Exploring the relationship between cognitive performance and gut and salivary microbial communities in Beagle dogs

Transpharmation
Science that translates into results

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INTRODUCTION

There are several biomarker and brain changes associated with canine aging that parallel neuropathological changes seen in human Alzheimer's Disease (AD); the canine microbiome is no exception. Many inflammatory diseases are linked to the microbiome, often with changes in microbial communities preceding the onset of such diseases.

This study aimed to explore the relationship between cognitive performance of aged dogs and both gut and salivary microbiota.

METHODS

The cognitive domains specifically examined in this study included executive function and short-term visuospatial working memory. Sixteen dogs, aged 6 to 15 years, were tested on an associative learning task (object discrimination), a performance-based visuospatial selective attention task, and a variable delay (20 and 90-second) non-matching to position task (varDNMP), then ranked on performance. Subjects were divided into two groups classified as either high performers, or low performers based on their accuracy for each task.

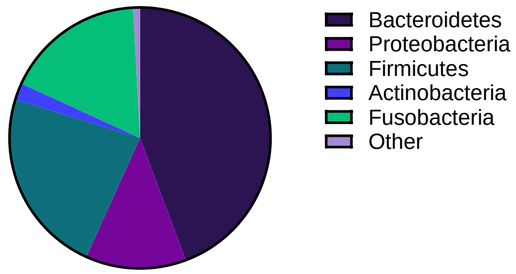
Fecal and saliva samples were collected from all subjects, prepared fixed in a Zymo DNA/RNA shield. DNA was extracted and DNA quality was evaluated visually via gel electrophoresis and quantified. Libraries were prepared using an Illumina Nextera library preparation kit with an in-house protocol. Fecal samples were analysed using paired-end sequencing. Shotgun metagenomic sequence reads were processed with the Sunbeam pipeline[1]. High-quality reads were mapped to the reference dog genome (Canis lupus familiaris, version UU_Cfam_GSD_1.0, accession number GCA_011100685.1). The remaining reads were taxonomically classified[2].

Negative binomial models were used for differential abundance testing of taxonomic and subsystem level 3 features. Group differences were looked for after controlling for sex, and for sex:group interactions; in both cases adjusted p-values (< 0.01) were used. A permutational multivariate analysis was used to estimate the effects of experimental factors on both taxonomic and functional profiles. Differences in groups were explored using a general model (Sex*Group). The remaining variation was used to look for differences in groups. Alpha diversity was calculated from taxonomic profiles (Species level) using Shannon's diversity index.

RESULTS

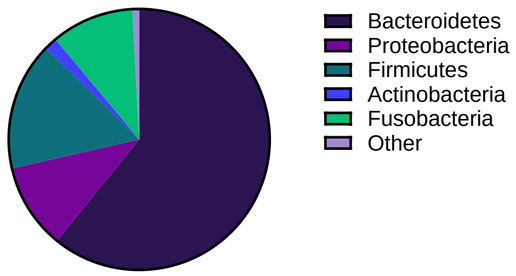
Overall salivary and fecal microbial communities were well correlated. In all subjects regardless of cognitive status, bacterial profiles were dominated by the Bacteroidetes, Firmicutes, and Fusobacteria phyla, accounting for 51.5%, 19%, and 13.5%, respectively.

Relative Abundance (%) of Lower Performers



Total=97.409

Relative Abundance (%) of Higher Performers



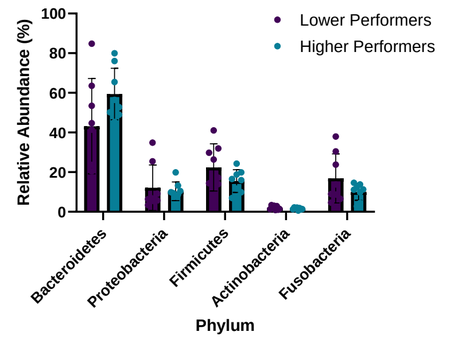
Total=97.597

No significant taxa or functions associated with group differences were found. However, the % abundance of Bacteroidetes was slightly increased in the group of higher performers and the Firmicutes and Fusobacteria were slightly increased in the group of low performers. These trends suggest that significant group differences may be observed if a larger sample size was used. Human gut-brain axis studies suggest that throughout aging, Firmicutes decrease and Proteobacteria increase. A larger sample size across a wider age range will help verify if this is a consistent finding in dogs and how it relates to cognitive function.

