeneity than cats with experimentally induced disease.[81,96] However, like cats with experimental asthma, cats with spontaneous asthma also had almost complete loss of *Pseudomonadaceae*. Most asthmatic cats (69%) in this study had a predominance based on sequencing of either *Filobacterium* (ie, cilia-associated respiratory [CAR] bacteria) or *Acinetobacter* spp. Both taxa could be considered pathobionts, because they are part of the commensal microbiota in health but have been associated with disease.[132,133] Additionally, *Mycoplasma felis*, a known lower airway pathogen of cats,[134,135] was identified in 35% of asthmatic but not in healthy cats, supporting that in addition to pathobionts, some opportunistic bacteria also may contribute to, or thrive in, airways with altered microbial communities.

In both experimental and spontaneous asthma in cats, dysbiosis in the lower airways is characterized by the near absence of *Pseudomonadaceae*, which has been consistently associated with health. Future studies including larger populations of healthy cats and those with respiratory disease and longitudinal assessments of their airway microbiota after manipulation of the microbiota with corticosteroids, antibiotics, or probiotics would be warranted to determine if the airway microbiota can be restored to a healthy state.

8.3 | Parenchymal diseases in dogs

8.3.1 | Bacterial pneumonia

Bacterial pneumonia, a common condition in dogs associated with morbidity and increased risk of mortality, is defined as inflammation of

the pulmonary parenchyma caused by bacterial infection.[136,137] The most common causes of bacterial pneumonia in dogs are community-acquired pneumonia (CAP) and secondary bacterial pneumonia (SBP).[138] Bacterial and viral pathogens contribute to canine infectious respiratory disease complex (CIRDC), with CAP leading to bacterial pneumonia after close contact with another dog harboring a contagious bacterial pathogen such as *B. bronchiseptica*, *Mycoplasma cynos*, or *Streptococcus equi* subspecies *zooepidemicus*.[139] Secondary bacterial pneumonia most commonly occurs from damage associated with aspiration of OP or gastric materials, which subsequently may allow for colonization of bacteria, leading to SBP. Diagnosis of CAP or SBP is based on history, clinical findings, and bacterial culture results.[140] The development of culture-independent methods has highlighted that standard practices are selective, capable of identifying only cultivable bacteria, and may not adequately represent the full picture of microbial involvement in dogs with pneumonia.

Discrepancies between bacteria identified by culture and sequencing were noted when comparing 20 samples collected from 15 dogs diagnosed with bacterial pneumonia.[113] Cultures of BALF grossly underestimated presence of microbial communities, identifying between 0 and 5 bacterial species, compared to 22-185 distinct taxa at the species level identified by DNA sequencing. In dogs with CAP ($n = 6$), 4/6 had 1 dominant taxon at a relative abundance >95%, whereas dogs with SBP ($n = 9$ dogs with 14 samples) were more likely to have more taxa sequenced at lower relative abundances. Agreement was found between standard culture results and the most abundant taxa identified in sequencing in only 3/6 samples from dogs with CAP and 4/14 samples from dogs with SBP. In the 2 remaining cases of CAP, *B. bronchiseptica* was identified on culture, but it was not detected on sequencing. Instead, taxa from the family *Mycoplasmacetae* (*Mycoplasma* and *Ureaplasma* spp.) were the most abundant. Coinfection is common in dogs with CIRDC.[137,141] Another study of the microbiota analyzed BALF from 20 pet dogs diagnosed with *B. bronchiseptica* by culture or quantitative PCR (qPCR) and documented that 7/18 dogs for which *M. cynos* qPCR results were available were positive for this second pathogen.[97] In contrast to a previous study,[113] all dogs in the diseased group had *B. bronchiseptica* identified on sequencing, with 13/20 having it at >50% relative abundance.[97]

Dogs with CAP had significantly lower microbial richness and diversity in the lower airways than dogs with SBP.[113] Oropharyngeal swabs also were sequenced in 11/20 samples. In dogs from which OP swabs were obtained, higher intrasubject variability was observed among dogs with CAP than among those with SBP. Additionally, OP and lower airway communities differed based on the presence or absence of certain taxa, but not regarding the relative abundance of shared taxa. Because the most common cause of SBP in this population was aspiration pneumonia, it is not surprising that the upper and lower airway community composition in those dogs shared more similarities than in dogs with CAP. As in other studies in dogs and humans,[21] significantly decreased richness and diversity were observed in bacterial pneumonia compared to health.[113,114] Dogs with CAP were more likely to have 1 or 2 dominant taxa compared to healthy dogs.[97]

Many clinicians may consider collection of BALF invasive with increased risk in dogs with respiratory compromise from diseases such as bacterial pneumonia. However, a recent study provided evidence to support that advanced imaging and BALF collection have been shown to have low morbidity, with no mortality directly attributed to the advanced diagnostic testing.[142] Regardless, many clinicians may not have the training or access to equipment necessary to perform these procedures, bringing into question the utility of less invasive surrogates for culture and molecular analysis. A recent study assessed the utility of blood cultures and sequencing, and OP 16S sequencing as potential surrogates for microbes in BALF in 9 dogs with bacterial pneumonia (2 with CAP and 7 with SBP).[114] This evaluation was accomplished by determining the proportion of agreement between cultivable bacteria in BALF and blood and by characterizing and comparing BALF, OP, and blood microbial communities. Disregarding 1 false positive result, blood cultures were positive in 2/9 dogs, yielding 5 isolates. All 5 isolates were present in BALF cultures, which yielded 16 isolates. Based on sequencing data, all 3 sites had rich and diverse microbial communities. Comparing cultured BALF bacterial genera with sequenced taxa, all dogs had ≥1 cultured isolate present in their microbiota: cultured BALF isolates were found in microbiota of BALF (12/16 isolates), blood (7/16 isolates), and OP (6/11 isolates; with only 7 dogs having OP swabs). Blood cultures were insensitive (22%) but specific for cultured BALF bacteria in dogs with bacterial pneumonia. Although all 3 sites were significantly different from each other in terms of microbial community composition, cultivable bacteria were present in BALF, blood, and OP microbiota to different degrees. Based on the available data, sequencing of surrogates such as blood and OP swabs may not be representative of changes in the microbial community composition and structure in the lower airways. Although advanced diagnostic testing (including collection of BALF) is more invasive, it can be performed safely, and has been shown to have low morbidity, with no mortality directly attributed to the advanced diagnostic testing itself.[142]

Several studies to date have shown that respiratory dysbiosis occurs in dogs with bacterial pneumonia, emphasizing the importance of understanding how cultivable bacterial species, presumed to cause disease, interact with other members of the respiratory microbiota. Airway dysbiosis observed in dogs with CAP and SBP may be a result of alterations in the microenvironment that can allow for pathogen invasion or for opportunistic pathogens or pathobionts to thrive. Based on these data, it is important to consider that >1 organism may be contributing to airway dysbiosis, clinical disease manifestations, or both. Furthermore, it is clinically relevant to recognize that most of those organisms may not be cultivable, such as *Mycoplasma* spp.

8.3.2 | Fibrotic lung disease

Canine idiopathic pulmonary fibrosis (CIPF) is a familial progressive, fibrotic interstitial lung disease most commonly reported in middle-aged to older WHWTs.[143] Affected dogs have a history of exercise intolerance alone or in association with cough and inspiratory crackles

evident on thoracic auscultation.[144] In more advanced or severe cases, affected dogs can exhibit respiratory distress or syncope associated with pulmonary hypertension.[144] Histologically, CIPF is characterized by collagenous thickening of the pulmonary interstitium leading to impaired gas exchange.[145] This disorder shares some features with 2 subtypes of idiopathic interstitial pneumonias in humans: usual interstitial pneumonia and nonspecific interstitial pneumonia.[145] The pathogenesis of CIPF and idiopathic pulmonary fibrosis (IPF) in humans is unknown.[146,147] Although familial IPF is uncommon in humans,[148] the predisposition of WHWT for CIPF raises suspicion for a genetic cause.[143] Although there is no evidence for causality, several studies have established that the lower airway microbiota of humans with IPF can be correlated to disease status, alveolar inflammation, and host genotype, among other factors.[149] Additionally, variation in the lung microbiota can predict disease progression and mortality among human patients with IPF.[150-152]

