



Characterization of the Shelter Feline Gut Microbiota and Optimization of Sample Collection Techniques

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Background

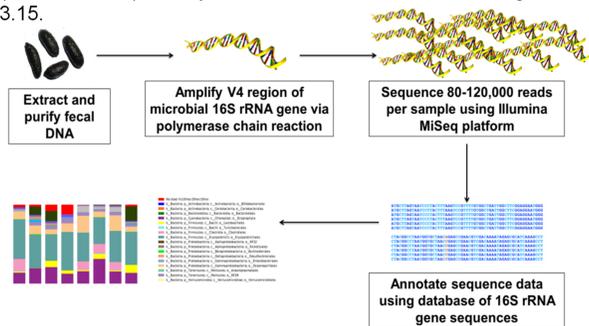
- The microbiome refers to the collection of organisms, their genomes, and the surrounding environmental conditions.
- Past research has explored the relationship between the gut microbiota (GM) and host health in humans, however, study of the feline microbiome is still in its infancy and more research is needed to investigate how environmental factors and disease influence the GM.
- We hypothesize that (1) felines exposed to related environments and disease states will have similar GM composition and (2) cat litter contains PCR inhibitors that are detrimental to obtaining quality metagenomics data.

Objectives

- The purposes of the present study are to:
 - characterize the GM of shelter and feral cats to determine what environmental or host health factors influence composition
 - assess whether litter contains components that interfere with the ability to obtain quality metagenomics data

Methods

- Fecal samples were collected and DNA was extracted with a commercially available PowerFecal[®] kit, amplified by PCR using conserved bacterial primers and subjected to next generation sequencing.
- Shelter and feral cats were assessed for parameters including shelter location, fecal score, and sex.
- To evaluate the impact of litter contamination, house cat fecal samples obtained from paper towel-lined litter pans are being spiked with litter and metagenomics data compared to that of unspiked samples.
- OTU's with reads <10,000 were excluded from the data. Differences between groups in beta-diversity were determined via one-way permutational multivariate analysis of variance (PERMANOVA) of Bray-Curtis and Jaccard distances using Past 3.15.



