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Short Communication

Histiocytic typhlocolitis in two colony Beagle dogs

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ABSTRACT

Two young female Beagle dogs in a laboratory colony with clinical signs of loose stools and fecal blood were confirmed to have histiocytic ulcerative colitis by histologic evaluation. This syndrome is well recognized in other dog breeds such as Boxers and related French Bulldogs, Mastiffs, Alaskan malamutes and Doberman Pinschers. Formalin-fixed paraffin sections of large intestine from one dog demonstrated the presence of *Escherichia coli* strain LF82 by immunohistochemistry and 16S ribosomal RNA gene sequencing. *E. coli* strain LF82 has been implicated in the pathogenesis of Crohn's disease and similar bacteria have been cultured from cases of histiocytic ulcerative colitis in Boxer dogs. Spontaneous histiocytic ulcerative colitis must be differentiated from test article-related findings in nonclinical toxicity studies in Beagle dogs.

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1. Introduction

Histiocytic ulcerative colitis (first described as granulomatous colitis and also called histiocytic typhlocolitis) is a well recognized syndrome of young Boxers and related French Bulldogs that causes mucoid and bloody diarrhea that may result in cachexia (Brown et al., 2007). This syndrome has been occasionally described in other breeds, such as Mastiff, Alaskan Malamute and Doberman Pinscher (Stokes et al., 2001). This report describes two cases of histiocytic typhlocolitis in young Beagle dogs in a laboratory colony. All procedures were approved by the animal care and use committee in accordance with federal regulations and the Guide for the Care and Use of Laboratory Animals (National Research Council, 2013).

2. Material and methods

2.1. Clinical history

Case 1 consisted of an 18-month-old female colony-Beagle dog (Female 1) presented with a 4-month clinical history of

intermittent loose stool containing mucus and blood. This dog was in good body condition and had unremarkable standard hematology and clinical chemistry parameters. This dog was treated with metronidazole (125 mg BID orally for 3 or 4 days) and fenbendazole (50 mg/kg PO for 3 days) with no apparent improvement. A colonoscopy revealed several multifocal to coalescing areas of mucosal erosion extending from the proximal rectum to the descending colon. Case 2 consisted of a 14-month old Female Beagle dog (Female 2) presented with soft stool mixed with blood of several weeks duration. This dog had good body condition and was also nonresponsive to metronidazole (250 mg PO SID × 4 days) treatment. A tentative diagnosis of chronic idiopathic colitis was made and these dogs were euthanized prior to submitting for necropsy.

2.2. Necropsy and histopathology

A full necropsy was performed for both animals following a previously described procedure (Van Kruiningen, 1971). For both cases, samples of colon, cecum, small intestine, stomach, liver, pancreas, spleen, kidneys, urinary bladder, lung, heart and brain were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Four-micrometer sections were stained with hematoxylin and eosin. Sections of colon and cecum were stained by using the Periodic Acid-Schiff (PAS) method, and Brown and Brenn's Gram stain.

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2.3. Immunohistochemistry

Formalin-fixed, paraffin embedded specimens of colon from Female 1 were submitted to the University of Connecticut for immunohistochemical identification of *Escherichia coli*, using primary antibody to *E. coli* (1:1000 Rabbit anti *E. coli*. GenWay Biotech, San Diego, CA). To detect primary antigens, a Dual Link system – horseradish peroxidase was used, and revealed with 3,3'-diaminobenzidine substrate chromagen (Envision – Dual link System HRP-DAB+, Dako, Carpinteria, CA).

16S ribosomal RNA gene sequencing: formalin-fixed paraffin-embedded colon tissue was submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University (University Park, PA) for DNA extraction and bacterial 16S ribosomal RNA (RNA) gene sequencing. Bacterial genomic DNA from the mucosa of the paraffin-embedded tissue was extracted using the DNeasy Blood and Tissue Kit according to manufacturer's instructions for "purification of total DNA from fixed, paraffin-embedded tissues (Qiagen, Valencia, CA). The bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primers p515FPL (5'-GCG GAT CCT CTA GAC TGC AGT GCC AGC AGC CGC GGT AA-3') and p13B (5'-CGG GAT CCC AGG CCC GGG AAC GTA TTC AC-3'). These primers have been designed to recognize two highly conserved regions of the bacterial 16S rRNA gene. PCR was performed in a Master Cycler Pro thermal cycler (Eppendorf North America, Hauppauge, NY) and had initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and elongation at 72 °C for 1 min, which was followed by a final extension at 72 °C for 10 min. PCR product was analyzed by electrophoresis in 1.5% SeaKem LE Agarose gel (Lonza Inc., Allendale, NJ) at 100 volts for 1 h followed by staining with 1% solution of ethidium bromide (50 µl/l). rDNA on agarose gel was visualized by UV transillumination (Alpha Imaging HP System, ProteinSimple, Santa Clara, CA) and the DNA band corresponding to 904-bp fragment was excised from the gel and was purified using MinElute Gel Extraction Kit (Qiagen, Valencia, CA). Purified PCR product was sequenced in both directions at the Genomics Core Facility (The Pennsylvania State University, University Park, PA) using the same primers used in the PCR. DNA sequences were compared with known 16S rRNA gene sequences deposited in GenBank using nucleotide BLAST tool (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>).

