REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO
JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

ORIGINAL ARTICLE

http://doi.org/10.1590/S1678-9946202365024

Can domestic dogs be considered a good reservoir of *Leishmania (L.) infantum chagasi* in an endemic area of nonulcerated cutaneous leishmaniasis in Southern Honduras?

Gabriela Beatriz Rodriguez Segura[1,2], Wilfredo Humberto Sosa Ochoa[1], Vânia Lúcia Ribeiro da Matta[3], Mercedes Martínez[2], Carol Rodriguez Tercero[1], Raquel Romero Gonzalez[1], Carmen M. Sandoval Pacheco[4], Gabriela V. Araujo Flores[4], Fernando Tobias Silveira[5,6], Maria Mercedes Rueda Henriquez[1*], Márcia Dalastra Laurenti[4*]

ABSTRACT

Dogs are considered to be the main domestic reservoir associated with the transmission of *Leishmania (L.) infantum chagasi* to humans in endemic areas of visceral leishmaniasis in America. However, little is known about the role of canines as a source of infection in endemic areas of nonulcerated cutaneous leishmaniasis (NUCL). Therefore, the objective of the present study was to investigate the role of dogs as a possible reservoir of the parasite in Southern Honduras. Dogs (n = 107) living with individuals affected by NUCL were clinically examined and biological material was collected for parasitological and immunological diagnosis. Most animals showed a healthy appearance and a few presented slight weight loss (64%), alopecia (7%), onychogryphosis (5%) and skin lesions (1%). The overall seroprevalence of *Leishmania* infection based on the DDP® quick test and/or in-house ELISA serological test was 41%. The presence of the parasite's DNA was confirmed in 94% of the dogs; however, the average parasite load in the buffy coat was low at 6.09 parasites/μL, ranging between 0.221 and 50.2. The skin of seropositive dogs examined by histopathology using paraffin sections stained by hematoxylin and immunohistochemistry did not show cutaneous lesions or parasite amastigotes. Based on the absence of parasites in the skin and the low parasite load detected in the buffy coat, it seems that the dog does not represent a good source of infection for the vector in the endemic area of NUCL transmission in Southern Honduras. Other domestic and/or wild animals should be investigated.

KEYWORDS: Canine leishmaniasis. *Leishmania (L.) infantum chagasi.* Nonulcerated cutaneous leishmaniasis. Diagnosis. Honduras.

INTRODUCTION

Leishmania (L.) infantum chagasi is a protozoan transmitted by a phlebotomine vector that is responsible for causing visceral leishmaniasis (VL) in the Americas[1-3], it becomes a fatal disease if not diagnosed in time and treated properly. However, in some Central American countries, such as Costa Rica, El Salvador, Nicaragua and Honduras, this species of protozoan causes, in addition to VL, an atypical cutaneous form called nonulcerated cutaneous leishmaniasis (NUCL)[4]. In Honduras, leishmaniasis is a notifiable disease and occurs in different clinical forms caused by different parasite species. In arid or semiarid zones found in Southern regions,

*These authors were co-principal investigators

[1]Universidad Nacional Autónoma de Honduras, Instituto de Investigaciones en Microbiologia, Tegucigalpa, Honduras

[2]Universidad Nacional Autónoma de Honduras, Posgrado en Salud Pública, Tegucigalpa, Honduras

[3]Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, Laboratório de Investigação Médica (LIM-50), São Paulo, São Paulo, Brazil

[4]Universidade de São Paulo, Faculdade de Medicina, Laboratório de Patologia de Moléstias Infecciosas, São Paulo, São Paulo, Brazil

[5]Instituto Evandro Chagas, Laboratório de Leishmanioses, Belém, Pará, Brazil

[6]Universidade Federal do Pará, Núcleo de Medicina Tropical, Belém, Pará, Brazil

Correspondence to: Márcia Dalastra Laurenti
Universidade de São Paulo, Faculdade de Medicina, Laboratório de Patologia de Moléstias Infecciosas, Av. Dr. Arnaldo, 455, sala 1209, Cerqueira César, CEP 01246-903, São Paulo, SP, Brazil
Tel: +55 11 3061-8614

E-mail: mdlauren@usp.br

Received: 28 October 2022

Accepted: 3 February 2023

nonulcerated or atypical cutaneous leishmaniasis and visceral leishmaniasis, both caused by *Leishmania* (*L.*) *infantum chagasi* are endemic; conversely, in the Northern region of the country with a predominance of a more humid climate, ulcerated cutaneous leishmaniasis and mucocutaneous leishmaniasis, both caused by parasites of the subgenus *Viannia* [*Leishmania* (*V.*) *braziliensis* and *Leishmania* (*V.*) *panamensis*] are prevalent[5,6].

