Interference Journal
ISSN 2009-3578
DOI
A2 QUALIS SUCUPIRA
2017-2020

Leishmaniasis research by parasitological lymph node examination, PCR and species characterization by RFLP-PCR in municipal shelter dogs previously submitted to immunochromatographic testing

Élida de Oliveira Ribeiro [1], Rodrigo Buzinaro Suzuki, [1] Caio Ferreira Repik[1] , Raquel Richter Nazari[2] , Márcia Aparecida Sperança[3], Isabela Bazzo da Costa[1]; Patricia Cincotto dos Santos Bueno[1], Rodrigo Prevedello Franco[1], Camila Dias Porto[1]

https://doi.org/10.36557/2009-3578.2025v11n2p2275-2298
Artigo recebido em 26 de Julho e publicado em 6 de Setembro de 2025

ARTIGO ORIGINAL

ABSTRACT

This study investigated *Leishmania* spp. infection in 153 dogs from a municipal shelter in an endemic area, previously tested as non-reactive by immunochromatography. Parasitological lymph node examination and PCR-RFLP directed to the chitinase gene were used for diagnosis and characterization of the species. The parasitological examination detected amastigotes in 3.3% of the dogs, while the PCR confirmed the infection in 1.3%, with high agreement between the methods (98%; Kappa=0.563; p<0.001). RFLP analysis identified the species as *Leishmania infantum chagasi*. The presence of clinical signs was not significantly associated with positive results. The results demonstrate the circulation of the parasite even in dogs with negative serological screening, evidencing the limitation of the isolated use of rapid tests and the need to adopt combined and more sensitive diagnostic protocols in shelters in endemic areas for the effective control of Canine Visceral Leishmaniasis.

Keywords: single health, epidemiological surveillance, chitinase gene, animal shelter.

Pesquisa de leishmaniose por exame parasitológico de linfonodos, PCR e caracterização de espécies por RFLP-PCR em cães de abrigos municipais previamente submetidos ao teste imunocromatográfico.

RESUMO

Este estudo investigou a infecção por *Leishmania* spp. em 153 cães de um abrigo municipal localizado em uma área endêmica, previamente testados como não reagentes por imunocromatografia. Foram utilizados o exame parasitológico de linfonodos e a PCR-RFLP direcionada ao gene da quitinase para diagnóstico e caracterização da espécie. O exame parasitológico detectou amastigotas em 3,3% dos cães, enquanto a PCR confirmou a infecção em 1,3%, com alta concordância entre os métodos (98%; Kappa=0,563; p<0,001). A análise por RFLP identificou a espécie como *Leishmania infantum chagasi*. A presença de sinais clínicos não esteve significativamente associada aos resultados positivos. Os resultados demonstram a circulação do parasito mesmo em cães com triagem sorológica negativa, evidenciando a limitação do uso isolado de testes rápidos e a necessidade de adoção de protocolos diagnósticos combinados e mais sensíveis em abrigos localizados em áreas endêmicas, para o controle eficaz da Leishmaniose Visceral Canina.

Palavras-chave: saúde única, vigilância epidemiológica, gene da quitinase, abrigo de animais.

Instituição afiliada –1- UNIVERSIDADE DE MARILIA - UNIMAR , 2-CENTRO UNIVERSITÁRIO DAS FACULDADES INTEGRADAS DE OURINHOS 3- UNIVERSIDADE FEDERAL DO ABC

Autor correspondente: Patricia Cincotto dos Santos Bueno -pcincotto@gmail.com

INTRODUCTION

The world population of domestic dogs is estimated at approximately 700 million individuals, comprising animals living with their owners and abandoned, living in shelters or freely in urban and rural environments. Of these, 75% roam freely, meaning they are unsupervised by a human (WOAH, 2023; SMITH et al., 2019).

Domestic dogs are dependent on people for food, directly or indirectly, and their presence and movement is closely linked to human actions. Thus, the size of the canine population is subject to human behavior, and consequently, the problems correlated to it (TAYLOR et al, 2017).

According to Smith et al (2019), loose dogs are associated with zoonoses, bites, and accidents in road traffic. In developing countries in Latin America, these dogs have inadequate veterinary care, representing a complex public health problem, in which they play the role of a potentially uncontrolled reservoir for zoonoses (GARDE et al, 2013).

It is important for municipalities to have a plan for the proper management of stray dogs, aiming to reduce the number of these animals. There are several models that are currently used, such as reproductive control, referral to shelters, euthanasia, and adoption and responsible ownership campaigns (SMITH et al., 2022).

Among the zoonoses of global prominence is leishmaniasis, with a high mortality rate in humans if left untreated (ASFARAM et al., 2022), which can lead to death in up to 90% of cases (KASZAK et al., 2015). Brazil stands out in the chain of transmission in the Americas, where it is seen as a neglected disease by the World Health Organization, predominating in poor and peripheral areas of the country (LEWGOY et. al., 2020).

Braz et al. (2021) cite that in Brazil the transmission of the *Leishmania* SPP occurs mainly through the blood meal of sandflies *Lutzomyia longipalpis*, being coyotes and foxes (*Dusicyon vetulus* and *Cerdocyon thous*) the most common wild reservoirs, and in the domestic environment dogs preserve the transmission cycle.

The infection in dogs usually presents in the visceral form, and can be classified into three distinct presentations: asymptomatic, in which the characteristic clinical signs of the disease are not observed, oligosymptomatic, when the clinical signs presented

are mild, and the symptomatic form, represented by more than three severe clinical signs (DADALTO, 2020). Clinical signs appear in variable proportions in symptomatic dogs, but most affected animals do not have clinical symptoms, making diagnosis difficult and facilitating the transmission of the disease (REGUERA et al., 2016).

In contrast to the historical characteristic of being considered a disease of rural areas, Nina et al. (2023) observed that between 2007 and 2020 there were large urban centers with a very intense risk for transmission of visceral leishmaniasis, evidencing the growth of the disease in urban areas.

As for the municipality of Marilia, located in the central-west region of the State of Sao Paulo, the city was classified as silent, receptive and vulnerable because it was close to other endemic municipalities until 2011. However, due to a case that affected a child in October 2011, the Ministry of Health changed its classification to municipality with transmission of the disease (PIRAJA, 2013). According to Pigozzi, Pigozzi Filho and Medeiros (2023), only in 2013 was the protozoan isolated in a canine reservoir and, according to the Epidemiological Classification of Municipalities and the Leishmaniasis Surveillance and Control Program in the State of Sao Paulo, in December 2014, the municipality of Marilia was classified as a municipality of moderate transmission and for the 2016-18 triennium as of intense transmission. Thus, by 2024, Marilia was classified as a municipality with canine and human transmission (CVE, 2025 A), being endemic for the disease.

