THE FECAL MICROBIOME OF DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY

A Isaiah, JC Parambeth, JM Steiner, JS Suchodolski

Gastrointestinal Laboratory, Texas A&M University, College Station, Texas, USA

Abstract

Exocrine pancreatic insufficiency (EPI) in dogs is a syndrome of inadequate synthesis and secretion of pancreatic enzymes, which leads to clinical signs of maldigestion. Previous reports show that small intestinal bacterial dysbiosis occurs in dogs with EPI and is reversed with pancreatic enzyme therapy. However, there are no studies evaluating the fecal microbiome of dogs with EPI. The objective of this study was to compare the fecal microbiome of healthy dogs (n=18), untreated (n=7) dogs with EPI, and dogs with EPI treated with enzyme replacement therapy (n=19).

To be included into the study, the dogs had to be at least 1 year of age, have clinical signs of EPI, a serum cTLI concentration ≤2.5 µg/L, and be free from any other concurrent disease. Three naturally voided fecal samples collected over three consecutive days were frozen immediately after collection and pooled. Fecal samples were collected in a similar manner from healthy dogs without any clinical signs of gastrointestinal disease. Extracted DNA from fecal samples was used for Illumina sequencing of the bacterial 16S rRNA gene and analyzed using Quantitative Insights Into Microbial Ecology (QIIME). The analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Luton, UK) was used on the unweighted UniFrac distance matrix to determine if any groups of samples contained significantly different bacterial communities. There was a significant difference in fecal microbial communities when healthy dogs were compared to treated (p=0.001) and untreated (p=0.001) dogs with EPI. Quantitative Insights Into Microbial Ecology (QIIME). The analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Luton, UK) was used on the unweighted UniFrac distance matrix to determine if any groups of samples contained significantly different bacterial communities. There was a significant difference in fecal microbial communities when healthy dogs were compared to treated (p=0.001) and untreated (p=0.001) dogs with EPI. Alpha diversity was significantly decreased in untreated and treated EPI dogs when compared to the healthy dogs (p<0.01). The families Bifidobacteriaceae (p=0.006), Enterococcaceae (p=0.035), and Lactobacillaceae (p=0.001) were significantly increased in the untreated and treated dogs with EPI when compared to healthy dogs. In contrast, Lachnospiraceae (p<0.001), and Blautia (p<0.001) were significantly decreased in dogs with EPI.

In conclusion, this study suggests that the fecal microbiome of dogs with EPI (both treated and untreated) is different from that of healthy dogs.

Background

- Exocrine pancreatic insufficiency (EPI) is a syndrome characterized by the inadequate synthesis and secretion of pancreatic digestive enzymes resulting in maldigestion.[1]
- Previous reports show that small intestinal bacterial dysbiosis occurs in dogs with EPI and is reversed with pancreatic enzyme therapy. [2]

Objective

The objective of this study was to compare the fecal microbiome of healthy dogs (n=18), untreated (n=7) dogs with EPI, and dogs with EPI treated with enzyme replacement therapy (n=19).

Materials and methods

Sampling population

- Surplus fecal samples from healthy dogs (n=18), untreated (n=7) dogs with EPI, and dogs with EPI treated with enzyme replacement therapy (n=19), enrolled in an unrelated clinical trial at the Gastrointestinal Laboratory were utilized.
- Inclusion criteria:
  - > 1 year of age
  - clinical signs of EPI (i.e., polyphagia, weight loss, steatorrhea, and/or loose, voluminous and/or malodorous stools)
  - serum cTLI concentration ≤2.5 µg/L
  - not be pregnant or lactating
  - free from any clinically apparent disease other than EPI.
- Three naturally voided fecal samples collected over three consecutive days were used.
  - fecal samples were frozen immediately after collection.

Sample analysis

- DNA was extracted with MoBio Power soil® DNA isolation kit (MoBio Laboratories, USA) following the manufacturer's instructions.

Materials and methods

Sample analysis

- Extracted DNA from fecal samples was used for Illumina sequencing of the bacterial 16S rRNA gene and analyzed using Quantitative Insights Into Microbial Ecology (QIIME).
- The analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Luton, UK) was used on the unweighted UniFrac distance matrix to determine if any groups of samples contained significantly different bacterial communities.

Results

- There was a significant difference in fecal microbial communities when healthy dogs were compared to treated (p=0.001) and untreated (p=0.001) dogs with EPI.
- Alpha diversity was significantly decreased in untreated and treated EPI dogs when compared to the healthy dogs (p<0.01).
- The families Bifidobacteriaceae (p=0.006), Enterococcaceae (p=0.035), and Lactobacillaceae (p=0.001) were significantly increased in the untreated and treated dogs with EPI when compared to healthy dogs.
- In contrast, Lachnospiraceae (p<0.001), and Blautia (p<0.001) were significantly decreased in dogs with EPI.

