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Histopathological and parasitological study of the gastrointestinal tract of dogs naturally infected with *Leishmania infantum*

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Abstract

Background: The aim of this study was to provide a systematic pathological and parasitological overview of the gastrointestinal tract (GIT), including the stomach, duodenum, jejunum, ileum, caecum and colon, of dogs naturally infected with *Leishmania*.

Methods: Twenty mongrel dogs naturally infected with *Leishmania (Leishmania) infantum* and obtained from the Control Zoonosis Center of the Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil, were analyzed. The dogs were divided into two groups: Group 1 comprised nine clinically normal dogs and group 2 comprised 11 clinically affected dogs. After necropsy, one sample was collected from each GIT segment, namely the stomach, duodenum, jejunum, ileum, caecum and colon. Furthermore, paraffin-embedded samples were used for histological and parasitological (immunohistochemistry) evaluation and a morphometrical study were carried out to determine the parasite load (immunolabeled amastigote forms of *Leishmania*). The Friedman and the Mann Whitney tests were used for statistical analysis. The Friedman test was used to analyze each segment of the GIT within each group of dogs and the Mann Whitney test was used to compare the GIT segments between clinically unaffected and affected dogs.

Results: The infected dogs had an increased number of macrophages, plasma cells and lymphocytes, but lesions were generally mild. Parasite distribution in the GIT was evident in all intestinal segments and layers of the intestinal wall (mucosal, muscular and submucosal) irrespective of the clinical status of the dogs. However, the parasite load was statistically higher in the caecum and colon than in other segments of the GIT.

Conclusion: The high parasite burden evident throughout the GIT mucosa with only mild pathological alterations led us to consider whether *Leishmania* gains an advantage from the intestinal immunoregulatory response (immunological tolerance).

Background

Canine visceral leishmaniasis (CVL) is a worldwide zoonosis prevalent in approximately 50 countries in the Mediterranean basin, Middle East and South America [1]. In Brazil, the parasite *Leishmania infantum* is the cause of CVL [2,3] and the sand fly *Lutzomyia longipalpis* is the principal blood-sucking vector. High infection rates are evident in areas of the country where environmental degradation and disorderly migration of people are related to the urbanization process [4,5]. Dogs are the principal reservoir for the parasite, playing a central role in transmission to humans, and therefore present a serious public health concern [6]. CVL infection is considerably more prevalent than clinical illness in endemic areas; this has been reported both in Brazil and Europe (Mediterranean basin countries) [7-13]. Furthermore, clinical signs of the disease are highly variable and can include lymphadenopathy [14,15], skin lesions [16-18], progressive weight loss [19], hepatosplenomegaly [20,21], ocular

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and musculoskeletal abnormalities, renal disease and epistaxis [22-24].

Gastrointestinal tract (GIT) disorders occur in human visceral leishmaniasis (HVL) [25] and in dogs both in natural [26-31] and experimental infection [32,33]. Hervás et al. [34] described pathoanatomical alterations in a hemorrhagic stomach during necropsy of a sylvester reservoir in the jackal (*Canis aureus*); diagnosis was confirmed by the presence of macrophages parasitized with amastigotes forms of *Leishmania*. The majority of studies have described clinical signs and the subsequent inflammatory response in canidae predominantly infected with *Leishmania infantum* from the Mediterranean basin. In Brazil, Silva et al. [35] described inflammatory lesions in one clinically unaffected dog that were comparable to those described in the literature. A chronic inflammatory exudate composed of macrophages, plasma cells and lymphocytes, with rare neutrophils and eosinophils, was found throughout the mucosa of the small and large intestine. Immunohistochemical analysis revealed that macrophages of the lamina propria were parasitized with immunolabelled intracellular amastigote forms of *Leishmania*. The authors reported a chronic inflammatory reaction along segments of the GIT involving all histological layers. Subsequently, Toplu and Aydogan [36] carried out a study in 22 dogs naturally infected with *Leishmania* in Turkey and observed a mild to severe mononuclear cell infiltration, predominantly in the lamina propria, in the small and large intestines of all dogs as described by Silva et al. [35]. However, parasites were evident in only 30 percent of cases.

The aim of this study was to provide a systematic pathological and parasitological overview of the GIT of dogs naturally infected with *L. infantum* from an endemic metropolitan area of Belo Horizonte, Minas Gerais, Brazil.

Methods

Naturally infected animals

Twenty adult mongrel dogs of unknown age naturally infected with *Leishmania* were identified during an epidemiological survey of CVL carried out by the municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil, using indirect immunofluorescence antibody titers (IFAT) and enzyme-linked immunosorbent assay (ELISA). All dogs were positive for IgG when tested using IFAT (titers > 1:40) and ELISA (Optical Density > 100 > 1:400 dilutions). Clinical examinations were carried out on all the infected dogs, which were subsequently divided into two groups; Group I contained nine dogs that were healthy on clinical inspection (four males and five females), while Group II contained eleven dogs (five males and six females) that exhibited classical signs of CVL, including lymphadenopathy, cutaneous changes (alopecia, dry exfoliative dermatitis or ulcers), onychogryphosis, keratoconjunctivitis, cachexia and anemia.

Control dogs

Five dogs of unknown age were obtained from the Control Zoonosis Center of the Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil. Serological (IFAT, ELISA) and parasitological (immunohistochemistry) examinations were negative for *Leishmania* infection.

Ethical committee approve

In Brazil, animals with canine visceral leishmaniasis are usually not treated. So far, the treatment is prohibited followed by Brazilian Healthy Ministry (Portaria Interministerial 1.426 de 11 de Julho de 2008). According to the Ministry of Health Policy (recommended by World Health Organization) seropositive dogs (ELISA by using Biomanguinhos Test-FIOCRUZ-RJ) are eliminated. Otherwise, we do not euthanize dogs without Ethics Committee approval, particularly control dogs (uninfected dogs). Infected and uninfected dogs were obtained from the Control Zoonosis Center of the Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais state, Brazil. The study was submitted to and approved by the CETEA/UFMG (Comite de Etica em Experimentação Animal/Universidade Federal de Minas Gerais), protocol 218/2009 (valid to March 12, 2013). All procedures involving animals were conducted according to the guidelines of the Colégio Brasileiro de Experimentação Animal (COBEA).

Necropsy, parasitological diagnosis and histopathology

Dogs were sacrificed with 2.5% (1.0 ml/kg) thiopental (intravenous) and T61™ (0.3 ml/kg). During necropsy, tissue touch preparations (smears) of liver, spleen and lymph nodes were obtained to confirm *Leishmania* infection. Smears were air-dried and stained with Giemsa. Amastigotes forms of *Leishmania* were detected in all animals using oil immersion light microscopy (1000 × magnification). In addition, one sample of each GIT segment, including stomach, duodenum, jejunum, ileum, caecum and colon, were collected, fixed in 10% buffered formalin, dehydrated, cleared, embedded in paraffin, sectioned (3-4 μm thick) and stained with hematoxylin and eosin for histopathological studies. Each GIT sample segment was macroscopically obtained by one fragment. Therefore, each fragment of one TGI sample segment was sliced in other three new samples (transversal sections). All these three transversal sections were paraffin-embedded, cut and disposable in individual histological slide for histological analysis. Blind histological analysis of slides was carried out by a minimum of two pathologists.

Immunohistochemistry

Immunohistochemistry using the streptoavidin peroxidase reaction was carried out in accordance with Tafuri et al. [37] to demonstrate amastigote forms of *Leishmania* in paraffin-embedded GIT segment tissue samples.

Morphometrical study

For the histomorphometric study, twenty randomly chosen images (horizontal and vertical movements were carried out using the microscope stage - XY translation in order to avoid overlapping fields) from histological slides of GIT tissue fragments were used to assess the area of immunolabelled amastigotes. A Carl Zeiss image analyzer (KS300 software) as described by Caliari [38] and Lima et al. [39] was utilized, as was an Axiolab light microscope (Zeiss) at a resolution of x440.

Statistical analysis

The Friedman test was carried out for each segment of GIT within Group I and Group II for statistical analysis.

The Mann Whitney test was performed for comparison of segments between the two groups. The GraphPad Instat 3.0 and Prism 4.0 software's programs were used for these comparisons. In all cases the statistical difference were considered significant when the probabilities of equality p-values were < 0,05.

Results

Gross examination revealed no severe changes in any part of the GIT mucosa of the dogs included in the study. However, five dogs (20%) (two asymptomatic and three symptomatic dogs) contained helminthes and showed minor focal hyperemia, but no hemorrhages or ulcers were evident.

In infected animals, an increased number of cells, focal or diffuse, was observed in lamina propria, muscularis mucosae and submucosa of GIT, while no changes were found in the control dogs. A chronic cellular exudate was observed in all cases of Groups I and II and was composed predominately by macrophages, plasma cells and lymphocytes with rare polymorphonuclear neutrophils or eosinophils (Figure 1A-E). Macrophages containing many *Leishmania* amastigotes were easily found in lamina propria of the mucosa and the submucosa in the majority of the cases. Many of the macrophages showed a pale and abundant cytoplasm and less dense nucleus typical of epithelioid cells (Figure 1F and 1G). Multinucleated giant cells and epithelioid cells were associated with areas in which mononuclear cells were more concentrated, but no typical granulomatous reaction was evident in the lamina propria (Figure 1H).

Under microscopic analysis, we also observed that in spite of the parasitism we did not find mucosal erosions. Independently of the defined clinical status of the infected dogs, microscopic analysis revealed the presence of amastigote forms of *Leishmania* in all GIT layers, particularly in the cells (macrophages) of lamina propria (Figure 2A-D) without severe GIT mucosal alteration. In fact, immunolabeled amastigotes were evident in all intestinal segments, but the parasitism was more evidenced (frequency) in the caecum and colon than in other GIT segments. In addition a higher parasite load of amastigote forms of *Leishmania* were evident in these segments (Table 1). However, no statistical difference was observed between Groups I and II (Table 2). Thus, in clinically unaffected as well as the affected dogs, parasites were most numerous in the colon, followed by the caecum, while the other GIT segments contained relatively few parasites (Tables 3 and 4, respectively).

