Analysis on the Effects of Dog Ownership on the Gut Microbiota of their Owners

by

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Submitted to the Department of Biochemistry

School of Natural and Social Sciences

in partial fulfillment of the requirements

for the degree of Bachelor of Arts

Purchase College

State University of New York

May 2022

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Abstract

Pet ownership is an environmental exposure that may influence human health. This study aimed to explore the effects of dog ownership on gut microbial diversity utilizing data from the American Gut Project. A total of 2,239 samples were analyzed, 672 that owned a dog compared to 1567 that did not. Diversity metrics produced data plots that indicated no major differences in gut microbiota diversity or richness between both study groups. Taxonomic compositions portrayed the main phyla and families, besides a decrease in gram-negative bacteria, *Pseudomonadaceae,* in the dog group. This project demonstrated a possible decrease in significance of dog ownership on the owners' microbiome.

Introduction

The microbiome is the complex genetic material of microorganisms that live in and on the human body. Trillions of bacteria, fungi and viruses mainly reside in the gut, but can also be found in and on the skin, nose, and mouth. Healthy bacteria are essential to the body because they aid in human development, the immune system, and a healthy metabolism (Genome). Vitamins, amino acids, bile acids, and short-chain fatty acids (SCFAs) are some of the numerous metabolites produced and absorbed by commensal bacteria. For this reason, many researchers like to consider the microbiome as an organ. Microbiota taxonomic compositions are unique to the individual, but an abundance of studies and large projects suggest there is a general balance between symbiotic and pathogenic bacteria in healthy people. The most abundant phyla are Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, and Actinobacteria. Some of the most common genera belong to Firmicutes such as *Lactobacillus, Bacillus,* and *Clostridium*. *Bacteroidetes* and *Bifidobacterium* are also genera that make up the Bacteroidetes and Actinobacteria phyla in the gut, respectively (Nutrition Source).

Variations of the gut microbiota between individuals can be affected by several outside factors such as diet, disease, antibiotics, and more. A decline in bacterial diversity is naturally observed as one

ages. A high-fat, high-sugar diet is associated with negative changes in the gut and development of inflammatory bowel disease (IBD). Antibiotic usage is generally associated with a decrease in *Lactobacillus* and *Clostridium*, and an increase in Proteobacteria (Rinninella, Emanuele et al.). These are all examples of dysbiosis, an imbalance in microbiota homeostasis that eventually alters microbial function. With that, more environmental exposures should be considered and studied more frequently, such as pet ownership. According to the Insurance Information Institute, seventy percent of U.S. households have pets, a number that has certainly increased due to the pandemic (Facts + Statistics). Previous research suggests that-, pet owners have similar microbiomes to their pets and overall increased diversity (Researching Germany). One study compared the gut microbiotas of cat and non-cat owners, by using raw data from the American Gut Project (AGP). The microbial compositions of 111 female cat owners and 111 female non-cat owners were analyzed using bioinformatics software, QIIME2 specifically. PICRUSt2 predicted more than 50 metabolic pathways were changed in the cat group, such as an increase in metabolism of amino acids and nucleotides. The study reported alterations in the *Alcaligenaceae*, *Pseudomonadaceae* and *Enterobacteriaceae* families and concluded cats could have a significant influence on the gut microbiotas of females (Du G).

This study will use raw data from the American Gut Project to perform statistical analysis on the effects of dog ownership on the gut microbiota of their owners. The AGP is a large study that collects microbiome samples from volunteers worldwide for education and research (McDonald D, et al.). The consistent methods used makes it unique and important for individual studies on the microbiome. It is ideal for more studies on the different types of pets owned to provide a general understanding of their impact on the human body. The data is found on QIITA, an open-source microbial database (Antonio Gonzalez, et al.). The analyses were run on QIIME2 which measures species diversity and richness (Bolyen E, et al.). Taxa bar plots were also generated for dog owners and nondog owners to examine any differences at each taxonomic level. The influence of pet ownership was determined by comparing gut microbiota composition of dog owners and nondog owners.

Materials and Methods

To begin the study, 16S raw sequencing data from 30,612 samples of the American Gut Project were selected and downloaded via an open-source, microbial study database, QIITA. 16S refers to the subunit of the ribosome where sequence information is obtained, because it is found in all prokaryotic cells. QIITA platform allows for reanalysis of multiple datasets by transferring raw data to a bioinformatics software plugin that performs statistical analyses on microbial communities, QIIME 2. Once the samples were transferred, they were rarefied to a sampling depth of 8000 to fix samples with very long reads that may skew diversity results. Sampling depth is the amount of sequenced bases for a sample.

The metadata was then filtered by age, sex, location, BMI, and pet ownership. Only fecal samples were kept and anyone under the age of 20 was removed. A large percentage of the samples were from the United States and the United Kingdom, specifically California and England. Therefore, samples from other countries were filtered out. There was also a lack of racial diversity as well as most of the samples were predominantly Caucasian. The nondog study group was filtered so that the samples included did not have any pets at all and the dog study group only owned dogs. Any samples that did not provide information for all the categories mentioned previously were removed. In the early stages of filtering, each command ran for at least 5 hours due to the large number initially selected. There was also a lot of trial and error executing each command due to improper syntax for each filtering step. The final number of samples came to 2,602. The data was filtered strategically to ensure the two study groups were represented correctly.

