# Study on the zoonotic cycle of tegumentary leishmaniasis in an endemic area of a metropolitan region in the Northeastern region of Brazil

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## ABSTRACT

This study was conducted to characterize the transmission cycle of the tegumentary leishmaniasis (TL) in an old colonization area at Pernambuco State, Brazil. The aims were to identify autochthonous cases, sandflies fauna, domestic animals as possible reservoir hosts and the *Leishmania* species involved in this endemic area. A total of 168 suspected human cases of TL and 272 domestic animals (canine, feline, equine, goat, and sheep) were included. The sandflies were captured and identified by species. Patients were predominantly male and the average age was 37±18.1 years old. Of 85 patients who had skin lesions, 25.6% of them had direct positive smears for TL and 34 isolates were identified as *Leishmania* (*Viannia*) *braziliensis.* The confirmation for TL diagnosed by molecular detection (PCR) was almost three times more sensitive than the direct test [p < 0.001; PR = 2.72] associated with clinical examination. The Kappa test on PCR between two different specimens, biopsy, and skin lesion *swab* was 60.8% (p < 0.001). More than 200 specimens of sandflies (80 males and 159 females) were captured and identified as *Lutzomyia whitmani* (99.6%) and *Lu. evandroi* (0.4%). The detection of *L. (V.) braziliensis* by Real-Time PCR in the blood of a captured fed female was positive in 59.3% of *Lu. whitmani*. Of the 272 domestic animals included, 61.76% were male (n = 168). Thirty-six animals (13.2%) had lesions compatible with TL (34 dogs, 1 cat and 1 sheep) and 3 of them, all dogs, had lesions on the snout, showing destruction of cartilage and mucosa. The study suggests the participation of domestic animals as possible reservoirs. However, further studies are necessary to better understand the transmission cycle and take recommended measures in order to control the disease.

**KEYWORDS:** Transmission cycle. Eco-epidemiology. Tegumentary leishmaniasis. *Leishmania (Viannia) braziliensis*.

## INTRODUCTION

Tegumentary leishmaniasis (TL) is a zoonotic parasitic disease disseminated in the Old and New Worlds, endemic in 92 countries, with an estimate of one million new cases per year[1,2]. In Brazil, there were 15,484 new cases in 2019[3]. In the Americas, TL is transmitted through the bite of female phlebotomine sandflies of the *Lutzomyia* genus. The clinical forms of the disease are cutaneous (localized,

disseminated, diffuse, *recidivans* cutis) and mucocutaneous, and in some cases it may evolve to spontaneous healing, except for the diffuse form[1,4].

Between 2007 and 2017, 235,301 cases were reported in all regions of Brazil and the North and Northeastern regions were the most affected with 101,332 and 72,395 cases, respectively[5]. In the Northeast, the predominance of cases occurs in locations with different agricultural plantations, some remnants of the Atlantic rainforest and secondary vegetation, whose aspects contribute to the spread of phlebotomines and the enzootic maintenance cycle with the involvement of wild and synanthropic reservoir hosts[6]. The modification of the natural environment caused by anthropic action leads to the adaptation of vectors and reservoirs with the zoonotic disease cycle in the peridomicile areas, affecting both humans and domestic animals[7,8].

In Brazil, there are seven different predominant species of *Leishmania,* and *Leishmania (Viannia) braziliensis* is the predominant species. This species causes the most severe form of the disease, which can compromise the patient's mucosa and cartilage. It is also the predominant etiologic agent of the disease in Pernambuco (PE)[4,6,8,9].

The clinical diagnosis is based on anamnesis of the patient with clinical suspicion, epidemiological data (individual residing or transiting in an endemic area) and laboratory tests[4,6]. The gold standard method for the laboratory diagnosis of TL is the direct parasitological examination by microscopy on a slide with impression of the biopsy or scraping of the lesion[6]. Molecular tests, such as PCR and Real-Time PCR (qPCR), are very important in diagnosis because of their high sensitivity and specificity[10].