Discussion

Histology demonstrated an increased number of macrophages frequently parasitized with *Leishmania* amastigotes, plasma cells and lymphocytes throughout the GIT layers of all segments of the Groups I and II. However, parasites were predominantly located in the mucosa (lamina propria) of the GIT layers. These results are in accordance with Anderson et al. [28], Longstaffe and Guy [40] and Toplu et al. [36].

When comparing the GIT segments of all infected dogs we demonstrated the highest parasite load in the ceacum and colon. These results are consistent with Keenan et al. [41] González et al. [33] Ferrer et al. [30] and Adamama-Moraitou et al. [31]. Keenan et al. [41] observed comparable parasitological loading in all GIT segments of two distinct groups of German shepherd dogs experimentally infected with *L. chagasi* and *L. donovani*. González et al. [33] observed a severe chronic inflammatory process in the mucosa and submucosa of the colon and rectum of infected beagles where they described the surface and the epithelium of the crypts of Lieberkühn with progressive degeneration characterized by cellular swelling. As a result, focal micro-erosions developed in the mucosal surface reducing the area of the large bowel available for absorption and causing diarrhea. Therefore, the authors concluded that chronic colitis in beagles was caused by *L. infantum*, and that the diarrhea was consistent with a disorder prevailing in the large bowel. However, Ferrer et al. [30] reported chronic colitis and diarrhea in two dogs naturally infected with *L. infantum*, but questioned whether the concurrent lympho-plasmacytic infiltrate of the colonic mucosa was caused by *Leishmania* (detected by immunohistochemistry) or was an incidental finding, particularly relevant if the parasite load is low. The authors concluded that there was no evidence of a definitive pathogenic correlation between CVL and chronic colitis. Moreover, Adamama-Moraitou et al. [31] demonstrated that 32.3% of clinically affected dogs naturally

