

serum incubation with *E. coli* isolates was not different between IBD and healthy animals.

In addition, when compared to bacteria from normal control animals, IBD fecal bacteria significantly stimulated higher levels of phagocytosis in canine primary macrophage ($P = 0.02$) as well as in DH82 cells; canine macrophage tumor cell line ($P = 0.04$). The upregulation of surface activation markers (MHC II, CD40, CD86 and CD80) in response from both groups was comparable. Phagocytosis of IBD gut bacteria activated macrophages and stimulated higher production of TNF- α than normal gut bacteria ($P = 0.047$). In contrast, IL-10 production from primary macrophages incubated with IBD fecal bacteria was significantly decreased compared to normal flora from healthy dogs ($P = 0.045$).

Overall, the results showed that IBD dogs had higher Ig-bound fecal bacteria than normal healthy controls and potentially stimulates greater immune responses as determined by macrophage phagocytosis and activation. This suggested that the local humoral response against gut bacteria in IBD plays a crucial role in the pathogenesis resulting in chronically active inflammation scenario characteristic of IBD.

GI34

Altered Fecal Fatty Acid, Sterol, and Bile Acid Metabolism in Dogs with Acute Diarrhea

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While many studies of canine gastrointestinal diseases have focused on changes of the fecal microbiota, few have examined functional changes by studying fecal metabolites. Some of these metabolites, such as fatty acids and primary bile acids, have been associated with diarrhea in humans. Therefore, the aim of this study was to quantify several metabolites and associated bacterial groups in feces of dogs with acute diarrhea.

Fecal samples were obtained from healthy dogs ($n = 24$) and dogs with acute diarrhea ($n = 11$). DNA was extracted for analysis of major bacterial groups by qPCR, and a targeted gas chromatography-mass spectrometry assay was used to quantify fatty acid, sterol, and bile acid concentrations in feces. A dysbiosis index was calculated based on the abundances of major bacterial taxa (DI; reference limit < 0 with values ≥ 0 indicating dysbiosis). Statistical analysis was performed using Mann Whitney tests with significance set at $p < 0.05$.

Fecal concentrations of stearic acid ($p = 0.018$), arachidonic acid ($p < 0.001$), and nervonic acid ($p = 0.037$) were increased in dogs with acute diarrhea compared to healthy dogs. Cholesterol ($p = 0.018$)

concentration was increased, while coprostanol ($p = 0.047$), campesterol ($p = 0.002$), stigmasterol ($p = 0.029$), fucosterol ($p < 0.001$), β -sitosterol ($p < 0.001$), and sitostanol ($p < 0.001$) concentrations were all decreased in dogs with acute diarrhea. The ratio of secondary bile acids to total bile acids concentration was decreased in dogs with acute diarrhea ($p = 0.039$). The dysbiosis index was significantly increased in dogs with acute diarrhea ($p < 0.001$) compared to healthy dogs.

In conclusion, our results suggest that numerous metabolic changes occur concurrently with alterations of the microbiota in dogs with acute diarrhea. Fecal metabolite patterns in some dogs with acute diarrhea resemble those found in humans with bile acid or fatty acid diarrhea.

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Characterization of Paneth-like Cells in the Canine Small Intestine

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In humans, mice and pigs, Paneth cells locate in crypts close to intestinal stem cells (ISC), and contain large granules staining positively for lysozyme. These cells are involved in the production of growth factors necessary for ISC function, and produce antimicrobial peptides. Defects of Paneth cell function are involved in the pathogenesis of different intestinal diseases in humans, such as inflammatory bowel disease and colorectal cancer. In dogs and cats, the small intestine consistently stains negative for lysozyme, and cells that morphologically resemble Paneth cells have not been identified by light microscopy. We therefore sought to characterize cells that functionally represent Paneth cells in the dog small intestine, using full thickness biopsies as well as primary 3D cultures of epithelial cells (so-called enteroids).

Ten-centimeter tissue pieces were acquired from the jejunum of healthy dogs which had been euthanized for an unrelated project. Full thickness tissues were fixed in 10% formalin saline, routinely processed and embedded in paraffin. For enteroid culture, minced samples were washed and crypts were enriched, using EDTA chelation, embedded in matrigel, and grown in intestinal stem cell media. RNA in situ hybridization (RNA ISH) and immunohistochemistry (IHC) was used to identify cells staining positive for 7 previously reported markers of Paneth cells for mouse and human: lysozyme (LYS), canine interleukin 17 (IL-17), canine beta defensin 103 (CBD 103), canine cathelicidin (CATH), frizzled class receptor 5 (FZD5), neurogenin 3 (NEUROG3) and ephrin receptor tyrosine kinase B2 (EPHB2). In addition, ISC markers were used to characterize the stem cell niche: