patients with diarrhea. Future efforts to identify mechanisms responsible for susceptibility to EPEC-induced diarrhea will lead to advanced understanding of EPEC pathogenesis in kittens and children.

GI33

DEVELOPMENT AND ANALYTICAL VALIDATION OF AN ASSAY FOR THE QUANTIFICATION OF CANINE FECAL BILE ACIDS. B.C. Guard[1], M.M. Jonika[2], J.B. Honneffer[1], J.A. Lidbury[2], J.M. Steiner[1], J.S. Suchodolski[2]. [1]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College station, Texas, USA, College Station, TX, USA, [2]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA

The gut microbiota is important in maintaining intestinal health. Bile acids are increasingly appreciated to play a role in regulation of gut microbial composition and intestinal health. Bile acids are synthesized from cholesterol, conjugated in the liver, and once secreted into the gastrointestinal tract (GIT), undergo modification by certain members of the intestinal microbiota. Numerous bile acid receptors (e.g., farnesoid X receptor and G protein-coupled membrane receptor) have been identified along the GIT and are responsible for regulating metabolism and maintaining an anti-inflammatory environment in the gut. The aim of this study was to develop and analytically validate a gas chromatography/mass spectrometry (GC/MS) assay for the identification and quantification of bile acids in canine feces.

Fecal bile acids (cholic acid [CA], chenodeoxycholic acid [CDCA], lithocholic acid [LCA], deoxycholic acid [DCA], and ursodeoxycholic acid [UDCA]) were measured in their unconjugated form after undergoing butyl esterification for chromatographic separation. A capillary DB-1 ms Ultra Inert column was used with a 20:1 split sample injection ratio. Validation parameters included the lower and upper limits of quantification (LLOQ and ULOQ, respectively). Additionally, precision of the assay was calculated by assaying 6 aliquots taken from a single fecal sample from 4 dogs on the same run/day followed by calculating intra-assay coefficients of variation (CV%). Reproducibility of the assay was determined by analyzing 6 aliquots taken from a single fecal sample from 4 dogs on 6 consecutive days followed by calculating inter-assay variation (CV%).

The LLOQ and ULOQ in μg/mL were as follows for each compound: cholic acid (3.9 and 1000), chenodeoxycholic acid (6.25 and 200), lithocholic acid (1.9 and 500), deoxycholic acid (31.3 and 1000), and ursodeoxycholic acid (0.78 and 50). For intra-assay variability, the average CV%s were: 6.0, 5.6, 7.1, 7.3, and 8.8% for CA, CDCA, LCA, DCA, and UDCA, respectively. For inter-assay variability, the average CV%s were: 8.3, 8.0, 4.8, 8.6, and 13.2% for CA, CDCA, LCA, DCA, and UDCA, respectively.

In conclusion, the present assay was found to be both reproducible and precise for the quantification of select bile acids in canine feces.

GI34

LONGITUDINAL CHARACTERIZATION OF THE FECAL METABOLOME IN DOGS WITH INFLAMMATORY BOWEL DISEASE. B.C. Guard[1], M.M. Jonika[2], J.B. Honneffer[1], J.A. Lidbury[2], J.M. Steiner[1], A.E. Jergens[3], J.S. Suchodolski[2]. [1]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College station, Texas, USA, College Station, TX, USA, [2]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, [3]Iowa State University, College of Veterinary Medicine, Ames, IA, USA

Canine inflammatory bowel disease (IBD) is a multifactorial disease, the pathogenesis of which includes alterations in gut microbiota and improper activation of the immune system. Recent studies have used untargeted metabolomics of serum and feces to investigate patients with chronic gastrointestinal (GI) disease. However, evidence is lacking about how GI inflammation and ongoing microbial dysbiosis affect metabolites long-term in patients that undergo immunosuppressive therapy. Therefore, the purpose of this study was to characterize the fecal metabolome in dogs with IBD upon initial diagnosis and after therapy, using an untargeted approach.