After filtering, alpha and beta diversity metrics were measured in QIIME 2. Alpha diversity is a small-scale measure that describes the species evenness and richness in an ecosystem. On the other

hand, beta diversity is a large-scale measure that compares the diversity between two ecosystems (CosmosID). The alpha diversity box plots were individually calculated, Shannon's Index and Chao 1 Index. For beta diversity, a heat map showing the distance between pairs of samples was generated, and then from there a Principle of Coordinate Analysis plot was created. The plot provides a three-dimensional visual of the similarities between samples.

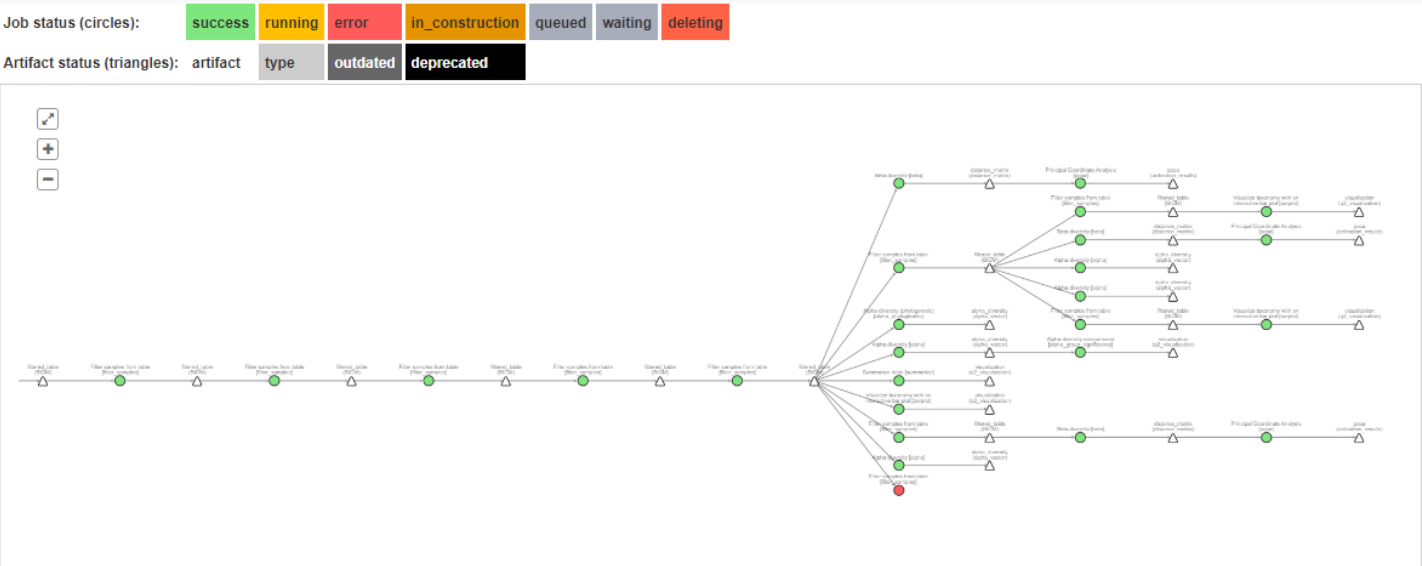
Taxonomic bar blots for each study group were also formed at each taxonomic level, but there were obstacles along the way. The filtering process began with 30,612 deblurred samples but taxonomic bar plots can only be generated using OTU (operational taxonomic units) samples. So initially when executing the command, an error message was produced. After searching several online forums, the decision to go back and restart the filtering process, but with 30,612 OTU samples was made. Once that mistake was corrected, another error was found in the taxa bar plots. There were duplicated samples spotted throughout the graph. After close examination of the csv file, several artifact ids were repeating samples. The artifact ids that repeated the samples were removed (363 total) and the final number of samples was instead 2,239. All diversity box plots, bar graphs, and heat maps were re-executed to reflect the revised number of samples.

Participant Demographics

Study Groups:	**Dog**	**No Dog**
Total Number: 2239	672	1567
Sex:		
Female	395 (59%)	775 (49%)
Male	277 (41%)	792 (51%)
Age Category:		
20s	97 (14%)	226 (15%)
30s	126 (19%)	445 (28%)
40s	134 (20%)	289 (18%)
50s	199 (30%)	245 (16%)
60s	116 (17%)	362 (23%)
BMI:		
Normal	435 (65%)	1014 (65%)
Overweight	212 (31%)	499 (32%)
Underweight	25 (4%)	54 (3%)

Antibiotic History:		
Week	24 (3%)	18 (1%)
Month	54 (8%)	43 (3%)
6 Months	62 (9%)	195 (12%)
Year	86 (13%)	181 (11%)
Has not taken antibiotics in the past year	445 (66%)	1122 (72%)
Not provided	1 (<1%)	8 (<1%)
Race:		
Caucasian	613 (91%)	1368 (87%)
African American	0	7 (<1%)
Hispanic	10 (2%)	38 (2%)
Asian or Pacific Islander	20 (3%)	108 (7%)
Other	29 (4%)	46 (3%)
Country:		
USA	490 (73%)	882 (56%)
United Kingdom	182 (27%)	685 (44%)

