DOI: 10.5433/1679-0359.2019v40n2p665

Histopathological alterations in the reproductive tract of male dogs with Canine Visceral Leishmaniasis

Alterações histopatológicas no trato reprodutivo de cães machos com Leishmaniose Visceral Canina

Andrea Lantieri Correa de Barros[1]; Ariane Pereira Benites[1]; Nathália Lopes Fontoura Mateus[2]; Carlos Eurico Fernandes[3*]

Abstract

Dogs are considered the main domestic host of *Leishmania infantum*. The transmission between vertebrate hosts normally occurs during blood feeding of *Lutzomyia* sp., a genus of phlebotomine sand flies. However, other forms of transmission without a vector have been reported. Presence of amastigotes of *Leishmania* sp. in different organs can cause several pathologies depending on the immune status of the host and the parasite load. The aim of this study was to evaluate histopathological changes in the reproductive tract of male dogs. Samples of testes, epididymis, and prostate gland were collected from 70 animals that were grouped according to serology test results for visceral leishmaniasis and clinical symptoms. Three experimental groups were formed based on serological results and clinical presentation: asymptomatic, symptomatic and control. Prostatic changes were seen regardless of the serological results; however, the changes were more severe in symptomatic animals. In the testes and epididymis, lesions such as interstitial infiltrate, degeneration, and fibrosis were seen more frequently and were more severe in positive animals when compared to the control group. These results demonstrate that dogs infected with *Leishmania* sp. can develop nonspecific changes in the reproductive tract, which are more severe in symptomatic animals.

Key words: Amastigotes. Epididymis. *Leishmania* sp. Prostate. Testis.

Resumo

Os cães são considerados os principais hospedeiros domésticos de *Leishmania infantum*. A transmissão entre hospedeiros vertebrados normalmente ocorre durante o repasto sanguíneo de *Lutzomyia* sp., gênero de flebotomíneo. Entretanto, outras formas de transmissão sem a presença do vetor têm sido relatadas. A presença de amastigotas de *Leishmania* sp. em diferentes órgãos pode causar diversas patologias de acordo com o status imune do hospedeiro e a carga parasitária. O objetivo deste trabalho foi avaliar as alterações histopatológicas no trato reprodutivo de cães machos. Amostras de testículo, epidídimo e próstata foram coletadas de 70 animais, os quais foram agrupados conforme o resultado da sorologia para leishmaniose visceral e os sinais clínicos. Três grupos experimentais foram formados baseados nos resultados sorológicos e manifestações clínicas: assintomático, sintomático e controle. Alterações prostáticas foram observadas independentemente dos resultados sorológicos; entretanto, foram mais

[1] Laboratório de Patologia Experimental, LAPEX, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Mato Grosso do Sul, UFMS, Campo Grande, MS, Brasil. E-mail: andrealcb@hotmail.com; arianebenites@hotmail.com

[2] Profª, Centro Universitário da Grande Dourados, UNIGRAN, Faculdade de Ciências Exatas e Agrárias, Curso de Medicina Veterinária, Dourados, MS, Brasil, E-mail: nathaliafontouraveterinaria@gmail.com

[3] Prof., Laboratório de Patologia Experimental, LAPEX, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Mato Grosso do Sul, UFMS, Campo Grande, MS, Brasil. E-mail: carlos.fernandes@ufms.br

* Author for correspondence

Received: June 25, 2017 - Approved: Nov. 25, 2018

acentuadas nos animais sintomáticos. Nos testículos e epidídimos, lesões como infiltrado intersticial, degeneração e fibrose foram observadas mais frequentemente e de forma mais severa nos animais positivos quando comparados ao grupo controle. Estes resultados demonstram que cães infectados com *Leishmania* sp. podem desenvolver alterações não específicas no trato reprodutivo, as quais são mais severas nos animais sintomáticos.
Palavras-chave: Amastigotas. Epidídimo. *Leishmania* sp. Próstata. Testículo.

Introduction

Leishmaniasis is the most important and prevalent emerging disease in countries of Latin America (REIS et al., 2009). Previously, this disease was restricted to rustic environments; but the unplanned urban expansion into deforested areas with accumulation of organic matter associated with poor sanitary conditions contributed to an increased incidence in the urban environment (WERNECK et al., 2007).

Dogs have been known to be the main domestic host of *Leishmania* spp. (GRAMICCIA; GRADONI, 2005). The species present in the Americas, including Brazil, is *Leishmania infantum* (DEANE; DEANE, 1962). Sandflies of the genus *Lutzomyia* are responsible for the transmission of visceral form, named Canine Visceral Leishmaniasis (CVL). The infection mostly transmits from an invertebrate host to a vertebrate host; however, direct transmission through canine blood transfusion (OWENS et al., 2001) and sexual transmission of *L. infantum* from naturally infected male dogs to susceptible females has also been reported (SILVA et al., 2009).

Studies have shown that the reproductive tract of dogs that are serologically CVL-positive can work as a reservoir for infecting forms of *Leishmania* sp. (DINIZ et al., 2005; BENITES et al., 2011). Epididymitis associated with CVL can result in the presence of amastigotes in the semen, which can result in venereal transmission, as described in humans (SYMMERS, 1960; CABELLO et al., 2002). Dog mating often results in injuries in both male and female dogs, increasing the chance of transferring amastigotes through the external genitalia as well as through the amastigotes that are present in the semen.

