

Longitudinal Investigation of the Fecal Microbiome in Healthy Dogs

Veterinary Research
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Background

- Most chemotherapy-induced gastrointestinal and hematologic side effects occur 3-4 days and 7 days post-treatment, respectively.
- Patients undergoing chemotherapy are at increased risk of sepsis due to damage of epithelial barrier, decreased white blood cells, and possibly dysbiosis.
- The affect of chemotherapy on the fecal microbiome of dogs is currently unknown, and to study this, we must establish the stability of the healthy dog microbiome over time
- Studies in humans have shown that rectal swabs provide information similar to fecal samples for assessing the gut microbiome, but no such studies have been done in dogs.

Methods

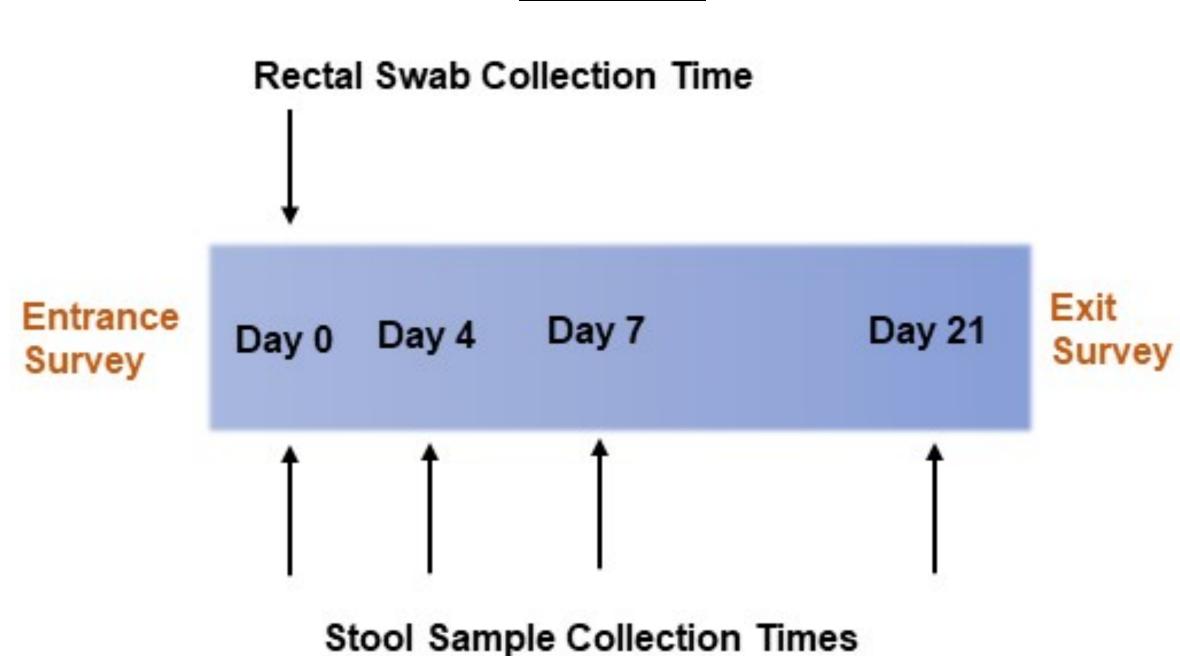


Figure 1. Schematic of the study design. Stool samples were collected on Days 0 (rectal exam and rectal swab), 4, 7 and 21 and kept frozen @ -20°C (home collections) or -80°C until DNA extraction. Entrance and Exit Surveys conducted at the beginning and end of the study period. Diet change, probiotics or antibiotics prohibited 7 days prior to or during the study period.

