doi: 10.5123/S2176-6223202501671

Distribution and occurrence of *Leishmania infantum chagasi* causing canine visceral leishmaniasis in an endemic area of Brazil

Distribuição e ocorrência de *Leishmania infantum chagasi* causadora da leishmaniose visceral canina em área endêmica do Brasil

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ABSTRACT

Leishmaniasis exhibits a spatial distribution strongly influenced by multiple factors. Epidemiological studies conducted in locations close to endemic areas are mandatory to monitor disease spread. Porto Nacional, Tocantins State, Brazil, is an endemic area for canine visceral leishmaniasis (CVL) with high transmission rates, yet few studies have examined parasite isolation in dogs. OBJECTIVES: To determine the impact of seropositive dog euthanasia on the incidence of human visceral leishmaniasis (HVL) cases and identify the *Leishmania* species circulating in dogs with CVL in Porto Nacional from 2010 to 2019. MATERIALS AND METHODS: Seropositive dogs for CVL from Porto Nacional were tested for parasite DNA using polymerase chain reaction (PCR) and immunohistochemistry for species characterization. Data on animals were obtained from the local Center for Zoonosis Control, and HVL notifications (2010–2019) were retrieved from the National Disease Notification System. RESULTS: *Leishmania infantum chagasi* was the only species detected by PCR in infected dogs. The number of seropositive dogs increased over time, with the highest number in 2019. The most frequent clinical signs were lymphadenomegaly (73%), skin lesions (70%), and hepatomegaly (63%). No positive correlation was found between euthanasia of seropositive dogs and HVL incidence. CONCLUSION: *L. infantum chagasi* was confirmed in infected dogs, underscoring the need to revise and adapt control strategies to local contexts in order to better assess the role of infected dogs in leishmaniasis endemic areas.

Keywords: Visceral Leishmaniasis; *Leishmania infantum chagasi*; Dogs; Domestic Animals; Animal Euthanasia; Animal Protozoan Infections.

RESUMO

A leishmaniose apresenta distribuição espacial fortemente influenciada por múltiplos fatores. Estudos epidemiológicos em áreas próximas a regiões endêmicas são essenciais para monitorar a disseminação da doença. Porto Nacional, no estado do Tocantins, é uma área endêmica para leishmaniose visceral canina (LVC), com altas taxas de transmissão, mas poucos estudos abordaram o isolamento do parasito em cães. OBJETIVOS: Determinar o impacto da eutanásia de cães soropositivos na incidência de casos de leishmaniose visceral humana (LVH) e identificar as espécies de *Leishmania* circulantes em cães com LVC em Porto Nacional, de 2010 a 2019. MATERIAIS E MÉTODOS: Cães soropositivos para LVC foram testados para detecção de DNA do parasito por reação em cadeia da polimerase (PCR) e caracterização por imunohistoquímica. Os dados referentes aos animais foram obtidos no Centro de Controle de Zoonoses local, enquanto as notificações de LVH (2010–2019) foram extraídas do Sistema de Informação de Agravos de Notificação. RESULTADOS: *Leishmania infantum chagasi* foi a única espécie detectada por PCR em cães infectados. O número de cães soropositivos aumentou ao longo do período, atingindo o maior valor em 2019. Os sinais clínicos mais frequentes foram linfadenomegalia (73%), lesões cutâneas (70%) e hepatomegalia (63%). Não foi observada correlação positiva entre a eutanásia de cães soropositivos e a incidência de LVH. CONCLUSÃO: A presença de *L. infantum chagasi* foi confirmada em cães infectados, ressaltando a necessidade de revisão e adaptação das estratégias de controle aos contextos regionais, a fim de melhor avaliar o papel desses animais em áreas endêmicas de leishmaniose.

Palavras-chave: Leishmaniose Visceral; *Leishmania infantum chagasi*; Cães; Animais Domésticos; Eutanásia Animal; Infecções por Protozoários em Animais.

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INTRODUCTION

Protozoan parasites of the genus *Leishmania* cause neglected vector-borne diseases collectively known as leishmaniasis. Phlebotomine sandflies transmit these parasites to mammals, including humans, through bites during their blood meals. Because sandflies require warm temperatures for survival and breeding, most clinical cases are concentrated in tropical and subtropical regions, particularly in South and Central America, Africa, and the Middle East Asia[1]. Leishmaniasis and other vector-borne diseases affect impoverished populations due to malnutrition and limited access to essential health services. Unplanned urban growth, deficient waste collection, and inadequate surface-water drainage systems further promote vector proliferation[2]. These socioeconomic and environmental conditions, associated with the disease's complexity, culminate in an estimated global prevalence of 12 million cases – considering all clinical forms – with approximately 2 million new cases and up to 40,000 deaths annually[3]. Current chemotherapy toxicity and resistance-related issues raise more concerns[4], although recent research has advanced the development of alternative therapeutic strategies[5,6,7,8,9].

Human visceral leishmaniasis (HVL) is the deadliest clinical form, with mortality rates reaching 90% in untreated cases[3]. The systemic infection is characterized by long-term fever, progressive weight loss, hepatosplenomegaly, and anemia[10]. HVL is endemic in 13 countries in the Americas, but Brazil has historically accounted for the majority of the cases, reporting 97% of all regional cases in 2019[11]. In the same year, Brazil also exhibited the highest global case-fatality rate for HVL (7.7%), followed by South Sudan (5%), and Ethiopia (2.5%)[11].