This clinical form is characterized by nodular and/or papular lesions surrounded by a hypopigmented halo with slow evolution that does not ulcerate regardless of the time of infection. VL and NUCL are independent clinical forms; one does not precede the other[4,7]. The skin lesions in NUCL are characterized by a mononuclear inflammatory infiltrate[7], with a predominance of IFN-γ-producing CD8+ T lymphocytes and M1-type macrophages. This immune response could be responsible for controlling tissue parasitism and the progression of skin lesions[8,9]. Nonetheless, the presence of Treg cells in these lesions could be responsible for the maintenance of low tissue parasitism. Thus, these cells collaborate to maintain a memory cellular immune response[10], as confirmed by the strong late hypersensitivity response observed in these patients[11].

Dogs are considered to be the main domestic reservoir and their skin parasitism has been associated with the transmission of *Leishmania (L.) infantum chagasi* to humans[12]. However, controversial results concerning the transmission potential of symptomatic and asymptomatic dogs have been reported. While some authors have shown that asymptomatic dogs are unable to infect vectors[13] or infect similar proportions to oligosymptomatic animals[14], others have shown that asymptomatic dogs are highly competent to transmit *Leishmania (L.) infantum chagasi* to the natural vector, and the skin parasitism was not crucial for the ability to infect *Lutzomyia longipalpis,* but the presence of *Leishmania* in lymph nodes was significantly related to a positive xenodiagnosis[15].

In the early 1990s, when Ponce *et al*.[4] described the nonulcerated cutaneous form caused by *Leishmania (L.) infantum chagasi* in El Tigre island, Amapala city, Southern Honduras, they suggested the dog as a possible reservoir for the parasite, since 16% (7/45) of the examined animals showed positive serology. However, since then, no further studies have been carried out to assess the role of the dog as a possible reservoir for *Leishmania (L.) infantum chagasi* in this region. Therefore, the main objective of this study was to investigate the presence of dogs with clinical signs and/or positive parasitological and immunological diagnosis in residences where individuals affected by NUCL live, and to correlate these findings with the role of the dog as a possible reservoir for the parasite.

MATERIALS AND METHODS

Study design

Mongrel dogs of different ages and sexes living in endemic areas of nonulcerated cutaneous leishmaniasis (NUCL) caused by *Leishmania (L.) infantum chagasi*[4,7] in El Tigre island, Amapala city, Southern Honduras, were investigated from February to March 2019.

A selection was made with the support of the Amapala Health Unit, according to the convenience of the houses whose inhabitants agreed to participate in the study, and where the dogs and at least one patient with NUCL lived together or close by. The diagnosis of the patients was made by direct examination of the skin lesion scraping and stained with Giemsa; later, it was confirmed by molecular tests, including the characterization of the parasite species by PCR-RFLP using *HSP*-70 followed by *Hae*II digestion[11,16]. A total of 50 houses were visited and 107 dogs were clinically examined and submitted to collection of biological material for parasitological and immunological diagnosis. The animals had access to intra, peri- and extradomiciles throughout the day. Before the collection of biological samples, the animals were submitted to a clinical examination performed by a veterinarian who considered relevant clinical signs suggestive of canine leishmaniasis, such as skin lesions, alopecia, lymphadenomegaly, onychogryphosis, apathy, anorexia, weight loss, and epistaxis, among others[17].

With the help of the owners, the animals were submitted to the Leishmanin Skin Test (LST), followed by the collection of 4 mL of whole blood by venipuncture, which was placed in tubes with EDTA. After centrifugation, the plasma was collected and stored in a -20 °C freezer until use. Plasma was used for serological tests: Rapid Test Dual Path Platform – RT-DPP (Bio-Manguinhos, BR) and in-house Enzyme-linked Immunosorbent Assay (ELISA). The buffy coat was also collected and stored in a -70 °C freezer for DNA extraction and PCR. The biological material collected was stored at the Genetic Investigation Center of the Autonomous University of Honduras and later sent to the Laboratory of Pathology of Infectious Diseases (LIM-50) of the Faculty of Medicine of the University of Sao Paulo, where it was processed.