The aim of this study was to characterize the zoonotic cycle through the description of several aspects of the eco-epidemiology of TL in an endemic area of the Northeast of Brazil. It also aimed to identify autochthonous cases, characterize phlebotomine fauna and domestic animals as possible involved reservoir hosts.

## MATERIALS AND METHODS

A cross-sectional study was conducted in the Moreno municipality (Metropolitan Region of Recife, PE, Brazil), in the Atlantic Forest area, located at an altitude of 96 meters, with a rainy tropical climate and an average annual rainfall of 1,309.9 mm, and approximately 63,000 inhabitants, where 17% are in urban households[11]. The majority of the studied region is covered by a monoculture of sugar cane or banana plantations.

The sample of autochthonous human cases included residents in endemic locations, who sought the primary health care provided by Programa Saude da Familia (PSF - Family Health Program) between 2015 and 2018. After the anamnesis, data were collected for clinical and epidemiological diagnosis, and clinical samples by biopsy or lesion scraping. The samples were analysed for diagnostic confirmation at the Laboratory of Immunoparasitology of Aggeu Magalhaes Institute/Fiocruz-PE.

Case suspect: individuals with compatible clinical signs, such as the presence of a cutaneous ulcer, with a granular background and infiltrated edges, from an endemic area or someone who had visited it.

Case definition: individuals with compatible clinical signs, epidemiological data and positive parasitological or molecular tests.

The parasitological diagnosis was performed by visualizing the parasite in the smear through an optical microscope[6], and the lesion fragments or scrapings obtained were inoculated in culture medium and in hamsters (*Mesocricetus auratus*) for the *in vivo* isolation of *Leishmania* spp.[8]. The identification and characterization of the parasite was previously carried out through the analysis of the electrophoretic profile with isoenzymes (MLEE)[8,12], Leishmania Collection of the Oswaldo Cruz Institute/ FIOCRUZ, Rio de Janeiro - CLIOC/RJ. The samples of domestic animals residing in Engenho Jardim and Engenho Cumaru (Moreno) included canines, felines, horses, goats, and sheep examined by a veterinarian from the Municipal Health Surveillance Center between 2017 and 2018. Blood and *swab* samples from ocular conjunctiva were collected and, for those who had active lesion, scarification, puncture, or swab of skin lesion was performed.

The sandflies were captured between January 2017 and December 2018 inside the houses where human or suspected cases of TL were notified. For these captures, the light traps model CDC (Centers for Disease Control) was installed in areas of banana plantations and in the remnants of the Atlantic Forest, from the twilight period onwards[13]. The specimens were separated by gender and morphological identification according to Young and Duncan[14] and stored in ethanol 70% at room temperature until the DNA extraction.

Each entire female body specimen was submitted for molecular testing by qPCR to detect natural infection. For the molecular tests, DNA extraction and purification from human biological samples were performed using the commercial kit QIamp® DNA mini kit (Qiagen, Valencia, USA) according to the protocol of the manufacturer. The extraction of DNA was performed individually according to Solano *et al.*[15].

In order to confirm and verify the extraction efficiency, some random samples had DNA quantified on the NanoDrop spectrophotometer (Themo Scientific®, model 2000/2000c, Waltham, Massachusetts, USA).

The DNA from human samples and from the reference strain CLIOC IOC/L0566 (MHOM/BR/75/M2903) of *L. (V.) braziliensis* was amplified by PCR, using oligonucleotides B1/B2 target of *Leishmania* kDNA of the *Viannia* subgenus, performed according to de Bruijn and Barker[16]. Visualization of the amplification products was done by electrophoresis in 1% agarose gel, stained with ethidium bromide[17]. Visualization of the DNA bands occurred in a photo documentation system for electrophoresis gel (L-PIX Touch–Loccus, Cotia, SP, Brazil).

For molecular diagnosis in domestic animals, DNA from blood samples, *swab* from lesion secretion and from ocular conjunctiva were amplified by qPCR, as well as for the phlebotomine. The kDNA fragments of the subgenus (*Viannia*) used as qPCR target had 138 base pairs and were amplified using a reference thermocycler (QuantiStudio 5 Applied Biosystems®, Waltham, Massachusetts, USA), using Software 7500 (version 2.0.5, Applied Biosystems®). The qPCR reactions followed the conditions of Cavalcanti *et al.*[18].