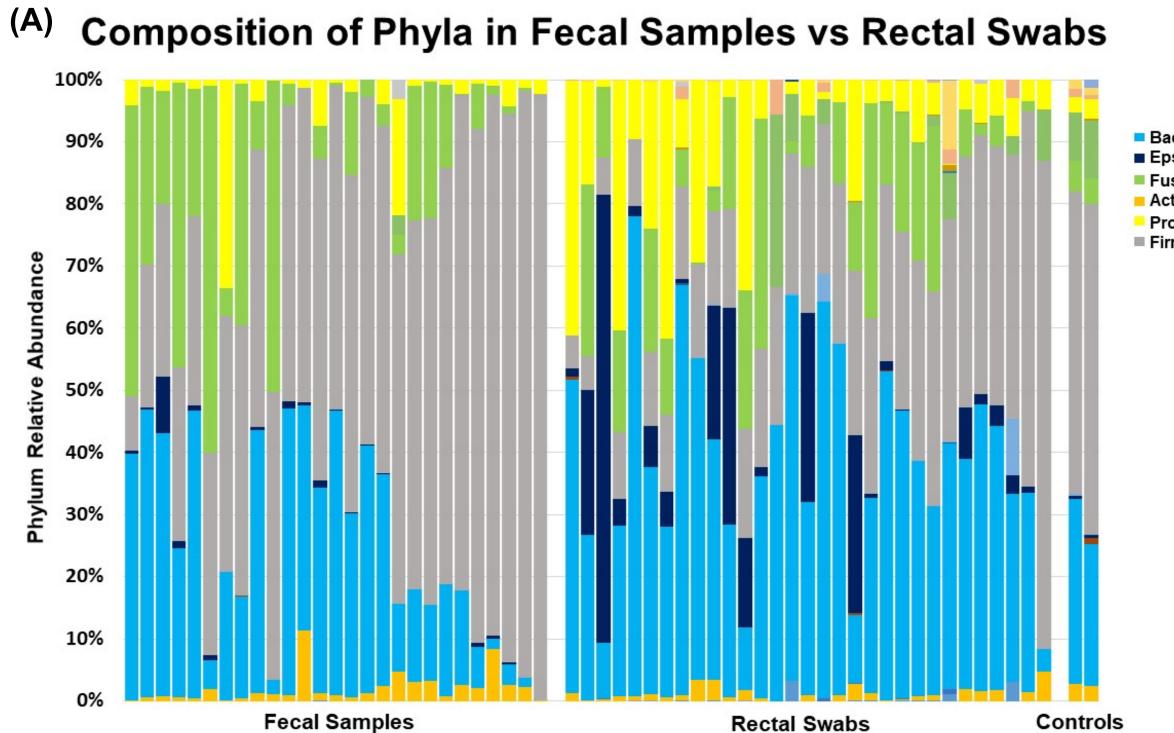
Sex	
Male, neutered	9
Male, intact	1
Female, spayed	16
Female, intact	1

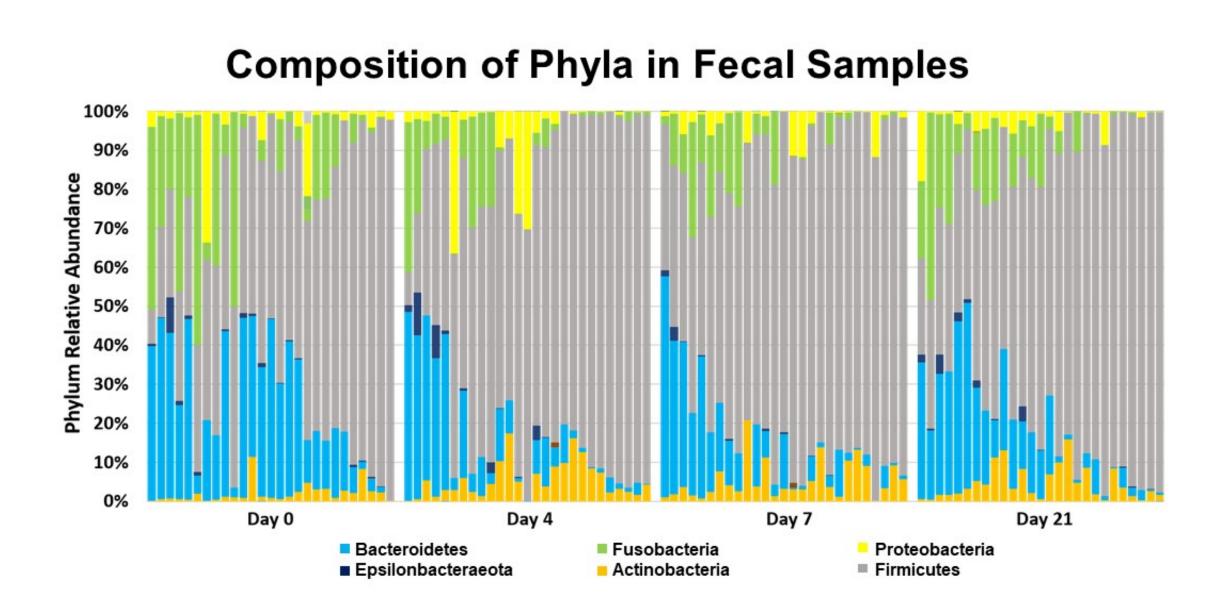
Figure 2. Table displaying the sex of participants in the longitudinal study in the left column and number of participants of that sex in the right column.

