Artigo

Cellular immune response in popliteal lymph nodes and liver of dogs with visceral leishmaniasis

Resposta imunológica celular em linfonodo poplíteo e fígado de cães com leishmaniose visceral

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Abstract: Canine visceral leishmaniasis (CVL) is a zoonosis caused by *Leishmania (Leishmania) infantumchagasi* in the American continent. There are few studies detailing the immunopathological characteristics in lymph nodes and liver in infected dogs in endemic area from Teresina city, Piauí State, Brazil. Thus, we have evaluated histopathological changes and the presence of CD4$^+$ and CD8$^+$ T cells in popliteal lymph nodes and liver of nine dogs with clinical manifestation (G1) and seven dogs without clinical manifestation (G2) naturally infected with *L. (L.) infantumchagasi*. Histopathological and immunohistochemical analysis were performed on samples liver and lymph nodes tissues. The important clinical changes were lymphadenopathy (80%) and skin lesions (70%). Histopathological analysis of the popliteal lymph node revealed higher depletion cell of the paracortical region. The number of CD4$^+$ and CD8$^+$ T cells was no significant between the groups but there was positive correlation between CD4$^+$ and CD8$^+$ T cells in G1 and G2. In the liver, the inflammatory infiltrate was focal consisting of mononuclear cell in the periportal and intralobular regions, in both groups. Inflammatory infiltrate of mononuclear cells showed positive staining for CD4 and CD8 T cells but there was no significant difference between groups. However, there was a positive correlation between CD4$^+$ and CD8$^+$ T cells in the G1. Immunoperoxidase analysis also revealed more amastigotes and antigen of *Leishmania* in G1 in liver and lymph nodes than in G2. The role of CD4$^+$ and CD8$^+$ T cells seems is not related to the clinical status of dogs with CVL.

Keywords: *Leishmania*, canine, CD4, CD8.

Resumo: Leishmaniose visceral canina (LVC) é uma zoonose causada por *Leishmania (Leishmania) infantumchagasi*no continente Americano. Poucos estudos demonstraram as características imunopatológicas em linfonodo poplíteo e fígado de cães infectados em área endêmica de Teresina, Piauí, Brasil. Assim, nós avaliamos as alterações histopatológicas e a presença de células T CD4$^+$ e CD8$^+$ em linfonodo poplíteo e fígado de nove cães com manifestações clínicas (G1) e sete cães sem manifestações clínicas (G2) naturalmente infectados com *L. (L.) infantumchagasi*. Fragmentos de linfonodo poplíteo e fígado foram

obtidos para análise histopatológica e imunoistoquímica. As principais manifestações clínicas foram linfadenopatia (80%) e lesões de pele (70%). Na análise histopatológica do linfonodo foi observada uma depleção de células da região cortical. Não havia diferença significante entre os grupos entre células T CD4$^+$ e CD8$^+$ mas havia uma correlação positiva entre essas células em G1 e G2. No fígado, o infiltrado inflamatório era focal consistindo de células mononucleares nas regiões periportal e intralobular, em ambos os grupos. O infiltrado inflamatório era positivo na imunomarcação para células T CD4 e CD8, mas sem diferença significante entre os grupos. Entretanto, havia uma correlação positiva entre as células T CD4$^+$ e CD8$^+$ no G1. Na análise da imunomarcação também mostrou mais amastigotas e antígenos de *Leishmania* no fígado e linfonodo poplíteo em G1 em relação ao G2. O papel das células T CD4$^+$ e CD8$^+$ parece não estar relacionada ao estado clínico dos cães com LVC.

Paravras-chave: *Leishmania*, canino, CD4, CD8.

Introduction

Canine visceral leishmaniasis (CVL) is a systemicandchronic disease caused by *L. (L.) infantumchagasi*in the American continent (MAURÍCIO et al., 2000). The dog is the most important reservoir of visceral leishmaniasis (VL) in urban and periurban environment, because they have high susceptibility to infection and high cutaneous parasitism, making them the main source of infection for insect vectors (DANTAS-TORRES, 2007). Thus, the presence of seropositive dogs in human dwellings is seen as a possible risk factor for *L. (L.) infantum* infection (CUNHA et al., 1995).

The clinic-pathologic spectrum of CVL is very diverse (CIARAMELLAet al.,

Mechanism of VL (ALVES et al., 2009; BEATTIE et al., 2010). The lesions

1997; TAFURI et al., 2001). The histological changes found in skin, lymph nodes, liver, spleen and bone marrow are similar to those described in human disease (Keenan et al., 1984).

Although skin lesions are a characteristic of dogs (Brachelenteet al., 2005), these can be seen also in man in cases of co-infection with *Leishmania*and HIV post-treatment (FAROOQ et al., 2013).

There are few studies detailing the histopathologic characteristics in lymph nodes and liver of the infected dog in Teresina, (Piauí state, Brazil). These organs are considered as initial targets and thus are important for understanding of immunopathologica.

in the liver contributes to several clinicopathological changes as

hypoalbuminemia, increased levels of transaminases (ALT, AST) and total protein (Abranches et al., 1991; Reis et al., 2006), but rarely causes liver failure (RALLIS et al., 2005).

The interaction between the Kupffer cells and CD8^{+} T cells within granulomas has important implications for the identification of novel antigens vaccine candidates that may serve as new model of immuno-therapeutic intervention (BEATTIE et al., 2010).

The early response of popliteal lymph node is the increase of volume (Costaet al., 2008; Giunchettiet al., 2008), with a humoral and cellular responserelated to the evolution of the infection (PINELLI et al., 1994).

There are few studies showing cell profiles in lymph nodes of dogs with VL, but the main change immunophenotypic is an increased in the frequency of T lymphocytes, particularly CD8^{+}, an increased expression of MCH-II and a decrease in the levels of cells B CD2[+](GIUNCHETTI et al., 2008).

In the CVL is not clear whether T lymphocytes and their subpopulations have an important role in modulating the immune response (MORENO et al., 1999; MORENO & ALVAR, 2002; MIRANDA et al., 2007). CD4^{+} T lymphocytes in peripheral blood may be inversely

correlated with disease severity (MIRANDA et al., 2007).

Studies have shown that macrophages of infected dogs are deficient in co-stimulatory molecules, reducing the ability to activate T cells (PINELLIet al., 1999).

It has not been shown a pattern of response associated with clinicopathological status among subpopulations of helper T lymphocytes in murine, human and/or canine models of VL. It is known that both Th1 and Th2 responses are present in dogs resistant, sometimes with a predominance of the first (PINELLIet al., 1994; MAIA & CAMPINO, 2012).