Weather data on temperature, relative humidity and precipitation from the Pernambuco Water and Climate Agency were analyzed and compared with the monthly density of the collected sandflies.

Statistical analysis of frequencies, Kappa tests, Pearson's and Yates' chi-square tests and Fisher's Exact tests were performed using the IBM SPSS Statistical Software (Version 20.0, IBM SPSS, Armonk, NY, USA). Comparison of proportions was calculated in MedCalc Statistical software (version 20.110 MedCalc Software, Ostend, Belgium), considering $\alpha = 5\%$. The risk of positivity (PR) was calculated using the prevalence ratio and respective 95% confidence intervals (CI). The calculation to analyze the leukocyte and plasma samples submitted to qPCR was performed according to Medronho *et al.*[19].

The study in humans was approved by the Ethics Committee of University Fernando Pessoa (SSD-18072016), with written informed consent. The study on domestic animals was approved by the Ethics Committee on the Use of Animals of the Aggeu Magalhaes Institute/Oswaldo Cruz Foundation in Pernambuco (115/2017), with free and informed consent of the research by the tutors.

## RESULTS

The aspect of the endemic region studied can be viewed in Figure 1, where a house can be seen among remains of the Atlantic Forest and sugarcane and banana plantations.

168 human cases with suspected TL were included, aged between 5 and 74 years old (mean 37±18.1), with the majority (63.1%) being male. Of the suspected cases,



**Figure 1 -** Panoramic view of Cumaru Mill in Moreno municipality.

112 (66.7%) were confirmed to have TL, 48 (28.6%) did not have the disease and 8 (4.8%) had no laboratorial confirmation.

The clinical forms found were localized cutaneous (92.9%), *recidivans cutis* (3.6%), disseminated (2.7%), and one that evolved to scar (0.8%). Most patients had a single skin lesion (81.2%) and the remaining had more than ten (multiple) lesions, with diameters between 0.5×0.5 cm and 9.0×7.5 cm and evolution periods between 15 days and 10 months. As for the location of the lesion, the majority (75.9%) occurred in the lower and upper limbs, while the others were affected in different regions of the body. Lesions of the type ulcerated (75.6%) were presented as plaque, crusted, verrucous, erythematous, disseminated, *recidivans* cutis and mucocutaneous, while the others healed spontaneously. Two patients had coinfection (*pyoderma gangrenosum* and psoriasis). Some patients had other diseases, such as vascular disease, stasis ulcer, tuberculosis, impetigo, fungi, chronic eczema, and folliculitis. After the diagnosis of TL, all patients received specific treatment.

The results of the laboratorial tests are shown in Table 1. It was found that the molecular test by PCR identified 54.8% and the parasitological test by direct research was found in 25.6% of cases, respectively. The concordance of the PCR test between samples collected by tissue fragment (biopsy) and lesion *swab* was 60.8% (Kappa value; p < 0.001).

The relation between gender, age, and skin color did not show any statistical differences in PR. Having complete or incomplete primary education is a protective factor for infection by *L. (V.) braziliensis* compared to illiterates (PR = 0.75; [CI 95%: 0.58-0.99]). The type of occupation seems to be associated with an increased risk of having the disease, but it did not reach statistical significance (Table 2).

Of all the 239 specimens (80 males and 159 females) of phlebotomine sandfly captured, almost all were identified as *Lu. whitmani*, and one female was *Lu. evandroi*.