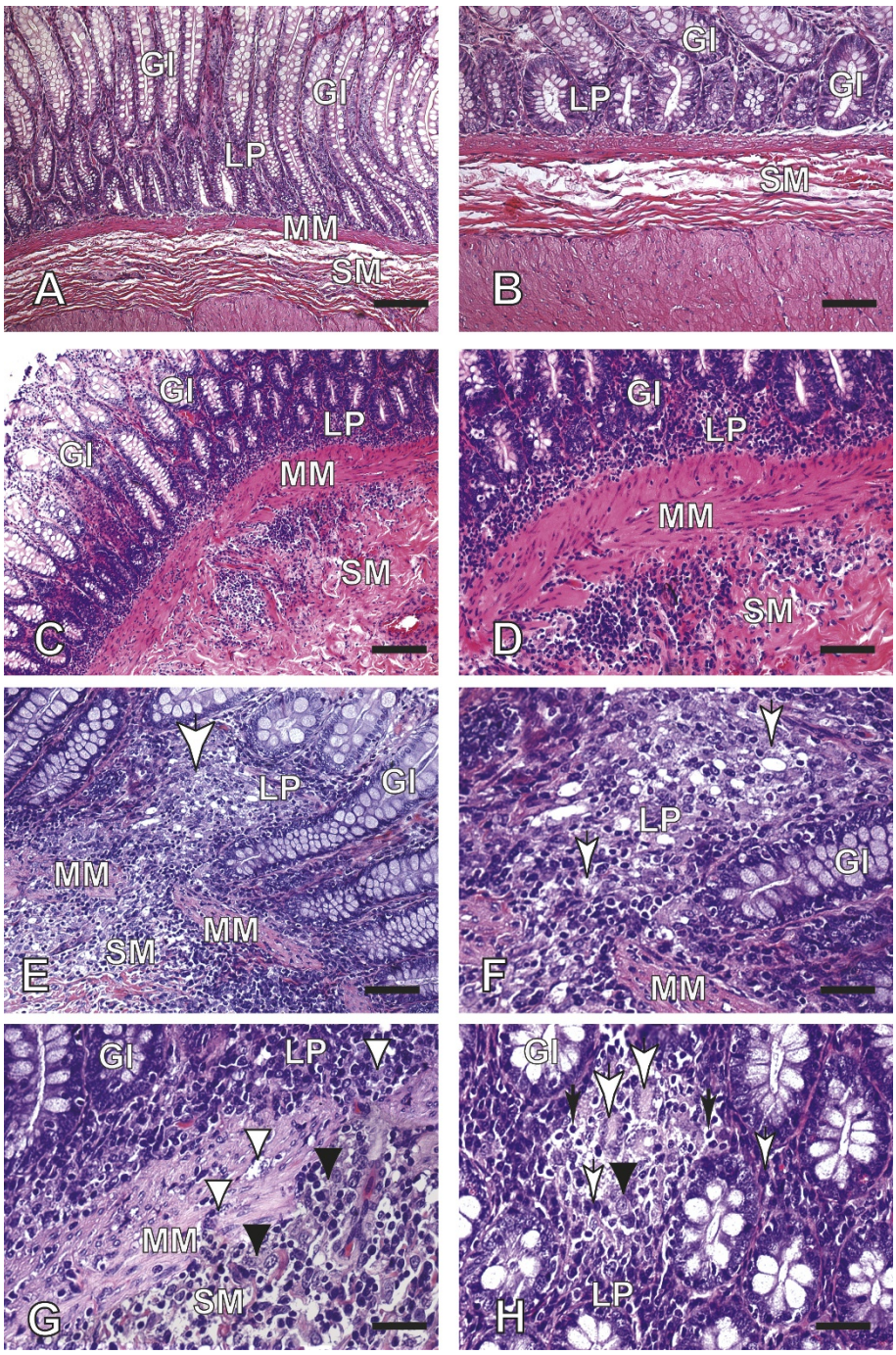

Figure 1 (A and B) Caecum sections of dogs uninfected (control); (C-H) Caecum sections of dogs naturally infected with *L. infantum* (A and B) Observe a histological normal picture of the mucosal (lamina propria), muscularis mucosae and submucosal layers, HE (Bars = 62 μm and 32 μm, respectively); (C-H) Symptomatic dog: (C and D) Note an increased number of cells of all gastrointestinal layers, HE (Bars = 62 μm and 32 μm, respectively); (E) Similarly, we can note an increased number of cells of all gastrointestinal layers where a focal cellular exudates (white arrow) where it becomes contiguous into the three gastrointestinal layers (lamina propria, muscularis mucosae and submucosal), HE (Bar = 32 μm); (F and G) Higher magnification of the previous picture: In (F) note parasitized macrophages in the lamina propria (mucosa layer) (white arrows), (Bar = 16 μm) and in (G) observe macrophages loaded with *Leishmania* in the muscularis mucosae (white arrowheads) and submucosal layers (black arrowheads) (Bar = 16 μm); (H) Multinucleated giant cells (white arrows) or epithelioid cells (black arrowhead) formation can be seen associated to the exudate of mononuclear concentrated in lamina propria (plasma cells - small white arrow, lymphocytes - small black arrow), HE (Bar = 16 μm); LP: Lamina Propria; MM: Muscularis Mucosae; SM: Submucosal; GI: Glands of Intestine Mucosal) and Hematoxilin-Eosin (HE).

