META-ANALYSIS OF MICROBIAL DIVERSITY IN YOUNG CATS USING T-BIOINFO SERVER

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Abstract

The present computational study re-analyzes microbial diversity in young felines using NCBI data to explore site-specific and gender-related patterns. Microbial communities across four anatomical sites-the gut, oropharynx, bronchoalveolar lavage fluid (BALF), and blood-were examined using the T-BioInfo server. Gut microbiota showed high abundances of *Bacteroidaceae* and *Lachnospiraceae*, with gender-based variations, such as increased *Bacteroidaceae* in females. In the oropharynx, *Pasteurellaceae* predominated in males, while *Moraxellaceae* was higher in females. BALF analysis revealed *Pseudomonadaceae* as the most abundant family, with *Sphingobacteriaceae* more prevalent in females and *Bradyrhizobiaceae* in males. Blood samples contained *Sphingobacteriaceae* and *Bradyrhizobiaceae*, with notable gender differences: *Sphingobacteriaceae* was more abundant in females, and *Bacteroidaceae* was exclusive to female samples. These findings emphasize individual and gender-based microbiota diversity across body sites, highlighting the importance of such variations for future research and veterinary care.

Keywords: **Feline microbiota; Gender-specific microbiota; Gut microbiome; Bronchoalveolar lavage fluid (BALF); Oropharyngeal microbiota**

Introduction

The microbiome of cats has been a topic of interest in recent years due to its potential impact on feline health and disease (Suchodolski, 2011). The study of the feline microbiome is crucial as it can provide insights into the role of microorganisms in feline health and disease. The gut microbiome, in particular, has been shown to play a crucial role in the regulation of host health (Suchodolski, 2011). The gut microbiome of cats is influenced by various factors such as diet, age, and environment (Tun *et al*., 2012). The microbiome of healthy cats is dominated by the phyla *Firmicutes* and Bacteroidetes, with *Proteobacteria*, *Fusobacteria*, and *Actinobacteria* also featuring prominently (Moon *et al*., 2018). The gut mycobiome, which is less studied than the bacterial microbiome, also plays a significant role in feline gastrointestinal health and disease (Tay *et al*., 2022). The oral microbiome of cats has also been studied, with previous research suggesting the involvement of

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viral and bacterial components in the initiation and progression of feline chronic gingivostomatitis (Krumbeck *et al*., 2021).

Moreover, the respiratory microbiota of cats has also been found to have rich and diverse microbiota, and studies have demonstrated differences in the composition of airway microbiota in health versus disease suggesting respiratory dysbiosis occurs Traditionally, the lungs were thought to be devoid of bacteria, largely due to the limitations of standard culture methods that fail to detect most bacteria. However, the use of culture-independent techniques, such as 16S rRNA sequencing, has revealed that healthy lungs in humans, dogs, and other animals host complex microbial communities. This discovery has led to the concept of a 'gut-lung axis,' a proposed mechanism of interaction and cross-talk between the gut and lung microbiota that contributes to overall health and modulates responses to disease. Cats and dogs, which share respiratory conditions resembling chronic obstructive pulmonary disease (COPD) and asthma in humans, respectively, present valuable models for studying these host-microbe interactions. (Vientos-Plotts, 2022).

In this study, we aim to fill the knowledge gap by analyzing the microbial communities in different body sites of six cat species. Furthermore, we will investigate whether there are any gender-based differences in the microbial composition of cats (Huang *et al*., 2022). In conclusion, this study will provide valuable insights into the feline microbiome and its impact on health and disease. The findings of this study will have significant implications for the diagnosis and treatment of various feline diseases, including urinary tract infections, periodontitis, and chronic kidney disease, which have been linked to dysbiosis of the microbiome (Kerr *et al*., 2014; Rodrigues *et al*., 2019; Kim *et al.*, 2021). The study will also contribute to our understanding of the further role of the microbiome in maintaining the health of cats and may lead to the development of new strategies for promoting feline health and well-being.

Materials And Methods

Dataset & Experimental Design:

This study exclusively utilized publicly available data from the Bioprojects section on NCBI, specifically from PLOS ONE under the article titled "Dynamic changes of the respiratory microbiota and its relationship to fecal and blood microbiota in healthy young cats" (accession code PRJNA350163) (Vientós-Plotts *et al*., 2017). The dataset includes microbial samples collected from young, sexually intact cats under one year of age (aged 25-35 weeks by the end of the study), organized into two litters: Litter A, consisting of three females (Belle, Elsa, and Hermione), and Litter B, with two males (Harry and Ron) and one female (Jasmine). At the outset of the original study, cats in Litters A and B were 24 and 14 weeks old, respectively.

Sample collection in the original dataset covered fecal swabs, oropharyngeal (OP) swabs, and bronchoalveolar lavage fluid (BALF) taken at three time points: day 0, week 2, and week 10, with blood samples additionally collected at week 10. In our study, we exclusively performed statistical analyses on this publicly available dataset to investigate microbial diversity and composition across these body sites, without conducting any new experimental procedures.

Pipeline:

For metagenomic analysis of paired end 16S rRNA the DADA2 pipeline is recommended since it can identify between species even with just one nucleotide difference (Callahan *et al*., 2016). T-BioInfo server (Tauber Bioinformatics: Making sense of big data in BIOINFORMATICS (tbio.info)) (https://server.t-bio.info/) was used to analyze the samples obtained. DADA2 was used to calculate the operational taxonomic units (OTU); whereas PHYLOSEQ was used for other quantitative analyses like Shannon Index. The parameters set for expected number of reads forwards and backwards was 238. The expected errors were estimated as 2 and the min/max value for overlaps was approximated as 12/0. The mean abundance was established as 150 after optimization. Once the OTU table was generated the results were scanned for changes in diversity. The process of the pipeline is illustrated in Figure 1.

Data Preparation:

The bacterial composition data for fecal, oropharyngeal, bronchoalveolar, and blood samples are collected and compiled into separate tables. Each table represented one type of sample and contained the relative abundance of different bacterial families identified in the samples. Each row in the tables corresponded to a bacterial family, and each column corresponded to a unique sample. Missing data were replaced with zero to indicate a non-detect for that bacterial family in the corresponding sample.

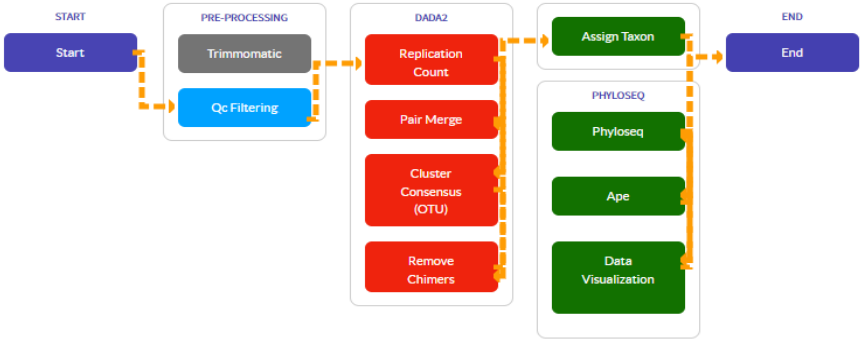

Figure 1. Pipeline graph of T-BioInfo server

All statistical analyses were conducted to assess differences in microbial diversity and composition across body sites (gut, oropharynx, bronchoalveolar lavage fluid, and blood) and between genders. Alpha diversity metrics, such as the Shannon index, were calculated to evaluate microbial diversity within each sample. Beta diversity, reflecting compositional differences between samples, was assessed using Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity.

Principal Component Analysis (PCA):

Principal Component Analysis (PCA) is performed on each datasets to reduce the conditionality and to visualize the variation in bacterial composition among samples. The PCA is carried out using the PCA function from the sklearn.decomposition module in Python. The input to the PCA is the transposed data matrix, with samples as rows and bacterial families as columns. The PCA results are then plotted as scatter plots with the first principal component (PC1) on the x-axis and the second principal component (PC2) on the y-axis. Each point on the scatter plot represented a sample, and its position was determined by its coordinates along PC1 and PC2. The scatter plots are further generated using the matplotlib.pyplot module in Python.

Principal Coordinates Analysis (PCoA):

Principal Coordinates Analysis (PCoA) is executed on the dataset to visualize the dissimilarity in microbial composition among different samples. The PCoA is implemented using the PCoA function from the skbio.stats.ordination module in Python, with the Bray-Curtis dissimilarity matrix as the input. The dissimilarity matrix is calculated from the original data matrix, with samples as rows and bacterial families as columns, using the braycurtis function from the scipy.spatial.distance module. The PCoA results are then plotted as scatter plots, with the first principal coordinate (PCo1) on the x-axis and the second principal coordinate (PCo2) on the y-axis. Each point on the scatter plot represents a sample, and its position is determined by its coordinates along PCo1 and PCo2. The scatter plots are produced using the matplotlib.pyplot module in Python.

Heatmap Generation:

Heatmaps are generated to visualize the relative abundance of bacterial families in each sample. The heatmaps are created using the heatmap function from the seaborn library in Python. The input to the heatmap function is the original data matrix, with bacterial families as rows and samples as columns. The color in the heatmap represented the relative abundance of a bacterial family in a sample, with darker colors indicating higher values.

Results

OTU Classification:

The fundamental step that was executed in this research was to identify which bacterial families the aforementioned OTUs belong to. The bar plot shown in the Figure 2 and Figure 3 displays the increase and decrease of abundances of different families of bacteria present in samples including fecal, oropharyngeal swabs, bronchoalveolar lavage fluid (BALF) and blood.

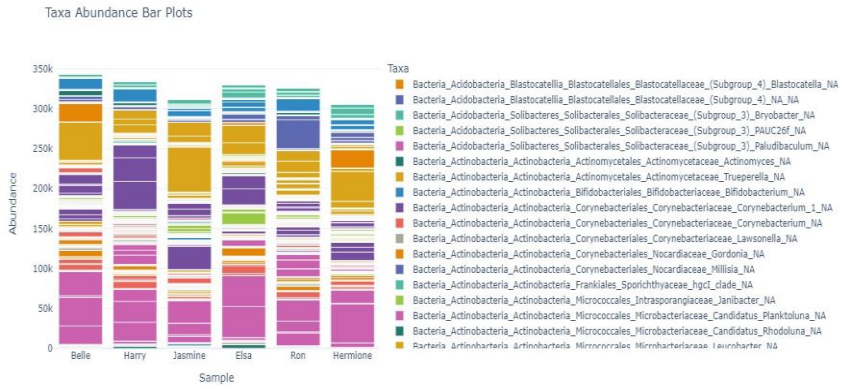

Figure 2. Taxa abundance bar plot generated from T-BioInfo server

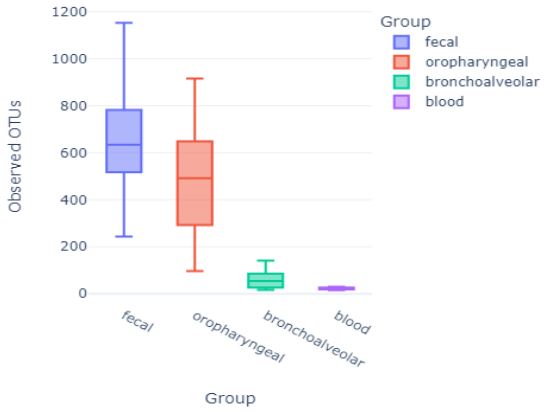

Figure 3. Observed OTUs generated from T-BioInfo server

Microbial Diversity Analysis of the Feline Gut Microbiota:

The present analysis shed lights to the fact that the gut microbiome of cats is composed of a diverse community of microorganisms that play an important role in various physiological processes such as digestion, immune system regulation, and energy metabolism (Nealon *et al.*, 2023). The study found that relative abundant bacterial families found in fecal samples include *Bacteroidaceae*, *Fusobacteriaceae*, *Lachnospiraceae*, *Tissierellaceae*, *Campylobacteraceae*, *Enterobacteriaceae*, *Streptococcaceae*. The remaining is further listed in the Table.1 These bacterial families are involved in the fermentation of dietary fibers, production of short-chain fatty acids, and regulation of the immune system (Nealon *et al.*, 2023).

The study also found that the relative abundance of these bacterial families can be influenced by various factors such as age, and gender of the cat. The analysis further shows that the microbiota in the gut of cats can differ significantly depending on the individual cat. This can be further seen from the bacterial communities in the fecal samples of cats, Belle and Hermione. The results indicate that Belle's microbiota exhibited variations in *Bacteroidaceae*, *Campylobacteraceae*, and *Staphylococcaceae*, while Hermione's microbiota showed variations in *Fusobacteriaceae*, *Lachnospiraceae*, *Succinivibrionaceae*, *Pseudomonadaceae*, and *Porphyromonadaceae*.

The *Bacteroidaceae* family was more abundant in female fecal samples compared to male fecal samples. This family is involved in the breakdown of complex carbohydrates, and the higher abundance in female samples may be related to differences in dietary habits between males and females (Nealon *et al.*, 2023). The *Campylobacteraceae* family was more abundant in female fecal samples in some instances, but more abundant in male fecal samples in others. This family contains pathogenic bacteria that are known to cause gastrointestinal infections (Nealon *et al.*, 2023). The *Succinivibrionaceae* family is also more abundant in female fecal samples compared to male fecal samples. This family plays a role in the fermentation of dietary fiber and is associated with gut health (Ma *et al.*, 2022). The *Lachnospiraceae* family was more abundant in female fecal samples in some instances, but more abundant in male fecal samples in others. This family is involved in the production of short-chain fatty acids, which can have important implications for gut health. The *Fusobacteriaceae* family was more abundant in male fecal samples compared to female fecal samples. This family is associated with various diseases, including colorectal cancer (Taddese *et al.*, 2020). Overall, these variations suggest that there may be differences in the microbial communities in the gut of males and females cats, which may be related to differences in dietary

habits, hormonal differences, or other factors. The microbial diversity analysis of the feline Gut microbiota is visually represented using Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), heatmaps, and pie charts in Figures 4, 8, 12, and 16, respectively.

