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Journal of Veterinary Medicine and Research

Case Report

Chronic Small Bowel Diarrhea Due to Granulomatous Duodenitis by *Leishmania* in two Dogs

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Submitted: 03 January 2016
Accepted: 04 January 2016
Published: 17 January 2016
ISSN: 2378-931X

Keywords
- Leishmania
- Granulomatous duodenitis
- Protein-losing enteropathy
- Small bowell diarrea

Abstract

Canine gastrointestinal infection by *Leishmania* presenting large bowel involvement has been reported to date. This case series describes two dogs suffering from severe chronic small bowel diarrhea. Duodenal biopsies demonstrated granulomatous infiltration with intracytoplasmatic corpuscles of *Leishmania* amastigotes. Furthermore one of the dogs was diagnosed with protein-losing enteropathy (PLE) based on the presence of multifocal dilation of intestinal crypts and hypoalbuminemia. To the author's knowledge, PLE associated to canine granulomatous duodenitis by *Leishmania* had not been previously reported. Dogs were successfully treated with meglumine antimoniate for at least one month and allopurinol as a long-term single agent. These findings suggest that leishmaniosis should be included in the differential diagnosis for dogs presenting chronic small bowel diarrhea or PLE, especially in endemic areas. Close monitoring of the dogs affected is highly recommended.

ABBREVIATIONS

PLE: Protein-Losing Enteropathy; BCS: Body Condition Score; **SBP**: **S**erum Biochemical Profile; **CBC**: Complete Blood Count; **TLI**: Serum Trypsin-Like Immunoreactivity; **SBA**: Serum Bile Acids; **USG**: Urinalysis Showed Urine Specific Gravity; **UPC**: Urinary Creatinine-Protein Ratio

INTRODUCTION

Clinical features associated with canine leishmaniosis vary widely and the most frequent findings include progressive weight loss, cutaneous, ocular and musculoskeletal signs, renal and liver disease, systemic lymphadenomegaly, hepatomegaly, splenomegaly and epistaxis [1-3].

Gastrointestinal infection by *Leishmania* has been rarely documented in dogs. Previous reported cases describe chronic hepatitis and large bowel involvement [4-6].The presence of parasites through all intestinal segments and layers of the gastrointestinal tract of dogs naturally infected by *Leishmania* regardless of their clinical status has been described [7]. However, the highest parasite load was found in caecum and colon. The cause of Leishmania's irregular distribution over the gastrointestinal tract and pathophysiology of associated clinical signs remains unclear [8-10].

Small intestinal involvement causing overt malabsorption in visceral leishmaniosis has been reported more frequently in immunosuppressed human patients [11-14]. Two cases of visceral human leishmaniosis where the main findings were chronic diarrhea and malabsorption have been published [15]. In both cases, duodenal and colonic mucosa was loaded with *Leishmania* bodies and one of them had diffuse colonic atrophy and discrete ulcerations in colon.

To the authors knowledge only 12 cases of canine granulomatous duodenitis by *Leishmania* independently of the clinical status of the infected dogs have been reported so far [7,16]. However, protein-losing enteropathy (PLE) associated with the presence of leishmaniosis had not been previously described in dogs.

This paper aims to describe two dogs suffering from clinically patent granulomatous duodenitis due to *Leishmania* with overt PLE in one of them.

CASE PRESENTATION

Case 1

A 6-year-old, 33 kg intact male Rottweiler was referred for

evaluation of a 4 month duration chronic small bowel diarrhea. Severe weight loss was observed in the meantime with appetite remaining normal. Several diet and drug therapies had been performed with no clinical improvement. The dog had been diagnosed with leishmaniosis 3 years before and already treated with allopurinol (10 mg/kg BID PO).

At referral, physical examination showed no abnormalities, except poor (1/9) body condition score (BCS) [17]. Serum biochemical profile (SBP) revealed severe panhypoproteinemia (total protein 3.2 g/dl (5.4-7.1), with hypoalbuminemia 1.1 g/dl (2.6-3.3) and hypoglobulinemia 2.1 g/dl (2.8-3.8) and, hypocholesterolemia 99 mg/dl (135-270). *Leishmania* serum antibody titer (ELISA) was low positive 89% (11-300). No other abnormalities in the SBP were found. Complete blood count (CBC), serum trypsin-like immunoreactivity (TLI) and serum bile acids (SBA) were within reference intervals. Fecal flotation was negative. Urinalysis showed urine specific gravity (USG) of 1.040, inactive sediment and urinary creatinine-protein ratio (UPC) of 0.3 (<0.5). Abdominal ultrasound revealed whole-intestinal thickening (up to 6.9 mm) and abnormalities in colonic layer stratification as main findings. (**Figure 1**)

Based on the results, a presumptive diagnosis of PLE was made and gastroduodenoscopy was performed. No gross mucosal lesions were observed on endoscopy. Several gastric and duodenal samples were obtained for histopathological examination.