QIITA/QIIME2 Workflow

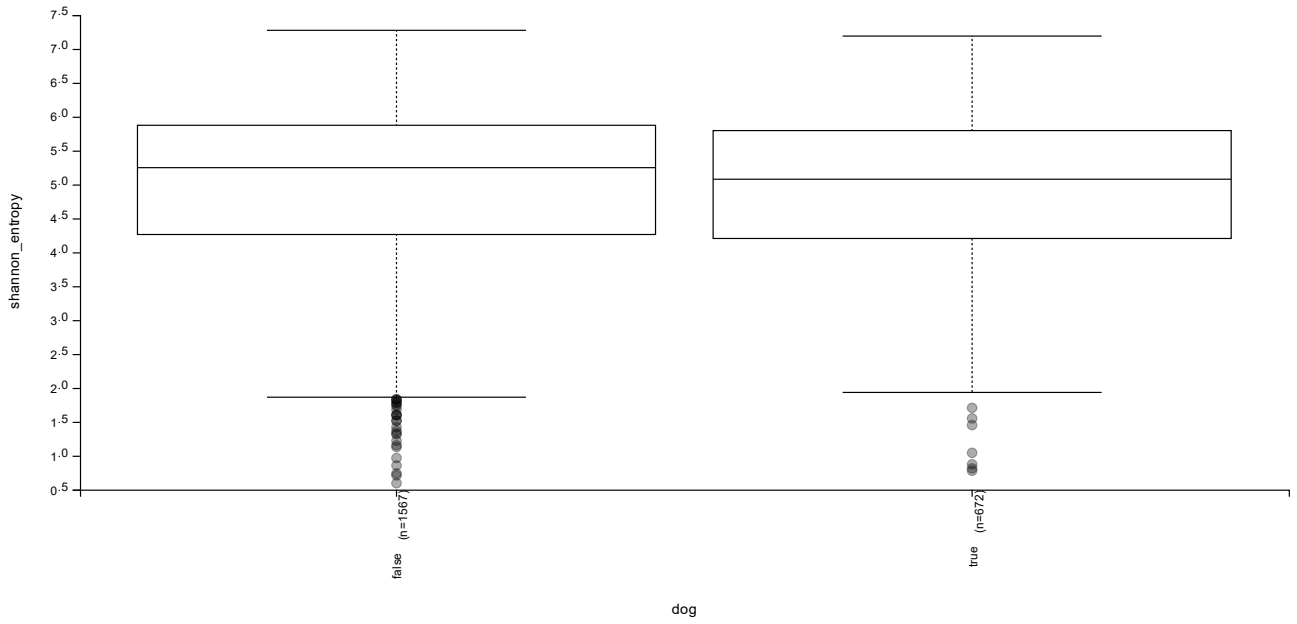


The QIIME2 workflow built out for this experiment.

