We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Relationship of Parasitic Index and Cytokine Profile in Canine Visceral Leishmaniasis

José Nivaldo da Silva, Valéria Régia Franco Sousa, Arleana do Bom Parto Ferreira de Almeida, Adenilda Cristina Honorio-França and Eduardo Luzía França

Abstract

Visceral leishmaniasis (VL) is a zoonotic parasitic disease caused by *Leishmania infantum (L. chagasi)* that infects cells of the monocyte-phagocyte system. This work aims to describe the bone marrow parasitism in dogs naturally infected by *L. chagasi*, and to correlate with serum concentrations of cytokines and antibody level. It evaluated 42 dogs, 21 uninfected and 21 infected by *L. infantum*, of both sexes and of different ages; dogs were classified into three clinical stages: stage I, mild disease; stage II, moderate disease; and stage III, severe disease. Parasitic index was determined by real-time polymerase chain reaction (PCR) and cytokine serum concentration by flow cytometry. The average parasitic index of infected dogs was 4.59 × 1010 copies/μl. IL-4 and TNF-α concentrations were higher in infected dogs than in the control group. Antibody levels were positively correlated with IL-4 expression. There was a significant positive correlation of IL-6 cytokine levels with the evolution of stages I and III. Antibody levels were positively correlated with IL-4 expression. There was a significant positive correlation of IL-6 cytokine levels with the evolution of stages I and III. However, this cytokine can be used as a marker to distinguish between different clinical stages.

Keywords: *Leishmania infantum*, dogs, cytokines, parasitic index, cytometry

1. Introduction

Visceral leishmaniasis (VL) is a parasitic zoonotic disease caused by the protozoan *Leishmania infantum* (syn. *L. chagasi)*, an intracellular parasite of the phagocytic mononuclear system [1, 2]. In Brazil, VL is transmitted by sandflies, *Lutzomyia longipalpis* [1, 3, 4].

In a global scenario, it is estimated that 300,000 new cases of VL occur with a rate of 20,000 deaths each year, with 94% new cases reported in Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan [5]. While in Latin America, LV spreads from Mexico to Argentina, with the largest number of cases concentrated in Brazil [6]. With the urbanization of VL in Brazil, annually, the country records approximately 3500 new cases, mainly in medium and large cities; probably, it is due to the disordered anthropic occupation of the geographic space [7].

Despite scientific advances, cases of VL are expanding, which has a major impact on public health, as dogs are the main reservoirs in the urban environment and therefore play an important role in the transmission cycle [8, 9].

Canine visceral leishmaniasis (CVL) is characterized by a broad clinical spectrum, from mild and moderate to fatal clinical manifestations. Major clinical signs in dogs include hepatosplenomegaly, lymphadenopathy, exfoliative dermatitis, alopecia, onychogryphosis, keratoconjunctivitis, apathy, anorexia, and severe weight loss [10–13].

The clinical manifestation of CVL depends on the interaction of the parasite with the host immune response [2]. In susceptible dogs, clinicopathological abnormalities are preceded by an evident humoral response and depression of the cellular response, mediated by a non-protective Th2 immune response associated with cytokines IL-4, IL-5, IL-6, and IL-10 [14, 15]. Dogs that do not develop the disease have a protective cellular response (Th1) [16, 17], related to INF-γ, TNF-α, IL-2, and IL-12 cytokines.

Different procedures are used for the diagnosis of CVL [18]. The Brazilian Ministry of Health recommends serology in the investigation of canine disease by the Dual-Path Platform (DPP®) rapid method as a screening test and ELISA as confirmatory test [19]. Other tests are used to demonstrate infection, such as cytology, histopathology [20], and real-time PCR (RT-PCR) [21].

Similarly, determination of parasitic index has become important for early detection, but also evaluation of treatment efficacy and monitoring of relapses [22]. Thus, the aim of this study was to associate parasitic index to serum cytokine concentration in dogs naturally infected by *L. infantum* at different clinical stages of infection.

2. Methodological aspects

The procedures were previously approved by the Ethics Committee on the Use of Animals (ECUA)/UFMT, Brazil (n° 23108.019567/14-1), and collection of clinical samples was authorized by the dog owners by signing the informed consent form.

2.1 Animals

This study was conducted over a 16-month period, evaluating 42 male and female dogs of different ages and breeds from Barra do Garças, Mato Grosso State, Brazil (latitude, −15.893; longitude, 52.2599; south,15° 53′ 35″; west 52° 15′ 36″). Dogs with canine visceral leishmaniasis (n = 21) were classified into clinical stages at diagnosis as described by Solano et al. [23] and confirmed using the Dual-Path Platform Rapid Test (RT DPP®) and polymerase chain reaction (PCR). A control group (n = 21) was also formed, comprising dogs with no clinical changes and negative results for RT DPP® and conventional PCR.