Costa et al. (2021) mention that the diagnosis of canine visceral leishmaniasis (CVL) is complex, as clinical findings are common to other diseases, and laboratory abnormalities found in the blood count or biochemical tests are also nonspecific. They also point out that early detection of infected dogs is essential to prevent the expansion of the disease and control it.

Among the tests that can be used for the diagnosis of CVL are immunochromatographic tests, serological methods BRAZIL, 2014).

The Ministry of Health, through the Visceral Leishmaniasis Control Program (PCLV), has instituted diagnostic methods, such as the Dual-path Platform chromatographic immunoassay (rapid TR-DPP Biomanguinhos test, Rio de Janeiro, Brazil), which works as a screening, and the ELISA being confirmatory. However, they have some limitations, especially in the diagnosis of asymptomatic dogs (AYRES et. al.,

2022; DIAS et. al., 2021; PEIXOTO et al, 2014; Funed, 2010). However, in addition to the detection of vaccine antibodies, serological techniques can result in nonspecific reactions with other parasitic diseases, as cited by Alves and Bevilacqua (2004).

The parasitological test is one of the most used by veterinarians for the diagnosis of CVL. Silva (2009) mentions that intracellular forms of *Leishmania spp* can be identified in different preparations, such as skin, spleen, liver, lymph node prints, and blood and bone marrow smears, although there is a great possibility of false-negative results, as the number of parasites can be low, especially in asymptomatic animals. Thus, Laurenti (2009) reports that the sensitivity of this test depends on the degree of parasitism, the type of biological material collected, its processing and coloration, in addition to the observer, and can be from 50 to 83% in bone marrow samples, between 30 and 85% in lymph node samples and between 71 and 91% when both tissues are combined.

Polymerase Chain Reaction (PCR) consists of the identification of the genetic material of the parasite, and small amounts of material obtained from various types of biological samples can be used, producing a reliable result, fast, at low cost, showing high sensitivity and specificity (DANTAS-TORRES et al, 2017).

In order for control measures to be effective, the Ministry of Health recommends that actions be differentiated in each region, according to its epidemiological situation, and priority should be given to areas with the most serious epidemiological situation (BRASIL, 2014).

The contact of pets with their owners becomes more frequent and closer every day. According to Belchior and Dias (2019), the concept of "multispecies family" constitutes a new social affective reality. Due to the tightening of ties, the coexistence between humans and animals has become more relevant to public health, raising concerns about the transmission of zoonotic diseases (CHOMEL & SUN, 2011), such as leishmaniasis.

Considering that the dog infected with *Leishmania* is an important risk factor for the occurrence of human VL, and that the immunochromatographic test can often be the only diagnostic resource in shelters due to the cost of the tests, the objective of this work is to investigate with the RFLP-PCR technique the presence of the parasite in dogs in a shelter, rescued from abandonment, and compare it with the parasitological test, as well as characterize the *Leishmania species*.

MATERIAL AND METHODS

The project was approved by the Ethics Committee on Animal Use, protocol 02/2022.

Male and female, adult dogs of all breeds were used. The animals came from a shelter for animals rescued from abandonment, located in the city of Marilia, State of Sao Paulo.

The clinical signs evaluated for symptomatic characterization of the animals are described in Chart 1.

Table 1. Clinical signs evaluated for symptomatic characterization of rescued dogs to be tested for *Leishmania spp.*

Alopecia	Thoracolumbar or limb skin ulcerations
Skin peeling	Low body score
Hyperkeratosis	Cachexia
Onychogryphosis	Paw edema
Ear, muzzle, or periocular ulcers	Enlarged lymph nodes

For direct parasitological examination, fine-needle lymph node capillarity puncture was performed. By default, material was collected from the popliteal lymph node, and in small dogs, submandibular lymph nodes. This was followed by the smear on a slide, dried in the open air, fixation in methanol and staining with Giemsa. The slides were evaluated under light microscopy at 1000x magnification with the aid of immersion oil to identify amastigotes.

The molecular diagnosis was performed from the collection of 3.0 mL of whole blood, and the preferred route for collection was the cephalic vein. The jugular vein was used in low weight or dehydrated animals. The blood was stored in a sterile tube with EDTA k3, kept refrigerated at 4ºC in a thermal box. From the centrigugation at 4000 rpm for 10 minutes, red series and plasma were stored separately in microtubes and frozen at -20ºC until the moment of PCR.

Genomic DNA was extracted using the commercial PureLink™ Genomic DNA Mini

Kit (Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions. DNA quantification was verified by electrophoresis on 1% agarose gel stained with ethidium bromide, determined by comparing the intensity of the band with the intensity of the Low DNA Mass Ladder molecular mass pattern (Thermo Fischer Scientific, USA) following the manufacturer's instructions.

The diagnostic method for detecting *Leishmania* spp was performed as described by JORDÃO et al (2021).

The oligonucleotides used in this method are Lquit224F (5' GTTCMACTACGAGGCCTTCTTCAA 3') and Lquit1182R (5'CAGATCATTATCCCAGACAAGTT 3'). The PCR reaction was performed with the GoTaq® DNA polymerase enzyme (PROMEGA Corporation, Wisconsin, USA). PCR amplification resulted in a 953 bp product corresponding to the chitinase gene and was analyzed by electrophoresis in 1% agarose gel, stained with ethidium bromide, and observed in an ultraviolet light transilluminator.

For the diagnosis of *Leishmania spp* from the samples collected, samples of the parasite isolated in culture, provided by the Parasitology Laboratory of the Faculty of Medicine of Marilia, were used as a positive standard.

After molecular diagnosis, the amplified product corresponding to the chitinase gene of *Leishmania* spp was submitted to fragment size restriction analysis (RFLP) using PstI endonuclease (New England biolabs Inc., Ipswich, MA, USA) according to the manufacturer's instructions. The fragments obtained were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and observed in ultraviolet light transilluminator.

The sizes of the fragments obtained after digestion with PstI were 390 bp, 258 bp, 192 bp, and 113 bp, which corresponds to the specific restriction pattern of *the L. infantum chagasi species*, according to the method described by Jordão et al. (2021).

Qualitative variables were presented as absolute and relative frequency distributions. The Kappa test was applied for the association between the parasitological test and clinical signs, and also to evaluate the agreement between the diagnostic methods (GAMER, JIM LEMON & SINGH, 2019). The association between symptomatic dogs and parasitological test results was performed by the chi-square test (R Core Team, 2023). The tests applied are recommended by ARANGO (2005). Statistical analyseswere

performed using the Jamovi software (version2.5; THE JAMOVI PROJECT, 2024).