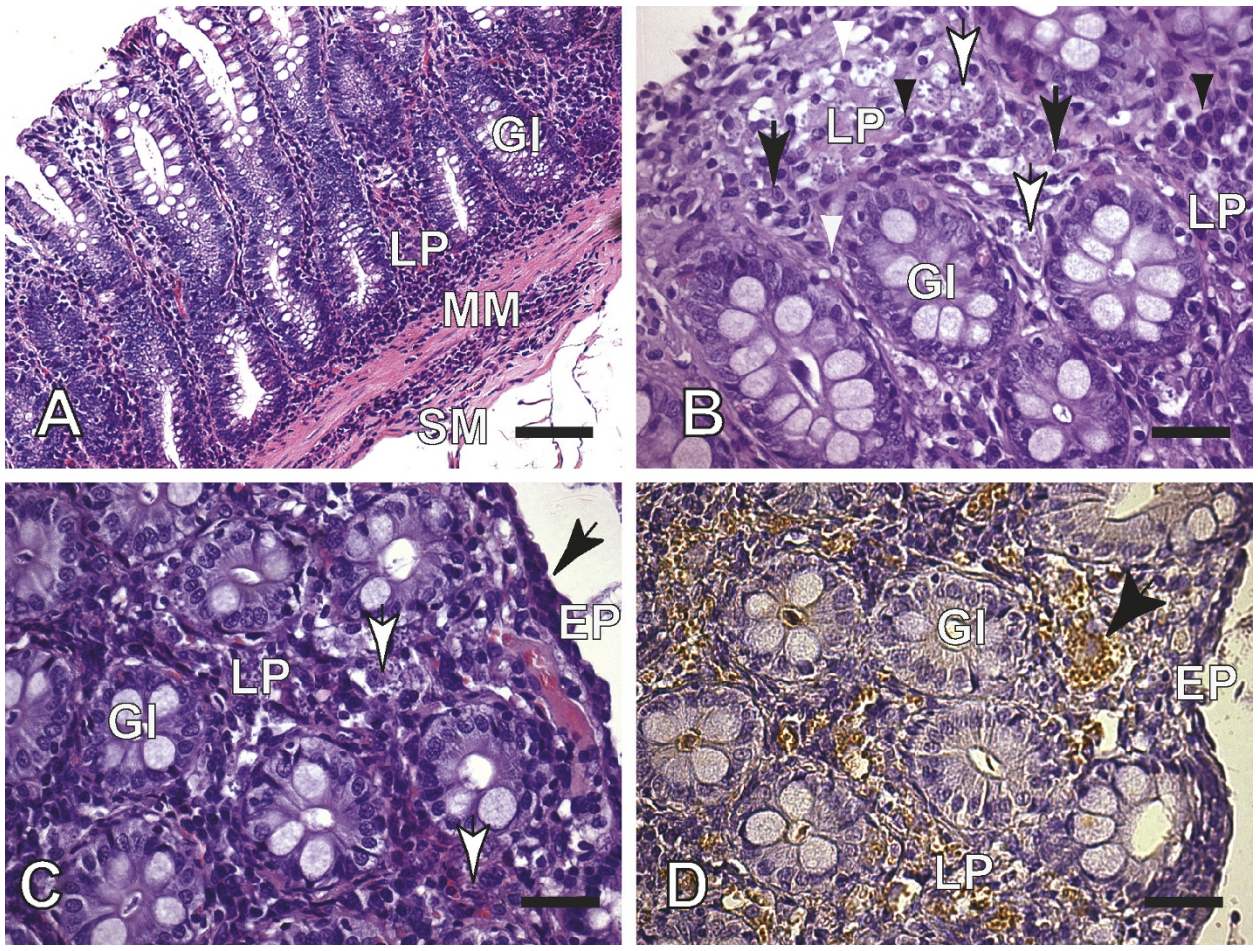

Figure 2 (A-D) Colon sections of an asymptomatic dog naturally infected with *L. infantum*: (A and B) (A) Panoramic view of mucosal (lamina propria), muscularis mucosae and submucosal layers of caecum fragment showing an intense and diffuse cellular exudates, HE (Bar = 32 μm); (B) Higher magnification showing mononuclear cells represented by parasitized macrophages with *Leishmania* (white arrow) or not (black arrow), lymphocytes (white arrowheads) and plasma cells (black arrowheads) without ulcer or erosions of the epithelium (Bar = 16 μm); (C) Mucosa (Lamina propria) layer showing an increased number of cells. Macrophages with *Leishmania* can be noted (white arrows). Only a discrete atrophy (flatting) of the epithelial cells can be observed (black arrow), HE (Bar = 16 μm); (D) Same fragment of colon showing higher parasitism tissue load. Observe a hipertrophic macrophages with many intracellular forms of *Leishmania* (black arrow), Streptoavidin-peroxidase, (Bar = 16 μm). LP: Lamina Propria; MM: Muscularis mucosae; SM: Submucosal; GI: Glands of Intestine Mucosal and Hematoxilin-Eosin (HE).

infected with *L. infantum* presented with asymptomatic colitis. Endoscopic examination of colonic mucosa demonstrated focal hyperemia, edema and mild erosions in infected dogs, which were comparable to those observed in dogs with CVL or other infections including *Histoplasma, Salmonella* and *Yersinia*, or infected with parasites such as *Giardia, Trichuris, Ancylostoma, Entamoeba* and *Balantidium*. However, the authors excluded these infections after fecal examination. As reported by Chiapella [42], it is important to consider idiopathic large bowel inflammatory diseases such as plasmacytic-lymphocytic colitis and histiocytic-ulcerative colitis before making a diagnosis.

No severe macroscopic and microscopic lesions in the mucosa of the GIT during necropsy were evident during the present study. In some dogs the mucosa was somewhat hyperemic, and helminth parasites were observed in less than 20% of cases (three clinically affected and two unaffected dogs). Despite the presence of helminth parasites in the guts of some dogs, it was concluded that this did not interfere with the histological analysis of GIT segments from dogs with CVL, as no severe macroscopic or microscopic related alterations were evident, such as hemorrhages, ulcers or pyogenic granulomas. In the present study, an increased number of macrophages, frequently parasitized with *Leishmania* amastigotes, and plasma cells and lymphocytes, were observed throughout the GIT layers. However, as depicted in Figure 2, despite the presence of *Leishmania* amastigotes in macrophages of the lamina propria, only

Table 1 Frequency of amastigote forms of *Leishmania* in the gastrointestinal tract (GIT) of asymptomatic and symptomatic naturally infected dogs with *Leishmania infantum* from Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil

Gastrintestinal fragments	Frequence (n = 20)	
	Asymptomatic (n = 9)	Symptomatic (n = 11)
Stomach	3 (33,3%)	1 (9,0%)
Duodenum	4 (44,4%)	6 (54,5%)
Jejune	5 (55,5%)	7 (63,6%)
Ileum	5 (55,5%)	7 (63,6%)
Cecum	8 (88,8%)	9 (81,8%)
Colon	6 (66,6%)	10 (90,9%)

Table 2 Morphometrical analysis (μm^2) to quantify amastigotes forms of *Leishmania* in the gastrointestinal tract (GIT) of asymptomatic and symptomatic naturally infected dogs with *Leishmania infantum* from Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil

Gastrintestinal fragments	Clinic status (n = 20)		Statistical analysis (Mann Whitney Test)
	Asymtomatic	Symptomatic	(p < 0,05)
Stomach	0,21	0,15	0,9671
Duodenum	0,22	0,43	0,4029
Jejune	0,10	0,05	0,6474
Ileum	0,04	0,13	0,2443
Cecum	0,76	3,03	0,7725
Colon	0,41	4,73	0,1165