2.2 Blood and bone marrow sample

Blood samples (5 mL) were collected by cephalic or jugular venipuncture, placed in tubes without anticoagulant to obtain serum. Serum was obtained by centrifuging the blood sample at 300× *g* for 5 minutes and was then transferred to 2 mL microtubes and stored at −80°C for cytokine dosing.

After dog restraint and local anesthesia with 2% lidocaine, bone marrow samples were obtained from the sternal manubrium, placed in microtubes with 0.5 mL 0.9% sterile NaCl solution, and stored at −20°C for subsequent molecular techniques.

2.3 Immunochromatographic rapid test: RT DPP® kit

The immunochromatographic rapid test for detection of anti-*Leishmania infantum* antibodies (DPP®—Canine Visceral Leishmaniasis-Bio-Manguinhos/FIOCRUZ, Rio de Janeiro, Brazil) that uses the recombinant protein K39 (rK39) as an antigen, a cloned 39 amino acid sequence of the specific *L. infantum* kinase region, was performed according to the manufacturer's guidance.

2.4 DNA extraction, conventional PCR, and qPCR

DNA extraction from blood samples was performed by the phenol-chloroform method. The polymerase chain reaction assay was performed using the primers RV1 (sense) 5′-CTT TTC TGG TCC CGC GGG TAG G-3′ and RV2 (antisense) 5′-CCA CCT GGC TAT TTT ACA CCA-3′ [24], which amplifies the DNA fragment of a 145 bp region of conserved kDNA present in *L. infantum*. Amplification used 200 mM dNTP, 1 pM from each primer, a buffer solution (10 mM Tris–HCl and 50 mM KCl, pH 8.3), 2 mM MgCl2, 1.5 U Taq DNA polymerase, and 2 μl of the DNA sample in the final volume of 25 μl. Assays were performed for one cycle at 94°C for 4 minutes, followed by 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and final extension of one cycle at 72°C for 10 minutes. The amplification product was fractionated by 2.0% agarose gel electrophoresis, stained with red gel spot, and visualized on a transilluminator (UV, 300 nm).

Quantitative PCR (qPCR) was performed in triplicate using the StepOne™ Real-Time PCR System Sequence Detection System (Applied Biosystems) targeting RV1–5′-CTT TTC TGG TCC GGG TAG G-3′ primers and RV2–5′-CCA CCT GGC TAT TTT ACA CCA-3′ amplifying a 145 bp sequence of *L. infantum*-specific kDNA [24]. Reactions were prepared in a 25 μl final volume containing SYBR Green Master Mix, 0.3 μM of each primer, and 2 μl of target DNA. Amplification conditions included an initial incubation step at 94°C for 10 minutes, followed by 40 cycles of amplification, 94°C for 15 seconds, and 60°C for 60 seconds. The standard curve was established for each assay using known amounts of TOPO PCR 2.1 plasmid (Invitrogen Corp.) containing *L. infantum* kDNA gene. Serial (10×) dilutions of the recombinant plasmid containing 2.9×10^4–2.9×10^8 copies of the plasmid were performed and used on the standard curve.

2.5 Cytokine quantification by flow cytometry

Serum cytokine concentration (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17) was assessed using the Cytometric Bead Array (CBA) Kit (BD Bioscience, USA) and evaluated by a flow cytometer (FACSCalibur®, BD Bioscience, USA). The reading was done using the CellQuest. Data were analyzed in FCAP array software version 5.0.

2.6 Determination of serum immunoglobulins

Immunoglobulin concentrations (IgM and IgG) in the sera were determined by turbidimetric method. For 1:11 (v/v) IgM and 1:15 (v/v) IgG, antibody concentrations were determined using IgM (Bioclin®, Brazil, Ref K063) and IgG (Bioclin®, Brazil, Ref K062) antiserum diluted with 1:12 (v/v). The calibration curve obtained from the Multical calibrator (Bioclin®, Brazil, Ref K064) was used to determine the standard curve for each immunoglobulin. Positive and negative serum samples, standards, and controls were placed in 500 μl buffer solution (0.15 mol/L sodium

chloride, Tris 50 mmol/L, 6.0000 PEG 50 g/L, and sodium azide 15.38 nmol/L). The suspensions were mixed and incubated at 37°C for 10 minutes. Reactions were read on a spectrophotometer at 340 nm.

2.7 Statistical analysis

For the analysis of the concentration of cytokines and immunoglobulins (IgG and IgM), the Student t-test independent samples were used. For the quantification of parasitic index of the bone marrow and cytokines when compared by clinical stage, Kruskal-Wallis analysis of variance was used. Parasite load correlation analysis of IgG in the presence of cytokines was also performed by calculating the Spearman correlation coefficient. Data were expressed as mean ± standard error. Values less than 0.05 ($p < 0.05$) were considered significant.

