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Original full paper

Description of Visceral, Lymphatic and Central Nervous System Lesions in Dogs Infected with *Leishmania* spp.

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Abstract

Leishmaniases are a group of chronic systemic infectious diseases caused by intracellular protozoa of the genus *Leishmania*. In the present study the authors aimed to investigate *Leishmania* spp-induced lesions mainly to detect the presence of the parasite within the CNS and lymph nodes in dogs from the Araguaina region, Tocantins State, Northern Brazil. Descriptive statistical analysis of variance and correlation were conducted, indicating that gross signs and visceral lesions do not correlate positively. Therefore we conclude that the diagnosis cannot be based on macroscopic signs alone presented by the infected dogs.

Key Words: Leshmania spp; Central nervous system; dogs

Introduction

Leishmaniases are a group of chronic systemic infectious diseases caused by intracellular protozoa of the genus *Leishmania*. Reservoir hosts include domestic and wild animals. They affect people and domestic and wild animals and as zoonosis are a matter of great concern in many areas of the world. The disease is endemic in many South American countries (12), and the canine disease is prevalent in many Brazilian areas (4; 7, 10) where infected dogs act as reservoirs for people.

The infection is transmitted by members of the genus *Phlebotomus* in the Old World and *Lutzomya* in the New World. Transmission occurs when the hematophagous female sandfly first bites infected individuals – usually in crepuscular and nocturnal hours- and ingests infected macrophages; this vector harbors promastigotes in their gut which are transmitted during a blood meal to people, domestic or wild animals where the amastigote form develops (8).

Leishmania spp are intracellular microorganisms which, without the host´s efficient immune response, multiply and migrate from lymphoid organs to other organs inducing clinicopathological changes that may lead to death of the host. *Leishmania* spp also survive by modulating the host´s immune system when they either promote immunosuppression or pro parasitism functions (1). Thus, depending on the animal´s immune state, signs of the disease could be observed after months or even years post infections. Yet they may never be detected and the animal might be an unsuspected infecting reservoir.

A study compared parasitic *Leishmania* spp density in dog´s skin, lymph nodes and viscera of symptomatic and asymptomatic seropositive animals and found no differences between them, with the skin being the most parasitized organ, round the place of the bite (13). The finding suggests that animals symptomatic and asymptomatic can be permanent source of the infection.

In order to correlate facial skin lesions with parasitic load in regional lymph nodes, a previous study analyzed infected dogs with and without clinical signs. It was observed that dogs with facial lesions had larger loads in cervical lymph nodes than dogs without facial lesions, although the latter harbored chronic skin inflammation with macrophages infiltration, plasma cells and lymphocytes, thus confirming the fact that apparently normal skin may host the parasite (5).

The disease develops as a progressive chronic illness 2 to 4 months or even 7 years after the infection (2) characterized as chronic and debilitating (caquexia), with skin lesions (hyperkeratosis, dry scaling dermatitis, ulcerations and alopecia), generalized lymphadenomegaly, hepatosplenomegaly and anemia (4, 23).

Regarding the prevalence of the parasites within the central nervous system (CNS), the matter is not settled, although neurological signs are not frequent. A previous histopathological study in dogs revealed some morphological changes associated with a chronic meningeal inflammation with lymphoplasmocytic infiltrate (23).

A previous study found similar changes in the CNS, i.e. lymphoplasmocytic and histiocytic meningitis, perivascular cuffing and gliotic foci in small numbers in relation to the total number of seropositive animals. Only 30,76% from 39 samples showed changes, and the presence of parasites was detected in only 5,12% from all animals. This finding reveals the importance of investigating the CNS involvement in leishmaniasis cases (18).

There are many diagnostic methods for leishmaniasis. None of them has, as yet, 100% sensitivity. Clinical diagnosis encounters many difficulties in detecting the disease, since one individual may manifest specific and non specific signs of leishmaniasis (21). This indicates the need to develop new diagnostic methods.

In the present study the authors aimed to correlate *Leishmania* spp-induced lesions among lymph nodes, viscera and the CNS, and to detect the presence of the parasite within the CNS and lymph nodes in dogs from the Araguaina region, Tocantins State, Northern Brazil.

Material and Methods

Samples

Tissue samples were collected from 23 dogs euthanized at the Center for Zoonosis Control (CZC) of Araguaina County, State of Tocantins, Brazil. Necropsies of all dogs were performed to withdraw lymph nodes, brain, kidney, spleen, liver, heart, lungs and skin samples.

