ASYMPTOMATIC COLITIS IN NATURALLY INFECTED DOGS WITH LEISHMANIA INFANTUM: A PROSPECTIVE STUDY

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Abstract. A total of 31 dogs with naturally occurring and symptomatic leishmaniasis (Leishmania infantum), but without historical or clinical evidence of overt colitis, were included in this study. With owners’ consent, a colonoscopy was performed in all these dogs, revealing patches of hyperemic, edematous, irregular, and mildly erosive colonic mucosa in 25.8% of the animals. Biopsies were obtained from the colonic mucosa and stained with hematoxylin-eosin (histopathology) and avidin-biotin-peroxidase technique (immunohistochemical detection of parasites). Leishmania amastigotes were detected immunohistochemically in 32.3% of the dogs. The most common inflammatory pattern in the colonic mucosa of these dogs was pyogranulomatous (90%), whereas in the dogs without Leishmania amastigotes immunohistochemically detected in the colonic mucosa (67.7%), there was no evidence of gross and microscopic lesions. Also, in 2 of the 10 dogs in which parasites were detected immunohistochemically in the colonic mucosa, no lesions could be detected on colonoscopy. There was no correlation between the dogs with or without parasites detected in the colonic mucosa regarding the sex, age, or the type of diet of these animals. However, the positive correlation ($P < 0.001$) found between colonic parasitism and gross lesions detected on colonoscopy would justify the inclusion of canine leishmaniasis in the list of differentials of canine chronic or recurrent colitis.

INTRODUCTION

Canine leishmaniasis (CanL), caused by the protozoan parasite Leishmania infantum, is a common infectious disease in the countries of the Mediterranean basin and Portugal. CanL is a disease with variable clinical picture, where almost every organ system is involved. In particular, it may include progressive loss of body weight, cutaneous, ocular, and musculoskeletal signs, renal and liver disease, peripheral lymphadenomegaly, hepatosplenomegaly, and epistaxis.

Diarrhea, irrespective of being originated from the small or large intestine, has been included among the anamnestic signs of CanL, with a prevalence as high as 30%, whereas it was much lower in other studies. The occurrence of diarrhea has been mostly associated with chronic renal or hepatic failure that may develop in the course of the disease. In a few cases, small bowel diarrhea has been attributed to the infestation of intestinal mucosa by parasitized cells or to emaciation. Moreover, in one CanL case, diarrhea was associated with acute pancreatitis.

Chronic colitis is generally considered an unusual clinical presentation of CanL. In two naturally occurring CanL cases admitted with chronic large bowel diarrhea, the gross appearance of the colonic mucosa was similar to what is usually seen in idiopathic chronic colitis cases. Colonic histopathology of these cases revealed a mixed-cell inflammatory infiltration by macrophages, plasma cells, lymphocytes, and neutrophils, along with the presence of L. infantum amastigotes, subsequently confirmed by immunohistochemistry; nevertheless, the authors did not manage to accomplish a definitive pathogenetic correlation between CanL and chronic colitis.

To the authors’ knowledge, no attempt has been made to access the prevalence of L. infantum parasitism in the colonic mucosa and to establish a cause-and-effect relationship with subclinical colitis in symptomatic dogs residing the endemic areas of the disease. In dogs, but also in humans, only case reports have been published showing the rarity of large intestinal symptoms in leishmaniasis. However, it has been suggested that rectal biopsy may be of possible value for the diagnosis of the disease, even in the absence of colitis signs. Therefore, the aims of this study were the investigation of the prevalence of L. infantum parasitism in canine colonic mucosa and its correlation with the potential colonscopic and histopathologic findings in CanL cases presenting no historical or clinical evidence of overt colitis, such as increased frequency of defecation, decreased fecal volume per attempt, urgency along with unproductive attempts, excessive fecal mucus, and hematochezia.

MATERIALS AND METHODS

A total of 31 dogs with symptomatic CanL, admitted to the Companion Animal Clinic (Medicine), Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, between January 1998 and December 2001, were included in the study. Diagnosis was confirmed by the direct observation of Leishmania amastigotes in Giemsa-stained lymph node and/or bone marrow aspiration smears, coupled with either immunofluorescent antibody test (IFAT) or enzyme-linked immunosorbent assay (ELISA) positive serology. None of the 31 dogs had been treated for CanL, at least in the past 6 months, and there was no historical, clinical, and laboratory evidence of concomitant diseases or acute, chronic, or recurrent colitis.