3. Results

Most of the 21 dogs in the control group were mongrel dog (15/71%), Labrador retriever (1/5%), dachshund (1/5%), pinscher (3/14%), and rottweiler (1/5%). Age ranged from 14 months to 8 years (average 3.4 years). Thirteen dogs were female (13/62%) and eight dogs were male (8/38%). Most of the 21 dogs with leishmaniasis were dogs from mongrel dog (12/57%), Labrador retriever (1/05%), American pit bull (1/05%), poodle (1/05%), and shih tzu (6/28%). Age ranged from 12 months to 11 years (mean 4.3 years). Six dogs were female (6/29%) and 15 dogs were male (15/72%).

At the time of clinical evaluation, all dogs diagnosed with VL had several clinicopathological findings typical of the disease. Clinical symptoms in seropositive animals (CVL) included lymphadenopathy (17/13%), skin ulcers (12/10%), onychogryphosis (11/09%), ear ulceration (11/09%), scaling (10/08%), weight loss (9/07%), dermatopathy (8/06%), ophthalmopathy (8/06%), muscle atrophy (4/03%), splenomegaly (7/06%), alopecia (6/05%), lethargy (5/04%), periocular alopecia (4/03%), skin nodules (3/02%), hepatomegaly (3/02%), cachexia (3/02%), and hyperkeratosis (2/01%).

Dogs were classified into three clinical stages: stage I, mild disease (n = 5/24%); stage II, moderate disease (n = 9/43%); and stage III, severe disease (n = 7/33%). Stage II dogs were not subclassified.

Leishmania infantum DNA was detected in all dogs of the group with CVL up to a concentration of 1 fg/μl. Real-time PCR of bone marrow samples was positive in all dogs in the CVL group (100%). There was no statistical difference in the distribution between clinical stages and parasitic index, as shown in **Table 1**.

The mean and standard error of concentrations (pg/ml) of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17 cytokines based on clinical staging in CVL-infected dogs are shown in **Table 1**. It was observed that IL-6 and TNF-α concentrations increased in serum of infected dogs with significant statistical difference between the clinical stages of CVL, although most infected dogs had moderate and severe clinical manifestations of the disease.

Among dogs with CVL and uninfected dogs, an increase of IL-4 and TNF-α concentrations in serum from dogs infected with CVL was observed. Similar serum concentrations of IL-2, IL-10, IL-17, and IFN-γ were observed between the groups studied (**Table 2**).

When comparing immunoglobulin means, IgG levels were elevated in the CVL group when compared to IgM levels. A significant difference ($p = <0.0001$)

was observed. Similarly, IgG concentration between the control and CVL groups was evaluated. IgG levels were found to be higher in serum from dogs with CVL (2300.75 ± 678.463) when compared to control group IgG concentrations (636.94 ± 312.8 mg/dl), showing a significant difference between groups ($p = <0.0001$). Regarding the comparison of IgM concentration (mg/dl) in the CVL group (279.74 ± 37.755) compared to the control group (241.12 ± 59.835), there was no difference (**Table 3**).

Correlations of IL-6 and TNF-α concentrations were analyzed according to clinical staging with parasitic index according to stage I, IL-6 ($rs = 0.400$, $p = 0.5046$) and TNF-α ($rs = 0.700$, $p = 0.1881$); stage II, IL-6 ($rs = 0.7000$, $p = 0.1881$) and TNF-α ($rs = -0.1590$, $p = 0.6828$); and stage III, IL-6 ($rs = -0.3571$, $p = 0.4316$) and TNF-α ($rs = -0.4643$, $p = 0.2939$). There was no correlation between the other parameters evaluated.

The correlation between the parasitic index of dogs with CVL in the presence of cytokine IL-4 and TNF-α in the blood of dogs infected with CVL presented the IL-4 ($rs = 0.0240$, $p = 0.9176$) and TNF-α ($rs = 0.0825$, $p = 0.7221$). No additional significant correlations were found. Antibody levels were positively correlated with IL-4 expression ($rs = 0.5997$, $p = 0.0040$) (**Table 4**).

Cytokines/parasitemia	I	II	III	p-Value
IL-2	6.62 ± 1.18	12.01 ± 7.99	15.09 ± 6.34	0.152
IL-4	10.50 ± 2.05	11.38 ± 3.81	9.90 ± 2.73	0.9044
IL-6	2.14 ± 0.57	2.72 ± 0.66	3.12 ± 0.50	0.0350
IL-10	2.47 ± 0.97	2.85 ± 0.96	2.39 ± 0.84	0.8973
IL-17	2.22 ± 0.22	12.38 ± 9.63	13.27 ± 7.51	0.4345
TNF-α	4.52 ± 2.12	4.65 ± 2.31	6.14 ± 1.43	0.0462
IFN	3.07 ± 0.99	28.19 ± 23.21	2.58 ± 0.28	0.4648
Parasite copy number ($\times 10^7$)/ml	4.96 ± 1.00	4.63 ± 1.37	4.55 ± 1.49	0.9467

The results were expressed in mean and standard error.