Duodenal biopsies demonstrated a diffuse severe granulomatous infiltration of the lamina propria by macrophages, lymphocytes and few neutrophils. Some macrophages were filled with intracytoplasmatic corpuscles consistent with *Leishmania* amastigotes. Specific indirect immunoperoxidase staining on paraffin-embedded sections was positive. Multifocal dilation of intestinal crypts filled with necrotic debris and mucus was also observed (**Figure 2,3**).

Granulomatous duodenitis due to *Leishmania* infection was diagnosed. Meglumine antimoniate (50 mg/kg BID SC for 45 days) was initiated and allopurinol (10 mg/kg BID PO) was maintained. Clinical signs resolved and serum proteins reached normal values in 4 weeks. The referring veterinarian did the follow up and 1 year after diagnosis the dog was free of clinical signs showing a normal BCS [17].

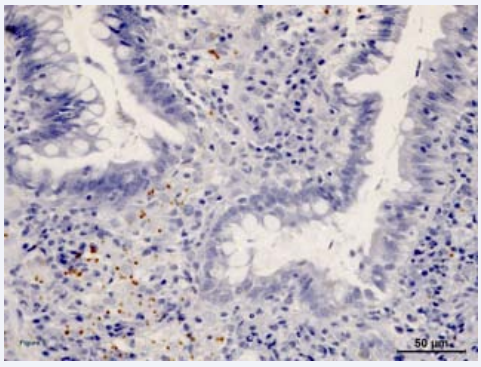

Figure 1 Abdominal ultrasound revealed diffuse small intestinal thickening (up to 6.9 mm) and abnormalities in layer stratification. No other abnormalities were found.

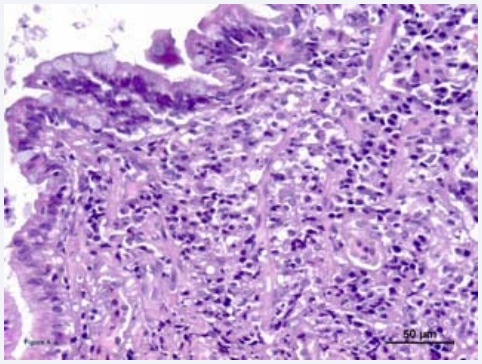

Figure 2 Duodenal biopsies demonstrated diffuse severe granulomatous infiltration of the lamina propia by macrophages, lymphocytes and few neutrophils. Note some macrophages concentrated in the lamina propia were filled with intracytoplasmatic corpusculesconsistent with *Leishmania* amastigotes. Multifocal dilation of intestinal crypts filled with necrotic debris and mucus were also seen. HE. Bar 50µm.

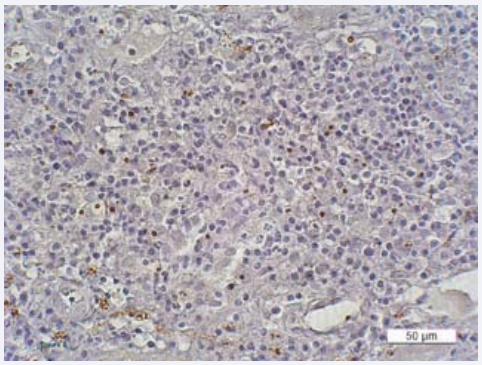

Figure 3 Macrophages with numerous intracytoplasmatic *Leishmania* amastigotes are evident. Specific indirect immunoperoxidase staining on paraffin-embedded sections. Bar50µm.

Case 2

A 2-year-old, 22.6 kg female mixed breed dog was referred with a chronic history of hemorrhagic small bowel diarrhea of 7 months duration. During that period the dog had presented intermittent diarrhea with partial responses to several prescription diets, metronidazole and fenbendazole treatments. Pre-referral abdominal ultrasound was compatible with diffuse intestinal inflammation with no abnormalities in layer stratification. The dog had been diagnosed with leishmaniosis and treated with allopurinol (10 mg/kg BID PO) for 10 months.

On admission, physical examination showed no abnormalities, except poor (3/9) BCS [17]. SBP revealed mild hypoalbuminemia 2.5 g/dl (2.6-3.3) and mild hyperglobulinemia 4.0 g/dl (2.8-3.8). CBC was unremarkable. *Leishmania* serum antibody titer (ELISA) was high positive 200% (11-300). Fecal flotation was negative. Urinalysis revealed a USG of 1.045, inactive sediment and UPC 0.1 (<0.5).