CD8^{+} T lymphocytes play an essential role in protecting the host because they can lyse macrophages infected with *Leishmania*(PINELLI et al., 1994). But, studies show that their number both peripheral blood and lymph nodes may be increased in susceptible dogs (MORENO et al., 1999; GIUNCHETTIet al., 2008). The immunology of VL is closely related to the modulatory role of host immune response. Thus, the aim of this study was correlate the morphological changes and the presence of CD4^{+} and CD8^{+} T cells in liver and popliteallymphnode, with the clinical status of dogs naturally infected with *Leishmania (L.) infantumchagasi.*

Material and Methods

Animals and diagnosis of VL

16 mongrel dogs, males and females, adults, naturally infected by *L. (L.) infantumchagasi* from Teresina, Piaui, an endemic area in Brazil, kindly provided by Management Control of Zoonosis – Fundação Municipal de Saúde, were included in this study. The animals were divided in two groups: group 1 (G1), with nine animals with serology and parasitological positive with clinical manifestations of VL, and group 2 (G2) with seven animals with positive parasitological and serological examination without clinical manifestations of VL. The diagnosis of VL was confirmed by indirect immunofluorescence (IFAT), combined with parasitological examination and/or culture of sternal bone marrow, spleen and popliteal lymph node.

Sample and processing of the material

After general anesthesia with thiopental sodium, the animals were euthanized with potassium chloride 20%. Fragments were collected from liver and popliteal lymph node of 0.5 cm thick to touch imprint cytology. Samples were stained with Giemsa for parasite count in 50 random fields under the light microscope using oil immersion at 100X.

Others fragments of tissue were fixed in 10% formalin buffered with 0.01 M phosphate pH 7.4 and processed by routine technique and stained with hematoxylin-eosin for light microscopy and other fragments were adhered to slides with organosilane for immunohistochemistry.

The detection of parasite antigens was performed using mouse polyclonal, anti-*Leishmaniaamazonensis* antibody, diluted 1:1600 (v/v). CD4$^+$ and CD8$^+$ T cells were detected with mouse monoclonal anti-canine CD4$^+$ (VMRD. Hybridoma DH29A, Lot 1089, Pullman, USA) and CD8$^+$ (VMRD, Inc., hybridoma CAD046A, Lot 0395-0598, Pullman, USA) antibodies, diluted 1:800. The reaction proceeded using the EnVision$^+$ peroxidase system (DAKO Corporation, Carpinteria, CA, USA, Code 4001 k). The reaction was developed using 0.06% hydrogen peroxide and 0.3 mg/ml 3,3'diaminobenzidine (Sigma Chemical, USA) in PBS. Counterstaining was performed using Harry's haematoxylin (Sigma Chemical, USA).

We used as positive control kidney tissue of a dog with LV where the marking of T cells was already well established (COSTA et al., 2010). As negative control the primary antibody was omitted.

Morphometric analysis

Quantification of $CD4^+$ and $CD8^+$ T cells was performed in popliteal lymph node and liver by immunoperoxidase using image analyzer computerized Leica Qwin D-1000, version 4.1 (Cambridge, UK), Departamento de Patologia Animal (BIOLAI), Centro de CiênciasAgrárias, Universidade Federal do Piauí. 20 random fields were captured by lymph nodes and liver of each animal for counting of the number of $CD4^+$ and $CD8^+$ T cells.

Ethical Principles

The procedures involving animals were performed according to the Brazilian Guide for Care of Laboratory Animals (Bill No. 3.964/97 - http//: www.planalto.gov.br) and the experimental protocol followed the criteria established by Research Ethics Committee in Animal Experimentation of Universidade Federal do Piauí.

Statistical Analysis

In the analysis comparative of data, Mann Whitney U test was used to compare two groups and for linear regression were used Sigma Stat software (Jandel Corporation, USA). The level of significance was set at $P<0.05$.

The semi-quantitative analysis were performed to measure the distribution and intensity of lesions in the scale 0-4 where 0 = normal, 1 = minimal or doubtful, 2 = average, 3 = moderate 4 = moderately severe, 5 = severe.

RESULTS AND DISCUSSION

In areas endemic for VL, few studies have demonstrated the association of lesions in different organs affected by parasite and cellular immune response. In the present study we seek to understand the morphology changes associated with the presence of $CD4^+$ and $CD8^+$ T cells in popliteal lymph node and liver and its relationship to the severity clinical status in dogs naturally infected by *L. (L.) infantumchagasi*.In dogs with clinical manifestations to VL, the most frequent changes were local or generalized lymphadenopathy (80%), skinlesions (70%), weight loss (45%), fever (35%) and anemia (10%) similar other studies (CIARAMELLA et al., 1997; SOLANO-GALLEGO et al., 2011). In this group of dogs (G1) popliteal lymph node and hepatic tissue stained with Giemsa and H-E showed that amastigotes were present at the same intensity when compared to animals positive for VL, but without clinical manifestations (G2). However, immunoperoxidase analysis revealed that there were more amastigotes and antigen of *Leishmania* in G1 than in G2 in both, lymph node (P = 0.0255, Mann Whitney test) and liver (P = 0.0076, Mann Whitney test) (Figure 1A-B), respectively.

The presence of antigen was significantly higher in hepatocytes in G1 (P = 0.0382, Mann Whitney test) as well as in the periportal inflammatory cells (P = 0.0139, Mann Whitney test) (Figure 1C-D) (Table 1). This result is related to the immune response of dogs against infection by *Leishmania*, because dogs without clinical manifestations exhibit a profile mixed Th1/Th2, but in many cases, the

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Th1-resistent profile is predominant (Pinelli et al., 1994; Cabral et al., 1998) and, therefore dogs with higher parasitic load, as observed in G1 compared to G2, showed greater power to infect the vector, although this fact is also present in infected animals without clinical manifestations, but to a lower degree (MICHALSKY et al., 2007; SOARES et al., 2011).