Although various aspects of the pathogenesis, immunopathology, clinical signs, and pathological manifestations of CVL are relatively well understood, the presence of amastigotes in the reproductive tract and the specific lesions associated with it have not been widely studied. Therefore, given the epidemiological importance of the dog, the objective of this study was to characterize the histopathological changes in the prostate, testis, and epididymis of serologically positive dogs with and without clinical symptoms of CVL and to establish the relation of these changes with the presence of amastigote forms in the evaluated organs.

Materials and Method

Animals and experimental groups

Seventy male mongrel dogs from the Zoonosis Control Center (ZCC) of Campo Grande, MS, Midwest, Brazil (20°26′34″S, 54°38′47″W) were used in this study. Dogs were tested using enzyme-linked immunosorbent assay (ELISA; EIE Leishmaniose Visceral Canina - Bio-Manguinhos, FIOCRUZ) and indirect immunofluorescence (IIF; IFI Leishmaniose Visceral Canina - Bio-Manguinhos, FIOCRUZ) and categorized according to positive results in both serological tests, considering the manufacturer's instructions, and clinical signs observed. The following three groups were formed: asymptomatic (n=25), serologically positive dogs without clinical manifestation; symptomatic (n=25), serologically positive dogs with one or more clinical manifestation regardless of the presence of signs in the reproductive tract; and control (n=20), serologically negative animals. The dogs were euthanized by intravenous administration

of the anesthetic sodium Thiopental (50 mg/Kg) and subsequent administration of potassium chloride (191 mg/Kg). Given to the origin of the dogs that were used, no information regarding age or prior illness was obtained and the breed was not taken into account. All the procedures of this study were in accordance to Research Ethics Committee, UFMS (protocol 203/2009).

Parasitological examination and histopathology

After euthanasia, the prostate gland and the right testis and epididymis were removed from each dog. Immediately after excision, the organs were longitudinally sectioned. Randomly obtained fragments were used to prepare impression smears. The impression smears were stained with diff-quick and parasitological examination was performed (BENITES et al., 2011). Prostate and testis fragments were fixed in Bouin's (RUSSEL et al., 1990), epididymal fragments were fixed in 10% buffered formalin-saline. After time, these fragments were transferred for 70% alcohol. The samples were dehydrated, embedded in paraffin blocks and cut at a maximum thickness of 4µm. All samples were stained with Hematoxylin & Eosin (TOLOSA et al., 2003).

During histopathological analysis of the prostate gland was assessed the presence of glandular parenchyma atrophy, fibrosis, and inflammatory infiltrate in the interstitial space. In the testis were assessed the tubular degeneration and hypospermatogenesis, edema, inflammatory infiltrates, and interstitial fibrosis. In the epididymis, the presence of inflammatory infiltrate and intertubular fibrosis were determined per region (caput, corpus, and cauda). In all samples, the severity of inflammatory infiltrates and the presence of fibrosis, were classified as absent, mild, moderate or severe (SLAUSON; COOPER, 2002).

Statistical analysis

Two statistical analyses models were performed. In the first, the effect of each group on the different histopathological findings (presence or absence) in the prostate, testis, caput, corpus, and cauda of the epididymis were verified. The chi-square test was used for contingency tables or Fisher´s exact test (2 x 2 tables). The severity of inflammatory infiltration and fibrosis was analyzed using the non-parametric ANOVA (Kruskal Wallis) and Mann-Whitney test for pairwise comparison. In the second statistical analysis, the results from the parasitological examination (presence or absence of amastigotes) were added to the previously adopted experimental groups to analyze the interactive effects between these two predictive variables. Categorical data analysis was performed using a saturated nonhierarchical Log-linear model with backwards elimination. All statistical analyses were performed through the statistical package SPSS 17.0.

Results

Analysis of the histopathology results in the different parts of the reproductive tract revealed differences in the frequency of histopathological changes between the groups and even more pronounced differences in the severity scores between the groups. In all parts of the tract, lymphomononuclear infiltrate was the predominant finding. Granulomatous formation was not detected. Only two samples (2/25; 8.0%) of prostatic tissue obtained from the symptomatic group, showed acute infiltrate in combination with liquefactive necrosis. Samples from these testis and epididymis showed no neutrophilic infiltrate.

The frequency of histopathological changes detected in the prostate and testis are shown in Tables 1 and 2, respectively. In the prostatic tissue, there was a significant difference ($p < 0.05$) between the asymptomatic and the symptomatic group in

occurrence of atrophy, intensity of fibrosis and inflammatory infiltrate. The control group differed from the symptomatic group in severity of fibrosis and inflammatory infiltrate, but not of asymptomatic group. Although histopathological changes have occurred in the control group, these were minor when compared to the other groups. In the testicular tissue, fibrosis was only detected in the samples of symptomatic dogs (5/70; 7.0%). There was no difference between the groups for the presence of edema. The presence of amastigote forms in the fragments that were analyzed by histopathology was not associated with lesions in the reproductive tract. Additionally, were not found granulomatous inflammatory reaction, include the epididymis. The infiltrate was characterized by lymphocytes, macrophages and plasma cells, occasionally, suggesting non-specific inflammation (Figure 1).

