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Rafael Cosme Silva

Leishmaniose canina: estudo sobre flebotomíneos no Brasil e a experiência em município do semiárido

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Leishmaniose Canina: Estudo dos flebotomíneos e taxa de infecção em município do semiárido paraibano

Dissertação submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Mestre em Ciência e Saúde Animal.

Prof.ª Dr.ª Marcia Almeida de Melo

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RAFAEL COSME SILVA

LEISHMANIOSE CANINA: ESTUDO SOBRE FLEBOTOMÍNEOS NO BRASIL E A EXPERIÊNCIA EM MUNICÍPIO DO SEMIÁRIDO

Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Mestre em Ciência e Saúde Animal.

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RESUMO

A Leishmaniose é uma doença zoonótica amplamente distribuída pelo país e de grande importância para saúde pública. No Brasil, tem como principal agente etiológico a *Leishmania infantum* para a Leishmaniose Visceral (LV) e *Leishmania braziliensis* para a Leishmaniose Tegumentar (LT). A doença tem como vetor de transmissão os flebotomíneos, pequenos insetos alados de hábitos noturnos, conhecidos popularmente como "mosquito-palha". A LV tem o cão como principal elo em seu ciclo doméstico. O presente estudo teve como objetivo avaliar a distribuição e abundância da fauna de flebotomíneos do Brasil e também a incidência de Leishmaniose Visceral Canina em um município do semiárido paraibano. Para isso, foi realizada uma revisão sistemática dos dados quantitativos obtidos sobre a fauna de flebotomíneos no Brasil, e a coleta de sangue de 211 cães do município de Mãe d'Água/PB. Da coleta de sangue dos animais de Mãe d'Água foram realizados exames sorológicos (DPP® (Dual Path Platform), ELISA S7®, ELISA-EIE®) e molecular (qPCR), obtendo-se a incidência da doença quando comparada ao último levantamento feito no município, os fatores de risco foram determinados a partir dos dados levantados no questionário epidemiológico e, para a avaliação da distribuição da doença no município, foi utilizado o programa QGIS. Na revisão sistemática foram analisados 35 trabalhos na íntegra e, através de análise qualitativa, observou-se um total de 94 espécies de flebotomíneos distribuídas por todas as 5 regiões do Brasil. O gênero *Lutzomyia* foi o de maior diversidade com um total de 48 espécies, e o subgênero *Nyssomyia* foi o de maior abundância em 4 das 5 regiões do país. O levantamento epidemiológico realizado no município de Mãe d'Água apresentou uma incidência de 3,3 cães infectados a cada 100 e prevalência 11,3%. Apesar da queda da prevalência se faz necessário a intensificação das medidas de controle para leishmaniose no município, devido a zonas de alta densidade da doença observadas na análise espacial.

Palavras-chave: cão, epidemiologia, fauna, flebotomíneos, leishmaniose

ABSTRACT

Leishmaniasis is a zoonotic disease widely distributed across the country and of great importance for public health. In Brazil, its main etiological agent is Leishmania infantum for Visceral Leishmaniasis (VL) and Leishmania braziliensis for Cutaneous Leishmaniasis (TL). The disease is transmitted by sandflies, small winged insects with nocturnal habits, popularly known as "straw mosquito". LV has the dog as the main link in its domestic cycle. The present study aimed to evaluate the distribution and abundance of the sandfly fauna in Brazil and also the incidence of Canine Visceral Leishmaniasis in a municipality in the semi-arid region of Paraíba. For this, a systematic review of the quantitative data obtained on the sandfly fauna in Brazil was carried out, and the blood collection of 211 dogs in the municipality of Mãe d'Água/PB. Serological tests (DPP® (Dual Path Platform), ELISA S7®, ELISA-EIE®) and molecular tests (qPCR) were performed from the blood collection of the animals of Mãe d'Água, obtaining the incidence of the disease when compared to the last In a survey carried out in the municipality, the risk factors were determined from the data collected in the epidemiological questionnaire and, to assess the distribution of the disease in the municipality, the QGIS program was used. Altogether, 35 works were analyzed in full during the systematic review and, through qualitative analysis, a total of 94 species of sandflies distributed across all 5 regions of Brazil was observed. The genus Lutzomyia was the most diverse with a total of 48 species, and the subgenus Nyssomyia was the most abundant in 4 of the 5 regions of the country. The municipality of Mãe d'Água had an incidence of 3.3 infected dogs per 100 and a prevalence of 11.3%. Despite the drop in prevalence, it is necessary to intensify the control measures for leishmaniasis in the municipality, due to the high-density zones of the disease observed in the spatial analysis.

Keywords: dog, epidemiology, fauna, sandflies, leishmaniasis

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CAPÍTULO 2

LISTA DE ABREVIATURAS E SIGLAS

CA	Califórnia
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEUA	Comissão de Ética no Uso de Animais
CRD	Com raça definida
DP	Desvio padrão
DPP	*Dual path platform*
EDTA-K3	Anticoagulante ácido etilenodiaminotetracético potássico
ELISA	Ensaio imunoenzimático
EUA	Estados Unidos da América
GN	Grupo negativo
GP	Grupo positivo
GPS	*Global Position System*
HVU/UFCG	Hospital Veterinário Universitário Prof. Dr. Ivon Macedo
LV	Leishmaniose visceral
LVA	Leishmaniose visceral americana
LVC	Leishmaniose visceral canina
LT	Leishmaniose tegumentar
LTA	Leishmaniose tegumentar americana
MAPA	Ministério da Agricultura Pecuária e Abastecimento
NI	Não informado
PB	Paraíba
PCR	Reação em cadeia da polimerase
PRISMA	Itens de Relatório Preferenciais para Revisões Sistemáticas e Meta-Análise
qPCR	Reação em cadeia da polimerase em tempo real
SRD	Sem raça definida
SIG	Sistemas de Informações Geográficas
UFCG	Universidade Federal de Campina Grande

LISTA DE SÍMBOLOS

%	Percentual
<	Menor que
>	Maior que
≤	Menor ou igual que
ºC	Graus Celsius
g/dL	Grama por decilitro
Nº	Número
m²	Metro quadrado
mg/dL	Miligrama por decilitro
mm	Milímetros
mL	Mililitro
rpm	Rotações por minuto
U/L	Unidade por litro

INTRODUÇÃO GERAL

A Leishmaniose Visceral (LV) é uma patologia potencialmente fatal, de grande importância para saúde pública, sendo considerada uma doença negligenciada que possui como agente etiológico a *Leishmania infantum* (WHO, 2010; PAHO, 2021).

A LV tem ampla distribuição geográfica, ocorrendo na Europa, Oriente Médio, África, Ásia e Américas, sendo transmitida, principalmente, através do repasto sanguíneo de insetos flebotomíneos infectados (LAINSON, 1987; GRIMALDI, 1991; MISSAWA, 2006; BRASIL, 2014; WHO, 2014). Os flebótomos são dípteros com considerável importância na sanidade animal e na saúde pública nas Américas, além de outras partes do mundo. Seu destaque se dá pelo envolvimento na infecção e transmissão de doenças como bartonelose, arboviroses e as leishmanioses podendo transmitir a leishmaniose visceral (LV) ou a leishmaniose tegumentar americana (LTA). A transmissão ocorre tanto no meio urbano como no rural, podendo atingir animais e os humanos (ALEXANDER, 2000; DANTAS-TORRES, 2009).

A LV é uma doença reemergente que ocorre em diferentes áreas urbanas e rurais do Brasil, tendo o Nordeste como a região mais afetada, apresentando quase 50% de todos os casos de LV registrados no país. No Brasil, as estratégias de controle da LV estão baseadas no diagnóstico e tratamento precoce de casos humanos, no controle dos vetores com uso de inseticidas e na detecção dos cães infectados através de análises sorológicas, com posterior eutanásia dos animais positivos (BRASIL, 2014; COSTA, 2007; MARINS, 2011).

O diagnóstico da LV pode ser realizado por técnicas sorológicas e moleculares. No entanto, em áreas de sobreposição de tripanossomatídeos (como *Leishmania* spp. e *T.cruzi*), os testes sorológicos estão sujeitos à ocorrência de reatividade cruzada, uma vez que estes parasitos compartilham determinantes antigênicos, devido sua proximidade filogenética, o que prejudica a especificidade destes testes. Assim, para um diagnóstico mais preciso, técnicas com alta especificidade como a PCR, devem ser utilizadas (LUCIANO et al., 2009; ALVES, 2012).