Nine dogs that were non-responsive to dietary or antibiotic therapy and had histologically confirmed intestinal inflammation were enrolled. Fecal samples were collected at baseline, 3 weeks, and 8 weeks. Patients received immunosuppressive therapy after initial diagnosis and clinical signs were scored according to the canine IBD activity index (CIBDAI). Fecal samples were also collected from healthy dogs ($n = 13$) to serve as controls. Fecal samples were analyzed by an untargeted metabolomics platform using gas chromatography coupled with mass spectrometry. Data were found to be non-parametric. Therefore, comparisons were made across time points using a Friedman's test for repeated measures. A Dunn's post-test was used where appropriate. *P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg False Discovery Rate and significance was set at $P < 0.05$.

Multivariate testing consisted of principal component analysis that revealed significant differences between healthy controls and dogs with IBD at baseline, 3 weeks, and 8 weeks after therapy. The median CIBDAI score for patients 8 weeks following initial diagnosis was 0 compared to a median CIBDAI score of 7 at enrollment. Univariate analysis revealed that of the 233 metabolites measured, trans-4-hydroxyproline, sinapinic acid, glycine, 2-methylglyceric acid, stearic acid, heptadecanoic acid, myo-inositol, and dehydroabietic acid were found to be significantly different among the repeated time points sampled from dogs with IBD ($P < 0.05$).

In conclusion, distinct changes in metabolic profiles were observed between healthy dogs and dogs with IBD. Despite improvement in clinical activity scores, several metabolites remained altered at 8 weeks follow up.

GI35

LONGITUDINAL ASSESSMENT OF FECAL STEROL AND FATTY ACID CONCENTRATIONS IN DOGS WITH DIARRHEAL DISEASES. J.B. Honneffer[1], B.C. Guard[1], S. Unterer[2], F. Bresciani[3], S.A. Wennogle[4], J.A. Lidbury[5], J.M. Steiner[1], A.E. Jergens[6], J.S. Suchodolski[5]. [1]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [2]Clinic of Small Animal Medicine, LMU Munich, Germany, Munich, Bayern, Germany, [3]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Emilia-Romagna, Italy, [4]College of Veterinary Medicine, Colorado State University, Fort Collins, CO, Fort Collins, CO, USA, [5]Gastrointestinal Laboratory, Texas A&M University, College station, TX, USA, [6]Iowa State University, College of Veterinary Medicine, Ames, IA, USA

Sterols and fatty acids play an essential role as building blocks for structural components, as signaling molecules, and in energy metabolism, yet can also be toxic to cells at increased concentrations. Increased cholesterol and decreased phytosterol concentrations in the feces of dogs with chronic enteropathy have previously been reported. This study aimed to explore concentrations of fecal sterols and fatty acids in dogs exhibiting a wide variety of gastrointestinal disease phenotypes.

Baseline fecal samples were collected from dogs with acute hemorrhagic diarrhea syndrome (AHDS, $n = 22$), food-responsive diarrhea (FRD, $n = 10$), steroid-responsive diarrhea (SRD, $n = 24$), and healthy control dogs ($n = 82$). In a subset of diseased dogs, follow-up samples were collected 2–3 months after the baseline sample (AHDS, $n = 7$; FRD, $n = 6$; SRD, $n = 9$). Diagnoses of FRD and SRD were based on response to therapy. All dogs with FRD were successfully managed with the same vegetarian diet. Feces were analyzed by gas chromatography/mass spectrometry (GC/MS) using an in-house assay. At each of the two timepoints, a Kruskal-Wallis test and the Benjamini-Hochberg step-up method to adjust for multiple comparisons were used to identify significantly altered compounds. Dunn's test was used to compare groups, and statistical significance was set at $P < 0.05$.

At baseline, dogs with AHDS exhibited decreased fecal phytosterol (i.e., β-sitosterol, campesterol, sitostanol, fucosterol, and