Table 1. The frequency of histopathological alterations in the prostate of asymptomatic, symptomatic and control dogs.

Histopathological changes	Groups		
	Asymptomatic (n=25)	Symptomatic (n=25)	Control (n=20)
Atrophy	14 (20,3%)[a]	20 (29,0%)[b]	16 (23,2%)[a]
Fibrosis	18 (26,1%)[a]	20 (29,0%)[a]	16 (23,2%)[a]
Intensity of fibrosis	1,2[a]	2,1[b]	1,4[a]
Inflammatory infiltrate	19 (27,2%)[a]	21 (30,4%)[a]	18 (26,1%)[a]
Intensity of infiltrate	1,3[a]	2,0[b]	1,5[a]

Distinct letter among groups represent significant difference (p<0,05).

Table 2. The frequency of histopathological alterations in the testis of asymptomatic, symptomatic and control dogs.

Groups	Histopathological changes					
	Degeneration	Hypoplasia	Inflammatory infiltrate	Fibrosis	Infiltrate (intensity)	Edema
Asymptomatic (n=25)	15 (21,4%)[a]	8 (11,6%)[a]	9 (12,9%)[a]	0 (0%)[a]	0.7[a]	4 (5,7%)[a]
Symptomatic (n=25)	19 (27,1%)[a]	15 (21,7%)[a]	16 (22,9%)[a]	5 (7,1%)[b]	1.6[a]	3 (4,3%)[a]
Control (n=20)	10 (14,3%)[b]	1 (1,4%)[b]	0 (0%)[b]	0 (0%)[a]	0.0[b]	1 (1,4%)[a]

Distinct letter among groups represent significant difference (p < 0,05).

The presence of lymphomononuclear infiltrate and fibrosis in the three regions of the epididymis are shown in Figure 2. There was no difference between the control group and the asymptomatic group in the severity of the inflammatory infiltrate in any of the epididymal segments. Overall, fewer symptomatic dogs were categorized as "absent" regarding the inflammatory infiltrate, and higher numbers were seen in the mild, moderate, and severe categories when compared to the asymptomatic and control groups.

Figure 1. Histopathological sections of prostate (A-B) and testis (C-F) of symptomatic dogs with Visceral Leishmaniasis. **A**. Marked atrophy of duct-acinar zones with diffuse mononuclear (macrophages and lymphocytes) infiltrate. **B**. High magnification of image A; tiny distorted glands with cystic atrophy; ductal and fibromuscular stroma infiltrate inflammatory. **C.** Marked degeneration of seminiferous tubules sections; some basal germinative cells are observed. **D.** Peritubular orchitis and severe chronic inflammation (mononuclear infiltrate) associated to tubular degeneration. **E.** Peritubular infiltrate characterized by macrophages and lymphocytes. **F.** Interstitial zone with infiltrate rich in lymphocytes and plasma cells. Haemotoxylin & Esosin stain. Bar 100µm: E, F; 200µm: B, C, D; 500µm: A.