Figure 1. Presence of amastigotes forms and antigen of *Leishmania* sp. in symptomatic and asymptomatic dog infected. (A) Lymph Nodes and (B) liver. Antigen of *Leishmania* sp. in (C) hepatocyte and (D) periportal inflammatory cels of liver of symptomatic and asymptomatic dog infected. Immunoperoxidase staining. *P<0.05, Mann-Whitney test.
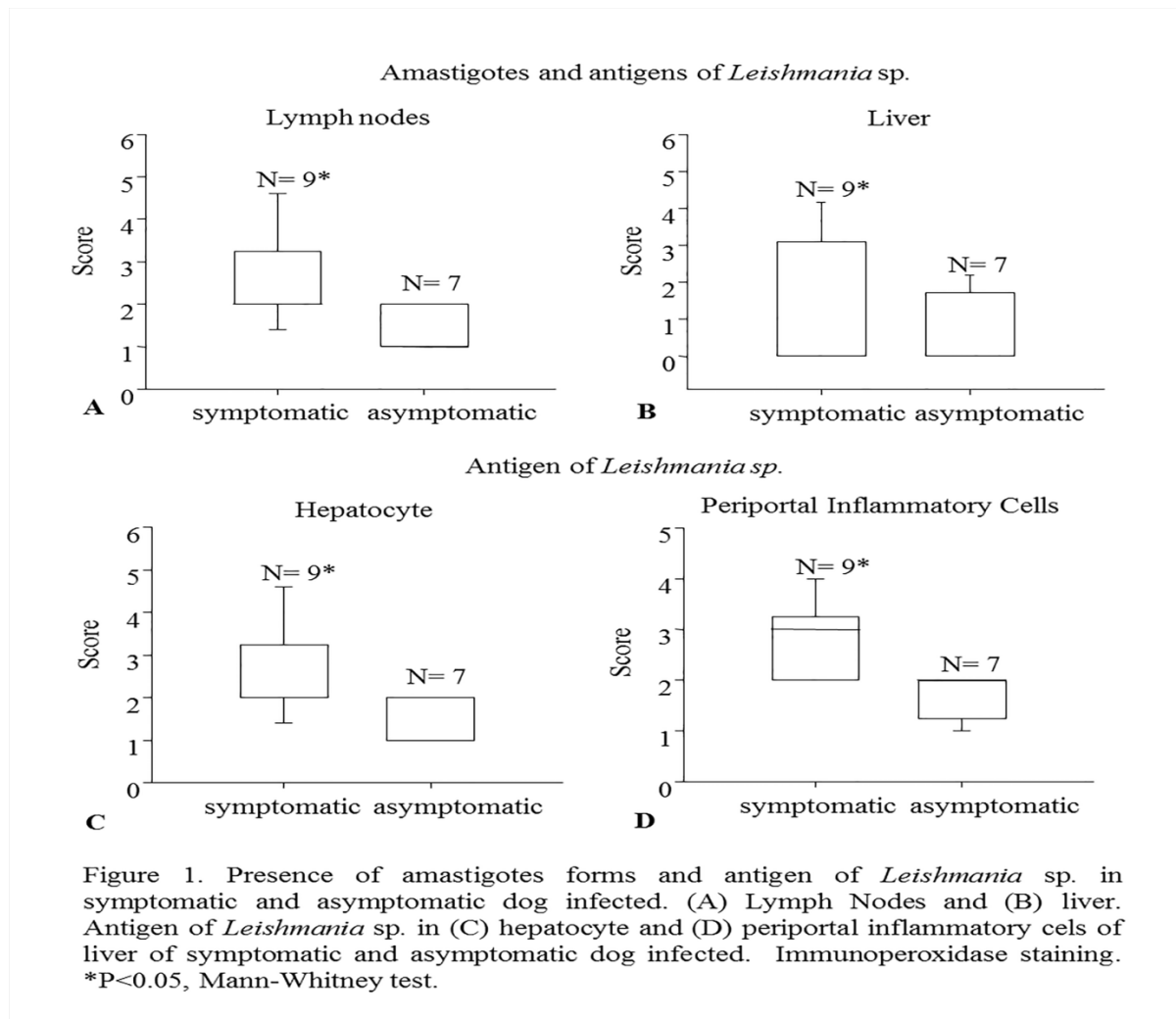
Table 1. Clinical and pathological parameters of the lymph nodes and liver of 16 seropositive dogs infected naturally with *L. (L.) infantum chagasi* and testing positive for the parasite

Animals	Clinical signs	Serology(IFI)	Parasitology	Cytology		IMH				Inflammatory Infiltrate	Depletion of cells in the paracortical area
				LN	Liver	CD4+		CD8+		Liver	Lymph nodes
						LN	Liver	LN	Liver		
1	Asymptomatic	ND	Positive	1	0	0.80	9.80	6.75	4.60	++	N
2	Asymptomatic	1:160	Positive	2	0	47.65	1.60	43.30	0.00	+	N
3	Asymptomatic	1:320	Positive	ND	1	7.20	0.25	10.50	0.85	+	N
4	Asymptomatic	1:40	Positive	0	0	ND	1.05	ND	4.80	+	-
5	Asymptomatic	1:80	Positive	1	0	0.45	ND	0.14	0.30	++	N
6	Asymptomatic	Negative	Positive	0	0	4.20	ND	5.7	ND	+++	N
7	Asymptomatic	1:80	Positive	1	0	ND	0.00	ND	ND	++	N
8	Symptomatic	Negative	Positive	152	7	280.9	ND	302.7	ND	+++	+
9	Symptomatic	Negative	Positive	0	2	39.05	5.05	26.50	5.30	++	N
10	Symptomatic	Negative	Positive	0	0	ND	3.70	ND	4.40	++	-
11	Symptomatic	1:80	Positive	0	0	12.85	ND	19.80	ND	++	+
12	Symptomatic	1:160	Positive	0	0	ND	0.06	ND	3.40	++	++
13	Symptomatic	Negative	Positive	0	1	ND	0.05	ND	0.05	++	++
14	Symptomatic	1:80	Positive	11	3	ND	0.00	ND	0.00	++	+++
15	Symptomatic	1:40	Positive	0	0	ND	0.00	ND	0.00	++	N
16	Symptomatic	ND	Positive	0	0	64.65	ND	41.9	ND	++	+++