3. Results

The most significant necropsy finding in both dogs included multifocal to coalescing, reddened, slightly raised, well demarcated circular or linear mucosal lesions in the cecum and distal colon (Fig. 1). These areas consisted histologically of expansion of the lamina propria and submucosa of the large intestine with a dense inflammatory infiltrate composed of large, eosinophilic, foamy macrophages and variable numbers of neutrophils. The cellular infiltrate separated the mucosal glands and expanded the submucosa and displaced the lymphoid aggregates (Fig. 2). A few bacteria were recognized in macrophages located near the apical portion of the mucosa, whereas necrotic debris was present in macrophages deep in the mucosa and submucosa. Macrophages were PAS positive and bacteria inside macrophages were Gram negative. In these sections, numerous bacteria staining positively for *E. coli* by immunohistochemistry were present free in the mucosa and in macrophages (Fig. 3). Finally, the DNA sequence obtained from the intestinal mucosa matched 100% with *E. coli* LF82 (GenBank CU651637).

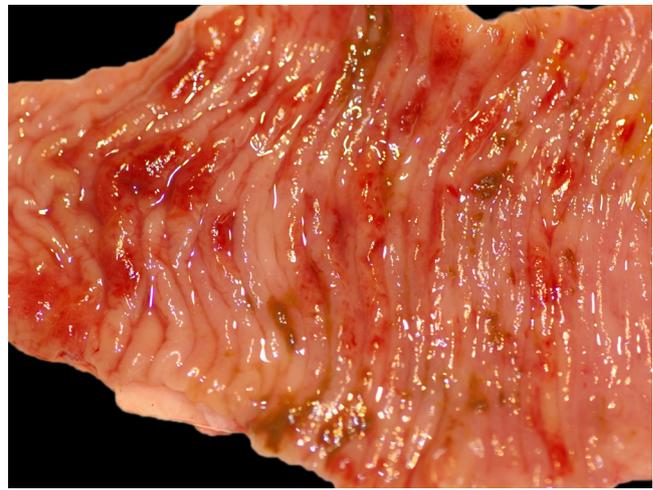


Fig. 1. Cecum (female 1): the mucosa had multifocal to coalescing, 0.3–0.6 cm in diameter erosions. Similar lesions were observed in the distal colon.

4. Discussion

The first cases of granulomatous colitis were described in a kennel of Boxer dogs with chronic diarrhea accompanied by large bowel hemorrhages and weight loss (Van Kruiningen et al., 1965). Necropsy findings include eccentric thickening of the cecal and distal colonic walls with islands and plaques of reddened epithelium. Intervening regions are often unaffected (Sander and Langham, 1968). Histologically, histiocytic ulcerative colitis is characterized by transmural inflammation and distortion of the normal glandular architecture of the colon and cecum, with accumulations of foamy PAS-positive macrophages in the mucosa, submucosa and draining lymph nodes. The earliest discernible histologic changes are focal epithelial cell degeneration and acute inflammation occurring along the luminal surface of the large intestine. Progressive mucosal destruction leads to focal ulcers which enlarge and coalesce (Russell et al., 1971). As the lesions become chronic, mostly macrophages and fewer lymphocytes, plasma cells and mast cells can be found (Van Kruiningen et al., 1965; Sander and Langham, 1968). An infectious agent was proposed as the cause of this disease, but early attempts to culture the cause did not produce a single consistent etiological agent. Other authors suggested that bacteria present in