To date, a single peer-reviewed study has been published characterizing the lower airway microbiota of WHWTs with CIPF.[94] This study compared the lower airway microbiota of WHWTs with CIPF (n = 11) with healthy pet dogs of a variety of breeds (shepherds [n = 11], terriers [n = 10], brachycephalics [n = 9], healthy research dogs [n = 9], and WHWT [n = 6]). Albeit at low relative abundance, 5 genera were only present in healthy WHWTs (*Limnohabitans*, *Rhodoluna*, *Curvibacter*, *Pseudarcicella*, and *Sporochthyaceae*) and 1 genus was only present in WHWTs and healthy research dogs (*Brochothrix*) but not in any other breeds in this study. When comparing healthy pet dogs, healthy WHWTs, and WHWTs with CIPF, a significant difference in β-diversity was found between healthy dogs (excluding healthy WHWTs) and diseased WHWT. This significant difference did not extend to comparisons between healthy WHWTs and diseased WHWTs. Given the evidence supporting an association between the lung microbiota and IPF in humans,[111] larger studies in WHWTs with and without CIPF are needed. Not only could such studies provide insight into the pathogenesis, disease progression, and prognosis of dogs with CIPF but WHWT with CIPF also may be of relevance as a large animal model for humans with IPF.

9 | CONCLUSIONS

In conclusion, studies of the respiratory microbiota in dogs and cats have found low biomass but rich and diverse microbial populations which are altered in disease. Compared to people, dogs and cats have similarities in anatomy, physiology, and immunology; have complex genetics; share their environments and thus exposures; and develop similar naturally occurring respiratory disorders. Additional investigation of the role of microbial communities in patients with respiratory disease will have bidirectional value between dogs and cats and humans.

10 | FUTURE DIRECTIONS

The study of the respiratory microbiota in dogs and cats is still in its infancy. Many knowledge gaps still exist, especially with respect to interactions between the respiratory microbiota and the host immune system and gut-lung axis. Some questions that remain unanswered include: (1) Does a core or healthy airway microbiota exist? Longitudinal studies, with broader age- and breed-matched populations, evaluating the influence of factors such as maturation, sex, diet, environment, and geographic location would be helpful to gain a better understanding of the respiratory microbiota in health. (2) Are specific taxa, groups of taxa, or metabolites involved in the pathogenesis of inflammatory or infectious airway or parenchymal diseases, or specific types of nasal or lung neoplasia? Larger studies in these populations are warranted to attempt to answer these questions. (3) What are the mechanisms involved in host-lung microbiome interactions and in the gut-lung axis? Does crosstalk occur via metabolite production or consumption, or both and immune modulation? (4) Can we modulate the airway microbiota with probiotics, prebiotics, or antibiotics to restore homeostasis? Future studies characterizing the metabolome, virome, and mycobiome could provide details to better understand interactions within the lung microbiota. Similarly, the low biomass of airway samples makes whole metagenome sequencing approaches inherently challenging and highlights the need for research and technical advances in terms of sample and library preparation. By gaining a better understanding of airway bacterial communities in health and disease, their interactions with the immune system, and bacterial communities in other sites such as the GIT, insight may be gained into alternative strategies to treat, manage, or prevent respiratory diseases in dogs, cats, and humans.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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Authors declare human ethics approval was not needed for this study.

ORCID

Aida I. Vientós-Plotts https://orcid.org/0000-0002-8146-0580
Carol R. Reinero https://orcid.org/0000-0002-6382-5582

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