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# Characterization of the Canine Aural Microbiome

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## Introduction

- Ear infections are consistently ranked as a top health concern for dogs in veterinary clinics and shelters.
- Standard diagnostic methods for directing treatment of ear infections focus on single identifiable agents, and may be misleading.
- More reliable methods to characterize other microbial inhabitants of the ear are commonly called for in the literature.
- Understanding the canine aural microbiome and its role in ear health and disease will improve our ability to treat ear infections and, in turn, canine welfare.

## Hypothesis

- Different DNA extraction methods will differ in ability to adequately prepare aural swab samples for sequencing analysis.
- Variables such as breed, environment, and health status will affect the canine aural microbiome.

## Objectives

- Compare established DNA extraction methods for preparation of aural swab samples for DNA sequencing
- Characterize the canine aural microbiome

## Methods

- The University of Missouri IACUC approved our protocol.
- Care was taken to minimize discomfort for the dogs – if signs of distress were noted, sampling ended immediately.
- Our sampling technique was chosen for its ease and feasibility in normal clinical practice, as well as similarity to established methods for aural sample collection.

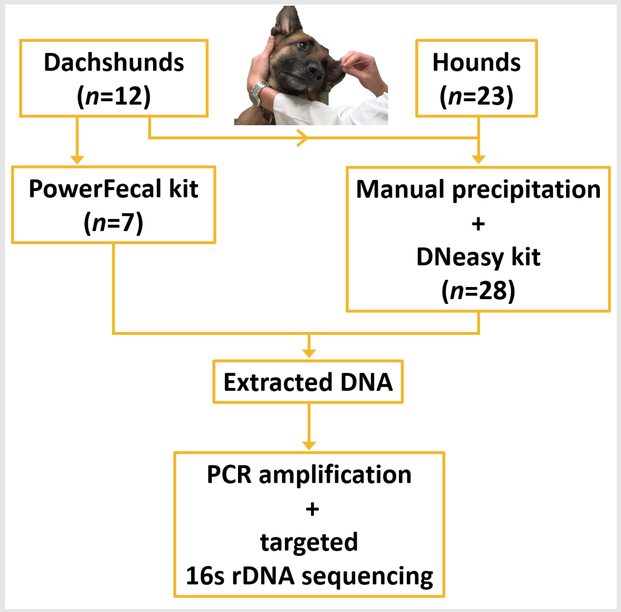


**Figure 1.** Flowchart depicting sample processing. A polyester-tipped swab was used to sample the deepest possible part of the vertical canal. Samples were then prepared for sequencing using one of two different methods (Qiagen PowerFecal kit or benchtop manual DNA precipitation followed by Qiagen DNeasy kit). Subsequent processing of PCR amplification and targeted 16s rDNA sequencing was identical between groups.

## Read Counts between Methods

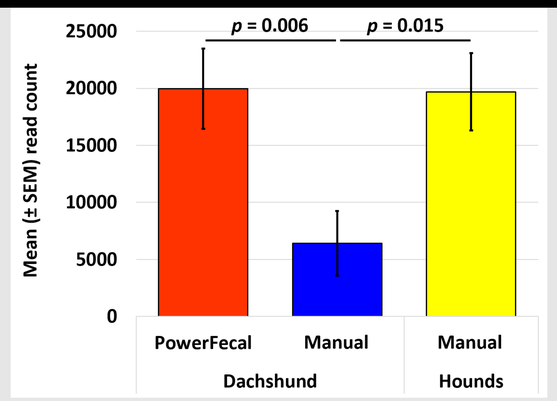


**Figure 2.** Mean (± SEM) read count of samples derived from different processing methods, and from different dog breeds. *p* values represent pairwise comparisons using Kruskal-Wallis one-way ANOVA on ranks with Dunn's methods for pairwise comparisons. PowerFecal preparation resulted in significantly higher mean read counts compared to manual preparation in the Dachshund population, but was similar in mean read count to the manual-processed samples from the hounds.

## Family Relative Abundance, Dachshunds

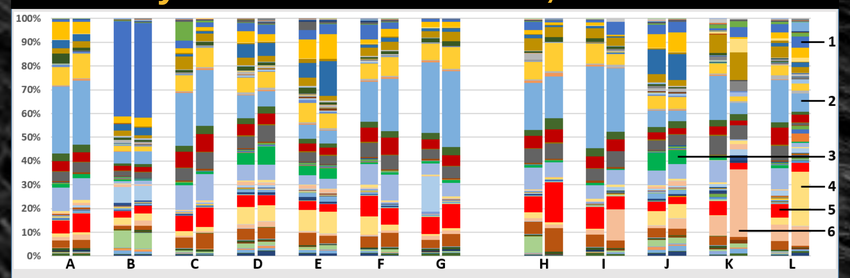


**Figure 3.** Stacked bar charts showing relative abundance of all families detected in samples from Dachshunds, extracted using either PowerFecal kits (dogs A to G) or a manual nucleic acid precipitation followed by purification using DNeasy kits (dogs H to L). Each dog is labeled with a letter A-G, with individuals' left and right ears grouped together. Labels at right indicate families of interest: 1 = *Moraxellaceae*, 2 = *Erysipelotrichaceae*, 3 = *Streptococcaceae*, 4 = *Porphyromonadaceae*, 5 = *Prevotellaceae*, 6 = *Muribaculaceae*.

## Family Relative Abundance, Hounds

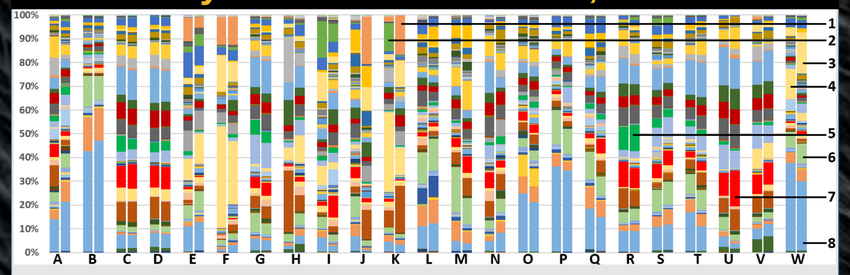


**Figure 4.** Stacked bar charts showing relative abundance of all families detected in samples from hound mixes, extracted using manual nucleic acid precipitation followed by purification with DNeasy kits. Each dog is identified with a letter A-W, with individuals' left and right ears grouped together. Labels at right indicate families of interest: 1 = *Anaeroplasmataceae*, 2 = *Pseudomonadaceae*, 3 = *Enterobacteriaceae*, 4 = *Enterococcaceae*, 5 = *Streptococcaceae*, 6 = *Micrococcaceae*, 7 = *Prevotellaceae*, 8 = *Corynebacteriaceae*.

## Family Relative Abundance, All Samples

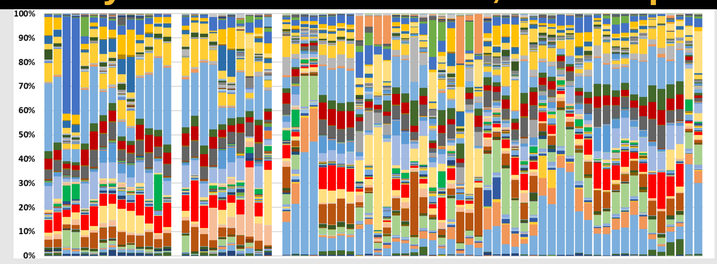


**Figure 5.** Stacked bar charts showing relative abundance of all families detected in all samples (Dachshunds, left and middle groups; hounds, right group).

## Similarities between Groups, Unweighted

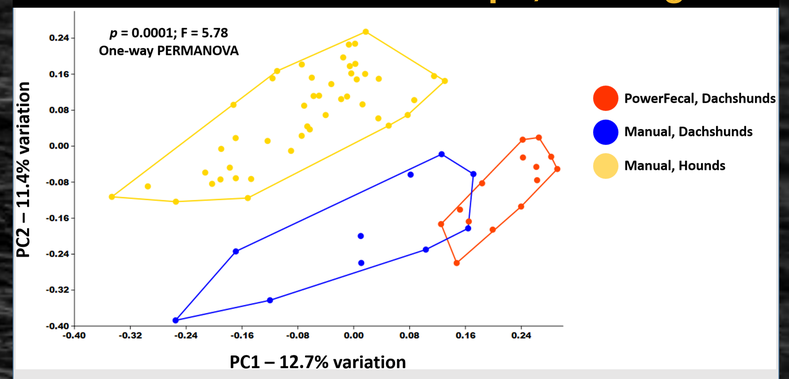


**Figure 6.** Principal coordinate analysis performed using Jaccard (unweighted) similarity demonstrates distinct community composition among groups (one-way PERMANOVA). Legend shown at right.

## Similarities between Groups, Weighted

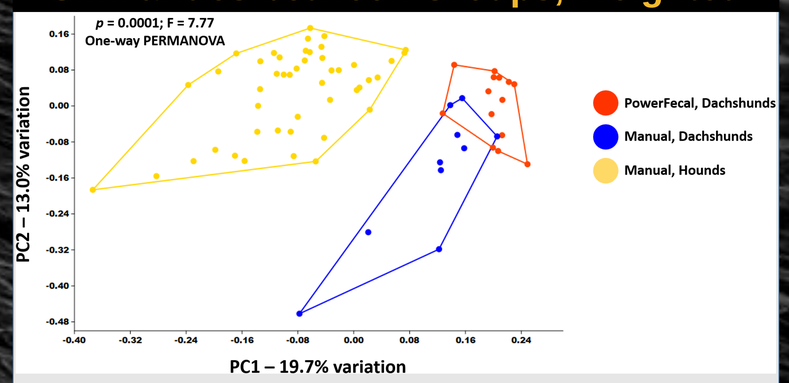


**Figure 7.** Principal coordinate analysis performed using Bray-Curtis (weighted) similarity demonstrates distinct community composition among groups (one-way PERMANOVA). Legend shown at right.

## Conclusions

- Choice of DNA extraction method for aural samples may require consideration of breed and other factors.
- Canine aural microbiome composition is highly variable among individuals but may cluster with factors such as breed.

## Future Work

- Compare efficacy of the two methods of DNA extraction in additional populations
- Identify potential factors contributing to the variation observed in these aural microbiome populations
- Assess the impact of ear health, treatments, and environment on the aural microbiome through sampling of other research laboratory and shelter populations
- Determine the diagnostic utility of aural microbiome profiles

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