Parasitological diagnosis

Histology

Skin biopsies from the pinna were collected using a 3-mm punch under aseptic conditions and local anesthesia. The biopsies were fixed in 10% formalin solution buffered

with 0.01 mol/L phosphate and processed by using the usual histological techniques. Paraffin sections of 4-5 µm were stained with hematoxylin and eosin (HE). The sections were observed under an optical microscope to characterize the histological features and tissue parasitism.

Immunohistochemistry

Paraffin histological sections of the skin biopsies were subjected to immunohistochemical reactions for the detection of parasites, according to Moreira *et al*.[18], using the total serum of mice chronically infected with *Leishmania* as a primary antibody and the LSAB kit (DakoCytomation, USA) for the development of the reaction and detection of parasites *in situ*.

PCR

Real-time PCR was performed in the buffy coat (BC) to quantify and identify the infectious agents[2].

DNA extraction from BC was performed with the Illustra Blood Genomic Mini Prep (GE Healthcare, USA) following the kit protocol. The DNA was stored at -20 °C until use. Primers targeting a small fragment (120 bp) of *Leishmania* kDNA, LEISH-1 (5'-AACTTTTCTGGTCCTCCGGGTAG-3') and LEISH-2 (5'-ACCCCCAGTTTCCCGCC-3') were used[7,19]. The amplification conditions were as follows: 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing of primers at 58 °C for 20 s and extension at 72 °C for 8 s. The negative results were checked by using mammalian β-actin primers. The cutoff (Ct) value of each sample was plotted against a standard curve generated with serial dilutions of *Leishmania (L.) infantum chagasi* DNA (MHOM/BR/72/BH-46) from 1×10^{6} to 1×10^{-4} parasites/µL to estimate the parasite load/µL.

After 35 cycles of amplification, a melting curve was generated with a ramp speed of 2.0 °C/s between 70 °C and 95 °C and analyzed by using Eppendorf Realplex software (version 2.0, Merck KGaA, Darmstadt, Germany). The species identification was based on the characteristic melting temperature (Tm) for *Leishmania (L.) infantum chagasi* (Tm = 83.5 ± 0.5°C, one peak)[7].

Immunological diagnosis

Leishmanin Skin Test (LST)

The leishmanin skin test was performed according to Silveira *et al*.[20]. An antigenic suspension was prepared from *Leishmania (L.) infantum chagasi* promastigotes (MCAO/BR/2003/M22697) at a concentration of 4×10^{8} parasites/mL in merthiolate solution (1:10,000). A volume of 100 µL was injected intradermally into the abdominal region of each dog. After 48–72 h, the area of erythema or nodules was measured and the dogs that presented erythema or nodules with a diameter ≥ 5 mm were considered to have a positive reaction for LST. As a negative control, Merthiolate solution (1: 10,000) was applied intradermally at an adjacent point at least 5 cm away from the leishmanin inoculation site.

DPP®

A chromatographic immunoassay based on Dual Path Platform technology (DPP® CVL rapid test, BioManguinhos, Rio de Janeiro, Brazil) was developed according to the manufacturer's recommendations[21].

In-house ELISA

An enzyme-linked immunosorbent assay was performed according to Laurenti *et al*.[21]. Briefly, a microtiter of 96-well flat bottoms was sensitized with 100 mL of crude total antigen, 10 mg/mL of the protein of *Leishmania (L.) infantum chagasi* promastigotes in a carbonate-bicarbonate buffer 0.1 M pH 9.5 and incubated in a humid chamber overnight at 4 °C. Then, the microtiter was washed with a 0.15 M pH 7.2 phosphate buffer containing 0.05% Tween-20 (PBS-T). The plates were blocked with a solution of 10% skimmed milk powder in PBS-T and incubated in a humid chamber for 2 h at 37 °C. After washing three times with PBS-T, the samples of the test sera and positive and negative controls were added in duplicate at a dilution of 1:400 in PBS-T and incubated at 37 °C for 1 h. Following a new wash, anti-canine IgG (A40-123AP) conjugated to alkaline phosphatase (Bethyl, USA) was added at a 1:2000 dilution in PBS-T and incubated at 37 °C for 45 min in a humid chamber. The development of the color reaction was performed with a chromogenic substrate (1.0 mg/mL pNPP, Sigma, USA) in a 0.1 M pH 9.5 carbonate-bicarbonate buffer with incubation at room temperature for 30 min. The reaction was stopped with 3 M NaOH, and the absorbance was read with a 405-nm filter. The cutoff of the reaction was calculated from the mean absorbance of the negative sera added to three standard deviations.

