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The fecal microbiome of dogs with exocrine pancreatic insufficiency

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ABSTRACT

Exocrine pancreatic insufficiency (EPI) in dogs is a syndrome of inadequate synthesis and secretion of pancreatic enzymes. 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 evaluate the fecal microbiome of dogs with EPI. Three day pooled fecal samples were collected from healthy dogs (n = 18), untreated (n = 7) dogs with EPI, and dogs with EPI treated with enzyme replacement therapy (n = 19). 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) and PICRUSt was used to predict the functional gene content of the microbiome. Linear discriminant analysis effect size (LEfSe) revealed significant differences in bacterial groups and functional genes between the healthy dogs and dogs with EPI.

There was a significant difference in fecal microbial communities when healthy dogs were compared to treated and untreated dogs with EPI (unweighted UniFrac distance, ANOSIM $P = 0.001$, and 0.001 respectively). Alpha diversity was significantly decreased in untreated and treated EPI dogs when compared to the healthy dogs with respect to Chao1, Observed OTU, and Shannon diversity ($P = 0.008$, 0.003, and 0.002 respectively). The families Bifidobacteriaceae ($P = 0.005$), Enterococcaceae ($P = 0.018$), 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 Ruminococcaceae ($P < 0.01$) were significantly decreased in dogs with EPI. Dogs with EPI (before treatment) had significant increases in functional genes associated with secretion system, fatty acid metabolism, and phosphotransferase system. In contrast, healthy dogs had a significant increase in genes related to phenylalanine, tyrosine and tryptophan biosynthesis, transcription machinery and sporulation.

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

1. Introduction

Exocrine pancreatic insufficiency (EPI) in dogs is characterized by the inadequate production of digestive enzymes by pancreatic acinar cells, which leads to maldigestion and malabsorption of nutrients. Clinical signs of dogs with EPI include weight loss despite polyphagia, steatorrhea, loose and voluminous, and/or malodorous stools [1–4]. A clinical suspicion is confirmed by the measurement of canine serum trypsin like immunoreactivity (cTLI), and a concentration of less than or equal to 2.5 μg/L is diagnostic for EPI [5]. EPI is a relatively common pancreatic disease in dogs with an estimated prevalence of approximately 7–9% of dogs tested using the canine trypsin like immunoreactivity assay (cTLI) [6–8]. While this disease can affect any breed, certain breeds like German shepherd dogs (GSD) and Rough-coated collies are predisposed [5,7].

The most common cause of EPI in dogs is pancreatic acinar atrophy. Other reasons include chronic pancreatitis and pancreatic neoplasia [9]. EPI in dogs seems to be a unique disease when compared to this disease in other species. Unlike in dogs, the most frequent cause of EPI in cats and humans is chronic pancreatitis [10,11]. EPI in humans has also been reported to co-occur with other conditions like cystic fibrosis, Johanson-Blizzard syndrome and Shwachman-Diamond syndrome [12–15], but these have not been reported in dogs so far.

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The main treatment for EPI in dogs is oral pancreatic enzyme replacement therapy, which is given with every meal. Affected dogs require lifelong therapy and about 60–65% of dogs have a good initial response to enzyme therapy alone. About 17–20% of dogs with EPI has a poor response to enzyme therapy alone [7,16]. Therefore additional measures such as, administration of antibiotics, antacids, and dietary interventions may be necessary based on the patient's initial response to enzyme supplementation [9,17]. Unfortunately euthanasia due to a failure to respond to treatment is a common outcome [18].

Culture based methods have shown that small intestinal dysbiosis previously referred to as small intestinal bacterial overgrowth (SIBO) does occur in dogs [1,4] and humans [19] with EPI. This has been attributed to the increased availability of undigested food material in the small intestinal lumen, lack of antibacterial factors in the pancreatic juice, changes in intestinal motility, and possibly altered gastrointestinal immune function [1,2]. Previous studies show that small intestinal dysbiosis improves with pancreatic enzyme supplementation [1] and in the absence of an adequate response, tylosin administration reduces the small intestinal dysbiosis [2]. Small intestinal bacterial overgrowth has been previously described to occur in GSDs [20]. The advent of culture independent molecular methods has deepened our understanding of the microbial alterations in various canine gastrointestinal diseases [21] and has identified numerous bacteria that were previously uncultureable from the gastrointestinal contents and feces of subjects using conventional culture based techniques. Previous studies have shown differences in the fecal microbiome of dogs with acute diarrhea and inflammatory bowel disease [22,23]. To our best knowledge, there are very few studies published that have used culture independent molecular methods to study the dysbiosis that occurs with EPI in dogs and other hosts. In this study, we aimed to describe the fecal microbiome and predict the functional potential of the microbiota in dogs with EPI when compared to healthy dogs, and to investigate if healthy German Shepherd dogs had a microbiome different from healthy dogs of other breeds.