Visceral leishmaniasis is a zoonosis. In Brazil, the primary reservoir is the domestic dog (*Canis lupus familiaris*), the etiological agent is the protozoan parasite *Leishmania infantum chagasi*[12], and the main vector is the phlebotomine sandfly *Lutzomyia longipalpis*[13]. Dogs are highly susceptible to *L. infantum chagasi* infection, presenting a wide range of clinical signs – including dermatitis, alopecia, onychogryphosis, hepatosplenomegaly, lymphadenomegaly, ophthalmic alterations, hyperthermia, and apathy[14] – characterizing canine visceral leishmaniasis (CVL). Infected dogs play a central role in the epidemiological chain of *L. infantum chagasi* in urban areas. However, other *Leishmania* species, such as *Leishmania braziliensis* and *Leishmania amazonensis* – causative agents of human cutaneous leishmaniasis (HCL) – may also infect dogs[15,16]. Additionally, *L. nfantum chagasi* has been reported in wild mammals in Brazil, including canids[17], primates[18], and felids[19].

The parasite's complex life cycle and broad clinical spectrum pose major challenges for public health, especially given the recent urbanization of leishmaniasis, followed by the reemergence and expansion of transmission areas[14,20].

HVL control strategies in Brazil have long relied on the euthanasia of infected canine reservoirs[14,21]. However, this approach requires reliable diagnostic methods, such as immunohistochemistry (IHC), to ensure accurate detection[22]. Cross-reactivity between *L. infantum chagasi* antigens and dogs infected with *L. braziliensis* has been observed[23,24], although visceralization of *L. braziliensis* in dogs appears rare, usually reported in cases of co-infection with other parasites[24]. In this scenario, molecular techniques offer a significant advancement in diagnosis, providing higher sensitivity for reservoir identification[25].

This study aimed to determine the impact of seropositive dog euthanasia on the incidence of HVL cases and identify the *Leishmania* species circulating in dogs with CVL in Porto Nacional, Tocantins State, Brazil, from 2010 to 2019.

MATERIALS AND METHODS

STUDY AREA AND DESIGN

Tocantins State has 139 municipalities grouped into eight micro-regions, including the Porto Nacional micro-region, located centrally. Among its 11 municipalities, Palmas is the State capital (10°10'08" S, 48°19'54" W; 255 m above sea level), while Porto Nacional (10°42'27" S, 48°24'51" W; 232 m above sea level) lies 60 km away. Porto Nacional has an area of 4,449,892 km^2, with 53,316 residents (2020 data estimation), a human development index (HDI) of 0.740 (2010 data), and 65.7% coverage of adequate sanitation services (2010 data)[26]. The region's average annual rainfall is 1,664 mm, mainly during the summer, with a mean temperature of 27.4 °C, and Cerrado (Brazilian savanna) as the predominant vegetation[27,28].

A retrospective study was conducted, combining temporal descriptive analysis of incidence and mortality with spatial methods.

DATA SOURCES

Data on screened and seropositive dogs for *Leishmania* spp. were obtained from the local Center for Zoonosis Control (CZC). Canine sera were first tested using a rapid diagnostic test, followed by ELISA confirmation. HVL data for 2010–2019 were retrieved from the Notifiable Diseases Information System (SINAN), distributed by the Department of Information Technology of the Brazilian Public Unified Health System (DATASUS)[29]. Population estimates for 2010–2019 were obtained from the Brazilian Institute of Geography and Statistics (IBGE)[27].

ANIMALS AND TISSUE COLLECTION

This study was conducted in strict accordance with the recommendations of the Brazilian College of Animal Experimentation to minimize animal suffering. It was approved by the Ethics Committee on the Use of Animals (CEUA) of the Instituto Tocantinense Presidente Antônio Carlos (ITPAC), in Porto Nacional, on July 24, 2015 (protocol no. 005/2015).

Thirty dogs captured by the CZC in 2016 and confirmed seropositive for *Leishmania* were included. A veterinarian examined the animals for clinical signs of leishmaniasis before euthanasia. Of these, 65% were young (6 months–3 years old), presenting diverse clinical manifestations consistent with CVL.

Euthanasia was performed under general anesthesia with ketamine (10 mg/kg) and xylazine (1 mg/kg, IV), followed by potassium chloride (75–150 mg/kg). During necropsy, the liver, spleen, and 22 lymph nodes were collected, weighed, and processed for IHC or molecular analysis.

IHC

Paraffin tissue samples were stained using the biotin-streptavidin peroxidase immunostaining technique. The primary antibody was a polyclonal anti-*L. amazonensis* serum (cross-reactive with *L. infantum chagasi* and *L. braziliensis*), produced in mice provided by Dr. Hiro Goto (Instituto de Medicina Tropical de São Paulo, Brazil). After dewaxing and tissue treatment, tissue sections were rehydrated and incubated three times in 3% hydrogen peroxide (H_2O_2) for 10 min to block endogenous peroxidase activity, followed by sequential washes in running water, distilled water, and phosphate-buffered saline (PBS) for 5 min each. Antigen exposure was carried out using Dako Target Retrieval Solution (pH 9.0) according to the manufacturer's instructions. Sections were incubated with the primary antibody (1:1000 dilution in 1% bovine serum albumin) overnight at 4 °C, followed by incubation with antibody anti-mouse IgG (biotinylated) for 30 min at 37 °C. After washing, slides were incubated with streptavidin-peroxidase complex (Dako LSAB® kit, code K690) for 30 min at 37 °C. The immunoreaction was visualized with 45 mg 3,3-diaminobenzidine in 100 mL PBS containing 0.03% H_2O_2 and counterstained with hematoxylin for 10 s.