Diante do exposto, o objetivo da presente dissertação foi identificar as espécies de flebotomineos presentes no Brasil, além de estimar a incidência da LVC no município paraibano de Mãe d'Água e analisar o desempenho das técnicas de diagnóstico por nós empregadas.

O capítulo I consiste em uma revisão sistemática sobre a biodiversidade de flebotomíneos no Brasil, a partir de artigos de periódicos indexados, na intenção de reunir informações sobre as possíveis espécies transmissoras de LV no Brasil para auxiliar nas estratégias de vigilância entomológica.

O capítulo II consiste em um estudo da LVC no município de Mãe d'Água, localizado na região do semiárido Paraibano, com o intuito de avaliar a incidência, distribuição espacial e fatores de risco associados à doença em cães domésticos.

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CAPÍTULO I:

FAUNA DE FLEBOTOMÍNEOS (DIPTERA: PSYCHODIDAE) NO BRASIL: UMA REVISÃO SISTEMÁTICA

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Phlebotomine (Diptera: Psychodidae) Fauna in Brazil: a systematic review

Fauna de flebotomíneos (Diptera: Psychodidae) no Brasil: Uma revisão sistemática

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Abstract

Sandflies are widely distributed insects in Brazil and are considered the main vectors of leishmaniasis in the country. Leishmaniasis is an extremely neglected disease, and an entomological survey is one of the main measures for disease control and prevention. The present study aimed to carry out a systematic review encompassing the entire country in order to know about the diversity and abundance of sand fly species in Brazil. For this, after a screening of articles searched in 5 databases, 35 works were analyzed in full. In a qualitative analysis, it was possible to observe a total of 94 species of sand flies distributed throughout all regions of the country, with the genus *Lutzomyia* being the most diverse with 48 species. There is a need for more studies throughout the country, so that more data can be collected regarding the interaction of coinfection between sandflies and *Leishmania spp.*, in addition to the intensification of entomological surveillance to control leishmaniasis and other diseases that sandflies act as main transmitters.

Keywords: epidemiology, fauna, leishmaniasis, sandflies.

Resumo

Os flebotomíneos são insetos de ampla distribuição no Brasil e são considerados os principais vetores de leishmanioses no país. As leishmanioses são doenças extremamente negligenciadas, e o levantamento entomológico é uma das principais medidas para controle e prevenção da doença. O presente estudo teve como objetivo realizar uma revisão sistemática englobando todo país com intuito de saber sobre a diversidade e abundância das espécies de flebotomíneos do Brasil. Para isso, após uma triagem de artigos pesquisados em 5 bases de dados, 35 trabalhos foram analisados na íntegra. Em uma análise qualitativa, foi possível observar um total de 94 espécies de flebotomíneos distribuídos por todas as regiões do país, com o gênero *Lutzomyia*

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sendo o de maior diversidade com 48 espécies. Ressalta-se a necessidade de mais estudos em todo país, a fim de que possam ser coletados mais dados a respeito da interação de coinfecção entre flebotomíneos e *Leishmania* spp., além da intensificação da vigilância entomológica para controle das leishmanioses e outras doenças que os flebótomos atuam como principais transmissores.

Palavras-chave: epidemiologia, fauna, flebotomíneos, leishmanioses.

INTRODUCTION

Sandflies are insects that belong to the Diptera order, Psychodidae family, and are widely distributed across the globe, occurring on all continents except Antarctica (Lewis, 1974). They present several subfamilies, of which only Phlebotominae includes hematophagous species with great medical interest because they are vectors of leishmaniasis (Desjeux, 2004; Souza et al., 2009;).

They are insects that show twilight or nocturnal activity and are often found in natural ecotopes, sheltering in tree trunks, animal burrows, fallen leaves on the ground, bushes and cracks in rocks or in caves (Galati et al., 2003). They are also found invading shelters for domestic animals (pens, pens, chicken coops) and in homes, taking shelter in dark places, such as in cracks in walls, which demonstrates their adaptability (Tolezano et al., 2001). The most important vector sand fly species belong to two genera: Phlebotomus, in the Old World and *Lutzomyia*, in the New World. The species of these genera are involved in the transmission of protozoa of the genus Leishmania spp., the etiological agents of leishmaniasis (Desjeux, 2004). Leishmaniasis has a great impact on public health and is characterized by being chronic, non-contagious infections that can assume a zoonotic character, involving humans, domestic and wild animals in their transmission cycle. They are caused by several species of leishmania and transmitted through the blood meal of infected female sandflies (Lainson & Shaw, 1987; Grimaldi et al., 1991; WHO, 2014). They are among the six endemics considered priority in the world with reported cases of human infection in about 100 countries, reaching all continents with the exception of Antarctica. It is estimated that approximately 350 million people are exposed to contracting the disease, and that two million new cases are recorded each year. Leishmaniasis manifests itself in two basic clinical forms, American Cutaneous Leishmaniasis (ACL) and American Visceral Leishmaniasis (AVL) (Alvar et al., 2012; WHO, 2019).

Brazil is the country with the highest estimated annual incidences of AVL and ATL, both forms of the disease are widespread in Brazilian territory, where their notification is mandatory. Recent data from the Ministry of Health revealed changes in the epidemiological

profile of cutaneous leishmaniasis due to its territorial expansion, making its urbanization increasingly evident (Alvar et al. 2012; Brasil 2006).

The changes in the environment, provoked by the intense migratory process, the process of growing urbanization and the socioeconomic pressures, cause the expansion of endemic areas and the appearance of new foci. In this way, sandfly species that resist adverse conditions are able to explore new environments, getting closer and closer to the peridomicile, facilitating the transmission of the disease (Marzochi & Marzochi, 1994; WHO, 1992). In Brazil, entomological surveillance is one of the control strategies for leishmaniasis and comprises, mainly, knowledge of the presence of the vector, its distribution and the monitoring of its dispersion (Brasil 2006).

Considering the complexity in the epidemiology of ATL and AVL, the present study aimed to gather the greatest amount of information on the abundance and diversity of sandflies in Brazil, in order to improve the understanding of the transmission cycles of leishmaniasis and, consequently, improve the entomological surveillance actions and disease control.

MATERIAL AND METHODS

This research consisted of a systematic review of the literature on the diversity of sand fly species in Brazil, based on data available in articles from indexed journals, being conducted in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Liberati et al., 2009).

Studies were considered appropriate for data extraction when they had the following inclusion criteria: complete articles that included information on the sandfly species captured in regions of Brazil. The following exclusion criteria were defined: studies that were not about sandfly fauna in Brazil; who were not in established housing areas (urban or rural); literature review articles; books or book chapters (for titles); abstracts of articles presented in proceedings of symposia or conferences, studies with inappropriate design (unrepresentative sample size, incorrect sampling approach, failure to use the appropriate identification technique) (for abstracts) or containing confusing text and incomprehensible analyzes (for the works in full). Once the inclusion and exclusion criteria were defined, the search was carried out in the electronic databases PubMed, Scopus, Web of Science, Science Direct and Scielo, using combinations of the keywords: phlebotomine AND species AND fauna AND Brazil. The data obtained were saved in BibTex format and exported to Mendeley's bibliographic manager, with subsequent rigorous removal of duplicates, followed by screening of titles and abstracts. The

screening and selection of studies was performed independently by two researchers and differences between them were resolved by discussion and consensus. After this step, the studies considered eligible had their text analyzed in full and the data recorded in an Excel spreadsheet. For the qualitative evaluation of data from the articles, the following information was used: name of authors, year of publication, study area and species found. In the analysis of qualitative data, descriptive statistics were used in order to group the largest number of species found in Brazil, in addition to identifying the most abundant sand fly species in each region.

RESULTS

During the search in the databases, 3419 articles were found. Of these, 35 met the eligibility criteria and were evaluated by qualitative analysis. A flowchart with the steps involved in carrying out the systematic review is shown in Figure 1.

After qualitative analysis of the 35 selected articles, the phlebotomine fauna consisted of 94 species distributed across the 5 regions of Brazil, in the following states: Acre, Alagoas, Amapá, Amazonas, Ceará, Espírito Santo, Maranhão, Mato Grosso, Mato Grosso do Sul , Minas Gerais, Pará, Paraná, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Rondônia, São Paulo and Tocantins.

The genus *Lutzomyia* was the one that presented the greatest diversity with 48 species, but species of other 7 (seven) genera/subgenera were also found. Only 2 (two) species were observed in all regions of Brazil: *Nyssomyia whitmani* and *Migonemyia migonei* (Table 1 and Figure 2).