2. Methods

2.1. Study population

Fecal samples were collected from client owned dogs with spontaneously occurring EPI and staff owned healthy dogs. This study was part of another clinical trial approved by the Clinical Research Review Committee at Texas A&M University and the study protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University (AUP 2011-84) & IACUC 2014-0094 CA.

Inclusion criteria for dogs with EPI were: a serum cTLI concentration $\leq$2.5 μg/L, which is considered to be diagnostic for EPI for this species, age $\geq$1 year, clinical signs of EPI (polyphagia, weight loss, steatorrhea, and/or loose, voluminous, and/or malodorous stools), and the absence of other concurrent diseases. The dogs with EPI were further divided into two groups; those that were treated with enzyme supplementation (EPI + E) and those that were untreated (EPI-E).

The control group consisted of healthy pet dogs; all the dogs were older than 1 year, free from any clinically apparent disease and were not pregnant or lactating. None of the healthy dogs had a history of gastrointestinal symptoms or antibiotic administration for at least a month prior sample collection, while five dogs in the EPI group (n = 5) were on antibiotics. Table 1 summarizes the basic characteristics of the dogs in the study.

2.2. Sample collection, DNA extraction and 16S rRNA sequencing

Three naturally voided fecal samples were collected on three consecutive days to account for variability. The samples were frozen immediately after collection, and transported while they were still frozen. On arrival to the laboratory, samples were thawed at room temperature, pooled, and then an aliquot was used for DNA extraction using a MoBio Power soil DNA isolation kit (MoBio Laboratories, USA) following the manufacturer's instructions. Illumina sequencing of the bacterial 16S rRNA genes was performed using primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) to 806R (5′-GGACTACVSGGGTATCTAAT-3″) at the MR DNA laboratory (www.mrdnalab.com, Shallowater, TX, USA).

2.3. Analysis of 16S rRNA genes

Sequences were processed and analyzed using Quantitative Insights Into Microbial Ecology (QIIME) v 1.8 [24]. The raw sequences were uploaded to NCBI Sequence Read Archive under the accession number SRP091334. The sequence data was demultiplexed, and then quality filtered using the default settings for QIIME. Chimeras were detected and filtered from the reads using USEARCH [25] against the 97% clustered representative sequences from the Greengenes v 13.8 database [26]. The remaining sequences were clustered into Operational Taxonomic Units (OTUs) by using an open reference approach in QIIME [26]. Prior to downstream analysis, sequences assigned as chloroplast, mitochondria, and low abundance OTUs, containing less than 0.01% of the total reads in the dataset were removed.

All samples were rarefied to 2,180 sequences per sample to account for unequal sequencing depth. The rarefaction depth was based on the lowest read depth of samples to have the optimum combination between number of sequences and number of samples in the diseased group. Alpha diversity was measured with the Chao1 (richness), Shannon diversity, and observed OTU metrics. Beta diversity was evaluated with the phylogeny based UniFrac [27] distance metric and visualized using Principal Coordinate Analysis (PCoA) plots.

2.4. PICRUSt

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was used to predict functional gene content based on 16S rRNA gene data present in the Greengenes database and the KEGG database [28]. PICRUSt was used in the

Table 1
Dog characteristics.

	Healthy	EPI- E	EPI + E
Number	18	7	19
Age in years (mean ± SD)	6.81 ± 3.6	2.44 ± 1.35	3.85 ± 3.21
Gender (male/female)	(8/10)	(5/2)	(5/14)
Breed	GSD = 7; other breeds = 11	GSD = 4; other breeds = 3	GSD = 8; other breeds = 11