MOLECULAR TECHNIQUES

DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen®, Brazil) and quantified with Nanodrop. The PCR protocol amplified the target DNA fragments in 25 µL final volume reaction containing 12.5 µL Gotaq® Green Master Mix (Promega®, Brazil), 80–150 ng DNA, 10 pmol of each primer, and ultrapure water (q.s.). Ultrapure water served as a negative control. Positive controls included: *L. infantum chagasi* (MHOM/BR2000/MER2, FIOCRUZ/BA – Merivaldo strain, IOC-LC2455, isolated from a patient with visceral leishmaniasis in an endemic area of Jequié, Bahia); *L. braziliensis* (MHOM/BR/1975/M2903) and *L. amazonensis* (IFLA/BR/1967/PH8), provided by Prof. Dr. Silma Regina (Genetics and Molecular Biology Laboratory, Department of Biology, Federal University of Maranhão). All reactions included positive and negative controls.

The primer sequences used were as follows: for *L. infantum chagasi*, RV1 (forward 5'-ctt ttc tgg tcc cgg ggg tag g-3') and RV2 (reverse 5'-cca cct ggc cta ttt tac acc a-3'); for *L. amazonensis*, LU-5A (forward 5'-ttt att ggt atg cga aac ttc-3') and LB-3C (reverse 5'-taa aag ccg ctc acc cac ag-3'); and for *L. braziliensis*, LU-5A (forward 5'-ttt att ggt atg cga aac ttc-3') and Lam (reverse 5'-cgt ccc gaa ccc cgt gtc-3'). The primers targeting the LT1 fragment of kDNA of *L. infantum chagasi* DNA amplified a 145 base pair (bp) fragment[30]. For *L. braziliensis* detection, a universal sense primer for *Leishmania* species (LU-5A) and an antisense primer specific to the *Viannia* subgenus (LB-3C) amplified a 149 bp fragment[31]. The LU-5A primer, combined with an antisense primer specific to *L. amazonensis* (Lam), produced a 220 bp fragment. Based on a sequence previously published[31], the Lam primers were redesigned using Primer Designer v.2.0 (IDT-DNA, USA) software, starting at the spliced leader region of RNA sequence (mini-exon).

PCR cycling conditions started with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 58 °C for 40 s for *L. infantum chagasi*. For *L. braziliensis* reactions, 10 pmol of each LU-5A and LBr primer were used, with 0.6 M betaine, 1 mM dithiothreitol (DTT), and 50 mM tetramethylammonium chloride (TMAC) in a final volume of 25 µL. Cycling conditions included an initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min and a hold at 4 °C for 5 min. For *L. amazonensis* reactions, 10 pmol of each LU-5A and Lam primer were used, with 0.6 M betaine, 1 mM DTT, 50 mM TMAC, and 10.5% dimethylsulfoxide (DMSO) in a final volume of 25 µL, following the same cycling program as *L. braziliensis*[31]. The amplified products were analyzed by electrophoresis on 2–3% agarose gel in 1x TAE buffer with 0.1 µg/mL ethidium bromide and visualized under an ultraviolet transilluminator.

STATISTICAL ANALYSIS

The percentage of positive dogs was calculated as the number of seropositive dogs divided by the total screened, multiplied by 100. HVL incidence was calculated as new cases per year divided by the corresponding population size and multiplied by 10,000 or 100,000. Thus, the results were expressed as the number of cases per 10,000 or 100,000 inhabitants. The G test was used to compare IHC results among different tissues. Regarding the comparative analysis between PCR and IHC, the Kappa test was applied to measure the agreement between two nominal observations of the same variable. Analyses were performed using BioEstat v.5.6. GraphPad Prism v.8.0.1 was applied to evaluate normality (Shapiro-Wilk test) and correlations (Spearman's r) between the number of euthanized dogs and HVL incidence. Normality was assessed before analysis. Differences pointed out as statistically significant imply $p \leq 0.05$.

RESULTS

HVL IN PORTO NACIONAL AND CORRELATION WITH DOG EUTHANASIA

HVL incidence in Porto Nacional showed two peaks, with case numbers above the period average, occurring in 2010–2011 and 2017–2018 (Table 1).

Table 1 – HVL and percentage of seropositive dogs in Porto Nacional, Tocantins State, Brazil, from 2010 to 2019

Year	HVL incidence (per 10,000) Shapiro-Wilk: 0.2865	No. HVL cases	No. examined dogs	No. seropositive dogs Shapiro-Wilk: 0.006	Seropositive dogs (%)
2010	6.5	32	3,570	526	14.7
2011	4.2	21	2,778	661	23.8
2012	1.8	9	2,928	463	15.8
2013	1.7	9	4,391	847	19.3
2014	0.4	2	3,429	770	22.5
2015	1.5	8	1,459	342	23.4
2016	1.3	7	1,624	402	24.8
2017	3.6	19	2,477	447	18.0
2018	3.4	18	2,923	640	21.9
2019	2.1	11	5,555	2,002	36.0
Total	–	136	31,134	7,100	22.8

Source: SINAN, 2010–2019.

