

RENAL HISTOPATHOLOGICAL FINDINGS IN DOGS WITH VISCERAL LEISHMANIASIS

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SUMMARY

Visceral leishmaniasis affects various organs including the kidneys; which can lead to renal failure and death. In order to verify this renal involvement, material was evaluated from 100 dogs naturally infected and with serological diagnosis of canine visceral leishmaniasis (CVL). Inflammatory changes were present in 25.3% of the tubules, in 67.0% of interstitium and in 52.0% of glomeruli. There was no significant difference ($p > 0.05$) between the presence of glomerulonephritis in symptomatic and oligosymptomatic dogs. The membranous and membranoproliferative glomerulonephritis were the most frequent, both with 18.0% frequency, followed by focal segmental glomerulosclerosis with 14.0%. Changes such as cylindruria, tubular and fibrosis hypertrophy, periglomerular inflammatory infiltrate, and multifocal and diffuse peritubular inflammatory infiltrate were observed. The findings are consistent with those of other authors indicating that renal involvement is common in CVL and the standards of membranous and membranoproliferative glomerulonephritis, as well as the tubulointerstitial involvement, are frequent.

KEYWORDS: Renal histopathology; Visceral leishmaniasis; Dogs.

INTRODUCTION

The numerous species of *Leishmania* are geographically defined, with a high prevalence in regions of Latin America, Southern Europe and the Middle East. There are three forms of clinical manifestation of the disease: cutaneous, mucocutaneous and visceral.

Brazilian leishmaniasis became urbanized in the 90s, with the dog as the main component in the cycle of human transmission; the disease then became greatly important to public health⁴. Since it develops the disease in a similar manner to man, the dog can serve as model for human studies⁹.

Renal involvement is common as the cause of renal failure and death^{15,18}. Renal involvement is very common in dogs with canine visceral leishmaniasis (CVL) due to the deposition of immunocomplexes in the basal membranes of the renal parenchyma. This is caused by persistent infection associated with prolonged antigenemia¹⁷. Symptomatic and asymptomatic dogs can have varying degrees of renal lesions¹⁷, as some studies have shown that nearly 100% of dogs with CVL have macro- or microscopic renal lesions^{9,18}. Clinical signs of renal dysfunction, however, only appear with extensive tissue injury⁷. Canine visceral leishmaniasis is usually associated with hyperimmunoglobulinemia with high IgG levels, circulating immune complexes and high titers of rheumatoid factor and cryoglobulin. Together with the presence of immunoglobulins in the glomeruli, this suggests the pivotal role of polyclonal B-cell activation and "classical" B-cell activation in the pathogenesis of leishmanial nephritis²⁷.

The objective of this study was to demonstrate renal lesions caused

by this parasitic infection. Histopathological studies were carried out with renal tissue of dogs naturally infected by *Leishmania (L.) chagasi*, obtained from the Zoonosis Control Center in the city of Campo Grande, MS, Brazil, in July and August 2010.

MATERIAL AND METHODS

Kidney tissue was obtained from 100 dogs of different breeds, adults of both sexes, positive in ELISA and indirect immunofluorescence (IIF) tests for leishmaniasis, collected at the Zoonosis Control Center in the city of Campo Grande in the state of Mato Grosso do Sul (MS), Brazil, in July and August 2010. The dogs were euthanized with the application of intravenous thiopental 50 mg/kg, following anesthesia with a lethal dose of potassium chloride (2 mmol/kg, flask of 19.1% KCl: 2.56mEq/mL)⁷. The material was preserved in 10% formalin, embedded in paraffin, sliced with a microtome to a thickness of 5 μ m and stained using the following methods: hematoxylin and eosin for the visualization of nuclear and cytoplasmic alterations, PAS for changes of the glomerular basal membrane, and Masson's trichrome for fibrosis. The slides were evaluated with light microscopy, where the classification of the histopathological findings was made according to the criteria of the World Health Organization⁶ and the Banff classification 1997, updated in 2007²³.

In order to classify and grade the lesions in the renal fragments, those with 10 or more glomeruli, and two or more sections of arteries, were considered adequate. The interstitial areas were evaluated for the presence of fibrosis, tubular atrophy and mononuclear infiltrate, quantifying these alterations relative to the extension of the renal cortex.