Data analysis

Statistical analysis was performed with GraphPad Prism (version 8.0, GraphPad, Boston, MA, USA). We used the Kolmogorov–Smirnov's test for normality. To correlate the different tests for the diagnosis of canine leishmaniasis, the Pearson's test was used for data with a Gaussian distribution, and the Spearman's test was used for those without a normal distribution. A significant difference was considered when $p < 0.05$.

Table 1 - Clinical data of households with dogs that live with NUCL patients on El Tigre island, Amapala city, Honduras, 2019 (n = 107).

	Weight loss	Onychogryphosis	Skin lesions	Alopecia
Positive	69 (64%)	5 (5%)	1 (1%)	8 (7%)
Negative	38 (36%)	102 (95%)	106 (99%)	99 (93%)

Ethical statement

The project was approved by the Research Ethics Committee of the Master of Infectious and Zoonotic Diseases of the National Autonomous University of Honduras (Nº 03-2014) and the Committee on Ethics in the Use of Animals and the National Council for the Control of Animal Experimentation of the Faculty of Medicine of University of Sao Paulo (Nº 172/14).

RESULTS

Clinical data

An average of 3 dogs per household were observed, ranging from 1 to 12 animals. Of the 107 dogs evaluated in the present study, 65 (61%) were males and 42 (39%) were females. The age of the animals ranged from 1 to 10 years, with 50% of them aged less than or equal to 3 years. Most of the animals showed a healthy appearance and a few of them presented clinical signs suggestive of canine leishmaniasis, 5% (5/107) onychogryphosis, 1% (1/107) skin lesion and 7% (8/107) alopecia, in addition to a slight weight loss found in 64% (69/107) of the dogs (Table 1).

Parasitological diagnosis

Parasitological diagnosis was performed by searching for *Leishmania* DNA through polymerase chain reaction in the buffy coat (PCR-BC), as well as by cutaneous histopathological studies using paraffin sections stained by hematoxylin (HE) and immunohistochemistry (IHC). The results are shown in Table 2. By PCR-BC, we observed 56% (60/107) of the dogs were positive; however, none of them showed any histopathological changes in the skin or parasite amastigotes by immunohistochemistry in the cutaneous tissue. Considering at least two clinical signs, from nine animals, six presented positive results in PCR-BC (66.7% of them). The average parasite number detected in the buffy coat was 6.09 parasites/µL, ranging between 0.221 and 50.2. Considering at least two clinical signs (9/107), the average parasite load in the buffy coat was 17.9, ranging between 5.99 and 24.4. The parasites were characterized as *Leishmania (L.) infantum chagasi* based on their typical melting temperature.

Table 2 - Parasitological diagnosis of the household with dogs that live with NUCL patients on El Tigre island, Amapala city, Honduras, 2019 (n = 107).

	Parasitological Diagnosis		
	Peripheral Blood	Skin	
	PCR-BC	HE	IHC
Positive	60 (56%)	0 (0%)	0 (0%)
Negative	47 (44%)	107 (100%)	107 (100%)

PCR-BC = Polymerase chain reaction – buffy coat; HE = hematoxylin and eosin; IHC = immunohistochemistry.

Immunological diagnosis

The immunological diagnosis evaluated the humoral immunity by testing for IgG in the plasma, using serological tests (DPP and ELISA) and cellular immunity by delayed-type hypersensitivity test (LST). The data are shown in Table 3. Serological assays showed that 12% (13/107) of dogs were positive in the DPP test and 37% (39/107) were positive in the ELISA, but only 7 animals were positive in the DPP test, confirming the result by ELISA, presenting no correlation between the serological tests ($p > 0.05$). However, the positivity in ELISA showed a direct correlation (concordance = 0.5981; kappa = 0.2219) with PCR-BC ($p = 0.0065$), pointing to replicability between tests even if weak. In relation to LST, only one dog (1%) showed a diameter greater than or equal to 5 mm.