In 2019, a total of 2,827 confirmed HVL cases were reported in Brazil. Tocantins State presented the highest incidence in the country, with 11.8 cases per 100,000 inhabitants (Figure 1A). The municipality of Porto Nacional, located in the micro-region of the same name in the central area of Tocantins (Figure 1B), stood out with consistently high rates. Comparing Brazil, Tocantins, and Porto Nacional from 2010 to 2019, the data showed an overall increase in VL incidence in Tocantins and Porto Nacional, despite the yearly stability in the total incidence of Brazil (per 10,000 inhabitants). This suggests that the population of Porto Nacional bears the highest collective risk of getting sick, with three new cases per 10,000 inhabitants, followed by Tocantins and Brazil, according to the Wilcoxon test (Figure 1C).

A total of 136 HVL cases were registered in Porto Nacional during the study period, heterogeneously distributed across the years (Table 1). There were more HVL cases between 2010 and 2011, diminishing from 2012 to 2016. From 2017 onward, it is possible to visualize an increase in the number of cases, which is reflected in the incidence compared to previous years. Notably, in 2019, the number of examined dogs (5,555) was the highest recorded, correlating with an increased proportion of seropositive dogs (36%), compared to the lower (14.7%) in 2010, when 526 of 3,570 dogs tested positive (Figure 2A).

When analyzing the correlation between HVL incidence and the percentage of euthanized seropositive dogs, no direct equivalence was observed (Figure 2B). The highest incidence of human cases occurred in 2010 (6.5 new cases per 10,000 inhabitants), when the percentage of seropositive dogs was the lowest (14.7%). Conversely, the lowest incidence occurred in 2014, with 0.4 cases per 10,000 inhabitants (Table 1), despite 22.5% of examined dogs testing positive and being euthanized. It is worth mentioning that the highest percentage of euthanized dogs occurred in 2019 (36%) for an HVL incidence coefficient of 2.1 new cases per 10,000 inhabitants.

LOCATIONS OF INFECTED DOGS IN PORTO NACIONAL

Since Porto Nacional is an endemic area with a high prevalence of seropositive dogs, confirmation of *Leishmania* species in the surrounding area was required. Thirty dogs were collected from multiple sites across Porto Nacional, with sampling distributed to cover the entire urban area, which corresponds to 4,499 km^2 (Figure 3A). Most collection sites were residential backyards frequently characterized by rubble, debris, and organic waste (Figure 3B).