Results

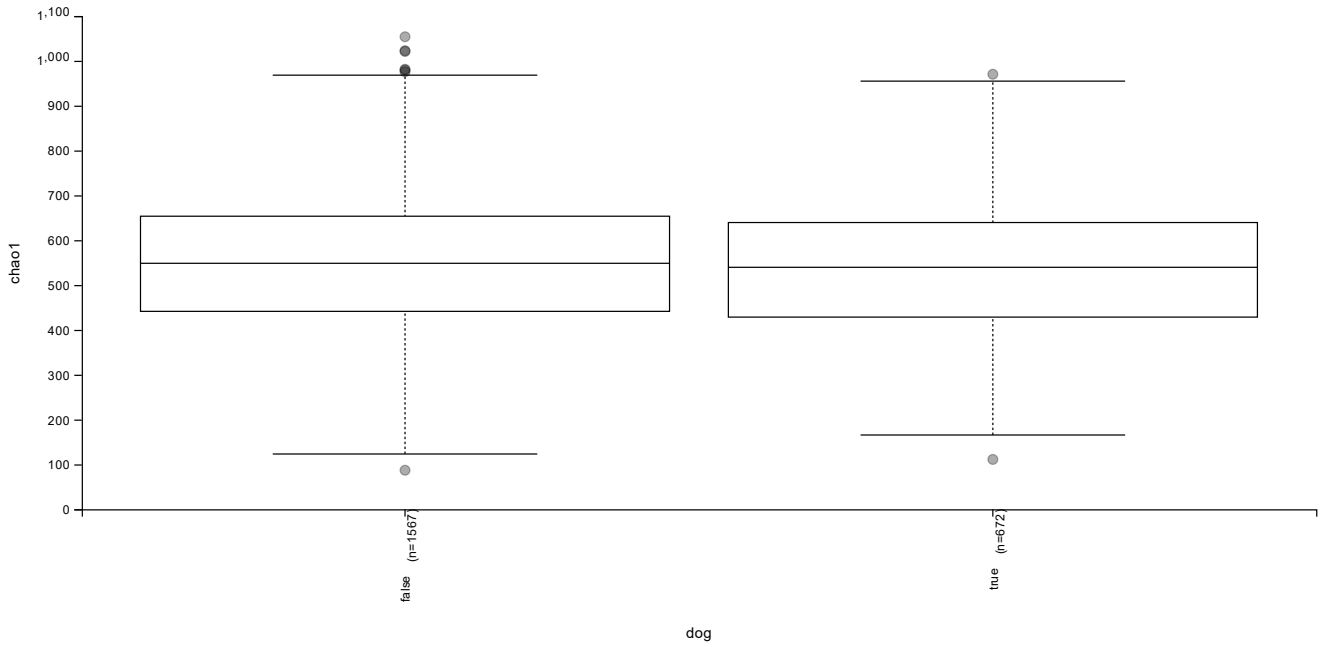
Alpha Diversity

Shannon's Index – Species Evenness



Nondog group is on the left, and the dog group is on the right. Shannon Index Diversity is used to measure the relative abundance of diverse species within each sample and gives more weight towards dominant species (CosmosID). The middle line represents the median and the box is the interquartile range. Dark circles represent outliers, which the nondog group has more of. Besides that, both groups produced almost identical plots, meaning the abundance of diversity of species is not affected by dogs. Had pet ownership been a factor, the box plots would vary in size and location.

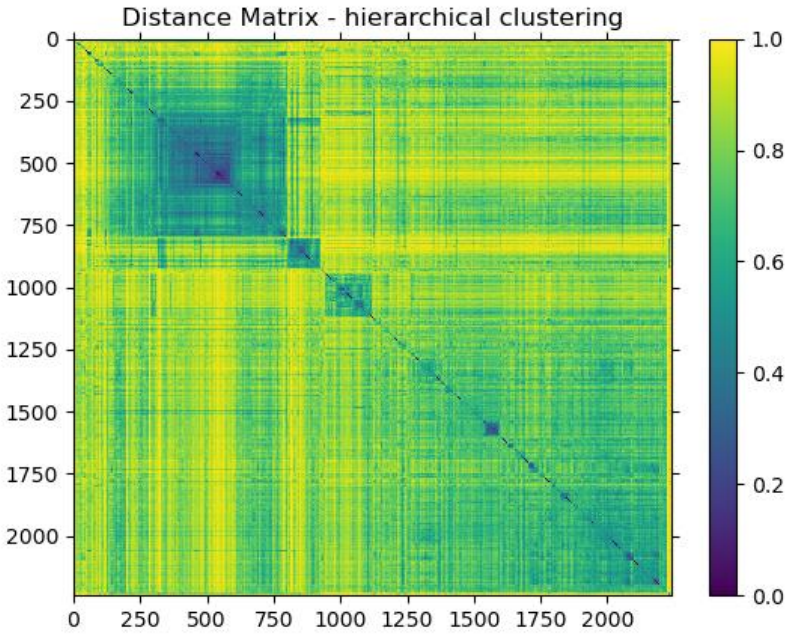
Chao 1 Index – Species Richness



Just like in the previous graph, Nondog group on the left and Dog group on the right. Similar data was produced for the Chao 1 index which measures the number of different species. In conclusion, non-pet owners and dog owners maintain similar gut microbiome data.

Beta Diversity

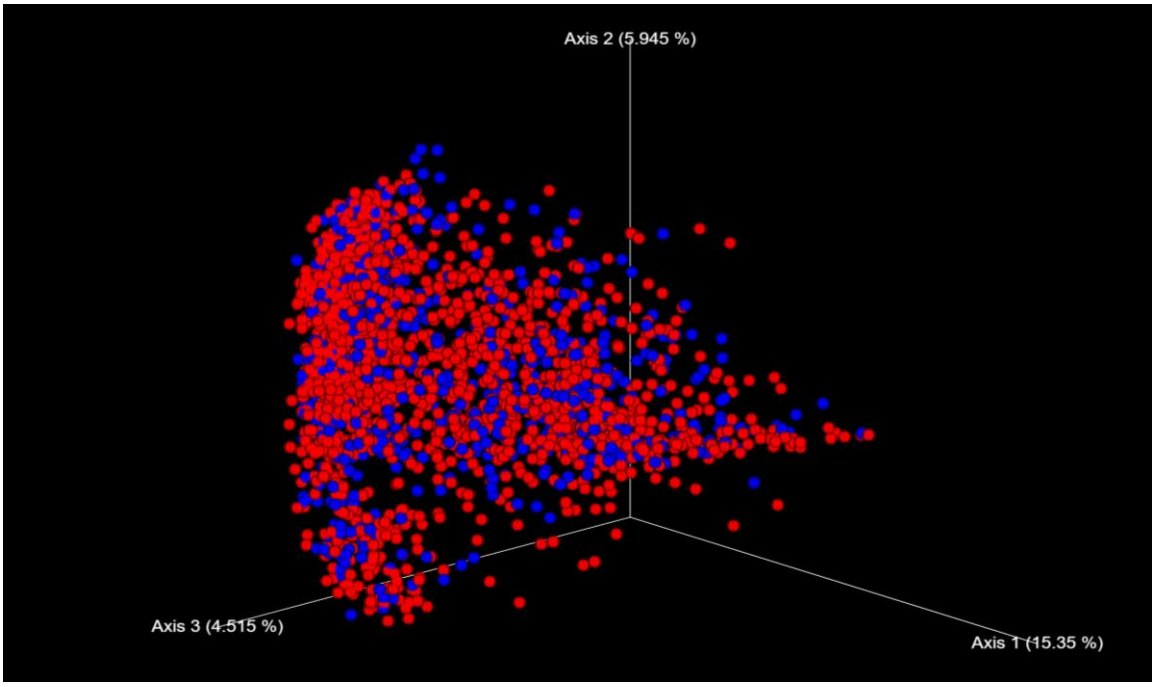
Distance Matrix



Number of samples: 2239
Minimum distance: 0.0376
Maximum distance: 1.0000
Mean distance: 0.7795
Median distance: 0.7935