Table 1.
Cytokine concentrations and parasite copy number ($\times 10^7$)/ml in dogs with visceral leishmaniasis in different clinical staging.

Cytokines	Control	CVL	p-Value
IL-2	9.18 ± 6.14	11.75 ± 6.89	0.3199
IL-4	7.43 ± 2.50	12.56 ± 5.37	0.0469
IL-6	2.87 ± 0.95	2.71 ± 0.67	0.3326
IL-10	2.98 ± 1.39	2.62 ± 0.87	0.2807
IL-17	11.12 ± 12.12	11.63 ± 9.66	0.4570
TNF-α	2.80 ± 0.52	5.12 ± 2.33	0.0009
IFN	13.26 ± 16.88	16.15 ± 19.01	0.3589

The results were expressed in mean and standard error.

Table 2.
Cytokine concentrations in dogs noninfected and dogs with canine visceral leishmaniasis.

Group	Control	CVL	p-Value
IgG	636.94 ± 255.52	2288.04 ± 610.08	<0.0001
IgM	241.12 ± 51.81	282.42 ± 33.99	0.0773

The results were expressed in mean and standard error.

Table 3.
Immunoglobulin concentrations (IgG and IgM) in serum from dogs with canine visceral leishmaniasis.

IgG		
Parameters	**rs**	**p-Value**
IL-4	0.5997	0.0040
TNF-α	0.4164	0.0603
Parasitic index	−0.2243	0.3282

rs, correlation coefficient of Spearman.

Table 4.
Correlation between IgG concentrations with IL-4 and TNF-α and parasitic index of dogs infected with CVL.

Stage	I and II		I and III		II and III	
	rs	**p**	**rs**	**p**	**rs**	**p**
IL-6	0.6031	0.0855	0.8469	0.0162	0.5630	0.1144
TNF-α	0.0350	0.9288	0.3784	0.4026	0.0168	0.9658

rs, correlation coefficient of Spearman.

Table 5.
Correlation of IL-6 and TNF-α cytokine levels of dogs with canine visceral leishmaniasis by clinical staging of serum from dogs of the CVL group.

In this study, as shown in **Table 5**, the correlation of the evolution of clinical signs between the stages presented below was analyzed. There was a significant positive correlation of IL-6 cytokine levels between stage I and stage III.

4. Discussion

In this study the most dogs in the control group and CVL were mixed breed. The clinical symptoms of seropositive dogs (CVL) included lymphadenopathy, skin ulcers, onychogryphosis, ear ulceration, scaling, weight loss, and others. Dogs were classified into three clinical stages: stage I, mild disease; stage II, moderate disease; and stage III, severe disease. There was no statistical difference in the distribution between clinical stages and parasitic index. IL-6 and TNF-α concentrations increased in serum from infected dogs with a statistically significant difference between the clinical stages of CVL. Between the dogs with CVL and the control group, there was a statistical difference in the serum concentrations of cytokines IL-4 and TNF-α. IgG levels were elevated in the CVL group when compared to IgM levels. Antibody levels were positively correlated with IL-4 expression (rs = 0.5997; p = 0.0040). There was a significant positive correlation of IL-6 cytokine levels between stage I and stage III.

The clinical signs of CVL are important for the diagnosis. In the present study, the most prevalent clinical signs were lymphadenopathy, skin ulcers, onychogryphosis, ear ulceration, and scaling. However, prevalence is highly variable across

studies, but generally these clinical signs are the most commonly reported in the literature. These results corroborate the findings of several authors [25, 26].

Regarding gender, there was a greater predominance of males in infected dogs and females in dogs in the control group. Regarding age, it did not present large variations. This fact seems to be associated with the higher risk of male exposure. However, the study shows no statistically significant differences for age and gender between healthy and sick dogs [27].

Bone marrow samples were taken from 21 dogs serologically positive for *L. infantum*. According to the clinical signs, dogs were classified as stages I, II, and III. Real-time PCR detected no parasite copies ($\times 10^{10}$)/μl *L. infantum* DNA in all animals of the CVL group, distributed as follows: stage I mean (4.964), stage II average (4.63), and stage III (4.55). No statistically significant difference was found in the average amount of DNA copy number between the different clinical stages ($p = 0.9467$). In bone marrow samples from dogs that are cytologically positive, a high parasitic index is detected [21].

Previous studies report that quantitative PCR on bone marrow samples from positive dogs in conventional tests contained a higher number of *Leishmania* kDNA copies than peripheral blood, although no significant differences were detected between symptomatic and asymptomatic dogs in terms of parasite load [28]. This literary quote converges with the findings of this study.