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Weight loss is an important clinical sign that accompanies the evolution of the disease, being part of the clinical diagnosis and used in the classification of the animals for the presence of clinical signs⁴. Since there was no record of weight loss in the records of the animals, their condition at the time of euthanasia was used to classify them into groups: **Group #1** oligosymptomatic, 35 animals, all with good corporal conditions, all being relatively mild and of a form common to leishmaniasis, such as: hyperkeratosis, local alopecia, popliteal lymphadenopathy and opacity of coat. **Group #2**, 65 symptomatic animals with poor corporal conditions, with the signs listed above but more advanced and associated with onychogryphosis, skin ulcers, increased abdominal volume, indicative of hepatomegaly and/or splenomegaly; clinical signs more characteristic of the disease.

Differences between the groups were analyzed by Chi-square and Fishers exact tests for a frequency ($p < 5$).

All procedures were carried out with the approval of the University Ethics Committee, protocol #74/2008B.

RESULTS

In 74.7% of the material evaluated, there was no tubular inflammatory infiltrate, while in 67% there was some degree of interstitial involvement and in 52% glomerular lesions were found (Table 1).

Table 1

Distribution of renal morphological findings, according to Banff classification* in 100 dogs naturally infected with *Leishmania* sp, Campo Grande, MS, July-August 2010

Criteria	%
Quantitative of tubulitis	
No tubular mononuclear cells	74.7
Foci with 1-4 mononuclear cells per tubular section	21.7
Foci with 5-10 mononuclear cells per tubular section	0.0
Foci with > 10 mononuclear cells per tubular section	3.6
Quantitative of interstitial inflammation	
No significant inflammation	33.0
10-25% of cortical interstitial inflammation	43.0
26-50% of cortical interstitial inflammation	11.0
> 50% of cortical interstitial inflammation	13.0
Quantitative of glomerulonephritis	
No glomerulonephritis	48.0
Glomerulonephritis in up to 25% of glomeruli	4.0
Glomerulonephritis in within 25-75% of glomeruli	21.0
Glomerulonephritis in up to > 75% of glomeruli	27.0

* Banff Classification 97/ Banff 2007 update.

Figure 1 demonstrates the principal glomerulonephritis type encountered. In membranoproliferative glomerulonephritis, an increase in the cells in the glomerular tuft and an increase in the thickness of the basal glomerular membrane were observed, with a characteristic doubled outline. In the case of membranous glomerulonephritis the basal membrane was thickened, without an increase in the cells in the glomerular tuft. In the case of segmental and focal glomerulonephritis, the capillary loops collapsed, present as a partial form within the same

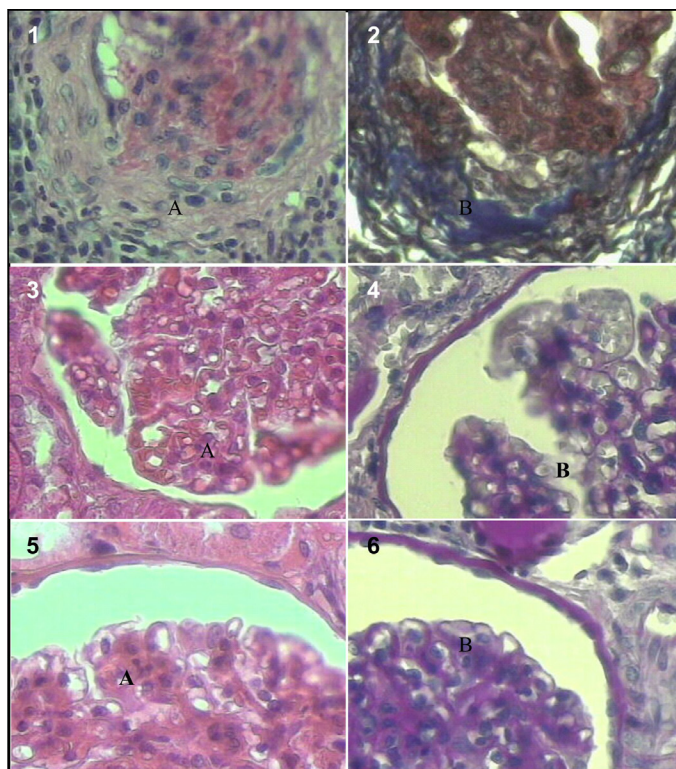


Fig. 1 - 1 and 2 - Segmental and focal glomerulosclerosis – GESF (40x), showing the collapse of the capillary loop, A with HE and B with Masson's trichrome; 3 and 4 - Membranoproliferative glomerulonephritis – GNMP (40x), thickening of the glomerular basal membrane. 3A - (HE) and 4B - (PAS), hypertrophy of the capillary tuft in 3A; 5 and 6 - Membranous glomerulonephritis- GNMB (10x) showing thickening of the basal glomerular membrane without increase in glomerular cells, A with HE and B with PAS.

glomerulus (segmental) or in only a few glomeruli (focal).