Microbial Diversity Analysis of the Feline oropharyngeal Microbiota:

Our analysis has revealed that the oropharyngeal microbiota of cats is predominantly composed of *Pasteurellaceae*, followed by other families such as *Moraxellaceae*, *Porphyromonadaceae*, *Pseudomonadaceae* and several others. The remaining is further listed in the Table 1. The analysis further shows that just like the microbiota in the gut the oropharyngeal of cats varies significantly depending on the individual cat. Ron showed variations in *Pasteurellaceae* and *Pseudomonadaceae*. *Pseudomonadaceae* variations were also found in Harry's sample, while *Alcaligenaceae* variation was present in Belle's sample. Elsa showed variations in *Staphylococcaceae*. These results indicate that the microbiota in the upper respiratory tract of cats can also vary between cats.

Table 1. Table showing microbial distribution and its function

Sl.No	Sample	Microbe	Role
1	Fecal	Bacteroidaceae	aiding in the digestion of complex carbohydrates and the production of short-chain fatty acids (SCFAs) such as butyrate, regulating the immune system and preventing inflammation in the gut, produce anti-inflammatory molecules that help to suppress harmful immune responses and promote the growth of beneficial bacteria in the gut
2	Fecal	*Fusobacteriaceae*	associated with inflammatory bowel disease (IBD) and colorectal cancer, promote inflammation in the gut, leading to damage to the intestinal lining and an increased risk of cancer
3	Fecal	*Lachnospiraceae*	produce short-chain fatty acids (SCFAs) through fermentation of dietary fibers, plays an important role in gut health by performing a variety of functions, including the production of butyrate, which serves as a primary energy source for colonocytes, the cells that line the colon, help to maintain gut barrier function and prevent the colonization of harmful bacteria by producing antimicrobial peptides and competing for nutrients with pathogenic bacteria, regulate the immune system, reducing inflammation and preventing the development of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis
4	Oropharyngeal	*Pasteurellaceae*	play a role in maintaining the balance of the microbial community, involved in the breakdown of complex carbohydrates and the production of short-chain fatty acidswhich are essential for the health of the intestinal epithelium, and play a role in the development of the immune system by regulating the expression of immune-related genes
5	Oropharyngeal	*Moraxellaceae*	play a role in maintaining the balance of the microbial communities in the oropharyngeal region
6	Oropharyngeal	*Porphyromonadaceae*	involved in the degradation of complex polysaccharides, including those found in the oral biofilm, modulate the host immune response by inducing the expression of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-8 (IL-8), and by promoting the differentiation of regulatory T cells
7	Bronchovascular fluid	*Pseudomonadaceae*	associated with a variety of infections, including lung infections in people with cystic fibrosis and other underlying lung conditions, and play a role in respiratory infections or inflammatory processes
8	Bronchovascular fluid	*Sphingobacteriaceae*	associated with respiratory infections, including pneumonia and bronchitis
9	Bronchovascular fluid	*Bradyrhizobiaceae*	play a role in the nitrogen cycle, specifically in nitrogen fixation
10	Blood	*Sphingobacteriaceae*	cause infections in individuals with weakened immune systems, which can lead to conditions such as sepsis, endocarditis, and bacteremia
11	Blood	*Bradyrhizobiaceae*	excat function is not known, can play a role in nitrogen fixation
12	Blood	*Bacteroidaceae*	In blood, the presence of Bacteroidaceae could be an indication of gut dysbiosis or bacterial translocation from the gut to the bloodstream. Bacterial translocation occurs when bacteria or bacterial products cross the intestinal mucosal barrier and enter the bloodstream, which can trigger an inflammatory response and potentially lead to systemic infections

The abundance of *Pasteurellaceae* in the oropharynx is higher in females than males, with an average relative abundance of 18.17% in female samples compared to 20.22% in male samples. The relative abundance of *Moraxellaceae* is higher in female oropharyngeal samples than in males, with an average of 16.7% compared to 14.5% in male samples. The abundance of *Pseudomonadaceae* is higher in male oropharyngeal samples than in females, with an average relative abundance of 30.3% compared to 16.2% in females. The relative abundance of *Neisseriaceae* in the oropharynx is higher in females than males, with an average relative abundance of 10.2% compared to 6.5% in males. The microbial diversity analysis of the feline oropharyngeal microbiota is visually represented using Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), heatmaps, and pie charts in Figures 5, 9, 13, and 17, respectively.

Microbial Diversity Analysis of the Feline bronchoalveolar lavage fluid (BALF) Microbiota:

Our analysis has revealed that in the bronchoalveolar lavage fluid, the most abundant family was *Pseudomonadaceae*, followed by other families such as *Sphingobacteriaceae*, *Bradyrhizobiaceae*, *Moraxellaceae*, and others. These bacteria are well adapted to the low-oxygen, high-moisture environment of the lungs and can be pathogenic if they colonize and grow unchecked. Notably, we observed variations in the relative abundance of specific bacterial families among individual cats. For instance, Hermione's sample showed variations in the relative abundance of *Pseudomonadaceae*, while Ron exhibited variations in *Sphingobacteriaceae* and *Bradyrhizobiaceae*. Belle's sample, on the other hand, showed variations in *Alcaligenaceae*.

Interestingly, the bronchoalveolar fluid samples showed some gender-based differences in the relative abundance of certain bacterial families. Specifically, we observed a higher abundance of *Pseudomonadaceae* in both male and female bronchoalveolar fluid samples. However, *Sphingobacteriaceae* was more abundant in female bronchoalveolar fluid samples, while *Bradyrhizobiaceae* was more abundant in male bronchoalveolar fluid samples. The microbial diversity analysis of the feline bronchoalveolar lavage fluid (BALF) microbiota is visually represented using Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), heatmaps, and pie charts in Figures 6, 10, 14, and 18, respectively.

Microbial Diversity Analysis of the Feline blood Microbiota:

Our analysis has revealed that in the blood samples, *Sphingobacteriaceae*, *Bradyrhizobiaceae*, and *Bacteroidaceae* were the most abundant families, followed by other families such as *Bacillaceae*, *Methylobacteriaceae*, S24-7, *Rikenellaceae*, and others. For instance, Hermione exhibited irregularities in the relative abundance of *Bradyrhizobiaceae* compared to the blood samples from other cats.

Furthermore, we found significant differences in the microbial communities between female and male blood samples. The *Sphingobacteriaceae* family was more prevalent in female blood samples, with an average percentage of 65.4%, compared to 60.2% in male blood samples. Conversely, the *Bradyrhizobiaceae* family showed a higher abundance in male blood samples, with an average percentage of 27.4%, compared to 21.7% in female blood samples. Interestingly, the *Bacteroidaceae* family was only found in female blood samples, with an average percentage of 3.5%, while the *Methylobacteriaceae* family was more prevalent in male blood samples, with an average percentage of 2.2%. Additionally, the S24-7 family was only detected in male blood samples, with an average percentage of 1.6%. The microbial diversity analysis of the feline blood microbiota is visually represented using Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), heatmaps, and pie charts in Figures 7, 11, 15, and 19, respectively.