Figure 3. Table of breeds represented in the longitudinal study in the left column with number of dogs belonging that breed in the right column (n=27).

Breed	
Mixed Breed	13
Greyhound	2
Great Dane	2
Australian Shepherd	1
Border Collie	1
Australian Cattle Dog	1
Chihuahua	1
German Shepherd Dog	1
Golden Retriever	1
Jack Russel	1
Miniature Pinscher	1
Newfoundland	1
Portuguese Water Dog	1
Total	27

<u>Results</u>





(B) Composition of OTUs in Fecal Samples vs Rectal Swabs

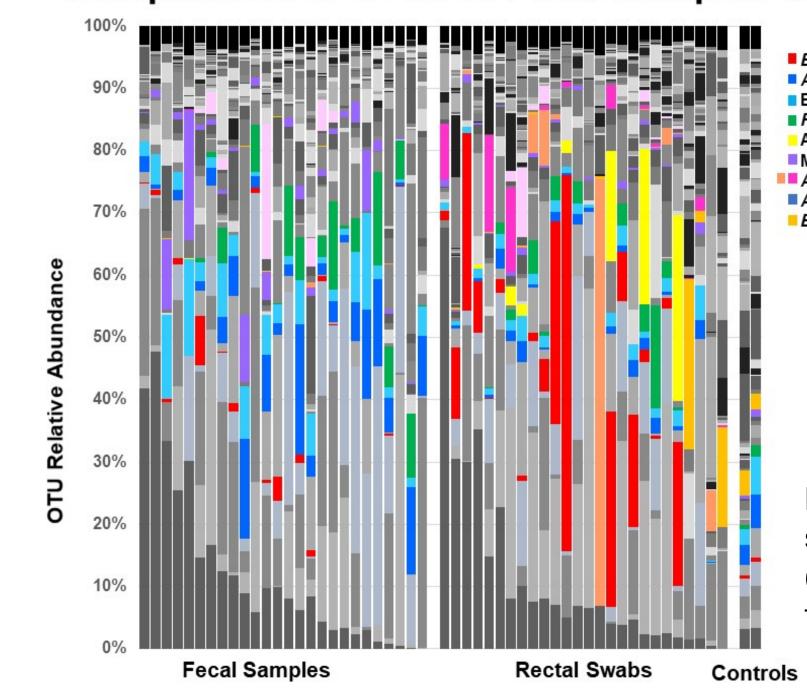


Figure 4. Stacked bar charts showing the composition of phyla (A) and OTUs (B) in rectal vs. fecal samples.