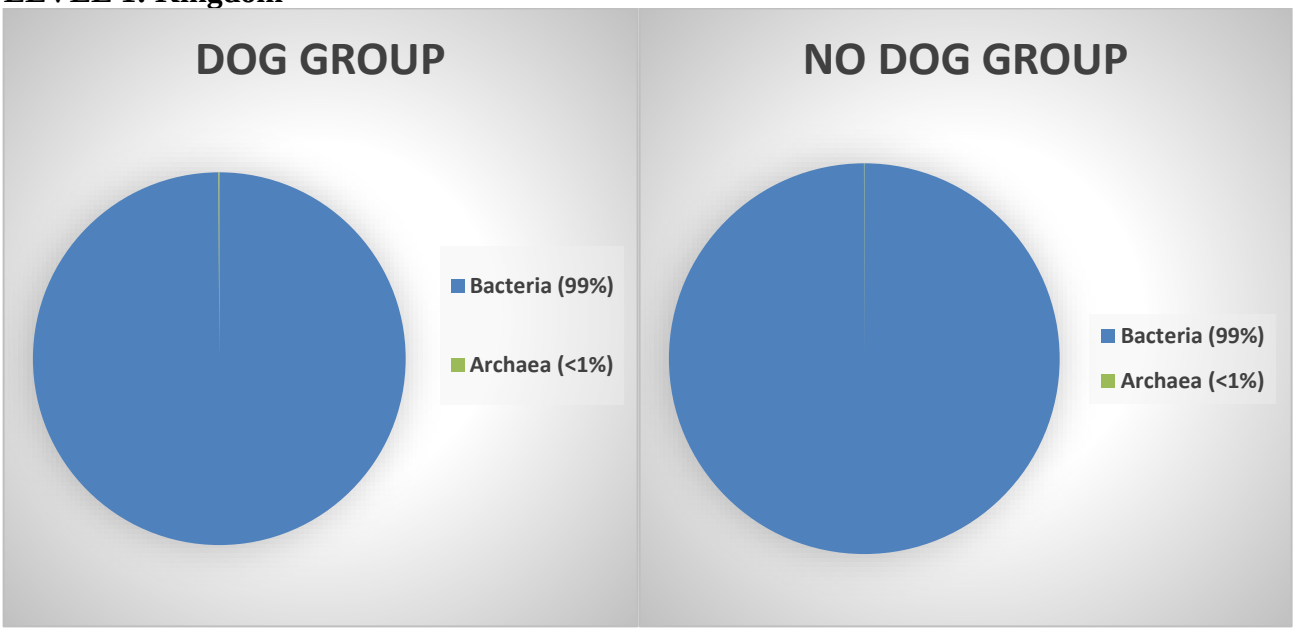
Distance Matrix measures similarities and dissimilarities between samples and then the data is transformed into a 3D PCoA plot. Axes represent the number of samples.

Principle Coordinate of Analysis (PCoA)

