BJGH Brazilian Journal of Global Health
Revista Brasileira de Saúde Global

Risk factors and seroprevalence of asymptomatic human visceral leishmaniasis in an area of intense transmission in Petrolina/PE, Brazil

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ABSTRACT

OBJECTIVE

To identify the seroprevalence of asymptomatic individuals in areas of disease transmission and analyze the environmental, socioeconomic and demographic risk factors in the municipality of Petrolina/PE, Brazil.

METHODS

We approached 92 individuals in 19 localities with VL cases recorded in the Information System for Notifiable Diseases (Sinan) between 2016 and 2017. In each visit, two questionnaires were applied seeking to obtain clinical, social, economic, and environmental variables. Viable blood samples collected during the visits (n=90) were submitted to the Indirect Immunofluorescence Reaction to identify anti-Leishmania antibodies. For data analysis, descriptive statistical tests were performed using Stata® 14.0 software.

RESULTS

The presence of antibodies was detected in 2.2% (2/90) of the samples, in which one individual presented symptoms and the other was classified as asymptomatic. No risk factors related to the presence of anti-Leishmania antibodies were identified.

CONCLUSION

The identification of one asymptomatic individual reinforces the importance of expanding human prevalence studies in endemic areas. Despite the low prevalence, it was possible to identify a possible case with manifestation of the clinical form of chronic VL that needs proper care.

DESCRIPTORS

Leishmania, Healthy carrier, IFT.

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INTRODUCTION

Visceral leishmaniasis (VL) is a neglected tropical zoonosis caused by the protozoan *Leishmania infantum chagasi* and transmitted by the sand fly *Lutzomyia longipalpis*[1]. It is distributed worldwide, and Brazil accounts for more than 90% of cases in Latin America[2], with the Northeast region concentrating the main endemic areas[3], responsible for approximately 45% of the human cases reported in the country in 2017[4]. In Pernambuco, autochthonous cases have been recorded in 130 of the 185 municipalities, demonstrating the geographical expansion of the disease in the state[5]. An increase in endemicity was also evidenced in the municipality of Petrolina, Pernambuco, with the emergence of new foci of the disease[6].

This etiologic agent has a complex biological cycle, making the disease of great magnitude, clinical, biological, and epidemiological complexity, and low vulnerability to current control measures[7,8]. The main prevention and control strategies are focused on early diagnosis and treatment of human cases, vector control with residual insecticides, identification and population control of domestic reservoirs, and health education activities[1]. However, it is known that several environmental, socioeconomic, and demographic factors can be conditioning factors for the expansion and maintenance of the disease[9,10,11,12,13], showing the degree of influence that the environment and the living conditions of individuals have on the transmission cycle.

Research with asymptomatic infected individuals has become increasingly important in recent years because it allows us to know the real dimension of the disease in a region, contributing to the prevention and control of VL[14]. Thus, the objective of this study was to determine the prevalence of asymptomatic patients and identify the risk factors involved in the transmission of the disease in endemic areas of the city of Petrolina, located in the state of Pernambuco, Brazil.

METHODS

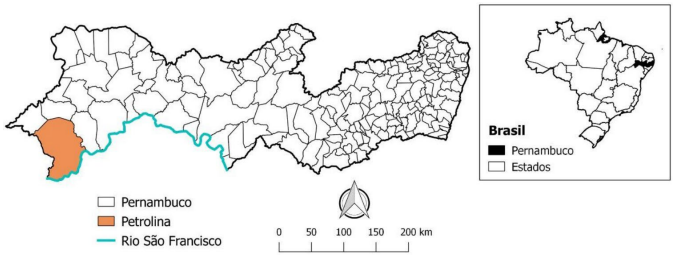
Ethical aspects

The present study was approved by the Research Ethics Committee of the Universidade Federal do Vale do São Francisco - CEP/Univasf (protocol 2.209.233).