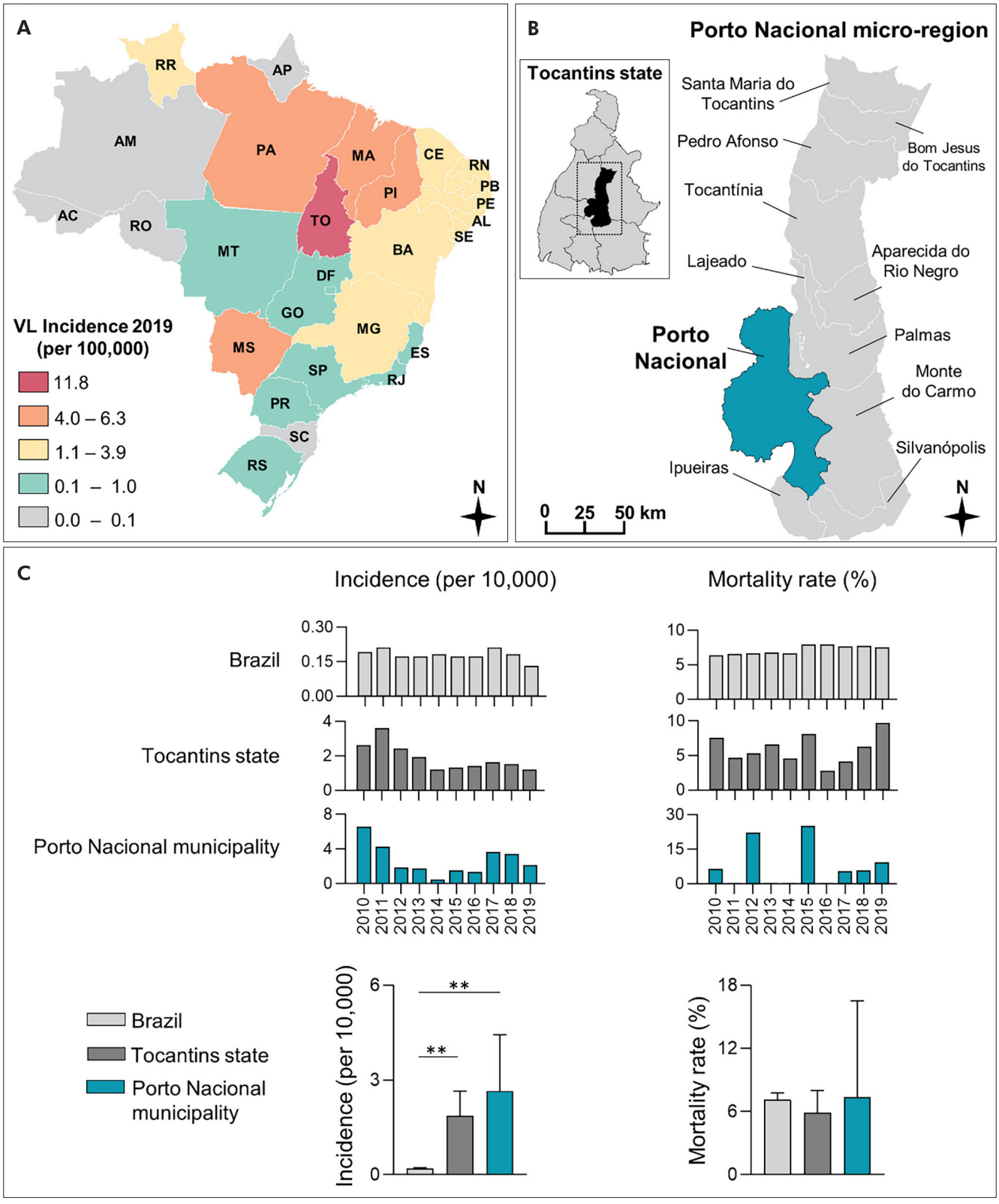
CLINICAL SIGNS IN SEROPOSITIVE DOGS

The clinical phenotype, an important parameter for sample collection, revealed lymphadenomegaly in 73% of the animals, followed by skin lesions such as abrasions (70%), hepatomegaly (63%), skin ulcerations or apathy (53%), and splenomegaly (50%). Less frequent signs included hyperthermia and weight loss (47%), pale mucous membranes (43%), onychogryphosis (30%), and ophthalmic alterations, such as conjunctivitis, in 17% of the dogs evaluated (Figure 4A). Tissue samples collected included liver, spleen, and lymph nodes from different anatomical regions, such as accessory, inguinal, mandibular, popliteal, and prescapular (Figure 4B).

Species identification was performed by PCR using specific primers detecting the three main *Leishmania* reference species (*Lc* for *L. infantum chagasi*; *La* for *L. amazonensis*; *Lb* for *L. braziliensis*). The reference species showed different patterns in PCR amplicon band size in agarose gel (*Lc* = 145 bp; *La* = 149 bp; *Lb* = 220 bp). As expected for endemic areas, 100% of samples tested positive for *L. infantum chagasi* (RV1/RV2 primer region) in the seropositive dogs (Figure 4C).

Among the 17 seropositive dog samples analyzed by IHC, 12 (70.5%) showed immunoreactivity for

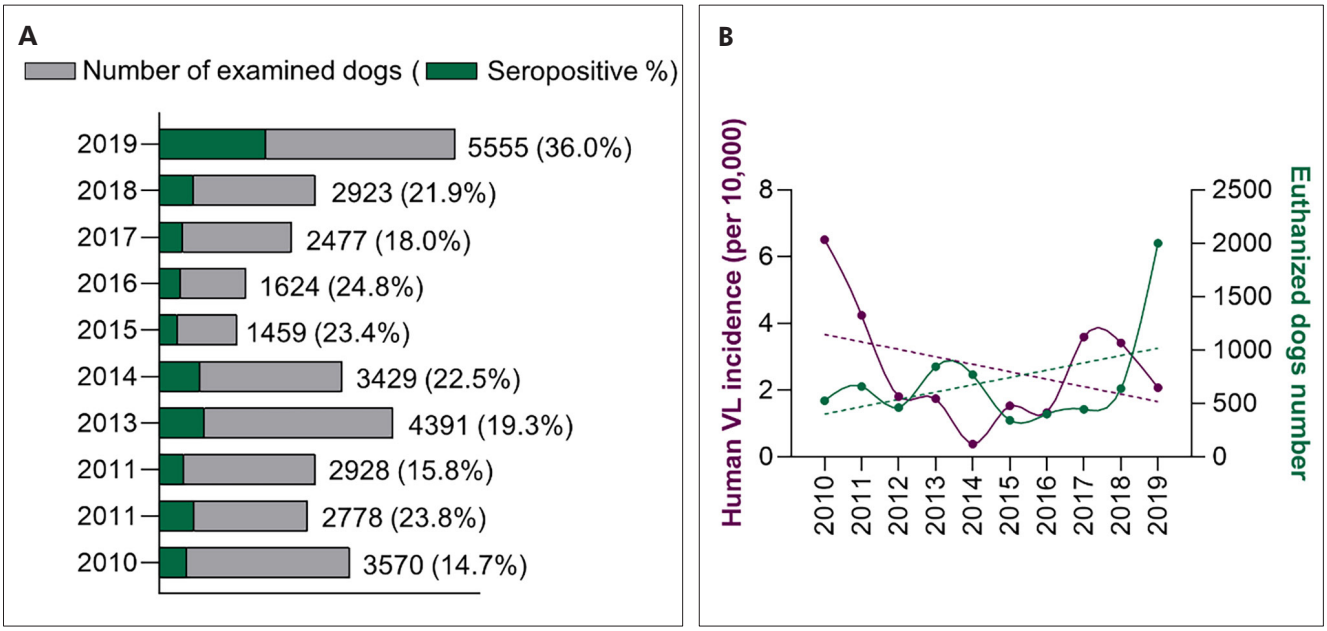
Leishmania spp. in at least one evaluated tissue (Figure 4D). Moreover, related to the tissues used for the IHC detection, 58.8% of the spleen samples and 47% of the liver samples analyzed showed positive immunoreaction. Despite these differences, G-test analysis indicated a significant association between IHC positivity, independent of tissue type (spleen or liver), with $p = 0.0280$ (p-value < 0.05), not denoting differences between the results. However, Kappa test results ($\kappa = 0.2941$) revealed poor agreement between PCR and IHC, suggesting low replicability across the two detection methods.