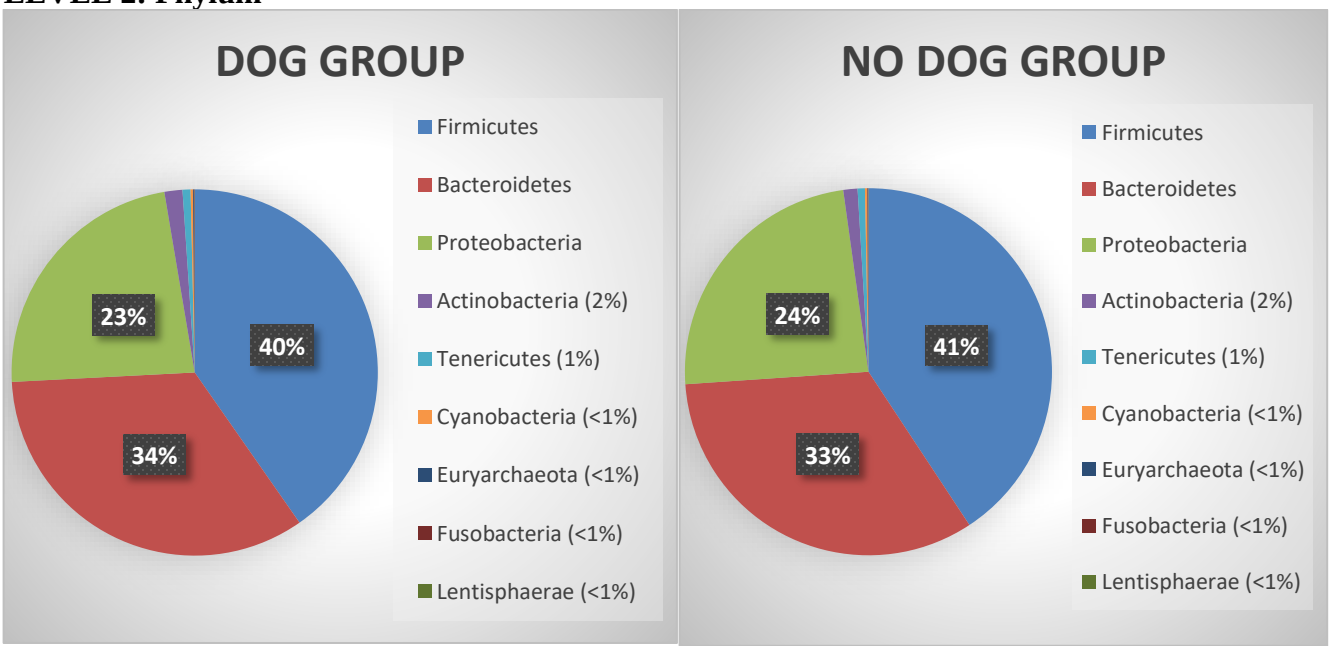


The blue samples represent the Dog group, and the red samples are the Nondog group. PCoA graphs help analyze any similarities between sample groups. The axes represent % variance and are not based on real values. There is obvious clustering between Axes 2 and 3 but, this was not a result of owning dogs, or the separation of the two groups would be more distinct.