Gross lesions evaluated

Onychogryphosis, lymphadenomegaly, hepatomegaly, splenomegaly, skin lesions, and conjunctivitis were evaluated in order to classify the dogs as symptomatic, oligosymptomatic or asymptomatic. (20)

Findings were submitted to descriptive statistics. Width and length of lymph nodes were measured with a caliper ruler and the area was calculated applying the ellipse formula (Area=JI.[width/2]. [length/2]).

Tukey test was used for variance analysis of lymph nodes-parasitic load, and Spearman correlation to evaluate putative correlation between data.

Histological methods

The collected samples were fixed in 10% neutral formalin and routinely processed for paraffin embedding. The organs, except the brain, were sliced in coronal sections 1 cm thick and after fixation further sliced in sections 2 mm thick prior to paraffin embedding. Histological sections 3 µm thick were cut from the paraffin blocks and stained with Hematoxylin and Eosin.

Histological slides were scanned to detect the agent and the induced lesions. The lesions were classified according to the intensity as: absent (-), mild (+), moderate (++), marked (+++).

Results and Discussion

Twenty three *Leishmania* spp-seropositive dogs from the CZC (Araguaina county, TO) were necropsied and samples collected. During necropsy, external as well as internal features usually present in infected animals were looked for, i.e. onychogryphosis, lymphadenomegaly, hepatomegaly, splenomegaly, skin lesions and conjunctivitis.

Eighteen out of 23 dogs were symptomatic and showed at least one of the gross lesions listed above. Nine of them were classified as polysymptomatic with at least three gross lesions, nine were oligosymptomatic, showing between one and three gross lesions and five of the animals were asymptomatic.

Gross lesions detected: onychogryphosis (12 dogs), lymphadenomegaly (8 dogs), hepatomegaly (11 dogs), splenomegaly (11 dogs), facial skin lesions (3 dogs), conjunctivitis (3 dogs) are summarized on Table 1.

Main changes detected in the collected samples from the 23 infected dogs were related to the inflammation induced by the agent as formerly reported by Mendonça & Ramos (2011).

Splenic lesions were characterized by massive macrophagic infiltration in 17 dogs; same change has been reported by Xavier (2006). From the 17 dogs, in two the

presence of the agent was conspicuous, in four macrophages had a marked hemossiderin load, and three had lost their follicular structure. Lung alterations were edema (6 dogs), vascular congestion (11 dogs), and lymphoplasmocytic and histiocytic bronchitis (4 dogs), same changes described by LUVIZOTTO, (2006). Kidney changes (9 dogs) were of lymphoplasmocytic and histiocytic infiltrate; seven of those also had vascular congestion. *Leishmania* spp-induced kidney lesions frequently lead to renal failure, one of the major causes of death in the disease (9).

Table 1. Gross lesions detected in 23 *Leishmania* spp-seropositive dogs

Gross lesions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Onychogryphosis	X	X		X						X		X		X	X	X			X	X	X	X	
Lymphadenomegaly	X				X					X	X	X		X		X							
Hepatomegaly	X					X	X		X		X			X	X				X		X	X	X
Splenomegaly	X				X					X	X			X	X	X				X	X	X	X
Facial skin lesions					X					X	X												
Conjunctivitis		X			X									X									

The liver was one of the more affected organs and the lesions were those of lymphoplasmocytic and histiocytic hepatitis (17 dogs), vascular congestion (20 dogs) and swollen cells (4 dogs). The majority of these animals present granulomatous lesions. However, mononuclear infiltrates are also a common finding (22).

Only few dogs had brain changes, all of which of inflammatory nature: focal lymphoplasmocytic meningitis and lymphocytes infiltration (4 dogs), lymphohistiocytic meningitis (Figure 1) (2 dogs), and vascular congestion (5 dogs). In none of the sections the agent was detected. It has been previously reported lymphocytic choroiditis and meningitis, with the presence of the parasite (*Leishmania infantum [= L. chagasi]* inside and outside macrophages in dogs). In contrast, no parasite was detected in HE or IHC histopathological examinations in this study. Parasites were also not detected in other studies (17), although high anti-Leishmania antibody titers were detected, including in the cerebrospinal fluid. Pathological reactions (leptomeningitis, satelitosis, neurophagitis and dark neurons) were previously reported in the CNS of dogs with leishmaniasis without neurological symptoms. In patients with neurological signs, histopathological alterations were more frequent and intense (11). This condition is known as cerebral leishmaniasis (17). The brain appears somehow protected in dogs infected with *Leishmania* spp. It has been suggested that it is because of the metalloproteases that take part in the blood brain barrier (16).