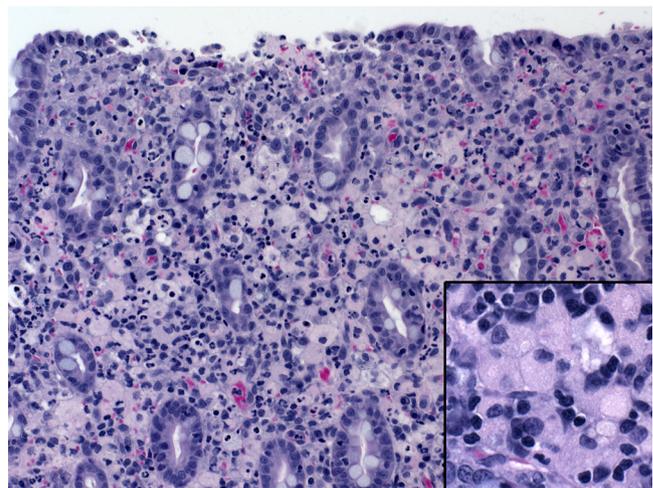


Fig. 2. Cecum (female 1): the mucosa and the submucosa (not shown) were expanded by numerous macrophages and some neutrophils. Macrophages had abundant eosinophilic to foamy cytoplasm (inset). Inflammatory cells separate the glands.

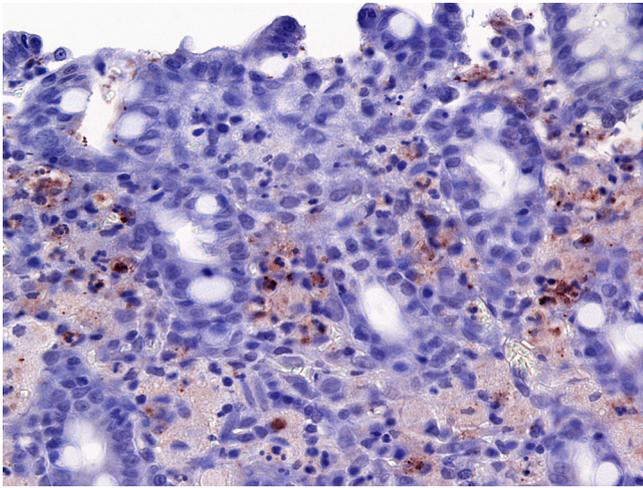


Fig. 3. Cecum (female 1): polyclonal anti-*Escherichia coli* immunohistochemical stain demonstrated numerous bacilli free in the upper lamina propria and in the cytoplasm of macrophages.

the lesions were likely to be secondary invaders, but could play a causative role in the mucosal inflammation (Fiocchi, 1998). Parallel to this, histiocytic ulcerative colitis has been regarded as an autoimmune disease with poor response to treatment (Churcher and Watson, 1997). Later, it was demonstrated that an adherent and invasive *E. coli* is responsible for this disease (Simpson et al., 2006), and that treatment with enrofloxacin results in complete remission of the disease and marked histological improvement of the lesions (Hostutler et al., 2004; Mansfield et al., 2009).

Beagle dogs are commonly used for nonclinical safety testing in the drug development process (Peckham and Thomassen, 2007). To the authors knowledge histiocytic ulcerative colitis has never been reported in Beagle dogs. In this report, molecular analyses and immunohistochemical staining confirmed the presence of *E. coli* strain LF82 in one Beagle dog. *E. coli* strain LF82 has been implicated in the pathogenesis of Crohn's disease and similar *E. coli* have been cultured from cases of histiocytic ulcerative colitis in Boxer dogs (Simpson et al., 2006). *E. coli* LF82 is an adherent and invasive *E. coli* (AIEC) that is able to attach and colonize the gut mucosa, through invasion and survival within epithelial cells and macrophages. AIEC replicates in macrophages without triggering cell death or interferon- γ secretion by these cells (Barnich et al., 2007). It has also been associated with abnormal expression of the specific host receptor CEACAM6 responsible for the binding of AIEC to the gut mucosa (Barnich et al., 2007; Lapaquette and Darfeuille-Michaud, 2010).

Female 1 had clinical signs and histopathologic findings consistent with histiocytic ulcerative colitis reported in Boxers and French Bulldogs. Female 2 had clinical and histopathologic findings similar to those observed in female 1 and was included in this report, although molecular and IHC analyses were not performed. These results indicate that *E. coli* strain LF82 can cause histiocytic ulcerative colitis in Beagle dogs and spontaneous histiocytic ulcerative colitis must be differentiated from test article-related findings in nonclinical toxicity studies in Laboratory Beagle dogs.

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