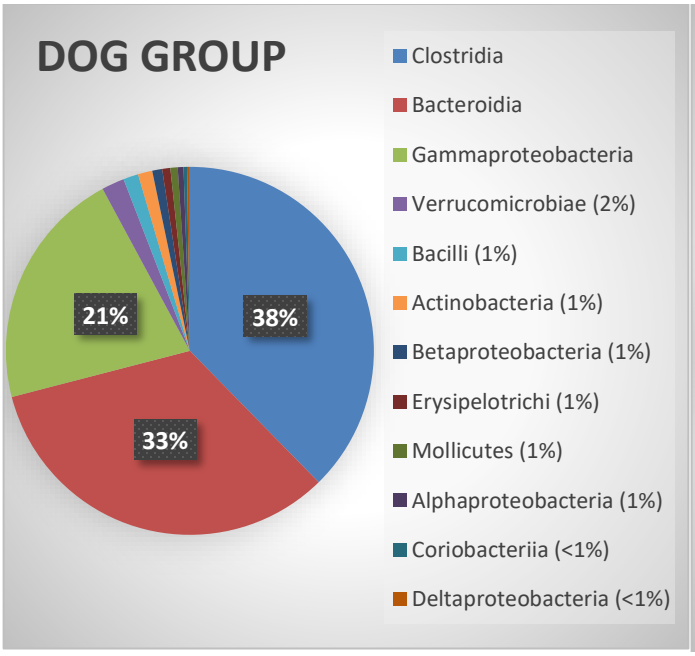
LEVEL 1: Kingdom

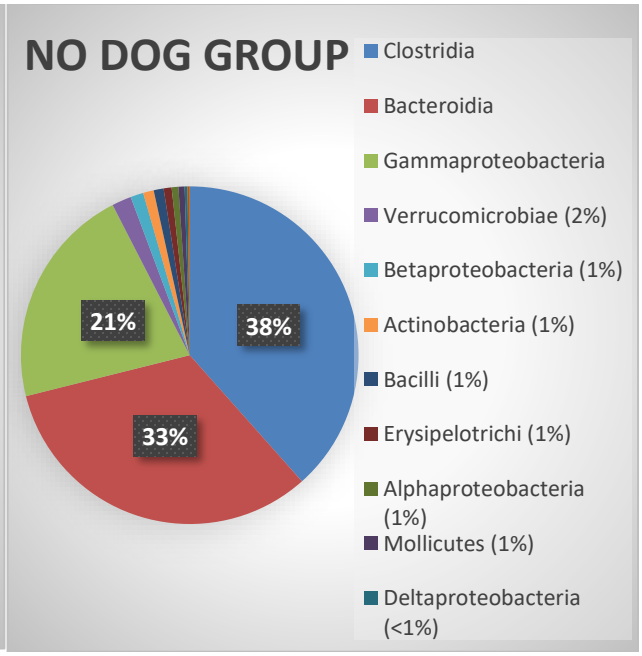


LEVEL 2: Phylum

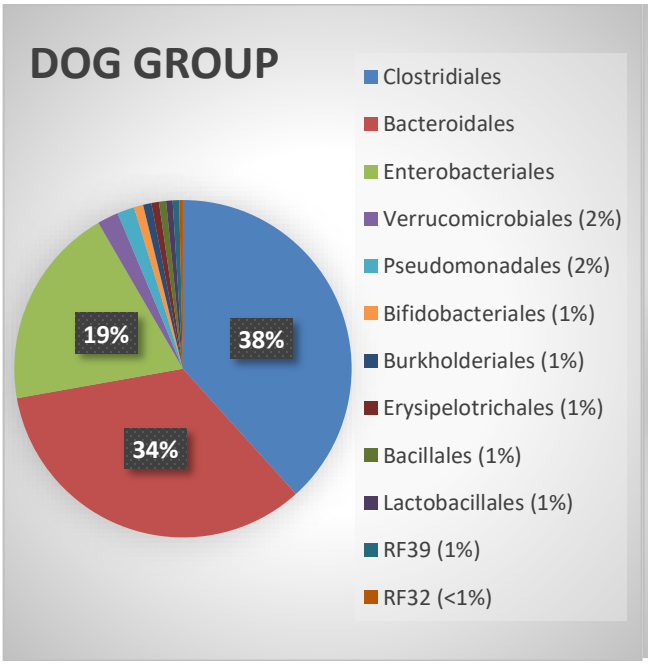


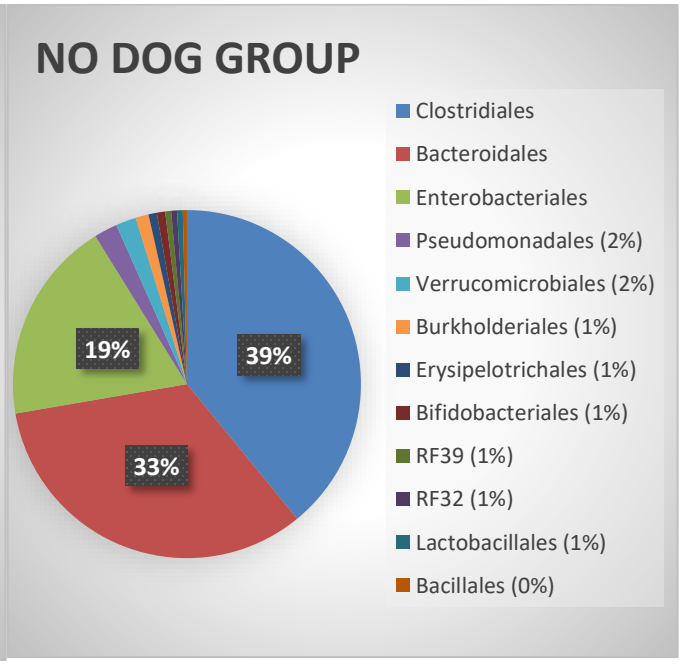
LEVEL 3: Class



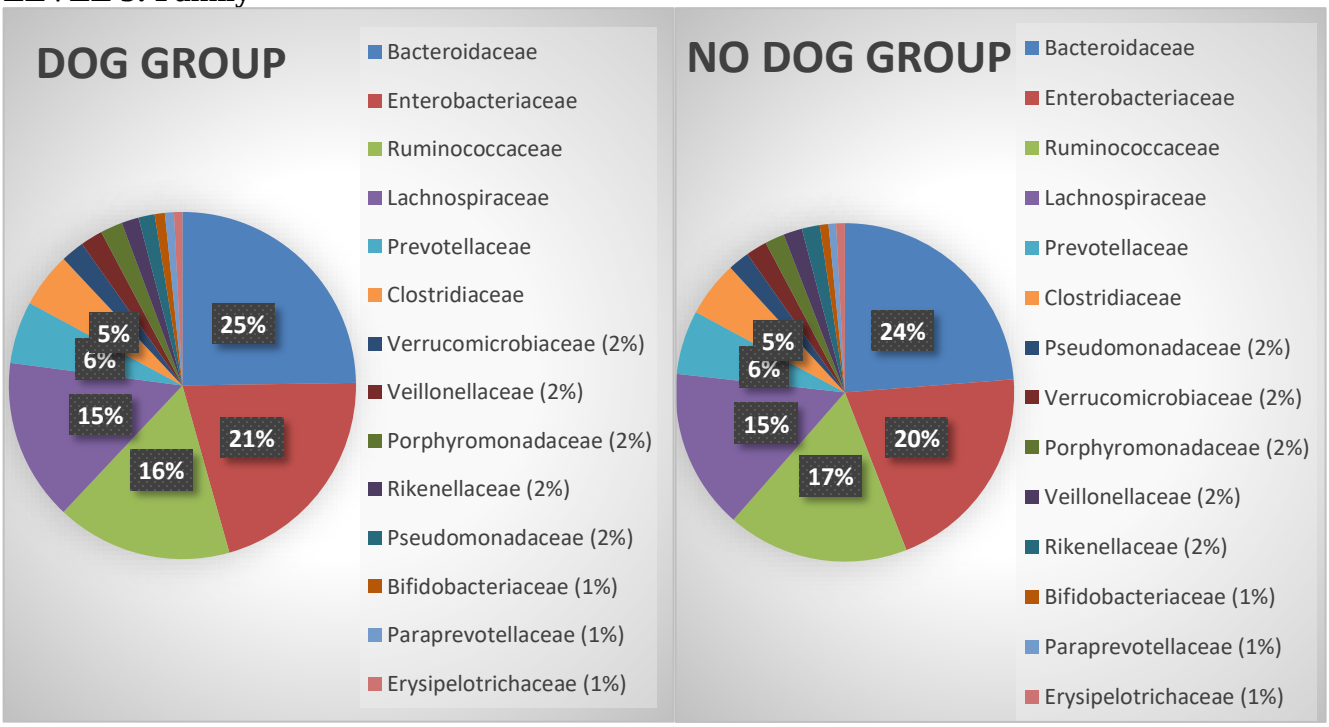


LEVEL 4: Order





LEVEL 5: Family



Pie charts were made for only five taxonomic levels because genus and species data were not present for all samples. Mean averages were calculated for each kingdom, phylum, class, order, and family. The averages always added up to the sampling depth of 8000 and with that information a decrease in *Pseudomonadaceae* in the dog group compared to the nondog group was calculated to be26%. Furthermore, the charts are almost identical besides minor percent changes.

