Title: Investigation of the fecal microbiome of raccoons (*Procyon lotor*) in a captive environment

Authors: Kelsey Holland, Mikaela Jahncke, Lisanework Ayalew, Shivani Ojha

Affiliations: Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE (Holland, Ayalew, Ojha).
Hope for wildlife rehabilitation and education center, Seaforth, Nova Scotia (Jahncke)

Abstract

The gut microbiome of raccoons is rarely studied despite its importance in the health of raccoons and its implications on the wild and urban ecosystems. Thus, the goal of this study was to explore the fecal microbiome of raccoons based on 16S rRNA metagenomic sequencing. A short longitudinal study was conducted in two phases, involving 15 raccoons housed at a wildlife facility in Nova Scotia. The first phase included juvenile raccoons, while the second phase was comprised of 12-14 weeks old animals that received medications. The study animals received different food formulations. Naturally voided fecal samples were collected in sterile containers and stored at -80ºC until further processing. Genomic DNA was extracted from each fecal sample. 16S rRNA gene sequence libraries were prepared using the Oxford Nanopre Rapid Sequencing DNA-16S barcoding kit as per the company's instructions. DNA libraries were sequenced using the Mk1b MinIon instrument. Sequence processing and taxonomic classification were performed using the EPI2ME bioinformatic platform. No significant differences in alpha or beta diversity were observed between samples collected from male and female raccoons ($p > 0.05$). However, beta-diversity comparisons showed significant difference in bacterial composition in animals fed Formula-bowls versus Formula diet ($p < 0.05$). The majority of animals that were fed Formula-bowls had a higher proportion of *Ligilactobacillus* and *Streptococcus* and less numbers of anaerobes. In contrast, most of the Formula-fed raccoons carried a higher proportion of *Escherichia*, *Shigella* and *Clostridium* species. Our results highlight the significant impact of diet on the gut microbial composition of raccoons. Further studies are required to understand type of diet and associated differences in microbial diversity in the context of host health. When completed, the gut microbiome analyses of phase 2 of the study will further our understanding of the bacterial community dynamics of raccoons in a captive environment.

Financial support: AVC research fund
Student Support: VetSRA and AVC research fund