Study site

The study was conducted in the municipality of Petrolina (9°23'55" S, 40°30'3" W), located in the hinterland of the state of Pernambuco, a region endemic for VL (Figure 1). It has a territorial area of 4,561,872 km² and an estimated population, in the year 2018, of 343,865 inhabitants15. The climate is tropical semi-arid, hot, with an average annual temperature of 26°C.

Figure 1. Map of Pernambuco, highlighting the city of Petrolina (PE).



Sampling

A cross-sectional descriptive study with a quantitative approach was conducted. Participants in the study included individuals living in localities with VL cases recorded in 2016 and 2017, totalling 25 notifications, however, 23 presented valid addresses belonging to 19 localities. These addresses were taken from the Sistema de Informação de Agravos de Notificação (SINAN) and made available by the Municipal Health Secretariat of Petrolina. Confirmed cases are those suspected individuals who met at least one of the confirmation criteria adopted by the Ministry of Health, which are clinical, laboratory, or clinical-epidemiological[1].

The home visits in the 19 locations were conducted during business hours and the ones that were closest to the residence of the notified confirmed case and that had residents at the time were selected. All of them occurred between October 2017 and April 2019, totalling in 62 homes visited and 92 studied individuals over the age of 12 years, distributed by place of residence (zone and locality). In each locality, the visits were conducted respecting a radius of 200 meters starting from the residence in which there was the confirmed case, in which is the average flight space of the vector *Lutzomiya longipalpis*[16].

Sample collection and processing

Blood samples were obtained by cephalic venipuncture, using 5 ml disposable needles and syringes, with proper hygiene and aseptic care, performed by nurses and nursing technicians. The blood was stored in 5 ml test tubes, without anticoagulant, properly identified and previously packed in isothermal boxes with recyclable ice and a thermometer for temperature control. After collection, the samples were taken to the Laboratory of the VIII Regional Health Management of Pernambuco or to the Immunology Laboratory of the Universidade Federal do Vale do São Francisco (Univasf) - Campus Centro Petrolina for processing. To obtain the blood serum, the collected material was centrifuged at 3,500 rpm for 15 minutes and then transferred to a new test tube. The samples were stored in a freezer at -20°C until the moment of the analyses.

Serological analysis

Serological diagnosis was performed using the Bio-Manguinhos® Human Leishmaniasis Indirect Immunofluorescence Reaction (IFT) kit donated by the manufacturer's laboratory and was carried out by researchers from the Laboratory of Parasitology and Parasitic Diseases of the Univasf - Agrarian Sciences Campus, following the manufacturer's instructions. The reading was performed with the aid of a fluorescence microscope, being considered reagent the sera presenting fluorescence; and non-reagent, the sera with absence of fluorescence, taking as reference the positive and negative controls included in each slide[1].

Clinical, socioeconomic, demographic, and environmental evaluation

At the time of each visit, two questionnaires adapted from Pedrosa and Ximenes[17] were applied; one was structured for individual questions, answered by each participant (individuals under 18 years of age answered under the supervision of a responsible adult), and the other was semi-structured for the group of residents of the same household, answered by only one resident of the household, over 18 years of age. Both questions included the possible manifestation of the main clinical findings of the disease (fever, weight loss, weakness, edema, cough and/or diarrhea, pallor, increased abdominal volume, hemorrhagic phenomena, jaundice, and others); demographic and socioeconomic variables (sex, skin colour, age, education, occupation/profession, monthly family income, per capita income, material used in the external wall of the house,

material used in the roof of the house, screens on doors and windows, bathroom in the house, source of water used); and environmental factors (forest near the house, river or stream near the house, presence of vacant land near the house, organic material in the soil, presence of a domestic animal in the house, presence of other animals in the house, existence of animals around the house, garbage collection, destination of waste, paving of the streets, drainage of the streets).

Statistical analysis

Data were analyzed using descriptive statistics with estimates of frequencies, proportions, confidence intervals, means, standard deviations, minimum and maximum values using Stata® software version 14.0. Ignored responses were excluded from the analyses, so the N (sample size) alternated between the variables analyzed.