DISCUSSION

The species *Lutzomyia longipalpis* has its distribution in Central and South America and is considered the main vector of Leishmania infantum. It is a species that can be found both in dry climate regions and in forested regions with high humidity, but its main occurrence is in urban and rural areas with proximity to humans. The species showed great adaptability to these human-inhabited regions and successfully established itself (Brasil 2013, Dvorak et al. 2018, Lainson & Rangel 2005, Rêgo & Soares 2021, Rodrigues et al. 2014). In this systematic review, *Lutzomyia longipalpis* was the most abundant species in the Northeast region, being also reported in large numbers in the Center-West and Southeast regions. The species was found in anthropic environments where animals are raised, but it is also common in other environments, both rural and urban, where AVL and canine visceral leishmaniasis (CVL) occur, which

reinforces the fact that this species is the vector responsible for the transmission of the disease in these regions (Andrade et al. 2009, Colla-Jacques et al. 2010, Krauspenhar et al. 2007, Nunes et al. 2008, Oliveira et al. 2006). Another factor observed in these regions of high concentration of Lu. longipalpis was the similarity in climate/temperature. The regions studied were located more than 100 km from the coast, characterized by a dry climate and high average annual temperature, factors that favor the high abundance of the species (Nieto et al. 2006, Thompson et al. 2002). The genus *Nysomia* is of great importance for surveillance studies and understanding of the ecopidemiology of ATL, occurring from North America (Mexico) to South America (Argentina), with a total of seven proven or suspected ATL transmission species (Marcondes et al. 1998, Rangel & Lainson 2003). The species *Nyssomyia whitmani* found in all regions of Brazil and, predominantly, in the Center-West region, is the most important vector involved in the transmission of ATL in the country, standing out both in the peridomiciliary and in the anthropic environment (Costa et al. 2007; Rangel & Lainson 2003, Shimabukuro et al. 2010). Infection of *Nyssomyia whitmani* by *Leishmania amazonensis*, *Leishmania infantum* and *Leishmania brasiliensis* has already been reported in studies (Azevedo et al. 1990, Luz et al. 2000, Margonari et al. 2010, Machado et al. 2017), and has been linked to transmission of *Leishmania spp.* in 4 of the 5 regions of Brazil, except for the North. (Costa et al. 2007, Fonteles et al. 2015). This species is often found in animal breeding grounds such as chicken coops and also in primary vegetation near farms (Casanova et al. 2013, Souza et al. 2014). Despite being a species that adapts well to different climatic conditions, it is more abundant in the colder months, from June to August (Souza et al. 2002, Teodoro et al. 2003). Nyssomyia intermedia, the most abundant species in the Southeast region, is also associated with the transmission of *Leishmania braziliensis*, the agent of American cutaneous leishmaniasis, and was captured in almost all studies in the region (Barata et al. 2011, Brazil et al. 2015, Ferreira et al. 2001, Galati et al. 2010, Pinto et al. 2012). *Ny. intermedia* can also be found in other countries in the Americas, such as Suriname, French Guinea, Peru, Bolivia, Paraguay and Argentina (Aguiar & Vieira 2003, Shimabukuro et al. 2017). This species, which has greater adaptability, has benefited from the devastation caused by humans and has had its density greatly increased in environments with modified or degraded vegetation, which has been associated with reported human cases of ACL (Andrade Filho et al. 2007, Gomes et al. 1986). *Nyssomyia intermedia* has anthropophilic behavior and a tendency to invade homes to obtain blood and shelter. Studies demonstrate the decline in the external abundance of this species after midnight, as they would have entered homes by that time, becoming one of the most important factors in the transmission of ATL (Campbell-Lemdrum et al 1999). *Nyssomyia umbratilis* occurs in northern

South America, including Bolivia, Brazil, Colombia, French Guiana, Guyana, Peru, Suriname, and Venezuela (Young & Duncan 1994, Azevedo et al 2002). It is found in regions of high prevalence of ATL, being considered one of the main vectors of *Leishmania guyanensis* in most of Latin America (Lainson 2010). In Brazil, its highest occurrence is reported in the Amazon region (Brasil 2019), and it has been identified as the main vector of this parasite in the states of Amazonas, Amapá, Pará and Roraima (Figueira et al 2013). Studies show that the Rio Negro is directly linked to a vicariance process of this species, acting as a barrier that isolates two groups of *Ny. umbratilis*, which, for this reason, end up showing different characteristics despite belonging to the same species (Arias & Freitas 1978, Justiniano et al. 2004).

The sandflies of the *Nyssomyia neivai* species are usually found in more restricted locations, such as dry and cold areas in Bolivia and Paraguay. In Brazil, it is more abundant in the south and southeast regions (Aguiar & Vieira 2003, Shimabukuro et al. 2017). Like those of its sympatric species (*Ny. Intermedia*), it has easy adaptation to degraded residual forests and peridomiciles. (Aguiar & Medeiros 2003). This species has already been found naturally infected by *Leishmania braziliensis* and is considered one of the main vectors of ATL (Neves et al. 2002, Pereira et al. 2009). A risk factor that could be observed in collections carried out in the South region, which indicated a predominance of the specie *Ny neivai*, was the presence of livestock, such as cattle, pigs and horses, which cause the accumulation of organic matter with their waste, creating an ideal environment for the sandfly, in addition to favoring the contact of the human population with the vectors (Cella et al. al. 2011, Cruz et al. 2012, Silva et al. 2008).

Currently, it is still difficult to control populations of sandflies that are vectors of *Leishmania spp*., however, important tools have emerged to improve control strategies for these insects. Volatile compounds based on male pheromones and kairomones have shown good efficacy when used in combination with automatic light traps, improving capture rates. In addition, synthetic pheromones can improve the effectiveness of sand fly control programs when used in conjunction with insecticides. This combined strategy attracts and kills both sexes, preventing host-seeking females from transmitting *Leishmania spp*. and males to establish alternative aggregation sites (Bray et al. 2009). The decrease in the number of sandflies attracted usually occurs as a consequence of insecticide treatments, however, the application of synthetic pheromones in insecticide-sprayed sheds seems to prevent and reverse this, improving capture rates (Bray et al. 2010).

CONCLUSION

Our results highlight the need for permanent entomological and parasitological surveillance. In fact, further studies are still needed to accurately determine the behavioral characteristics and seasonal population dynamics of these vectors, as well as infection by Leishmania spp. in sandflies, guiding the choice of additional measures to control and prevent cases of leishmaniasis. The need to adopt policies that involve health education, environmental management and understanding of the basic concepts of the disease are essential to ensure the success of an entomological surveillance and leishmaniasis control program in Brazil. It is worth noting an important aspect in the epidemiology of leishmaniasis, which is the existence of a population of hosts responsible for the maintenance and distribution of the disease. Therefore, knowledge of the reservoirs is also important for the effective control of leishmaniasis.

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Legendas das Tabelas e Figuras

Figure 1. Flowchart of the systematic review steps

Figure 2. Main sand fly species found by region of Brazil, in order of abundance

Table 1. Species of sandflies in greater abundance of each study

Figure 1.