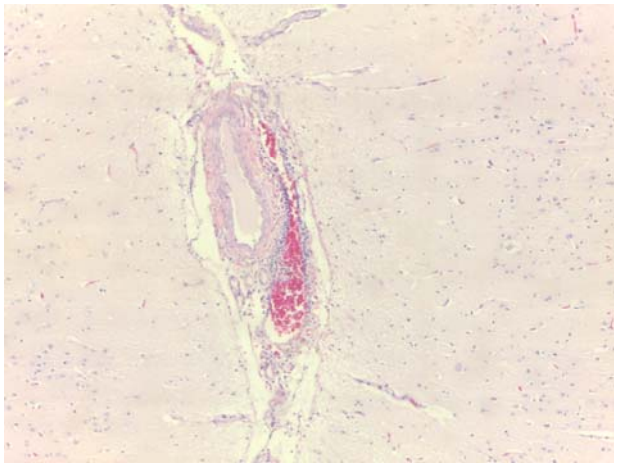

Figure 1. Brain cortex, mild lymphohistiocytic meningitis. HE Stain, Objective: 10X.

No significant correlation was found among the lesions described in all the organs investigated (e.g., higher degrees of hepatic lesions were not necessarily accompanied by higher degrees of kidney lesions).

There was no correlation (0,1612; p=0,46) between the number of parasites found within lymph nodes and the degree of visceral lesions. Therefore, asymptomatic dogs are a source of infection for the sandfly vector and have a very important part in the transmission of the disease (19).

When comparing the number of parasites in lymph nodes with CNS lesions, there was a negative correlation between them (-0,1705; p=0,43).

Another lack of correlation was observed when comparing the number of parasites with de size of the corresponding infected submandibular, prescapular and popliteal lymph nodes (Table 2).

Table 2. Correlation data between lymph node sectional area and the number of counted parasites.

Lymphnode	Area mm^2	Number of protozoa	Correlation	P
Submandibular	0,6471	18,1695a	0.32732	0.1274
Prescapular	0,9608	14,9956ab	0.19328	0.3769
Popliteal	0,9067	9,0000b	0.01261	0.9545
Total	0,8382	14,0550	0.24111	0.2677

Tukey test among lymph nodes; P<0,05

When comparing the amount of visceral lesions and CNS lesions, no correlation was found (0,0788; p=0,7207). Thus an animal with many visceral lesions will not necessarily have CNS lesions and vice versa. In fact, CNS lesions were observed in only a few dogs (10/23), suggesting that the system does not favor *Leishmania* spp infection. Some previous investigations have pointed to that fact (11). Also, brain lesions when present were mild (Table 3).

Table 3. Intensity of brain lesions in Leishmania spp-seropositive dogs in Araguaina County, TO.

Change/ Animal	L1	L5	L7	L8	L9	L11	L12	L14	L18	L20
Lymphoplasmocytic meningitis	+	-	-	+	-	-	-	-	-	+
Lymphocytes infiltration	-	-	+	-	+	-	-	-	+	-
Lymphohistiocytic meningitis	+	-	-	-	-	-	-	+	-	-
Protozoa presence	-	-	-	-	-		-	-	-	-
Vascular congestion	-	+	-	+	-	+	+	-	+	-

-: absent, +: mild, ++ moderate, +++: marked

There was neither correlation between gross changes and intensity of histological lesions (0,2890; p=0,1810) nor between gross changes and amount of parasites within lymph nodes of all 23 dogs (0,1187; p=0,5893). Those data demonstrate that each animal has a peculiar form of reacting to *Leishmania* spp infection, which may change according to the geographical region, climate (3) and breed (20). Thus, a negative diagnosis cannot be based solely on the absence of signs.

Mean of the amount of parasites per regional lymph node (Giemsa stain) are summarized in Figure 4. It is confirmed that the greatest load is found in cranial lymph nodes (Figure 2 and 3). A previous study (5) reported the differences between parasite load in cranial lymph nodes due to differences in the regions drained by these lymph nodes.

