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.

G133 DEVELOPMENT AND ANALYTICAL VALIDATION OF AN ASSAY FOR THE QUANTIFICATION OF CANINE FECAL BILE ACIDS. B.C. Guard1, M.M. Jonika2, J.B. Honnelfer1, J.A. Lidbury2, J.M. Steiner1, A.E. Jergens3, J.S. Suchodolski2. 1Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA, 2College Station, TX, USA, 3Gastrointestinal 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% 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% 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.

G134 LONGITUDINAL CHARACTERIZATION OF THE FECAL METABOLOME IN DOGS WITH INFLAMMATORY BOWEL DISEASE. B.C. Guard1, M.M. Jonika2, J.B. Honnelfer1, J.A. Lidbury2, J.M. Steiner1, A.E. Jergens3, J.S. Suchodolski2. 1Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA, 2College Station, TX, USA, 3Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, 4Towa 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 differences 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.

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.

G135 LONGITUDINAL ASSESSMENT OF FECAL STEROL AND FATTY ACID CONCENTRATIONS IN DOGS WITH DIARRHEAL DISEASES. J.B. Honnelfer1, B.C. Guard2, S. Unterter1, P. Brese1, A. Wömmelhu1, J.M. Steiner1, A.E. Jergens3, J.S. Suchodolski2. 1Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, 2Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, 3Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell’Emilia, Emilia-Romagna, Italy, 4College of Veterinary Medicine, Colorado State University, Fort Collins, CO, Fort Collins, CO, USA, 5College of Veterinary Medicine, Colorado State University, College Station, TX, USA, 6Towa 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 IBD and FRD were based on response to therapy. All AHDS and 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...