Table 3 Morphometrical analysis (μm^2) to quantify amastigotes forms of *Leishmania* in the gastrointestinal tract of asymptomatic naturally infected dogs with *Leishmania infantum* from Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil

Gastrintestinal fragments	μm^2	Gastrintestinal fragments	μm^2	Statistical analysis (Friedman test) (p < 0,05)
Stomach	0,21	Duodenum	0,22	0,3383
Stomach	0,21	Jejune	0,11	0,4911
Stomach	0,21	Ileum	0,04	0,3678
Stomach	0,21	Cecum	0,76	0,2475
Stomach	0,21	Colon	0,41	0,9579
Duodenum	0,22	Jejune	0,10	0,5577
Duodenum	0,22	Ileum	0,04	0,7091
Duodenum	0,22	Cecum $^+$	0,76	0,0354*
Duodenum	0,22	Colon	0,41	0,2892
Jejune	0,10	Ileum	0,04	0,5589
Jejune	0,10	Cecum $^+$	0,76	0,0403*
Jejune	0,10	Colon	0,41	0,5607
Ileum	0,04	Cecum $^+$	0,76	0,0180*
Ileum	0,04	Colon	0,41	0,2667
Cecum	0,76	Colon	0,41	0,2928

discrete atrophy of the mucosa epithelium cells without any severe lesions (necrotic or erosive mucosal lesions) was observed. González et al. [33] described the surface and the epithelium of the crypts of Lieberkühn as showing progressive degeneration characterized by cellular swelling, but with only focal micro-erosions, in the mucosa of the colon and rectum. Epithelioid and multinuclear cell formations were observed in some cases, although typical granulomas were not observed. However, Adamama-Moraitou et al. [31] reported that a

Table 4 Morphometrical analysis (μm²) to quantify amastigote forms of *Leishmania* in the gastrointestinal tract of symptomatic naturally infected dogs with *Leishmania infantum* from Municipality of Ribeirão das Neves, Belo Horizonte Metropolitan area, Minas Gerais (MG) state, Brazil

Gastrintestinal fragments	μm²	Gastrintestinal fragments	μm²	Statistical analysis (Friedman test) (p < 0,05)
Stomach	0,15	Duodenum	0,43	0,4687
Stomach	0,15	Jejune	0,05	0,1387
Stomach	0,15	Ileum	0,05	0,6451
Stomach	0,15	Cecum	0,05	0,0761
Stomach	0,15	Colon $^{+}$	0,05	0,0302*
Duodenum	0,43	Jejune	0,05	0,7917
Duodenum	0,43	Ileum	0,13	0,6919
Duodenum	0,43	Cecum	3,03	0,0565
Duodenum	0,43	Colon $^{+}$	4,73	0,0138*
Jejune	0,05	Ileum	0,13	0,3912
Jejune	0,05	Cecum^{+}	3,03	0,0125*
Jejune	0,05	Colon^{+}	4,73	0,0031*
Ileum	0,13	Cecum^{+}	3,03	0,0486*
Ileum	0,13	Colon^{+}	4,73	0,0417*
Cecum	3,03	Colon	4,73	0,6457

granulomatous inflammatory pattern was the most common histological feature in the colonic mucosa and that this could be associated with a mildly eroded colonic mucosa.

In our study, clinically affected dogs harbored more parasites in the caecum and colon than in other GIT segments. This is consistent with other reports Anderson et al. [28]; Ferrer et al. [30]; González et al. [33]; Silva et al. [35]; Longstaffe and Guy [40]; Keenan et al. [41] where the difference in parasitic loading between specific GIT segments was observed in clinically affected dogs. However, it is important to reiterate that in our studies also clinically unaffected dogs harbored parasites in all GIT segments.

Conclusion

We observed a high parasite burden throughout the GIT mucosa, but the pathological changes were relatively mild. Thus, this may led us to consider whether *Leishmania* gains an advantage from the intestinal immunoregulatory response (immunological tolerance). Such question will require further profound research and will help to elucidate the mechanisms underlying *Leishmania* infection.

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Authors' contributions

AJWP, MMF and FLS did all the clinical exams, necropsies and histology. AJWP, MMF, TM did morphometrical and statistical analysis. MSMM was responsible for all serological exams. WLT, WLT advisors, revise all the histological analysis and the manuscript.
All authors read and approved the final manuscript.

Competing interests

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References

1. Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L: **Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one.** *Trends Parasitol* 2008, **24**:324-330.
2. Maurício IL, Stothard JR, Miles MA: **The strange case of *Leishmania chagasi*.** *Parasitol Today* 2000, **16**:188-189.
3. Lukes J, Mauricio IL, Schönian G, Dujardin JC, Soteriadou K, Dedet JP, Tintaya KW, Jirku M, Chocholová E, Haralambous C, Pratlong F, Oborník M, Horák A, Ayala FJ, Miles MA: **Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy.** *Proc Natl Acad Sci USA* 2008, **29**:9375-9380.
4. França-Silva JC, Barata RA, Costa RT, Monteiro EM, Machado-Coelho GL, Vieira EP, Prata A, Mayrink W, Nascimento E, Fortes-Dias CL, Silva JC, Dias ES: **Importance of Lutzomyia longipalpis in the dynamics of transmission of canine visceral leishmaniasis in the endemic area of Porteirinha Municipality, Minas Gerais, Brazil.** *Vet Parasitol* 2005, **131**:213-220.