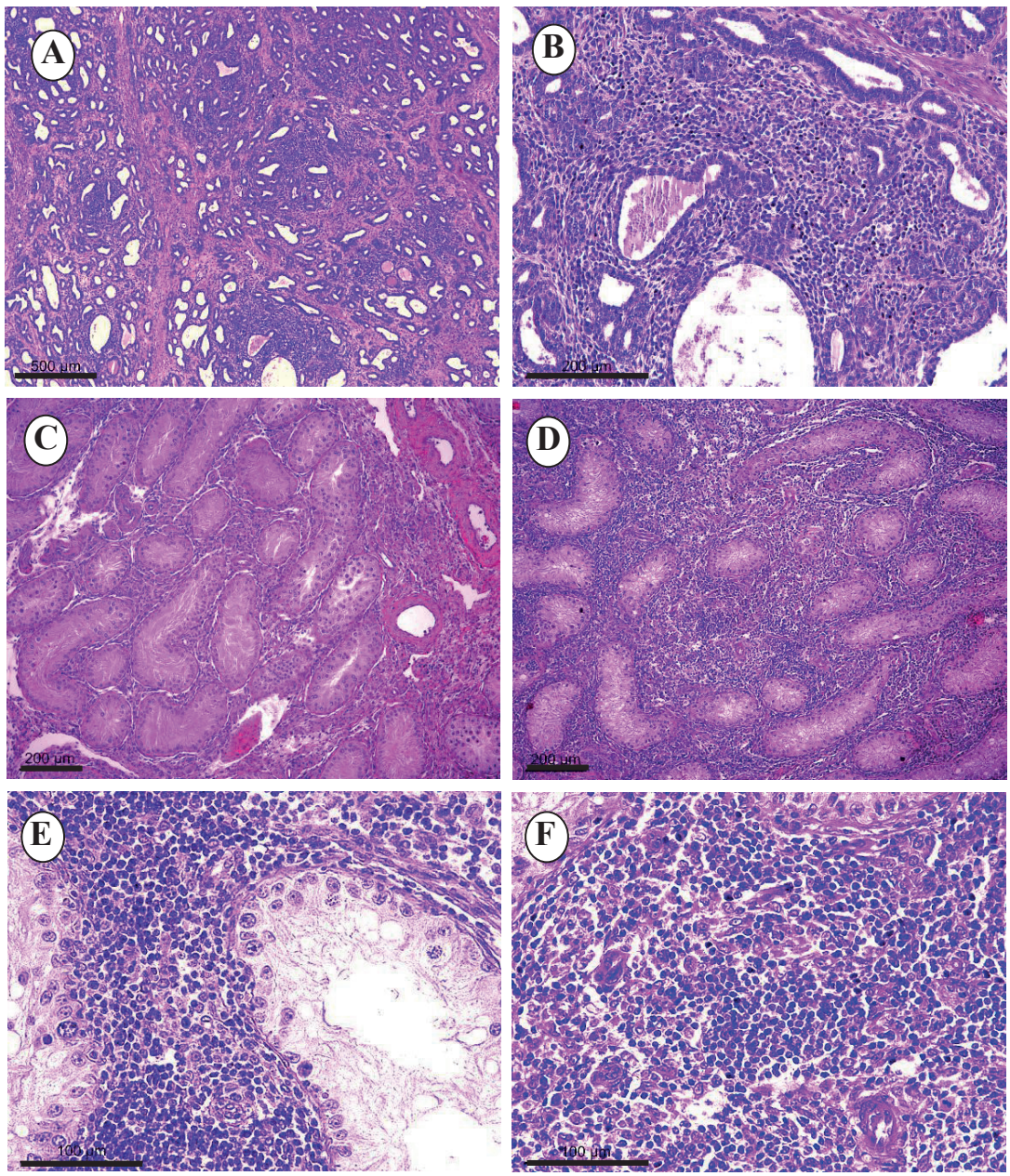
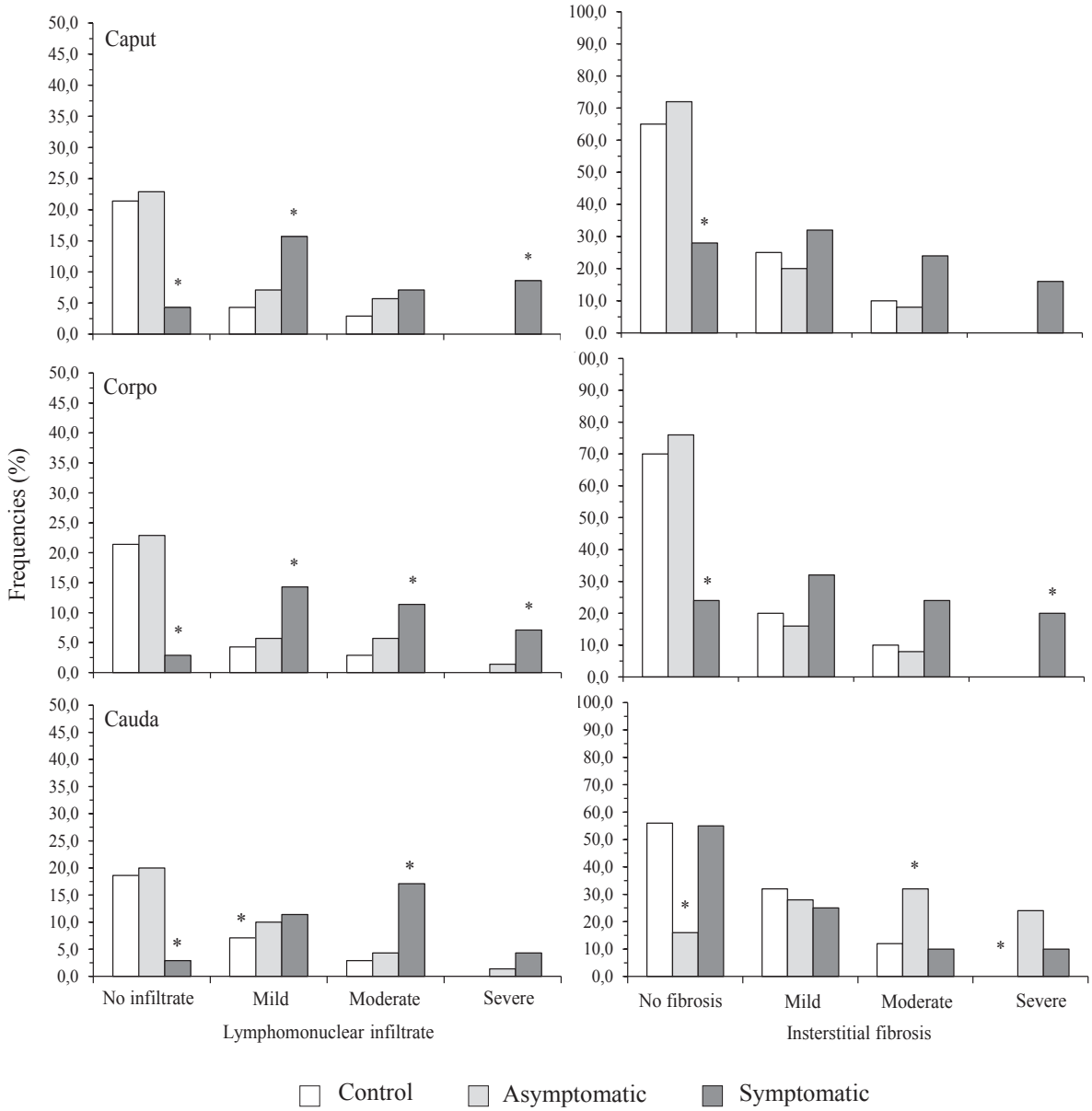


Figure 2. Frequencies (%) of lymphomononuclear infiltrate interstitial fibrosis and severity in different regions of epididymis in asymptomatic, symptomatic and control dogs.