PCR can be used for detection of *Leishmania* in naturally infected dog samples, and PCR-RFLP (restriction fragment length polymorphism) is sensitive for identification of *Leishmania* species [28]. In addition, qPCR is effective in quantifying *Leishmania* DNA loading in clinical samples [29]. The blood sample from dogs infected by *L. infantum* was found by real-time PCR to have a sensitivity of 100% and specificity of 96.4% [30].

Most cytokines remain partially conserved between species; in this sense, the amino acid sequence of humans and canine cytokines shows 49–96% homology, suggesting a high probability of cross-reactivity between monoclonal antibodies; thus antibodies against human cytokines may be recommended as immunological biomarkers under pathological conditions by flow cytometry in human [31] and dogs [32] as used in this study.

In the present work, the serum concentration of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17) was compared between the control groups and the group with CVL. In addition, cytokine levels were compared within the CVL group with clinical staging I, II, and III. When comparing the groups, IL-4 and TNF-α were higher in infected dogs than in the control group, showing significant difference between IL-4 ($p = 0.0469$) and TNF-α ($p = 0.0009$) groups. In the group with CVL there were differences between stages I and III with significant differences only for cytokines IL-6 ($p = 0.0350$) and TNF-α ($p = 0.0462$).

Elevated levels of IL-6 were found in serum from dogs with active leishmaniasis compared to healthy dogs [33]. These results corroborate the findings of this study. However, other authors reported that IL-6 production did not vary significantly between the groups studied [34]. On the other hand were described in the literature that elevated levels of IL-6 in dogs without clinical signs or symptoms in CVL dogs [35], and also highlights that, among other factors, it may indicate a balance between the parasite elimination effort and the active disease. Increased IL-6 levels suggest a restricted ability to control infection [36]. Even in the absence of clinical signs or symptoms, the animals showed granulomas on histopathological evaluation, suggesting chronicity and therefore a longtime course of infection [35]. Innate immune effector cells primarily neutrophils, monocytes, and macrophages produce and respond to IL-6, which may result in amplification of inflammation and a change from an acute inflammatory state to a chronic state [37].

IL-6 expression increases in dogs with active visceral leishmaniasis and may be a useful marker for active disease [33, 35]. Increased IL-6 production is not directly related to anti-*Leishmania* antibody titers, suggesting that other cytokines may be involved with hypergammaglobulinemia [33].

As shown in this work, it was observed that there was correlation of IL-6 expression between stages I and III of bone marrow aspirate of dogs infected with CVL. IL-6 production in dogs with active leishmaniasis appears to be associated with severe disease [33]. This statement converges with the findings in this study, as the dogs used in the control group were mostly stage II and III. IL-6 is essential for terminal B-cell differentiation and immunoglobulin production [38].

TNF-α concentration was higher in infected dogs than in the control group, as detected by de Lima et al. [33]. CVL susceptibility is closely associated with downregulation of key cytokines such as IFN-γ, TNF-α, and IL-17A, thus impairing iNOS activation and NO production and favoring parasite replication and disease development [39].

The increased activity of TNF-α in the liver of infected dogs compared to healthy canines has been reported [37, 40]. Higher TNF-α levels in infected dogs indicate that the presence of *L. infantum* induces an immune response with relevant TNF-α expression when the protozoan is present [40].

Studies suggest that decreased survival of L. *infantum* in canine macrophages is associated with increased TNF-α and IFN-γ production and decreased IL-10 production [41].

In dogs naturally infected with *L. infantum*, increased hepatic TNF-α may be associated with increased parasite load on this organ [42]. The cytokines IL-2, IL-4, IL-10, IFN-γ, TNF-α, and IL-12 may be used as markers in epidemiological studies conducted in endemic areas to distinguish between different clinical forms of VL [15]. However, Lima et al. [33] indicate that TNF-α is not considered a good marker of active disease in dogs with VL.

A study has reported a significant relationship between bone marrow IL-4 detection in naturally infected dogs with and without clinical signs and disease severity, suggesting that IL-4 production is associated with pathology [43]. Increased expression of IL-4 cytokine is associated with both severe clinical signs and a high parasitic index on skin lesions [44]. In bone marrow aspirates, IL-4 was elevated in naturally infected dogs with more severe symptoms [43].

The study points to evidence that IL-4 cytokine polymorphism may contribute to innate immunity to *L. infantum* infection [45].

Antibody levels were positively correlated with IL-4 expression ($rs = 0.5997$; $p = 0.0040$). IgG is also linked to chronic infection in patients with VL, where high levels of IgG are predictive of the disease. This finding is in line with the study by Lima et al. [33] suggesting that other cytokines, such as IL-10 or IL-4, may be associated with hypergammaglobulinemia observed in dogs with CVL. Previous studies have detected increased serum IgG levels in symptomatic dogs compared with healthy dogs and are related to pathophysiological disorders and active disease [33].