Serology (IFI) = indirect immunofluorescence. Positive parasitology was determined by the presence of leishmania amastigotes in smears of bone marrow and/or lymph node. Cytology scale was the number of amastigotes in 50 random fields under a light microscope using oil immersion at a magnification of 100x. IMH= immunohistochemistry. Scale used for IMH and inflammatory infiltrate; N= normal; += minimum or doubtful; ++ = mild; +++ = moderate; ++++ = severe=moderate; +++++=severe; ND = not determined; LN = popliteal lymph node.
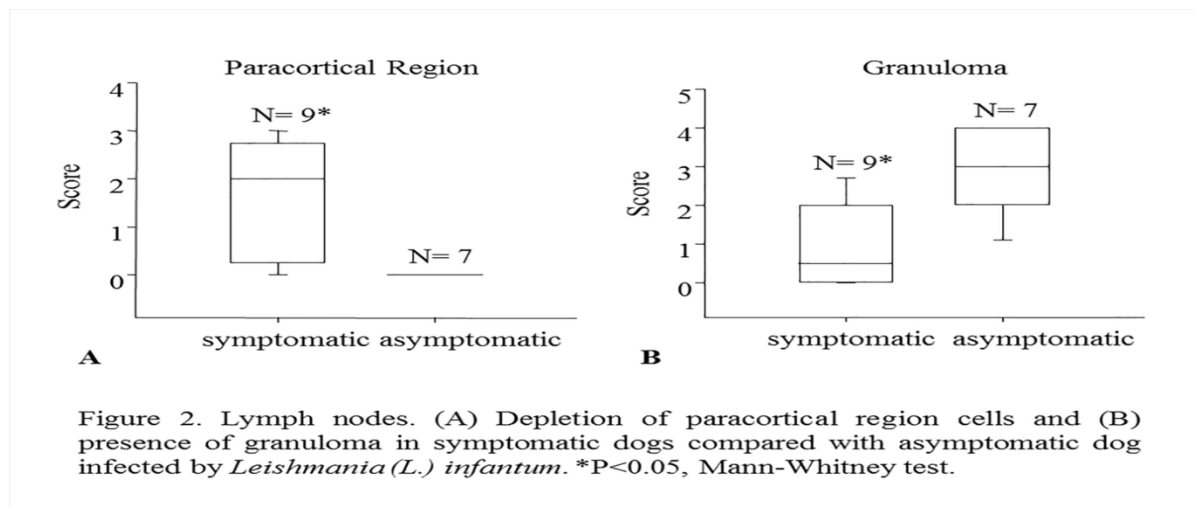
The immunology of CVL is complex and requires understanding of the cellular immune response involving CD4^{+} and CD8^{+} T cells in organs of the mononuclear phagocytic system as lymph nodes and liver which were the organs selected for this study because these cells actively participate of the modulation the canine infection (Reis et al., 2006; Beattie et al., 2010) and it is known that the organic response is compartmentalized (REIS et al., 2009). Histopathological analysis of the popliteal lymph node revealed higher intensity of depletion cell of the paracortical region (P = 0.03, Mann Whitney test) and granuloma (P = 0.03, Mann Whitney test) (Figure 2A-B) in G1 when compared with G2. The depletion of follicles was more frequently observed in G1 (Figure 4A-C). The inflammatory infiltrate consisted mainly of macrophages and lymphocytes, but in some cases, a high presence of plasma cells in both groups.

In one animal in G1 was observed an intense presence of fibroblasts as component of the inflammatory infiltrate. In capsule and subcapsular sinus, the inflammatory response varied from minimal to severe intensity, the presence of eosinophils also was observed, particularly in G1. The cell hyperplasia in the medullar sinus was more evident in G1.

Scarce neutrophils were found in the inflammatory infiltrate in lymph nodes in both groups. Immunohistochemical analysis revealed in lymph nodes that most cells of the paracortical region showed positive staining for CD4^{+} and CD8^{+} T

cells (Figure 4E), but without significant difference between G1 and G2. We observed positive correlation between CD4^{+} and CD8^{+} T cells, both in G1 (P = 0.001, r = 0.99 – Spearman correlation) and G2 (P = 0.005, r = 0.99 - Spearman correlation) (Figure 3A-B) (Table 1).

Figure 2. Lymph nodes. (A) Depletion of paracortical region cells and (B) presence of granuloma in symptomatic dogs compared with asymptomatic dog infected by *Leishmania (L.) infantum*. *P<0.05, Mann-Whitney test.

Depletion of the paracortical region is an important morphological aspect in popliteal lymph nodes because the paracortical region is rich in T cells (ABBAS et al., 2012) and sub-population of CD4^{+} T cells which can be directly related to the immune response associated with activation of Th1 cytokines leading to control of infection (PINELLI et al., 1994; PINELLI et al., 1999). This aspect has been better observed in murine model of experimental cutaneous leishmaniasis (HEINZEL et al., 1989; ROGERS et al., 2002). In the present study, however, this confirmation was not observed because the animals of G1 and G2 did not differ in number of T cells in the organs analyzed. It is known that the dog with VL does not show a clear dichotomy Th1/Th2 (STRAUSS-AYALI et al., 2007). The

infection seems to induce a mixed response and the control of parasite replication, progression or cure of the disease is determined by a balance between the two dichotomous patterns (BANETH et al., 2008). Thus, T cells may be present in the same proportion in dogs with and without clinical manifestations for VL, as observed in this study.
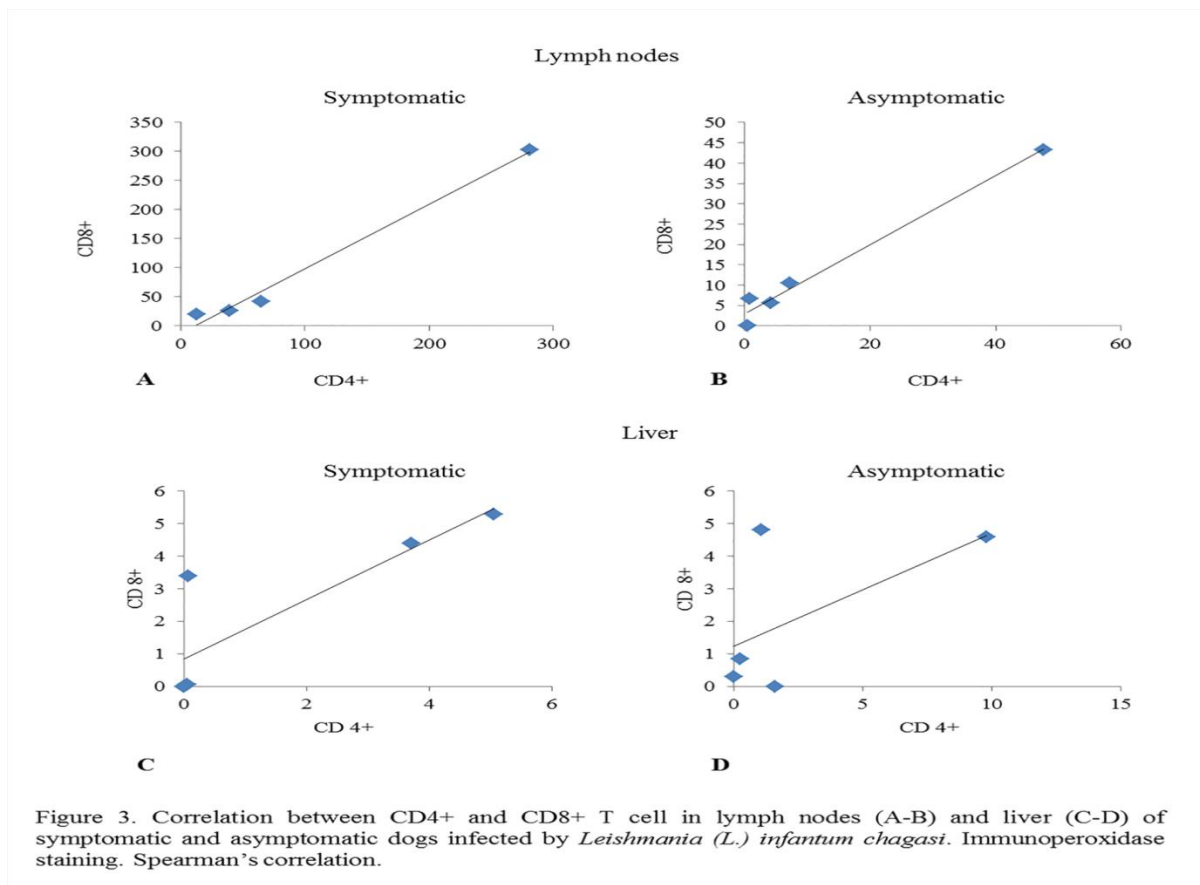
A fact that needs better understanding it is the cell depletion of the paracortical region in dogs of G1 and sub-populations of T cells. This probably resulted from the action of the parasite on the extracellular matrix and in the host cells, taking to the death by apoptosis of the infected cells as has been demonstrated in experimental infection (Abreu-Silva et al., 2004) but not in T cells which are not target of *Leishmania* infection. Probably it