Complete blood count (CBC), serum biochemistry (total protein, albumin, glucose, alanine aminotransferase, alkaline phosphatase, urea nitrogen, creatinine, phosphorus, potassium), urinalysis (specific gravity, Heller test, dip stick, sediment evaluation), and fecal parasitologic examination (direct plus zinc sulfate flotation techniques) were performed in all 31 dogs. Dogs with biochemical abnormalities other than hyperproteinemia and/or intestinal parasitism were excluded from the study.
Of the 31 dogs, which belonged to various breeds, 20 (64.5%) were intact males and 11 (35.5%) were intact females, with ages ranging from 1 to 11 years (median: 4 years). Nineteen of 31 (61.3%) dogs were being fed commercial and homemade diets, whereas the rest were consuming either commercial (8 of 31; 25.8%) or homemade (4 of 31; 12.9%) diets exclusively. All the dogs were current on vaccination and anthelmintic prophylaxis.

Colonoscopy was performed with the owners’ consent and after the approval of the study by the Ethics Committee of the Faculty of Veterinary Medicine and the National Veterinary Authority. All the animals were routinely prepared for colonoscopy (48-hour fasting period followed by two warm water enemas 12 and 2 hours before the procedure), sedated with intramuscular xylazine (Rompun; Bayer, Leverkusen, Germany) at a dose of 1 mg/kg body weight and atropine sulfate (Atropine Sulfate; Demo, Athens, Greece) at a dose of 0.044 mg/kg body weight, and placed on left lateral recumbency. With the aid of a flexible endoscope (Type XP20; Olympus Optical, Hamburg, Germany), the entire large intestine was examined, and all possible visible lesions, such as hyperemic and edematous mucosa, mucus accumulation, mucosal irregularity, increased friability, and erosions or ulcers, were recorded. At the same time, a total of six to eight biopsies, measuring 1.8 mm in diameter, were obtained from all three segments of the colon and the rectum of each dog, fixed in neutral-buffered 10% formalin solution, embedded in paraffin, sectioned at 4–5 μm, and stained with hematoxylin and eosin (HE) and Gram methods. Also, tissue sections from all the animals were processed for immunohistochemistry by applying the avidin-biotin-peroxidase technique as proposed by Bourdoiseau and others14 to facilitate the detection of *Leishmania* amastigotes. Briefly, serum from a dog with CanL (IFAT titer > 1/1600) was used as primary and sheep anti-canine IgG conjugated with horseradish peroxidase (code AA109P; Serotec, Oxford, UK) as secondary antibody, both commercial (8 of 31; 25.8%) or homemade (4 of 31; 12.9%) diets exclusively. All the dogs were consuming either commercial versus homemade diets, whereas the rest were consuming either mixed diet, and colonoscopy (48-hour fasting period followed by two warm water enemas 12 and 2 hours before the procedure), sedated with intramuscular xylazine (Rompun; Bayer, Leverkusen, Germany) at a dose of 1 mg/kg body weight and atropine sulfate (Atropine Sulfate; Demo, Athens, Greece) at a dose of 0.044 mg/kg body weight, and placed on left lateral recumbency. With the aid of a flexible endoscope (Type XP20; Olympus Optical, Hamburg, Germany), the entire large intestine was examined, and all possible visible lesions, such as hyperemic and edematous mucosa, mucus accumulation, mucosal irregularity, increased friability, and erosions or ulcers, were recorded. At the same time, a total of six to eight biopsies, measuring 1.8 mm in diameter, were obtained from all three segments of the colon and the rectum of each dog, fixed in neutral-buffered 10% formalin solution, embedded in paraffin, sectioned at 4–5 μm, and stained with hematoxylin and eosin (HE) and Gram methods. Also, tissue sections from all the animals were processed for immunohistochemistry by applying the avidin-biotin-peroxidase technique as proposed by Bourdoiseau and others14 to facilitate the detection of *Leishmania* amastigotes. Briefly, serum from a dog with CanL (IFAT titer > 1/1600) was used as primary and sheep anti-canine IgG conjugated with horseradish peroxidase (code AA109P; Serotec, Oxford, UK) as secondary antibody, both diluted at 1:500. Canine lymph node sections heavily parasitized with *L. infantum* served as positive controls, whereas tissue sections incubated only with phosphate-buffered saline (PBS) served as negative controls. Diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO) was used as chromogen lightly counterstained with Mayer hematoxylin.

The possible associations between the dogs with or without immunohistochemically detected *Leishmania* spp. amastigotes in the colonic mucosa and the sex (males versus females), age (adults versus middle-aged to elderly), diet type (commercial versus homemade–mixed diet), and colonoscopy findings of the dogs were examined by χ² test. The level of significance was set at 5%.

**RESULTS**

**Clinical signs and laboratory findings.** Physical examination revealed peripheral lymphadenomegaly (23 of 31; 74.1%), focal, multifocal, or diffuse exfoliative dermatitis (16 of 31; 51.6%), nasodigital hyperkeratosis (4 of 31; 12.9%), and onychogryposis (2 of 31; 6.5%).