Response to natural infection of *L. infantum* is linked to the presence of IgG [43] and *Leishmania*-specific IgM antibodies that can be detected in infected dogs [46]. Some studies have reported that increased total protein is frequent in dogs infected with visceral leishmaniasis due to increased antibody production [47, 48].

5. Conclusion

These results may contribute to a better understanding of the immune response in dogs infected with *L. infantum*. Antibody levels were positively correlated with

IL-4 expression. There was a significant positive correlation of IL-6 cytokine levels with the evolution of stages I and III. However, this cytokine can be used as a marker to distinguish between different clinical stages.

Acknowledgements

This research received grants from the Mato Grosso Research Support Foundation (FAPEMAT No. 299032/2010) and from the National Council for Scientific and Technological Development (CNPq No. 447218/2014–0 and No. 305725/2018–1), in Brazil.

Conflict of interest

The authors declare that there is no conflict of interest and nonfinancial competitors.

Author details

José Nivaldo da Silva[1], Valéria Régia Franco Sousa[1], Arleana do Bom Parto Ferreira de Almeida[1], Adenilda Cristina Honorio-França[2] and Eduardo Luzía França[2*]

1 Faculty of Veterinary Medicine, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil

2 Institute of Biological and Health Sciences, Federal University of Mato Grosso, Barra do Garças, Mato Grosso, Brazil

*Address all correspondence to: elfranca@ufmt.br

References

[1] Ferreira GE, dos Santos BN, Dorval ME, Ramos TP, Porrozzi R, Peixoto AA, et al. The genetic structure of *Leishmania infantum* populations in Brazil and its possible association with the transmission cycle of visceral leishmaniasis. PLoS One. 2012;**7**:e36242

[2] Saporito L, Giammanco GM, De Grazia S, Colomba C. Visceral leishmaniasis: Host-parasite interactions and clinical presentation in the immunocompetent and in the immunocompromised host. International Journal of Infectious Diseases. 2013;**17**:e572-e576

[3] Missawa NA, Lima GB. Spatial distribution of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) and *Lutzomyia cruzi* (Mangabeira, 1938) in the state of Mato Grosso. Revista da Sociedade Brasileira de Medicina Tropical. 2006;**39**:337-340

[4] Missawa NA, Veloso MA, Maciel GB, Michalsky EM, Dias ES. Evidence of transmission of visceral leishmaniasis by *Lutzomyia cruzi* in the municipality of Jaciara, state of Mato Grosso, Brazil. Revista da Sociedade Brasileira de Medicina Tropical. 2011;**44**:76-78

[5] World Health Organization. 2019. Available from: http://www.who.ch [Accessed: November 18, 2019]

[6] Feitosa MM, Day MJ. Current status and management of canine leishmaniasis in Latin America. Research in Veterinary Science. 2019;**123**:261-272

[7] Carvalho AG, Luz JGG, Rodrigues LD, Dias JVL, Fontes CJF. Factors associated with *Leishmania* spp. infection in domestic dogs from an emerging area of high endemicity for visceral leishmaniasis in Central-Western Brazil. Research in Veterinary Science. 2019;**125**:205-211

[8] Reis AB, Martins-Filho OA, Teixeira-Carvalho A, Giunchetti RC, Carneiro CM, Mayrink W, et al. Systemic and compartmentalized immune response in canine visceral leishmaniasis. Veterinary Immunology and Immunopathology. 2009;**128**:87-95

[9] Travi BL, Cordeiro-da-Silva A, Dantas-Torres F, Miró G. Canine visceral leishmaniasis: Diagnosis and management of the reservoir living among us. PLoS Neglected Tropical Diseases. 2018;**12**:e0006082

[10] Reis AB, Teixeira-Carvalho A, Giunchetti RC, Guerra LL, Carvalho MG, Mayrink W, et al. Phenotypic features of circulating leucocytes as immunological markers for clinical status and bone marrow parasite density in dogs naturally infected by *Leishmania chagasi*. Clinical and Experimental Immunology. 2006;**146**:303-311

[11] Freitas JC, Nunes-Pinheiro DC, Lopes Neto BE, Santos GJ, Abreu CR, Braga RR, et al. Clinical and laboratory alterations in dogs naturally infected by *Leishmania chagasi*. Revista da Sociedade Brasileira de Medicina Tropical. 2012;**45**:24-29

[12] Nascimento MSL, Albuquerque TDR, Do-Valle-Matta MA, Caldas IS, Diniz LF, Talvani A, et al. Naturally *Leishmania infantum*-infected dogs display an overall impairment of chemokine and chemokine receptor expression during visceral leishmaniasis. Veterinary Immunology and Immunopathology. 2013;**153**:202-208