happened because the parasite load in G1 was higher than in G2 (P= 0.0255) as demonstrated by immunoperoxidase technique to detection of parasite and antigen.

In the liver, the inflammatory infiltrate was focal consisting of mononuclear cell located in the periportal region, corresponding the zone 3 of the hepatic lobe of Rappaport (Thung & Gerber, 1994), and intralobular of mild intensity to moderate with granulomatous organization in both groups of dogs. There was a tendency to have more hyperplasia of Kupffer cells (Figure 4D) and fatty

metamorphosis in G2 of minimal intensity to severe, but without significant difference between groups.

Inflammatory infiltrate of mononuclear cells showed positive staining for CD4^{+} and CD8^{+} T cells (Figure 4F), but similar to lymph nodes, there was no significant difference between G1 and G2. It was found that there was a positive correlation between CD4^{+} and CD8^{+} T cells in the G1 (P = 0.03, r = 0.8 - Spearman correlation), but this correlation was not observed in G2 (P = 0.28, r = 0.6 - Spearman correlation) (Figure 3C-D) (Table 1).

Figure 3. Correlation between CD4+ and CD8+ T cell in lymph nodes (A-B) and liver (C-D) of symptomatic and asymptomatic dogs infected by *Leishmania (L.) infantum chagasi*. Immunoperoxidase staining. Spearman's correlation.
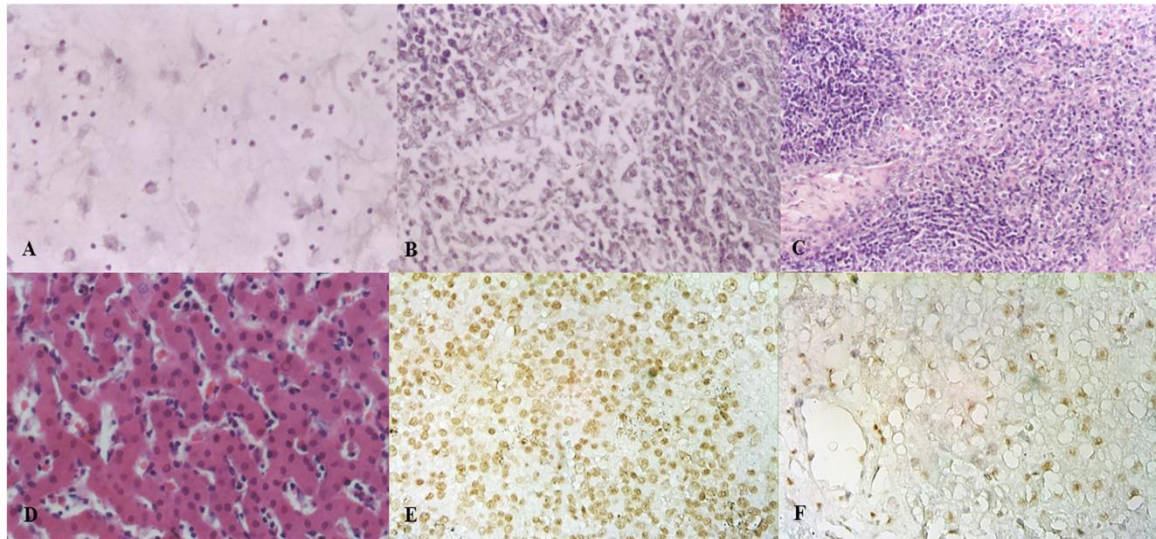
Figure 4. Histological sections from dogs infected naturally with *Leishmania (L.) infantum chagasi*. Lymph nodes: (A) Depletion of follicle and (B) paracortical region cells in symptomatic dogs and (C) hypercellularity of paracortical region cells of asymptomatic dogs. Liver: (D) Kupffer cells hyperplasia in asymptomatic dogs. Hematoxylin-Eosin staining. Bar= 25 μm. Immunohistochemical localization of CD4+ in lymph nodes (E) and liver (F). Immunoperoxidase staining. Bar= 25 μm.

In the liver, the morphological changes were similar in G1 and G2. However, it was observed that there was a slight predominance of Kupffer cells in dogs without clinical manifestation and granuloma in dogs with clinical manifestations, but with populations of CD4$^+$ and CD8$^+$ T cells in similar proportions. In this case, it is possible that the Kupffer cells in the animals without clinical manifestations have a role in infection control. Regarding the granuloma, studies in BALB/c and C57BL/6 mice infected with *Leishmania donovani* showed that the inflammatory reaction is organ-specific (Murray, 2001) and is not always associated with the infection control (LEMOS DE SOUZA et al., 2000). That is what seems to happen in the dog, because liver granulomas were presented similar in both animals with clinical signs and without clinical manifestations, as observed also for number of CD4$^+$ and CD8$^+$ T cells.