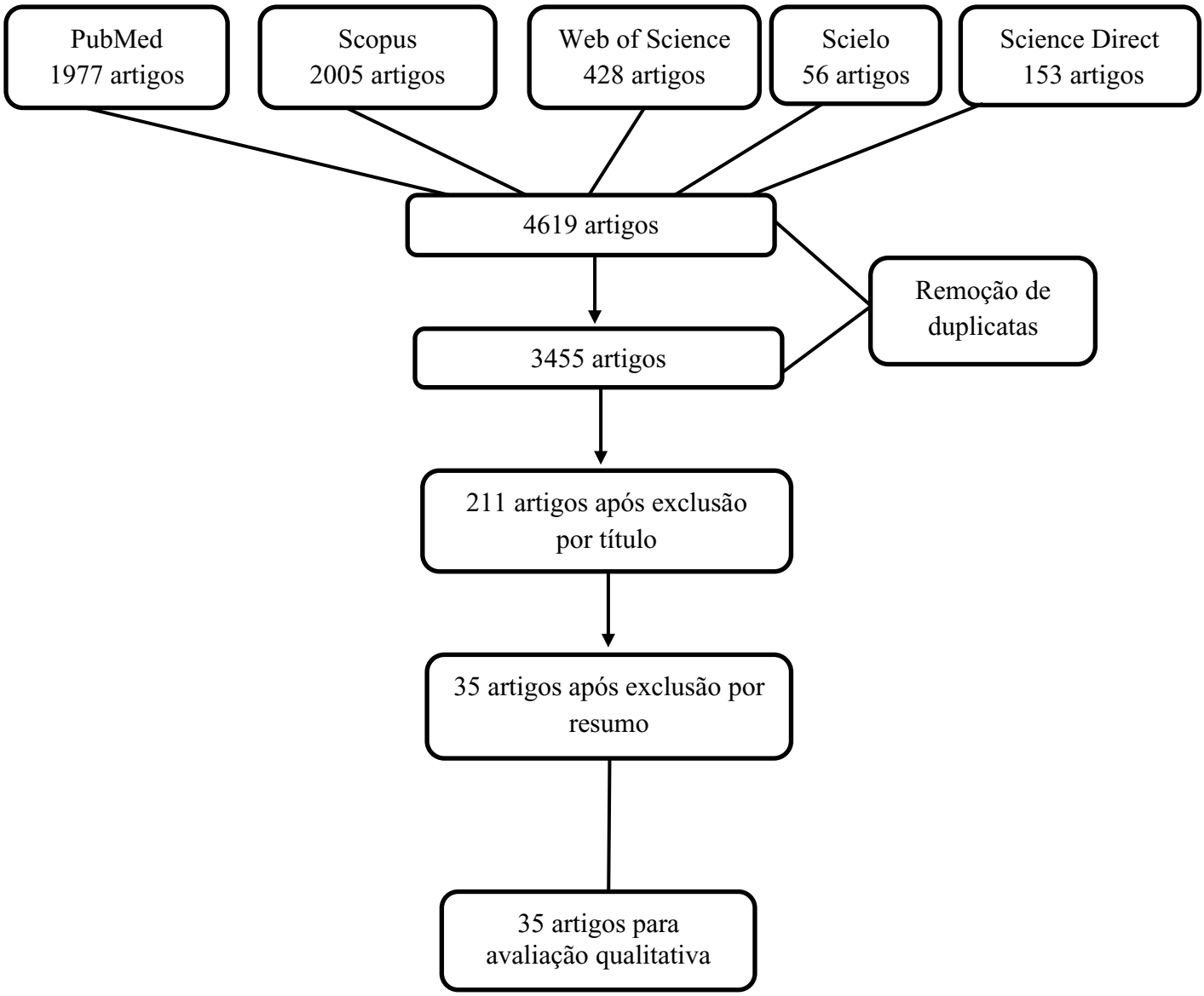
PubMed
1977 artigos
Scopus
2005 artigos
Web of Science
428 artigos
Scielo
56 artigos
Science Direct
153 artigos
4619 artigos
Remoção de duplicatas
3455 artigos
211 artigos após exclusão por título
35 artigos após exclusão por resumo
35 artigos para avaliação qualitativa

Figure 2.


1 Nyssomyia umbratilis
2 Nyssomyia whitmani
3 Evandromyia carmelinoi
1 Lutzomyia longipalpis
2 Evandromyia evandroi
3 Nyssomyia whitmani
1 Nyssomyia whitmani
2 Lutzomyia longipalpis
3 Lutzomyia cruzi
1 Nyssomyia intermedia
2 Migonemyia migonei
3 Lutzomyia longipalpis
1 Nyssomyia neivai
2 Nyssomyia whitmani
3 Nyssomyia intermedia
500 0 500 1.000 1.500 km
Legenda
BRASIL
Centro-oeste
Nordeste
Norte
Sudeste
Sul

Table 1.

Autors	Local	Main Species Found	%
Abreu et al. 2008	Maranhão	*Lutzomyia longipalpis*	86,9
		Evandromyia evandroi	9,6
		Lutzomyia choti	2,1
Afonso et al. 2007	Rio de Janeiro	*Pintomyia monticola*	33,8
		Lutzomyia matosi	20,9
		Lutzomyia ayrozai	17,6
Alencar et al. 2007	Amazonas	*Nyssomyia umbratilis*	37,1
		Lutzomyia monstruosa	14,8
		3 espécies	11,1
Almeida et al. 2010	Mato Grosso do Sul	*Lutzomyia longipalpis*	56,3
		Lutzomyia cruzi	37,9
		Nyssomyia whitmani	1,1
Alves et al. 2012	Mato Grosso	*Ny. whitmani*	26,5
		Evandromyia evandroi	16,0
		Evandromyia termitophila	12,7
Amaral et al. 2011	Mato Grosso	*Brumptomyia brumpti*	23,5
		Lutzomyia sallesi	18,7
		Lutzomyia carmelinoi	15,4
Amorim et al. 2021	Ceará	*Lutzomyia longipalpis*	66,9
		Nyssomyia intermedia	17,7
		Nyssomyia whitmani	6,8
Andrade-Filho 2001	Tocantins	*Nyssomyia whitmani*	52,9
		Lutzomyia longipalpis	25,2
		Evandromyia carmelinoi	6,9
Andrade-Filho 2009	Alagoas	*Migonemyia migonei*	75,2
		Nyssomyia intermedia	10,2
		Lutzomyia longipalpis	6,8
Azevedo et al 2002	Mato Grosso	*Nyssomyia whitmani*	35,4
		Nyssomyia antunesi	18,1
		Lutzomyia llanosmartinsi	9,6
Azevedo et al 2008	Acre	*Nyssomyia antunesi*	30,4
		Nyssomyia whitmani	27,2
		Psychodopygus davisi	21,2

Balbino et al. 2005	Pernambuco	*Nyssomyia whitmani*	75,9
		Nyssomyia umbratilis	11,9
		Lutzomyia complexa	5,2
Barata et al. 2011	Minas Gerais	*Nyssomyia intermedia*	29,9
		Evandromyia cortelezzii	19,5
		Lutzomyia longipalpis	11,9
Barbosa et al. 2008	Amazonas	*Nyssomyia umbratilis*	32,3
		Nyssomyia anduzei	20,0
		Trichophoromyia eurypyga	12,0
Brazil et al. 2011	Rio de Janeiro	*Nyssomyia intermedia*	77,5
		Pintomyia fischeri	15,1
		Migonemyia migonei	3,4
Brilhante et al. 2015	Mato Grosso do Sul	*Ny. whitmani*	49,3
		Psathyromyia bigeniculata	26,9
		Lu. longipalpis	13,8
Campos et al. 2013	Maranhão	*Lutzomyia longipalpis*	61,5
		Evandromyia evandroi	12,5
		Lutzomyia flaviscutellata	5,6
Cella et al. 2011	Paraná	*Nyssomyia neivai*	89,7
		Nyssomyia whitmani	9,5
		Pintomyia pessoai	0,3
Cerino et al. 2009	Paraná	*Nyssomyia whitmani*	84,0
		Nyssomyia neivai	12,7
		Pintomyia monticola	1,8
Colla-Jacques et al. 2010	São Paulo	*Nyssomyia whitmani*	35,9
		Lutzomyia longipalpis	32,9
		Pintomyia pessoai	14,8
Costa et al. 2021	Rondônia	*Nyssomyia antunesi*	45,5
		Psychodopygus davisi	10,8
		Psathyromyia hermanlenti	6,6
Costa et al. 2022	Amapá	*Nyssomyia umbratilis*	13,2
		Trichopygomya trychopyga	11,4
		Trichophoromya ubiquitalis	9,5
Cruz et al. 2012	Paraná	*Nyssomyia neivai*	34,4
		Pintomyia pessoai	32,6

		Migonemyia migonei	11,6
Feitosa et al. 2012	Pará	*Lutzomyia longipalpis*	95,4
		Evandromyia carmelinoi	3,7
		Nyssomyia whitmani	0,3
Ferreira et al. 2001	Espírito Santo	*Nyssomyia intermedia*	24,3
		Migonemyia migonei	22,3
		Nyssomyia whitmani	15,4
Galati et al. 2010	São Paulo	*Nyssomyia intermedia*	70,9
		Nyssomyia neivai	26,9
		Pintomyia fischeri	1,1
Godoy et al. 2017	Tocantins	*Psathyromyia complexus*	51,0
		Psathyromyia llanosmartinsi	18,4
		Nyssomyia antunesi	17,9
Machado et al. 2012	Tocantins	*Micropygomyia goiana*	25,8
		Siopemyia sordellii	13,7
		Evandromyia carmelinoi	11,5
Oliveira et al. 2016	Mato Grosso	*Lutzomyia cruzi*	93,9
		Lutzomyia forattinii	3,2
		Evandromyia corumbaensis	1,8
Pereira et al. 2014	Acre	*Trichophoromyia auraensis*	28,5
		Nyssomyia whitmani	10,1
		Nyssomyia antunesi	2,5
Pinto et al. 2012	Espírito Santo	*Nyssomyia intermedia*	73,8
		Lutzomyia longipalpis	11,3
		Migonemyia migonei	5,2
Silva et al. 1999	Rio Grande do Sul	*Migonemyia migonei*	50,9
		Pintomyia pessoai	17,5
		Psathromyia lanei	9,5
Silva et al. 2008	Paraná	*Nyssomyia neivai*	75,6
		Nyssomyia whitmani	10,1
		Migonemyia migonei	7,8
Ximenes et al. 2000	Rio Grande do Norte	*Lutzomyia longipalpis*	85,6
		Evandromyia evandroi	10,8
		Lutzomyia oswaldoi	0,9