*Represent significant difference among experimental groups.

The results of the second set of statistical analyses are shown in Tables 3 and 4. There was no significant interaction between the different epididymal regions (experimental group × parasitological examination × lesion) and the severity of the inflammatory infiltrate in the testis and the prostate. In the prostate gland, other histopathological findings did not reveal any significant interactions (Table 3). In contrast, significant second order interactions for testicular degeneration (parasitological exam × experimental group), lymphomononuclear infiltrate (infiltrate × experimental group; parasitological exam × experimental group) and fibrosis (parasitological exam × experimental group; fibrosis × experimental group) were found in the testis.

Table 3. The interaction between the frequencies (%) of prostatic histological changes, parasitological examination, and experimental groups.

Parasitologic	Atrophy	Groups			Interactive effect*	χ^2	p
		Control n (%)	Asymptomatic n (%)	Symptomatic n (%)			
(+)	(+)	0 (0,0)	0 (0,0)	3 (4,3)	Atrophy X Group	3,904	0,142
(–)		16 (23,2)	14 (20,3)	16 (23,4)	Paras[1] X Group	5,328	0,070
(+)	(–)	0 (0,0)	2 (2,9)	0 (0,0)	Paras. X Atrophy.	0,444	0,505
(–)		4 (5,8)	9 (13,0)	4 (5,8)			
	Fibrosis						
(+)	(+)	0 (0,0)	1 (1,4)	3 (4,3)	Paras. X Group	3,847	0,146
(–)		16 (23,2)	17 (24,6)	16 (23,2)	Fibrosis X Group	0,959	0,619
(+)	(–)	0 (0,0)	1 (1,4)	0 (0,0)	Paras. X Fibrosis	0,005	0,944
(–)		4 (5,8)	6 (8,7)	4 (5,8)			
	Infiltrate[2]						
(+)	(+)	0 (0,0)	0 (0,0)	3 (4,3)	Paras. X Group	3,756	0,153
(–)		18 (26,1)	19 (27,5)	18 (26,1)	Infiltrate X Group	1,804	0,406
(+)	(–)	0 (0,0)	2 (2,9)	0 (0,0)	Paras. X Infiltrate	1,736	0,188
(–)		2 (2,9)	4 (5,8)	3 (4,3)			

*Log linear nonhierarchical model; ordered interactive effect of two order (n=70); [1]Parasitologic exam (+, presence of at least one form amastigote in one imprinting of tissue; – absence of amastigote forms); [2]Inflammatory infiltrate.

Table 4. The interaction between the frequencies (%) of testicular histological changes, parasitological examination, and experimental group.

Parasitologic	Degeneration	Groups			Interactive effect*	χ^2	p
		Control n (%)	Asymptomatic n (%)	Symptomatic n (%)			
(+)	(+)	0 (0,0)	10 (14,3)	4(5,7)	Paras.[1] X Group	25,055	0,001
(–)		10 (14,3)	9 (12,9)	11 (15,7)	Degen.[2] X Group	3,497	0,174
(+)	(–)	0 (0,0)	5 (7,1)	1 (1,4)	Paras. X Degen.	0,135	0,713
(–)		11 (15,7)	1 (1,4)	8 (11,4)			
	Infiltrate[3]						
(+)	(+)	0 (0,0)	9 (12,8)	4 (5,7)	Infilt. X Group	25,352	0,001
(–)		0 (0,0)	7 (10,0)	8 (11,4)	Paras. X Group	24,076	0,001
(+)	(–)	0 (0,0)	6 (8,5)	4 (5,7)	Paras. X Infilt.	0,966	0,326
(–)		20 (28,6)	3 (4,3)	11 (15,7)			
	Fibrosis						
(+)	(+)	0 (0,0)	3 (4,3)	0 (0,0)	Paras. X Group	23,222	0,001
(–)		0 (0,0)	2 (2,8)	8 (11,4)	Fibrosis X Group	8,683	0,013
(+)	(–)	0 (0,0)	2 (2,8)	0 (0,0)	Paras. X Fibrosis	0,000	-
(–)		20 (28,6)	8 (11,4)	19 (27,1)			

*Loglinear nonhierarchical model; ordered interactive effect of two order (n=70); [1]Parasitologic exam (+, presence of at least one form amastigote in one imprinting of tissue; – absence of amastigote forms); [2]Testis degeneration; [3]Lymphomononuclear infiltrate.