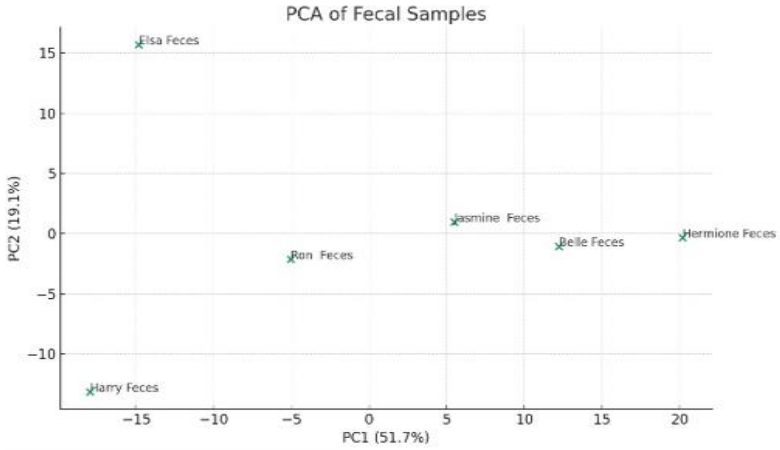

Figure 4. Principal Component Analysis (PCA)F of fecal samples

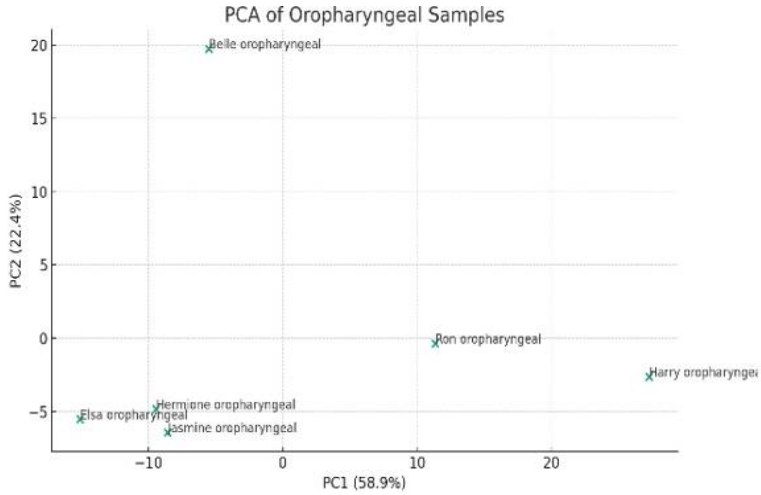

Figure 5. Principal Component Analysis (PCA) of oropharyngeal samples

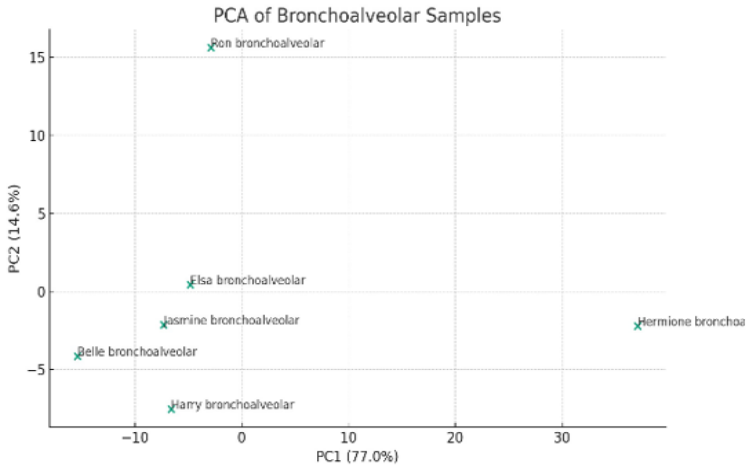

Figure 6. Principal Component Analysis (PCA) of bronchoalveolar samples

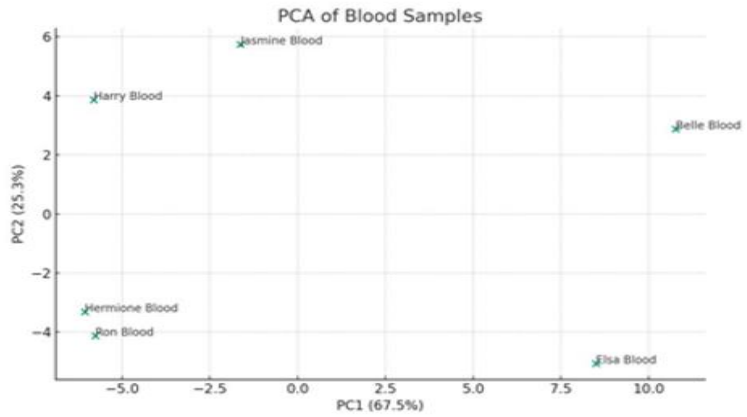

Figure 7. Principal Component Analysis (PCA) of blood samples

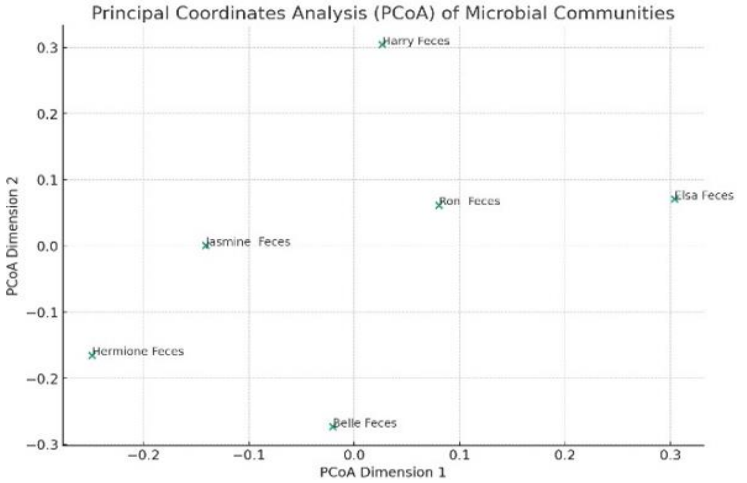

Figure 8. Principal Coordinate Analysis (PCoA) of fecal samples

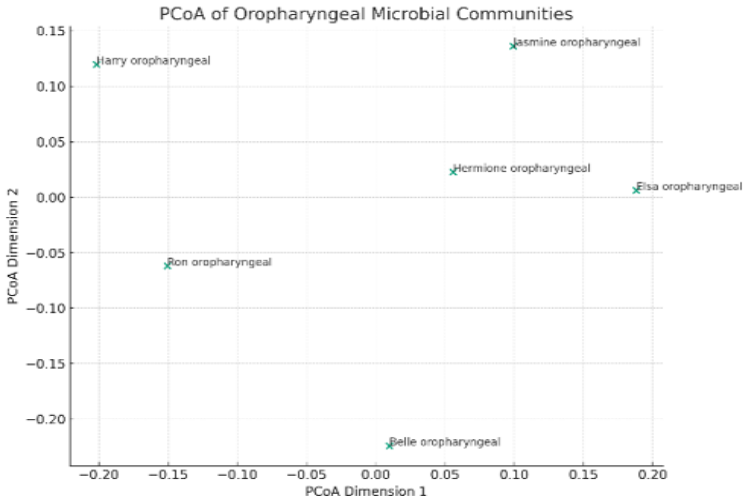

Figure 9. Principal Coordinate Analysis (PCoA) of oropharyngeal samples

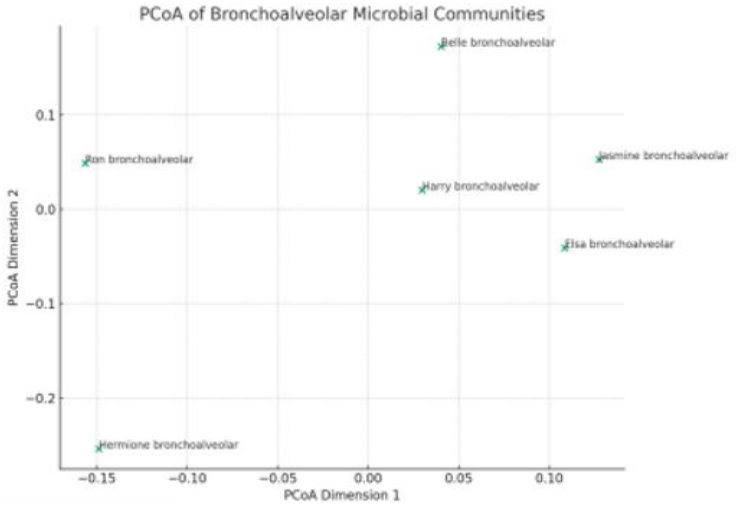

Figure 10. Principal Coordinate Analysis (PCoA) of bronchoalveolar samples

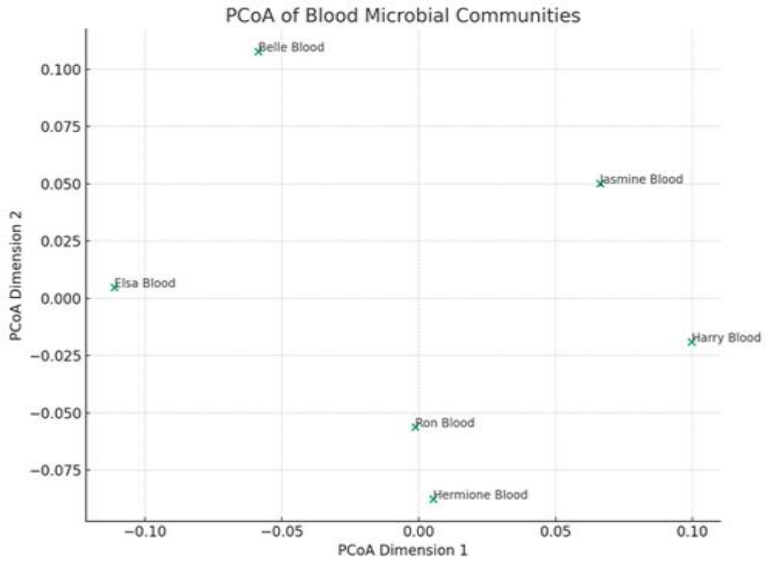

Figure 11. Principal Coordinate Analysis (PCoA) of blood samples

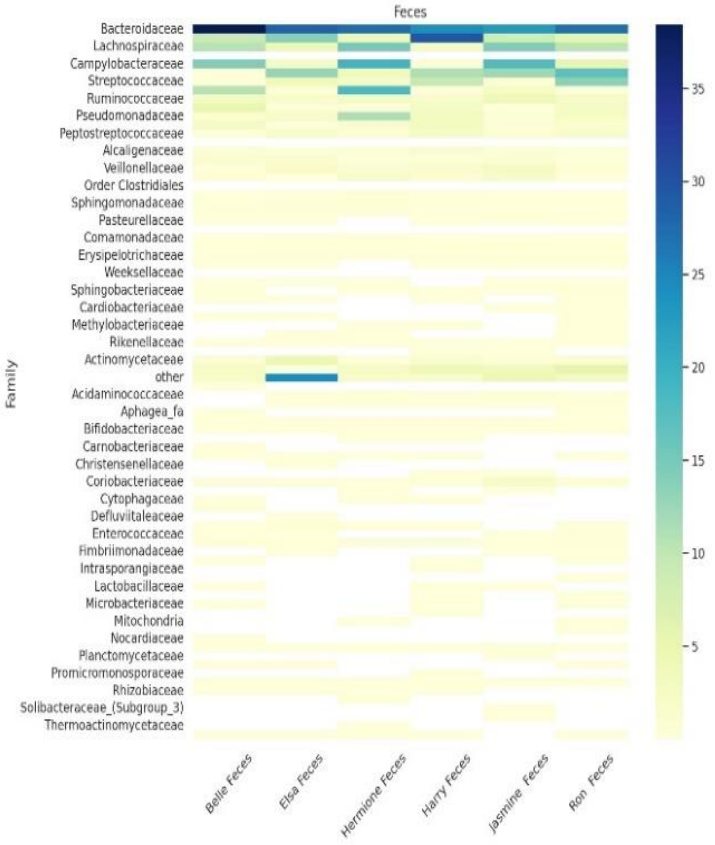

Figure 12. Heatmap showing microbial distribution in fecal samples

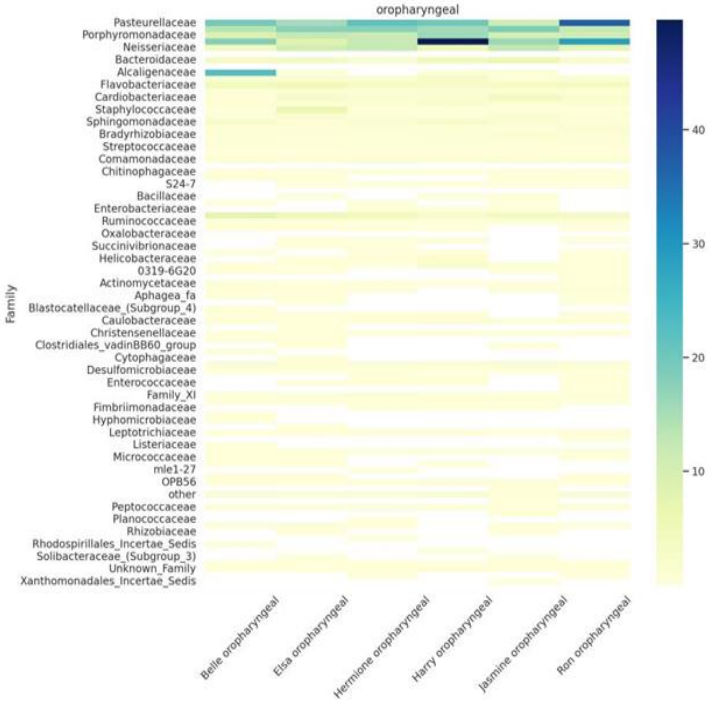

Figure 13. Heatmap showing microbial distribution in oropharyngeal samples

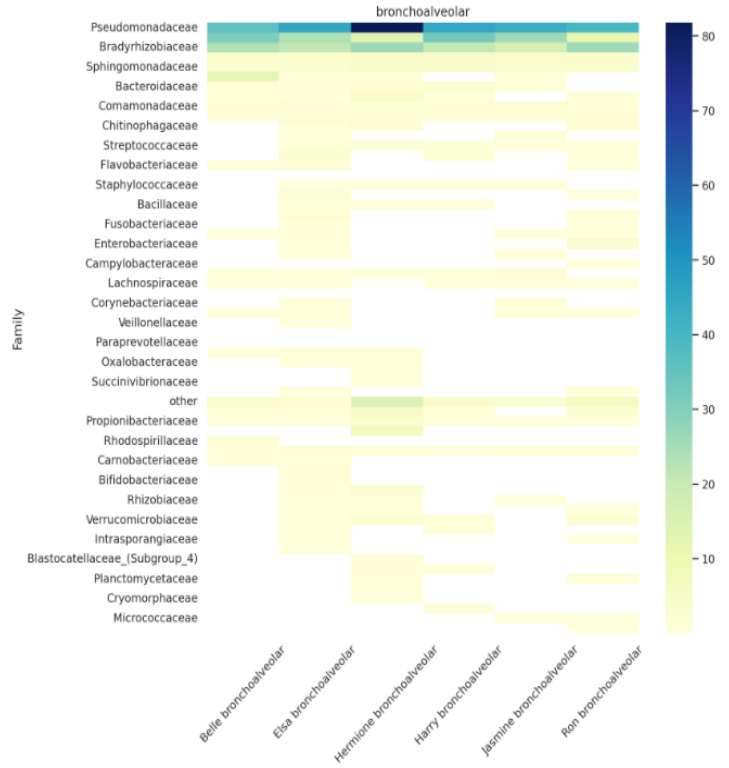

Figure 14. Heatmap showing microbial distribution in bronchoalveolar samples

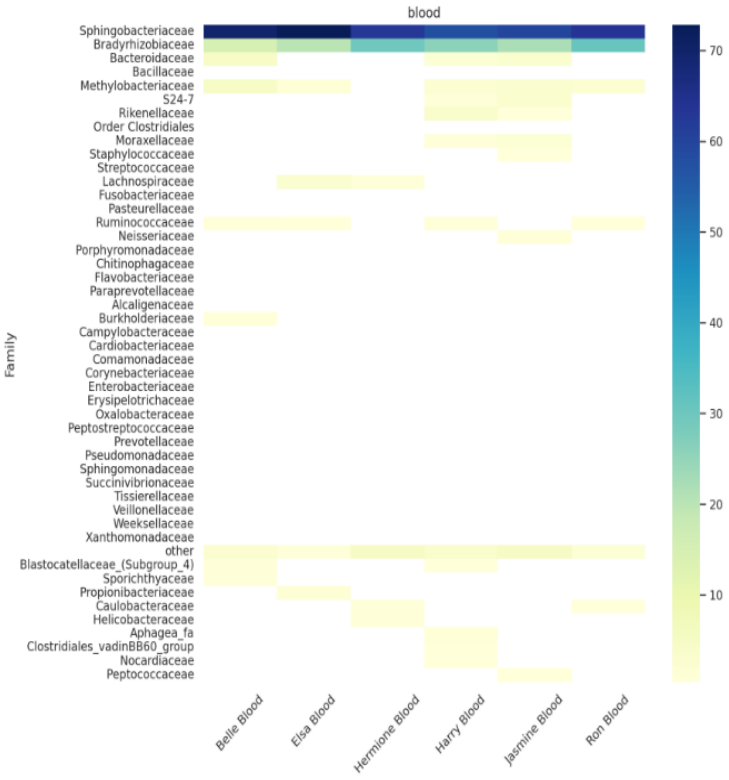

Figure 15. Heatmap showing microbial distribution in blood samples

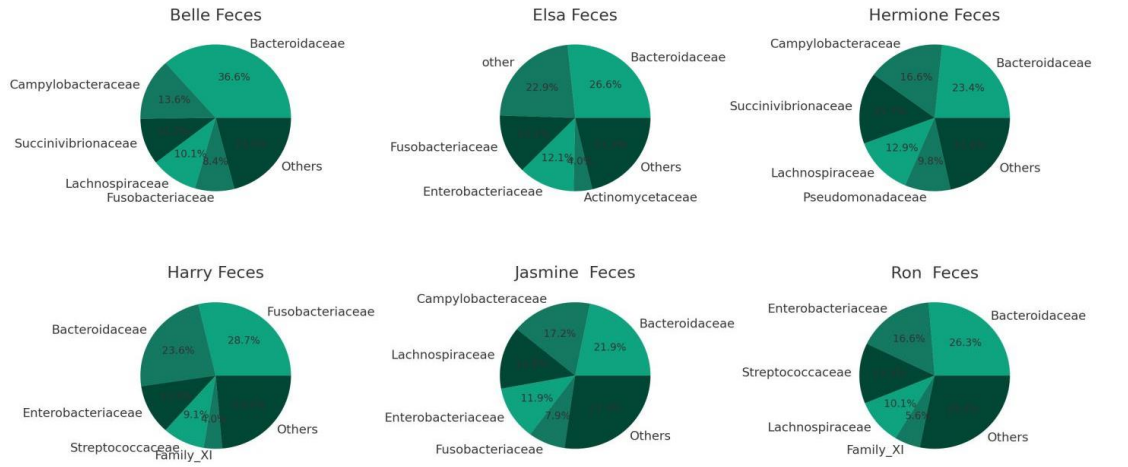

Figure 16. Pie charts showing microbial diversity in the fecal samples

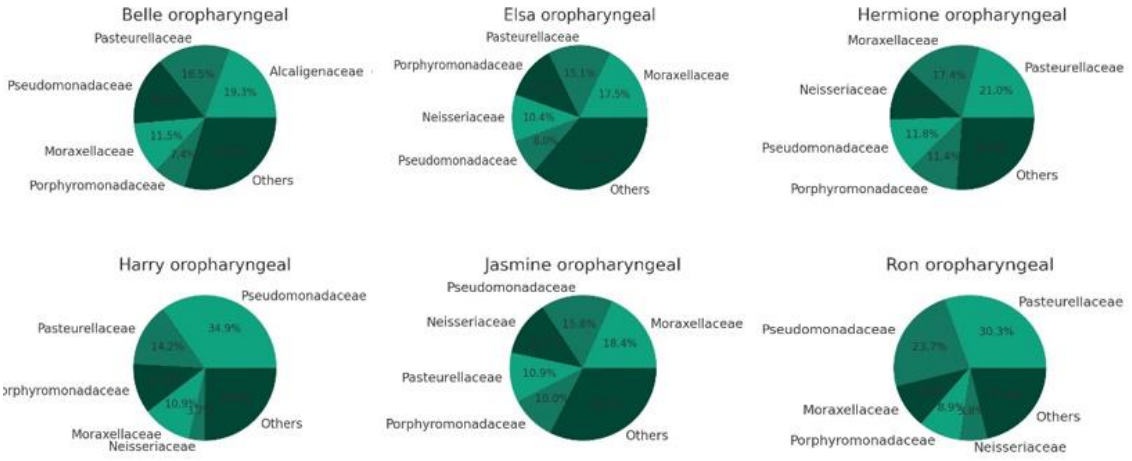

Figure 17. Pie charts showing microbial diversity in the oropharyngeal samples

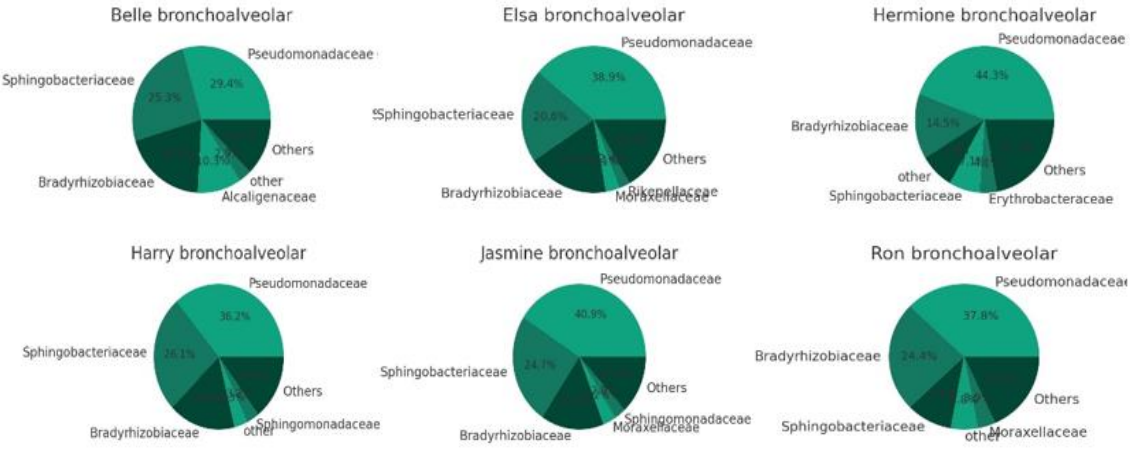