CAPÍTULO II:

ASPECTOS EPIDEMIOLÓGICOS DA LEISHMANIOSE VISCERAL CANINA EM MUNICÍPIO DO SEMIÁRIDO PARAIBANO, BRASIL

Trabalho submetido à Revista Brasileira de Parasitologia Veterinária (ISSN: 1984-2961) Jaboticabal /SP, Qualis A2

Epidemiological Aspects of Canine Visceral Leishmaniasis in a Municipality in the Semiarid Region of Paraiba, Brazil

Aspectos Epidemiológicos da Leishmaniose Visceral Canina em Município do Semiárido Paraibano, Brasil

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ABSTRACT

Visceral Leishmaniasis (VL) is an endemic zoonotic disease with wide distribution in northeastern Brazil, considered of great importance for public health. This research aimed to estimate the incidence of canine visceral leishmaniasis (CVL) in the municipality of Mãe d'Água, based on studies carried out in the city in previous years. In 2021, 211 blood samples were collected from dogs (*Canis lupus familiaris*) domiciled in rural and urban areas of the municipality. Dogs were diagnosed by serological (DPP®, ELISA/S7®, ELISA-EIE®) and molecular (qPCR) tests, and dogs reactive in at least 2 (two) serological tests were considered positive. From the data obtained in the epidemiological questionnaire, the factors associated with the infection were determined. The spatial distribution was performed in the QGIS program using the coordinates obtained by GPS (Global Position System) during the application of the questionnaire. The incidence for the municipality was 3.3 infected dogs per 100, no factor associated with the infection was identified and in the spatial analysis it was possible to observe a concentration of cases in the urban area. After a few years, it is possible to verify the same rate of infection among dogs for CVL, suggesting an intensification of control measures so that there is a drop in the numbers of the disease

Keywords: Epidemiology, dogs, leishmaniasis.

RESUMO

A Leishmaniose Visceral (LV) é uma doença zoonótica endêmica e de ampla distribuição no nordeste brasileiro, considerada de grande importância para a saúde pública. Esta pesquisa teve como objetivo estimar a incidência da leishmaniose visceral canina (LVC) no município de Mãe d'Água, tendo como base estudos realizados na cidade em anos anteriores. No ano de 2021, foram coletadas 211 amostras de sangue de cães (*Canis lupus familiaris*) domiciliados na zona rural e urbana do município. Os cães foram diagnosticados através de testes sorológicos (DPP®, ELISA/S7®, ELISA-EIE®) e molecular (qPCR) e os cães reagentes em, pelo menos, 2

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(dois) testes sorológicos foram considerados positivos. A partir dos dados obtidos no questionário epidemiológico, foram determinados os fatores associados à infecção. A distribuição espacial foi realizada no programa QGIS utilizando as coordenadas obtidas por GPS (*Global Position System*) durante a aplicação do questionário. A incidência para o município foi de 3,3 cães infectados a cada 100, nenhum fator associado à infecção foi identificado e na análise espacial foi possível observar uma concentração de casos na zona urbana. Após alguns anos, é possível verificar uma mesma taxa de infecção entre os cães para LVC, sendo sugerido uma intensificação nas medidas de controle para que haja uma queda dos números da doença.

Palavras-chave: Epidemiologia, cães, leishmaniose.

INTRODUCTION

Visceral leishmaniasis (VL) is a neglected tropical disease, of zoonotic character, caused by the protozoan Leishmania infantum (synonym L. chagasi). In Brazil, the main transmitting agent is the sand fly *Lutzomyia longipalpis* (WHO, 2010). The disease has a wide worldwide distribution and, in Latin America, 90% of cases occur in Brazil, mainly in the Northeast region (Brasil, 2014). Although VL affects several mammals, the domestic dog has an important epidemiological role in the disease, being considered the main link in the chain of transmission in the domestic environment, with reports of its infection in foci of human disease (Melo, 2004; Dantas-Torres, 2007; Ursine et al. 2016). Strategies to control this zoonosis are based on early diagnosis and treatment of human cases, vector control, population health education and detection of infected dogs through serological tests, with subsequent euthanasia of positive animals (Brasil, 2014), since the dog is a food source for the sand fly and canine infection usually precedes human cases in endemic urban areas (Bevilacqua et al., 2001; Afonso et al., 2012). In 2019, epidemiological surveys of VL were carried out in Mãe d'Água, showing the presence of this disease in the canine population (Braz et al., 2021). Due to the fact that the dog is an important link for the dissemination of this zoonosis (Melo, 2004; Luciano et al., 2009; Souza et al., 2009), epidemiological surveillance in the canine population must be permanent. In view of this, the objective of the present study was to evaluate the incidence, prevalence, spatial distribution and risk factors associated with the disease in domestic dogs in the year 2021.

MATERIAL AND METHODS

Study Location

The methodological protocols adopted in this research were submitted to the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Campina Grande

(UFCG), under protocol number 06/2021. The study was carried out in the urban and rural areas of the municipality of Mãe d'Água, located in the intermediate and immediate geographic region of Patos (Fig. 1). The population is approximately 4009 inhabitants, with a territorial extension of 228.7 km² (IBGE, 2019). The sample size was determined using the formula for simple random samples, based on an expected prevalence of 50% (in order to maximize sampling), a confidence level of 95% and a sampling error of 5% (Thursfield, 2007), the which resulted in a sample population of at least 152 dogs. Blood samples were collected both in urban and rural areas, from dogs over six months old, males and females, without distinction of breed and always with the consent of the tutors, with the signature of the Free and Informed Consent Term.

Sample collection

To obtain blood, cephalic or jugular venipuncture was performed, with the aid of a 5mL syringe and 25x8mm needles, sterile and for individual use. The collected blood was immediately transferred to tubes containing clot separator gel and ethylenediaminetetraacetic acid potassium anticoagulant (EDTA-K3 - Vacutte do Brasil Ltda), stored under refrigeration and sent to the Laboratory of Molecular Biology of the Semiarid Region (UFCG - Patos/PB). The blood collected without anticoagulant was centrifuged at 2000rpm for 5 minutes to separate the serum, stored in 1.5mL microtubes, identified and stored at -20ºC until the serological tests were performed. The blood collected with EDTA-K3 was stored at -20ºC until the molecular assay was performed. At the time of collection of biological material, a physical evaluation of the animals was performed, recording the clinical changes observed and the tutors answered an epidemiological questionnaire in order to verify socio-environmental factors that could act as possible risk factors for the disease under study.

Serological and Molecular

Tests Serological tests were performed at the Laboratory of Molecular Biology of the Semi-Arid Region (UFCG – Patos/PB) and at the Central Public Health Laboratory of Paraíba (LACEN/PB). The serum of the sampled animals was used to perform the DPP (Dual Path Platform - Chembio Diagnostic Systems, INC.), ELISA/S7 (Biogene Indústria e Comércio Ltda.) and ELISA/EIE (Canine Visceral Leishmaniasis, Bio-Manguinhos, Rio de Janeiro, Brazil), for the diagnosis of leishmaniasis, according to the manufacturer's instructions. As for the qPCR, performed at the Laboratory of Molecular Biology of the Semi-Arid Region (UFCG – Patos/PB) from samples of positive animals in at least 2 serological tests, the protocol

described by Silva et al. (2016), by extracting DNA from 100 µL of blood, using the DNeasy blood and tissue kit (Qiagen®, Hilden, Germany), following the manufacturer's recommendations, and using the Linf kDNA-F primers 5' GGCGTTCTGCAAAATCGGAAA-3', Linf kDNA-R 5' CCGATTTTTGGCATTTTTGGTCGAT-3' and Linf kDNA_FAM-5' TTTTGAACGGGATTTCTG-3' to amplify the L. infantum kinetoplast minicircle gene (kDNA). A culture of L. infantum was used as a positive control and ultrapure water as a negative control.