RESULTS

All dogs in the shelter, per the company's protocol, were tested by immunochromatography for leishmaniasis, and the reagents were euthanized.

Only animals that presented paired parasitological and molecular examinations were considered for the results, totaling 153 animals. Of these, 27 were considered symptomatic. The results of the distribution of dogs according to sex and presentation of clinical signs are shown in Figure 1.

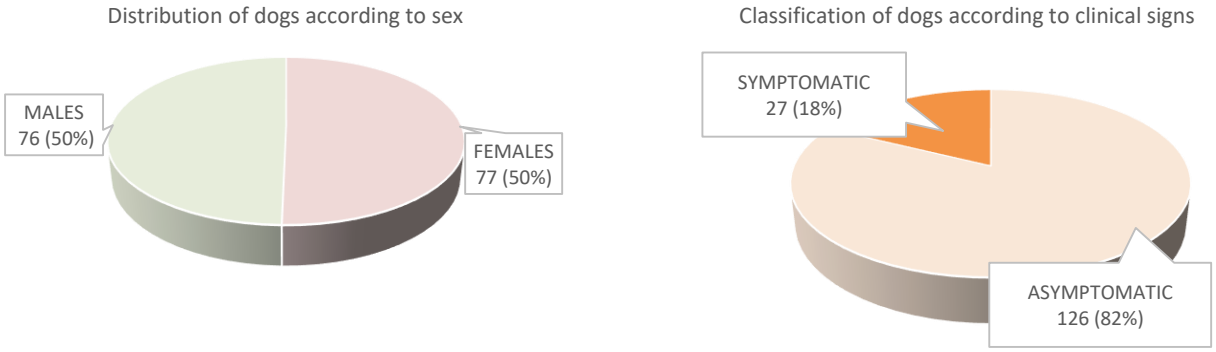

Figure 1. Distribution of sheltered dogs according to sex and presentation of clinical signs.

Among the clinical signs presented in the 27 symptomatic animals, the most observed were alopecia, onychogryphosis and ear ulcers. All animals were considered oligosymptomatic, and the majority (20; 74%) manifested only one clinical sign. These data are represented in Figures 2 and 3.

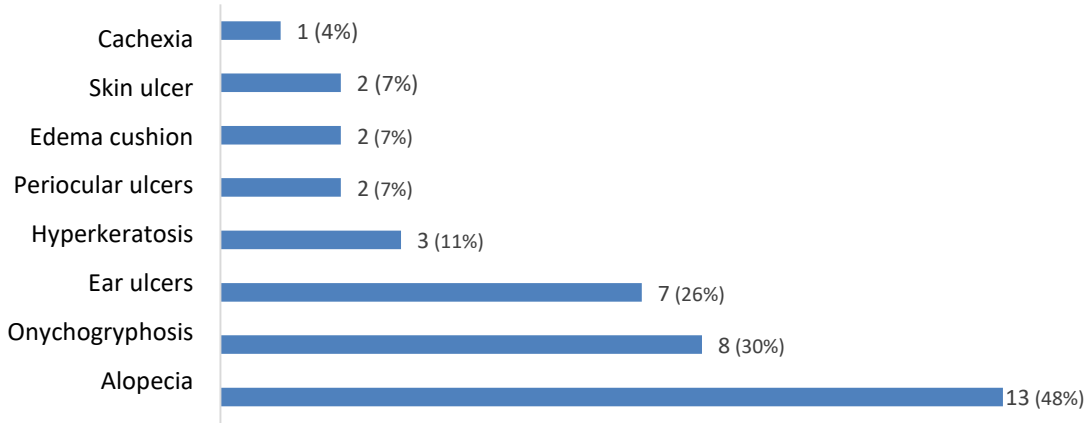

Figure 2. Distribution of sheltered dogs according to the clinical signs observed.

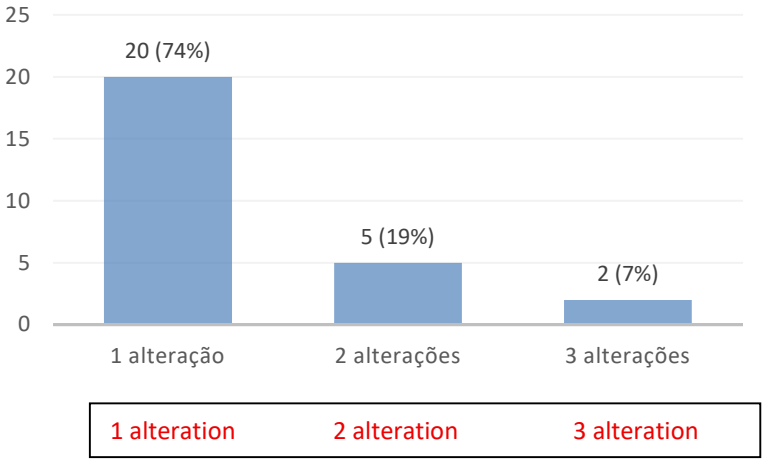

Figure 3. Distribution of sheltered dogs according to the number of clinical signs presented.

Of the 153 lymph node samples, five animals (3%) had amastigotes (Figure 5). Among the positive dogs, 40% were classified as symptomatic. These data are shown in

Figures 6 and 7.