The results obtained in this study demonstrated that the morphological changes in lymph node and liver were not associated with a predominant type of infiltration of T cells (CD4$^+$ and/or CD8$^+$), because these cells were labeled in a similar intensity in both organs (lymph node and liver) and in both groups (G1 and G2) despite the difference in the parasite load.

Alexandre-Pires et al., (2010) also demonstrated that symptomatic and asymptomatic dogs with VL not have difference between CD4$^+$ and CD8$^+$ T cells in lymph nodes. These results are different of peripheral blood compartment, where

the presence of T cells is inversely correlated with disease severity (MIRANDA et al., 2007). Our results suggest that this systemic response can be different from the response observed *in situ*. Furthermore, it is likely that the group of animals with clinical manifestations had a prevalence of other sub-populations of T cells, as Tr1 or regulatory T adaptive and these are sub-populations of T cells which express also the molecule CD4$^+$ and can therefore be marked. It is known that these sub-populations of T cells are important for producing IL-10 which is associated with susceptibility to infection in experimental and human VL (Nylénet al., 2007). In addition, macrophages can also express the CD4 molecule on the surface (CROCKER et al. , 1987; KAZAZI et al., 1989), which probably contributed to the increased number of cells expressing CD4$^+$ on dogs of the G1 group, since the inflammatory infiltrate was formed also by macrophages. Thus, it is observed that for a better understanding of the cellular response in organs of the mononuclear phagocytic system, it is necessary to identify other lymphocyte populations. This aspect is being goal of studies conducted by our group.

Moreover, considering that the population of CD4$^+$ and CD8$^+$ T cells in both animal groups (G1 and G2) were quantitatively similar we believe that small differences in the number of cell populations may have no statistical significance, but what matters most is the level of cell activation, even in small numbers, that may have a decisive influence on the biological modulation of pathological processes. That is what this study suggests. Possibly the occurrence or absence of disease was caused by slight mismatch between the activation of these two cell populations among dogs with and without clinical manifestations of VL.

The protective role of CD8$^+$ T cells in CVL suggests that the preferential migration of these cells may occur in the parasitized spleen, with the aim of promoting the lysis of infected macrophages (GUERRA et al., 2009). We did not observe this behavior in lymph nodes, as well as no difference between G1 and G2, since animals with clinical signs had a higher number of CD8$^+$ T cells. Thus, our results suggest that CD8$^+$ T cells have an undefined role in the CVL, similar to those found in other studies (GUARGA et al., 2000). There is evidence that cytotoxic T cells are not activated by the presence of *Leishmania* antigen, remaining mainly as inactivated cells (MÜLLER et al., 1994; MENDONÇA et al., 1995).

Although no difference was observed between CD4$^+$ and CD8$^+$ T cells, we found a positive correlation between these two cell populations in G1 and G2

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(P= 0.005, r = 0.99) in lymph node and a positive correlation between $CD4^+$ and $CD8^+$ T cells only in G1 in the liver. Studies using mice athymic BALB/c (nu/nu) and experiments of cell depletion in BALB/c (nu/+) using monoclonal anti-CD4 or anti-CD8 showed the necessity of both sub-populations $CD4^+$ and $CD8^+$ T cells in protection against infection by *L. donovani* (STERN et al., 1988). In the initial phase of infection of mice infected with *L. donovani* was observed that $L3T4^+$ ($CD4^+$) cells are important in the presence of the parasite, mainly with the formation of hepatic granulomas. With the evolution of the infection, the cell population declines and is replaced by $Lyt-2^+$ ($CD8^+$) cells, when the infectious process is controlled (MCELRATH et al., 1988).

In lymph node from treated dogs was observed lower $CD8^+$ T cells when compared with asymptomatic dogs (ALEXANDRE-PIRES et al., 2010). Furthermore, $CD4^+$ T cells of lymph node in asymptomatic dogs and two treated dogs were significantly greater than in dogs not infected. Together, these results suggest that activation of lymphocytes in the lymph nodes with the expansion of $CD4^+$ T cells may facilitate the control of infection by *Leishmania* because of a local reduction in replication of the parasite and/or parasite elimination, while an increase in $CD8^+$ T cells appears to be related to the persistence of the parasite and activity immunomodulating of the cells.

Conclusions

The present study shows that the response of T cells in lymph nodes and liver differs from the systemic response and reveal that the role of $CD4^+$ T cells and $CD8^+$ seems not related to the clinical status of dogs with VL. On the other hand, further studies on phenotypic marking are needed for better evaluation of compartmentalized cellular immunity in CVL.

Acknowledgment

References

1.ABBAS, A.K.; LICHTMAN, A.H.; PILLAI, S. Cellular and molecular immunobiology. 7th ed. Rio de Janeiro: Elsevier, 2011. 592p.

2.ABRANCHES, P.; SANTO-GOMES, G.; RACHAMIM, N.; CAMPINO, L.; SCHUNUR, L.F.; JAFFE, C.L. An experimental model for canine visceral leishmaniasis. **Parasite Immunoly,** v. 13, n. 5, p. 537-50, 1991.

3.ABREU-SILVA, A. L.; CALABRESE, K.S.; MORTARA, R. A.; TEDESCO, R. C.; CARDOSO, F. O.; CARVALHO, L. O.; GONÇALVES DA COSTA, S. C. Extracellular matrix alterations in experimental murine *Leishmania (L.) amazonensis* infection. **Parasitology,** v. 128, n. 4, p. 385-90, 2004.

4.ALEXANDRE-PIRES, G.; DE BRITO, M. T.; ALGUERÓ, C.; MARTINS, C.; RODRIGUES, O. R.; DA FONSECA, I. P.; SANTOS-GOMES, G. Canine leishmaniasis. Immunophenotypic profile of leukocytes in different compartments of symptomatic, asymptomatic and treated dogs. **Veterinary Immunology and Immunopathology,** v. 137, n. 3-4, p. 275-83, 2010.

5.ALVES, C.F.; DE AMORIM, I. F.; MOURA, E.P.; RIBEIRO, R.R.; ALVES, C.F.; MICHALICK, M. S.; KALAPOTHAKIS, E.; BRUNA-ROMERO, O.; TAFURI, W.L.; TEIXEIRA, M.M.; MELO, M.N. Expression of IFN-gamma, TNF-alpha, IL-10 and TGF-beta in lymph nodes associates with parasite load and clinical form of disease in dogs naturally infected with *Leishmania (Leishmania) chagasi*. **Veterinary Immunology and Immunopatholy,** v. 128, n. 4, p. 349-58, 2009.

6.BANETH, G.; KOUTINAS, A. F.; SOLANO-GALLEGO, L.; BOURDEAU, P.; FERRER, L. Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. **Trends in Parasitology,** v. 24, n. 7, p. 324-30, 2008.