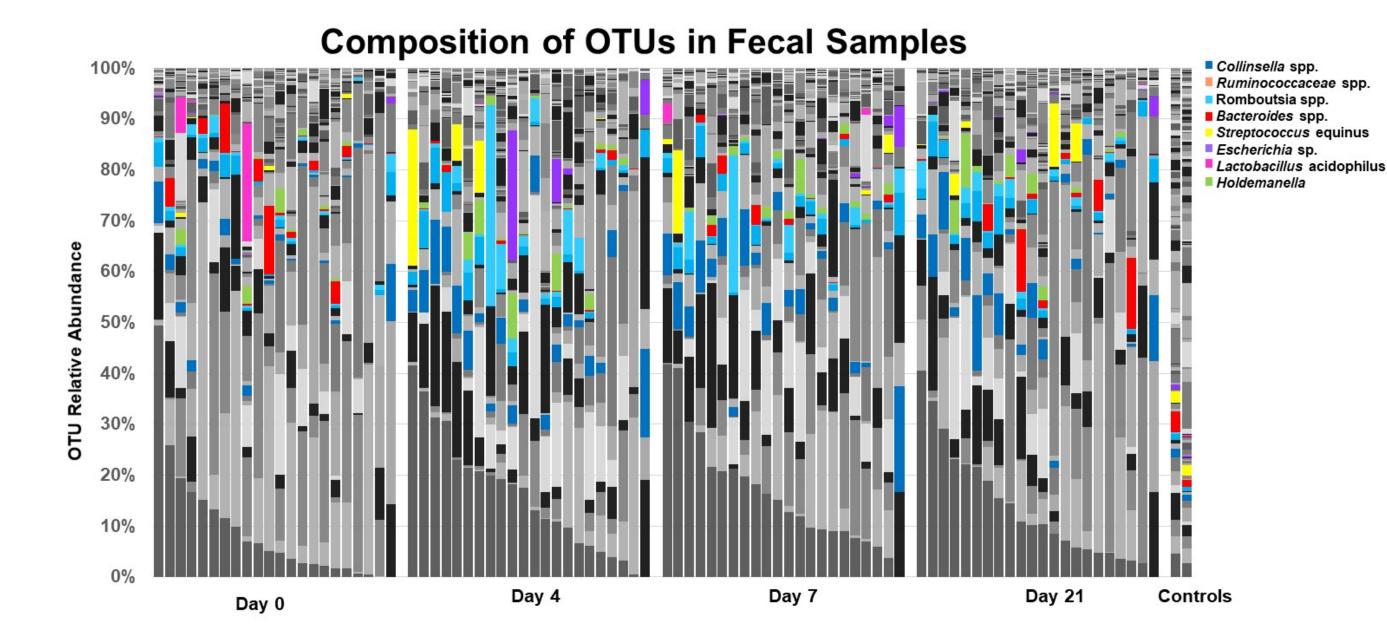


Figure 5. Stacked bar chart depicting the phyla (A) and OTUs (B) represented in fecal samples taken on day 0, 4, 7 and 21. Firmicutes were the most abundant phylum, followed by Bacteroidetes, Proteobacteria and Fusobacteria. Samples collected on Day 0 represent a different microbial diversity, but Days 4, 7 and 21 were stable.

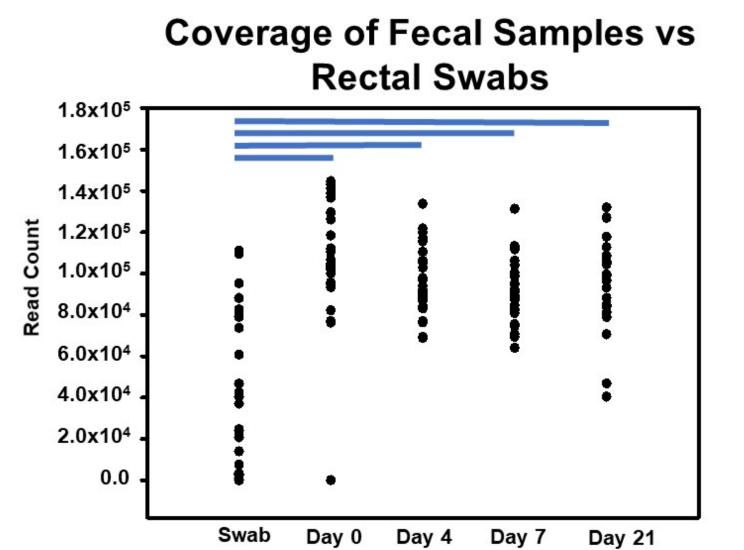
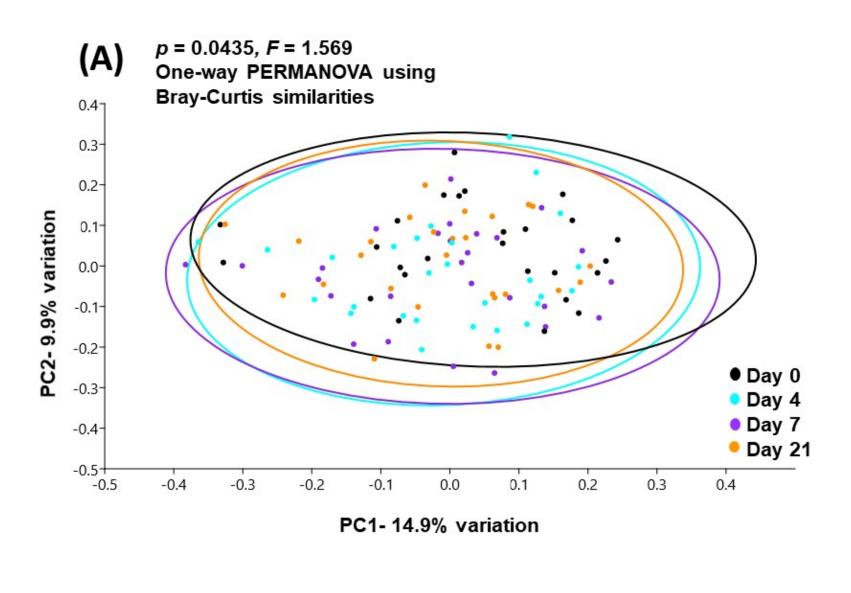