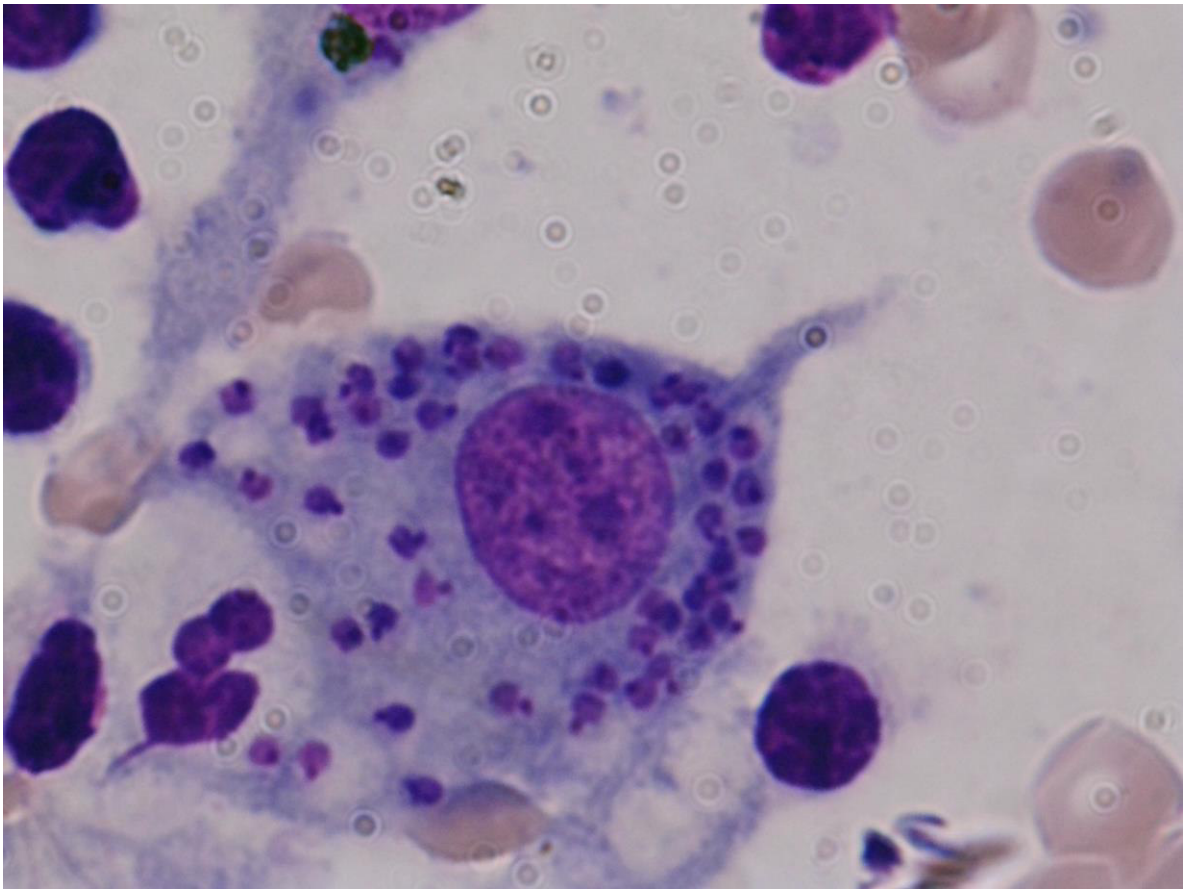

Figure 5. Lymph node. Amastigotes are observed in macrophages. Giemsa. 100x objective.

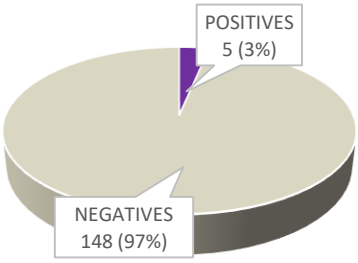

Figure 6. Distribution of sheltered dogs according to the parasitological test.

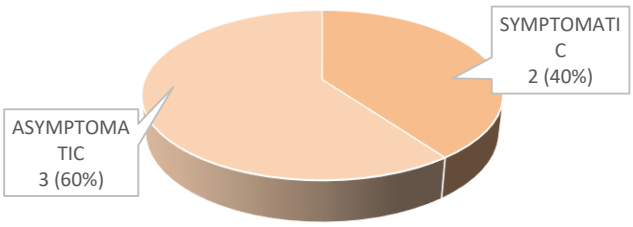

Figure 7. Distribution of sheltered dogs with *Leishmania spp*, according to the presence of clinical signs.

Among the animals in the study, only two were reactive for PCR, as shown in Figure 8. The reagent tests and standardization of the technique for characterization of the *Leishmania* species by RFLP obtained from the PCR reaction with PstI endonuclease showed a restriction pattern that characterizes the *Leishmania infantum chagasi species.* These results are shown in Figure 9.

All animals reactive in the PCR proved to be positive in the parasitological test.

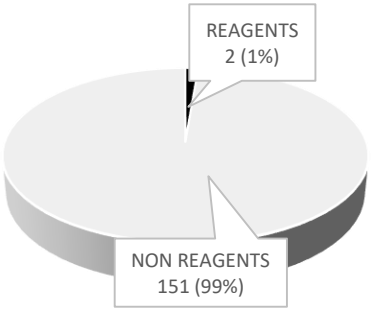

Figure 8. Distribution of sheltered dogs according to the PCR test.

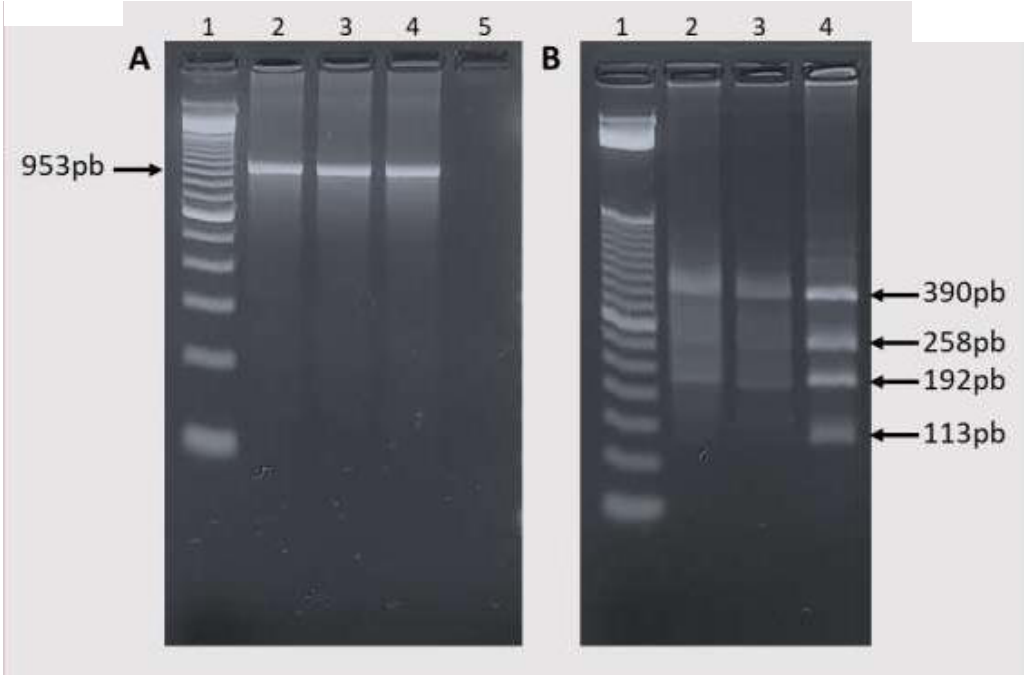

Figure 9. Reaction for the diagnosis of *Leishmania spp* by the PCR technique and species characterization by the PCR-RFLP technique. In A, amplification analysis of a 953 bp fragment, corresponding to the chitinase gene. 1 – 1kb DNA Ladder Marker (Invitrogen, Thermo Fisher Scientific, US); 2 to 5 – PCR reaction with 3µl of DNA positive for Leishmania ssp, and 5 – Negative control. In B, analysis of the method of characterization of species with digestion of the product of 953 obtained from the PCR reaction. 1 – 50bp DNA Ladder Marker (Invitrogen, Thermo Fisher Scientific, US); 2 – Digestion of 3µl of the PCR product; 3 – Digestion of 4µl of the PCR product; and 4 – Digestion of 3µl of the PCR product, showing the restriction pattern that characterizes the species *Leishmania infantum chagasi*.