Discussion

This study showed that dog ownership does not significantly affect gut microbiota. Alpha and beta diversity metrics calculated minor differences in the amount of microbial diversity and richness between the gut microbiota of dog owners and non-dog owners. Shannon and Chao 1 Index box plots both produced similar medians and interquartile ranges did not vary. Next, the PCoA plot displayed obvious clustering in between Axis 2 and 3, but the colored data plots indicated that the clustering was

not a unique result of either study group. Finally, pie charts were produced for five taxonomic levels for both dog and nondog groups. The main phyla were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Tenericutes and there was no variation between the major families, *Bacteroidaceae, Enterobacteriaceae, Ruminococcaceae, and Lachnospiraceae*. The dog group saw a 26% decrease in the *Pseudomonadaceae* family. Overall, this data demonstrated that the microbial compositions of both study groups were more alike than different.

These results contradict previous research that suggests pets have a major significance on gut microbiota. Important factors such as age and diet were filtered, but ultimately could not be accounted for in the calculations only utilizing the QIITA and QIIME2 software. In the study analyzing the effects of cat ownership, cat and noncat groups were further grouped by sex and BMI, and then individually analyzed. In addition, the number of samples for each group were kept the same which is a factor that should be considered henceforth. By analyzing cat ownership, the researchers also predicted changes in metabolic pathways controlled by the microbiota with a more advanced analytical tool. This particularly provided them with the knowledge of specific pathways that might be affected by the animal exposure. In the female cat group, they reported the metabolism of amino acids, carbohydrates, vitamins, and lipids was significantly increased (Du G, et al.). Perhaps this type of further analysis can indicate whether dogs are affecting the human body differently than cats.

Linear modeling or a t-test would also provide further insight on the data. Linear modeling is useful for predicting behaviors of complex systems like the microbiome while factoring other known variables. These variables could be the same metadata columns used in this experiment. In the demographics chart, it was noticeable that more females owned dogs than males which may have impacted the results. In addition, 61% of samples lived in the U.S., where the diet is known to be dominated by high-sugar and high-fats. For antibiotic usage, 70% of samples reported to have not taken any antibiotics in the past year. This data can be factored or calculated into future analyses,

utilizing more complex bioinformatic tools, so there is a better understanding of pet ownership on gut microbiota.

Conclusion

Pet ownership is one of the many environmental exposures being investigated today for its effects on the gut microbiota. Dogs owners do not have a major difference in their microbial diversity or richness, and carry similar taxonomic profiles compared to those who do not own any pets at all. The effects of animals on their owner's health might need to be reconsidered or dogs might affect the gut microbiota in a different manner compared to cats.

References

"Alpha Diversity." *CosmosID*, https://docs.cosmosid.com/docs/alpha-diversity.

"Beta Diversity." *CosmosID*, https://docs.cosmosid.com/docs/beta-diversity#:~:text=Alpha%20diversity%20is%20how%20many%20different%20taxa%20are,the%20differences%20in%20microbial%20composition%20between%20the%20samples.

Bolyen E, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 852–857. https://doi.org/10.1038/s41587-019-0209-9

"Dog microbiomes closer to humans' than expected". *Research in Germany, Federal Ministry of Education and Research.* www.researchingermany.org. Published on April 20, 2018. Accessed May 11, 2020.

Du G, Huang H, Zhu Q, Ying L (2021) Effects of cat ownership on the gut microbiota of owners. PLoS ONE 16(6): e0253133. https://doi.org/10.1371/journal.pone.0253133

"Facts + Statistics: Pet Ownership and Insurance." *III*, https://www.iii.org/fact-statistic/facts-statistics-pet-ownership-and-insurance.

"Linear Models." *Brilliant Math & Science Wiki*, https://brilliant.org/wiki/linear-models/.

"Microbiome." Genome.gov, https://www.genome.gov/genetics-glossary/Microbiome.

"The Microbiome." *The Nutrition Source*, 1 May 2020, https://www.hsph.harvard.edu/nutritionsource/microbiome/.

McDonald D, et al. American Gut: an Open Platform for Citizen Science Microbiome Research. mSystems. 2018 May 15;3(3):e00031-18. doi: 10.1128/mSystems.00031-18. PMID: 29795809; PMCID: PMC5954204.

Qiita: rapid, web-enabled microbiome meta-analysis. Antonio Gonzalez, et al. Nature Methods, volume 15, pages 796–798 (2018); https://doi.org/10.1038/s41592-018-0141-9.

"Researching Global Microbiomes." *The Microsetta Initiative*, 2 Feb. 2022, https://microsetta.ucsd.edu/.

Rinninella, Emanuele et al. "What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases." *Microorganisms* vol. 7,1 14. 10 Jan. 2019, doi:10.3390/microorganisms7010014