7. BEATTIE, L.; PELTAN, A.; MAROOF, A.; KIRBY, A.; BROWN, N.; COLES, M.; SMITH, D.F.; KAYE, P.M. Dynamic imaging of experimental *Leishmania donovani*-induced hepatic granulomas detects Kupffer cell-restricted antigen presentation to antigen-specific CD8 T cells. **PLoS Pathogens,** v. 6, n. 3, p. e1000805, 2010.

8.BRACHELENTE, C.; MÜLLER, N.; DOHERR, M.G.; SATTLER, U.; WELLE, M. Cutaneous leishmaniasis in naturally infected dogs is associated with a T helper-2-based immune response. **Veterinary Patholology,** v. 42, n. 2, p. 166-75, 2005.

9.CABRAL, M.; O'GRADY, J.E.; GOMES, S.; SOUSA, J.C.; THOMPSON, H.; ALEXANDER, J. The immunology of canine leishmaniosis: strong evidence for a developing disease spectrum from asymptomatic dogs. **Veterinary Parasitology,** v. 76, n. 3, p. 173-80, 1998.

10.CIARAMELLA, P.; OLIVA, G.; LUNA, R.D.; 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. **Veterinary Record,** v. 141, n. 21, p. 539-43, 1997.

11.COSTA, F.A.; PRIANTI, M.G.; SILVA, T.C.; SILVA, S.M.; GUERRA, J.L.; GOTO, H. T cells, adhesion molecules and modulation of apoptosis in visceral leishmaniasis glomerulonephritis. **BMC Infectious Disease,** v. 10, p. 112, 2010.

12.COSTA, M.M.; LIMA, W. G.; FIGUEIREDO, M.M.; MICHALICK, M.S.; TAFURI, W.L.; TAFURI, W.L. Cervical, mandibular, and parotid lymph nodes of dogs naturally infected with *Leishmania infantum*: a histopathologic and immuno histochemistry study and its correlation with facial skin lesions. **Veterinary Patholology,** v. 45, n. 5, p. 613-6, 2008.

13.CROCKER, P.R.; DAVIES, E.V.; BLACKWELL, J.M. Variable expression of the murine natural resistance gene Lsh in different macrophage populations infected in vitro with Leishmania donovani. **Parasite Immunology,** v. 9, n. 6, p. 705-19, 1987.

14.CUNHA, S.; FREIRE, M.; EULALIO, C.; CRITOSVAO, J.; NETTO, E.; JOHNSON, W.D. J.R.; REED, S.G.; BADARO, R. Visceral leishmaniasis in a new ecological niche near a major metropolitan area of Brazil. **Transactions of the Royal Society of Tropical Medicine and Hygiene,** v. 89, n. 2, p. 155-8, 1995.

15.DANTAS-TORRES, F. The role of dogs as reservoirs of Leishmania parasites, with emphasis on *Leishmania (Leishmania) infantum* and *Leishmania (Viannia) braziliensis*. **Veterinary Parasitology,** v. 149, n. 3-4, p. 139-46, 2007.

16.FAROOQ, U.; CHOUDHARY, S.; CHACON, A.H.; LEBRUN, E.; SHIMAN, M.I.; HERNANDEZ, J.; MILIKOWSKI, C.; KERDEL, F.A.; ROMANELLI, P. Post-kala-azar dermal leishmaniasis in HIV-infected patients with AIDS: a report of two cases

diagnosed in the USA. **International journal of dermatology,** v. 52, n. 9, p. 1098-104, 2013.

17.GIUNCHETTI, R.C.; MARTINS-FILHO, O. A.; CARNEIRO, C.M.; MAYRINK, W.; MARQUES, M.J.; TAFURI, W. L.; CORRÊA-OLIVEIRA, R.; REIS, A.B. Histopathology, parasite density and cell phenotypes of the popliteal lymph node in canine visceral leishmaniasis. **Veterinary Immunology and Immunopathology,** v. 121, n. 1-2, p. 23-33, 2008.

18.GUARGA, J.L.; LUCIENTES, J.; PERIBÁÑEZ, M.A.; MOLINA, R.; GRACIA, M.J.; CASTILLO, J.A. Experimental infection of *Phlebotomus perniciosus* and determination of the natural infection rates of *Leishmania infantum* in dogs. **Acta Tropica,** v. 77, n. 2, p. 203-7, 2000.

19.GUERRA, L.L.; TEIXEIRA-CARVALHO, A.; GIUNCHETTI, R. C.; MARTINS-FILHO, O.A.; REIS, A.B.; CORRÊA-OLIVEIRA, R. Evaluation of the influence of tissue parasite density on hematological and phenotypic cellular parameters of circulating leukocytes and splenocytes during ongoing canine visceral leishmaniasis. **Parasitology Research,** v. 104, n. 3, p. 611-22, 2009.

20.HEINZEL, F.P.; SADICK, M. D.; HOLADAY, B.J.; COFFMAN, R. L.; LOCKSLEY, R.M. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. **TheJournal of Experimental Medicine,** v. 169, n. 1, p. 59-72, 1989.

21.KAZAZI, F.; MATHIJS, J.M.; FOLEY, P.; CUNNINGHAM, A.L. Variations in CD4 expression by human monocytes and macrophages and their relationships to infection with the human immunodeficiency virus. **The Journal of general virology,** v. 70, p. 2661-72, 1989.

22.KEENAN, C.M.; HENDRICKS, L. D.; LIGHTNER, L.; WEBSTER, H. K.; JOHNSON, A.J. Visceral leishmaniasis in the German shepherd dog. I. Infection, clinical disease, and clinical pathology. **Veterinary Pathology,** v. 21, n. 1, p. 74-9, 1984.

23.LEMOS DE SOUZA, V.; ASCENÇÃO SOUZA, J.; CORREIA SILVA, T. M.; SAMPAIO TAVARES VERAS, P.; RODRIGUES DE-FREITAS, L.A. Different *Leishmania* species determine distinct profiles of immune and histopathological responses in CBA mice. **Microbes and infection / Institut Pasteur,** v. 2, n. 15, p. 1807-15, 2000.

24.MAIA, C.; CAMPINO, L. Cytokine and Phenotypic Cell Profiles of Leishmania infantum Infection in the Dog. **Journal of Tropical Medicine,** v. 2012, p. 541571, 2012.

25.MAURÍCIO, I.L.; STOTHARD, J.R.; MILES, M.A. The strange case of Leishmania chagasi. **Parasitology Today,** v. 16, n. 5, p. 188-9, 2000.