During the study period, the presence of the sandfly species occurred predominantly in the months of August 2017 and July 2018. At first, the room temperature varied between 20 and 28 ºC, the relative humidity between 51 and 70.4% and the rainfall was 123.2 mm (with annual average

**Table 1** - Results of laboratory tests on human samples.

| Laboratory tests (n = 168) | Positive result n (%) | Negative result n (%) | Not carried out or contaminated n (%) |
|---|---|---|---|
| **Parasitological** | | | |
| - Direct search (smear slide) | 43 (25.6) | 42 (25.0) | 83 (49.4) |
| - *In vivo* culture | 20 (11.9) | 23 (13.7) | 125 (74.4) |
| - *In vitro* culture | 14 (8.3) | 5 (3.0) | 149 (88.7) |
| **Molecular** | | | |
| - Conventional PCR | 92 (54.8) | 55 (32.7) | 21 (12.5) |

**Table 2 -** Characterization of the sample of cases with suspected TL according to the diagnosis and respective risk of positivity with a confidence interval of 95%.

| Characteristics | TL diagnosis | | | | |
|---|---|---|---|---|---|
| | Positive n (%) | Negative n (%) | Value of p | PR** [CI: 95%] | Total (%) |
| **Gender (n = 160)** | | | | | |
| Male | 69 (61.6) | 29 (60.4) | 0.971 | 1.02 [0.82-1.25] | 98 (61.2) |
| Female | 43 (38.4) | 19 (39.6) | | 1.0 | 62 (38.8) |
| **Age group (n = 158)*** | | | | | |
| 5 to 18 | 19 (17.0) | 10 (21.8) | | 1.0 | 29 (18.4) |
| 19 to 29 | 24 (21.4) | 7 (15.2) | 0.773 | 1.18 [0.85-1.64] | 31 (19.6) |
| 30 to 49 | 41 (36.6) | 18 (39.1) | | 1.06 [0.78-1.45] | 59 (37.3) |
| 50 to 74 | 28 (25.0) | 11 (23.9) | | 1.10 [0.79-1.52] | 39 (24.7) |
| **Skin color (n = 160)** | | | | | |
| White | 16 (14.3) | 8 (16.7) | | 1.0 | 24 (15.0) |
| Mixed ethnicity | 84 (75) | 33 (68.8) | 0.694 | 1.27 [0.5-3.26] | 117 (73.1) |
| Black | 12 (10.7) | 7 (14.6) | | 0.86 [0.24-3.02] | 19 (11.9) |
| **Level of education (n = 160)** | | | | | |
| Illiterate | 27 (24.1) | 8 (16.7) | | 1.0 | 35 (21.9) |
| Incomplete or complete elementary school | 39 (34.8) | 28 (58.3) | 0.021 | 0.75 [0.58-0.99] | 67 (41.9) |
| Completed high school or more | 46 (41.1) | 12 (25.0) | | 1.03 [0.82-1.29] | 58 (36.2) |
| **Occupation (n = 160)** | | | | | |
| Agricultural professions | 34 (30.3) | 18 (37.5) | | 1.08 [0.43-2.68] | 52 (32.5) |
| Other professions (indoor or mixed environment) | 57 (50.9) | 18 (37.5) | 0.293 | 1.81 [0.75-4.39] | 75 (46.9) |
| No profession (children and students) | 21 (18.8) | 12 (25.0) | | 1.0 | 33 (20.6) |

*There was no information for 2 individuals; **PR – risk of positivity calculated as prevalence ratio.

of 1,619.8 mm). Temperature average in the capture of July 2018, varied between 22 ºC and 26 ºC, the relative humidity between 57 and 79% and the rainfall was 4.0 mm (with an annual average of 1,331.4 mm).

In the peridomicile, phlebotomine infestation was more common in chicken coops (92%) than in stables, but, in general, shelters for domestic animals were close to homes and with poor hygiene conditions.

The specimens collected were submitted to qPCR for confirmation of the infection by *L. (V.) braziliensis*. Among those classified as fed (with visible blood in the abdomen, n = 27), 59.3% (n = 16) were positive for the parasite. Among unfed females (no visible blood on the abdomen, n = 132), 33.3% were positive (n = 44) for *L. (V.) braziliensis*. The average concentration level of DNA

of *L. (V.) braziliensis* among the fed female sandflies was 10.07±28.28 fg (ranging from 0 to 119.669 fg) and, in the non-fed, 2.79±11.63 fg (ranging from 0 to 119.669 fg). Among all captured females (visibly fed and not fed), the infection rate was 37.7%.