Discussion

Only a small number of studies have reported changes in the reproductive tract of male dogs with CVL. The relationship between changes in the reproductive tract, and the presence of amastigotes is not well understood. Studies have revealed that 84.6% to 100% of dogs have some type of change in the prostate. The most common changes are prostatitis and hyperplasia, which are mostly seen in middle-aged dogs (OLIVEIRA, 1996; OLIVEIRA et al., 2007). In the present study, the frequency of inflammatory infiltrate and deposition of fibrous connective tissue indicative of an inflammatory process, were not different between the groups for the prostatic tissue. Nonetheless, the severity of both processes was greater in serologically positive animals of the symptomatic group (Table 1). Repair of any type of tissue injury can take place by regeneration or cicatrization (replacement by fibrous connective tissue). This repair is usually associated with a nonspecific chronic infiltrate. Lee et al. (1997) reported that prostatic infiltration by lymphocytes and macrophages increases as age advances, and this process is usually unrelated to infection.

Dogs suffering from leishmaniasis present a variety of inflammatory responses depending on the parasite load and the clinical signs of the disease, which are typically related to the immunological characteristics of an individual (REIS et al., 2006, 2009). It has been suggested that symptomatic animals have a greater density of amastigote forms than asymptomatic animals, which leads to a higher chance of migrating infected macrophages, intensifying the inflammatory response (BENITES et al., 2011). Diniz et al. (2005) did not observe this relation in dogs with visceral leishmaniasis. In the present study, a significant difference was shown between the symptomatic and the asymptomatic group regarding the presence of prostatic atrophy; however, there was no difference between the asymptomatic and the control group.

Prostatic atrophy can occur in response to changes in androgen production or due to the presence of inflammation directly affecting the glandular epithelium (FOSTER, 2012). According to Toledo et al. (2010), inflammation, particularly the mononuclear type (lymphocytes, macrophages and occasional plasma cells), in the interstitial region of tubule-alveolar segments influences the occurrence of atrophy in the glandular epithelium (Proliferative Inflammatory Atrophy - PIA). In our study, no information was available regarding the age of the dogs that were used. Age-related changes and change related to hormonal stimuli could have been present in the prostate of these animals. Therefore, hyperplasia was not considered a relevant input when comparing the experimental groups. Prostate hyperplasia is a common alteration in dogs over three years old due to changes in the androgen and estrogen ratio in the secretion of the testicles (SMITH, 2008).

Orchitis and epididymitis is a common manifestation in dogs and has a variety of causal factors (THOMÉ et al., 2007). Inflammatory lesions are more common in the epididymis than in the testis and can be caused by an ascending secondary infection through the ductus deferens via the lymphatic vessels of the spermatic cord, by hematogenous spread or in combination with a primary urinary tract infection (FOSTER, 2012). Symptomatic and asymptomatic dogs that are positive for CVL, present with amastigote forms in most of the lymph nodes and the density of the parasites varies depending on the circulating antibody levels (LIMA et al., 2010). Given that testis have a rich vasculature and a complex lymphatic network, one possible explanation for the observed changes in the testicular tissue is the presence of amastigote forms derived from the draining lymph nodes. Another possibility is that a currently unknown mechanism causes the changes in the testicular tissue (DINIZ et al., 2005). However, in the present study, these hypotheses could not be confirmed.

The statistical model to detect the interaction between predictive variables (parasitological exam, experimental groups, and lesions) revealed that the lesions were not caused by the presence of amastigotes in the prostate gland and testes organs. However, the clinical condition, possibly related to the individual debility, predominantly seen in the symptomatic and asymptomatic groups, may have been determinant to the presence of these lesions. A few aspects should be considered regarding this finding. The first is the possibility that the amastigotes were an incidental finding in the impression smears. The second is the vascular leukocyte transmigration in the reproductive tract, especially that of testicular macrophages. These cells are phagocytes that ingest the amastigotes and act as modulators of the inflammatory process, possibly dependent of the local parasite load (REIS et al., 2009; WINNALL; HEDGER, 2013). It is known that in vitro, Sertoli cells secrete immunosuppressive molecules capable of inhibiting proliferation and specific activity of B and T lymphocytes, and reducing the secretion of interleukin-2 (DE CESARIS et al., 1992). The testicular degeneration process can exemplify the difficulty in establishing this relation. This pathology, predominantly common in canines (NASCIMENTO; SANTOS, 1997) is multifactorial and is associated with systemic processes including fever and metabolic, traumatic, and infectious diseases (FOSTER, 2012). In the case of leishmaniasis, it is possible that the presence of amastigotes in the testicular environment triggers an inflammatory response that, depending on the lesion degree, results in slow and progressive degeneration of the germinal epithelium and azoospermy (AMARA et al., 2009; LABAT et al., 2010). However, it is possible that inflammatory and regenerative changes were already present before the invasion of the parasitic forms.

In the epididymis, due to low prevalence of amastigote forms (only three dogs were positive, two asymptomatic and one symptomatic), it was not possible to evaluate the interaction between the presence of amastigotes and the experimental groups; however, the lesions were different between the regions. The findings in this study are consistent with those of other studies reporting epididymitis as the most important pathological process. The epididymis cauda is more affected compared to corpus and caput segments (FOSTER; LADDS, 2007; FOSTER, 2012).

