Blood and Fecal Microbiota in Healthy Dogs

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INTRODUCTION

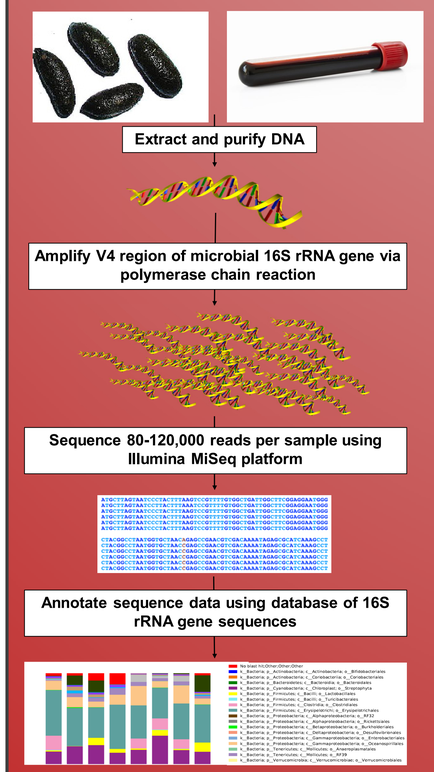
- Microbial identification utilizing metagenomics has allowed characterization of communities of bacteria present in healthy individuals. Further, the microbiota differ in healthy versus inflammatory diseases. Our laboratory is interested in investigating the relationship between microbial communities present in the gastrointestinal tract and the blood. Recent data from our laboratory and others using sequencing of the microbial 16S rRNA gene challenges the dogma that blood is free of bacteria in healthy individuals. The origin of these microbes is thought to predominantly be the gut; however, especially in illness, microbes may translocate from other regions of the body. Clarity of where blood microbes are derived is needed; a better understanding of the healthy blood microbiota would allow for comparison to the microbiota of diseased states.
- The study objective was to document the presence of and characterize the healthy canine microbiota from blood and fecal samples.

HYPOTHESIS

- We hypothesized that using 16S rRNA amplicon sequencing of microbial DNA from the blood of healthy dogs there would exist a rich and diverse blood microbiota that would be similar to the gut microbiota.

MATERIALS & METHODS

- Paired blood and fecal samples were collected from 13 healthy dogs, not receiving drugs or antibiotics known to affect the microbiome. Two control samples were taken in dogs after a sterile preparation for blood draw by inserting the needle through the skin without withdrawing blood. DNA was extracted from samples and amplified via PCR, then sequenced utilizing Illumina MiSeq platform. Operational taxonomic units (OTU) were then determined and assessed. Principal component analysis (PCA) allowed for visualization of relatedness of samples; PERMANOVA was used to test for significant differences in microbial community composition.



RESULTS

- Coverage varied substantially in blood with 5 dogs having very low reads/sample similar to controls (Fig.1). Mean±SD richness was significantly lower in blood than feces (69±20 versus 188±37 unique sequences, respectively; p<0.001; Fig. 2a). The blood had a significantly lower α-diversity than feces (Shannon index 2.5±0.6 vs 3.1±0.5, respectively; p=0.016; Fig. 2b). The relative abundance at the level of the OTU for blood, controls and feces is depicted in Fig 3. Three different microbial profiles were noted in blood. One contained primarily *Acinetobacter* sp., one was similar to feces with *Megamonas* and *Fusobacterium* species predominating, and one resembled the controls with an abundance of the unclassified *Bradyrhizobiaceae* family. PCA plots showed microbial communities between blood and feces were significantly different (p=0.0001; Fig 4).

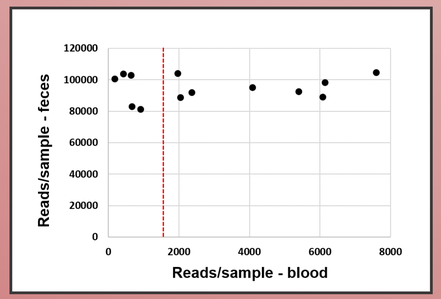


Figure 1. Dot plot showing relationship between read counts obtained from paired blood and fecal samples; red line indicates 2 standard deviations above the mean coverage obtained from two needle stick control samples.