STATISTICS

The Kappa test showed an agreement of 81.7% between the animals positive in the parasitological examination and the presence of one or more clinical signs. However, the relationship between the presence of clinical signs and positive CVL test by parasitological examination was not significant (p = 0.650). These results are shown in Table 1.

Table 1. Testing of association between parasitological examination (standard) and

presentation of clinical signs of dogs sheltered by the Kappa method.

	N	Raters	Kappa	Z	p
Light's Kappa	153	2	0.0739	0.454	0.650

Simple agreement percentage.

	Subjects	Raters	Agreement(%)
Value	153	2	81.7

The Kappa test showed an agreement of 98.0%, and the p-value <0.001 (highly significant) indicates that there is a proven Good Agreement between the PCR and the parasitological test. These results are shown in Table 2.

Table 2. Test of agreement between parasitological examination (standard) and PCR by the Kappa test of sheltered dogs.

Method	Kappa coefficient
Agreement % (Trust)	98
Kappa	0.563 (0 to 1, the closer to 1, the greater the agreement between the tests)
p-value	<.001 (Values less than 0.05 indicate significance of 95% and values less than 0.01 indicate significance at the level of 99%

As for symptomatic animals, there was no relationship between dogs positive for leishmaniasis in cytology and the presentation of clinical signs (Table 3).

Table 3. x2 association test between symptomatic animals and parasitological examination of sheltered dogs.

Contingency Tables

	Symptomatology		
Parasitological	**ASYMPTOMATIC**	**SYMPTOMATIC**	**Total**
NEGATIVE	123	25	148
POSITIVE	3	2	5
Total	126	27	153

χ^2 Tests

	Value	GI	p
χ^2	1.78	1	**0.183** (not significant)
N	153		

DISCUSSION

According to the report of the Sao Paulo State Health Department, Marilia is among the municipalities where leishmaniasis has canine and human transmission (CVE, 2025 A). Leishmaniasis is a vector-borne disease, and vector control is a major challenge for public health, mainly due to the characteristics of the sandfly *Lutzomyia* spp, the environmental and social factors that favor its proliferation(PAHO, 2023). Thus, the dogs in the shelter have an increased risk of transmitting and contracting leishmaniasis, since many have possibly already arrived sick and/or immunocompromised in an environment with high population density and limited space, in addition to the presence of the vector in the endemic municipality. It is noteworthy that Marilia had 39 cases of visceral leishmaniasis in humans from 2017 to July 2025, with three cases of death in this period (CVE, 2025 B)

Although the shelter studied screened for canine visceral leishmaniasis (CVL) and housed mostly non-reactive dogs, the identification of individuals presenting clinical signs strongly associated with the disease – such as cachexia (4%) and characteristic dermatological alterations, such as alopecia (48%), onychogryphosis (8%), ear ulcers (26%), hyperkeratosis (11%) and periocular ulcers (7%) – introduces an element of

complexity. This finding suggests the potential presence of false-negatives in the initial screening, infections acquired within the shelter itself after screening, or the late manifestation of signs in previously infected animals with low parasite load and slow seroconversion, requiring a more in-depth diagnostic investigation in these specific cases. Even though it can be stated that the presence of characteristic clinical signs is not a reliable indicator of infection, since 25 of the 27 symptomatic dogs were negative in the parasitological examination, it is noteworthy that of the total number of animals evaluated, 80% were asymptomatic, and without adequate diagnostic procedures they represent an epidemiological risk, as they can be silent reservoirs. In this study, the lack of a relationship between the presence of clinical signs and animals diagnosed by the tests used may be related to the euthanasia of animals reactive by the rapid test.

Thus, the needto use combined methods for the diagnosis of leishmaniasis in dogs was confirmed, since immunochromatography has limitationsin relation to other techniques, including serological ones (GUZMAN et al., 2023; SILVA et al., 2021; DANTAS-TORRES, 2018).

In the meantime, we performed two diagnostic techniques for comparison in this population: lymph node cytology, as a parasitological technique, and PCR, since the isolated use of immunochromatography excluded false negatives, but dispensed with diagnostic confirmationin reagent animals. The procedure performed by the shelter indicates that the scarcity of financial resources is a limiting factor for the adoption of an appropriate diagnostic protocol, directly impacting the strategies that veterinarians will practice for disease control. Most shelters do not have regular government funding, and they also have financial support from donationsfrom individuals and companies.

The chitinase gene was chosen in this research as a molecular target for the diagnosis of *Leishmania* spp by PCR because it is exclusive to the Leishmania *gene*, not being found in other trypanosomides, which significantly increases the specificity of the test and minimizes cross-reactions with other agents (CABRAL, et al., 2020; SUZUKI et al., 2016). In addition, the methodology employed – the combination of the PCR reaction for amplification of the specific fragment and the RFLP (Restriction Fragment Length Polymorphism-PCR) technique for the typification of thespecies – allows not only to detect the presence of the parasite, but also to accurately identify the species *Leishmania infantum chagasi* (PRADELLA et al., 2022; JORDÃO et al., 2021). This

differentiation is crucial to guide therapeutic decisions, such as clinical management or the indication of euthanasia in dogs, considering the epidemiological and public health implications. The set of procedures – from the extraction and verification of DNA, through the standardization of the PCR protocol with specific primers, to the analysis of the patterns of fragments generated by enzymatic digestion – proved that the proposed methodology is effective and accurate to diagnose *Leishmania infection* and identify the species involved. This is a relatively simple, fast, and affordable technique, which makes it suitable for epidemiological studies and for the laboratory diagnosis of leishmaniasis.

Moreira et al. (2007), comparing the parasitological test with serological test and genetic identification of *Leishmania* in dogs, concluded that the cytological examination of the popliteal lymph node was more sensitive in symptomatic animals, and that in this material the PCR was highly sensitive and effective for the diagnosis of positives. In the present study, we can state that the high percentage of agreement (98%) reinforced the reliability of PCR in confirming positive cases. However, discordance was also observedin non-reactive animals in PCR and a positive parasitological test, although without significant difference. Thus, even though it is a reliable method, these results suggest that PCR should not replace other methodologies. The type of tissue used to perform this technique may have influenced the result, since previous studies (DANTAS-TORRES et al, 2017) indicate that the parasite load in the peripheral blood of patients with visceral leishmaniasis can be variable and often low, and is not the material of choice for this diagnosis. Furthermore, by obtaining 100% specificity, it was corroborated that PCR did not generate false positives.