Of the 272 domestic animals included in the study, 212 were dogs (*Canis familiaris*), 21 were cats (*Felis cattus*), 33 were horses/donkeys (*Equus caballus /Equus asinus*), 5 were goats (*Capra aegagrus hircus*) and 1 was a sheep (*Ovis aries*). They had different ages, breeds, and sizes. More than 70% of all animals were medium or large and only 13.2% (34 dogs, 1 cat and 1 sheep) had skin lesions.

Of the 272 animals in the study, 255 blood samples and 192 ocular conjunctival *swabs* were collected and submitted to qPCR. The positivity of the skin lesion smear in qPCR

from domestic animals is described in Table 3. It was found that 174 animals (64%) were positive for *L. (V.) braziliensis*, of which 52.6% were asymptomatic. Of the animals with a suspicious lesion, 5 dogs were positive for sporotrichosis and 31 were positive for TL. In the parallel analysis between the qPCR results and the presence or absence of a skin lesion, there was 87.3% concordance of the negative results and 12.6% of the positive results (Table 4).

The comparison of positivity between the different biological samples collected from domestic animals showed greater positivity in the *swab* from the skin lesion (85.7%) than in whole blood (73.3%) (Table 5).

## DISCUSSION

According to the Health Surveillance Secretary of the Ministry of Health[20], the Pernambuco State recorded an average of 38 cases of TL annually. In 2015, there was a doubling of the number of cases in the Moreno municipality, which on average had 50 cases per year (unpublished data). The municipality is considered a low-risk area for TL in terms of infection: from 2018 to 2020, 35 cases were confirmed in the region. It had an average incidence of 11.67/year (between 2018 and 2020), with a ranging from 8 (in 2020) cases to 16 (in 2018). Despite the low-risk area for TL, Moreno is among the seven municipalities with the highest incidence of annual cases in the Pernambuco State[20].

In the present study, the individuals were mostly male, of an economically active age, with a pattern of populations vulnerable to infection by *Leishmania* spp. in rural areas[21]. The disease affects different ages, including children[9,22,23]. A previous study in the same population found a positivity

**Table 3 -** qPCR results for detection of *Leishmania* spp. in blood samples, conjunctival swab or lesion swab from domestic animals residing in the study regions.

| Domestic animal | Biological sample | Positive Result n (%) | Not detected n (%) | Not collected n (%) | Total |
|---|---|---|---|---|---|
| Dog | Blood | 141 (66.5) | 63 (29.7) | 8 (3.8) | 212 |
| | *Swab* | 38 (17.9) | 115 (54.3) | 59 (27.8) | |
| Cat | Blood | 11 (52.4) | 1 (4.8) | 9 (42.8) | 21 |
| | *Swab* | 5 (23.8) | 8 (38.1) | 8 (38.1) | |
| Horse Donkey | Blood | 19 (57.6) | 14 (42.4) | 0 (0.0) | 33 |
| | *Swab* | 5 (15.1) | 16 (48.5) | 12 (36.4) | |
| Goat | Blood | 2 (40) | 3 (60) | 0 (0.0) | 5 |
| | *Swab* | 1 (20) | 3 (60) | 1 (20) | |
| Sheep | Blood | 1 (100) | 0 (0.0) | 0 (0.0) | 1 |
| | *Swab* | 0 (0.0) | 1 (100) | 0 (0.0) | |
| Total | Blood | 174 (64) | 81 (29.8) | 17 (6.2) | 272 |
| | *Swab* | 49 (18) | 143 (52.6) | 80 (29.4) | |

**Table 4 -** qPCR result as a function of the existence, or not, of a skin lesion compatible with TL.

| qPCR (parallel analysis)* | Skin lesion | | |
|---|---|---|---|
| | Yes n (%) | No n (%) | Total n (%) |
| Positive | 24 (12.6) | 167 (87.4) | 191 (100) |
| Negative | 9 (12.7) | 62 (87.3) | 70 (100) |
| Total | 33 (12.6) | 229 (87.4) | 262** (100) |

*Parallel analysis means that every type of biological sample was analyzed in parallel: when all qPCR results were negative, the overall "qPCR" result was negative; when at least one qPCR result in any biological sample was positive, the overall "qPCR" result was positive; **it was not possible to obtain qPCR results of 10 animals.