On CBC, anemia (range: 23.5–36.5%; mean: 31.1%; reference range: 37–55%) and thrombocytopenia (range: 78 × 10³–188 × 10³/μL; mean: 121 × 10³/μL; reference range: 200 × 10³–500 × 10³/μL) were detected in 23 of 31 (74.2%) and 12 of 31 (38.7%) dogs, respectively. Hyperproteinemia was detected in 18 of 31 (58.1%) dogs (range: 8.2–11.6 mg/100 mL; mean: 9 mg/100 mL; reference range: 6–8 mg/100 mL). Urinalysis disclosed glomerular proteinuria in 18 of 31 (68.1%) cases, whereas fecal examination was negative for endoparasites in all 31 dogs.

**Colonoscopy findings.** Colonoscopy revealed hyperemic areas with or without irregular and/or edematous colonic mucosa, and a few erosions in 8 of 31 (25.8%) dogs (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Colonoscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mongrel</td>
<td>♂</td>
<td>3</td>
<td>Focal hyperemia</td>
</tr>
<tr>
<td>2</td>
<td>Jura des Alpes</td>
<td>♂</td>
<td>4</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Mongrel</td>
<td>♂</td>
<td>7</td>
<td>Focal hyperemia and edema</td>
</tr>
<tr>
<td>4</td>
<td>Hellenic hound</td>
<td>♀</td>
<td>3</td>
<td>Focal hyperemia and irregularity</td>
</tr>
<tr>
<td>5</td>
<td>Mongrel</td>
<td>♀</td>
<td>2</td>
<td>Focal hyperemia and irregularity</td>
</tr>
<tr>
<td>6</td>
<td>German shepherd</td>
<td>♂</td>
<td>5</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>Jura des Alpes cross</td>
<td>♂</td>
<td>2</td>
<td>Focal hyperemia and edema</td>
</tr>
<tr>
<td>8</td>
<td>Mongrel</td>
<td>♂</td>
<td>5</td>
<td>Focal hyperemia and edema</td>
</tr>
<tr>
<td>9</td>
<td>Hellenic hound</td>
<td>♂</td>
<td>1.5</td>
<td>Focal hyperemia, edema, and irregularity</td>
</tr>
<tr>
<td>10</td>
<td>Golden retriever</td>
<td>♂</td>
<td>2.5</td>
<td>Focal hyperemia with few erosions</td>
</tr>
</tbody>
</table>

**Immunohistochemical and histopathologic findings.** On immunohistochemistry, intracellular and extracellular (macrophages) *Leishmania* amastigotes were amply visible within the colonic mucosa of 10 of 31 (32.3%) dogs; lamina propria was more parasitized (Figures 1 and 2) compared with muscularis mucosa and submucosa. In all eight dogs showing

![Figure 1](https://www.ajtmh.org)
gastrointestinal disease. In this study, the colonic mucosa of 8 of 31 (25.8%) dogs appeared patchily hyperemic, granular, edematous, and mildly erosive. These endoscopic findings were similar to those visualized in dogs with either CanL-associated colitis\textsuperscript{10} or that of other etiology.\textsuperscript{15} In idiopathic lymphocytic-plasmacytic colitis, reportedly the most common inflammatory large intestinal disease in the dog, colonoscopy usually reveals mucosal hyperemia, edema, irregularity, granularity, and friability, as well as a submucosal vascular pattern, mucus accumulation, and the presence of erosions or ulcerations, all appearing in various combinations and degrees.\textsuperscript{15,16} Patchy areas with mucosal hyperemia, edema and irregularity, and a few erosions, noticed in 8 of 10 dogs, were highly suggestive of an underlying histopathology. Nevertheless, despite the histologic lesions in two dogs with parasites detected on colonic immunohistochemistry, the large intestinal mucosa appeared quite normal on endoscopy. Similarly, \textit{Leishmania} \textit{amastigotes} were also detected in apparently normal canine skin\textsuperscript{17,18} and in the hepatic tissue of symptomatic CanL cases showing no clinical or laboratory evidence of liver disease.\textsuperscript{19}

The absence of clinical signs characterizing the chronic or recurrent colitis in dogs can be explained by the fact that endoscopic abnormalities and/or histologic lesions do not always correlate with the severity of the clinical disease.\textsuperscript{15} Interestingly, despite the complete clinical remission, colonic inflammation still persisted in re-biopsied dogs and humans with chronic colitis.\textsuperscript{15,20} Therefore, it is assumed that other factors lowering the “clinical threshold” of the colonic mucosa may be responsible for asymptomatic dogs turning symptomatic. No gastrointestinal signs were observed in a woman with visceral leishmaniasis, the diagnosis of which was based on rectal histopathology, thus suggesting only focal large intestinal disease.\textsuperscript{12} The same was also true in a dog naturally infected with \textit{L. chagasi},\textsuperscript{11} the genotype of which is identical.
to that of L. infantum. Symptomatic colitis was not observed in any of the dogs with the experimental disease, in contrast to what was witnessed in 3 of 6 (50%) dogs of another experimental study, where the microscopic lesions were more severe in the large intestine. Although it has not been documented in the dog, in the murine model of the L. major-induced disease, interferon (IFN)-γ is needed to suppress the progression of the disease, whereas interleukin (IL)-10 is the main pro-inflammatory cytokine responsible for its expansion and persistence. Nevertheless, IL-10 has been reported to suppress the development of colitis in laboratory mice.