[13] Silva KL, de Andrade MM, Melo LM, Perosso J, Vasconcelos RO, Munari DP, et al. CD4+FOXP3+ cells produce IL-10 in the spleens of dogs with visceral leishmaniasis. Veterinary Parasitology. 2014;**202**:313-318

[14] Rogers KA, DeKrey GK, Mbow ML, Gillespie RD, Brodskyn CI, Titus RG. Type 1 and type 2 responses to *leishmania major*. FEMS Microbiology Letters. 2002;**209**:1-7

[15] Costa ASA, Costa GC, Aquino DMC, Mendonça VRR, Barral A, Barral-Netto M, et al. Cytokines and visceral leishmaniasis: A comparison of plasma cytokine profiles between the clinical forms of visceral leishmaniasis. Memórias do Instituto Oswaldo Cruz. 2012;**107**:735-739

[16] Ferrer L, Solano-Gallego L, Arboix M, Alberola J. Evaluation of the specific immune response in dogs infected by *Leishmania infantum*. Blackwell Science. 2002:92-99

[17] Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L. Canine leishmaniosis—New concepts and insights on an expanding zoonosis: Part one. Trends in Parasitology. 2008;**24**:324-330

[18] Dantas-Torres F, Solano-Gallego L, Baneth G, Ribeiro VM, de Paiva-Cavalcanti M, Otranto D. Canine leishmaniosis in the old and new worlds: Unveiled similarities and differences. Trends in Parasitology. 2012;**28**:531-538

[19] MS. Secretaria de Vigilância em Saúde. Departamento de Análise de Situação de Saúde.Saúde Brasil 2011: uma análise da situação de saúde e a vigilância da saúde da mulher/Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Análise de Situação de Saúde. – Brasília: 444 p. il. Editora do Ministério da Saúde;2012

[20] Maia C, Campino L. Methods for diagnosis of canine leishmaniasis and immune response to infection. Veterinary Parasitology. 2008;**158**:274-287

[21] Ramos RA, Ramos CA, Santos EM, de Araújo FR, de Carvalho GA, Faustino MA, et al. Quantification of *Leishmania infantum* DNA in the bone marrow, lymph node and spleen of dogs. Revista Brasileira de Parasitologia Veterinária. 2013;**22**:346-350

[22] Paiva-Cavalcanti M, de Morais RC, Pessoa-E-Silva R, Trajano-Silva LA, Gonçalves-de-Albuquerque SAC, Tavares DEH, et al. Leishmaniases diagnosis: An update on the use of immunological and molecular tools. Cell and Bioscience. 2015;**5**:31

[23] Solano-Gallego L, Koutinas A, Miró G, Cardoso L, Pennisi MG, Ferrer L, et al. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. Veterinary Parasitology. 2009;**165**:1-18

[24] Lachaud L, Marchergui-Hammami S, Chabbert E, Dereure J, Dedet JP, Bastien P. Comparison of six PCR methods using peripheral blood for detection of canine visceral leishmaniasis. Journal of Clinical Microbiology. 2002;**40**:210-215

[25] Noli C, Saridomichelakis MN. An update on the diagnosis and treatment of canine leishmaniosis caused by *Leishmania infantum* (syn. *L. chagasi*). Veterinary Journal. 2014;**202**:425-435

[26] Silva KR, Mendonça VR, Silva KM, Nascimento LF, Mendes-Sousa AF, Pinho FA, et al. Scoring clinical signs can help diagnose canine visceral leishmaniasis in a highly endemic area in Brazil. Memórias do Instituto Oswaldo Cruz. 2017;**112**:53-63

[27] Meléndez-Lazo A, Ordeix L, Planellas M, Pastor J, Solano-Gallego L. Clinicopathological findings in sick dogs naturally infected with *Leishmania infantum*: Comparison of five different clinical classification systems. Research in Veterinary Science. 2018;**117**:18-27

[28] Quaresma PF, Murta SMF, de Castro Ferreira E, da Rocha ACVM,

Xavier AAP, Gontijo CMF. Molecular diagnosis of canine visceral leishmaniasis: Identification of *Leishmania* species by PCR-RFLP and quantification of parasite DNA by real-time PCR. Acta Tropica. 2009;**111**:289-294

[29] Morais RCS, Costa Oliveira CN, Albuquerque SDCG, Silva LAMT, Pessoa-Silva R, Cruz HLA, et al. Real-time PCR for *Leishmania* species identification: Evaluation and comparison with classical techniques. Experimental Parasitology. 2016;**165**:43-50

[30] Mohammadiha A, Mohebali M, Haghighi A, Mahdian R, Abadi AR, Zarei Z, et al. Comparison of real-time PCR and conventional PCR with two DNA targets for detection of *Leishmania (Leishmania) infantum* infection in human and dog blood samples. Experimental Parasitology. 2013;**133**:89-94

