The Impact of the Gut-Microbiome on Canine Personality

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Abstract

Personality is largely influenced through the **gut-microbiome** via the **gut-brain axis** (GBA) and vagus nerve signaling. In the past, evaluations of the canine and human microbiomes have shown the former to be a good predictor of personality due to great similarities between the two microbiomes and substantial overlap between metagenomic genes. Further analysis of microbiome composition in humans and canines has evidenced the link between personality traits and mental health to gut-microbiota. The phylogenetic contents of fecal samples (n = 17) were prepared for 16S application using **MinION** sequencing and compared to the results of a personality index (**C-BARQ Survey**). The resulting correlation coefficients did not indicate an association of the genera Blautia, Fusobacterium, and Streptococcus with personality traits as indicated in prior studies addressing aggressive and phobic behavior. Further evaluation of the microbiome is required to determine higher confidence in these correlations and diversity levels.

Introduction

Hypothesis: Gut health and phylogenetic diversity directly cause positive personality traits in canines, while an over/under representation of certain bacteria cause mental health disorders as well as phobic and aggressive behaviors.

Background: Human personality can be defined by the five major dimensions of the NEO personality index (neuroticism, extroversion, openness, agreeableness, and conscientiousness) and influenced by the composition of the gut microbiome (**H.-N. Kim et al., 2017**). These traits can be modeled in canines to study the role of the gut bacteria in personality determination.

The gut-brain axis (GBA) is composed of a two-way communication network between the microbiome and the CNS through vagus nerve signaling (**Heym et al., 2019**). Studies performing fecal transplants between two mice have shown a near-complete transfer of personalities (**Kelly, 2016**). A study of 1,247,407 dog, pig, mice, and human metagenomic genes related to the GBA showed a 63% overlap between dog and human samples (**Coelho, 2016**). Others have found a common prevalence of *Bacteroides* and *Lactobacillus* in both canine and human microbiomes which should be expected in our results (**Mondo et al., 2020**).

Approximately 17.3 million adults had at least one major depressive episode per year and 6.8 million adults are affected by Generalized Anxiety Disorder every year (**National Institute of Mental Health, 2017**). Decreases in brain-derived neurotrophic factor mRNA, produced in the microbiome, have also been linked to depressive/anxiety-like disorders (**Foster and Neufield, 2013**). Future research may prove a further association between microbiota and an increased risk of depression and anxiety.

Further investigation of this relationship in canines will advance knowledge in the adjacent realm of human mental health and depressive disorders. Understanding the role of canine personality on gut microbiota will aid the scientific community in understanding the correlation between mental health and gut microbiota.

Methodology

The University of Pennsylvania's **C-BARQ Survey** was administered by the owners of the canine subjects who also produced fecal samples. A **probiotic/antibiotic supplementation survey** was also administered to the study participants to identify underlying variables.

DNA extraction of the fecal samples was performed using Qiagen's **DNeasy Powersoil DNA Extraction Kit** according to the manufacturer's user protocol. **Nanodrop** spectral images were taken to analyze the concentration and purity of DNA. Beckman Coulter **AMPure XP Bead Cleanup** was conducted on any extracted DNA with significant impurities.

Quantities were then verified by a **Qubit Fluorometer** using dsDNA Broad Range assay and diluted to 1 ng/µl. Oxford Nanopore Technologies' **16S Barcoding Kit** was used to prepare the 16S rRNA gene library for each sample and all barcodes were pooled to a total of approximately 100 femtomoles.

Raw data obtained from MinION sequencing runs was analyzed using NCBI BLAST and Oxford Nanopore Technologies' FASTQ application within the **EPI2ME** platform in order to create phylogenetic trees for each sample.

Results

n	Shannon index (H')	Pielou Evenness (J)
2	1.06	0.39
3	1.21	0.50
4	1.25	0.50
5	1.17	0.53
6	0.53	0.30
7	1.22	0.41
8	0.85	0.31
9	0.88	0.34
10	0.96	0.37
11	1.25	0.50
12	0.84	0.29
13	1.35	0.44
14	0.59	0.21
15	1.23	0.39
16	1.00	0.34
17	0.93	0.32
18	1.42	0.43

n	260/230	260/280	[ng/µl]
2	2.18	1.86	495.6
3	0.65	1.87	170.4
4	2.11	1.87	178.1
5	2.33	1.89	15.2
6	2.02	1.83	79.5
7	2.32	1.90	161.9
8	2.01	1.87	95.4
9	2.06	1.88	449.2
10	2.30	1.87	148.6
11	2.00	1.88	439.0
12	2.37	1.89	290.6
13	2.40	1.87	234.1
14	2.49	1.86	118.5
15	1.99	1.87	854.5
16	1.96	1.96	9.4
17	2.07	1.88	52.2
18	2.24	1.84	522.7