Due to parasite tropism by tissue macrophages, especially in the bone marrow , spleen, and lymph nodes in visceral leishmaniasis, comparative studies often demonstrate substantially higher parasite loads in these tissues than in circulating blood (FATTAHI-BAFGHI et al, 2020; MARTÍNEZ-RONDÁN et al, 2018; MEMBRILLO et al., 2015). This characteristic of infection impacts the sensitivity of diagnostic methods based on blood samples, particularly those less sensitive than qPCR. Therefore, variability and often low parasite load in peripheral blood may limit the diagnostic efficacy of methods based on blood samples. This underscores the importance of considering other sample sources, such as lymph node aspirates or bone marrow , which may have higher sensitivity due to the higher concentration of parasites in these tissues.

Even though there was no significant difference between the parasitological and PCR results, this study is in line with the data already present in the literature. However, even today, many laboratories offer PCR of blood samples as a diagnosis, which may need to be reevaluated by veterinarians.

From the results obtained, it is evident that the use of the rapid test alone may not be sufficient for the conclusive diagnosis of CVL, especially considering its limited sensitivity in asymptomatic dogs or those with initial infection. As pointed out in the study by Costa (2024) and Estevam et al. (2022), our data reinforced the need for a combined diagnostic approach, using PCR-RFLP for confirmation and differentiation of the species involved. Also, the use of RFLP can characterize a geographic region according to the species that affects dogs and humans. In this way, the study of georeferencing can assist in the implementation of more effective and targeted interventions by health authorities (COSTA, 2024; FRANCO et al, 2013; BARATA et al., 2013; FERROGLIO et al., 2006). The city of Marilia is an endemic area, and the detection of the species in dogs reinforces the need for integrated surveillance.

This study evaluated only dogs that were not reactive in immunochromatography for LCV, since the reagents had been euthanized according to the health policyof the establishment. This restriction prevented the analysis of the agreement between serological, parasitological and molecular methods in seropositive animals and may have underestimated theactual prevalence of infection. In addition, it limits the direct comparison with studies that included both groups, such as that of SILVA (2009). Even so, the detection of parasites in the cytological and PCR positive tests in dogs that did not reactive to the rapid test evidenced infections that were not identified serologically and reinforcedthe need to associate different methods with serological screening to improve diagnostic sensitivity.

The policy of compulsory euthanasia of dogs positive for visceral leishmaniasis, although historically adopted in Brazil (BRASIL, 2014), faces increasing technical and ethical criticism. The persistence of this measure reflects structural flaws in public policies: the outdated norms in the face of scientific evidence, the underuse of health education, and the negligence in the environmental control of the vector. As Costa et al. (2024) conclude, an integrated approach – combining epidemiological surveillance, vector prevention, animal treatment, and community engagement – is not only more

ethical, but also more effective in the sustainable control of leishmaniasis.

CONCLUSION

Leishmaniasis can be considered a major challenge for health professionals in both the human and veterinary areas and constitutes a serious problem in single health. There is a difficulty in diagnosing the disease, especially due to the large number of tests available, none of which are fully sensitive. It can be said that the most reliable diagnostic tests are parasitological and PCR, as they are 100% specific, however their sensitivity depends on the parasite load of the animal and the analyzer technique. Chitinase PCR is a promising molecular technique for the diagnosis and monitoring of canine leishmaniasis, with high sensitivity and specificity. However, more research is needed to evaluate the applicability of the technique on a large scale and its standardization in different tissue samples. Because it is a complex disease, with nonspecific clinical signs and the presence of asymptomatic carrier animals, it is necessary to make associations between various diagnostic techniques.

REFERENCES

ALVES, Waneska, A; Bevilacqua, Paula Dias. Reflections on the quality of the diagnosis of canine visceral leishmaniasis in epidemiological surveys: the case of the epidemic in Belo Horizonte, Minas Gerais State, Brazil, 1993–1997. Cadernos de Saúde Pública, Rio de Janeiro, 20(1):259–265, jan-feb, 2004.

ARANGO, H.G. *Theoretical and computational biostatistics*. 2ed. Rio de Janeiro: Guanabara Koogan, 2005. 423p.

ASFARAM, S., Fakhar, M., Mohebali, M. et al. A Convenient and Sensitive kDNA-PCR for Screening of Leishmania infantum Latent Infection Among Blood Donors in a Highly Endemic Focus, Northwestern Iran. Acta Parasitologica, 67, 842–850, 2022. https://doi.org/10.1007/s11686-022-00528-2

AYRES, Eveline da Cruz Boa Sorte; GOMES, Ana Paula; MOREIRA, Jéssica. Clinical and parasitological impact of short-term treatment using miltefosine and allopurinol monotherapy or combination therapy in canine visceral leishmaniasis. Brazilian Journal of Veterinary Parasitology, v. 31, 2022.

BARATA, R. A.; PEIXOTO, J. C.; TANURE, A.; GOMES, M. E.; APOLINÁRIO, E. C.; BODEVAN, E. C.; DIAS, E. S. Epidemiology of visceral leishmaniasis in a reemerging focus of intense transmission in Minas Gerais State, Brazil. BioMed Research International, 2013.

BELCHIOR, GPN, DIAS, MRMS. The responsible custody of pets in the multispecies family. Rev. Bras. Direito Anim. [Internet]. v.14, n. 02, p. 64–79, 2019. Available at: https://periodicos.ufba.br/index.php/RBDA/article/view/33325

BRAZIL. Ministry of Health. Health Surveillance Secretariat. Surveillance Department Epidemiological. Manual for the surveillance and control of visceral leishmaniasis / Ministry of Health, Secretariat
of Health Surveillance, Department of Epidemiological Surveillance. 1st ed., 5. reprint. Brasilia: Ministry of
Health, 2014. 120p.