The most frequent types of glomerulonephritis were membranoproliferative glomerulonephritis and membranous glomerulonephritis, 18.0% respectively (Table 2).

In the group of oligosymptomatic dogs, 54.3% had some degree of glomerular lesions, and in the symptomatic dogs, 50.8% had no significant difference between the two groups ($p > 0.05$).

When using the histopathological classification of glomerular lesions, there was a significant difference ($p < 0.05$) between the groups; in cases of focal segmental glomerulosclerosis and chronic glomerulonephritis.

Table 3 shows that in the presence of membranous glomerulonephritis there was tubular hypertrophy, isolated or associated with intratubular cylinders, in 50.0% of the samples; in 16.0% there was the presence of fibrosis, and in 11.0% associated of the fibrosis and inflammatory infiltration in the sample. In membranoproliferative glomerulonephritis there was a greater presence of cylinders, isolated or associated with hypertrophy, and inflammatory infiltration, 27.8% and 22.2% respectively. In cases of focal segmental glomerulosclerosis there was a 64.3% incidence of cylinders, isolated or associated with tubular hypertrophy, and fibrosis areas, isolated or associated with inflammatory

Table 2

Percentage distribution of glomerulonephritis found in dogs naturally infected with *Leishmania* sp, according to the presence of clinical symptoms, Campo Grande, MS, July-August 2010

Classification of glomerulonephritis	Symptoms Presence		Total (n=100)
	Oligosymptomatic (n=35)	Symptomatic (n=65)	
Without glomerulonephritis	45.7	49.2	48.0
Membranous glomerulonephritis	22.9	15.4	18.0
Membranoproliferative glomerulonephritis	22.9	15.4	18.0
Focal segmental glomerulosclerosis	8.6	16.9	14.0
Chronic glomerulonephritis	0.0	3.1	2.0

Table 3

Tubulointerstitial findings, according to the glomerulonephritis found in dogs naturally infected with *Leishmania* sp, Campo Grande, MS, July-August 2010

Structure/Injury	% by glomerulonephritis type			
	MGN (n=18)	PMGN (n=18)	FSGS (n=14)	Normal (n=48)
Tubules				
Hypertrophy	33.3	11.1	14.3	10.4
Cylinders	5.6	22.2	14.3	22.9
Hypertrophy and cylinders	16.7	5.6	50.0	25.0
Interstice				
Fibrosis	16.6	11.1	29.6	4.2
Infiltrate	11.1	22.2	21.4	27.1
Infiltrate and fibrosis	11.1	0.0	28.6	6.3

MGN: Membranous glomerulonephritis; PMGN: Membranoproliferative glomerulonephritis; FSGS: Focal segmental glomerulosclerosis; Normal: Normal glomeruli.

foci, were observed in 58.2%. Even in the materials of normal glomeruli there was a presence of inflammatory infiltration cylinders in 47.9% and 33.4%, respectively.

DISCUSSION

Regardless of the presence of clinical signs, the kidneys are often affected in canine visceral leishmaniasis⁷. TAFURI *et al.*²⁷ stated that the occurrence of glomerulonephritis was considered the principal cause of mortality in VCL – these findings confirm the importance of renal damage in leishmaniasis.

In a study of kidney material from 12 dogs, 91.7% of interstitial inflammatory alterations and 83.3% of glomerulosclerosis were seen¹; rates higher than those found in this study - 24.7% of interstitial alterations and 31.3% of glomerular alterations. Histological alterations found in host animals depend on the inflammatory response to the infection, influenced

by the ability of the parasite to resist macrophage action and the capacity to inhibit the cellular immune response of the host¹⁵. Histochemical studies are more suitable to confirm these responses, but microscopic observations of the renal tissue studied here, with inflammatory infiltrate in 25.3% of the samples, suggest the participation of the immunological response in the pathology of the disease.