5. Rondon FC, Bevilaqua CM, Franke CR, Barros RS, Oliveira FR, Alcântara AC, Diniz AT: **Cross-sectional serological study of canine *Leishmania* infection in Fortaleza, Ceara state, Brazil.** *Vet Parasitol* 2008, **155**:24-31.
6. Deane LM, Deane MP, Alencar JE: **Control of *Phlebotomus longipalpis* by DDT house spraying endemic foci of kala-azar in Ceará.** *Rev Bras Malariol Doenças Trop* 1955, **7**:131-141.
7. Tesh RB: **Control of zoonotic visceral leishmaniasis: is it time to change strategies?** *Am J Trop Med Hyg* 1995, **52**:287-292.
8. Abranches P, Campino L, Santos-Gomes GM: **Canine leishmaniasis. New concepts of epidemiology and immunopathology: their impact in the control of human visceral leishmaniasis.** *Acta Med Port* 1998, **11**:871-875.
9. Solano-Gallego L, Morell P, Arboix M, Alberola J, Ferrer L: **Prevalence of Leishmania infantum infection in dogs living in an area of canine leishmaniasis endemicity using PCR on several tissues and serology.** *J Clinical Microbiol* 2001, **39**:560-563.
10. Solano-Gallego L, Koutinas A, Miro G, Cardoso L, Pennisi MG, Ferrer L, Bourdeau P, Oliva G, Baneth G: **Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniasis.** *Vet Parasitol* 2009, **165**:1-18.
11. Silva ES, Gontijo CM, Pacheco RS, Fiúza VO, Brazil RP: **Visceral leishmaniasis in the Metropolitan Region of Belo Horizonte, State of Minas Gerais, Brazil.** *Memórias do Instituto Oswaldo Cruz* 2001, **96**:285-291.
12. Michalsky EM, Rocha MF, Rocha-Lima AC, França-Silva JC, Pires MQ, Oliveira FS, Pacheco RS, Santos SL, Barata RA, Romanha AJ, Fortes-Dias CL, Dias ES: **Infectivity of seropositive dogs, showing different clinical forms of leishmaniasis, to *Lutzomyia longipalpis* phlebotomine sand flies.** *Vet Parasitol* 2007, **147**:67-76.
13. Queiroz PV, Monteiro GR, Macedo VP, Rocha MA, Batista LM, Queiroz JW, Jerônimo SM, Ximenes MF: **Canine visceral leishmaniasis in urban and rural areas of Northeast Brazil.** *Res Vet Sci* 2009, **86**:267-273.
14. Lima WG, Michalick MSM, Melo MN, Tafuri WL, Tafuri WL: **Canine visceral leishmaniasis: a histopathological study of lymph nodes.** *Acta Trop* 2004, **92**:43-53.
15. Costa MM, Lima WG, Figueiredo MM, Michalick MSM, Tafuri WL, Tafuri WL: **Cervical, mandibular, and parotid lymph nodes of dogs naturally infected with Leishmania infantum: a histopathologic and immunohistochemistry study and its correlation with facial skin lesions.** *Vet Pathol* 2008, **45**:613-616.
16. Ferrer L, Rabanal RM, Domingo M, Ramos JA, Fondevila D: **Identification of *Leishmania donovani* amastigotes in canine tissues by immunoperoxidase staining.** *Res Vet Sci* 1988, **44**:194-196.
17. Xavier SC, Andrade HM, Monte SJ, Chiarelli IM, Lima WG, Michalick MSM, Tafuri WL, Tafuri WL: **Comparison of paraffin-embedded skin biopsies from different anatomical regions as sampling methods for detection of *Leishmania* infection in dogs using histological, immunohistochemical and PCR methods.** *BMC Vet Res* 2006, **2**:17.
18. Figueiredo MM, Moura EP, Costa MM, Ribeiro VM, Michalick MSM, Tafuri WL, Tafuri WL: **Histopathological and parasitological investigations of ear healthy skin of dogs naturally and experimentally infected with *Leishmania (Leishmania) chagasi*.** *Histol Histopathol* 2010, **25**.
19. Ciaramella P, Oliva G, Luna RD, Gradoni L, Ambrosio R, Cortese L, Scalone A, Persechino A: **A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*.** *Vet Rec* 1997, **141**:539-543.
20. Tafuri WL, Tafuri WL, Barbosa AJ, Michalick MSM, Genaro O, França-Silva JC, Mayrink W, Nascimento E: **Histopathology and immunocytochemical study of type 3 and type 4 complement receptors in the liver and spleen of dogs naturally and experimentally infected with Leishmania (Leishmania) chagasi.** *Rev Inst Med Trop São Paulo* 1996, **38**:81-89.
21. Melo F, Amaral M, Oliveira P, Lima WG, Andrade M, Michalick MSM, Raso P, Tafuri WL, Tafuri WL: **Diffuse intralobular liver fibrosis in dogs naturally infected with Leishmania (Leishmania) chagasi.** *Am J Trop Med Hyg* 2008, **79**:198-204.