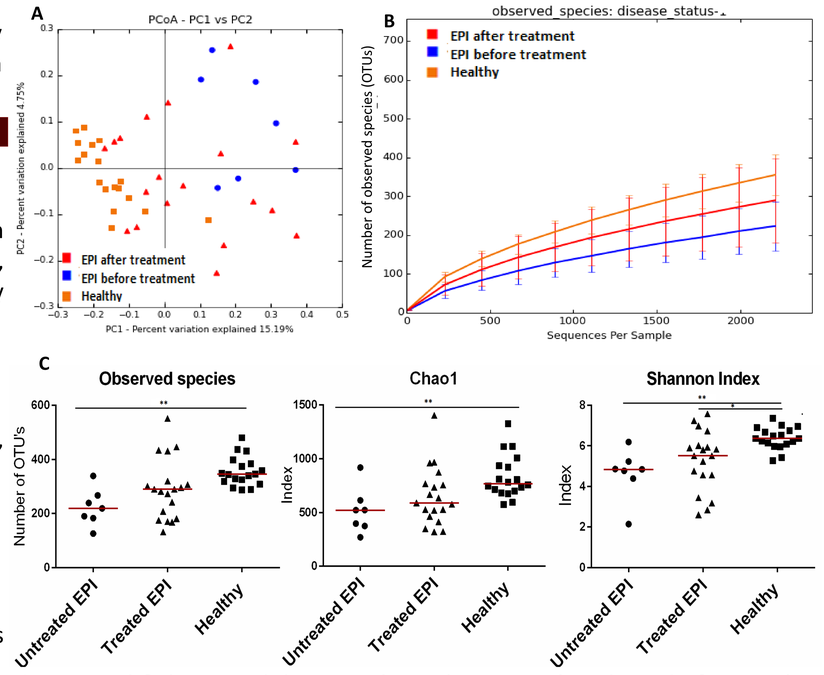

Figure 1: A) β diversity: (A) principal coordinates analysis (PCoA) of unweighted UniFrac distances of 16S rRNA genes. (B)Alpha rarefaction curve showing number of observed species at a depth of 2200 sequences/sample, error bars show standard deviation. (C) Comparisons of α diversity. Red lines represent the median for each measure. *p < 0.05; and **P < 0.01

Results

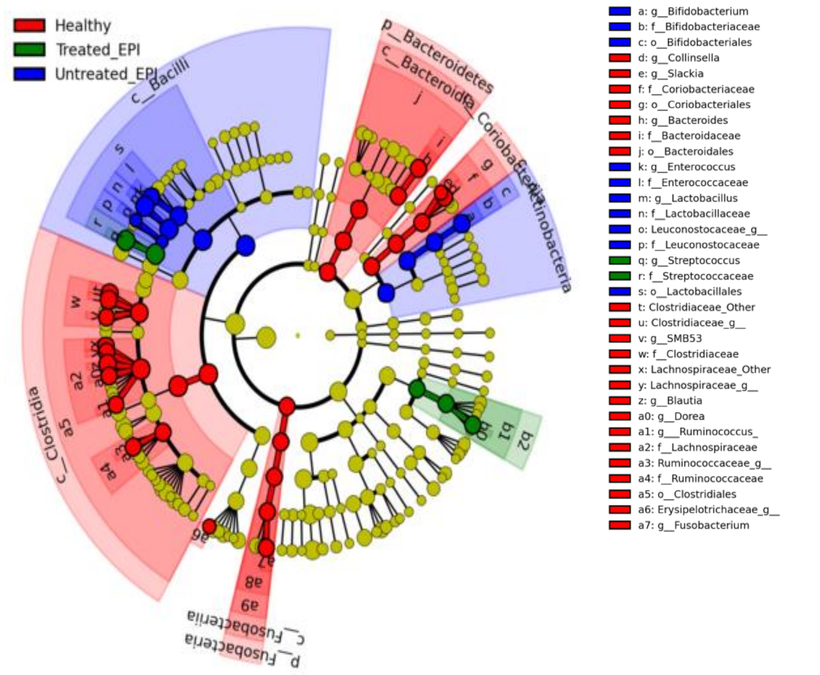

Figure 2: Taxonomic cladogram obtained using Linear discriminant analysis (LDA) effect size (LEFSe) based on 16S rRNA gene sequences and identifies the most differentially abundant taxa in treated and untreated dogs with EPI and healthy dogs.

Discussion and Conclusion

- This study suggests that the fecal microbiome of dogs with EPI (both treated and untreated) is different from that of healthy dogs.
- Further studies are necessary to elucidate the mechanisms that are responsible

References

1. Steiner JM. Exocrine Pancreas. In: Steiner JM, ed. *Small Animal Gastroenterology*, Schlütersche Verlagsgesellschaft mbH & Co. KG, Hannover, 294-299, 2008.
2. Westermarck E, Wiberg M, Exocrine pancreatic insufficiency in dogs. Vet Clin North Am Small Anim Pract 33, 1165-1179, 2003.

Disclosure

The authors disclose no conflicts of interest.

Anitha Isaiah
Graduate Assistant Research
Gastrointestinal Laboratory
AIsaiah@cvm.tamu.edu

VETERINARY MEDICINE & BIOMEDICAL SCIENCES
TEXAS A&M UNIVERSITY