In the present study, it was noted that fibrosis was seen more frequently than the presence of lymphomononuclear infiltration, suggesting the presence of additional chronic processes. The effect of CVL on the epididymal function is still controversial. Serologically positive dogs may have an unsatisfactory semen quality. When treated, these alterations can be reversed partially, improving sperm quality in affected dogs (ASSIS et al., 2010). Leukocyte profiles in the reproductive tract of serologically negative and positive dogs, in both symptomatic and asymptomatic individuals, are different according to the location and the response to the amastigotes. The profiles are characterized by the number of neutrophils, macrophages, and lymphocytes and it has been suggested that changes caused by CVL can enhance preexisting inflammatory processes in the male reproductive tract of dogs (BENITES et al., 2011).

In conclusion, the histopathological changes found in the male reproductive tract of naturally infected dogs with *Leishmania* sp., were nonspecific but they were associated with the presence of systemic clinical signs. There is no relationship between the presence of amastigotes and histological lesions in the different segments of the reproductive tract.

Acknowledgement

The authors thank the FUNDECT-MS for financial support for this study.

References

AMARA, A.; MRAD, I.; MELKI, M. K.; MRAD, M. B.; REJEB, A. Etude histologique des lésions testiculaires chez les chiens leishmaniens. *Revue de Médecine Vétérinaire*, Toulouse, v. 160, n. 1, p. 54-60, 2009.

ASSIS, V. P.; RIBEIRO, V. M.; RACHID, M. A.; CASTRO, A. C. S.; VALLE, G. R. Dogs with *Leishmania chagasi* infection have semen abnormalities that partially revert during 150 days of Allopurinol and Amphotericin B therapy. *Animal Reproduction Science*, Filadélfia, v. 117, n. 1, p. 183-186, 2010.

BENITES, A. P.; FERNANDES, C. E.; BRUM, K. B.; MARIA AUXILIADORA, G. S.; ABDO, M. A. G. S. Presença de formas amastigotas de *Leishmania chagasi* e perfil leucocitário no aparelho reprodutivo de cães. *Revista Pesquisa Veterinária Brasileira*, Seropédica, v. 31, n. 1, p. 72-77, 2011.

CABELLO, I.; CARABALLO, A.; MILLÁN, Y. Leishmaniasis in the genital area. *Revista do Instituto de Medicina Tropical de São Paulo*, São Paulo, v. 44, n. 2, p. 105-107, 2002.

DE CESARIS, P.; FILIPPINI, A.; CERVELLI, C.; RICCIOLI, A.; MUCI, S.; STARACE, G.; STEFANINI, M.; ZIPARO, E. Immunosuppressive molecules produced by Sertoli cells cultured in vitro: biological effects on lymphocytes. *Biochemical and Biophysical Research Communications*, Orlando, v. 186, n. 3, p. 1639-1646, 1992.

DEANE, L. M.; DEANE, M. P. Visceral leishmaniasis in Brazil: geographical distribution and transmission. *Revista do Instituto de Medicina Tropical de São Paulo*, São Paulo, v. 4, n. 3, p. 198-212, 1962.

DINIZ, S. A.; MELO, M. S.; BORGES, A. M.; BUENO, R.; REIS, B. P.; TAFURI, W. L.; NASCIMENTO, E. F.; SANTOS, R. L. Genital lesions associated with visceral leishmaniasis and shedding of *Leishmania* sp. in the semen of naturally infected dogs. *Veterinary Pathology*, Thousand Oaks, v. 42, n. 2, p. 650-658, 2005.

FOSTER, R. A. Common lesions in the male reproductive tract of cats and dogs. *Veterinary Clinics of North America Small Animal Practice*, Maryland Heights, v. 42, n. 3, p. 527-545, 2012.

FOSTER, R.; LADDS, P. W. Male genital system. In: MAXIE, M. G. (Eds.). *Jubb, Kennedy, and Palmer's:* pathology of domestic animals. Philadelphia: Saunders Ltd., 2007. v. 3, 465-510.

GRAMICCIA, M.; GRADONI, L. The current status of zoonotic leishmaniases and approaches to disease control. *International Journal for Parasitology*, Melbourne, v. 35, n. 11-12, p. 1169-1180, 2005.

LABAT, É.; CARREIRAJ, T.; MATSUKUMA, B. H.; MARTINS, M. T. A.; LIMA, V. M. F.; BOMFIM, S. R. M.; PERRI, S. H. V.; KOIVISTO, M. B. Qualidade espermática de sêmen de cães naturalmente infectados por *Leishmania* sp. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 62, n. 3, p. 609-614, 2010.

LEE, C.; KOZLOWSKI, J. M.; GRAYHACK, J. T. Intrinsic and extrinsic factors controlling benign prostatic growth. *The Prostate*, Medford, v. 31, n. 1, p. 131-138, 1997.

LIMA, L. V. R.; CARNEIRO, L. A.; CAMPOS, M. B.; CHAGAS, E. J.; LAURENTI, M. D.; CORBETT, C. E. P.; LAINSON, R.; SILVEIRA, F. T. Canine visceral leishmaniasis due to *Leishmania* (*L.*) *infantum chagasi* in Amazonian Brazil: comparison of the parasite density from the skin, lymph node and visceral tissues between symptomatic and asymptomatic, seropositive dogs. *Revista do Instituto de Medicina Tropical de São Paulo*, São Paulo, v. 52, n. 5, p. 259-265, 2010.

NASCIMENTO, E. F.; SANTOS, R. L. *Patologia da reprodução dos animais domésticos*. 2. ed. Rio de Janeiro: Guanabara Koogan, 1997. 108 p.