22. Tafuri WL, Michalick MSM, Dias M, Genaro O, Leite VH, Barbosa AJ, Bambirra EA, Costa CA, Melo MN, Mayrink W: **Optical and electron microscopic study of the kidney of dogs naturally and experimentally infected with *Leishmania (Leishmania) chagasi*.** *Rev Inst Med Trop Sao Paulo* 1989, **31**:139-145.
23. Koutinas AF, Polizopoulou ZS, Saridomichelakis MN, Argyriadis D, Fytianou A, Plevraki KG: **Clinical considerations on canine visceral leishmaniasis in Greece: a retrospective study of 158 cases 1989-1996.** *J Am Animal Hosp Association* 1999, **35**:376-383.
24. Alvar J, Canavate C, Molina R, Moreno J, Nieto J: **Canine leishmaniasis.** *Adv Parasitol* 2004, **57**:1-88.
25. Veress B, Malik MO, Satir AA, Hassan AM: **Morphological observations on visceral leishmaniasis in the Sudan.** *Trop Geog Med* 1974, **26**:198-203.
26. Lennox WJ, Smart ME, Little PB: **Canine leishmaniasis in Canada.** *The Can Vet J* 1972, **13**:188-190.
27. Tryphonas L, Zawidzka Z, Bernard MA, Janzen EA: **Visceral leishmaniasis in a dog: clinical, hematological and pathological observations.** *Can J Comp Med* 1977, **41**:1-12.
28. Anderson DC, Buckner RG, Glenn BL, MacVean DW: **Endemic canine leishmaniasis.** *Vet Pathol* 1980, **17**:94-96.
29. Slappendel RJ: **Canine leishmaniasis. A review based on 95 cases in The Netherlands.** *Vet Quart* 1988, **10**:1-16.
30. Ferrer L, Juanola B, Ramos JA, Ramis A: **Chronic colitis due to *Leishmania* infection in two dogs.** *Vet Pathol* 1991, **28**:342-343.
31. Adamama-Moraitou KK, Rallis TS, Koytinas AF, Tontis D, Plevraki K, Kritsepi M: **Asymptomatic colitis in naturally infected dogs with *Leishmania infantum*: a prospective study.** *Am J Trop Med Hyg* 2007, **76**:53-57.
32. Keenan CM, Hendricks LD, Lightner L, Webster HK, Johnson AJ: **Visceral leishmaniasis in the German shepherd dog. I. Infection, clinical disease, and clinical pathology.** *Vet Pathol* 1984, **21**:74-79.
33. González JL, Fermin ML, Garcia P, Rollan E, Castano M: **Erosive colitis in experimental canine Leishmaniasis.** *J Vet Med* 1990, **37**:377-382.
34. Hervás J, Mendez A, Carrasco L, Gomez-Villamandos JC: **Pathological study of visceral leishmaniasis in a jackal (*Canis aureus*).** *Vet Rec* 1996, **139**:293-295.
35. Silva FL, Tafuri WL, Oliveira MR, Tafuri WL: **Histopathological and immunohistochemical study of the gastrointestinal tract from a dog naturally infected with *Leishmania (Leishmania) chagasi*: A case report.** *Arq Bras Med Vet Zootec* 2002, **54(4)**.
36. Toplu N, Aydogan A: **An immunohistochemical study in cases with usual and unusual clinicopathological findings of canine visceral leishmaniosis.** *Parasitol Res* 2011, DOI10.1007/s00436-011-2345-0.
37. Tafuri WL, Santos RL, Arantes RM, Gonçalves R, Melo MN, Michalick MSM, Tafuri WL: **An alternative immunohistochemical method for detecting *Leishmania* amastigotes in paraffin-embedded canine tissues.** *J Immunol Methods* 2004, **292**:17-23.
38. Caliari MV: **Princípios de Morfometria Digital: KS300 para iniciantes.** *Editora da UFMG* 1997, **148.**
39. Lima WG, Oliveira PS, Caliari MV, Gonçalves R, Michalick MSM, Melo MN, Tafuri WL, Tafuri WL: **Histopathological and immunohistochemical study of type 3 complement receptors (CD11b/CD18) in livers and spleens of asymptomatic and symptomatic dogs naturally infected with *Leishmania (Leishmania) chagasi*.** *Vet Immunol Immunopath* 2007, **117**:129-136.
40. Longstaffe JA, Guy MW: **Leishmaniasis in dogs.** *Vet Annu* 1985, **25**:358-367.
41. Keenan CM, Hendricks LD, Lightner L, Johnson AJ: **Visceral leishmaniasis in the German shepherd dog. II. Pathology.** *Vet Pathol* 1984, **21**:80-86.
42. Chiapella A: **Diagnosis and Management of chronic colitis in the dog and cat.** *Cur Vet Therapy* 1986, 267-274.

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