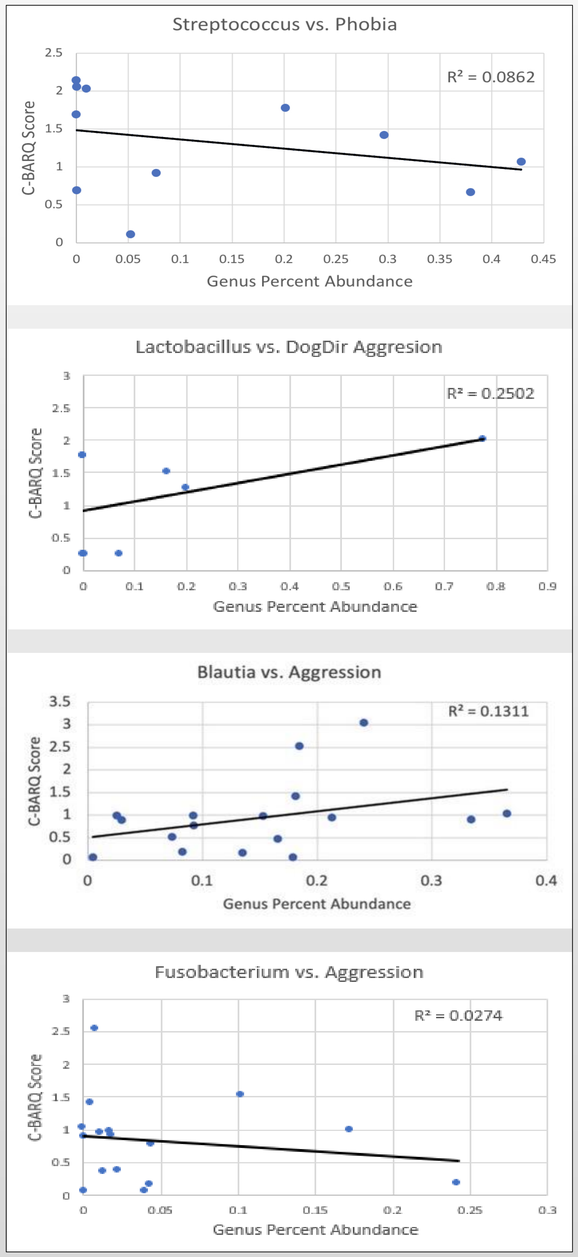

Figure 1. Trend lines relating the average scores for composite phobia, aggression, and dog-directed aggression were plotted on the y-axis to their respective bacterial genera *Streptococcus, Lactobacillus, Blautia*, and *Fusobacterium.*

Total Basecalls: **6.6 Gb**
Reads Classified: **4,243,223**
Average Sequence Length: **1,523 bp**
Average Quality Score: **9.19**

Total Basecalls: **5.8 Gb**
Reads Classified: **3,763,152**
Average Sequence Length: **1,520 bp**
Average Quality Score: **9.89**

Discussion

Samples with high abundances of *Blautia* were originally expected to express more aggressive traits. *Fusobacterium* and Aggression were expected to be inversely related. No previous literature was found regarding *Streptococcus'* role within the microbiome. However, Streptococcus is in the same class as *Lactobacillus* which has been tied to phobic traits (**Kirchoff et al., 2019; Mondo et al., 2020; Pilla and Suchodolski, 2020**). However, these correlations were not reflected in our data.

The Shannon Diversity Index and Pielou evenness were used to analyze how phylogenetic diversity contributed to overall health in canines. However, the indices provided confounding variables that made it difficult to draw conclusions and determine diversity within the samples.

Further analysis of each microbial ecosystem will involve the QIIME2 application and its core metrics which consist of alpha and beta diversity measures: Chao1 index, Faith's phylogenetic diversity, Fisher's index, Shannon's index, observed OTUs, Pielou's evenness, Jaccard distance, weighted and unweighted Unifrac distances, and Bray-Curtis dissimilarity. MaAsLin packages within RStudio and PICRUSt may also be used to compute multivariable analyses of "clinical metadata and microbial community abundance" (**Kim, 2018**).

In future research, variation in diet and breed would be accounted for and a larger sample size would be obtained. Further evaluation of the microbiome is needed to determine a higher level of confidence in these results.

By evaluating abundances of gut microbiota in relation to personality characteristics, veterinarians could then use this information to both determine reasons behind presence of certain personality traits and develop probiotics to treat negative traits such as aggression and phobia. This research can also be applied to humans in the realm of mental health treatment by developing probiotics to rebalance gut microbiota populations which have been shown to affect mental health disorders like anxiety and depression.

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