Source: Datasus, 2019.

A: HVL incidence across Brazilian states; **B**: Porto Nacional micro-region map showing the 11 municipalities, with Porto Nacional municipality highlighted in blue; **C**: Comparison of HVL incidence (per 10,000 inhabitants) and mortality rate in Brazil, Tocantins State, and Porto Nacional municipality. (Wilcoxon test: $W = p = 0.0020$).

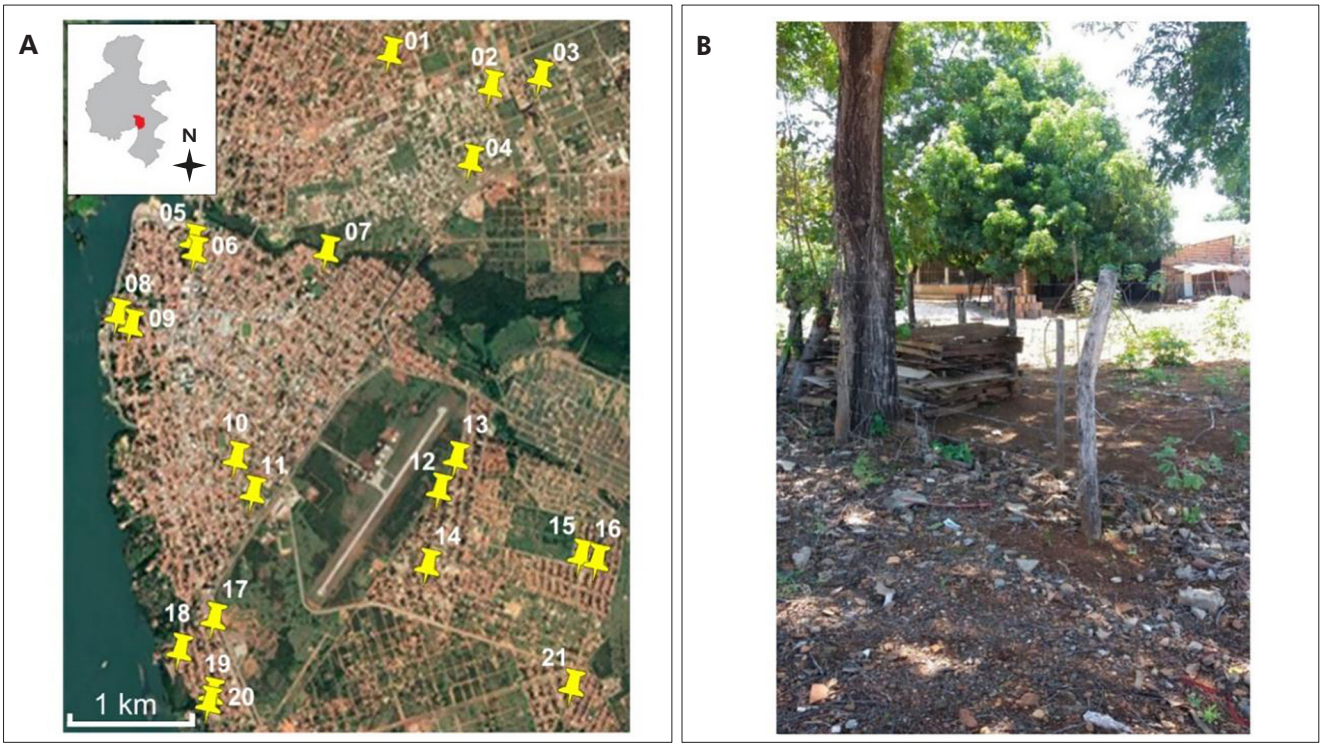
Figure 1 – HVL distribution in Brazil: incidence and mortality



Source: SINAN, 2019.