Figure 18. Pie charts showing microbial diversity in the bronchoalveolar lavage fluid (BALF) samples

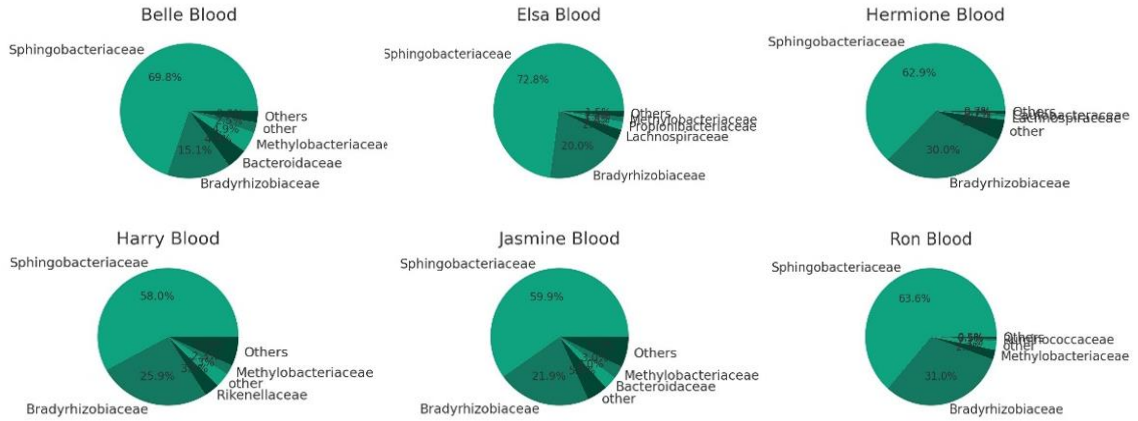

Figure 19. Pie charts showing microbial diversity in the blood samples

Discussion

This study builds upon the foundational dataset initially published in *PLOS ONE* under the title 'Dynamic changes of the respiratory microbiota and its relationship to fecal and blood microbiota in healthy young cats' (accession code PRJNA350163) (Vientós-Plotts *et al.*, 2017). The original study provided valuable insights into microbial compositions across multiple body sites in healthy young cats, focusing on temporal changes and general site-specific microbiota distributions (Vientós-Plotts *et al.*, 2017). However, the scope of the initial study was primarily descriptive, centered on broad compositional shifts without delving into detailed statistical or demographic analyses, particularly regarding individual and gender-specific variations.

In our present study, we reanalyzed this dataset with a focus on computational and statistical assessments of microbial diversity, paying particular attention to gender and individual differences across body sites, including fecal, oropharyngeal, bronchoalveolar lavage fluid (BALF), and blood samples. This approach allowed us to identify distinct gender-specific patterns in microbial composition at each site, highlighting significant differences in bacterial family abundances. For instance, *Bacteroidaceae* and *Succinivibrionaceae* were more prevalent in female fecal samples, which may be linked to metabolic or hormonal factors influencing these bacterial populationally, *Fusobacteriaceae* was found to be more abundant in male fecal samples, suggesting potential gender-driven variations in gut health and microbiome interactions (Santos-Marcos *et al.*, 2023; Zhang *et al*., 2023),