Beatriz Maria de Almeida **BRAZ,** Denny Parente de Sá Barreto Maia Leite, Raizza Barros Sousa Silva, Maria
Luana Cristiny Rodrigues da Silva, Ana Clara de França Silva Azevedo, Erotides Martins Filho, Walter
Massa Ramalho, Marcia Almeida de Melo. Factors associated with Leishmania infection in dogs and
geospatial analysis in the Sertão of Paraíba, Northeast Brazil. Rev. Bras. Parasitol. Vet. 30 (2) • 2021
https://doi.org/10.1590/S1984-29612021047

Aline Diniz **CABRAL**, Felipe Baena Garcia, Rodrigo Buzinaro Suzuki, Tanil Lacerda Góis Filho, Renata Torres da Costa, Ligia Marinho Pereira Vasconcelos, Edmar Silva Santos, Márcia Aparecida Sperança. Dataset on recombinant expression of an ancient chitinase gene from different species of Leishmania parasites in bacteria and in Spodoptera frugiperda cells using baculovirus. Date in Brief 32 (2020) 106259. https://doi.org/10.1016/j.dib.2020.106259

CHOMEL, BB; SUN, B. Zoonoses in the bedroom. Emerging Infectious Diseases, Vol. 17, No. 2, February 2011. P.167-172. doi: 10.3201/eid1702101070

COSTA, A.B.P. Epidemiological analysis of canine leishmaniasis in Paraguaçu Paulista: application of serological diagnoses and georeferencing of incidence areas. Dissertation (professional master's degree in Veterinary Medicine – Animal Health, Production and Environment). University of Marilia, Marilia, 2024.

COSTA, A. B. P., Repik, C. F., Lisbon, A. C. L. da C., Suzuki, R. B., Repetti, C. S. F., Manhoso, F. F. R., Costa, I. B. da, Bueno, P. dos S. C., & Porto, C. D. (2024). Euthanasia of dogs as control of Visceral Leishmaniasis: brazilian law from a technical and ethical-moral view. Pedagogical Notebook, 21(10), e9866. https://doi.org/10.54033/cadpedv21n10-368

COSTA, Graciele Pereira et al. METHODS OF DIAGNOSIS OF CANINE LEISHMANIASIS. Saber Científico (1982-792X), v. 9, n. 2, p. 95–104, 2021.

CVE (A) Center for Epidemiological Surveillance, Sao Paulo State Department of Health. Municipalities with human transmission according to the Visceral Leishmaniasis Surveillance and Control Program, by 2024.

https://www.saude.sp.gov.br/cve-centro-de-vigilancia-epidemiologica-prof.-alexandre-vranjac/areas-de-vigilancia/doencas-de-transmissao-por-vetores-e-zoonoses/agravos/leishmaniose-visceral/classificacao-epidemiologica-dos-municipios. Accessed on 28/08/2025.

CVE (B) Center for Epidemiological Surveillance, Sao Paulo State Department of Health. Confirmed cases of Visceral Leishmaniasis according to LPI and year of notification, State of Sao Paulo, 2017 to 2025.
https://www.saude.sp.gov.br/resources/cve-centro-de-vigilancia-epidemiologica/areas-de-vigilancia/doencas-de-transmissao-por-vetores-e-zoonoses/dados/leish/lv2017a2025_lpi.pdf. Accessed on 28/08/2025.

DADALTO, C. R. Doppler and elastographic aspects of the kidney and splenic in canine visceral leishmaniasis. 2020. 99 f. Thesis (Doctorate) – Veterinary Medicine Course, Sao Paulo State University Julio de Mesquita Filho, Botucatu, 2020.

DANTAS-TORRES, F. Canine leishmaniasis: diagnosis and management of the urban reservoir. Instituto Adolfo Lutz Magazine, Sao Paulo, 2018.

Filipe DANTAS-TORRES, Kamila Gaudêncio da Silva Sales, Lidiane Gomes da Silva, Domenico Otranto, Luciana Aguiar Figueredo. Leishmania-FAST15: A rapid, sensitive and low-cost real-time PCR assay for the detection of Leishmania infantum and Leishmania braziliensis kinetoplast DNA in canine blood samples. Molecular and Cellular Probes, Volume 31, 2017, Pages 65-69. https://doi.org/10.1016/j.mcp.2016.08.006

DIAS, Álvaro Felipe LR; ALMEIDA, E. M.; GONÇALVES, J. M. Molecular detection of visceral leishmaniasis in dogs from Barão de Melgaço, Pantanal region of Mato Grosso, Brazil. Brazilian Veterinary Research, v. 41, 2021.

ESTEVAM, Letícia G.T.M., Veloso, Laura B., Silva, Geralda G., Mori, Carina C., Franco, Paula F., Rocha Lima, Ana C.V.M., Assimos, Gabriela R., Reis, Ilka A., Andrade-Filho, Jose D., Araújo, Marcio S.S., Ribeiro, Vitor M., Almeida, Ana P.M.M., Paz, Gustavo F. Leishmania infantum infection rate in dogs housed in open-admission shelters is higher than of domiciled dogs in an endemic area of canine visceral leishmaniasis. Epidemiological implications. Acta Tropica, 232 (2022) 106492.

FATTAHI-Bafghi, A., AKHTARDANESH, B., & GHAFFARIFAR, F. (2020). Detection of Leishmania tropica DNA in the blood and skin of stray dogs using polymerase chain reaction. Annals of tropical medicine and public health, 23(5), 266–270.

And. **FERROGLIO**, A. Romano, A. Trisciuoglio, M. Poggi, E. Ghiggi, P. Sacchi, A. Biglino. Characterization of Leishmania infantum strains in blood samples from infected dogs and humans by PCR-RFLP. Transactions of the Royal Society of Tropical Medicine and Hygiene (2006) 100, 636–641.

FRANCO, A. O.; DAVIES, C. R.; MYLNE, A.; DEDET, J. P.; GÁLLEGO, M.; BALLART, C.; READY, P. D. Predicting the distribution of Phlebotomus papatasi vectors and the zoonotic

cutaneous leishmaniasis agent
Leishmania major in Europe. PLOS Neglected Tropical Diseases, v. 7, n. 7, e2322, 2013

FUNED. Ezequiel Dias Foundation. Funed validates faster test for canine visceral leishmaniasis. FUNED, Minas Gerais, Apr. 2010. Available at: http://www.isaude.net/pt-BR/notícias/6636/geral/funed-valida-teste-mais-rapido-para-leishmaniose-visceral-canina.htm. Accessed on: 29 ago. 2022.

Gamer, M., Jim Lemon, J., & Singh, I. (2019). *irr: Various Coefficients of Interrater Reliability and Agreement*. [R package]. URL https://CRAN.R-project.org/package=irr.

GARDE, Elena, Gerardo Acosta-Jamett, Barend Mark Bronsvoort. Review of the Risks of Some Canine Zoonoses from Free-Roaming Dogs in the Post-Disaster Setting of Latin America. Animals, 2013, 3, 855–865; doi:10.3390/ani3030855.

GUZMAN YCM, Hospinal-Santiani M, Soccol CR, Thomaz-Soccol V. Immunochromatographic tests for human and canine leishmaniasis diagnosis: a critical review and future prospects. Biotechnology Research and Innovation, 7(2): e2023012, 2023. doi:10.4322/biori.00122023.

