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Beatriz Maria de Almeida Braz

LEISHMANIOSE VISCERAL CANINA: ESTUDO EPIDEMIOLÓGICO E GEOESPACIAL, ASPECTOS ECOLÓGICOS E PESQUISA DE INFECÇÃO NATURAL EM *Lutzomyia longipalpis*

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Beatriz Maria de Almeida Braz

Leishmaniose visceral canina: estudo epidemiológico e geoespacial, aspectos ecológicos e pesquisa de infecção natural em *Lutzomyia longipalpis*

Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal da Universidade Federal de Campina Grande como requisito parcial para obtenção do título de Doutora em Ciência e Saúde Animal.

Profª Draª Marcia Almeida de Melo
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APROVADO EM 21/02/2020

EXAMINADORES

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Unidade Acadêmica de Medicina Veterinária/CSTR/UFCG
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Membro Externo

Profa. Dra. Carolina de Sousa Américo Batista Santos
Unidade Acadêmica de Medicina Veterinária/CSTR/UFCG
Membro Externo

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RESUMO

A leishmaniose visceral (LV) é uma doença tropical negligenciada, com 94% dos novos casos distribuídos em países como Etiópia, Índia, Quênia, Somália, Sudão, Sudão do Sul e Brasil. A infecção caninaé indicativa de risco para a infecção humana, e devido ao estreito convívio de cães no ambiente domiciliar, estes animais se tornam importantes reservatórios e fonte de infecção. Esta tese é composta por três capítulos. No capítulo I, foi determinada a prevalência de leishmaniose visceral canina (LVC) no município de Mãe D'Água e estudada a epidemiologia da zoonose por meio da análise dos fatores de associação e a sua distribuição através de georreferenciamento. Para isso, amostras de sangue de 150 cães provenientes das zonas urbana e rural do município foram coletadas. A prevalência de anticorpos anti- *Leishmania infantum* foi de 18,6% (28/150), sendo 10,6% (8/75) na zona urbana e 26,6% (20/75) na zona rural. A Zona rural foi apontada como fator de risco (*Odds ratio* = 2,93); a permanência do animal solto à noite (*Odds ratio* = 0,33) e a realização de vermifugação (*Odds ratio*= 0.30) foram classificados como fatores de proteção. A análise espacial demonstrou a formação de um *cluster* primário de risco na zona urbana (p= 0,010) localizado na região norte do município. No capítulo II, foi realizado levantamento entomológico e sazonal dos flebotomíneos também no município de Mãe D'Água. Foram capturados 567 flebotomíneos, sendo 146 (25,7%) fêmeas e 421 (74,2%) machos, e Junho de 2019 foi o mês com maior número de espécimes coletados (133/567- 23,4%). Foram identificadas as espécies *Lutzomyia lenti* e *Lutzomyia longipalpis,* sendo esta espécie de maior ocorrência na área rural (487/493- 98,7%). Foi detectada a presença de DNA de *Leishmania infantum* em 4 (15,3%) dos 26 *pool*s analisados. O cálculo da taxa de Razão de Infecção Mínima (MIR) revelou que 4% das fêmeas foram positivas. O capítulo III teve como objetivo descrever a distribuição temporal e espacial dos casos de leishmaniose visceral humana (LVH) no Estado de Alagoas, a fim de identificar as áreas de alto risco de transmissão da doença no período de 2007 a 2018. O Estado é composto por 102 municípios, destes, 68,62% (n= 70) notificaram pelo menos um caso de LVH nos 12 anos do estudo. Foram registrados 489 casos de leishmaniose visceral entre 2007 e 2018, com uma média de 40,7 casos por ano e incidência de 1,25/100 mil habitantes. Foi detectada dependência espacial no segundo, terceiro e quarto triênios e semelhança entre os municípios, porém com fraca correlação, com *clusters* de alta incidência no sertão. Ficou evidente que o

Estado de Alagoas apresentou uma acentuada expansão geográfica da LVH, sendo necessário priorizar áreas e massificar as ações de vigilância e controle epidemiológico.

Palavras-chave: leishmanioses; sorologia; qPCR; Nordeste; georreferenciamento.

ABSTRACT

Visceral leishmaniasis (VL) is a neglected tropical disease, with 94% of new cases distributed in countries like Ethiopia, India, Kenya, Somalia, Sudan, South Sudan and Brazil. Canine infection is indicative of risk for human infection, and due to the close interaction of dogs in the home environment, these animals become important reservoirs and source of infection. This thesis consists of three chapters. In chapter I, the prevalence of canine visceral leishmaniasis (CVL) in the municipality of Mãe D'Água was determined and the epidemiology of zoonosis was studied by analyzing the association factors and their distribution by georeferencing. For this, blood samples from 150 dogs, from urban and rural areas of the municipality, were collected. The prevalence of anti-*Leishmania infantum* antibodies was 18.6% (28/150), 10.6% (8/75) in the urban area and 26.6% (20/75) in the rural area. The rural area was identified as a risk factor (*Odds ratio* = 2.93); the permanence of the animal without restraint at night (*Odds ratio* = 0.33) and deworming (*Odds ratio* = 0.30) were classified as protective factors. The spatial analysis showed the formation of a primary risk cluster in the urban area (p = 0.010) located in the northern region of the municipality. In chapter II, an entomological and seasonal survey of sandflies was also carried out in the municipality of MãeD'Água. 567 sandflies were captured, 146 (25.7%) females and 421 (74.2%) males, and June 2019 was the month with the highest number of specimens collected (133 / 567- 23.4%). The species *Lutzomyia lenti* and *Lutzomyia longipalpis* were identified, this species being more prevalent in the rural area (487 / 493- 98.7%). The presence of *Leishmania infantum* DNA was detected in 4 (15.3%) of the 26 pools analyzed. The calculation of the Minimum Infection Ratio (MIR) rate demonstrated that 4% of females were positive. Chapter III aimed to describe the temporal and spatial distribution of cases of human visceral leishmaniasis (HVL) in the State of Alagoas, in order to identify areas of high risk of disease transmission from 2007 to 2018. The State is composed by 102 municipalities, of these, 68.62% (n = 70) reported at least one case of HVL in the 12 years studied. 489 cases of visceral leishmaniasis were recorded between 2007 and 2018, with an average of 40.7 cases per year and an incidence of 1.25/ 100 thousand inhabitants. Spatial dependence was detected in the second, third and fourth trienniums and similarity between the municipalities, but with a weak correlation and with high incidence clusters in the hinterland. It was evident that the

State of Alagoas presented a marked geographical expansion of the HVL, making it necessary to prioritize areas and massify the actions of surveillance and epidemiological control.

Keywords: leishmaniasis; serology; qPCR; Brazilian Northeast Region; georeferencing.

SUMÁRIO

LISTA DE ILUSTRAÇÕES

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

LISTA DE TABELAS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

1. INTRODUÇÃO GERAL

A leishmaniose visceral (LV) é uma doença tropical negligenciada, com 94% dos novos casos distribuídos em países como Etiópia, Índia, Quênia, Somália, Sudão, Sudão do Sul e Brasil (WHO, 2017). O Brasil possui 96% dos casos ocorridos nas Américas, alcançando em 2016 uma taxa de letalidade de 7,9%, sendo considerada a mais elevada quando comparada a outros países (OPAS, 2018).

No Brasil, a doença encontra-se distribuída nas cinco regiões, sendo a maioria dos casos, tanto de leishmaniose visceral humana (LVH) quanto canina (LVC), provenientes do Nordeste do país. De acordo com o Ministério da Saúde, de 2015 a 2017 foram regristrados 10.063 casos de leishmaniose visceral humana (LVH) no Brasil, sendo o Nordeste responsável por uma ampla distribuição de casos humanos (PAHO-WHO, 2019). A doença, antes limitada às áreas rurais e às pequenas localidades urbanas, encontra-se em franca expansão para grandes centros, em um processo deperiurbanização e urbanização que vem ocorrendo nas últimas décadas. Tem se tornado um importante problema de saúde pública, onde fatores sociais, econômicos e ambientais estão interligados, entre eles a urbanização acelerada e o desmatamento, que facilitam a sua ocorrência (BRASIL, 2017).

Apesar de na última décadater havido a descentralização das ações de controle da Fundação Nacional de Saúde (FUNASA) para as Secretarias de Saúde Estaduais e Municipais, não houve redução da incidência da doença, além de deficiência de investimentos em estudos de prevalência no Nordeste dirigidos pelas Secretarias de Saúde no âmbito estadual e municipal. De acordo com o Sistema Nacional de Agravos e Notificação (SINAN), no Estado da Paraíba foramnotificados 48 casos de LV em 2018 (TABNET, 2019a).

A prevalência da infecção canina é indicativa de risco para a infecção humana, e devido ao estreito convíviode cães no ambiente domiciliar, estes animais se tornam importantesreservatóriose fonte de infecção (RODRIGUES *et al.,* 2017). Os primeiros estudos de Leishmaniose visceral canina (LVC) na Paraíba foram descritos por Guedes et al. (1974) em João Pessoa, mas vários trabalhos foram realizados na última década com cães domiciliados residentes na zona urbana e/ou ruralem Brejo do Cruz,Patos, Sousa, Cajazeiras e Uiraúna, cujas prevalências variaram de 6% a 38,6%

(FERNANDES *et al.*, 2018; SILVA *et al.,*2016; SILVA *et al.,*2017; SILVA *et al.*, 2018). Trabalhos epidemiológicos de LVC no Brasil têm utilizado o georreferenciamento como ferramenta de estudo para, a partir da distribuição espacial ou temporal de casos, verificar o padrão de apresentação da doença (BORGES *et al.,* 2014; FONTOURA *et al*., 2016; MAIA *et al.,* 2014; NAVEDA *et al.,* 2006; URSINE *et al.,* 2016). Segundo Borges *et al.* (2014), as vantagens desse método são: visualizar áreas com maior risco de transmissão e, por conseguinte, direcionar e priorizar ações de prevenção e controle.

Nos últimos seis anos o município de Mãe D'Água, situado no Sertão paraibano, tem notificado casos humanos e caninos de LV, possivelmente devido a fatores como, a ineficácia das estratégias de controle conduzidas pela Vigilância Epidemiológica e a características edafoclimáticas do município favoráveis a sobrevivência do vetor, já que possui 370m de altitude, e está incluso num relevo denominado "Planície Sertaneja", formada por um conjunto de serras alongadas e alinhadas, apresentando uma vegetação predominante de caatinga bastante densa e conservada (SILVA *et al*., 2005). Para tanto, o objetivo do **Capítulo I** foi analisar a prevalência de LVC, determinar indicadores epidemiológicos da zoonose por meio da análise dos fatores de risco, e atravésdogeorreferenciamento, verificar a distribuição espacial da doença no município.

O principal vetor da leishmaniose visceral no Brasil é o flebotomíneo da espécie *Lutzomyia (Lutzomyia) longipalpis,* umdíptero pertencente à família *Psychodidae* e subfamília *Phlebotominae* (ROSÁRIO *et al*., 2005). Segundo Missawa e Lima (2006), há uma grande ocorrência de *L. longipalpis* em áreas com bioma de floresta, transição e de cerrado, dessa forma, indicando as áreas vulneráveis para a transmissão da doença. Estudos relacionados à ecologia e à curva de sazonalidade dos flebotomíneosvisam avaliar o comportamento das espéciesem relação aos seus ecótopos naturais, prevalência de infecção e a sua distribuição de acordo com as características climáticas da região estudada (COSTA et al. 2013; OLIVEIRA et al., 2013; SILVA et al. 2007).

Além disso, também se tem verificado a infecção natural por espécies de *Leishmania* em fêmeas de *L. longipalpis*, utilizando métodos moleculares, com o intuito de avaliar a efetividade das estratégias de controle e tratamento (BRIGHENT *et al.,* 2018; CUNHA *et al*., 2014; GONZÁLEZ *et al.,* 2017; MICHALSKY *et al*., 2011; RODRIGUES *et al*., 2014; SARAIVA *et al.,* 2015).

O monitoramento entomológico é realizado por meio de armadilhas de sucção ou luminosas, tipo CDC, instaladas mensalmente em pontos fixos por duas ou três noites consecutivas, e no caso de trabalhos que enfoquem a sazonalidade do vetor, é feito por no mínimo dois anos. Éuma das estratégias de controle recomendado em municípios com transmissão moderada ou intensa, mas é sabido que há uma flutuação das populações de flebotomíneos em cada região geográfica, ligada a fatores climáticos (temperatura, umidade relativa do ar, índice pluviométrico) e a fatores como composição do solo, altitude, relevo e tipo de vegetação (BRASIL, 2016). Em Mãe D'Água, é realizada a captura de flebótomos por meio de sucção pelos Agentes de endemias, mas somente para fins de identificação de espécie, sem que haja uma pesquisa a cercada infecção natural.

Haja vista a importância do vetor na manutenção do ciclo de transmissão da LV, o **Capítulo II** consiste em um levantamento entomológico da sazonalidade e da ocorrência de subpopulaçõesno município de Mãe D'Água, por meio de captura e identificação de espécies de flebotomíneos utilizando armadilhas luminosas, eatravés da Reação em Cadeia da Polimerase em Tempo Real (qPCR), identificar o DNA de *Leishmania infantum* nas fêmeas de *Lutzomyia longipalpis* infectadas.

O **Capítulo III** tem como objetivo descrever a distribuição temporal e espacial dos casos de LV no estado de Alagoas, a fim de identificar as áreas de alto risco de transmissão da doença no período de 2007 a 2018 em intervalos trienais. Situado no Nordeste do Brasil, o Estado de Alagoas é considerado endêmico, e têm apresentado elevação no número de casos humanos e caninos nos últimos 12 e dois anos, respectivamente (SESAU, 2018; TABNET, 2020b). Por isso, é notável a necessidade de se buscar ferramentas para estudar a disposição da doença.

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CAPÍTULO I:

FATORES ASSOCIADOS À LEISHMANIOSE VISCERAL CANINA E ANÁLISE ESPACIAL NO SERTÃO DA PARAÍBA, NORDESTE DO BRASIL

Factors associated with canine visceral leishmaniasis and geospatial analysis in the Sertão of Paraíba, Northeast Brazil

Beatriz Maria de Almeida Braz; Denny Parente de Sá Barreto Maia Leite; Raizza Barros Sousa Silva; Maria Luana Cristiny Rodrigues da Silva; Ana Clara de França Silva Azevedo; Erotides Martins Filho; Walter Massa Ramalho; Marcia Almeida de Melo

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Fatores associados à leishmaniose visceral canina e análise geoespacial no Sertão da Paraíba, Nordeste do Brasil

Factors associated with canine visceral leishmaniasis and geospatial analysis in the Sertão of Paraíba, Northeast Brazil

Beatriz Maria de Almeida Braz [1)*]; Denny Parente de Sá Barreto Maia Leite[1)]; Raizza Barros Sousa Silva[1)]; Maria Luana Cristiny Rodrigues da Silva[1)]; Ana Clara de França Silva Azevedo[1)]; Erotides Martins Filho[1)]; Walter Massa Ramalho[2)]; Marcia Almeida de Melo[1)]

[1]Laboratório de Biologia Molecular do Semiárido,Universidade Federal de Campina Grande – UFCG, Patos, PB, Brasil

[2]Universidade de Brasília (UnB - Faculdade de Ceilândia)

*Autor correspondente. Universidade Federal de Campina Grande, CSTR/ UAMV.Av. Universitária, s/n, Hospital Veterinário, Sta Cecília, CEP: 58700970 - Patos, PB –Brasil. Telefone: (83) 34239523. Brasil. marcia.melo@ufcg.edu.br

Abstract

Visceral leishmaniasis (VL) is a zoonosis distributed worldwide and has a major impact on public health. The objective of this study was to verify the prevalence of canine visceral leishmaniasis (CVL), and thus assess the factors associated with the disease as well as its spatial distribution in the municipality of Mãe D'Água, in the Sertão region of Paraíba state, Northeast Brazil. Blood samples collected from 150 dogs were subjected to DPP®, ELISA-S7®, and qPCR assays to diagnose. The prevalence was calculated by considering positivity in at least two tests or only in qPCR. SaTScan® software was used for the spatial analysis. The prevalence of CVL was 18.6% (28/150), and the rural area was identified as a risk factor (Odds ratio (OR) = 2.93). The permanence of dogs that were not restrained at night (OR = 0.33) and deworming (OR= 0.30) were classified as protective factors. A risk cluster was formed in the northern region of the urban area. The rural area of Mãe D'Água exhibited a pattern of active transmission; however, control measures also need to be implemented in the urban area, to prevent the spread of the disease and the occurrence of human cases in the risk zone.

Keywords: *cluster*, georeferencing, leishmaniasis, Real-time PCR, serology

Resumo
A leishmaniose visceral (LV) é uma zoonose com distribuição mundial de grande impacto na saúde pública. O objetivo deste estudo foi verificar a prevalência da leishmaniose visceral canina (LVC), os fatores associados à doença e sua distribuição espacial no município de Mãe D'Água, na região do Sertão da Paraíba, Nordeste do Brasil. Amostras de sangue foram coletadas de 150 cães para diagnóstico pelos ensaios, DPP®, ELISA-S7® e qPCR. A prevalência foi calculada considerando a positividade em pelo menos dois testes ou apenas na qPCR. O Satscan® foi utilizado para a análise espacial. A prevalência de LVC foi de 18,6% (28/150), sendo a zona rural identificada como fator de risco (Odds Ratio (OR) = 2,93). A permanência do cão solto durante a noite (OR = 0,33) e a vermifugação (OR = 0,30) foram classificados como fatores de proteção. Um *cluster* de risco foi formado na região Norte da area urbana. Mãe D'Água tem apresentado um padrão de transmissão ativa na área rural, porém, medidas de controle da LVC também precisam ser realizadas na area urbana, para evitar a propagação da doença e a presença de casos humanos na zona de risco.
Palavras-chave: *cluster*, georreferenciamento, leishmaniose, PCR em Tempo Real, sorologia.

Introduction

Visceral leishmaniasis (VL) is an important zoonosis distributed worldwide and has a great impact on public health. The WHO (2018) estimated that the global VL case count in 2018 increased from 50,000 to 90,000, with the majority of cases in Brazil, Eastern Africa, and Southeast Asia.

In Brazil, the disease is known as "kala-azar" and, currently, occurs in the five regions of the country, encompassing 19 states, in which 10,063 cases were reported from 2015 to 2017. According to Sousa et al.(2018), the majority of the cases were reported in the Northeast region, especially in the states of Maranhão and Ceará.

In Brazil, the transmission occurs mainly through the blood repast of infected females of the *Lutzomyia longipalpis* phlebotomine (Lainson & Rangel, 2005; Missawa & Lima, 2006) and, rarely, by means of blood transfusion (Cohen et al., 1991) or via the trans-placental route. In the wild, coyotes and foxes act as reservoirs, and in the domestic environment, dogs preserve the transmission cycle (WHO, 2014), thereby acting as important sources of infection for the disease vectors (Ashford, 1996); humans are accidental hosts of the parasite.

The Ministry of Health (Brasil, 2011) proposes the use of the DPP® rapid test and ELISA EIE-LVC® as a confirmatory test, as screening tools for the diagnosis of canine visceral leishmaniasis (CVL). When an animal tests positive for CVL, treatment is possible only after obtaining permission from the owner. The animal is thus administered with Milteforan, a miltefosine-based drug, which is registered by the Ministry of Agriculture, Livestock, and Supply (MAPA). However, the use of Milteforan constitutes a protective measure for individual animals, and is neither considered a control strategy of the disease nor does it protect public health,owing to its cost and long implementation time (Brasil, 2016). Based on an analysis of the local epidemiological situation, the Visceral Leishmaniasis Control Program (PCLV) in Brazil suggests that preventive and control measures should be adopted to tackle this zoonosis. These measures consist of entomological surveillance, confirmation,treatment of human cases, and the culling of CVL-positive dogs (Brasil, 2006); however, there is no confirmation that the latter measure can interrupt the dissemination of the disease (Werneck, 2014).

The prevalence of CVL is itself a risk indicator for its occurrence in humans, and its study indicates to the local surveillance organizations regarding the necessary control actions that should be implemented. However, owing to the decentralization of the control actions, the National Health Foundation (FUNASA) and the municipalities have not investigated the prevalence of the disease in dogs. Despite this, numerous studies have been carried out by the scientific community in the states of the Northeast region, such as Rio Grande do Norte (Amóra et al., 2006), Maranhão (Barbosa et al., 2010), Piauí (Figueiredo et al., 2017), Ceará (Rordrigues et al., 2017), and Paraíba (Brito et al., 2016; Fernandes et al., 2018; Silva et al., 2018); these studies have emphasized the prevalence of CVL. Nevertheless, over the last decades the surveys conducted by the municipal and state health departments are scarce.

Three cases of human visceral leishmaniasis HVL were reported between 2014 and 2018 in the municipality of Mãe D'Água in the state of Paraíba (Sinan, 2019); a new case was reported in December 2019, but it has still not been loggedin the national disease notification system (SINAN).Additionally, samples were collected from 55 stray dogs by the endemic disease control agents and tested at the Central Laboratory of

Public Health of Paraíba (LACEN), as recommended by the Ministry of Health; the results showed that the occurrence of CVL positive animals was 10.9% (6/55).

Mãe D'Água is located in the Sertão mesoregion of Paraíba state, in the metropolitan region of the municipality of Patos. The preserved xerophytic caatinga vegetation is set in a relief denominated "Sertanejo Plain," which constitutes an extensive pediplain surface. This surface is formed by a set of elongated and aligned mountains over the regional geological structure, which extends through the municipalities of Manaíra to Teixeira. Located in this region is the Jabre Peak, with an altitude of 1,197m, which constitutes the highest point of the state (CPRM/PRODEEM, 2005); this may favor the occurrence of disease vectors throughout the year as well asmaintenance of the disease cycle.

In view of this, the aim of this study was to analyze the prevalence of CVL in the municipality of Mãe D'Água, to study the epidemiology of the zoonosis by analyzing the associated factors, and to verify the spatial distribution of the disease using georeferencing. The results will allow us to identify priority areas and disease patterns, so that adequate prevention and control measures are implemented in the future.

Material and Methods

Study design and setting

The study was conducted in the urban and rural areas of the municipality of Mãe D'Água (7°15′10″S,37°25′58″ W), located in the semi-arid region of Paraíba State, in the Sertão mesoregion (Figure 1).The area has a hot and dry climate and high temperatures throughout the year, with annual variations between 23 °C and 30°C,while the average rainfall in 2018 was 746.9 mm (AESA, 2019) (Köppen climatic classification: BSh). The estimated population is 4,019 inhabitants, 1,569 and 2,450 of whomlive in the urban and rural areas, respectively, and this population is distributed over 243.75 km² (IBGE, 2020).The municipality does not have a territorial division of neighborhoods, and the central neighborhood is considered the urban area.

Ethical considerations

The work was approved by the Ethics Committee of the Health and Rural Technology Center (CSTR) of the Federal University of Campina Grande (UFCG), under protocol n. 156 059/2018.

Sample size

Taking into consideration that there is one dog for every seven inhabitants, the estimated canine population of the municipality is 574 animals. Using a simple random sampling formula, with an expected prevalence of 50%, a 99% significance level, and a sampling error of 7% (Thursfield, 2007), the minimum number of samples required was calculated as 146 animals (Savani et al., 2003).

Sample and data collection

The samples were collected between May and June 2018, in the urban and rural locations, and in points chosen using the municipality sketch. Home visits were carried out with the support of healthcare and endemic disease agents of the Municipal Healthcare Department. We collected blood samples from 150 male and female domestic dogs (75 each from the urban and rural areas), and these dogs were over six months old. The samples were collected by jugular, cephalic, or saphenous venipuncture, using 5-mL sterile syringes and 25 ×8mm needles. Then, they were added to tubes containing 4% sodium citrate, refrigerated in a thermal box, and forwarded to the Molecular Biology Laboratory (BiolMol/UFCG), Patos-PB Campus, located in the semi-arid region of Paraiba state to obtain blood plasma. The whole blood and plasma samples were stored in 1.5-mL microtubes, identified, and stored at -20 °C until the serological assays and molecular tests were performed.

The owners answered an epidemiological survey containing 31 variables related to the owner, the animals, and the environment, and this surveyaimed to analyze the main epidemiological indicators associated with the occurrence of the disease.

Serological tests

The prevalence of CVL was estimated by the immunochromatographic Dual Path Platform DP

(Rapid Test Canine Visceral Leishmaniasis, Bio-Manguinhos), and two enzyme-linked immunosorbent assays (ELISA-EIE®, Canine Visceral Leishmaniasis, Bio-Manguinhos; ELISA-S7® kit Biogene, Industry and Commerce Ltda) and the analyses were carried out by the team of the Municipal Laboratory of Patos/PB and of the Molecular Biology Laboratory of the Federal University of Campina Grande, respectively. The protocol followed the manufacturers' recommendations. The samples that reacted in the DPP® were sent to the Public Health Central Laboratory of Paraíba (LACEN), which is responsible for conducting the ELISA- EIE® test.

Real-time quantitative polymerase chain reaction (qPCR)

The DNA of the blood samples were extracted using U-Trinzol®, following the manufacturer's recommendations and qPCR was performed as previously described (Fernandes et al., 2018). *Leishmania infantum* culture was used as a positive control and ultra-pure water as a negative control. The initiators used for the amplification of the sequences of the minicircle of the kDNA of *L. Infantum* were Leish RV1 (5′-CTT TTCTGGTCCCGCGGGTAGG-3′) and Leish RV2 (5′-CCACCCGGCCTATTTTACACCA-3′). Thermocycling was conducted using a Bioer Technology® thermocycler. The prevalence was determined taking into consideration the positivity in at least two serological techniques or only in PCR.

Statistical analysis

The analysis of the risk factors associated with CVL was calculated in two stages using univariate and multivariate analysis, and the independent variables were categorized and codified. The categories, including income, level of schooling, cleaning frequency, and types of vaccine were aggregated in order to facilitate the statistical analysis dueto the small sample size. Either chi-squared or Fisher's exact tests (ZAR) were used in the univariate analysis for the variables with $p \leq 0.20$ and in the multivariate analysis through multiple logistic regression (HOSMER et al., 2000). The significance level adopted in the multiple analysis was 7%. The analysis was carried out with IBM SPSS® Statistics for Windows, version 22.0 (IBM Corp., Armonk, USA).

Georeferencing

The geographic coordinates of the residence point of each animal were obtained using the global positioning system (GPS) receiver GarmineTrex 30 when the questionnaire was answered by the dog owners, with the intention of analyzing and tracking the animals involved in the work. Using the obtained geographic coordinates, the CVL cases were inserted into the cartographic base of the municipality (Figure 3), using ageographic information system (GIS) and Google Earth 7.3.0; the maps were createdinquantum GIS (QGIS). A heatmap or quadratic Kernel estimation was constructed using QGIS 2.18.0 software, in an effort to observe the disease clusters. SaTScan® software was used to verify the areas with cases exceeding the expected number, by means of scan statistics for the detection of clusters,using a Bernoulli model.

Results

Of the 150 serum samples of the dogs analyzed, 19.3% (29/150), 41.3% (62/150), and 3.3% (5/150) tested positive in the DPP® screening test, ELISA-S7®,qPCR, respectively. As proposed by the Ministry of Health, only the positive samples in the DPP® rapid test were tested in the ELSA kit EIE-CVL®, which resulted in 12.6% (19/29) reactive samples.

The prevalence of CVL in the municipality of Mãe D'Água was 18.6% (28/150), with 10.6% (8/75) and 26.6% (20/75) accounting for the urban and rural area cases, respectively.

In the univariate analysis, we selected the following variables with a p-value $\leq$ 0.2 (Table1): origin, income, gender, breed, type of rearing, feeding, contact with wild animals, contact with felines and birds, rearing environment, cleanliness, frequency of cleaning, deworming, whether the dog was adopted, hunting activity, where the dog sleeps, and how does if spend the night (chained or not).

The categories that remained at the end of the multiple logistic regression model were the following: animals that spend the night free/without containment (OD = 0.33) and deworming (the animal has been dewormed at least once) (OD = 0.30). These categories were identified as protection factors for the occurrence of CVL; rural origin was identified as a risk factor (OD = 2.93) (Table 2). Dogs from the rural area were 2.93 times more likely to contract the disease. The dogs that were free/without containment during the night and dewormed ones were 67% and 70% more protected, respectively, than the contained ones.

The Kernel density estimator allowed us to determine the location for greater risk for the disease occurrence, and the areas with a higher concentration of cases are highlighted in red. These areas are situated in the North, Northwest, Northeast, central or urban area, and South of the municipality, with densities varying from 0.55 to 2.21 (Figure 2). The settlements situated in the Southeast region were not analyzed, as its accesses ion was impossible during the study period, while the endemic disease agents stated that there was an insignificant number of dogs in this area.

Using SaTScan, we calculated the clusters based on the relative risk. A primary cluster of significant risk ($p = 0.010$) was located in the northern area of the urban zone (Figure 3), where dogs were 17.45 times more likely to become infected than those in other areas of the municipality. This cluster expanded to areas of vegetation without residencies in the limits of the municipality, with the exception of a small portion in the Southeast extremity.

Discussion

Although the methodology used in our study differs from those of others, the CVL prevalence detected in this study is similar to that of several international studies conducted, e.g., in Turkey (10.9%) (Bakirci et al., 2016), Iran (15.4%) (Mahshid et al., 2014), and Brazil in Niterói-Rio de Janeiro (21.66%) (Abrantes et al., 2018) as well as in other municipalities of the Sertão region of the Paraíba State, such Cajazeiras (20%), Uiraúna (16.3%), Sousa (10.5%), and Patos (7.2%) (Silva et al., 2018). The highest or lowest prevalence may be attributed to the study region, the population characteristics, the statistical analysis used, and the sample size (Brito et al., 2016; Costa et al., 2018; Felipe et al., 2011; Silva et al., 2017; Wang et al., 2011).

In the present study, the prevalence of CVL was higher in the rural area (25.3%), and this variable was considered a risk factor for the occurrence of the disease, there by reinforcing the ancient dynamics of leishmaniasis as being endemic to rural areas (Amóra et al., 2006). Although the disease has expanded to urbanized areas since the 1950s (Figueiredo et al., 2016; Guimarães et al., 2017), this factor does not apply to the municipality studied, as the area is small and there is a close contact between the urban and rural areas.

The predominant vegetation in the municipality of Mãe D’Água is caatinga, is dense and preserved, and is thus considered a favorable environment for the survival of the disease vectors. It is important to mention that the constructions of rural areas or the proximity to woodlands facilitate the contact of dogs and humans with the disease vectors in their natural habitat, thereby enabling the maintenance of the transmission cycle and increasing the chances of these animals getting infected (Abrantes et al., 2018; Barbosa et al., 2010).

The occurrence of CVL is also dependent on social issues that enable the survival and reproduction of the vector; such factors are related to infrastructure, including migration and the disorderly construction of houses in the periphery, precariousness of basic sanitation, and climate changes resulting from deforestation (Camargo et al., 2007; Gontijo & Melo, 2004). The locations visited presented propitious environmental and social characteristics for the maintenance of the transmission cycle, such as the rearing of animals in the peridomicile and precarious sanitary conditions resulting from the organic matter produced.

In Mãe D’Água, 60% of the owners let their animals free during the night, and the category “free during the night/without a collar” was considered a protection factor, as these dogs were 67% more protected against CVL than those contained using collars during the study period. On the contrary,the prevalence of the zoonosis was higher in semi-domesticated dogs and those that spent the nights contained (Amóra et al., 2006).Our results showed that the dog’s permanence in the peridomicile was an important risk factor for CVL, and was associated with higher contact incidences with vectors (Almeida et al., 2009).

It is believed that animals that spend the night chained are restricted in a certain area, thus face a greater risk of being bitten by a vector. In contrast, an animal that is permitted to roam free during the night has higher mobility and can walk and sleep in different places, thereby decreasing its chances of becoming infected. Borges et al.(2009) observed that humans are infected more when the dogs are maintained indoors during the night, and this is the same time during which the vectors feed on hosts (from evening until dawn) (Felipe et al., 2011; Galati et al., 1996; Wang et al., 2011). The night also represents the time when most people and animals are in a restricted area inside their houses.

According to the Ministry of Health, prophylactic measures, such as vaccination and deworming, are essential to guarantee that animals are protected against diseases. In the present study,only10% (7/70) of the dewormed dogs (p= 0.01) were seroreactant, which was indicative of protection against *L. Infantum* infection, and may be associated with a better immune status of the evaluated dogs, as a result of greater care and wellbeing provided by the owner (Brasil, 2003).

Spatial analysis is a tool that has been used in CVL epidemiological studies, with the intention of identifying priority areas and disease patterns as well as helping identify control measures for the zoonosis (Margonari et al., 2006). Using SaTScan®, a risk cluster (p = 0.010) was identified in the northern region of the urban area, with a light expansion to uninhabited areas and with a region of preserved woodland in the urban perimeter.

Most (96.4%) of the urban residencies in the municipality of Mãe D'Água were located in public streets with afforestation, and only 15.2% were in areaswith adequate urban features, such as drains, sidewalks, and paving (Ibge, 2020). The houses in the urban area of the municipality were close to secondary vegetation and in areas with frequent accumulation of organic matter.

Rural and peripheral urban areas tend to have higher occurrence of VL when in proximity to abundant vegetation (Cerbino et al., 2009), which is proven to be the natural habitat of phlebotomine vectors, whose presence is indispensable for the maintenance of the transmission cycle; therefore, entomological monitoring is recommended in localities with active transmission (Borges et al., 2009).

In this study, the disease was more prevalent in the rural area, but was dispersed all over the municipality, a fact that, according to Mestre et al. (2011) and Paulan et al. (2012),is related to the adaptation of the vector to the peridomicile conditions.Such localities are often characterized by a lack of infrastructure or anthropogenic activities, orby the migration of dogs and infected individuals.

The pattern of active transmission of CVL in Mãe D'Água was more prevalent in the rural area. As human VL cases were recently reported, the adoption of immediate control measures, such as the use of repellent collars, control of vectors in household environments and in the peridomicile with the use of insecticides, removal of organic matter from the peridomicile, and refrainment of rearing domestic animals close to the domicile, are recommended. Additionally, these measures need to be implemented in the urban area to prevent the expansion of the disease and the occurrence of human cases in the risk zone.

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Figure 1. Map of the municipality of Mãe D'Água, Semi-arid region of Paraíba State, Brazil, 2018.

Figure 2. Kernel map showing areas with the greater distribution of canine visceral leishmaniasis cases in the municipality of Mãe D'Água, Semi-arid region of Paraíba State, 2018.

Figure 3. Satellite image showing the risk cluster for the occurrence of canine visceral leishmaniasis in the urban area of the municipality of Mãe D'Água, Paraíba, Brazil, 2018. Source: Google Earth.

Table 1. Univariate analysis of the risk factors associated with canine visceral leishmaniasis in the Municipality of Mãe D'Água, Semi-arid region of Paraíba State, between May and July 2018. Significant variables in the univariate analysis ($p \leq 0.2$).

Table 2. Multivariate analysis of the risk and protection factors associated with canine visceral leishmaniasis in the municipality of Mãe D'Água, Semi-arid region of Paraíba State between May and July 2018. Significant variables in the multivariate analysis ($p \leq 0.05$)

Table 1. Univariate analysis of the risk factors associated with canine visceral leishmaniasis in the Municipality of Mãe D'Água, Semi-arid region of Paraíba State, between May and July 2018. Significant variables in the univariate analysis (p ≤ 0.2). (Initial)

Variable	**Category**	**N. of animals**	**Positives**	***Odds ratio***	**Confidence interval**	**P-value**
Origin	Urban	75	8(10.6%)	0.11	0.05-0.23	
	Rural	75	20 (26.6%)	3.04	1.28-7.84	0.01*
Level of schooling of the owner	Iliterate	12	3(25%)	0.33	0.07-1.11	
	Any form of literacy	138	25(18.1%)	0.66	0.18 -3.14	0.55
Income of the owner	Less than 2MS	91	14 (15.3%)	0.18	0.09 -0.31	
	2 or >2 MS	59	14(23.7%)	1.71	0.74 -3.94	0.20*
Gender of the animal	Male	105	25(23.8%)	0.31	0.19 -0.48	
	Female	45	3(6.6%)	0.22	0.05 -0.70	0.02*
Age of the animal	6 months - 2 years	17	4 (23.5%)	0.30	0.08 -0.86	
	>2 Years	133	24(18%)	0.71	0.22 -2.71	0.58
Breed	Mixed breed	132	27(20.4%)	0.25	0.16 -0.38	
	Pure breed	18	1(5.5%)	0.22	0.01-1.19	0.16*
Type of rearing	Domiciliary	70	7(10%)	0.11	0.04 – 0.22	
	Semi-domiciliary	62	17(27.4%)	3.4	1.34 – 9.42	0.01*
	Free	18	4(22.2%)	2.57	1.60 – 9.79	0.17*
Type of feed	Commercial feed	13	1(7.7%)	0.08	0.04 – 0.42	
	Homemade food	125	26(20.8%)	3.15	0.57 – 58.71	0.28*
	Both	12	1(8.3%)	1.09	0.03 – 29.88	
Contact with other animals	Yes	125	26(20.8%)	3.02	0.81 – 19.57	0.15*
	No	25	2(8%)	0.08	0.01 – 0.29	
Contact with equines	Yes	12	3(25%)	1.50	0.31 – 5.47	0.55
	No	138	25(18.1%)	0.22	0.14 – 0.33	
Contact with wild animals	Yes	20	2(10%)	0.44	0.06 -1.67	0.29*
	No	130	26(20%)	0.25	0.15 -0.37	
Contact with felines	Yes	38	10(26.3%)	1.86	0.75 – 4.4	0.16*
	No	112	18(16%)	0.19	0.11 – 0.30	
Contact with dogs	Yes	85	17(20%)	1.22	0.53 – 2.90	0.63
	No	65	11(17%)	0.20	0.10 – 0.37	
Contact with swines	Yes	17	2(11.7%)	0.54	0.08 – 2.11	0.44
	No	133	26(19.5%)	0.24	0.15 -0.36	
Contact with small ruminants	Yes	15	3(20%)	1.1	0.23 – 3.78	0.88
	No	135	25(18.5%)	0.22	0.14 – 0.34	
Contact with bovines	Yes	15	4(26.6%)	1.68	0.43 -5.40	0.40
	No	135	24(17.7%)	0.21	0.13 -0.32	
Contact with birds	Yes	84	21(25%)	2.80	1.15 -7.57	0.02*
	No	66	7(10.6%)	0.11	0.04 -0.24	
In which environment is reared	Soil	93	21(22.5%)	0.20	0.17 -0.46	0.12*
	Cement	57	7(12.2%)	0.48	0.17 -1.16	
Execution of the cleansing of the environment	Yes	89	12(13.4%)	0.43	0.18 -1.00	0.05*
	No	61	16(26.2%)	0.35	0.19 -0.61	

Table 1. Univariate analysis of the risk factors associated with canine visceral leishmaniasis in the Municipality of Mãe D'Água, Semi-arid region of Paraíba State, between May and July 2018. Significant variables in the univariate analysis (p ≤ 0.2). **(Final)**

Variable	Category	N. of animals	Positives	*Odds ratio*	Confidence interval	P-value
Cleaning of the environment frequency	Does not perform	61	16(26.2%)	0.35	0.19 -0.61	
	Daily	63	8(12.6%)	0.40	0.15 -1.01	0.06*
	Weekly/Fortnightly /monthly	26	4(15.3%)	0.51	0.13 -1.59	0.27*
Vaccinated animal (Anti-rabies, viruses, CVL)	Yes	126	22(17.4%)	0.63	0.23 -1.91	
	No	24	6(25%)	0.33	0.12 -0.79	0.38
Vermifugated animal	Yes	70	7(10%)	0.31	0.11 -0.75	0.01*
	No	80	21(26.2%)	0.35	0.21 -0.57	
Presence of ticks	Yes	115	21(18.2%)	0.89	0.35- 2.46	0.81
	No	35	7(20%)	0.25	0.10 -0.54	
Has Always lived with the same owner	Yes	98	18(18.3%)	0.94	0.40 -2.29	0.89
	No	52	10(2%)	0.23	0.11 -0.45	
Adopted	No	86	14(16.2%)	0.19	0.10 – 0.33	
	From the streets	15	2(13.3%)	0.79	0.11 – 3.30	0.77
	Other owner	49	12(24.4%)	1.66	1.69 – 3.97	0.24*
Has Always lived in the same municipality	Yes	125	25(20%)	1.83	0.57 – 8.17	0.35
	No	25	3 (12%)	0.13	0.03 – 0.39	
The animal hunts	Yes	18	6(33.3%)	2.5	0.79 – 7.19	0.09*
	No	132	22(16.6%)	0.20	0.12 – 0.30	
Where does it sleep	Indoors	42	5(11.9%)	0.13	0.04 – 0.31	
	Peridomicile	98	21(21.4%)	2.01	0.75 – 6.41	0.19*
	Street	10	2(20%)	1.85	0.23 – 10.46	0.50
How spends the night	Free/without collar	90	11(12.2%)	0.39	0.21 -0.68	0.01*
	Tied	60	17(28.3%)	0.35	0.14 -0.81	
Travels	Yes	12	2(16.6%)	0.86	0.12 – 3.52	0.86
	No	138	26(18.8%)	0.23	0.14 -0.34	
Repellent collar	Yes	1	-	7.51	-	0.99
	No	149	28(18.8%)	0.23	0.15- 0.34	

Table 2 Multivariate analysis of the risk and protection factors associated with canine visceral leishmaniasis in the municipality of Mãe D'Água, Semi-arid region of Paraíba State between May and July 2018. Significant variables in the multivariate analysis (p ≤ 0.05)

	Variable	*Odds ratio* CI (95%)	P-value
Risk factor	Origin (rural)	2.93 (1.18 – 7.92)	0.02
Protection factor	Vermifugated animal (yes)	0.30 (0.10 – 0.77)	0.01
	How the animal spends the night (Free)	0.33 (0.13 – 0.80)	0.01

Figura 1 Map of the municipality of Mãe D'Água, Semi-arid region of Paraíba State, Brazil, 2018

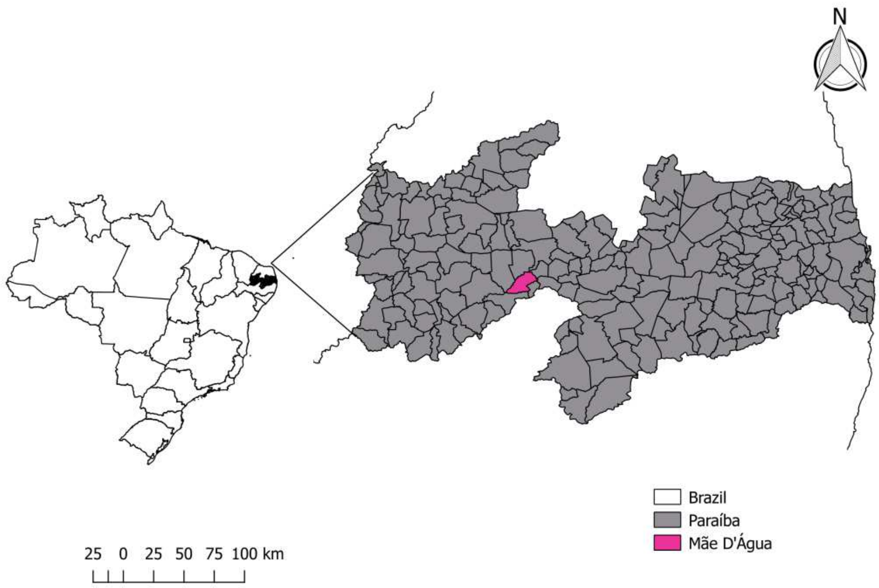


Figura 2 Kernel map showing areas with the greater distribution of canine visceral leishmaniasis cases in the municipality of Mãe D'Água, Semi-arid region of Paraíba State, 2018

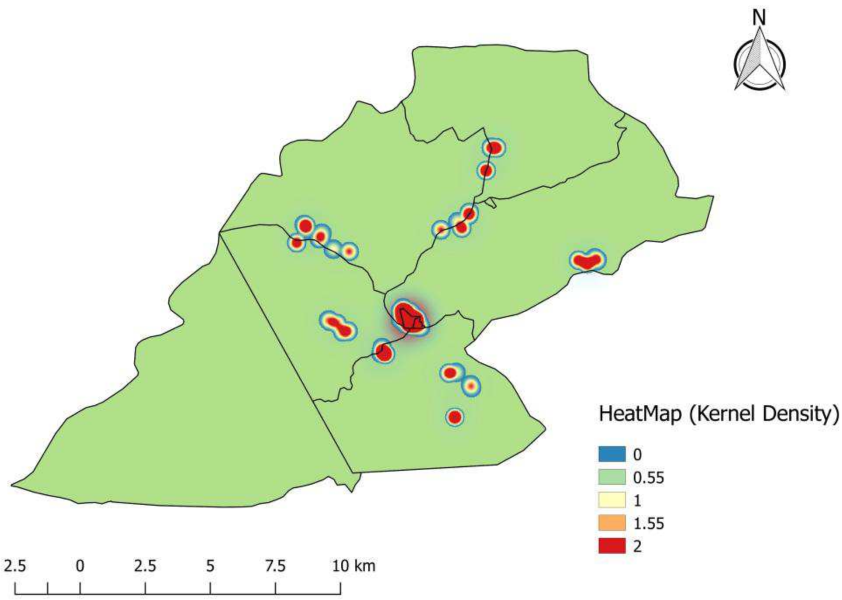
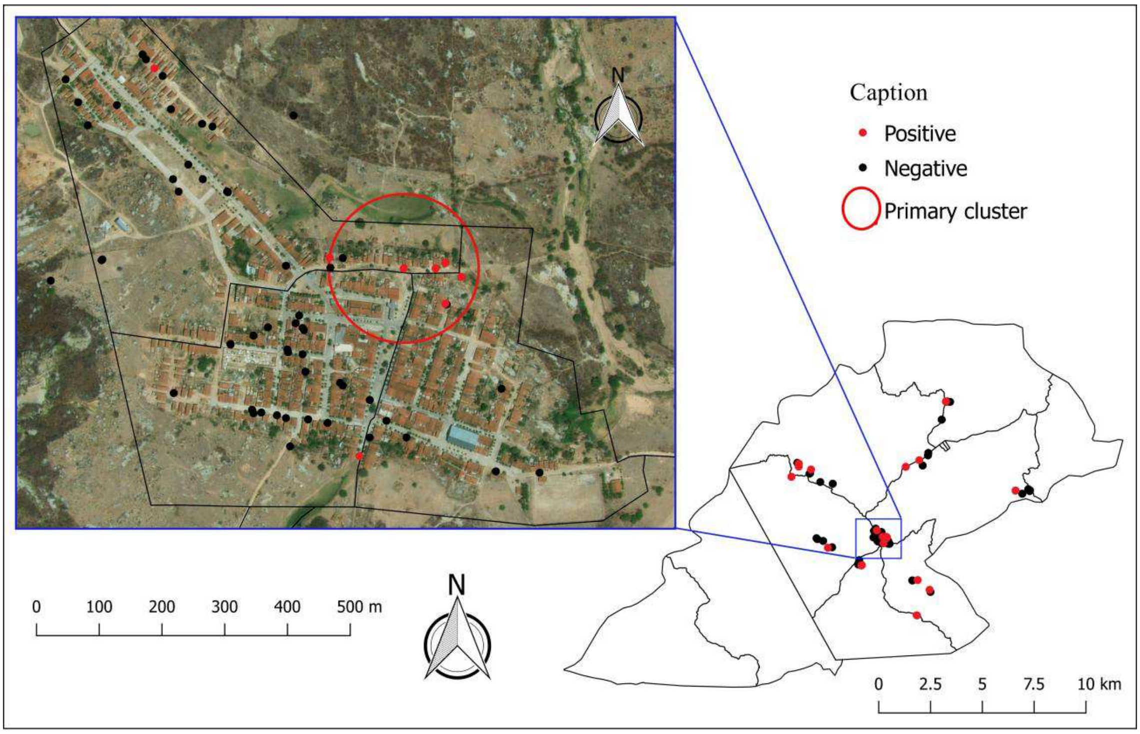


Figura 3. Satellite image showing the risk cluster for the occurrence of canine visceral leishmaniasis in the urban area of the municipality of Mãe D'Água, Paraíba, Brazil, 2018. Source: Google Earth.



CAPÍTULO II:

Pesquisa da infecção natural por Leishmania infantum em Lutzomyia longipalpis em área de transmissão da leishmaniose visceral no Sertão da Paraíba Nordeste do Brazil

Research of natural infection by *Leishmania infantum* in *Lutzomyia longipalpis* in a transmission area of visceral leishmaniasis in the Sertão region of Paraíba Northeast of Brazil.

Beatriz Maria de Almeida Braz; Denny Parente de Sá Barreto Maia Leite; Erotides Martins Filho; Raízza Barros Sousa Silva; João Pessoa Araújo Júnior; Maria Luana Cristiny Rodrigues da Silva; Leila Sabrina Ullmann; Camila Dantas Malossi; Walter Massa Ramalho; Marcia Almeida de Melo

Trabalho submetido à Veterinary Parasitology: Regional Studies and Reports (INSS: 2405-9390, Qualis A4)

Research of natural infection by *Leishmania infantum* in *Lutzomyia longipalpis* in a transmission area of visceral leishmaniasis in the Sertão region of Paraíba Northeast of Brazil.

Pesquisa da infecção natural por *Leishmania infantum* em *Lutzomyia longipalpis* em área de transmissão da leishmaniose visceral no Sertão da Paraíba Nordeste do Brazil

Beatriz Maria de Almeida Braz[a]*, Denny Parente de Sá Barreto Maia Leite[a], Erotides Martins Filho[a], Raízza Barros Sousa Silva[a], João Pessoa Araújo Júnior[c]; Maria Luana Cristiny Rodrigues da Silva[a]; Leila Sabrina Ullmann[c]; Camila Dantas Malossi[c], Walter Massa Ramalho[b]; Marcia Almeida de Melo[a]

[a]Laboratório de Biologia Molecular do Semiárido,Universidade Federal de Campina Grande – UFCG, Patos, PB, Brasil

[b]Universidade de Brasília (UnB - Faculdade de Ceilândia), Brasília, Brasil

[c]Universidade Estadual Paulista (Unesp), Botucatu, SP, Brasil

Abstract

Visceral leishmaniasis is a protozoonosis caused, in Brazil, by *Leishmania infantum*, transmitted to the susceptible hosts during the blood repast of infected phlebotomine females, belonging to the *Lutzomyia longipalpis* complex. The objective of this work was to conduct the entomological and seasonal survey of the phlebotomines in the municipality of Mãe D'Água in the state of Paraíba, Northeast of Brazil. The capture of the insects was carried out using luminous CDC traps, from October 2018 to October 2019. The specimens were separated by gender and the species were identified by means of an optical microscope. Pearson's Correlation Coefficient (r) was used to verify the association between the averages of the bioclimatic variables and the quantity of specimens captured each month. The positioning of the traps was obtained using the Global Positioning System (GPS) receiver Garmin ETrex 30x. The map of the capture sites was built using the QGIS software. Real-time PCR (qPCR) was performed for the detection of natural infection by *Leishmania infantum* in pools containing 10 *Lu. Longipalpis* females. 567 phlebotomines were captured, being 146 (25.75%) of them females and 421 (74.25%) males. In all the months of the study, captures occurred, and June 2019 was the month with the greatest number of insects collected (23.46% -

133/567). Were identified the species *Lu. lenti* and *Lu. longipalpis*. The former was captured in all the periods of study, with a highest occurrence in the rural area (98.78% - 487/493). The presence of *Leishmania infantum* DNA was detected in four (15.3%) of the 26 pools analyzed. By the calculation of the Minimum Infection Rate (MIR) was revealed that 4% of the females were positive. The findings of this study show the constant presence of *Lu. longipalpis*, which indicates the risk of transmission of the disease throughout the year, being greater after rainy periods, data which constitutes an instrument so that control strategies can be employed.

Keywords: leishmaniasis, seasonality, natural infection, neglected disease, zoonosis.

Resumo

A leishmaniose visceral é uma protozoonose causada, no Brasil, pela *Leishmania infantum*, transmitida aos hospedeiros susceptíveis durante o repasto sanguíneo de fêmeas infectadas de flebotomíneos, pertencentes ao complexo *Lutzomyia longipalpis*. O objetivo deste trabalho foi realizar o levantamento entomológico e sazonal dos flebotomíneos do município paraibano de Mãe D'Água, Nordeste do Brasil. A captura dos insetos foi realizada com armadilhas luminosas do tipo CDC, de outubro de 2018 a outubro de 2019. Os espécimes foram separados por sexo e as espécies foram identificadas por meio de microscópio óptico. O coeficiente de Correlação de Pearson (r) foi empregado para verificar a associação entre as médias das variáveis bioclimáticas e a quantidade de espécimes capturados em cada mês. O posicionamento das armadilhas foi obtido por receptor de Sistema de Posicionamento Global (GPS) GarmineeTrex 30x. O mapa dos locais de captura foi construído no software QGIS. Foi realizada a PCR em tempo real (qPCR) para a detecção de infecção natural por *Leishmania infantum* em pools contendo 10 fêmeas de *Lu. longipalpis*. Foram capturados 567 flebotomíneos, sendo 146 (25,75%) fêmeas e 421 (74,25%) machos. Em todos os meses do estudo, houve captura, sendo junho de 2019 o mês de maior número de insetos coletado (23,46% - 133/567). Foram identificadas as espécies *Lu. lenti* e *Lu. longipalpis*. A primeira foi capturada em todos os períodos de estudo, com maior ocorrência na área rural (98,78% - 487/493). Foi detectada a presença de DNA de *Leishmania infantum* em quatro (15,3%) dos 26 pools analisados. Através do cálculo da Razão de Infecção Mínima (MIR) revelou que 4% das fêmeas foram positivas. Os achados deste estudo

mostram a presença constante de *Lu. longipalpis*, o que indica risco de transmissão da doença ao longo de todo o ano, sendo maior após períodos chuvosos, dado que constitui uma ferramenta para que estratégias de controle sejam empregadas.

Palavras-chave: leishmaniose, sazonalidade, infecção natural, doença negligenciada, zoonose.

Introduction

Leishmaniasis integrates the group of neglected diseases as they occur in poorer countries, with precarious health services and of difficult access (OPAS, 2019). Visceral leishmaniasis (VL) is the most severe form of the disease, and is endemic in more than 80 countries of Africa, Asia and the Americas, with more than 90% of new cases being registered in Ethiopia, India, Somalia, Sudan, South Sudan and Brazil (WHO, 2019).

The main form of transmission of VL is by vectors and occurs by means of the crepuscular and nocturnal hematophagous activity of infected phlebotomine females, which are diptera of the family Psychodidae and subfamily Phlebotominae (Galati, 2003), considered to be important for public health, especially in tropical and subtropical regions. They are distributed in all the continents, except Antarctica, with more than a thousand species and subspecies described, being more abundant in the humid and hot regions of the planet, with 530 species located in the Neotropical region (Brazil et al, 2015; Shimabukuro et al., 2017).

In the Old World, the species and subspecies of the genus *Phlebotomus* are present and, *Lutzomyia* can be found in the New World (WHO, 2017). In South America, the Brazilian territory has the greatest diversity, with more than 267 catalogued species (Shimabukuro and Galati, 2011; Ready, 2013), and 134 registered only in Amazonian region (Alves et al., 2012; Figueira et al., 2013; Oliveira et al., 2013). In the Americas, the species *Lu. Longipalpis* (Lutz e Neiva, 1912) and *Lutzomyia* *cruzi* are important vectors of *Leishmania infantum*, the former especially in urban environments in all the Brazilian territory, and the latter in the central-west, mainly in Mato Grosso and Mato Grosso do Sul.

According to the Ministry of Health, it is possible that a third species, *Lu. migonei*, also participates in the transmission of VL, as in areas with the absence of *Lu. longipalpis* and/or *L. cruzi* there have been registers of autochthonous cases of the disease (Brasil, 2019). There are also other species, which are considered permissive vectors, as they can host *L. infantum*, but do not act in the transmission of the VL (Miskova et al., 2007). The *Lu. lenti* species is not involved in the cycle of the VL, however, can be transmitters of other trypanosomatids and arboviruses, therefore, its monitoring and control are necessary, since they are hematophagous of domestic animals (Sherlock, 2003).

Depending on the geographical region, in Brazil, *Lu. longipalpis* is known as *mosquito palha, birigui, tatuquira* and easily adapts to the temperature variations both in the peridomicile, and in the interior of houses and animal shelters (Brasil, 2006; Costa, 2011).

According to the Ministry of Health (2019), the Northeasten region of Brazil was responsible for 43.1% of the cases of VL in 2012, what makes this region relevant in the transmission of the disease, mainly due to the social and climate conditions that the region presents. In the State of Paraíba were notified 479 cases in the last eleven years, with an incidence coefficient of 1% per 100.000 inhabitants in 2015 (Brasil, 2016; Tabnet, 2019).

Situated in the *Sertão* region of the State of Paraíba, the municipality of Mãe D’Água notified in the last six years three cases of Human Visceral Leishmaniasis (HVL) (Tabnet, 2019) and, recently, in December 2019, presented a new case (data from the Health Department, personal communication). Furthermore, through serological tests, the occurrence of the disease in 10.99% of stray dogs (6/55) was diagnosed, by means of the action of the Municipality´s Health Department, and prevalence of 18.6% (28/150) in domiciled dogs (personal data in publication).

The municipality is situated in the mesoregion of the *Sertão* region of the State of Paraíba, metropolitan region of Patos, where the climate is hot and dry with high temperatures all the year round, with variation between 23 to 30ºC, and an average rainfall of 746.9 mm (AESA, 2019). According to Silva et al. (2005), the vegetation is composed of xerophytic *Caatinga* and is included in a relief denominated "*Sertaneja* Plain". This geographical scenario, that can favor the occurrence of the vector throughout the year and, the absence of studies in the region about the ecological aspects of the vector, motivated the development of this research, seeking to understand the seasonal fluctuation of the phlebotomines and the detection of natural infection by *L. infantum* in the *Lu. Longipalpis* species.

2. Material and Methods

2.1 Study area

The study was conducted in the municipality of Mãe D'Água (Figure 1) with the location coordinates 7°15′10″ South and 37°25′58 West. The estimated population is of 4.020 inhabitants, 1.569 originated from the urban area and 2.450 from the rural area, distributed in 243,75 km² of territorial area (IBGE, 2019). The municipality is situated at 341 km distance from the state capital João Pessoa, and is situated in the *Sertão* mesoregion (Köppen Climate Classification: BSh).

2.2.Capture of the phlebotomines

Between October 2018 and October 2019, were installed luminous Center on Disease Control (CDC) type traps, monthly, for two consecutive days, from 18h to 6h, totaling a monthly capture effort of 24 hours and a total of 288 hours for 12 months, in points of the urban and rural areas, in domicile and peridomicile. The selection of the capture point was based on criteria such as: the presence of phlebotomines, proximity with areas of vegetation, livestock farming, presence of organic matter, precarious socioeconomic conditions and existence of confirmed human and canine cases. Three traps were installed in the urban area (UA). Trap 1 (UA1 7.25902º S, 37.42500º W) in the interior of a domicile; trap 2 (UA2 7.27122 S, 37.43805° W) in a henhouse located in the backyard of the same house and trap 3 (UA3 7.25683° S, 37.42791º W) in the back of a warehouse used to store construction materials, close to an area of chicken and cattle farming. The UA1 and UA2 were about 300 m away from the city center and the UA3 120m.

In the rural area (RA), the fourth trap was installed at the "Dona Ninfa's" farm (RA1 - 724190º S, 37.47105º W) inside of a goat (*Capra hircus*) pen; the fifth trap in the "Covão" farm (RA2 - 7.23900º S, 37.47170º W) inside a *casa de taipa* (mud house) where hens (*Gallus gallus domesticus*) and an armadillo (*Euphractus sexcinctus*) were kept, and the sixth trap at the "Cariri" farm (RA3 - 7.27124° S, 37.43806° W), in an open area where hens (*Gallus gallus domesticus*), ducks (*Anas platyrhynchos domesticus*) and dogs (*Canis lúpus familiaris*) were kept, about 6 Km distance in relation to the two other farms. The traps RA1 and RA2 were about 5 Km, and RA3 about 3 Km away from the city center.

2.3 Taxonomic identification of the phlebotomines

After the collection, the insects were stored in a freezer, still in the traps to facilitate the triage and, subsequently, the separation by gender was carried out, by means of a stereoscopic magnifying glass. The specimens were maintained in microtubes containing alcohol 70% up to the moment of the identification, in the Molecular Biology Laboratory of the Semiarid of the Veterinary Hospital of the Federal University of Campina Grande (UFCG-Patos/PB) and in the Municipal Laboratory of Patos/PB. In order to be identified, they went through the clarification process, which consisted in the washing with distilled water for 15 minutes and immersion in Potassium Hydroxide (KOH) solution 10% for 14 hours, followed by washing in distilled water and storing in 95% glycerin solution and alcohol 70% until the analysis in optical microscope in slide. The identification of the species was carried out by means of the dichotomous keys described by Young and Duncan (1994).

2.4 Georeferencing and collection of climatic data

The traps were georeferenced using the Global Positioning System (GPS) GarmineTrex HC for subsequent plotting in map using the QGIS software. The bioclimatic data of minimum and maximum temperature (ºC), relative humidity of the air (%), wind speed (m/s) and rainfall (mm) were obtained in the database of the National Institute of Meteorology (INMET, 2019) from October 2018 to October 2019.

2.5 Detection of *Leishmania infantum* infection through the Real-Time Polymerase Chain Reaction (qPCR)

Lu. Longipalpis were used, fed and non-fed, grouped in pools of up to 10 specimens, taking into consideration the origin of the trap (rural or urban) and the months of collection. The extraction of the DNA from the phlebotomine samples was performed using the Dneasy Blood and Tissue (Qiagen Hilden, Alemanha) kit, following the recommendations of the manufacturer. The (qPCR) was performed as described by Silva et al. (2016), using the primers Linf KDNA-F 5'-GGCGTTCTGCAAAATCGGAAAA-3', and Linf KDNA-5'-CCGATTTTTGGCATTTTTGGTCGAT-3' and Linf KDNA-FAM-5'-TTTTGAACGGGATTTCTG-3' for the amplification of the mini-cycle of the kinetoplast of *L. infantum*. As positive control was used a culture of *L. infantum* and ultra-pure water DNAse, RNAse free as negative control.

2.6 Nucleotide sequencing

Sequencing reactions were performed with the primers described by Silva et al. (2016) using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA. Capillary electrophoresis was performed using a 3130xL Gene Analyzer and POP-7 polymer (Applied Biosystems, Foster City, CA, USA) (Platt et al., 2007).

The nucleotide sequence alignment was performed in BioEdit (Hall, 1999). The sequence was aligned with reference *Leishmania* strains obtained from GenBank™ (National Center for Biotechnology Information, Bethesda, MD, USA) (http.//www.ncbi.nlm.nih.gov), using the BLAST tool http://www.ncbi.nlm.nih.gov/BLAST/. Nucleotide sequence data reported in this paper are available in the GenBank™ databases under the accession numbers: MW245062.

2.7 Phylogenetic Analysis

A phylogenetic tree was generated using the o software Seaview 4 (Gouy et al., 2010). Phylogenetic trees were constructed, using the Bio Neighbor-Joining method and Jukes- Cantor model boots trap with 1,000 repetitions. The trees were visualized in Fig.Tree v1.4.4 (http://tree.bio.ed.ac.uk/). The phylogenetic reconstruction program included sequences of *Leishmania* for comparison.

2.8 Calculation of the natural infection rate

The rate of natural infection by *Leishmania* in the *Lu. longipalpis* females was expressed as Minimum Infection Rate (MIR) by the calculation of the ratio of the number of positive pools x 100/total of tested mosquitos according to Paiva et al. (2006).

2.9 Statistical analysis

The descriptive analysis was carried out of the number of males and females, total of specimens collected per month and per capture site (traps of the rural or urban areas). Pearson's correlation coefficient (r) was used to evaluate the correlation between the averages of the bioclimatic variables, the quantity and the species of phlebotomines captured each month. A hypothesis test was not used seen as the number of observations was small.

3. Results

From October 2018 to October 2019, 923 phlebotomines were captured, however, during the triage, were selected only the insects that had preserved anatomical structures, which enabled the identification of the species. Therefore, the total of identified specimens was of 567, being 25.7% of them females (146/567) and 74.3%, males (421/567) (Table 1).

The quantity of males collected represented the triple of females and the female/male ratio was of 0.35. The greatest quantity of specimens was captured in June 2019 (23.4% - 133/567), and the majority was originated from the rural area (98,6%) and belonged to the species *Lutzomyia longipalpis* (86.6%) and *Lutzomyia lenti* (13.05%).

The species *Lu. lenti* was captured in all the months of the experiment, except for December (Table 2), and 97.3% (72/74) were from the rural area and 2.7% (2/74) from the urban area. The months of May and September 2019 corresponded to the months of the highest (20.27 - 15/74) and lowest (1.35% - 1/74) of specimens captured, respectively.

The species *Lu. Longipalpis* was mainly captured in the rural area (98.78%-487/493). The month with the greatest capture was June 2019 (25.96%) (128/493), and the one with the lowest intensity was September (1.62%) (8/493) (Table 3).

Throughout the study period, the averages of rainfall and humidity had a greater variation between December 2018 and April 2019. The maximum average of rainfall occurred in February 2019 (6.31 mm) and of humidity in April of the same year (72.8%). The wind speed presented a marked reduction, reaching the lowest average in April 2019 (1.99 m/s). The minimum and maximum temperature averages were of 20° and 40° C, respectively.

The figures 2 and 3 present the behavior of *Lu. lenti* and *Lu. longipalpis*, respectively, when facing the changes of the climatic variables. Divergence 0 was adopted, in other words, the values for each bioclimatic variable correspond to the respective months of capture. *Lu. Lenti* was captured in a smaller quantity than the *Lu.*

longipalpis but, both presented the same behavior of population growth after the increase of the rainfall. According to Pearson's correlation coefficient, the species *Lu. lenti* (Figure 4) presented negative correlation with the increase in temperature (r = -0.16) and with wind speed (r = -0.46). For the other climatic variables, the correlation was positive: minimum temperature (r = 0.16); rainfall (r = 0.24) and humidity (r = 0.34). As for the species *Lu. longipalpis* (Figure 5), it presented a negative correlation with the maximum temperature (r = -0.27) and with wind speed (r = -0.32). For the other parameters, this data tended to nullity for minimum temperature (r = -0.07) and presented positive correlation with rainfall (r = 0.22) and humidity (r = 0.29).

The presence of *L. infantum* DNA was detected in 4 (15.3%) of the 26 pools analyzed in the qPCR, containing from 1 to 10 *Lu. longipalpis* females captured in the twelve-month study period, with a MIR value of 4%. The collection sites that presented positive pools were RA1 (2/9), RA2(1/8) and RA3(1/7). The RA1 presented the highest number of females captured and o positive pools, and the MIR values were of 10% in June and 9% in October 2019. The RA2 and RA3 presented a MIR of 50% in October 2019, and in November 2018, respectively. Natural infection was not detected in females originated from the traps of the urban area in the period studied. The sequencing of only one sample was performed due to the reduced quantity of amplicons of the samples.

4. Discussion

The total of phlebotomines captured in this study is within the range reported by other authors, which varies from 343 to 214.213 specimens (Silva et al., 2017; Godoy et al., 2018; Lana et al., 2018). It is important to highlight that the number of traps used was small, and may influence in the total of insects collected.

The oscillations in the number of insects captured may also occur due to differences in the methodologies employed, region studied, frequency of installation, quantity and type of trap used (Silva et al., 2014). It is also important to highlight the influence of environmental and bioclimatological characteristics of each study location, as they have direct effect on the biotopes where the phlebotomines are found (Oliveira-Pereira, 2008). According to Oliveira et al. (2010), the climatic differences and the speed of the winds influence in the seasonality of the insects, as well as the installation sites of the traps. The installation close to green areas, rich in fruit-bearing trees and which contain more than one species of domestic animals or livestock farming, especially gallinaceous, favors the presence of insects (Oliveira et al. 2013; Silva et al., 2014; Salomón et al., 2015), which is in accordance with what was found in this work.

In relation to the gender, the higher frequency of males captured, is in accordance with other surveys conducted in Brazil (Fernandes et al., 2017; Silva, K., et al., 2018; Faria et al., 2019; Sales et al., 2019). This can be explained by courtship behavior, which consists in the disposition in aggregates, making the males more susceptible to the capture during the displacement (Gomes et al., 1980). Also, the males are born before the females and these seek safe shelters to perform the digestion and maturation of the ovarian follicles after the blood repast, which reduces the chance of being captured (Casaril et al., 2019).

In this study, there was capture in all the months of the year (Silva et al., 2018), being the greatest occurrence in March (17.28 %) (98/567) and June (23.46%) (133/567), due to the increase of the rainfall, of the humidity and mild temperatures, creating favorable conditions for the hatching of the larvae in the soil (Souza et al., 2017). The majority of the species originated from the rural area (98.59% - 559/567) (Alves et al., 2012). In these locations, in addition to the presence of shady areas, the homes were located close to the vegetation cover and commonly there is rearing of domestic animals in the peridomicile, where there is organic matter in decomposition (Berrozpe et al., 2017).

The species *Lutzomyia lenti* was predominant in the rural area; and can be found distributed in locations in the process of urbanization in states of the Northeast, North, Central-west and Southeast (Sangiorgi et al., 2012; Silva, K.et al., 2017). This species has been frequently captured in henhouses, acting as hematophagous of domestic animals, but, still without the observation of infection by *Leishmania spp* (Guimarães-e-Silva et al., 2017).

The species *Lu. longipalpis* was the most found, representing 86.9% (493/567) of the total of specimens captured (Bastos et al., 2016; Sales et al., 2019). It is considered to be the most important transmitter of *L. infantum* (Rangel e Lainson, 2003; Gontijo e Melo, 2004) and in the regions North and Northeast is found in entomological studies also associated to the *caatinga* (Chagas et al., 2016; Pinheiro et al., 2016; Silva, K., et al., 2017).

The capture presented a consistent pattern and in a greater frequency in the rural area (Silva, K. R., et al., 2017; Silva, K. B. et al., 2018; Silva, R. et al., 2018). June was the month of greatest abundance, notably after the rainy period (Silva et al, 2017) and the lowest was the month of September, characterized by a period of drought (Sales et al., 2019). However, the reduction in the month of September may be associated to the destruction of part of the wall of the *casa de taipa* (mud house) where the trap RA5 was installed during all the study, increasing the wind circulation and the luminosity, making the location less attractive as a shelter. In the month of October, after the repairs, the total of insects collected had a substantial increase, even in the absence of rainfall.

Peaks of capture are described in months with low rainfall (Silva et al., 2015), because high rainfall interferes in the size of the population, as it destroys the daytime resting places of the adults and carry the pupae from the soil (Scandar et al., 2011). The seasonal variation of the phlebotomines is also influenced by abiotic factors, ecology of the species and geographical characteristics of the area is which the capture takes place, being possible that the same species present distinct seasonal patterns in the same geographical area, therefore, is is necessary to correlate climatic parameters and the density of phlebotomines for the construction of a seasonal curve (Saraiva et al.., 2017).

There was positive correlation with the rainfall and humidity, confirming that there is the need for the first rains and for the increase of the humidity for the eclosion of the eggs and the development of the immature forms (Bastos et al., 2016; Saraiva et al., 2017; Souza et al., 2017). After the finalization of the cycle, the adult and winged forms emerge increasing the number of specimens in the following months. According to Colla-Jacques et al. (2010), in anthropized environments, the *Lu. Longipalpis* is under more stable environmental conditions, which explains its occurrence in dry and humid periods and even in drought seasons, creating favorable conditions for an increase of the population in the following months.

The correlation with the relative humidity of the air was stronger than with the rainfall, which can explain the peak of *Lu. longipalpis* (51.03%) in October 2019, despite the absence of rain, the humidity rate was high, influencing in the next month's capture (Bastos et al., 2016), but, this peak can still be associated to the influence of the reconstruction of the *casa de taipa* (mud house) in the RA5 in the month of October.

The temperature parameter presented negative correlation with the number of *Lu. longipalpis*, in other words, the higher the temperature, lower its occurrence (Amorim et al., 2015). According to Medlock al. (2014), both low and high temperatures are limiting factors on the fluctuation of phlebotomines. The municipality of Mãe D'Água presents mild temperatures, varying between 24°C in the winter and 30°C in the summer, with minimum temperatures between 18°C and 20°C (AESA, 2019), however, the minimum temperature presented a weak correlation with *Lu. lenti*.

The effect of the wind speed had negative correlation on the density of the phlebotomines. These diptera have habits of mating and rest in protected places, as they are fragile, which makes them not search for food, sexual partners or carry out flying activities in situations of intense rainfall and high wind speed (Amorim et al., 2015).

In an endemic area for the occurrence of visceral leishmaniasis, the detection of natural infection in *Lu. Longipalpis* females is of vital importance (Anaguano et al., 2015; Saraiva et al. 2015; Mota et al., 2019), being the qPCR reaction a more sensitive and specific method when compared to the dissection of phlebotomines method. In this study, the technique was capable of detecting *L. infantum* in four pools of *Lu. Longipalpis* females, three from the year 2019 and one from 2018.

By the calculation of the minimum infection, was verified a natural infection rate of 4%, being two pools originated from the same collection site, but from different months, one containing 10 females and another formed by a sample of only one specimen. Demonstrating the efficiency of the technique in detecting DNA in a small number of phlebotomines (Cunha et al., 2014).

None of the samples originated from the urban area were positive, indicating that, despite the expansion of the disease to urbanized areas observed over the last years, in this study the rural area presented a greater potential of occurrence of the disease, in areas close to preserved native vegetation, and above all, for maintaining livestock farming in the peridomicile, possible food sources for the vector (Michalsky et al., 2009). Furthermore, two of three rural locations have already notified cases of the disease in humans and in dogs, and these present similar characteristics regarding the presence of shady areas and accumulation of organic matter.

The positive pool of June 2019 coincided with the greater abundance of the species, both of males and females, mainly as a result of the rainy period. Two of the four positive pools originated from the collection carried out in October 2019, period when there was a peak of *Lu. Longipalpis* males, probably due to the elevation of the humidity in the previous month.

5. Conclusion

The climatic conditions and the biome of the Municipality of Mãe D'Água favor the presence of phlebotomines throughout the year, presenting a high density of *Lu. lenti* and, especially, of *Lu. longipalpis*, the main vector species of visceral leishmaniasis in the area studied, which associated to the rate of infected females through the molecular analysis, indicates the need for a continuous vector control.

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Table 1. Phlebotomines captured from October 2018 to October 2019 in the urban and rural areas of the Municipality of Mãe D'Água, *Sertão* Region of the State of Paraíba, Brazil.

Table 2. Total of *Lutzomyia lenti* specimens captured from October 2018 to October 2019 in the Municipality of Mãe D'Água, *Sertão* Region of the State of Paraíba, Brazil.

Table 3. Total of *Lutzomyia longipalpis* specimens captured from October 2018 to October 2019 in the Municipality of Mãe D'Água, *Sertão* Region of the State of Paraíba, Brazil.

Figure 1. Location map of CDC traps in the municipality of Mãe D'Água, Paraíba, Brazil

Figure 2. Seasonal variation of the *Lutzomyia lenti* between October 2018 and October 2019 in Mãe D'Água, *Sertão* region of the State of Paraíba, Brazil

Figure 3. Seasonal variation of the *Lutzomyia longipalpis* between October 2018 and October 2019 in the Municipality of Mãe D'Água, *Sertão* region of the State of Paraíba, Brazil.

Figure 4. Correlation of the climatic variables and *Lutzomiya lenti* specimens captured in the Municipality of Mãe D'Água, PB, Brazil, between 2018 and 2019.

Figure 5. Correlation of the climatic variables and *Lutzomyia longipalpis* specimens captured in the Municipality of Mãe D'Água, PB, Brazil, between 2018 and 2019.

Figure 6. Phylogenetic tree based on the nucleotide sequence alignment of the kinetoplast minicircle DNA of *Leishmania* constructed using the Bio Neighbor-Joining method and Jukes- Cantor model bootstrap with 1,000 repetitions. ▲ Sequenced samples.

Table 1. Phlebotomines captured from October 2018 to October 2019 in the urban and rural areas of the Municipality of Mãe D'Água, Sertão Region of the State of Paraíba, Brazil.

	URBAN AREA		RURAL AREA		TOTAL
MONTHS/ SEX	Male	Female	Male	Female	
October/2018	0	0	10	15	**25**
November/ 2018	1	0	5	9	**15**
December/ 2018	0	0	14	3	**17**
February/ 2019	0	0	59	4	**63**
March/ 2019	0	0	75	23	**98**
April/ 2019	0	0	6	5	**11**
May/ 2019	0	1	20	10	**31**
June/ 2019	0	0	98	35	**133**
July/ 2019	2	1	19	7	**29**
August/ 2019	2	1	26	16	**45**
September/ 2019	0	0	7	2	**9**
October/ 2019	0	0	77	14	**91**
TOTAL	**5**	**3**	**416**	**143**	**567**

Table 2. Total of *Lutzomyia lenti* specimens captured from October 2018 to October 2019 in the Municipality of Mãe D'Água, Sertão Region of the State of Paraíba, Brazil.

	UA 1		**UA 2**		**UA 3**		**TOTAL**	**RA 1**		**RA 2**		**RA 3**		**TOTAL**
MONTHS/ SEX	Male	Female	Male	Female	Male	Female		Male	Female	Male	Female	Male	Female	
October/2018	0	0	0	0	0	0	**0**	0	0	1	13	0	0	**14**
November/2018	0	0	0	0	0	0	**0**	0	0	0	5	0	0	**5**
December/2018	0	0	0	0	0	0	**0**	0	0	0	0	0	0	**0**
February/2019	0	0	0	0	0	0	**0**	0	0	3	1	0	0	**4**
March/2019	0	0	0	0	0	0	**0**	0	0	6	7	0	0	**13**
April/2019	0	0	0	0	0	0	**0**	0	0	1	1	0	0	**2**
May/2019	0	1	0	0	0	0	**1**	0	0	5	8	1	0	**14**
June/2019	0	0	0	0	0	0	**0**	2	3	0	0	0	0	**5**
July/2019	0	0	0	0	0	0	**0**	0	1	0	1	0	1	**3**
August/2019	0	0	1	0	0	0	**1**	0	1	3	1	2	0	**7**
September/2019	0	0	0	0	0	0	**0**	1	0	0	0	0	0	**1**
October/2019	0	0	0	0	0	0	**0**	3	1	0	0	0	0	**4**
TOTAL	**0**	**1**	**1**	**0**	**0**	**0**	**2**	**6**	**6**	**19**	**37**	**3**	**1**	**72**

Table 3. Total of *Lutzomyia longipalpis* specimens captured from October 2018 to October 2019 in the Municipality of Mãe D'Água, Sertão Region of the State of Paraíba, Brazil.

	UA 1		UA 2		UA 3		TOTAL	RA 1		RA 2		RA3		TOTAL
MONTHS/ SEX	Male	Female	Male	Female	Male	Female		Male	Female	Male	Female	Male	Female	
October/2018	0	0	0	00	0	0	**0**	0	0	9	2	0	0	**11**
November/2018	1	0	0	0	0	0	**1**	0	0	3	2	2	2	**9**
December/2018	0	0	0	0	0	0	**0**	0	0	14	0	0	3	**17**
February/2019	0	0	0	0	0	0	**0**	47	1	9	1	0	1	**59**
March/2019	0	0	0	0	0	0	**0**	0	0	64	16	5	0	**85**
April/2019	0	0	0	0	0	0	**0**	0	0	5	3	0	1	**9**
May/2019	0	0	0	0	0	0	**0**	0	0	14	1	0	1	**16**
June/2019	0	0	0	0	0	0	**0**	85	31	3	0	8	1	**128**
July/2019	0	0	0	1	2	0	**3**	9	2	2	0	8	2	**23**
August/2019	0	0	0	0	1	1	**2**	11	1	5	3	5	10	**35**
September/2019	0	0	0	0	0	0	**0**	3	1	3	1	0	0	**8**
October/2019	0	0	0	0	0	0	**0**	74	13	0	0	0	0	**87**
TOTAL	**1**	**0**	**0**	**1**	**3**	**1**	**6**	**229**	**49**	**131**	**29**	**28**	**21**	**487**

Figura 4. Location map of CDC traps in the municipality of Mãe D'Água, Paraíba, Brazil

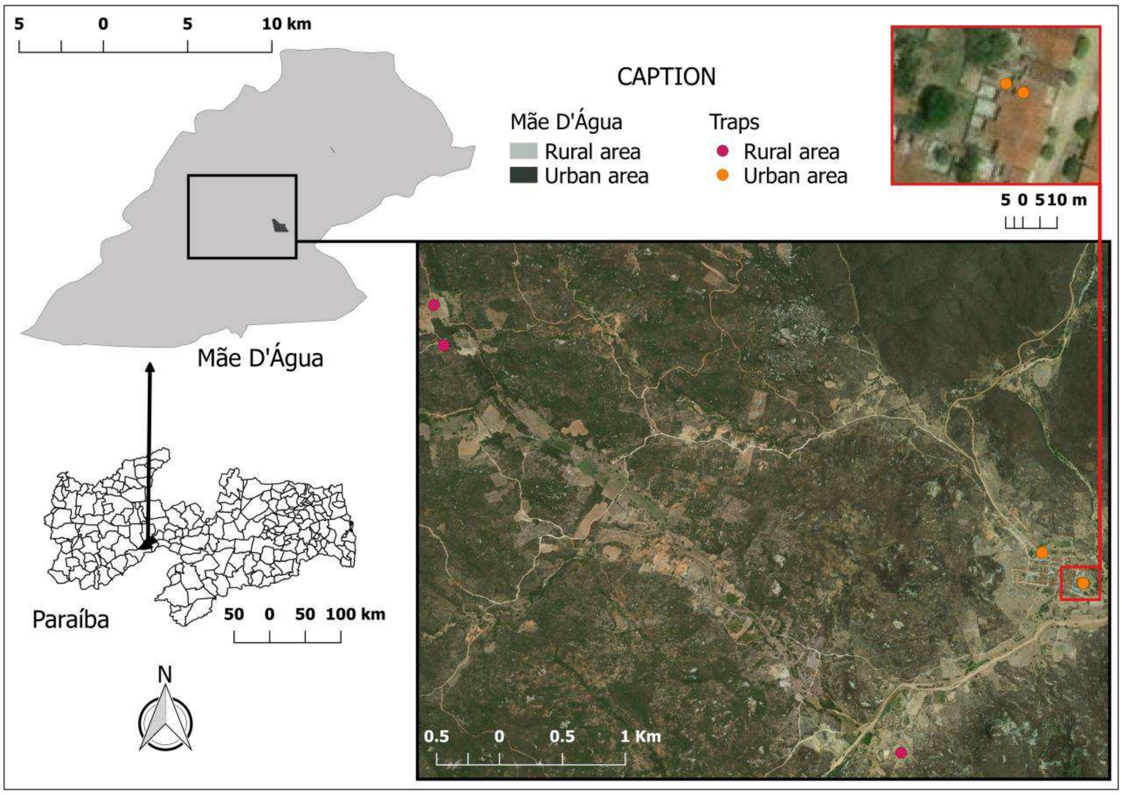


Figura 5. Seasonal variation of the *Lutzomyia lenti* between October 2018 and October 2019 in Mãe D'Água, Sertão region of the State of Paraíba, Brazil

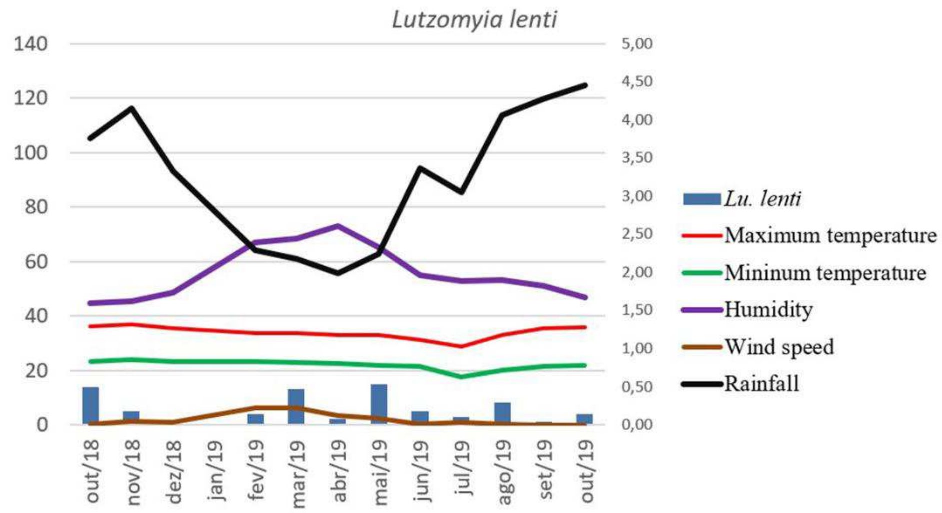


Figura 6. Seasonal variation of the *Lutzomyia longipalpis* between October 2018 and October 2019 in the Municipality of Mãe D'Água, Sertão region of the State of Paraíba, Brazil.

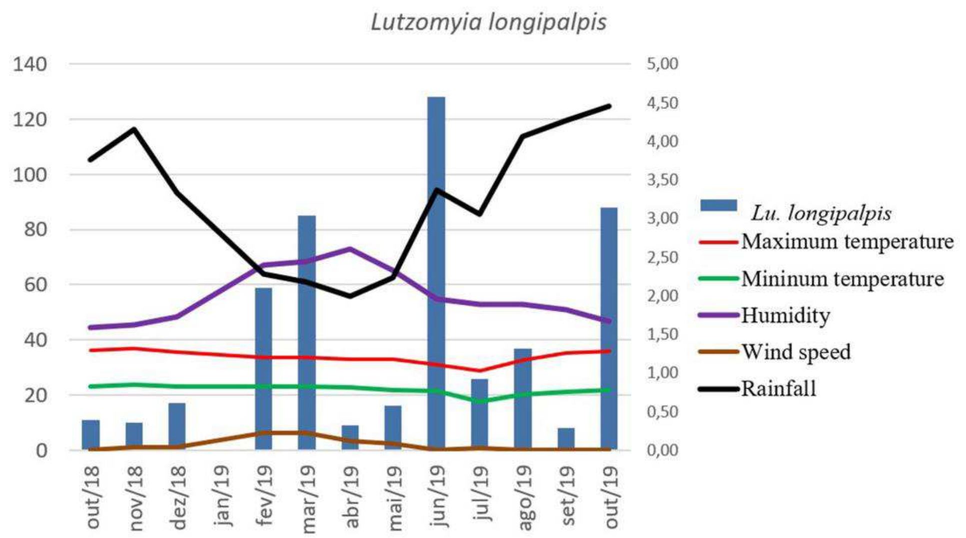


Figura 7. Correlation of the climatic variables and *Lutzomyia lenti* specimens captured in the Municipality of Mãe D'Água, PB, Brazil, between 2018 and 2019

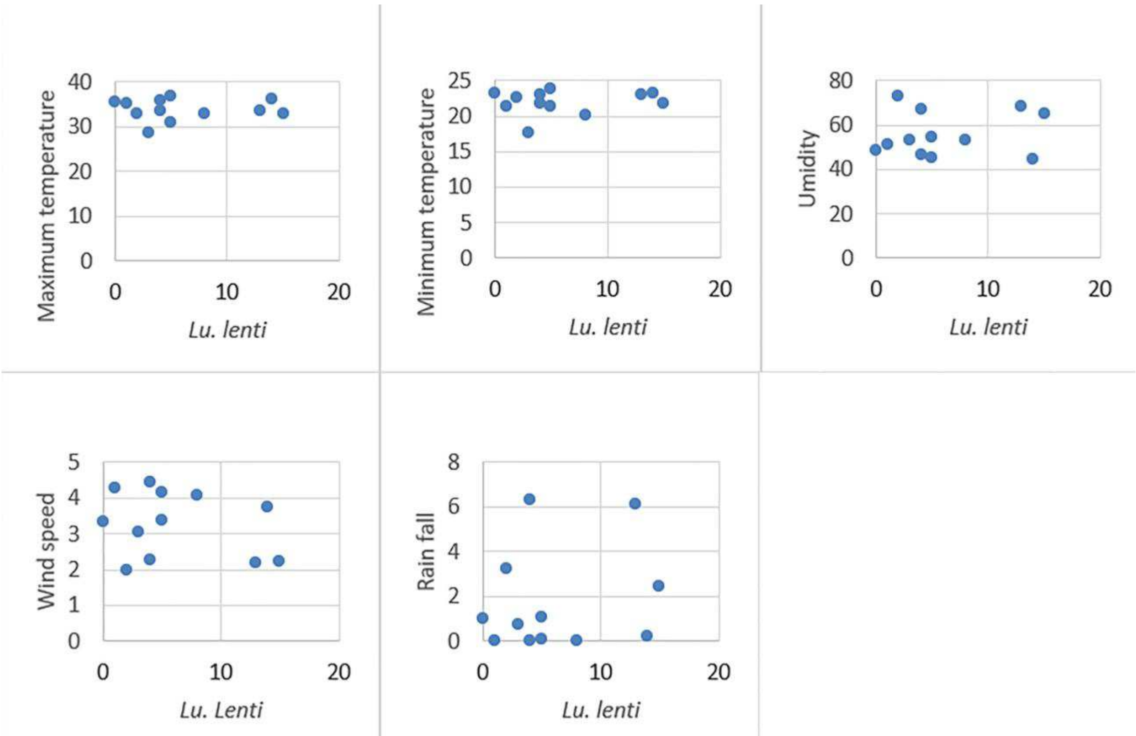


Figura 8. Correlation of the climatic variables and *Lutzomiya longipalpis* specimens captured in the Municipality of Mãe D'Água, PB, Brazil, between 2018 and 2019

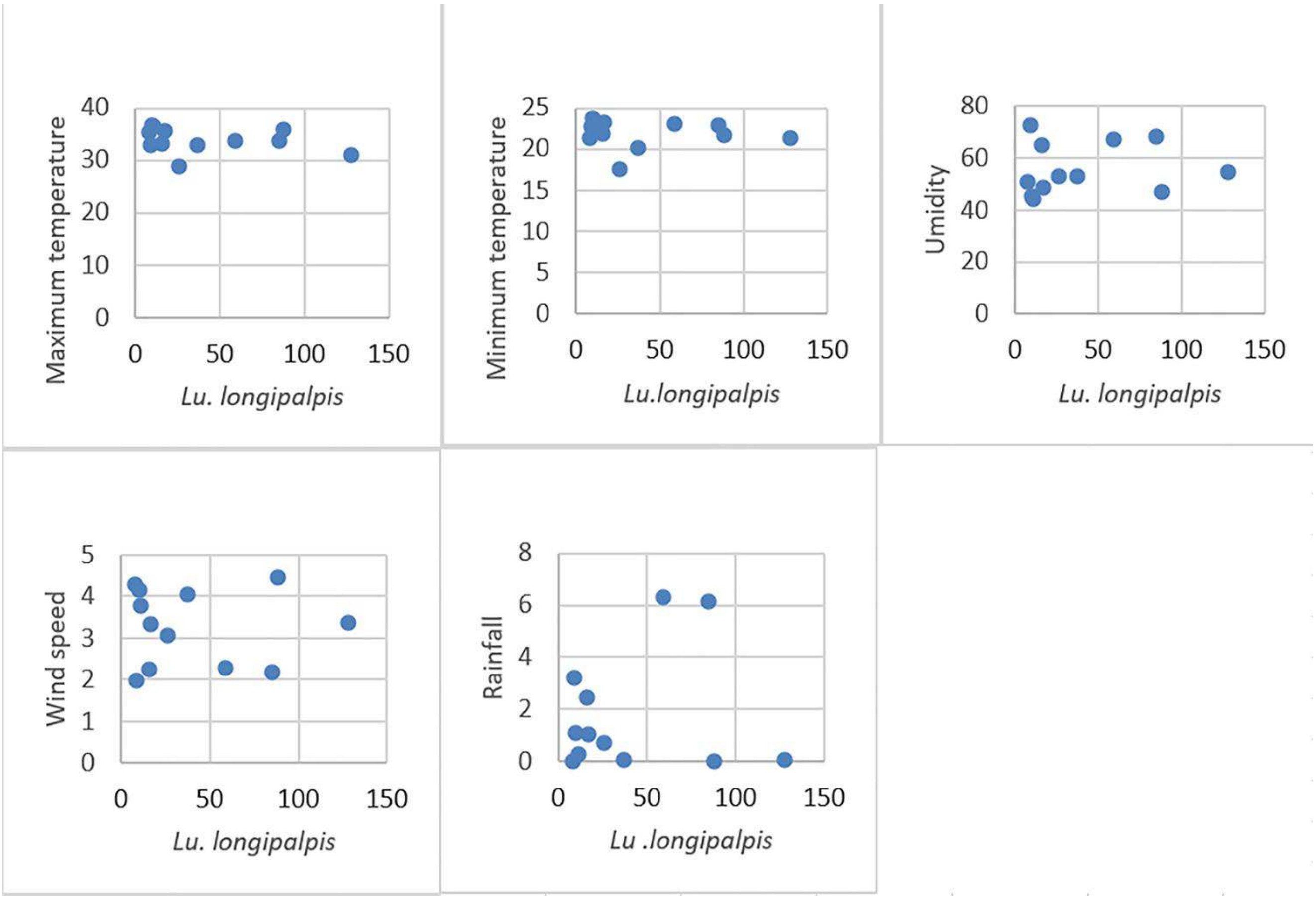
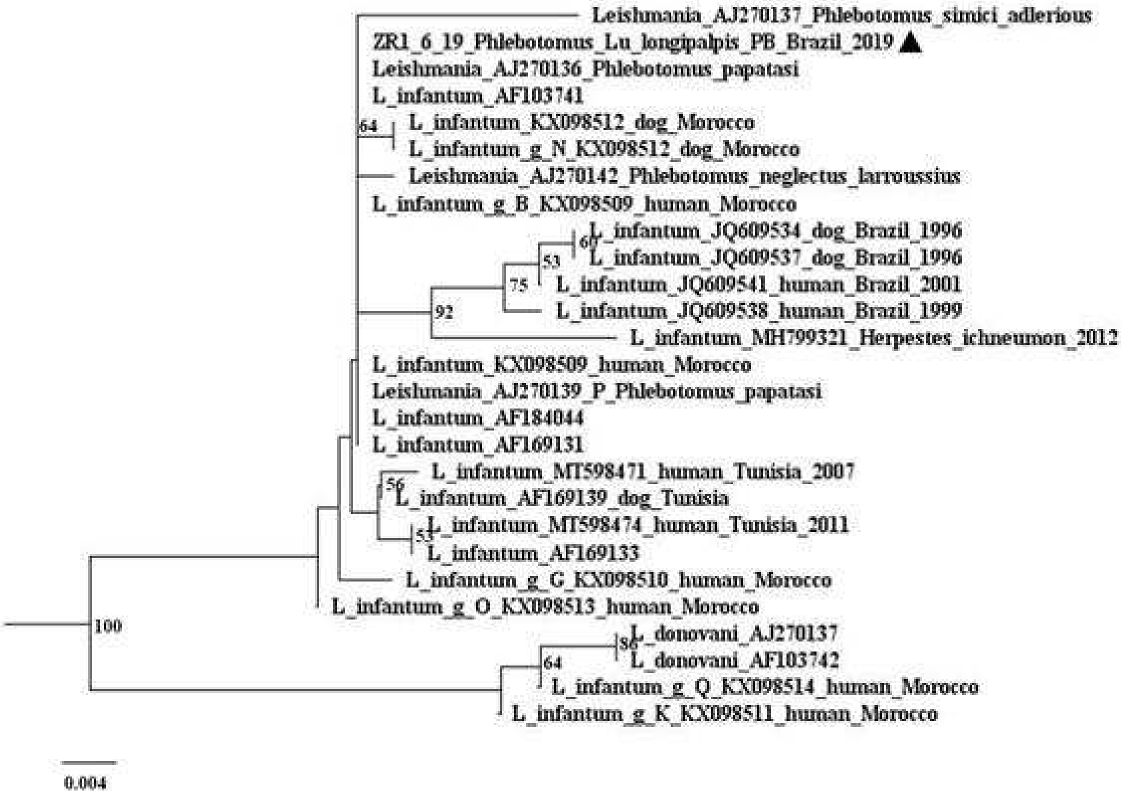


Figura 9. Phylogenetic tree based on the nucleotide sequence alignment of the kinetoplast minicircle DNA of Leishmania constructed using the Bio Neighbor-Joining method and Jukes- Cantor model bootstrap with 1,000 repetitions. ▲ Sequenced samples.



CAPÍTULO III:

ESTUDO DEMOGRÁFICO E ESPACIAL DA LEISHMANIOSE VISCERAL HUMANA NO ESTADO DE ALAGOAS, BRASIL ENTRE 2007 E 2018

Demographic and spatial study of human visceral leishmaniasis in the state of Alagoas, Brazil between 2007 and 2018

Beatriz Maria de Almeida Braz; Raizza Barros Sousa Silva; Diego Ricardo Xavier Silva; Walter Massa Ramalho; Suzanna Cavalcante Lins; Marcia Almeida de Melo

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Demographic and spatial study of human visceral leishmaniasis in the state of Alagoas, Brazil between 2007 and 2018

Estudo demográfico e espacial da leishmaniose human visceral no estado de Alagoas, Brasil entre 2007 e 2018

[1]Laboratório de Biologia Molecular do Semiárido, Programa de Pós-Graduação em Medicina Veterinária, Universidade Federal de Campina Grande – UFCG, Patos, PB, Brasil;
[2]Fundação Oswaldo Cruz, Instituto de Comunicação e Informação Científica Tecnológica, Rio de Janeiro, RJ, Brasil; [3]Universidade de Brasília (UnB - Faculdade de Ceilândia), Brasília, Brasil

Beatriz Maria de Almeida Braz[1]
Raizza Barros Sousa Silva[1]
Suzanna Cavalcante Lins[1]
Diego Ricardo Xavier Silva[2]
Walter Massa Ramalho[3]
Marcia Almeida de Melo[1]

Demographic and spatial study

Abstract

Introduction

Visceral leishamaniasis (VL) has a broad worldwide distribution and constitutes a public health problem in the Northeast of Brazil. Located in this region, the State of Alagoas is endemic to the disease in humans and has presented a significant increase in the number of positive dogs. The objective of this study was to describe the temporal and spatial distribution of the cases of human VL in the state of Alagoas with the aim of identifying the transmission risk areas in the period from 2007 to 2018.

Methods

The data available in the National Disease Notification System (SINAN-NET) was used. The Bayesian incidence rate and the Moran's global index were calculated by Terra View 4.2.2 program, and the maps were created using the QGIS*2.18.0*.

Results

From the 102 municipalities, 68.6% (n= 70) notified at least one case of VL in the years of study. 489 cases were registered, with an average of 40.7 cases per year and an incidence rate of 1.25/100 thousand inhabitants. In 2018, occurred the highest number of confirmed cases (105). Male individuals and children between 1-4 years old were the most affected, and 64% of the cases were of rural area. Spatial dependence was detected in all the intervals except for the first triennium, and clusters were formed in the West of the state.

Conclusion

It became evident that Alagoas presented an accentuated geographical expansion of VL, and it is necessary to prioritize areas and massify the surveillance actions and epidemiological control.

Keywords: Epidemiology; Georeferencing, Incidence, Epidemiological surveillance

Resumo

Introdução

A leishmaniose visceral (LV) possui ampla distribuição mundial e representa um problema de saúde pública no Nordeste do Brasil. Localizado nessa região, o Estado de Alagoas é endêmico para a doença no homem e tem apresentando significativa elevação no número de cães positivos. O objetivo desse estudo foi descrever a distribuição temporal e espacial dos casos de LV humana no estado de Alagoas com o objetivo de identificar as áreas de risco de transmissão no período de 2007 a 2018.

Métodos

Foram utilizados os dados disponíveis no Sistema de Informação de Agravos de Notificação (SINAN-NET). Através do TerraView 4.2.2,a incidência bayesiana e oíndice de Moran globalforam calculados, e os mapas foram elaborados no QGIS2.18.0.

Resultados

Dos 102 municípios, 68,6% (n= 70) notificaram pelo menos um caso de LV nos anos de estudo. Foram registrados 489 casos, com uma média de 40,7 casos por ano e incidência de 1,25/100 mil habitantes. Em 2018 ocorreu o maior número de casos confirmados (105). Foram mais acometidos indivíduos do sexo masculino e crianças com idade entre 1- 4 anos, e 64% dos casos tinha origem rural. Foi detectada dependência espacial em todos os intervalos, com exceção do primeiro triênio e clusters foram formados no oeste do estado.

Conclusão

Ficou evidente que Alagoas apresentou uma acentuada expansão geográfica da LV, sendo necessário priorizar áreas e massificar as ações de vigilância e controle epidemiológico.

Palavras-chave: Epidemiologia, Georreferenciamento, Incidência, Vigilância epidemiológica

Introduction

Visceral leishmaniasis (VL) is a neglected tropical disease caused by different species of the genus *Leishmania*[1] and transmitted through blood repast of females of the *Lutzomyia (Lutzomyia) longipalpis*[2] species. In Brazil, the specie *Leishmania infantum* is the etiological agent. The species *Lutzomyia longipalpis* and *Lutzomyia cruzi* are the vectors related to transmission[3,4]. Dogs (*Canis lupus familiaris*) are the main domestic reservoirs and play a major role in the maintenance of the transmission cycle between humans and the vectors[5].

Human visceral leishmaniasis (HVL) has a broad worldwide distribution with occurrence in Asia, Europe, Middle East, Africa and in at least 12 countries of the Americas[4], whose reported cases came from deforested, peripheral areas or those in process of territorial expansion[6]. The disease is endemic in Brazil, representing more than half of the cases of the Americas, and is distributed in the North, Central-West, Northeast, and Southeast regions, representing a public health problem[7,8].

Situated in the Northeast region, the state of Alagoas had the first cases of VL notified in 1934, originating from the coastal region and *Zona da Mata* (forest region)[9.] The disease is endemic in an area composed of 66 municipalities situated in the intermediate geographical regions of Maceió and Arapiraca[10], with a notorious increase in the number of confirmed cases over the last ten years[11]. In addition, there was a significant increase in the number of dogs positive for the disease diagnosed by serological methods, which resulted in the euthanasia of 909 positive animals in 2018[12].

Up to now, in Brazil, the Visceral Leishmaniasis Surveillance and Control Program (VLSCP) of the Health Ministry establishes the euthanasia of dogs with two positive serological or parasitological results as a control strategy, besides prevention measures aimed at the population at risk and the vector[13]. Therefore, it is becoming increasingly necessary to process the information available in public databases, including the National Disease Notification System (SINAN-NET) and the Geographic Information System (GIS), as health surveillance tools for reassessment of endemic control programs. Studies, which analyze the expansion process of the VL and the spatial and temporal variation of the incidence, are of great importance due to its dispersion over the last years in Brazil[14]. Therefore, the objective of this study was to describe the temporal and spatial distribution of cases of VL in the state of Alagoas, in order to identify the areas with high-risk of transmission of the disease in the period from 2007 to 2018 in triennial intervals.

Methods

Alagoas is one of the 27 federative units of Brazil, located in the Northeast region and has 102 municipalities. The approximate population is of 3.322.000 inhabitants in a territorial area of 27.778,506 km². Until 2017, the state was divided according to the climate into three mesoregions, composed of municipalities located in the Agreste, Sertão and Eastern regions of the state of Alagoas; the latter covers the municipalities of the coast and the forest zone (forest area). However, this division was replaced by the Brazilian Institute of Geography and Statistics (IBGE) by two intermediate geographical regions, Maceió and Arapiraca, and 11 immediate regions. This encompasses cities of the coast and *zona da mata* (forest area), however, this division was substituted by the Brazilian Institute of Geography and Statistics (IBGE) by two intermediate geographical regions, of Maceió and Arapiraca, and 11 immediate regions[15].

In this work, was carried out a descriptive evaluation with temporal and spatial analysis of the new confirmed cases and notified by means of the National Disease Notification System (SINAN-NET) obtained from the website of the National Health System Information Technology Department (DATASUS) between 2007 and 2018, which were disposed in four triennial intervals: 2007-2009; 2010-2012; 2013-2015 and 2016-2018. The data collection was carried out in February 2020. The parameters of distribution of the cases were evaluated by age, sex, level of education, zone of residence and evolution of the disease and presented in relative and absolute frequency.

The cartographic network of the state was obtained from the website of the IBGE and the data was inserted into spreadsheets in the Microsoft Excel2013®, in which the cumulative gross rate of incidence/municipality was calculated and imported to the Terra View 4.2.2 software. To calculate the smoothed incidence rate of each municipality, the local empirical Bayesian estimator was used, which uses the incidence rates of the neighboring municipalities converging to a local average, with the objective of correcting the generated rates, making them less unstable. The intervals of the used incidence rates considered the quartile, average, median, minimum and maximum values.

The classes of the incidence rates were categorized by adopting a quartile-based legend. To understand the spatial association patterns (*clusters*) and verify the extreme values (*outliers*), was used the Moran's global index, which informs the level of spatial interdependence, varying from -1 to 1[16]. The descriptive significance levels of the clusters were obtained from Moran's Global Index adopting a level of significance or p-value ≤0.05, indicating spatial dependence and demonstrated by Moran's Map. The municipalities with high priority of intervention were then identified. The QGIS 2.18.0 software was used for the elaboration of thematic maps.

Results

Between 2007 and 2018 were registered 489 cases of HVL in the State of Alagoas, with an average of 40.7 cases per year and an incidence of 1.25/100 per thousand inhabitants. The state is composed of 102 municipalities, of these, 68.6% (n= 70) notified at least one case of HVL in the 12 years of study. In the first triennium

(2007-2009), ninety-one (91) cases were notified in 37.2% (n= 38) of the municipalities, corresponding to 0.98 cases/100 thousand inhabitants. In the second (2010-2012) and third trienniums (2013-2015) occurred a discreet increase to 106 (1.12 cases/100 thousand inhabitants) and 115 cases (1.15 cases/100 thousand inhabitants), in 39.2% (n= 40) and 40.1% (n= 41) of the municipalities, respectively. In the fourth triennium (2016-2018), there was a significant increase to 177 cases (1.76 cases/100 thousand inhabitants), distributed in 41.1% (n=42) of the cities.

Most of the individuals affected were male (66.2%) and children aged between 1 and 4 years (28.6%), 87.7% of the cases were autochthonous and 64% of the total cases originated from rural areas. The lethality rate of VL was of 7.7% (38), with an average of 3.16 cases/100 thousand inhabitants; 46.21% evolved to cure (Table 1).

Through the incidence map, it is perceived that there were cases of the disease in all the geographical extension of the state during the four triennial intervals, however, the highest incidence rates were concentrated in the first triennium (15 to 30/100.000 inhabitants) (Figure 1), in municipalities of the Center-West, with some isolated cases on the East of the state, which was also confirmed in the in the map of smoothed cumulative incidence or Bayesian in the same period of the study (Figure 3).

In the second and third trienniums also occurred a similar distribution, however, high incidence rates were found in municipalities on the coast of the state (15 to 30/100.000 inhabitants), which presented a decline in the fourth interval of the study (1 to 5/100.000 inhabitants) (Figure 1 and 2), however the cases remained in the Center-West region with an increase of this rate when compared to the last three years (15 to 30/100.000 inhabitants) (Figure 1 and 2).

By means of the value of the Moran's index was observed that there was no spatial dependence in the first triennium and, therefore there were no significant clusters, but was observed a spatial dependence in the second (p= 0.015), third (p= 0,009) and fourth trienniums (p= 0.002), and a similarity between the municipalities, however, with a weak correlation (Table 2). Based on this index, Moran's Map was built (Figure 3). In the second triennium, a high incidence cluster was identified in cities near the municipalities of Monteirópolis and Olho D'Água das Flores, but these presented a low risk cluster.

In the East region, precisely in the municipalities of the *zona da mata* (forest region), an agglomerate was formed in the low-low category. During this period, even though it was inserted between the municipalities with low risk of occurrence of the disease, the Anadia municipality stood out as it presented a significant cluster.

In the third triennium, there was a reduction in the number of significant clusters, which were situated in municipalities of the semi-arid, and, even though it was close to the risk region, the municipality of Carneiros presented a low-risk cluster. Still in this study interval, a cluster of the high-low category was observed in the metropolitan region of Maceió, capital of the state. In the fourth triennium, a high significance cluster was formed in the West of the state of Alagoas, in the limits with the municipality of Minador do Negrão, and another, close to São Braz, but, despite this, they did not present a high risk for the occurrence of the disease.Still in this triennium, a low-incidence cluster was observed, starting from the Central-South to the East, surrounding the municipalities of Jacuípe to the Northeast and Marechal Deodoro to the Southeast of the state.

Discussion

The present study reported the temporal and spatial contribution of new cases of VL in the state of Alagoas, and the data was analyzed in a scale of triennial intervals, from 2007 to 2018, being the first report of this nature in the State. The incidence data revealed a progressive geographical expansion of the HVL in the studied period, showing a tendency to persist in municipalities which already had notified cases, strengthening the endemic status of Alagoas. According to Silveira et al.[17] and Rocha et al.[18], the disease in predominantly rural, and has been constantly registered in municipalities of the *Agreste* and *Sertão* regions, such as Arapiraca, Palmeira dos Índios, Traipú, Cacimbinhas, Igaci, Santana do Ipanema and São José da Tapera. In this study, Palmeira dos Índios and São José da Tapera, situated in the immediate geographical region of Arapiraca, presented the highest number of cases of the disease and, over the years has presented a status of moderate and intense transmission respectively[18]. This fact can be justified by the average (0.638) and low (0.527) Municipal Human Development Indexes (IDH-M) of Palmeira dos Índios and São José da Tapera[14], respectively, that these municipalities exhibit. Basically, the cases tended to progressively increase at each triennium in Palmeira dos Índios and to decrease in São José da Tapera, which may be an indication of the lack of an early diagnosis, shortcomings in the epidemiological surveillance and sub-notification.

The occurrence of the VL may be also associated to the environmental conditions of each geographical region, and these must be taken into consideration. As described by Leite et al.[19], in situations of high temperature and humidity, there was a decrease in the number of cases, in response to the low adaptation and reproduction of the vector. Additionally, Rocha et al.[18] justified that the emergence of cases in Alagoas may be due to socio-environmental issues, such as poor housing conditions, lack of basic sanitation and water supply, peridomicile waste accumulation both in urban and rural residences, presence of animal raising close to the houses and proximity to green areas. Associated, these factors interfere directly in the adaptation and reproduction of the vector[20], and is an aspect to be discussed, seen as the *Lu. longipalpis* species, involved in the transmission of the VL in Brazil, is completely adapted to this region, and can be found in all the territory of the state of Alagoas[21, 22]. It is important to report that, where there was a record of cases, municipal health agents were trained to act in entomological surveillance and in other actions within the scope of VL control. However, most municipalities did not continue their activities due to the lack of human resources[13].

Most of the cases affected children under the age of five and individuals of the male sex, which is a tendency also mentioned by other authors[17, 24, 25, 26]. The children are more susceptible to the infection due to the immaturity of the cellular immune system[27,28], that can be accentuated by malnutrition[29]. In Brazil, specifically in the Northeast, the *sertão* region is historically known for the precariousness caused by the drought, concerning housing and living conditions, where especially children live in conditions of malnutrition, favoring the installation and worsening of diseases[19, 30, 31], in contrast, when children present a good nutritional state and immunizations are up-to-date, the incidence of VL is reduced[25]. As for the individuals of the male sex, these have a greater tendency of developing occupational diseases[32].

VL was reported up to the 1980s as a rural endemic disease in Brazil, but it known that the disease has gone through a process of expansion and started to present a status of urban and peri-urban transmission. This is due to several factors such as, the difficulty to identify and eliminate the reservoirs, adaptation of the vector to the peridomicile, high cost of the control actions and insufficient control[15, 21, 29]. Despite this, in this study were notified more cases originated from rural areas, evidencing an old pattern of behavior of the disease in the State, arising from socio-economic and cultural issues of the populations of these areas, as well as the low educational level which indicate the lack of knowledge of the disease, the presence of the canine reservoir and sanitary precariousness.

Among the individuals diagnosed, 46.21% of them evolved to cure and 7.7% to death by VL. In these cases, the precocity in the diagnosis and treatment can reduce the lethality; however, there are operational difficulties in the basic healthcare network of the municipalities which prevent these developments[33]. Still on the evolution of the patient, it is important to pay attention that the percentage of ignored/blanks was equivalent to 33.12% of the total, which in a certain manner compromises the real knowledge of the number of deaths and cured individuals.

From the interpretation of the crude and smoothed incidence maps, it became evident that the disease tended to expand to the interior of the state, in a heterogeneous distribution, with the emergence of new outbreaks in the East and maintenance of the profile of active infection in former areas of occurrence in the *Sertão* region. The immediate geographical regions of Delmiro Gouveia, Santana do Ipanema and Pão de Açúcar - Olho D'Água das Flores - Batalha are situated in the driest area of the state territory, where the average annual rainfall is of 400 to 600 mm. The immediate geographical regions of Arapiraca and Palmeira dos Índios are situated between two distinct biomes, with small humid areas and wetlands, and precipitation varying between 600 mm and 900 mm[35].

According to Furtado et al.[35] and Oliveira & Montoni[11], these regions presented an elevation in the incidence over the years, as seen in the fourth triennium. However, these authors disagree with Pedrosa & Rocha[39] when they cite that the majority of the cases came from the immediate regions situated in the coastal stretch such as those of Maceió, Porto Calvo - São Luís do Quitunde and Penedo and, from the immediate regions of São Miguel dos Campos, União dos Palmares and Atalaia, restricted areas of

Atlantic forest, where the climate is rainy tropical with a dry Summer. In these non-endemic places, the presence of the disease was related to the migration of people and dogs from endemic areas[35].

According to the Health Ministry[14], the formation of clusters enables the knowledge of the distribution of the disease and to assess if the occurrence is related to factors such as the presence of the vector and migratory flow of individuals and infected humans and, with this, prioritize the areas at risk. In this study, there was the formation of high risk clusters, identified in the Moran's Map in the second, third and fourth trienniums, notably more expressive in the municipalities of the *Sertão* region. But, it is observed that, even situated in proximity to high risk clusters, the municipalities of Monteirópolis, Olho D'água das Flores, Carneiros, Minador do Negrão and São Braz presented low risk for the occurrence of the disease, which may be due to either the actions of surveillance and control of the VL in these cities, not disregarding the possibility of increase in the incidence during the next years, or for presenting particular bioclimatic characteristics that prevent the establishment of the cycle of the disease. In contrast, Anadia, Marechal Deodoro and Jacuípe, situated between the low-risk municipalities, presented risk clusters, which may be associated to possible intrinsic factors of each municipality, such as those of the economic sphere, and even those associated to the migration of individuals and to the non-execution of the measures of the Visceral Leishmaniasis Control Program.

Considering the importance of the dog in the transmission chain of the VL[10], it is important to mention that Marechal Deodoro, a municipality situated on the coastal stretch and inserted in the metropolitan region of Maceió, presented a low number of cases of the disease in humans, but in 2018 confirmed 105 cases of canine visceral leishmaniasis (CVL), published by means of the Informative Notice n. 51/2018 of the

Health Surveillance Superintendence (SUVISA)[13], pointing to the need of reviewing the measures of surveillance in regions either without the occurrence or with few human cases, seen as there are reports that the infection in the dog precedes the human infection when a critical value of infected animals is reached[37].

According to Anselin[38], by the calculation of the Global Moran's Index it is possible to visualize the spatial dependence between the municipalities.This was verified in the second, third and fourth trienniums, but with a weak correlation between the municipalities, in other words, presented few similarities in the distribution of the cases.

The Visceral Leishmaniasis Surveillance and Control Program (VLSCP)[14] cites that measures restricted only to the municipalities which present cases of HVL and CVL have not proved to be effective in the control of the disease, and for that, either transmission or areas at risk must be better defined, where municipalities and states without occurrence (or silent) must be incorporated in the actions of surveillance, with the aim of minimizing the consequences from this zoonosis in areas without transmission.

Several factors can be associated to the dissemination of the disease in Alagoas, such as the disorderly occupation of the outskirts of the cities, the precariousness of the sanitation system, the deforestation and the destruction of the vector's natural habitat, as well as the presence of the infected canine host[40]. Although the VL is of mandatory notification in Brazil, in this type of analysis in which are used passive secondary data, it is important to highlight that problems of sub-notification exist and can alter the results[41,42].

The state of Alagoas demonstrates an accentuated geographical expansion of the HVL over the last 12 years, emphasizing the need for the massification of surveillance actions and of epidemiological control, as well as those related to the levels of health education of the population, intrinsically connected to the knowledge of the disease.

For this reason, it is suggested the reassessment of the already known control measures, above all in situations in which there has been interruption or discontinuity by the local healthcare agencies, in association with health education strategies for the more vulnerable populations, such as, the sanitization of the places of shelter of the animals and the areas surrounding the homes; use of screens in doors and windows and of repellent at the vector's feeding time.

Conflict of interest statement

The authors declare that they have no competing interests.

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Table 1. Epidemiological variables of the cases of HVL in the state of Alagoas, from 2007 to 2018.

Table 2. Values of Moran´s index and the p-values for each triennium studied in the State of Alagoas**,** 2020

Figure 1. Incidence rate of human visceral leishmaniasis per municipality of Alagoas, Brazil, from 2007 to 2018 in triennial intervals. Data source: National Disease Notification System – SINAN, 2020

Figure 2. Bayesian incidence rate of human visceral leishmaniasis per municipality of Alagoas, Brazil, from 2007 to 2018 in triennial intervals. Data source: National Disease Notification System – SINAN, 2020

Figure 3. Moran's map for human visceral leishmaniasis per municipality of Alagoas, Brazil, from 2007 to 2018 in triennial intervals. Data source: National Disease Notification System – SINAN, 2020

Table 1. Epidemiological variables of the cases of HVL in the state of Alagoas, from 2007 to 2018.

Variable		n	Percentage %
Sex			
	Male	324	66.2
	Female	165	33.7
Age			
	<1 year	25	5.1
	1-4 years	140	28.6
	5-9 years	66	13.4
	10-14 years	48	9.8
	15-39	40	8.1
	20-39	112	22.9
	40-59	46	9.4
	60-79	12	2.4
Origin of the cases			
	Autochthonous	429	87.7
	Non-autochthonous	20	4
	Indeterminate	40	8.1
Zone of residence			
	Urban	132	26.9
	Rural	313	64
	Peri-urban	35	7.1
	Ignored/blank	9	1.8
Evolution			
	Ignored/blank	162	33.1
	Cure	226	46.2
	Abandonment	3	0.6
	Death dueto VL	38	7.7
	Death due to another cause	14	2.8
	Transference	46	9.4

Table 2. Values of Moran´s index and the p-values for each triennium studied in the State of Alagoas, 2020

Trienniums	Moran's index	p – value*
2007 – 2009	0.076	0.12
2010 – 2012	0.182	0.015*
2013 – 2015	0.190	0.009*
2016– 2018	0.336	0.002*

*p-value ≤0.05 indicates spatial dependence

Figure 1. Incidence rate of human visceral leishmaniasis per municipality of Alagoas, Brazil, from 2007 to 2018 in triennial intervals. Data source: National Disease Notification System – SINAN, 2020

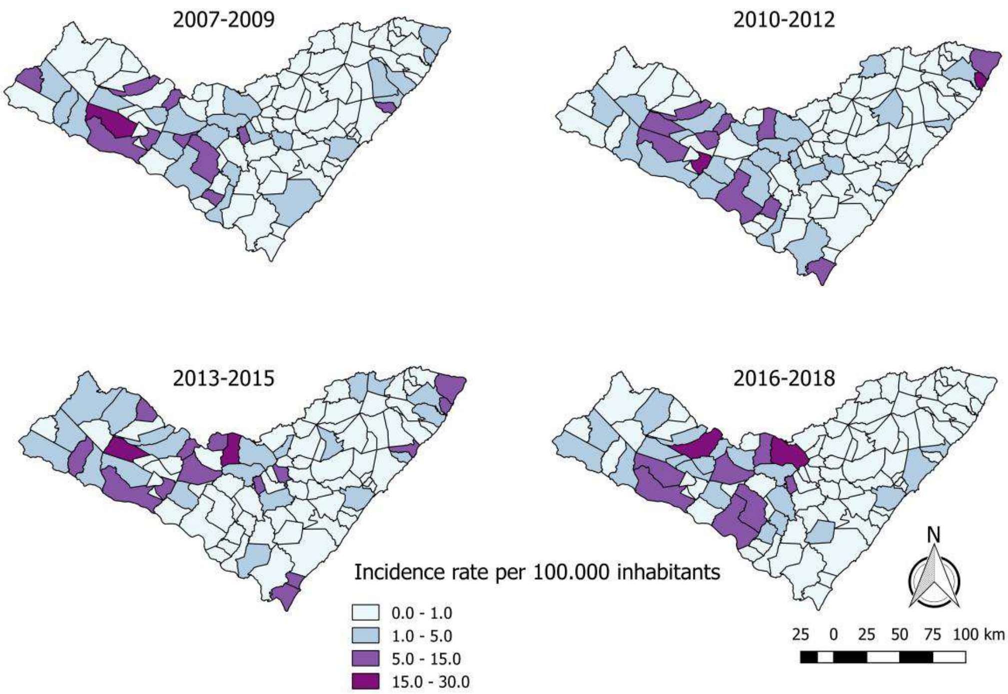


Figure 2. Bayesian incidence rate of human visceral leishmaniasis per municipality of Alagoas, Brazil, from 2007 to 2018 in triennial intervals. Data source: National Disease Notification System – SINAN, 2020

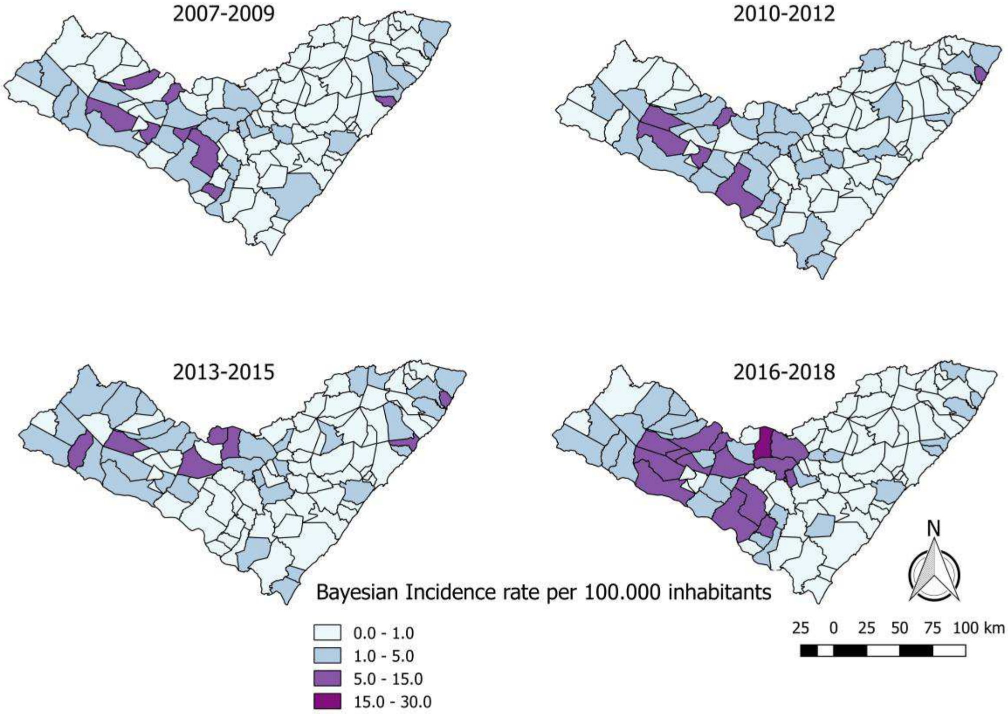
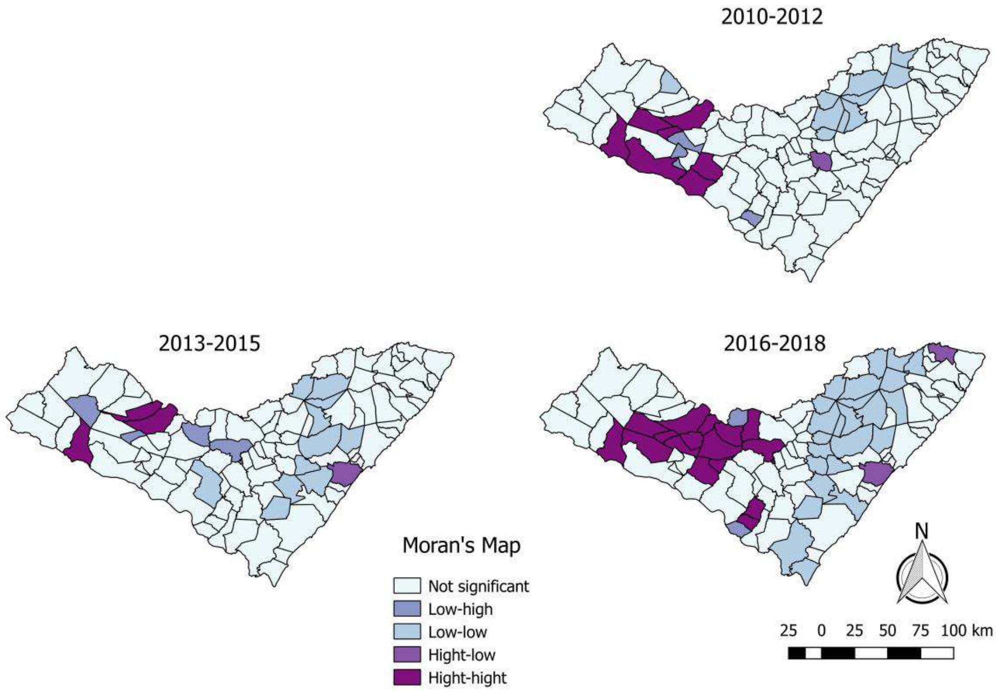


Figura 3. Moran's map for human visceral leishmaniasis per municipality of Alagoas, Brazil, from 2007 to 2018 in triennial intervals. Data source: National Disease Notification System – SINAN, 2020



CONCLUSÃO GERAL

Os três artigos que compõem esta tese levaram às seguintes conclusões:

- A leishmaniose visceral é ativa no município de Mãe D'Água e representa um risco para um aumento dos casos humanos, principalmente na zona rural, uma vez que esta apresentou a maior prevalência da leishmaniose visceral canina e flebótomos positivos;
- No município de Mãe D'Água se mantém o perfil de doença rural;
- No estado de Alagoas, houve expansão esporádica da LVH para o interior do Estado nos últimos anos.

CONSIDERAÇÃO GERAL

Sugere-se que as medidas de vigilância da LV sejam revistas no Brasil como um todo e, em especial, em estados do Nordeste, no que se refere à prevenção e controle da doença nos reservatórios domésticos e no emprego de medidas mais sistemáticas no controle do vetor através do controle químico, e no cuidado invidual por meio do uso de repelentes, mosquiteiros e telagem de portas e janelas.

ANEXOS

Anexo 1. Questionário epidemiológico aplicado aos proprietários durante a coleta de sangue dos cães.

I DADOS DO PROPRIETÁRIO	
1- Nome:	
2- Endereço:	Cidade:
3- Coordenadas:	
4- Telefone:	
5- Grau de escolaridade: ( ) Analfabeto ( ) 1° grau incompleto ( ) 1° grau completo ( ) 2° grau incompleto ( )2° grau completo ( )3° grau incompleto ( ) 3° grau completo	
6- Renda familiar: ( ) Menos de 2 salário mínimo ( ) 2 a 4 ( )5 a 6 ( ) + 6	
II DADOS DO ANIMAL	
7- Nome:	8- Sexo:( ) Macho ( ) Fêmea
9- Idade: ( )6 – 12m ( )13 – 24m ( ) 25 – 48m ( )4 – 6 anos ( ) +6 anos	
10- Raça: ( ) Sem raça definida ( ) Com raça definida. Qual?	
III MANEJO	
11- Tipo de criação: ( ) Domiciliar ( ) Semi-domiciliar ( ) Solto	
12- Alimentação: ( ) Ração comercial ( ) Alimento caseiro ( ) Ambos	
13- Tem contato com outros animais? ( ) não ( ) sim	
14 – Se sim, com quais?( )Equídeos ( )Caprinos ( )Ovinos ( )Bovinos ( ) Suínos ( )Aves ( )Felinos ( )Cães ( )Silvestres. Quais?	
15- Qual o ambiente onde o animal é criado? ( )Terra ( )Cimento ( ) Terra/cimento	
16- É realizada limpeza ou desinfecção do local? ( ) Não ( ) Sim	
17- Com que frequência? ( )Diária ( )Semanal ( )Quinzenal ( )Mensal	
18- O animal tomou alguma vacina?( ) Não ( ) Sim Qual?( )Anti-rábica ( )Viroses	
19- O animal já foi vermifugado? ( ) Não ( ) Sim Quando?	
20- O animal apresenta ou já apresentou carrapatos? Detalhar.	
21- O animal sempre morou com o proprietário?( ) Não ( ) Sim	
22- O animal foi adotado? ( )da rua ( )tinha outro proprietário.	
23- O animal sempre morou nessa cidade? ( ) Não ( ) Sim Se NÃO: Local anterior?__________________ Há quanto tempo mora aqui? __________	
24- O animal é usado para caça? ( ) Não( ) Sim	
25- Aonde o animal dorme?( )Dentro de casa ( )No Peridomicílio ( )Na rua	
26- Como passa a noite? ( )Amarrado ( )Solto	
27- Quando viaja, leva-o junto? ()Não ( ) Sim Há quanto tempo e Local?_____________	
28- O animal faz/fez uso de coleiras repelentes? ()Não ( ) Sim	
- Aspecto geral do animal:	

Anexo 2. Termo de consentimento de livre e esclarecido (TCLE) assinado pelos proprietários autorizando a coleta de sangue.

UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Convidamos o (a) Sr (a) para participar da Pesquisa intitulada **Prevalência, fatores de risco e análise geoespacial da leishmaniose visceral canina no sertão paraibano, Brasil** sob a responsabilidade do pesquisador Beatriz Maria de Almeida Braz, a qual pretende analisar a prevalência de LVC no município de Mãe D'Água , estudar a epidemiologia da zoonose por meio da análise dos fatores de risco e do uso do georreferenciamento Sua participação com seu animal (particular ou propriedade) é voluntária e se dará por meio de coleta de sangue por meio de venopunção cefálica

Se você aceitar participar, os resultados decorrentes do estudo com seu (s) animal (is) estará contribuindo para atestarmos que seu animal está infectado ou não com leishmaniose visceral canina.

Se depois de consentir em sua participação o (a) Sr (a) desistir de continuar participando, tem o direito e a liberdade de retirar seu consentimento em qualquer fase da pesquisa, seja antes ou depois da coleta dos dados, independente do motivo e sem prejuízo a sua pessoa.

O (a) Sr (a) não terá despesas e também não receberá remuneração. Os resultados da pesquisa serão analisados e publicados, mas sua identidade e de seu (s) animal (is) não serão divulgadas, sendo guardada em sigilo. Para qualquer outra informação, o (a) Sr (a) poderá entrar em contato com o pesquisador no endereço (Universidade Federal de Campina Grande, CSTR/ UAMV.Av. Universitária, s/n, Hospital VeterinárioSta Cecília58700970 - Patos, PB –BrasilTelefone: (83) 34239523. Brasil, pelo telefone (83.998918326).

Consentimento Pós–Informação

Eu,____, fui informado sobre o projeto **Prevalência, fatores de risco e análise geoespacial da leishmaniose visceral canina no sertão paraibano, Brasil** que o pesquisador quer fazer e porque precisa da minha colaboração, e entendi a explicação. Por isso, eu concordo em participar do projeto, sabendo que não vou ganhar nada e que posso sair quando quiser.

Este documento foi emitido em duas vias que serão ambas assinadas por mim e pelo pesquisador, ficando uma via com cada um de nós.

Data: ___/ ____/ _____

____
Assinatura do participante
Impressão do dedo polegar
Caso não saiba assinar

____

Assinatura do Pesquisador Responsável