Table 3 - Immunological diagnosis of the household with dogs that live with NUCL patients on El Tigre island, Amapala city, Honduras, 2019 (n = 107).

	Immunological Diagnosis		
	DPP	ELISA	LST
Positive	13 (12%)	39 (37%)	1 (1%)
Negative	94 (88%)	68 (63%)	106 (9%)

DPP = Dual Path Platform; ELISA = Enzyme-Linked Immunosorbent Assay; LST = Leishmanin Skin Test.

DISCUSSION

Dogs have been considered an important domestic reservoir for *Leishmania (L.) infantum chagasi* in urban

areas of transmission of human visceral leishmaniasis in the American continent, where the disease is zoonotic, since human cases have always been preceded by canine cases[12]. The vast majority of animals infected by *Leishmania (L.) infantum chagasi* remain asymptomatic for a long period of time, depending on their genetic and immunological background as well as their nutritional status, while some of those infected may progress to disease and present clinical signs suggestive of canine leishmaniasis, such as weight loss, apathy, skin lesions, alopecia, onychogryphosis, lymphadenomegaly, as well as laboratory alterations that indicate kidney and liver damage and hypergammaglobulinemia, a clinical picture that progresses to cachexia and death of the animal[22]. In a cross-sectional study carried out in an endemic area of high transmissibility in the Sao Paulo State, Brazil, a prevalence of 54.5% of canine infection by *Leishmania (L.) infantum chagasi* was observed based on direct parasitological diagnosis, with 67.5% presenting at least three clinical signs suggestive of canine leishmaniasis, characterizing a large number of animals as symptomatic[23]. However, another cross-sectional study in the Brazilian Amazon using an indirect fluorescent antibody test (IFAT-IgG), delayed-type hypersensitivity (DTH) and parasite research in popliteal lymph node aspiration determined the prevalence of canine *Leishmania (L.) infantum chagasi* infection in a cohort of 320 mongrel dogs living in an endemic area of visceral leishmaniasis transmission, and showed an overall prevalence of 43%. A higher prevalence of infection was observed in asymptomatic dogs, which was characterized by a majority of IFAT-IgG$^+$ showing the susceptible immunogenetic profile of canine infection[20].

In an endemic area of *Leishmania (L.) infantum chagasi* transmission in Southern Honduras, where nonulcerated cutaneous leishmaniasis is much more prevalent than visceral leishmaniasis, the presence of symptomatic dogs is not very evident. In this region, dogs, in general, do not show clinical signs suggestive of canine leishmaniasis, although previous seroepidemiological studies have shown a prevalence of 16%, suggesting the involvement of the dog in the transmission cycle of the parasite[4]. In this study, the clinical signs of canine leishmaniasis observed were very discreet, characterized by slight weight loss in 64% of the animals, which may not be very relevant considering the socioeconomic situation of the owners residing in the study area. In addition to slight weight loss, only 7% of the animals had areas of alopecia, 5% had onychogryphosis and 1% showed skin lesions; however, the majority of the dogs showed only one or two clinical signs, which does not characterize them as symptomatic animals[24]. Note that 21/69 (30%) of the dogs with weight loss, 6/8 (75%) of the dogs with alopecia, 2/5 (40%) of the dogs with onychogryphosis and 1/1 (100%) of the dogs with skin lesions showed the presence of parasite DNA in the buffy coat, suggesting that the clinical signs related to tegumentary injury could be associated with infection by *Leishmania (L.) infantum chagasi* as well as by another infectious agent or autoimmune disease, showing that the clinical signs are not pathognomonic of canine leishmaniasis.

Several conditions favoring the transmission of *Leishmania (L.) infantum chagasi* were observed in the study area; it was identified that 80% of the backyards of the houses visited had organic matter in decomposition and large amounts of common garbage; 24% of the dog tutors reported the presence of chicken coops in the peridomicile as well as other animals, including cats, pigs, rabbits, parrots and ducks. Furthermore, the circulation of Leishmania (L.) infantum chagasi in the sandfly species at El Tigre island was investigated. *Lutzo*myia longipalpis and Pintomyia evansi were the most abundant species, and parasite DNA was detected at rates of 9.4% and 2.7%, respectively[25].