**Table 5 -** Comparison of positivity between different biological samples of domestic animals.

| qPCR - Type of biological sample | Value of p | Chi-squared | Difference | Confidence interval 95% | Positivity in qPCR |
|---|---|---|---|---|---|
| | | | | | Whole blood* (leukocytes + plasma) = 73.3% |
| Whole blood vs. conjunctival swab | <0.001 | 98.40 | 47.5% | 38.71-55.12 | *Conjunctival Swab* = 25.8% |
| Whole blood vs. skin lesion swab | 0.464 | 0.54 | 12.4% | -24.97-25.46 | skin lesion swab 85.7% |

*Parallel analysis means that every type of biological sample was analyzed in parallel: when all qPCR results were negative, the overall "qPCR" result was negative; when at least one qPCR result in any biological sample was positive, the overall "qPCR" result was positive.

of 22.4% with a positive Montenegro skin test for previous infection[24]. Twenty years later, this study found a 66.7% positivity for TL infection (unpublished data).

Regarding the characteristics of the disease, several clinical forms of TL were identified, with a predominance of the simple ulcerated form, which is corroborated by other studies[4,21]. In Brazil, 90% of reported cases present the cutaneous form, whereas the mucocutaneous form varies from 3 to 6% of the cases, depending on the region[23,25]. Clinical manifestations are variable and dependent on the associated *Leishmania* species and the host's immune response[1,6]. Patients from the locations of this study presented co-infection with some bacteria species, *pyoderma gangrenosum*, psoriasis and other diseases, such as vascular diseases, stasis ulcer, impetigo, chronic eczema, folliculitis, STDs, tuberculosis — some with dermatological manifestations like TL. This makes the diagnosis of TL difficult[6], hence the need for specific and sensitive diagnostic tests for TL[25].

Direct examination results were higher than those from the *Leishmania* isolates obtained in culture[26]. The isolates obtained from hamsters (*Mesocricetus auratus*) had a higher positivity than the isolates from culture medium, but without statistical significance. The *Leishmania* species circulating in the region was identified in previous studies as *L. (V.) braziliensis*[4,8,27] and confirmed by specific qPCR tests. *L. (V.) braziliensis* is the predominant species throughout the country and is present in several municipalities in Pernambuco State[4,8,9,28].

The molecular tests used have high sensitivity and specificity, in addition to being fast and effective[16,17,29]. PCR has detection sensitivity up to 1 fentogram (10-15g) of *Leishmania* DNA, which corresponds to 1/10 of the parasite[16]. This method has been standardized in tissue samples (invasive methods) to identify *Leishmania* species in subclinical cases of the disease, or with low parasite load, following up on treatment and distinction between active and past infections[29].

It was demonstrated that PCR is better to define the patient diagnosis of TL in all types of the analyzed biological samples. Therefore, the combination of minimally invasive sample collection procedures (such as the *swab* of lesion, salivary fluid, and blood) with molecular PCR test for diagnostic confirmation of TL is very important[25,29-31]. Especially when compared with classic parasitological tests with lower positivity[10,26,32] and need for invasive collection (lesion puncture and biopsy).

The concordance in PCR between the tissue fragments and *swab* samples from the lesion had a very significant index according to Cohen's Kappa[31], which demonstrates the effectiveness of the *swab* collection technique in relation to biopsy[31]. The divergence of results can be attributed to the low number of parasites in the lesion or in the exudate, as the parasites are not evenly distributed in the lesion, which can lead to false negatives[32]. Amplification of *L. (V.) braziliensis* DNA by PCR in blood samples from patients was found to be low compared to the tissue biopsy samples, due to the scarcity of circulating parasites in this biological sample[33].