[31] Scherer EF, Cantarini DG, Siqueira R, Ribeiro EB, Braga É, Honório-França AC, et al. Cytokine modulation of human blood viscosity from vivax malaria patients. Acta Tropica. 2016;**158**:139-147

[32] Moreira ML, Dorneles EM, Soares RP, Magalhães CP, Costa-Pereira C, Lage AP, et al. Cross-reactivity of commercially available anti-human monoclonal antibodies with canine cytokines: Establishment of a reliable panel to detect the functional profile of peripheral blood lymphocytes by intracytoplasmic staining. Acta Veterinaria Scandinavica. 2015;**57**:51

[33] de Lima VM, Peiro JR, de Oliveira Vasconcelos R. IL-6 and TNF-alpha production during active canine visceral leishmaniasis. Veterinary Immunology and Immunopathology. 2007;**115**:189-193

[34] 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. 1994;**62**:229-235

[35] De Vasconcelos TC, Doyen N, Cavaillon JM, Bruno SF, de Campos MP, de Miranda LH, et al. Cytokine and iNOS profiles in lymph nodes of dogs naturally infected with *Leishmania infantum* and their association with the parasitic DNA load and clinical and histopathological features. Veterinary Parasitology. 2016;**227**:8-14

[36] Solcà MS, Andrade BB, Abbehusen MM, Teixeira CR, Khouri R, Valenzuela JG, et al. Circulating biomarkers of immune activation, oxidative stress and inflammation characterize severe canine visceral leishmaniasis. Scientific Reports. 2016;**6**:32619

[37] Choy E, Rose-John S. Interleukin-6 as a multifunctional regulator: Inflammation, immune response, and fibrosis. Journal of Scleroderma and Related Disorders. 2017;**2**:S1-S5

[38] Le JM, Vilcek J. Interleukin 6: A multifunctional cytokine regulating immune reactions and the acute phase protein response. Laboratory Investigation. 1989;**61**:588-602

[39] Nascimento MS, Albuquerque TD, Nascimento AF, Caldas IS, Do-Valle-Matta MA, Souto JT, et al. Impairment of interleukin-17A expression in canine visceral leishmaniosis is correlated with reduced interferon-γ and inducible nitric oxide synthase expression. Journal of Comparative Pathology. 2015;**153**:197-205

[40] Michelin FA, Perri SH, De Lima VM. Evaluation of TNF-α, IL-4, and IL-10 and parasite density in spleen and liver of *L. chagasi* naturally infected

dogs. Annals of Tropical Medicine and Parasitology. 2011;**105**:373-383

[41] Turchetti AP, da Costa LF, Romão EEL, Fujiwara RT, da Paixão TA, Santos RL. Transcription of innate immunity genes and cytokine secretion by canine macrophages resistant or susceptible to intracellular survival of *Leishmania infantum*. Veterinary Immunology and Immunopathology. 2015;**163**:67-76

[42] Giunchetti RC, Mayrink W, Carneiro CM, Corrêa-Oliveira R, Martins-Filho OA, Marques MJ, et al. Histopathological and immuno-histochemical investigations of the hepatic compartment associated with parasitism and serum biochemical changes in canine visceral leishmaniasis. Research in Veterinary Science. 2008;**84**:269-277

[43] Quinnell RJ, Courtenay O, Shaw MA, Day MJ, Garcez LM, Dye C, et al. Tissue cytokine responses in canine visceral leishmaniasis. The Journal of Infectious Diseases. 2001;**183**:1421-1424

[44] Brachelente C, Müller N, Doherr MG, Sattler U, Welle M. Cutaneous leishmaniasis in naturally infected dogs is associated with a T helper-2-biased immune response. Veterinary Pathology. 2005;**42**:166-175

[45] Jeronimo SMB, Holst AKB, Jamieson SE, Francis R, Martins DRA, Bezerra FL, et al. Genes at human chromosome 5q31.1 regulate delayed-type hypersensitivity responses associated with *Leishmania chagasi* infection. Genetics of Immunity. 2007;**8**:539

[46] Rodríguez A, Solano-Gallego L, Ojeda A, Quintana J, Riera C, Gállego M, et al. Dynamics of *Leishmania*-specific immunoglobulin isotypes in dogs with clinical leishmaniasis before and after treatment. Journal of Veterinary Internal Medicine. 2006;**20**:495-498

[47] Ciaramella P, Oliva G, Luna RD, Gradoni L, Ambrosio R, Cortese L, et al. A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. The Veterinary Record. 1997;**141**:539-543

[48] Medeiros CMO, Melo AGC, Lima AKF, Silva ING, Oliveira LCO, Silva MC. Haematological profile of dogs with visceral leishmaniasis in the city of Fortaleza, Ceará. Ciência Animal. 2008;**18**:43-50