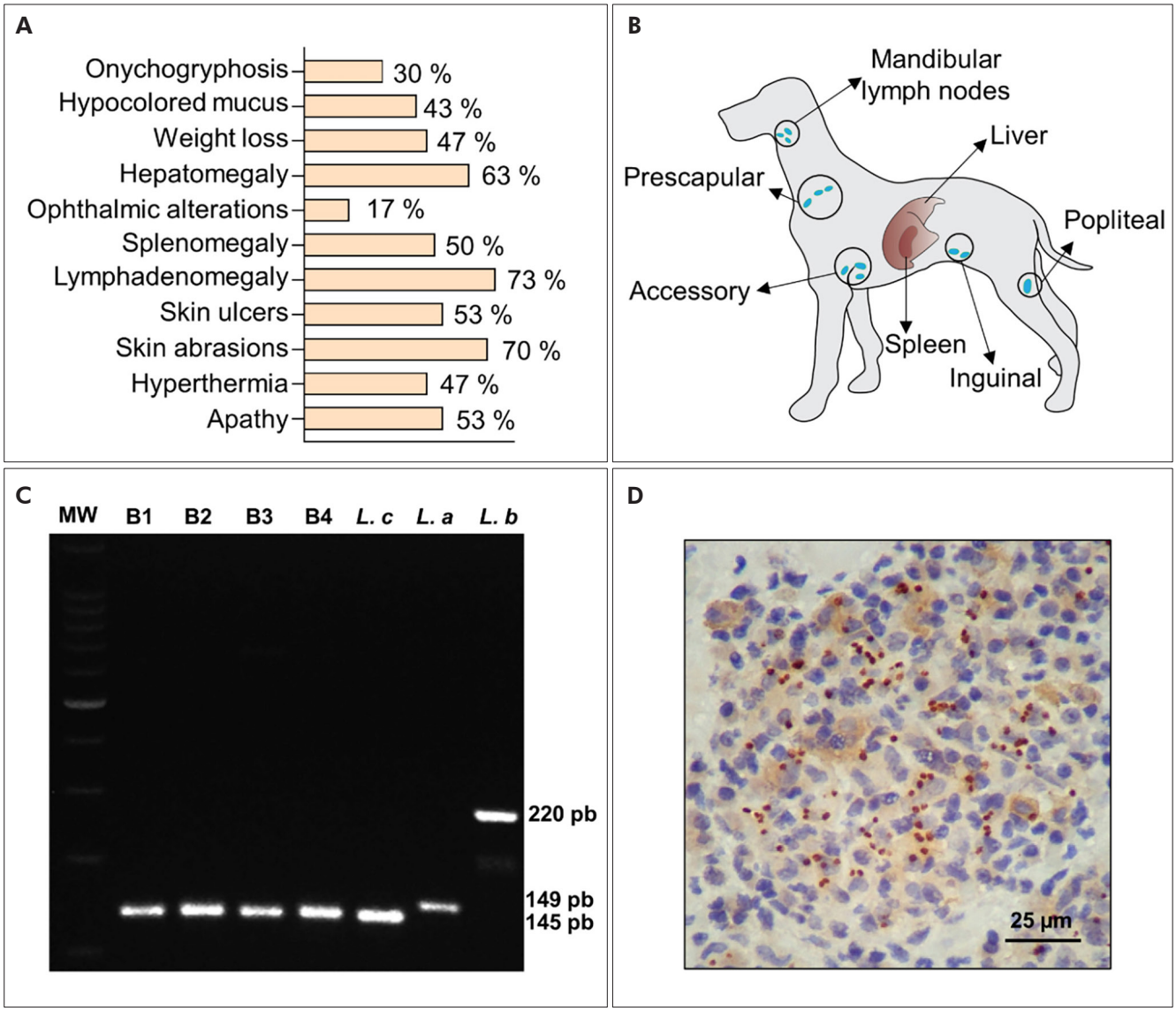
A: Number of examined dogs and percentage of seropositivity in Porto Nacional, from 2010 to 2019; **B**: Epidemiological indicators of canine euthanasia and HVL incidence from 2010 to 2019 (Spearman correlation test, r = 0.8113).

Figure 2 – Correlation between canine euthanasia surveillance and HVL incidence



A: Map indicating collection points in the urban area of Porto Nacional; **B**: Example of a collection site, showing an urban residential backyard with rubble.

Figure 3 – Dog collection sites



A: Clinical signs observed in dogs presenting CVL; **B**: Tissues collected post-euthanasia (spleen, liver, and lymph nodes) for *Leishmania* spp. detection; **C**: PCR results of four samples (B1 to B4), showing the molecular weight (MW, GeneRuler 100 bp DNA Ladder) on the left and positive controls for the different species on the right; **D**: IHC detection of stained amastigotes in a spleen section (bar size indicated in µm).

Figure 4 – Clinical signs of the *Leishmania* seropositive dogs and results of IHC and PCR analyses

DISCUSSION

The spatial distribution of leishmaniasis shows that HVL cases are concentrated in peripheral and central urban areas, typically associated with poor environments/urban infrastructure and worse sanitation[32]. Effective surveillance and epidemiological studies in areas close to highly endemic regions are mandatory to prevent disease spread, especially in areas of severe deforestation within the Cerrado biome (12,198 km^2), according to IBGE[27].

In 2001, the construction of the Luis Eduardo Magalhães Hydroelectric Power Plant delimited the Porto Nacional urban area by the Tocantins River damming reservoir. This project altered local vegetation and increased the demand for urban housing and the speculative interest in urban land. This urban area expansion without prior planning, combined with a historical deficit in infrastructure and public services, led to the proliferation of irregular waste dumpsites for domestic and commercial trash, rubble, and animal remains, which serve as vector proliferation sites[33]. Moreover, deforestation alters the natural behavior of *Leishmania*'s wildlife host, occasionally drawing them to urban areas, enabling phlebotomine fly vectors with feeding plasticity, i.e., *Lu. longipalpis*, which transmits the parasite from wildlife hosts to humans and domestic dogs[34]. Maximum entropy analysis has shown that the distribution of *Lu. longipalpis* is most prevalent in regions that combine urban and rural characteristics, characterized by the presence of vegetation associated with debris and the presence of animals, such as rodents, dogs, and chickens. The consequence of this is the unprecedented acceleration in the speed of dispersal of the vector during the 1990s and early 2000s[35].

A clear example of the association between distribution of leishmaniasis and urban infrastructure is provided by the surveillance data from the

Tocantins State Health Department, which attributed the leishmaniasis outbreaks in Porto Nacional (2001 and 2002) and the capital city, Palmas (2003), to environmental impacts caused by the power plant construction[36]. Although the study has limitations (use of secondary data sources and the absence of detailed vector distribution analyses), the present findings underscore the critical need for continuous surveillance and control of leishmaniasis in endemic regions such as Tocantins, by monitoring seropositive dogs. Therefore, efforts to optimize and improve the effectiveness of control measures remain critical. Different control strategies for VL in Brazil have been applied; however, they have shown limited success in reducing HVL incidence, leading to an increase in the number of cases, providing a geographic spread of the disease, mainly to the urban environment, establishing itself as a serious public health problem[37].