Dogs are considered the main domestic reservoir for the parasite and both symptomatic and asymptomatic infected dogs are highly able to transmit protozoa to the natural vector[15]. The skin is the tissue through which the etiological agent is delivered at the moment of a sandfly bite and it is considered the main source of parasites to the vectors[12]. However, little is known about the underlying mechanisms of parasite resistance in the skin of animals affected by VL, which is an important parasite reservoir tissue, both in clinically healthy and sick infected dogs[26]. The main histological changes observed in macroscopically uninjured ear skin in dogs affected by canine leishmaniasis included the presence of a nonspecific chronic inflammatory infiltrate in the superficial dermis, characterized by the presence of macrophages, T lymphocytes (CD4$^+$ and CD8$^+$) and cells expressing inducible nitric oxide synthase; however, the inflammatory response of the skin has been directly correlated to tissue parasitism, but not to the clinical status[27]. Notably, another report showed that the skin parasitism of symptomatic and asymptomatic dogs did not differ, and no correlation was found between skin parasite load and transmissibility to sandflies. However, the presence of Leishmania in lymph nodes was found to be the most related finding to a positive xenodiagnosis, which is very reasonable because parasites leave the lymph nodes through lymphatic vessels and enter into the bloodstream, the source for female vector feeding[15].

In the present study, the histological analysis of the dog's skin did not show microscopic changes and no amastigotes were observed by immunohistochemistry; however, *Leishmania* DNA was present in the buffy coat,

although the detection of parasite DNA does not necessarily mean the presence of viable amastigotes in the sample. In the first phases of infection, the parasites can be lysed by the host's innate immune response, and the parasite fragments, still detected by molecular tools, stimulate the adaptive immune response with the production of memory cells and specific antibodies. In this sense, the overall seroprevalence of *Leishmania* infection in the present study was 41% (44/107), as determined by both serological tests, DPP and/or ELISA, and the presence of parasite DNA was confirmed in 94% of them.

The average parasite load in the buffy coat was 6.09 parasites/μL, ranging between 0.221 and 50.2. In a previous study by our group in an endemic area with high transmission of *Leishmania (L.) infantum chagasi*, the average parasite load in the total blood was 153 parasites/μL and ranged between 1.0 and 1,000, indicating that the dog is a good reservoir for parasites[19]. It is important to mention that, similar to the present study, the blood parasitism was equivalent between symptomatic and asymptomatic dogs, suggesting that dogs with and without clinical signs are able to transmit parasites to the vector, as already shown by other authors[14,28]. However, the parasite load observed in the present study was very low, which led us to question the real role of dogs as efficient parasite reservoirs on El Tigre island. The hypothesis that other domestic or wild animals have a more relevant role in the maintenance of the parasite's biological cycle cannot be excluded.

Molecular clock analysis demonstrated that *Leishmania (L.) infantum chagasi* from Honduras is considerably more ancestral (approximately 382,800 years) than *Leishmania (L.) infantum chagasi* from Brazil (143,300 years) and *Leishmania (L.) infantum* from the Old World (13,000 years), despite high similarity (99.9%) when all genome chromosomes were compared[2]. Its ancestry may be correlated to a very balanced parasite-host relationship, where the parasite uses the vertebrate host to maintain its biological cycle without causing tissue damage. It must be happening in Southern Honduras, since both human and canine infections show a benign character. Human infection by *Leishmania (L.) infantum chagasi* is characterized mainly by small papular and/or nodular skin lesions with very slow evolution that are never ulcerated, showing a proinflammatory tissue response with the presence of M1 macrophages and IFN-γ producing lymphocytes that is related to slight skin parasitism[7-9]. On the other hand, the dog showed very discreet clinical signs and did not show any skin lesions or parasitism in the histopathological study, only discrete systemic parasitism determined by molecular diagnosis.

CONCLUSION

Despite all the limitations of this study due to the difficulties of access and infrastructure in the endemic area, which did not allow a randomized study, the opportunity to follow up with the dogs by clinical and laboratory exams, and xenodiagnosis to verify the possibility of parasite transmission from dogs to sandflies, the data obtained lead us to question the role of domestic dogs as a good source of parasites to the vector in Southern Honduras.

Based on the absence of parasites in the skin and the low parasite load detected in the buffy coat, it seems that dogs do not represent a good source of infection for the vector in endemic areas of NUCL transmission in Southern Honduras. However, more systematic studies are necessary to rule out dogs as an important reservoir for *Leishmania (L.) infantum chagasi* in the Gulf of Fonseca region, Central America, where atypical cutaneous leishmaniasis caused by *Leishmania (L.) infantum chagasi* is endemic and prevalent in countries such as Honduras, Nicaragua and El Salvador.