Our study also reveals substantial individual variability in microbial composition across different body sites, indicating environmental factors may shape unique microbiota profiles among individual cats (Zhou *et al*., 2024). For example, bacterial families varied distinctly between individual cats; *Bacteroidaceae*, *Campylobacteraceae*, and *Staphylococcaceae* were more prominent in one cat, while *Fusobacteriaceae*, *Lachnospiraceae*, and *Succinivibrionaceae* were more abundant in another.

In addition to the findings in fecal and respiratory samples, our study also provides new insights into blood microbiota, an area minimally addressed in the original publication. We observed significant gender-based differences in blood microbiota composition, with *Sphingobacteriaceae* being more abundant in females and *Bradyrhizobiaceae* more prevalent in males. Certain bacterial families, such as *Bacteroidaceae*, were exclusive to female blood samples, while *S24-7* and *Methylobacteriaceae* were unique to males. These gender-specific patterns in blood microbiota suggest that systemic physiological factors may play a role in shaping microbial presence beyond localized sites (Valeri and Endres, 2021).

The oropharyngeal microbiota, which was also a focus of our study, displayed significant gender-based and individual-specific trends. *Pasteurellaceae* was identified as the most abundant family, with variations in other bacterial families such as *Pseudomonadaceae* and *Alcaligenaceae* seen across different individual cats. Additionally, *Pasteurellaceae* was more prevalent in males, while *Moraxellaceae* and

Neisseriaceae were higher in females. These results reinforce the concept that respiratory microbiota, much like the gut, is subject to both individual and gender-specific influences, likely impacted by factors such as hormonal levels, environmental exposure, and immune responses (Karl *et al.*, 2018).

Lastly, in BALF samples, we noted a high prevalence of *Pseudomonadaceae* across both genders, along with significant gender-based variations in *Sphingobacteriaceae* (more abundant in females) and *Bradyrhizobiaceae* (more abundant in males). These findings suggest that respiratory microbiota composition can vary by gender, likely influenced by underlying immune or hormonal factors (Caputi *et al.*, 2022).

In summary, our study not only reaffirms the findings of the PLOS ONE article but also extends the dataset's utility through a more detailed analysis of gender- and individual-driven variability in feline microbiota using the T-BioInfo server (https://server.t-bio.info/). This computational platform enabled us to perform advanced analyses across multiple body sites, uncovering nuanced microbial patterns that highlight the importance of host-specific factors, such as gender, genetic background, and individual variability, in shaping the feline microbiome. These insights into unique microbial profiles across various anatomical sites in cats have valuable implications for developing personalized approaches in veterinary medicine and contribute to comparative biomedical research involving human and animal microbiota.