Figure 6. Dot plot of the read count of each sample grouped as swabs, Day 0, Day 4, Day 7 or Day 21. Bars indicate a significant difference between groups.



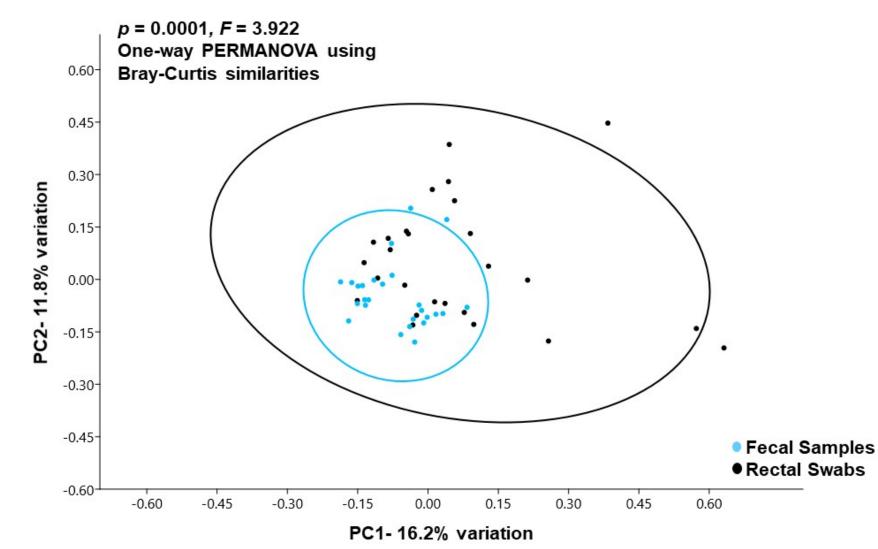


Figure 7. Principal coordinate analysis of rectal swabs vs. fecal samples.

	Day 0	Day 4	Day 7	Day 21
Day 0		0.004	0.006	0.156
Day 4	0.004		0.867	0.526
Day 7	0.006	0.867		0.930
Day 21	0.156	0.526	0.930	

Figure 8. Principal coordinate analysis plot **(A)** displaying only a significant difference in the composition of microbes on Day 0 to a *p*-value of at most 0.156 **(B)**. Days 4, 7 and 21 were all similar.

Conclusions

Rectal swabs reliably yielded poorer coverage than fecal samples and captured a different microbial diversity.

Rectal swabs contained higher percentages of Epsilonbacteraeota and Proteobacteria.

The fecal microbiome collected on Day 0 was different than those of Days 4, 7, and 21, possibly due to rectal exams performed on Day 0 or storage.

The fecal microbiome was stable over Days 4, 7 and 21.

Future Directions

- This study will serve as the healthy control group in a similar study design for comparison with dogs undergoing chemotherapy treatments.
- These additional investigations will aim to associate symptoms and treatment response with microbiome characteristics

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