RESULTS

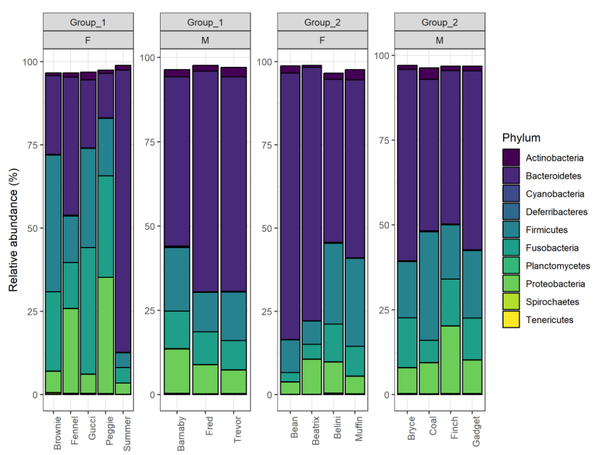
Both Actinobacteria and Proteobacteria were found in less abundance overall in our samples; but analyses of these taxa exhibited high individual variation. Overall, the relative abundance of both these taxa was inversely correlated with cognitive status. Previous canine studies identified fewer Actinobacteria in subjects with higher performance on memory tasks[3]; in humans, this phylum is increased with AD.

Relative Abundance (%) of Low vs High Performers



No significant group differences were found in either taxonomic or functional profiles after controlling for sex differences, though a sex effect was observed in the taxonomic profiles that explained roughly 15% of the variation (p=0.023). PERMANOVA did not find differences due to sex in the functional profiles however the p-value was 0.076 which again suggests that more replicates are needed to have enough statistical power.

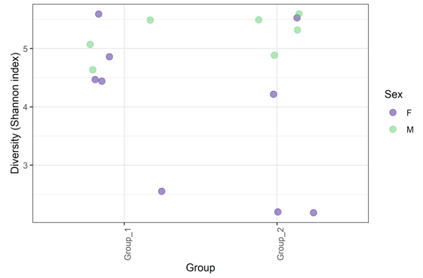
Bacterial Composition at Phylum Level as a Function of vDNMP Group and Sex



RESULTS

No significant group or sex differences were observed (Scheirer-Ray-Hare test), however, sex differences were approaching significance (p=0.051).

Alpha Diversity as a Function of vDNMP Group and Sex



CONCLUSIONS

- Salivary and fecal taxa are well correlated.
- Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Actinobacteria were the most abundant phyla, respectively.
- The relative abundance of Firmicutes, Fusobacteria, Actinobacteria and Proteobacteria in this study was inversely correlated with cognitive status.
- This study highlighted the possible benefit to running single sex cognitive studies when looking at the microbiome.
- Tracking cognition and microbiome composition over time is a valuable tool in identifying trends that will help further establish the relationship between cognitive performance and gut and salivary microbial communities.
- Further studies with larger samples sizes need to be conducted to better understand the relationship between cognitive status and the microbiome in the dog.

REFERENCES

1. Bioinformatics Group at the Babraham Institute. Software available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
2. Wood, D.E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken 2. Genome Biol 20, 257 (2019). https://doi.org/10.1186/s13059-019-1891-0
3. Kubinyi, E., Bel Rhali, S., Sándor, S., Szabó, A., & Felföldi, T. (2020). Gut microbiome composition is associated with age and memory performance in pet dogs. *Animals*, *10*(9), 1488. https://doi.org/10.3390/ani10091488

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