26.MCELRATH, M.J.; MURRAY, H.W.; COHN, Z.A. The dynamics of granuloma formation in experimental visceral leishmaniasis. **Journal of Experimental Medicine,** v. 167, n. 6, p. 1927-37, 1988.

27.MENDONCA, S.C.F.; LUCA, P.M.; MAYRINK, W.; RESTOM, T.G.; CONCEIÇÃO-SILVA, F.; DA-CRUZ, A. M.; BERTHO, A.L.; DA COSTA, C.A.; GENARO, O.; TOLEDO, A.P.C.P.; COUTINHO, S.G. Characterization of human T lymphocyte-mediated immune responses induced by a vaccine against American tegumentary leishmaniasis. **The American journal of tropical medicine and hygiene,** v. 53, n. 2, p. 195-201, 1995.

28.MICHALSKY, E.M.; ROCHA, M.F.; DA ROCHA LIMA, A.C.; FRANÇA-SILVA, J.C.; PIRES, M.Q.; OLIVEIRA, F. S.; PACHECO, R.S.; DOS SANTOS, S. L.; BARATA, R.A.; ROMANHA, A. J.; FORTES-DIAS, C.L.; DIAS, E.S. Infectivity of seropositive dogs, showing different clinical forms of leishmaniasis, to *Lutzomyia longipalpis* phlebotomine sand flies. **Veterinary Parasitology,** v. 147, n. 1-2, p. 67-76, 2007.

29.MIRANDA, S.; MARTORELL, S.; COSTA, M.; FERRER, L.; RAMIS, A. Characterization of circulating lymphocyte

subpopulations in canine leishmaniasis throughout treatment with antimonials and allopurinol. **Veterinary Parasitology,** v. 144, n. 3-4, p. 251-60, 2007.

30.MORENO, J.; ALVAR, J. Canine leishmaniasis: epidemiological risk and the experimental model. **Trends Parasitology,** v. 18, n. 9, p. 399-405, 2002.

31.MORENO, J.; NIETO, J.; CHAMIZO, C.; GONZÁLEZ, F.; BLANCO, F.; BARKER, D.C.; ALVAR, J. The immune response and PBMC subsets in canine visceral leishmaniasis before, and after, chemotherapy. **Veterinary Immunology and Immunopathology,** v. 71, n. 3-4, p. 181-95, 1999.

32.MURRAY, H.W. Tissue granuloma structure-function in experimental visceral leishmaniasis. **International journal of experimental pathology,** v. 82, n. 5, p. 249-67, 2001.

33.MÜLLER, I.; KROPF, P.; LOUIS, J. A.; MILON, G. Expansion of gamma interferon-producing CD8+ T cells following secondary infection of mice immune to Leishmania major. **Infection and Immunity,** v. 62, n. 6, p. 2575-81, 1994.

34.NYLÉN, S.; MAURYA, R.; EIDSMO, L.; MANANDHAR, K.D.; SUNDAR, S.; SACKS, D. Splenic accumulation of IL-10 mRNA in T cells distinct from CD4+CD25+ (Foxp3) regulatory T cells in human visceral leishmaniasis. **The Journal of Experimental Medicine,** v. 204, n. 4, p. 805-17, 2007.

35.PINELLI, E.; KILLICK-KENDRICK, R.; WAGENAAR, J.; BERNADINA, W.; DEL REAL, G.; RUITENBERG, J. Cellular and humoral immune responses in dogs experimentally and naturally infected with Leishmania infantum. **Infection and Immunity,** v. 62, n. 1, p. 229-35, 1994.

36.PINELLI, E.; RUTTEN, V. P.; BRUYSTERS, M.; MOORE, P. F.; RUITENBERG, E. J. Compensation for decreased expression of B7 molecules on Leishmania infantum-infected canine macrophages results in restoration of parasite-specific T-cell proliferation and gamma

interferon production. **Infection and Immunity,** v. 67, n. 1, p. 237-43, 1999.

37.RALLIS, T.; DAY, M. J.; SARIDOMICHELAKIS, M. N.; ADAMAMA-MORAITOU, K. K.; PAPAZOGLOU, L.; FYTIANOU, A.; KOUTINAS, A.F. Chronic hepatitis associated with canine leishmaniosis (*Leishmania infantum*): a clinicopathological study of 26 cases. **Journal of comparative pathology,** v. 132, n. 2-3, p. 145-52, 2005.

38.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,** v. 81, n. 1, p. 68-75, 2006.

39.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,** v. 128, n. 1-3, p. 87-95, 2009.

40.ROGERS, K.A.; DEKERY, G.K.; MBOW, M.L.; GILLESPIE, R.D.; BRODSKYN, C. I.; TITUS, R.G. Type 1 and type 2 responses to Leishmania major. **FEMS Microbiology Letters,** v. 209, n. 1, p. 1-7, 2002.

41.SOARES, M.R.; DE MENDONÇA, I. L.; DO BONFIM, J.M.; RODRIGUES, J. A.; WERNECK, G.L.; COSTA, CH. Canine visceral leishmaniasis in Teresina, Brazil: Relationship between clinical features and infectivity for sand flies. **Acta Tropica,** v. 117, n. 1, p. 6-9, 2011.

42.SOLANO-GALLEGO, L.; MIRÓ, G.; KOUTINAS, A.; CARDOSO, L.; PENNISI, M.G.; FERRER, L.; BOURDEAU, P.; OLIVA, G.; BANETH, G. LeishVet guidelines for the practical management of canine leishmaniosis. **Parasite and Vectors,** v. 4, p. 86, 2011.

43.STERN, J.J.; OCA, M.J.; RUBIN, B. Y.; ANDERSON, S.L.; MURRAY, H.W. Role of L3T4+ and LyT-2+ cells in experimental visceral leishmaniasis. **Journal of Immunology,** v. 140, n. 11, p. 3971-7, 1988.

44.STRAUSS-AYALI, D.; BANETH, G.; JAFFE, C.L. Splenic immune responses during canine visceral leishmaniasis. **Veterinary Research,** v. 38, n. 4, p. 547-64, 2007.

45.TAFURI, W.L.; DE OLIVEIRA, M.R.; MELO, M.N. Canine visceral leishmaniosis: a remarkable histopathological picture of one case reported from Brazil. **Veterinary Parasitology,** v. 96, n. 3, p. 203-12, 2001.

46.THUNG, S.N.; GERBER, F. Liver biopsy. MAC SWENN, R.N.M. et al. In: Pathology of the liver. 3th ed. London: Churchill Livingstone, 1994. p.787-796.