Variation Among Shelters

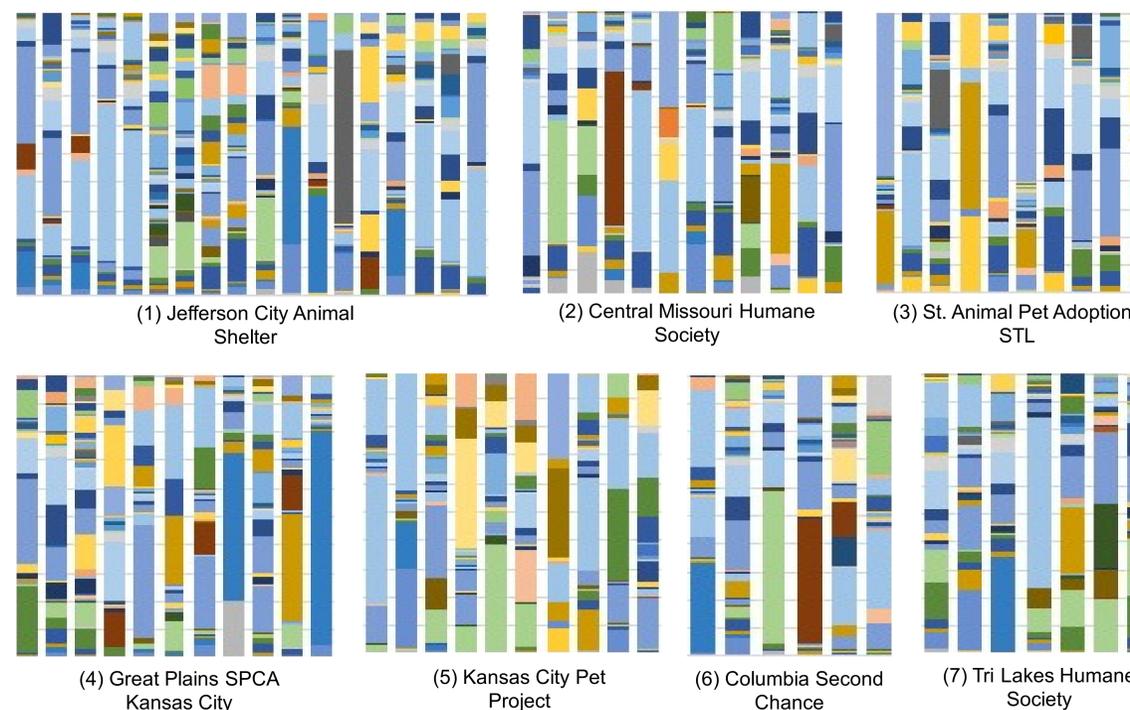


Figure 1: Stacked bar charts of 76 samples showing marked variation in GM at the operational taxonomic unit / species level.

Shelter Associated Differences in Fecal GM

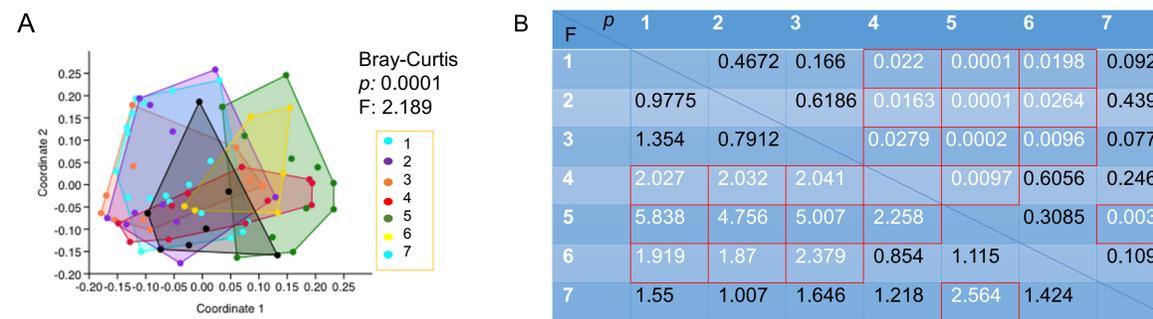


Figure 2: Analysis of samples collected at seven separate shelters. (A) Principal coordinate analysis shows clustering of samples taken at each location. (B) Results of a one-way PERMANOVA pairwise analysis revealed statistically significant differences between shelters, especially shelter 5 compared to shelters 1-7.

Fecal Score / Sex

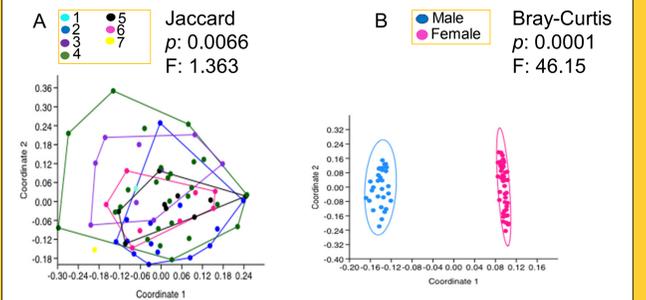


Figure 3: Principal coordinate analysis of fecal score (A) and sex (B). (A) shows no distinct clustering of samples suggesting that these factors do not strongly contribute to alteration or unique composition of GM in shelter felines while (B) shows distinct clustering indicating that sex significantly influences the GM composition of shelter felines.

Conclusions & Future Directions

- There was marked variation in fecal GM across all samples.
- GM composition was influenced by shelter and sex with the latter showing the most dramatic influence.
- it is unclear if other parameters measured such as behavior, body condition, diet, etc., correlate to changes in GM due to underpowered sample size.
- PowerFecal[®] extraction technique, compared to isopropanol, resulted in marked improvement of our ability to generate interpretable sequencing data.
- We are awaiting data on feral samples and house cat spiked samples to evaluate the presence of PCR inhibitors.
- This research will provide critical information about factors that can modulate GM in shelter and feral cats, which in turn may lead to novel strategies to prevent and treat intestinal diseases in these populations.

Acknowledgements

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