Conclusions

In conclusion, this study advances the understanding of the feline microbiome by providing a comprehensive analysis of microbial diversity across multiple body sites, revealing significant gender- and individual-specific variations. Utilizing the foundational dataset from *NCBI* and reanalyzing it with the T-BioInfo server enabled a nuanced exploration of bacterial communities in fecal, respiratory, and blood samples, emphasizing the dynamic nature of the feline microbiota. Findings such as the prevalence of *Bacteroidaceae* and *Succinivibrionaceae* in female gut samples and *Fusobacteriaceae* in male samples, as well as gender-specific patterns in blood and respiratory microbiota, underscore the importance of host-specific factors like gender and individual variability in shaping microbiome composition. A limitation of this study is its reliance on publicly available data from NCBI without direct experimental control, which restricts our ability to evaluate certain variables such as diet, environment, and age diversity. Nonetheless, this computational approach offers substantial benefits by providing a scalable method for in-depth microbial analysis and setting a foundation for personalized veterinary care. These insights contribute to the broader field of comparative biomedical research and highlight the potential for further investigation into microbiome-related health management in cats.

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References

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7):581-583. https://doi.org/10.1038/nmeth.3869

Caputi, V., Bastiaanssen, T.F., Peterson, V., Sajjad, J., Murphy, A., Stanton, C., and O'Mahony, S.M. (2022). Sex, pain, and the microbiome: The relationship between baseline gut microbiota composition, gender and somatic pain in healthy individuals. Brain, Behavior, and Immunity, 104:191-204. https://doi.org/10.1016/j.bbi.2022.02.006

Huang, W., Fu, K., Gao, Q., Tejo, R.P., Floeter-Winter, L.M., and Gutierrez, A.P. (Eds.). (2022). Host-microbe interaction and coevolution. Elsevier. https://doi.org/10.3389/978-2-88976-649-9

Karl, J.P., Hatch, A.M., Arcidiacono, S.M., Pearce, S.C., Pantoja-Feliciano, I.G., and Soares, J.W. (2018). Effects of psychological, environmental and physical stressors on the gut microbiota. Frontiers in Microbiology, 9:372026. https://doi.org/10.3389/fmicb.2018.00838

Kerr, K.R., Dowd, S.E., and Swanson, K.S. (2014). Faecal microbiota of domestic cats fed raw whole chicks v. an extruded chicken-based diet. Journal of Nutritional Science, 3:e22. https://doi.org/10.1017/jns.2014.22

Kim, Y., Carrai, M., Leung, M.H., Chin, J., Li, J., Lee, P. K., and Barrs, V.R. (2021). Dysbiosis of the urinary bladder microbiome in cats with chronic kidney disease. mSystems, 6(4):e00510-21. https://doi.org/10.1128/msystems.00510-21

Krumbeck, J.A., Reiter, A.M., Pohl, J.C., Tang, S., Kim, Y.J., Linde, A., and Melgarejo, T. (2021). Characterization of oral microbiota in cats: Novel insights on the potential role of fungi in feline chronic gingivostomatitis. Pathogens, 10(7):904. https://doi.org/10.3390/pathogens10070904

Ma, X., Brinker, E., Cao, W., Graff, E.C., and Wang, X. (2022). Effect of mineral oil as a lubricant to collect feces from cats for microbiome studies. Journal of Veterinary Internal Medicine, 36(5):1,728-1,736. https://doi.org/10.1111/jvim.16550

Moon, C.D., Young, W., Maclean, P.H., Cookson, A.L., and Bermingham, E.N. (2018). Metagenomic insights into the roles of Proteobacteria in the gastrointestinal microbiomes of healthy dogs and cats. MicrobiologyOpen, 7(5):e00677. https://doi.org/10.1002/mbo3.677

Nealon, N.J., Wood, A., Rudinsky, A.J., Klein, H., Salerno, M., Parker, V.J., and Winston, J.A. (2023). Fecal identification markers impact the feline fecal microbiota. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1039931

Rodrigues, M.X., Bicalho, R.C., Fiani, N., Lima, S.F., and Peralta, S. (2019). The subgingival microbial community of feline periodontitis and gingivostomatitis: Characterization and comparison between diseased and healthy cats. Scientific Reports, 9(1):1-10. https://doi.org/10.1038/s41598-019-48852-4

Santos-Marcos, J.A., Mora-Ortiz, M., Tena-Sempere, M., Lopez-Miranda, J., and Camargo, A. (2023). Interaction between gut microbiota and sex hormones and their relation to sexual dimorphism in metabolic diseases. Biology of Sex Differences, 14(1):4. https://doi.org/10.1186/s13293-023-00490-2

Suchodolski, J.S. (2011). Intestinal microbiota of dogs and cats: A bigger world than we thought. Veterinary Clinics of North America: Small Animal Practice, 41(2):261-272. https://doi.org/10.1016/j.cvsm.2010.12.006

Taddese, R., Garza, D.R., Ruiter, L.N., de Jonge, M.I., Belzer, C., Aalvink, S., and Boleij, A. (2020). Growth rate alterations of human colorectal cancer cells by 157 gut bacteria. Gut Microbes, 12(1):1799733. https://doi.org/10.1080/19490976.2020.1799733

Tay, D.D., Siew, S.W., Kamal, S.S., Razali, M.N., and Ahmad, H.F. (2022). Full-length ITS1 genes amplicon analysis of gut mycobiome using Nanopore Flongle sequencing reveals unique fungal communities between indoor and outdoor felines in Malaysia. Journal of Fungi. (In press) https://doi.org/10.21203/rs.3.rs-1456898/v1

Tun, H.M., Brar, M.S., Khin, N., Jun, L., Hui, R.K.H., Dowd, S.E., and Leung, F.C.C. (2012). Gene-centric metagenomics analysis of feline intestinal microbiome using 454 junior pyrosequencing. Journal of Microbiological Methods, 88(3):369-376. https://doi.org/10.1016/j.mimet.2011.12.014

Valeri, F. and Endres, K. (2021). How biological sex of the host shapes its gut microbiota. Frontiers in Neuroendocrinology, 61:100912. https://doi.org/10.1016/j.yfrne.2021.100912

Vientos-Plotts, A.I. (2022). The respiratory microbiota of dogs and cats in health and disease: Expanding our breadth of knowledge. (Doctoral dissertation). University of Missouri-Columbia.

Vientos-Plotts, A.I., Ericsson, A.C., Rindt, H., Grobman, M.E., Graham, A., Bishop, K., and Reinero, C.R. (2017). Dynamic changes of the respiratory microbiota and its relationship to fecal and blood microbiota in healthy young cats. PLOS ONE, 12(3):e0173818. https://doi.org/10.1371/journal.pone.0173818

Zhang, K., Liu, G., Wu, Y., Zhang, T., Guo, M., Lei, Y., and Chen, Y. (2023). Gut microbial succession patterns and metabolic profiling during pregnancy and lactation in a goat model. Microbiology Spectrum, 11(1):e02955-22. https://doi.org/10.1128/spectrum.02955-22

Zhou, X., Shen, X., Johnson, J.S., Spakowicz, D.J., Agnello, M., Zhou, W., and Snyder, M.P. (2024). Longitudinal profiling of the microbiome at four body sites reveals core stability and individualized dynamics during health and disease. Cell Host and Microbe, 32(4):506-526. https://doi.org/10.1016/j.chom.2023.09.012