Statistical analysis

The incidence/prevalence of visceral leishmaniasis was determined from samples that reacted to at least two serological tests used and evaluated using descriptive statistics. The analysis of possible risk factors associated with the positivity of the animals was carried out in two stages (univariate analysis and multivariate analysis) through the data collected with the questionnaires. Independent variables (possible factors associated with infection) were categorized and coded (Latorre, 2004). The variables that presented a value of $p \leq 0.20$ by the chi-square test or Fisher's exact test (Zar, 1999) were selected and used in the multivariate analysis, using multiple logistic regression (Hosmer and Lemeshow, 2000). The significance level adopted in the multiple analysis was 5%. The analyzes were performed using the SPSS 20.0 for Windows program.

Georeferencing

For the analysis and spatial representation of CVL cases in Mãe d'Água, the geographic coordinates were marked, obtaining the location of each property through the Garmin eTrex 30 GPS (Global Position System) receiver, at the time of collection of biological material. The georeferenced data were entered into the digitized cartographic database of the municipality. Free Geographic Information Systems (GIS) and QGIS were used to make maps and spatial visualization of LVC cases. The analyzes of the spatial distribution of CVL were carried out through a descriptive analysis of the epidemiological situation of the disease in the municipality, for that, a heat map was made through the quartic function of the Kernel estimate to observe clusters (hot areas) of cases of the disease, using QGIS software.

RESULTS AND DISCUSSION

From the collection of 211 blood samples from dogs in the municipality, an incidence rate of CVL in Mãe d'Água of 3.3 cases per 100 dogs was observed, considering that all positive

cases (24) are new cases in the municipality when compared to the last survey carried out at the site in 2018 (Braz et al., 2021), and a prevalence of 11.3% in the municipality, with 12.5% in the rural area and 9.9% in the urban area. . When considering each serological test, the percentage of positive animals was: DPP 31/211(14.7%), ELISA-S7 41/211(19.4%) and ELISA-EIE 20/211(9.4%). The 24 positive dogs in the serological tests underwent qPCR and 8.3% (2/24) were positive. The survey carried out showed a decrease in prevalence in the municipality when compared to the study carried out by Braz et. al. (2021) in the municipality, which was 18.6%. Other studies carried out in endemic areas of the Northeast also reported prevalences similar to those observed in the present study. Barbosa et al. (2015) identified a prevalence of 10.3% in areas of Natal-RN and Silva et al. (2016) of 11.3% in Patos-PB. However, in different regions of Brazil, the prevalence of CVL shows a wide variation, with values between 0.17% and 75.3% (Cortada et al. 2004; Barboza et al. 2009; Silva et al. 2010; De Nardo et al. 2011; Pimentel et al. 2015; Fernandes et al. 2016). Several factors imply the variation in prevalence observed in the different studies, such as the characteristics of the canine population, the methodology used in the evaluation (Azevedo et al. 2008; Dantas-Torres, 2009) and also climatic factors, since the sand fly population presents seasonal variation, and its dynamics are affected by rainfall and humidity (Barata et al. 2004). Table 1 shows the characteristics of the population studied. All positive animals were mixed breed (SRD), 75% had semi-domestic breeding (18/24), with 79.2% (19/24) males and 20.8% (5/24) females.

In the univariate analysis to identify factors associated with CVL, the selected factors were: sex, race, hair length, type of breeding, deworming and surroundings of the house (Table 1), however, none of these variables was identified as a risk factor. in the multivariate analysis. Although no statistical association was observed between seropositivity for Leishmania spp. and zone, sex and surroundings of the house, some studies have reported a 1.9 times greater risk of acquiring the disease in animals living in rural environments. In general, the occurrence of the disease in rural areas is higher than in urban areas, as seen in our study (Amóra et al., 2006; Almeida et al., 2012), although the prevalence in rural areas has shown a decrease. compared to the last study carried out in the city (Braz et al. 2021), which observed a prevalence of 26.6% of the disease in the region. Regarding the sex of the animals, Dantas-Torres et al. (2006) and Silva et al. (2016) also found a higher occurrence in males. Almeida et al. (2012) showed no sexual predisposition related to the infection. Some studies report a higher occurrence in females, with no statistically significant difference (Almeida et al., 2010; Brito et al., 2016). It was possible to verify that most of the positive cases (95.8%) were in residences with plants and/or livestock in their surroundings, some studies show that forest regions and

with a large amount of organic matter favor the development and proliferation of the vector, facilitating canine infection (Rondon et al., 2008; Barbosa et al., 2010; Von Zuben and Donalísio, 2016). In our study, we also observed positive dogs in households with a history of seropositive dogs for CVL that were removed by the municipality's Leishmaniasis control and surveillance program. This result corroborates the findings of Silva et al. (2012), who observed that in households with a history of dog removal by the program, the chance of having at least one infected dog was 5 times greater than in households with no history of dog removal. This usually occurs due to a favorable environment for the development of the vector in households, favoring the occurrence of new cases. As already observed in other studies, the simple removal of a positive dog from a household, without the adoption of vector control measures, does not prevent future infections in other animals inserted in the same place (Silva et al., 2012; Coura-Vital et al. 2013). In the municipality of Mãe d'Água, the socioeconomic conditions of the population, the lack of a routine survey and the scarcity of vector control measures contribute to this constant infection in some households. Despite the very close numbers of seropositive individuals between rural and urban areas, when analyzing the incidences for these areas, an infection rate is approximately 3x higher for dogs in the urban area (7.8 per 100 dogs) compared to those in rural areas (2.5 per 100 dogs). This difference may be associated with the fact that the majority of dogs from rural areas (83%) are bred in a "free-range" manner, and as already observed by Braz et al. (2021), this can be considered a protective factor for dogs in the municipality. In the analysis of spatial distribution, it was possible to observe positive cases distributed across all regions that were collected in the municipality, with a concentration of cases in the center (urban area) (Fig. 2). The pattern of distribution of positive cases in the periphery of the urban area of the municipality observed in our study (Fig. 3), was also seen in the survey carried out in 2018 in the municipality (Braz et al., 2021). As well mentioned by Braz et al. (2021), peripheral areas of urban areas close to the forest are perfect places for the proliferation of sandflies, which are an essential part of the transmission cycle (Neto et al., 2009).

Although the epidemiological profile of VL has been changing over the years and the urbanization of the disease has been observed in different regions of Brazil (Bevilacqua et al., 2001; Gontijo and Melo, 2004; Bavia et al., 2011), these conditions they do not apply to the municipality of Mãe d'Água, which, due to its small size, rural and urban areas are very close. One of the factors that may justify the distribution of the disease in the municipality is the recent and disorderly urban occupation with deforestation of native vegetation, causing environmental degradation, which increases the density of the vector in the periurban environment, making

the link between humans, dogs and sand flies becomes increasingly narrow (Almeida et al., 2012; Cesse et al., 2001; Maia et al., 2014; Marcondes and Rossi, 2013; Marzochi and Marzochi, 1994, Teles et al., 2015; Who, 2010).

CONCLUSION

The present work points to the occurrence of CVL in urban and rural areas in the municipality of Mãe d'Água. Although, statistically, no risk factors for CVL were observed, the use of spatial analysis techniques proved to be efficient in the observation of areas with clusters of cases of the disease, reaffirming its importance for a better understanding of the epidemiological situation of visceral leishmaniasis. Although the last notification of human VL in Mãe d'Água was in 2017, it is suggested that the municipality carry out educational campaigns for the population about the disease, preventing the formation of an environment conducive to the proliferation of vectors, as well as the spread of the disease in dogs and the resurgence of the disease in humans.

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Legends of Tables and Figures

Figure 1. Thematic map of the location of the municipality of Mãe d'Água in Paraíba, 2022.

Table 1. Univariate analysis of possible risk factors associated with canine leishmaniasis in Mãe d'Água/PB, 2021-2022.

Figure 2. A. Distribution of canine leishmaniasis cases. B. Heat map with demonstration of clusters of cases in the municipality of Mãe d'Água/PB, 2022.

Figure 3. A. Distribution of canine leishmaniasis cases in the urban area of the municipality of Mãe d'Água. B. Heat map with demonstration of clusters of cases in the urban area of the municipality of Mãe d'Água/PB, 2022

Figure 1.

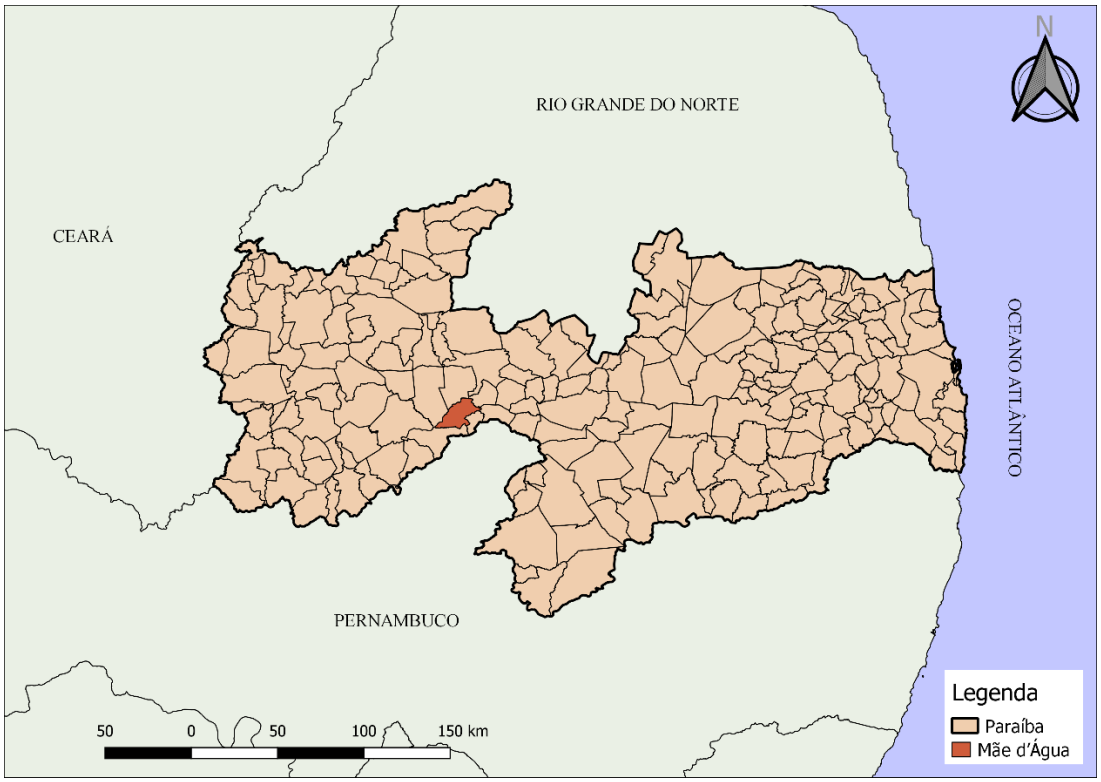


Table 1

Variável	Nº total de animais	Animais positivos (%)	Valor de p
Zona			
Urbana	91	9 (9,9%)	0,66
Rural	120	15 (12,5%)	
Grau de Escolaridade			
Analfabeto	32	5 (15,6%)	
Ensino fundamental completo/incompleto	75	9 (12%)	0,78
Ensino médio completo/incompleto	97	9 (9,3%)	
Ensino superior	7	1 (14,3%)	
Renda familiar			
Menos de 2 Salários Mínimos	176	23 (13,1%)	
2 a 4 Salários Mínimos	34	1 (2,9%)	0,22
5 a 6 Salários Mínimos	1	0 (0%)	
Mais de 6 Salários Mínimos	0	0 (0%)	
Sexo			
Macho	136	19 (14,0%)	0,12*
Fêmea	75	5 (6,7%)	
Idade (Meses)			
6 – 24	96	11 (11,5%)	
25 – 72	71	8 (11,3%)	0,99
> 72	44	5 (11,4%)	
Raça			
CRD	24	0 (0%)	0,08*

SRD	187	24 (12,8%)	
Comprimento do pelo			
Curto	165	22 (13,3%)	
Longo	46	2 (4,3%)	0,11*
Tipo de criação			
Domiciliar	65	3 (4,6%)	
Semidomiciliar	134	18 (13,4%)	0,05*
Solto	12	3 (25,0%)	
Alimentação			
Ração	16	1 (6,3%)	
Comida caseira	158	21 (13,3%)	0,31
Ambos	37	2 (5,4%)	
Contato com animais			
Não	38	2 (5,3%)	1,00
Sim	173	22 (12,7%)	
Contato com equídeos			
Não	204	23 (11,3%)	0,57
Sim	7	1 (14,3%)	
Contato com caprinos			
Não	200	24 (12,0%)	0,61
Sim	11	0 (0%)	
Contato com bovinos			
Não	206	24 (11,7%)	1,00
Sim	5	0 (0%)	
Contato com aves			
Não	129	14 (10,9%)	0,82
Sim	82	10 (12,2%)	
Contato com animais silvestres			
Não	184	21 (11,4%)	1,00
Sim	27	3 (11,1%)	
Contato com felinos			
Não	129	13 (10,1%)	0,51
Sim	82	11 (13,4%)	
Contato com cães			
Não	85	10 (11,8%)	1,00
Sim	126	14 (11,1%)	
Ambiente onde é criado			
Terra	85	11 (12,9%)	
Cimento	46	2 (4,3%)	0,23
Terra e Cimento	80	11 (13,8%)	
Limpeza do local			
Não	21	4 (19%)	0,27
Sim	190	20 (10,5%)	
Frequência de limpeza			
Nenhuma	19	4 (21,1%)	0,38
Diária ou Semanal	165	17 (10,3%)	
Quinzenal ou Mensal	27	3 (11,1%)	
Vermifugação			
Não	111	19 (17,1%)	0,01*
Sim	100	5 (5,0%)	

Vacinação			
Não	37	3 (8,1%)	0,77
Sim	174	21 (12,1)	
Qual Vacina?			
Nenhum	37	3 (8,1%)	
Antirrábica	153	21 (13,7%)	
Antirrábica + Viroses	9	0 (0%)	0,42
Antirrábica + Anticoncepcional	8	0 (0%)	
TODAS	4	0 (0%)	
Presença de carrapatos			
Nunca teve	44	2 (4,5%)	0,28
Está com carrapato	99	13 (13,1%)	
Já teve carrapato	68	9 (13,2	
Adotado			
Da rua	163	20 (12,3%)	0,61
De outro proprietário	48	4 (8,3%)	
Sempre morou nesse local			
Não	43	4 (9,3%,0)	0,79
Sim	168	20 (11,9%)	
Usado para caça			
Não	173	18 (10,4%)	0,40
Sim	38	6 (15,8%)	
Onde dorme			
Dentro de casa	34	2 (5,9%)	
Peridomicílio	174	22 (12,6%)	0,43
Rua	3	0 (0%)	
Como passa a noite			
Amarrado	74	7 (9,5 %)	0,65
Solto	137	17 (12,4 %)	
Uso de coleira repelente			
Não	209	24 (11,5%)	1,00
Sim	2	0 (0%)	
Entorno da casa			
Nada	14	0 (0%)	
Pedras	24	1 (4,2%)	
Plantas	22	0 (0%)	0,13*
Criação de animais	2	0 (0%)	
Pedra e criação de animas	9	1 (11,1%)	
Pedras e Plantas	69	10 (14,3%)	
Planta e criação de animais	1	0 (0%)	
Pedras, Plantas e criação de animais	70	12 (17,1%)	
Caso Humano no domicilio			
Não	202	23 (11,4%)	1,00
Sim	9	1 (11,1%)	

*Variáveis usadas na regressão logística múltipla.

Figure 2.

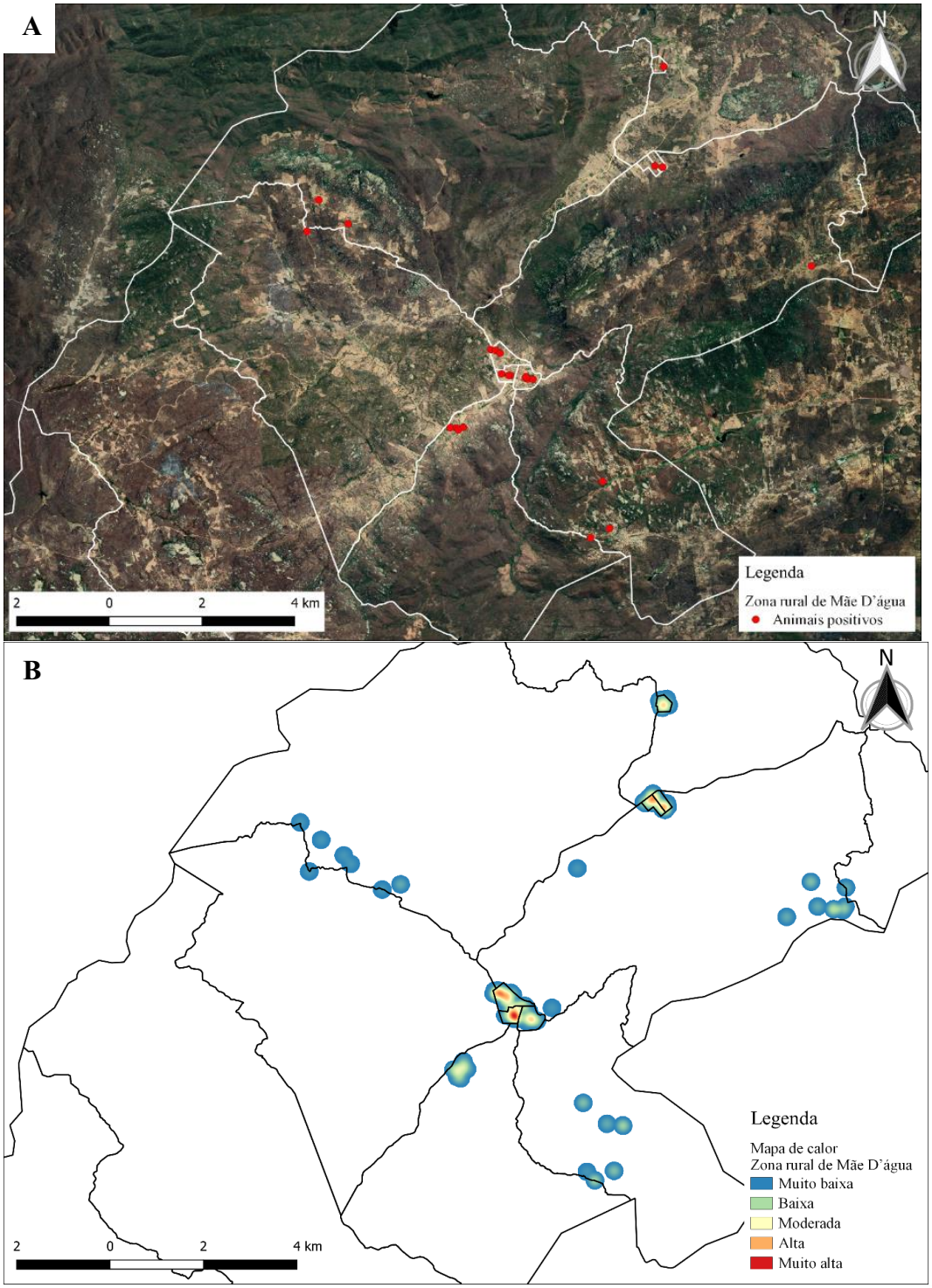
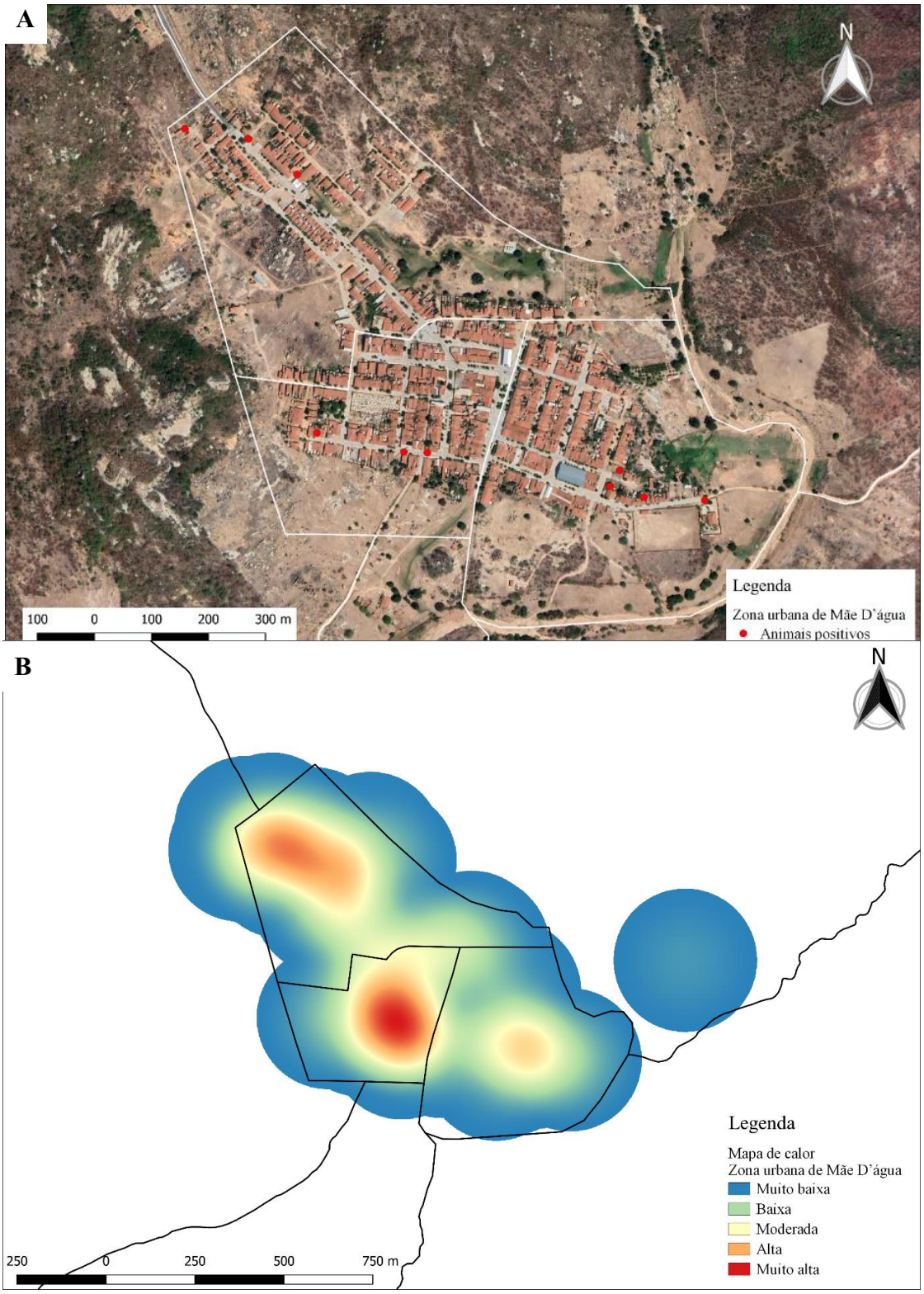

A
N
2 0 2 4 km
Legenda
Zona rural de Mãe D'água
Animais positivos
B
N
2 0 2 4 km
Legenda
Mapa de calor
Zona rural de Mãe D'água
Muito baixa
Baixa
Moderada
Alta
Muito alta

Figure 3.


A
N
100 0 100 200 300 m
Legenda
Zona urbana de Mãe D'água
Animais positivos
B
N
Legenda
Mapa de calor
Zona urbana de Mãe D'água
Muito baixa
Baixa
Moderada
Alta
Muito alta
250 0 250 500 750 m

CONCLUSÃO GERAL

A diversidade de espécies de flebotomíneos encontrada, demonstra a necessidade de intensa vigilância entomológica em todas as regiões do Brasil, visto que grande parte das espécies abundantes nestas regiões são transmissoras de doenças. As técnicas de combate aos flebotomíneos vetores precisam ser modernizadas, visando um melhor controle da dispersão desses insetos. Por fim, há a necessidade de mais trabalhos que identifiquem a relação de coinfecção de espécies de flebotomíneos com *leishmania spp.* para um melhor entendimento do ciclo da doença nas regiões do Brasil.

Em nosso trabalho, ainda foi possível identificar a incidência/prevalência para LVC no município de Mãe d'Água, que apresentou taxa ligeiramente inferior a estudos anteriores na região. Ainda assim, foi possível observar zonas de alta intensidade da doença através de analise espacial. É necessário que se intensifique as medidas de controle da região para que em pesquisas futuras, o número de cães infectados seja menor, logo apresentando um menor risco a população da cidade.