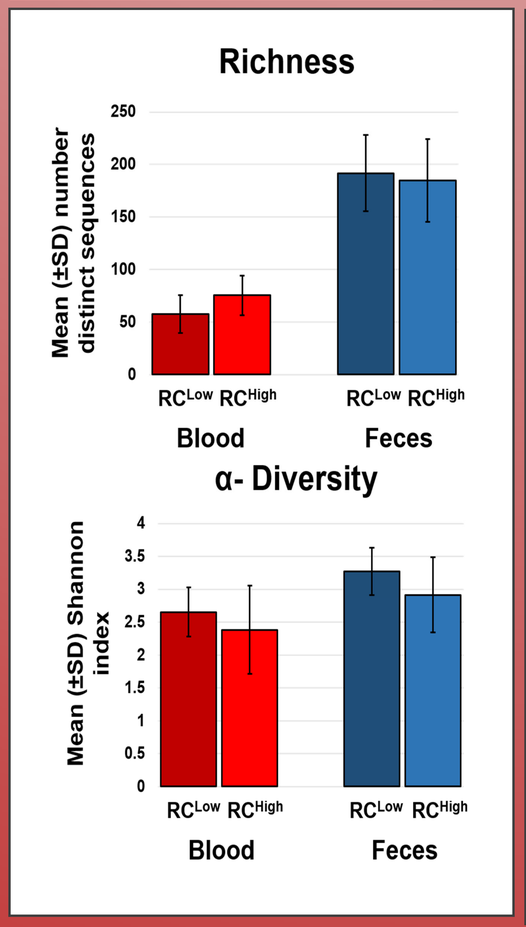


Figure 2a. Bar graph comparing richness in blood versus feces. Mean±SD richness was significantly lower in blood than feces (69±20 versus 188±37 unique sequences, respectively; p<0.001). RC^{Low} represents samples with read counts lower than 2 standard deviations away from the control samples. RC^{High} samples were above 2 standard deviations of the mean of controls.

Figure 2b. Bar graph comparing α-diversity in the blood and feces. Blood had a significantly lower α-diversity than feces (Shannon index 2.5±0.6 vs 3.1±0.5, respectively; p=0.016).

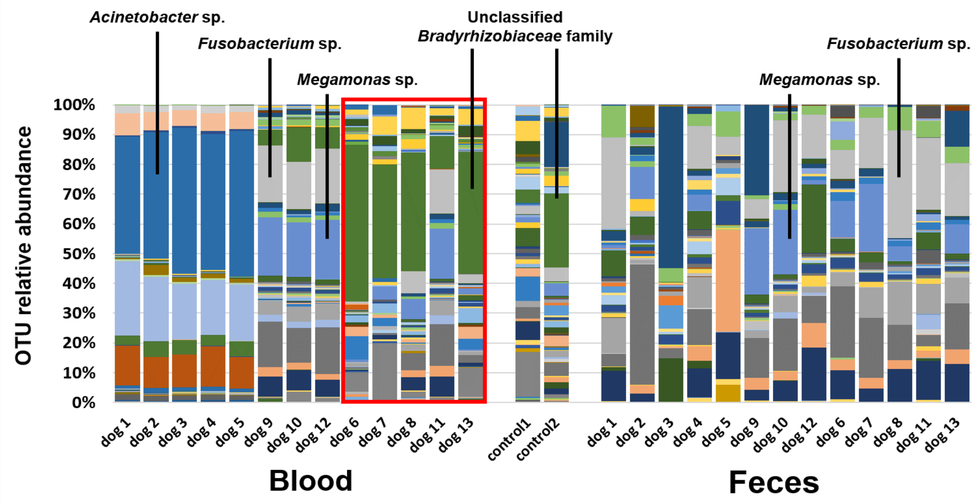


Figure 3. Stacked bar chart showing relative abundance at the level of OTU in blood and feces. Three microbial profiles were noted in blood. One contained predominantly *Acinetobacter* sp. (dogs 1-5), one was similar to feces with *Megamonas* and *Fusobacterium* species predominating (dogs 9, 10, 12), and the last resembled the controls and contained mainly unclassified *Bradyrhizobiaceae* family (dogs 6-13). The red box indicates blood samples with low read counts.

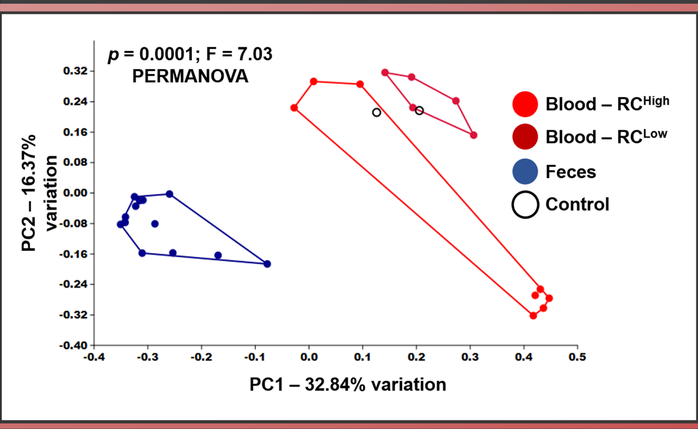


Figure 4. Principal component analysis plot comparing the microbial communities in the blood and feces. Microbial communities in blood and feces were significantly different (p=0.0001).

CONCLUSIONS

- This study, for the first time, documented the presence of a blood microbiome in the dog with healthy canine blood being significantly less rich and diverse when compared to feces. Although this was a small pilot study and there was variation between the blood OTU relative abundance between dogs, the microbial communities present within the blood and feces as documented by PCA plots are unique.

FUTURE DIRECTIONS

- Results of this study will allow for future comparisons of the microbiota in the blood in a variety of different disease states.
- To determine whether microbial DNA represents viable organisms or simply DNA.

ACKNOWLEDGEMENTS

- Student support for the VRSP program was provided by Mizzou Advantage Initiative in One Health/One Medicine.