AUTHORS' CONTRIBUTIONS

Conceptualization and methodology: ML, FTS, MMRH; investigation and resources: GBRS, WHSO, MMRH, ML, VLRM, CMSP, GVAF; formal analysis: ML, VLRM; writing – original draft: ML, VLRM; writing – review and editing: GBRS, WHSO, VLRM, MM, CRT, RRG, CMSP, GVAF, FTS, MMRH, ML; funding acquisition: ML, GBRS.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

FUNDING

This study was supported by the FAPESP, grant Nº 2014/50315-0; CNPq grant Nº 308817/2021-4; DICIHT-UNAH grant Nº PI-195-DICIHT; DSEP-UNAH and LIM50 HC-FMUSP.

REFERENCES

1. Silveira FT, Corbett CE. Leishmania chagasi Cunha & Chagas, 1937: indigenous or introduced? A brief review. Rev Pan-Amaz Saude. 2010;1:143-7.
2. Silveira FT, Sousa Junior EC, Silvestre RV, Costa-Martins AG, Pinheiro KC, Ochoa WS, et al. Whole-genome sequencing of Leishmania infantum chagasi isolates from Honduras and Brazil. Microbiol Resour Announc. 2021;10:e0047121.

3. Silveira FT, Sousa Junior EC, Silvestre RV, Vasconcelos dos Santos T, Sosa-Ochoa W, Valeriano CZ, et al. Comparative genomic analyses of new and old world viscerotropic leishmanine parasites: further insights into the origins of visceral leishmaniasis agents. Microorganisms. 2022;11:25.
4. Ponce C, Ponce E, Morrison A, Cruz A, Kreutzer R, McMahon-Pratt D, et al. Leishmania donovani chagasi: new clinical variant of cutaneous leishmaniasis in Honduras. Lancet. 1991;337:67-70.
5. Instituto de Enfermedades Infecciosas y Parasitología Antonio Vidal. Manual de manejo de enfermedades parasitarias prioritarias en Honduras. 2ª ed. Tegucigalpa: OPS; 2009.
6. Matute N, Espinoza C, Alger J, Padgett D, López E, Zúniga C. Caracterización clínico-epidemiológica de pacientes con leishmaniasis atendidos en el Hospital Escuela. Rev Med Hondur. 2009;77:7-15.
7. Sandoval Pacheco CM, Araujo Flores GV, Favero Ferreira A, Sosa Ochoa W, Ribeiro da Matta VL, Zúniga Valeriano C, et al. Histopathological features of skin lesions in patients affected by non-ulcerated or atypical cutaneous leishmaniasis in Honduras, Central America. Int J Exp Pathol. 2018;99:249-57.
8. Sandoval Pacheco CM, Araujo Flores GV, Gonzalez K, Gomes CM, Passero LF, Tomokane TY, et al. Macrophage polarization in the skin lesion caused by neotropical species of Leishmania sp. J Immunol Res. 2021;2021:5596876.
9. Sandoval C, Araujo G, Sosa W, Avalos S, Silveira F, Corbett C, et al. In situ cellular immune response in non-ulcerated skin lesions due to Leishmania (L.) infantum chagasi infection. J Venom Anim Toxins Incl Trop Dis. 2021;27:e20200149.
10. Araujo Flores GV, Sandoval Pacheco CM, Tomokane TY, Sosa Ochoa W, Zúniga Valeriano C, Castro Gomes CM, et al. Evaluation of regulatory immune response in skin lesions of patients affected by nonulcerated or atypical cutaneous leishmaniasis in Honduras, Central America. Mediators Inflamm. 2018;2018:3487591.
11. Sosa-Ochoa W, Zúniga C, Chaves LF, Araujo Flores GV, Sandoval Pacheco CM, Ribeiro da Matta VL, et al. Clinical and immunological features of human leishmania (L.) infantum-infection, novel insights Honduras, Central America. Pathogens. 2020;9:554.
12. Queiroz NM, Silveira RC, Noronha Jr AC, Oliveira TM, Machado RZ, Starke-Buzetti WA. Detection of Leishmania (L.) chagasi in canine skin. Vet Parasitol. 2011;178:1-8.
13. Verçosa BL, Lemos CM, Mendonça IL, Silva SM, Carvalho SM, Goto H, et al. Transmission potential, skin inflammatory response, and parasitism of symptomatic and asymptomatic dogs with visceral leishmaniasis. BMC Vet Res. 2008;4:45.