The sandflies species most involved in the transmission in Brazil are *Lu. whitmani*, *Lu. intermedia*, *Lu. umbratilis*, *Lu. wellcomei*, *Lu. flaviscutellata* and *Lu. migonei*[34]. In the present results, the predominance of *Lu. whitmani* was observed as the main species and probable vector in this region. Captured species were done in chicken coops and stables located in the peridomicile. The positivity to *L. (V.) braziliensis* in these sandflies measured by qPCR in the studied endemic region was very high. These results strongly indicate the natural infection by *L. (V.) braziliensis* in *Lu. migonei*. Among fed phlebotomine, positivity was 59.3% and in those without visible blood (not fed), 33.3%.

The low number of captured phlebotomine may be due to the use of herbicides and fire, as mentioned by Nasser and Will[35]. These factors cause changes in the feeding habits when adapting to the environment modified by anthropic action. The climate changes also interfere with the biological behaviour of the fauna and, consequently, with their feeding role, modifying the frequency of blood meals[36].

According to Brito *et al.*[24], the expansion of agriculture and construction of houses close to the forests favors the risk of infection due the presence of domestic and synanthropic animals naturally infected close to the houses. On the other hand, the adaptation of phlebotomine to the modified environment increases transmissibility, as well as the expansion of the zoonotic cycle of TL through the contact between these hosts and the human population more frequently.

*Lu. whitmani* is the most important species in the transmission of TL in Brazil. Our results showed positivity for *L. (V.) braziliensis* in specimens collected from *Lu. whitmani*. An entomological survey in 2001 collected 444 specimens of *Lu. whitmani* in this municipality, also showing the predominance of this species in eleven other locations in the municipality (unpublished data). Between 2011 and 2012, another study confirmed the presence of the species in the intradomicile and peridomicile, with 3,071 specimens collected, of which 2,919 *Lu. whitmani*, 122 *Lu. evandroi* and 30 *Lu. choti*. Of the 37 sandflies species identified in Pernambuco, *Lu. whitmani* was the most predominant[37].

The domestic animals examined in this study showed a high positivity rate for TL through qPCR, especially in blood samples, which confirms the high prevalence of infection in the region, even with some asymptomatic

animals. Dogs were the species with highest percentage of positivity in the blood. As they transit through more regions and different areas of the peridomicile, they may have a greater chance of being bitten. *Equidae* are also parasitized by *Leishmania* spp. Several studies have already confirmed the presence of *L. (V.) braziliensis* in these domestic animals residing in endemic areas[9,38,39].

A sheep with a lesion like TL (uncommon symptomatic condition) was also identified, confirmed by direct examination for TL. A similar case occurred in South Africa, where a sheep presented a lesion that evolved to spontaneous healing[7]. According to Dantas-Torres[40], the diagnosis of asymptomatic domestic animals is important, as they may be reservoirs of *Leishmania* spp.

The knowledge of eco-epidemiology of the studied region, transmission control through early diagnosis and treatment are measures which collaborate with prevalence and incidence's decrease of leishmaniasis, according to goals established by PAHO/WHO for 2030[3]. Also, the main measures of control for this disease, according to the Brazilian Ministry of Health are: avoiding living close to remnants of forest, netting on doors and windows, and applying repellent on the body[6].

## CONCLUSION

In conclusion, our findings demonstrate that the epidemiology of TL associated with *L. (V.) braziliensis* in this municipality of the Zona da Mata region has different characteristics, when compared with previous studies in the same region over time[4,25], to those observed in other regions of Brazil. In this sense, more studies are necessary, especially on the role of domestic animals, to support their real contribution to the disease transmission cycle and maintenance, and also to contribute to the adoption of TL control recommendations in endemic areas. It is also recommended that, within the scope of the Primary Health Care program and epidemiological surveillance, community health agents promote campaigns aimed to improve the spread of information on the transmission cycle, signs, and symptoms of disease and its care to the human populations living in the endemic areas of the country.

## CONFLICT OF INTERESTS

None to declare.

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