JORDÃO, F.T., et al. Evolutive History of Leishmania Genus and Differential Diagnosis of Clinical Important Species Based on A Unique Kinetoplastida Chitinase. International Journal of Medical Parasitology & Epidemiology Sciences, v.2, n.3, p.54–61, 2021.

KASZAK, I. et al. Review articles canine leishmaniasis - an emerging disease. Annals of Parasitology, v.61, n.2, p.69–76, 2015.

LAURENTI, Márcia Dalastra. Correlation between parasitological and serological diagnosis in canine American visceral leishmaniasis. BEPA. Boletim Epidemiológico Paulista, v. 6, n. 67, p. 13-23, 2009.

LEWGOY, Bernardo; MASTRANGELO, Andrea; BECK, Luiza. Thanatopolitics and biosafety: two regimes of government of life for canine visceral leishmaniasis in Brazil. Horizontes Antropológicos, v. 26, p. 145–176, 2020.

MARTÍNEZ-Rondán, F.J., Marín, C., Gómez-Nieto, L.C., García-Martínez, J.D., Martínez-Gallardo, F., & Miró, G. (2018). Comparison of four diagnostic methods for detection of Leishmania infantum infection in dogs. Veterinary parasitology: regional studies and reports, 14, 48–53.

MEMBRILLO, A., Rojas, E., Carrillo, E., Falconí, F., & Falcón, N. (2015). Detection of Leishmania infantum DNA in conjunctival swabs of dogs by quantitative real-time PCR. Veterinary Parasitology, 207(3–4), 214–219.

MOREIRA, M.A.B., Luvizotto, M.C.R., Garcia, J.F., Corbett, C.E.P., Laurenti, M.D. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. Veterinary Parasitology, 145 (2007)

245–252.

Moustapha NA, Magdanelo ELLH, Multari JN, Tellaroli G, Santos, EW. Approach to canine visceral leishmaniasis (CVL) byveterinarians. Vet. and Zootec. 2021; v28:001-012.

Nina LNS, Caldas AJM, Soeiro VMS, Ferreira TF, Silva TC, Rabelo PPC. Spatiotemporal distribution of visceral leishmaniasis in Brazil from 2007 to 2020. Rev Panam Salud Publica. 2023; 47:e160. https://doi.org/10.26633/RPSP.2023.160

PAHO. Pan American Health Organization. Leishmaniasis: Epidemiological Report of the Americas. n. 12, December 21, 2023. https://iris.paho.org/handle/10665.2/59170. Accessed on 02/18/2025.

PEIXOTO, H.M., DE OLIVEIRA, M.R., ROMERO, G.A. Serological diagnosis of canine visceral leishmaniasis in Brazil: systematic review and meta-analysis. Tropical Medicine & International Health, vol. 20, p. 334–352, 2014.

Pigozzi, RM, Pigozzi Filho, I, Medeiros, MO. Bioecological and legal aspects related to the urbanization of leishmaniasis in neighborhoods of the municipality of Marilia in the State of Sao Paulo. Revista Biodiversidade - v.22, n.1, 2023 - p. 98-120.

PIRAJÁ, G. V. Need for epidemiological surveillance for Leishmania infantum (syn. Leishmania chagasi) and Leishmania (viannia) braziliensis in sandflies and stray cats in the Municipal Forest of Marilia-SP. 2013. 155 f. Dissertation (Master's Degree) – Sao Paulo State University Júlio de Mesquita Filho, Faculty of Veterinary Medicine and Animal Science, 2013.

PRADELLA GD, Escobar TA, Santos TP, Vargas RC, Góss GC, Ferrareze PAG et al. PCR-RLFP characterization of Leishmania spp. in domestic animals from the south-western border of Brazil. Brazilian Journal of Veterinary Parasitology, 31(3): e005222, 2022. https://doi.org/10.1590/S1984-29612022035

R Core Team (2023). A: *A Language and environment for statistical computing*. (Version 4.3) [Computer software]. Retrieved from https://cran.r-project.org.

REGUERA, Rosa M., Miguel Morán, Yolanda Pérez-Pertejo, Carlos García-Estrada, Rafael Balaña-Fouce. Current status on prevention and treatment of canine leishmaniasis. Veterinary Parasitology, Volume 227, 2016, Pages 98–114, https://doi.org/10.1016/j.vetpar.2016.07.011.

SILVA, D, A., et al. Epidemiology of canine visceral leishmaniasis in a vulnerable region in Brazil. Revista Brasileira de Parasitologia Veterinária [online]. 2021, vol.30, n.3.

SILVA, S.R. Comparative analysis of parasitological, serological and molecular methods in confirming the diagnosis in dogs with positive serology for canine visceral leishmaniasis. Dissertation (master's degree). Oswaldo Cruz Foundation, René Rachou Research Center. Belo Horizonte, 2009. 110p.

SMITH, Lauren M., Sabine Hartmann, Alexandru M. Munteanu, Paolo Dalla Villa, Rupert J. Quinnell, Lisa M. Collins. The Effectiveness of Dog Population Management: A Systematic Review. Animals, 2019, 9, 1020; doi:10.3390/ani9121020.

SMITH LM, Quinnell RJ, Goold C, Munteanu AM, Hartmann S, Collins LM. Assessing the impact of free-roaming dog population management through systems modelling. Nature/Scientific Reports, 2022;12:11452. doi:10.1038/s41598-022-15049-1.

Suzuki RB, Cabral AD, Martins LP, Sperança MA. A highly sensitive Leishmania infantum chagasi isolation method from bone marrow and peripheral blood of adults and children. J Infect Dev Ctries. 2016 Nov 24; 10(11):1275-1277. doi: 10.3855/jidc.8022. PMID: 27886044.

TAYLOR, Louise H., Ryan M. Wallace, Deepashree Balaram, Joann M. Lindenmayer, Douglas C. Eckery, Beryl Mutonono-Watkiss, Ellie Parravani, Louis H. Nel. The Role of Dog Population Management in Rabies elimination—A Review of Current Approaches and Future Opportunities. Frontiers in Veterinary Science, July 2017, Volume 4. DOI: 10.3389/FVETS.2017.00109.

THE JAMOVI PROJECT. jamovi (Version 2.5) [Computer Software]. 2024. Available from: https://www.jamovi.org.

WOAH (World Organisation of Animal Health). Stepping up dog population management to achieve rabies elimination. 2023. https://www.woah.org/en/article/stepping-up-dog-population-management-to-achieve-rabies-elimination/. Accessed on 03/05/24.