Lymphocytes from symptomatic dogs proliferated against specific leishmania antigens and asymptomatic dogs can evolve to spontaneous healing, presenting a positive cellular immune response⁵. Canine visceral leishmaniasis is thought to be an immune-mediated disease; where the presence of a humoral response is associated with clinical disease, while the cellular response has been observed in asymptomatic animals^{1,17}. A study of dog kidney fragments showed significant differences between membranoproliferative glomerulonephritis and the presence or absence of symptoms²⁴. In the present study there was no significant difference between the presence of glomerulonephritis in symptomatic and oligosymptomatic groups, assuming that the renal alterations are more related to immune status and immune cell response of dogs than to the presence of signs and symptoms and exposure time¹. In visceral leishmaniasis, activation of the immune system occurs and lesions in non-lymphoid organs, including kidneys, occur not only by immune complex deposition, but also through the participation of macrophages, T cells, cytokines and immunoglobulins, by mechanisms which are not yet clear^{14,27}.

In the present study the histological pattern was found equal to membranous (18%) and membranoproliferative glomerulonephritis (18%), followed by segmental and focal glomerulosclerosis (14%). An experimental model showed histological alterations from 7-14 days post infection²¹. Since the animals in this study were naturally infected, there was no control over the time of infection, which may influence the degree of the alterations described here. The pattern of membranoproliferative glomerulonephritis was most frequent in studies on renal damage in CVL^{2,9,11,25}. POLI *et al.*²⁰ found 61.8% of proliferative mesangial focal or diffuse glomerulonephritis in 61.8% and 35.3% membranoproliferative in the animals studied. SOARES²⁵ found the mesangial proliferative pattern in 64.7% of asymptomatic dogs, while the symptomatic dogs showed 61.7% with membranoproliferative glomerulonephritis. A study by BENDERITTER *et al.*³ found 72.7% with glomerular lesions characterized by acute and membranous glomerulonephritis. Studies using mice²¹ and hamster²² models observed mesangial proliferative glomerulonephritis.

As in this study, others have observed segmental and focal in the second place^{9,11}. Studies with dogs naturally and experimentally infected with *Leishmania (L.) chagasi* found focal or diffuse mesangial glomerulonephritis and proliferation of mesangial cells, interstitial nephritis with heavy infiltration of plasma cells and tubular dilatation²⁶. Increased intensity of interstitial inflammatory infiltrate was observed in the cortical and medullary region, mainly in the kidneys of dogs with glomerulonephritis and membranoproliferative glomerulonephritis with minimal alterations¹³.

Alterations such as cylindruria, tubular hypertrophy and fibrosis, as well as periglomerular inflammatory infiltrates and multifocal and diffuse peritubular, were present, as reported in other studies^{1,9,13}.

The present study may indicate that renal involvement is common in CVL and that the membranous and membranoproliferative glomerulonephritis

patterns, as well as tubulointerstitial involvement, are frequent. Therefore renal lesions are important factors in the evolution of the disease¹². Using the dog as a model, this type of lesion should be evaluated in the diagnosis of the disease and taken into consideration in its treatment.

RESUMO

Achados histopatológicos renais em cães com leishmaniose visceral

A leishmaniose visceral acomete vários órgãos entre eles os rins; o que pode levar a insuficiência renal e a morte. Com o objetivo de verificar este acometimento renal foram avaliados materiais de 100 cães naturalmente infectados e com diagnósticos sorológicos de leishmaniose visceral canina - LVC. As alterações inflamatórias estavam presentes em 25,3% dos túbulos, em 67,0% do interstício e em 52,0% dos glomérulos. Não houve diferença significativa ($p > 0,05$) entre a presença de glomerulonefrite em cães sintomáticos e oligossintomáticos. As glomerulonefrites membranosa e membrano proliferativa foram as mais freqüentes, ambas com 18,0% de freqüência seguidas da glomerulosclerose segmentar e focal com 14,0%. Foram observadas alterações como cilindrúria, hipertrofia tubular e fibrose e infiltrados inflamatórios periglomerulares e peritubulares multifocais e difusos. Os achados concordam com os de outros autores indicando que o acometimento renal é comum na LVC e que os padrões de glomerulonefrites membranoproliferativa e membranosa; assim como o acometimento tubulointerstitial são freqüentes.

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