Gastroduodenoscopy revealed several abnormalities in the duodenal mucosa including hyperemia, hypertrophy, petechiae and increased mucous secretion. Colonoscopy showed similar but milder lesions in colonic mucosa. Diffuse severe granulomatous mucosal infiltration by macrophages, lymphocytes and neutrophils was observed through all duodenal and colonic segments microscopically examinated. (**Figure 4**,**5**) Macrophages had intracytoplasmatic corpuscles compatible with *Leishmania* amastigotes, which were also positive to immunoperoxidase staining. Severe granulomatous diffuse enterocolitis by *Leishmania* infection was diagnosed. The dog was treated with meglumine antimoniate (50 mg/kg BID SC for 30 days) and allopurinol (10 mg/kg BID PO for at least 12 months).

Weight gain and complete resolution of the clinical signs were obvious at one-month follow-up. Recheck 3 months later revealed a significant improvement of BCS (5-6/9) [17] without diarrhea and normal serum levels of albumin 3.1 g/dl (2.6-3.3) and globulins 3.3 g/dl (2.8-3.8). Clinical signs relapsed despite keeping the dog on allopurinol treatment, 10 months after initial diagnosis. At this time, BCS 3/9 [17], high positive *Leishmania* serum antibody titer (ELISA) 218% (11-300) and mild hypoalbuminemia 2.4 g/dl (2.6-3.3) were observed again. The treatment with meglumine antimoniate (50 mg/kg BID SC for 45 days) was reinitiated leading to an improvement of the clinical signs. 6 weeks later, BCS was 5/9 [17] and no clinical signs or clinic-pathological abnormalities were present. The dog was maintained on allopurinol treatment (10 mg/kg BID PO) for 12 months more. Follow-up including physical examination, CBC, serum biochemical profile, complete urinalysis and *Leishmania* serum antibody titer were performed every 4 months during the first year. No relapse was observed during this period.

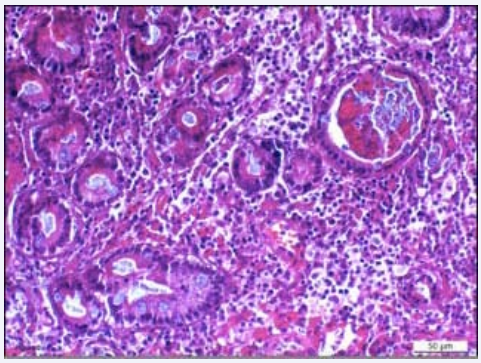

Figure 4 Inflammatory infiltrate consisting mostly of macrophages with some limphocytes, and neutrophils is present in the lamina propria was observed through all duodenal and colonic segments microscopically examinated. Some macrophages had intracytoplasmatic corpuscles compatible with *Leishmania* amastigotes. HE. Bar 50µm.

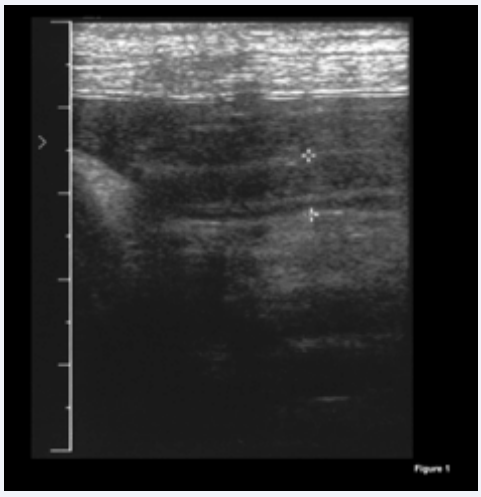

Figure 5 Severe granulomatous diffuse enterocolitis by *Leishmania* infection was diagnosed. Positive to immunoperoxidase staining. Bar 50µm.

DISCUSSION

This case report describes two dogs presenting small bowel chronic diarrhea and granulomatous duodenitis in which *Leishmania* parasites were histologically found. Both of them were successfully treated with meglumine antimoniate plus allopurinol. Interestingly, PLE was evidenced in one of the dogs. To the author's knowledge, PLE associated to granulomatous duodenitis by *Leishmania* infection had not been previously reported in the dog.

Clinically patent intestinal involvement by *Leishmania* is an unusual presentation. Recurrent colitis induced by the presence of parasites limited to colon and rectum has been the most common alteration documented [3,4,6,9,10,]. Only 12 naturally infected dogs with *Leishmania* presenting a high number of parasites in the duodenum have been documented; none of these dogs showed evident GI clinical signs [7,16]. The dogs presented here had a chronic history of small bowel diarrhea with severe weight loss that did not response to conventional treatments. Other clinical signs or clinicopathological abnormalities commonly found in clinically sick dogs with leishmaniosis such as proteinuria or non-regenerative anemia were not found. This is consistent with previous descriptions that pointed out the fact that the absence of classical clinical manifestations could contribute to making diagnosis difficult in dogs with leishmaniosis [2-4,18]. One study reported 32% of 31 dogs affected by leishmaniosis did not show any evidence of clinical signs although they presented *Leishmania* amastigotes and correlated lesions in colonic mucosa [5]. These data support that histological changes by *Leishmania* do not major correlate with the severity of the clinical signs.