In our study, Leishmania amastigotes were detected in the colonic mucosa of 10 of 31 (32.3%) dogs with symptomatic CanL. The fact that immunohistochemical staining is reported more often than HE or Giemsa staining for the detection of Leishmania amastigotes in various tissues was further confirmed by the results of this study, having shown that even very small numbers of intra- or extracellular immunolabeled amastigotes were easily recognizable. However, no correlations could be found between the dogs with and without parasites detected on colonic immunohistochemistry and the sex, age, or type of diet of these animals. Positive correlation was only found between colonic parasitism and colonscopic findings (P < 0.001), probably showing that the former is closely associated with the pathogenesis of the macroscopic mucosal lesions.

Pyogranulomatous, which was the most common histopathologic finding in this case series (90%), and granulomatous inflammation have been shown in various other organs, where the presence of amastigotes was accompanied by inflammatory reaction. Not surprisingly, the same type of inflammatory reaction has also been seen in CanL cases with exfoliative dermatitis or cutaneous nodules and in the recently described hepatic histopathology of CanL cases with asymptomatic chronic hepatitis. Dissemination and severity of inflammatory infiltrate have been associated with the load, species, and strain of the parasite and the immunologic status of the host. However, it has been shown that the severity of clinical skin lesions may be negatively correlated to parasite load.

In the canine model of the experimental disease, a moderate to severe infiltration of the colonic mucosa, mostly with parasitized macrophages, was detected in all the animals with L. infantum and in one third of the dogs with L. chagasi infection. In another experimental study, and in naturally occurring CanL cases, a diffuse mixed-cell infiltrate of the colonic mucosa and submucosa was also shown. Granulocytes act as a “Trojan horse,” facilitating the elusive invasion of macrophages by Leishmania organisms and subsequently the host response to infection. Granulomas are anatomically functional circumscribed structures that may also limit the spread of the infection (e.g., in human visceral leishmaniasis), where hepatic granulomas have resulted in limiting the infection. The concurrent lymphoplasmacytic infiltrate of the colonic mucosa also witnessed in naturally infected dogs raised the question of whether it was caused by the parasite or just an incidental finding. On the other hand, the histologic changes seen in the 10 dogs with Leishmania amastigotes in the colonic mucosa can also be detected in canine colitis cases of various etiologies. According to the authors’ observations, the glandular destruction amply seen in this study would be attributed to pyogranulomatous–granulomatous infiltration because it is a rather uncommon histologic finding in the much more reported idiopathic lymphoplasmacytic colitis of the dog.

At present, bone marrow and lymph node cytology, immunofluorescent antibody (IFA) serology, and real-time polymerase chain reaction (PCR) on skin biopsies or conjunctival swabs constitute the mainstay of the diagnosis in CanL. Cytology is the “gold standard” in achieving this goal, but the other methods should be applied, preferably in combination, when the results of the former are negative or in pursuing the response to various anti-leishmanial treatment.

It is assumed that the positive or negative cause-and-effect relationship of this study would have been further strengthened if all 10 dogs, presenting gross and/or microscopic lesions in their colonic mucosa, had been treated with a combination of meglumine antimonate and allopurinol and re-colonoscopy and re-biopsied after a reasonable period of time. Results undoubtedly would be more reliable if a blinded-placebo designated study had been applied. Sad to say, this opportunity was missed because of the owners’ denial to permit the repetition of the procedure 2–3 months after the initiation of the anti-leishmanial treatment.

In conclusion, the prevalence of Leishmania sp. parasitism in the colonic mucosa of dogs with symptomatic CanL, but without overt colitis, was surprisingly high, thus implying the inclusion of CanL in the list of differentials of canine chronic colitis, at least in endemic areas. The reason for the absence of symptoms characterizing colitis remains to be elucidated. The diagnosis of asymptomatic but infected dogs, also playing a significant role in the epidemiology of the disease, could benefit by the search of colonic biopsies. Confirmation of this hypothesis justifies further studies. Finally, other etiologies that may lead to overt subacute or chronic colitis in the dog should be pursued diagnostically even in symptomatic CanL cases with endoscopic and histopathologic evidence of colonic inflammation.

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