In this study, most of the dogs were symptomatic, with only one asymptomatic and eight oligosymptomatic. Nonetheless, many dogs with CVL can be asymptomatic and remain healthy throughout their lives, developing a cellular immune response[10] and becoming resistant to the disease[38]. Since dogs are domestic reservoirs of leishmaniasis, asymptomatic animals are important in monitoring and controlling disease spread[38]. Regardless of public control measures, clinical treatment or prophylactic measures, such as the use of insecticide collars, could help reduce the dissemination[39,40], since the application of dog euthanasia does not impact disease incidence, and its effectiveness is still insufficient, as confirmed by other studies[41,42]. Furthermore, these animals can be found roaming freely on the streets, especially in poor areas and outskirts, allowing vectors to thrive and increasing the risk of spreading CVL[43].

This study also shows that, despite the existence of a canine euthanasia program in Porto Nacional, there has been an increase in seropositive cases in recent years. The National Program for Surveillance and Control of Visceral *Leishmania* advocated serological surveys and euthanasia of seropositive dogs as strategies for controlling the canine reservoir, implemented at the public health level. However, managing the canine reservoir alone has not been sufficient to interrupt the transmission cycle of CVL[44]. Even given the recognized importance of dogs as reservoirs of the disease in urban areas, significant gaps remain in knowledge regarding the factors associated with phlebotomine infection in these animals[45]. It is worth noting that no single strategy for controlling leishmaniasis is effective. Therefore, alternative mechanisms have been proposed, including the use of insecticide-impregnated collars for sandflies, dog vaccination, and the proper management of vacant lots, which have been highly encouraged due to their effectiveness in reducing vector and parasite loads[46]. Environmental control, through the appropriate collection of solid waste, has a positive impact and should be encouraged as a recommended measure to reduce the burden of infection in the community. However, further investigation is still needed to determine the magnitude of its impact on CVL transmission[47].

Although *L. infantum chagasi*, *L. amazonensis*, and *L. braziliensis* have all been detected in infected dogs[16,23,41], only *L. infantum chagasi* is responsible for CVL[41,43]. For this reason, finding all the animals tested and infected by the visceral species was not surprising. Both PCR and IHC were used for parasite detection, as recommended by previous studies[48,49,50,51]. While IHC can identify the entire parasite in histological sections, PCR is more sensitive, fast, and specific, detecting only genetic material; but both techniques together are powerful tools for diagnosis[52]. In this study, IHC was less sensitive than PCR, since 100% of the samples resulted positive for *L. infantum chagasi* by PCR, but only 70% were positive by IHC. This discrepancy is explained by the high sensitivity of PCR, which can amplify small parts of the DNA molecule in tissue fragments. In contrast, IHC requires tissue fragments with sufficient parasites to detect them by antibodies[50] analyzed dog and horse tissues by real-time PCR, detecting low parasite loads in the tissues (0.016 amastigotes/µg of tissue), which required careful inspection by microscope of the whole tissue section to detect amastigotes by IHC. Moreover, a study comparing IHC and PCR in patients with cutaneous leishmaniasis verified 90% positivity by PCR in infected individuals and only 68.8% positivity by detecting *Leishmania* antigens[49]. These results are also confirmed when detecting *Leishmania* in dogs by different techniques, showing a 100% only when using PCR, which can discriminate negative from positive samples[51,52]. Nevertheless, the discovery of new molecules, such as protein biomarkers[53,54], can be more efficient and increase the performance qualities of each technique.

Thus, the leishmaniasis control strategies must be revised and optimized to establish the real impact of infected dogs in the endemic area studied.

CONCLUSION

Leishmania infantum chagasi circulates in dogs from Porto Nacional, confirmed by molecular and immunological methods. Most infected dogs were oligosymptomatic, and the number of canine cases increased from 2010 to 2019, underscoring the importance of dogs as reservoirs for human leishmaniasis.

FINANCIAL SUPPORT

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Grant nº 2012/24105-3; 2020/13562-0; 2024/05757-7 to MC; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant nº #443816/2014-0 to MC; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Finance Code nº 001 to MC.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

Conceptualization: K.W.O., P.J.S., and D.A.A.; Methodology: K.W.O., P.J.S., and D.A.A.; Investigation: K.W.O., W.P.O.J., and F.P.F.; Data curation: G.B.; Resources: K.W.O., P.J.S., D.A.A., and M.C.; Data compilation (SINAN and IBGE): T.C.S.F.; Writing – original draft preparation: T.C.S.F; Writing – review and editing: K.W.O. and M.C.; Formal analyses and visualization: T.C.S.F.; Supervision: P.J.S., D.A.A., and M.C; Project administration: K.W.O., P.J.S., D.A.A., and M.C. All authors have read and agreed to the published version of the manuscript.

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Received / Recebido em: 10/12/2024
Accepted / Aceito em: 22/6/2025

How to cite this article / Como citar este artigo:

Oliveira KW, Ferreira TCS, Spencer PJ, Oliveira Júnior WP, Frota FP, Oliveira JD, et al. Distribution and occurrence of *Leishmania infantum chagasi* causing canine visceral leishmaniasis in an endemic area of Brazil. Rev Pan Amaz Saude. 2025;16:e202501671. Doi: https://doi.org/10.5123/S2176-6223202501671