14. Michalsky EM, Rocha MF, Lima AC, França-Silva JC, Pires MQ, Oliveira FS, et al. Infectivity of seropositive dogs, showing different clinical forms of leishmaniasis, to Lutzomyia longipalpis phlebotomine sand flies. Vet Parasitol. 2007;147:67-76.
15. Laurenti MD, Rossi CN, Matta VL, Tomokane TY, Corbett CE, Secundino NF, et al. Asymptomatic dogs are highly competent to transmit Leishmania (Leishmania) infantum chagasi to the natural vector. Vet Parasitol. 2013;196:296-300.
16. Araujo Flores GV, Sandoval Pacheco CM, Ferreira AF, Tomokane TY, Nunes JB, Colombo FA, et al. Leishmania (L.) infantum chagasi isolated from skin lesions of patients affected by non-ulcerated cutaneous leishmaniasis lead to visceral lesion in hamsters. Parasitol Int. 2023;93:102723.
17. Paltrinieri S, Solano-Gallego L, Fondati A, Lubas G, Gradoni L, Castagnaro M, et al. Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. J Am Vet Med Assoc. 2010;236:1184-91.
18. Moreira MA, Luvizotto MC, Garcia JF, Corbett CE, Laurenti MD. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. Vet Parasitol. 2007;145:245-52.
19. Aschar M, Oliveira ET, Laurenti MD, Marcondes M, Tolezano JE, Hiramoto RM, et al. Value of the oral swab for the molecular diagnosis of dogs in different stages of infection with Leishmania infantum. Vet Parasitol. 2016;225:108-13.
20. Silveira FT, Carneiro LA, Ramos PK, Chagas EJ, Lima LV, Campos MB, et al. A cross-sectional study on canine Leishmania (L.) infantum chagasi infection in Amazonian Brazil ratifies a higher prevalence of specific IgG-antibody response than delayed-type hypersensitivity in symptomatic and asymptomatic dogs. Parasitol Res. 2012;111:1513-22.
21. Laurenti MD, Leandro Jr MV, Tomokane TY, De Lucca HR, Aschar M, Souza CS, et al. Comparative evaluation of the DPP® CVL rapid test for canine serodiagnosis in area of visceral leishmaniasis. Vet Parasitol. 2014;205:444-50.
22. Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L. Canine leishmaniosis-new concepts and insights on an expanding zoonosis: part one. Trends Parasitol. 2008;24:324-30.
23. Laranjeira DF, Matta VL, Tomokane TY, Marcondes M, Corbet CE, Laurenti MD. Serological and infection statuses of dogs from a visceral leishmaniasis-endemic area. Rev Saude Publica. 2014;48:563-71.
24. Mancianti F, Gramiccia M, Gradoni L, Pieri S. Studies on canine leishmaniasis control. 1. Evolution of infection of different clinical forms of canine leishmaniasis following antimonial treatment. Trans R Soc Trop Med Hyg. 1988;82:566-7.
25. Sosa-Ochoa W, Varela Amador J, Lozano-Sardaneta Y, Rodriguez Segura G, Zúniga Valeriano C, Araujo GV, et al. Detection of Leishmania infantum DNA in Pintomyia evansi and Lutzomyia longipalpis in Honduras. Parasit Vectors. 2020;13:593.
26. Solano-Gallego L, Riera C, Roura X, Iniesta L, Gallego M, Valladares JE, et al. Leishmania infantum-specific IgG, IgG1

and IgG2 antibody responses in healthy and ill dogs from endemic areas: evolution in the course of infection and after treatment. Vet Parasitol. 2001;96:265-76.

27. Rossi CN, Tomokane TY, Batista LF, Marcondes M, Larsson CE, Laurenti MD. In situ cutaneous cellular immune response in dogs naturally affected by visceral leishmaniasis. Rev Inst Med Trop Sao Paulo. 2016;58:48.

28. Soares MR, Mendonça IL, Bonfim JM, Rodrigues JA, Werneck GL, Costa CH. Canine visceral leishmaniasis in Teresina, Brazil: relationship between clinical features and infectivity for sand flies. Acta Trop. 2011;117:6-9.