OLIVEIRA, E. G. BANDARRA, E. P.; SIQUEIRA, J. L.; LAUFER, R.; CASTRO, A. P. Afecções da próstata em cães na região de Botucatu, estado de São Paulo. In: CONGRESSO DE INICIAÇÃO CIENTÍFICA DA UNESP, 8., 1996, Jaboticabal. *Anais...* Jaboticabal: UNESP, 1996. p. 327.

OLIVEIRA, K. S.; ARAÚJO, E. G.; SILVA, L. A. F.; MENEZES, L. B. Alterações prostáticas de cães adultos necropsiados na escola de veterinária da Universidade Federal de Goiás de maio a julho de 2004. *Revista Ciência Animal Brasileira*, Goiânia, v. 8, n. 2, p. 267-272, 2007.

OWENS, S. D.; OAKLEY, D. A.; MARRYOTT, K.; HATCHETT, W.; WALTON, R.; NOLAN, T. J.; NEWTON, A.; STEURER, F.; SCHANTZ, P.; GIGER, U. Transmission of visceral leishmaniasis through blood transfusions from infected English foxhounds to anemic dogs. *Journal of the American Veterinary Medical Association*, Schaumburg, v. 219, n. 8, p. 1076-1083, 2001.

REIS, A. B.; MARTINS-FILHO, O. A.; TEIXEIRA-CARVALHO, A.; CARVALHO, M. G.; MAYRINK, W.; FRANÇA-SILVA, J. C.; GIUNCHETTI, R. C.; GENARO, O.; CORRÊA-OLIVEIRA, R. Parasite density and impaired biochemical/hematological status

are associated with severe clinical aspects of canine visceral leishmaniasis. *Research in Veterinary Science*, Maryland Heights, v. 8, n. 1, p. 68-75, 2006.

REIS, A. B.; MARTINS-FILHO, O. A.; TEIXEIRA-CARVALHO, A.; GIUNCHETTI, R. C.; CARNEIRO, C. M.; MAYRINK, W.; TAFURI, W. L.; CORRÊA-OLIVEIRA, R. Systemic and compartmentalized immune response in canine visceral leishmaniasis. *Veterinary Immunology and Immunopathology*, Amsterdam, v. 128, n. 1-3, p. 87-95, 2009.

RUSSEL, L; ETTLIN, R. A.; SINHAHIKIM, A. P.; CLEGG, E. D. *Histological and histopatological evaluation of the testis*. Clearwater: Cache River Press, 1990. 286 p.

SILVA, F. L.; OLIVEIRA, R. G.; SILVA, T. M. A.; XAVIER, M. N.; NASCIMENTO, E. F.; SANTOS, R. L. Venereal transmission of canine visceral leishmaniasis. *Veterinary Parasitology*, Amsterdam, v. 160, n. 1-2, p. 55-59, 2009.

SLAUSON, D. O.; COOPER, B. J. *Mechanims of diseases.* A textbook of comparative general pathology. 3. ed. Philadelphia: Mosby, 2002. 445 p.

SMITH, J. Canine prostatic disease: a review of anatomy, pathology, diagnosis, and treatment. *Theriogenology*, Gainesville, v. 70, n. 3, p. 375-383, 2008.

SYMMERS, W. S. C. Leishmaniasis acquired by contagion: a case of marital infection in Britain. *The Lancet*, Londres, v. 16, n. 1, p. 127-132, 1960.

THOMÉ, H. E.; DI SANTIS, G. W.; MOURA, V. M. B. D.; AMORIM, R. L.; BANDARRA, E. P. Avaliação histopatológica testicular e epididimária em cães adultos sem raça definida (SRD) da região de São João da Boa Vista, SP. *Revista Ciência Animal Brasileira,* Goiânia, v. 8, n. 4, p. 745-755, 2007.

TOLEDO, D. C.; FALEIRO, M. B. R.; RODRIGUES, M. M. P.; SANTIS, G. W.; DI AMORIM, R. L.; MOURA, V. M. B. D. de. Caracterização histomorfológica da atrofia inflamatória proliferativa na próstata canina. *Ciência Rural*, Santa Maria, v. 40, n. 6, p. 1372-1377, 2010.

TOLOSA, E. M. C.; RODRIGUES, C. J.; BEHMER, O. A.; FREITAS NETO, A. G. *Manual de técnicas para histologia normal e patológica.* 2. ed. Barueri: Editora Manole, 2003. 331 p.

WERNECK, G. L.; PEREIRA, T. J. C. F.; FARIAS, G. C.; SILVA, F. O.; CHAVES, F. C.; GOUVEA, M. V.; COSTA, C. H. N.; CARVALHO, F. A. A. Avaliação da efetividade das estratégias de controle da leishmaniose visceral na cidade de Teresina, estado do Piauí, brasil: resultados do inquérito inicial - 2004. *Epidemiologia e Serviços de Saúde*, Brasília, v. 17, n. 2, p. 87-96, 2007.

WINNALL, W. R.; HEDGER, M. P. Phenotypic and functional heterogeneity of the testicular macrophage population: a new regulatory model. *Journal of Reproductive Immunology*, Amsterdam, v. 97, n. 2, p. 147-158, 2013.