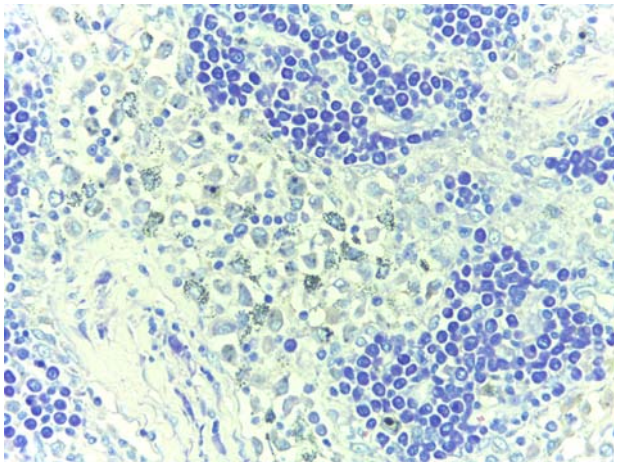

Figure 2. Leishmania amastigotes in prescapular lymph node. Giemsa Stain, Objective: 40X.

This mechanism would also explain the differences found in the present study due to the fact that lymph nodes with higher parasite load are located near the cranial region, where cutaneous lesions are more frequent and abundant.

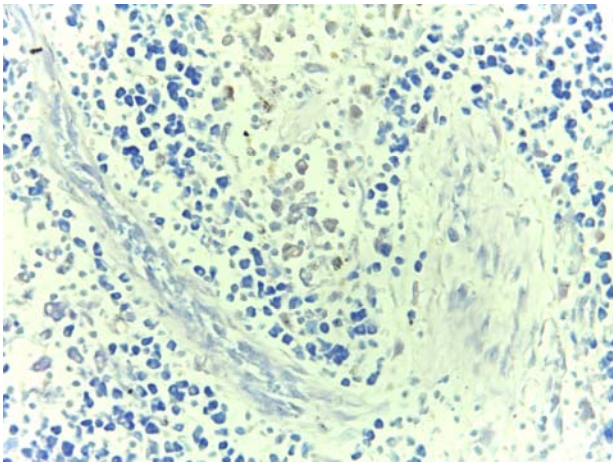

Figure 3. Leishmania amastigotes in popliteal lymphonode. Giemsa Stain, Objective: 40X.

The various forms of manifestation of the disease may be related mainly to variations in the resistance of the host, variations in the infectivity and antigenicity of *Leishmania infantum (= L. chagasi)* (6), with changes in the host immune response. Some dogs have predominantly macrophages and lymphocytes inflammatory infiltrate, and others only of mast cells (3).

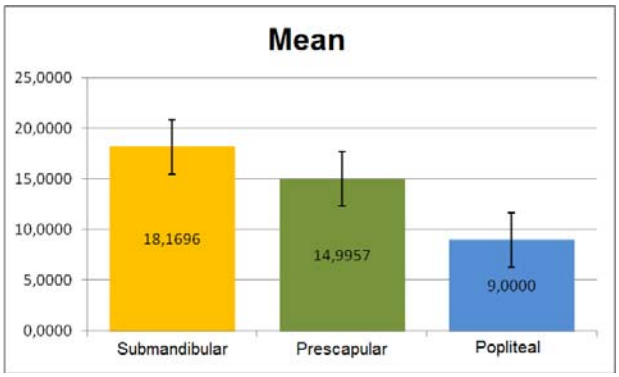

Figure 4. Mean of parasites in regional lymph nodes (Error=2,6914)

Conclusions

From the changes detected in samples obtained from 23 *Leishmania* spp-infected dogs, liver, spleen and cranial lymph nodes were the most affected organs with inflammatory lesions. Regarding lymph nodes, cranial lymph nodes had higher parasite load when compared to popliteal lymph nodes, indicating that the former would be the most suitable for parasite search.

An important data was obtained by comparing the presence of macroscopic signs with the degree of visceral lesions. It was possible to verify that animals with high degree of visceral lesions did not show gross signs and animals showing significant gross signs had a lower degree of visceral lesion.

Gross signs and visceral lesions do not correlate positively. Therefore, we conclude that leishmaniasis diagnosis cannot be based on macroscopic signs alone since they may not represent confirmative data of the disease.

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