According to previous publications, challenging diagnosis is also derived from endoscopic limitations, with about half of the cases showing normal appearance [4,5]. Similar to other cases previously described, in the first case reported here, endoscopy revealed no severe alterations of the mucosa in any gastrointestinal segment [16]. However, hyperemic and hypertrophic mucosa with petechiae and increase mucous secretion were detected in the second one, are common findings in other reported cases [3,5]. These lesions were similar to others caused by *Histoplasma, Salmonella, Yersinia, Giardia, Trichuris, Ancylostoma, Entamoeba* or *Balantidium* [1,16], but these other pathogens were ruled out in our patient by fecal analysis and histopathology.

In accordance with previous reports, if gastrointestinal signs are present in dogs where leishmaniosis is likely despite normal mucosa by endoscopy, biopsy needs to be performed, as the parasite load is independent of the clinical status of the dogs [5,7,9], and inmunohistochemistry is recommended, because it has higher sensitivity and specificity than histopathology alone to diagnose intestinal involvement of *Leishmania* [3,4,6].

As formerly described by other authors, an intense chronic granulomatous infiltration associated with *Leishmania* throughout the lamina propia was observed, but higher parasite load confirmed by inmunohistochemistry in duodenal segments was evident in both cases, which was different from those described in previous studies [4,5]. In the first case, histology revealed a multifocal dilation of intestinal crypts filled with necrotic debris and mucus. PLE has been associated with lesions in the intestinal crypts, because they do not retain protein, which is dropped into the intestinal lumen [19,20,15]. Although crypt disease has been proposed as an entity especially prevalent in Rottweilers [21], the favorable response associated to the treatment with meglumine antimoniate and allopurinol in the present case makes it unlikely that was the cause of PLE in this dog. Clinical diagnosis of PLE was made by the presence of chronic small bowel diarrhea and hypoalbuminemia with no evidence of inadequate food intake, protein losing nephropathy or liver failure. Focal micro-erosions and extended crypt lesions caused by *Leishmania* could induce PLE and reduce the area available for intestinal absorption [6,19,20]. Similar lesions have been described in the colonic mucosa and submucosa of *Leishmania* infected beagles with chronic colitis [22]. Mild hypoalbuminemia and hyperglobulinemia were found in the second case, possibly due to the inflammatory response. Infectious diseases have been associated to hypoalbuminemia plus hyperglobulinemia but there was no definitive confirmation that the hypoalbuminemia was induced by real PLE in these diseases [23,24].

The segmental distribution of the lesions demonstrates the need to biopsy multiple areas of the intestines [7,16,19]. Lower parasite load in the jejunum has been related with an increased expression of CD4, Foxp3 and CD8 receptors and IL-10, TGF, TNF, IFN cytokines, whereas increased levels of IL-4 in the colon were associated with a higher parasite load [9,10]. Based on these two cases, authors could hypothesize that different distribution and quantity of *Leishmania* parasites and presence of clinical signs could be affected by the variable immune response and cellular infiltration.

Treatment with meglumine antimoniate plus allopurinol remains the first elective therapy for clinical intestinal involvement of *Leishmania* [25]. In both cases GI signs disappeared immediately and serum titer of antibodies and proteins reached the reference intervals. Authors suspect that most of these chronic cases with unusual presentations maintain a positive or low positive titer of antibodies and temporary resolution of the clinical signs with a high rate of relapses despite maintaining allopurinol treatment. According to this hypothesis several human patients with small intestinal leishmaniosis tend to have a progressive worsening with response to treatment but with high relapse rate [11-15]. In the second case described here, clinical signs reappeared and *Leishmania* serum antibody titer was high positive again approximately 10 months after antimonial treatment; such event suggests that this unusual presentation of leishmaniosis probably needs to be managed as complicated cases with poor response to treatment: close monitoring and intermittent rescue treatment with antimonials [25].

CONCLUSIONS

This manuscript points out that leishmanios is should be included in the differential diagnosis of dogs presenting chronic small bowel diarrhea or PLE, especially in dogs living in endemic areas or with a travel history to high prevalence locations.

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Cite this article

Vila A, Movilla R, Lloret A, Majó N, Marco AJ, et al. (2016) Chronic Small Bowel Diarrhea Due to Granulomatous Duodenitis by Leishmania in two Dogs. J Vet Med Res 3(1): 1040.