RESULTS

The visits were carried out in 19 locations, totalling 92 people evaluated, 34 from urban areas, 17 from peri-urban areas and 41 from rural areas. Of the total number of blood samples collected, two were unviable, totalling 90 viable samples. Of these, 2.2% (2/90) were reagent for anti-Leishmania antibodies.

All the reactors were male and of mixed race/colour, 50% (1/2) were symptomatic, with the presence of clinical signs such as fever, weakness, edema, weight loss and increased abdominal volume, and 50% (1/2) were considered asymptomatic.

About the results obtained regarding risk factors in the studied population, it was observed that 67.4% (62/92) were female and 32.6% (30/92) were male; the most frequent race/colour was brown with 58.2% (53/91), followed by black with 22.0% (20/91) and white with 14.3% (13/91); 9.9% (9/91) reported being illiterate and the highest proportion, 37.4% (34/91) was of individuals who studied up to elementary school; the main occupation/profession was 21.7% (18/83) of students, 18.1% (15/83) of rural workers, 18.1% (15/83) of housewives, and 13.3% (11/83) of retirees (Table 1).

Table 1. Distribution of participants according to sex, race/color, level of education and occupation/profession in endemic areas for VL - Petrolina (PE), 2019.

VARIABLES	N	%	CI (95%)
Gender			
Female	62	67,4	57,0 - 76,3
Male	30	32,6	23,7 - 43,0
Total	92	100,0	
Race/Colour			
White	13	14,3	8,4 - 23,3
Brown	53	58,2	47,7 - 68,1
Black	20	22,0	14,5 - 31,8
Yellow	3	3,3	1,0 - 9,9
Red	1	1,1	0,1 - 7,6
Ignored	1	1,1	0,1 - 7,6
Total	91	100,0	
Education			
Illiterate	9	9,9	5,2 - 18,1
Up to elementary school	34	37,4	27,9 - 47,9
Up to high school	33	36,3	26,9 - 46,8
Higher and post-graduate education	15	16,5	10,1 - 25,8
Total	91	100,0	
Occupation/profession			
Rural worker	15	18,1	11,1 - 28,1
Retired	11	13,3	7,4 - 22,6
Student	18	21,7	14,0 - 32,1
Housewife	15	18,1	11,1 - 28,1
Unemployed	1	1,2	0,2 - 8,4
Teacher	3	3,6	1,1 - 10,8
Domestic Worker	6	7,2	3,2 - 15,4
Others	14	16,9	10,1 - 26,7
Total	83	100,0	

n: number of participants
*CI: Confidence Interval

Regarding the condition of the properties, it was observed that 100.0% (89/89) of the individuals lived in houses with walls made of durable materials, such as masonry, stone, concrete, clay or wood and 95.5% (85/89) had roofs also made of durable materials, such as tiles, asbestos-cement, aluminum-wood, wood or concrete slabs. The presence of screens on doors and windows was present in 13.3% (12/90) of the homes of the people questioned, against 86.7% (78/90) who did not have any. In addition, 88.8% (79/89) of the population reported having a bathroom inside the home, 7.8% (7/89) had the bathroom outside, and 3.4% (3/89) had no bathroom in their homes (Table 2).

Table 2. Distribution of participants according to household structuring, in endemic areas for VL - Petrolina (PE), 2019.

VARIABLES	N	%	CI (95%)
Predominantly wall material			
Durable	89	100,0	- -
Not durable	0	0,0	
Total	89	100,0	
Material predominantly used in the roof			
Durable	85	95,5	88,4 - 98,3
Not durable	4	4,5	1,7 - 11,6
Total	89	100,0	
Screens on doors and windows			
Yes	12	13,3	7,6 - 22,2
No	78	86,7	77,8 - 92,4
Total	90	100,0	
Bathroom in the house			
Absent	3	3,4	1,1 - 10,1
Present outside the dwelling	7	7,8	3,7 - 15,8
Present inside the dwelling	79	88,8	80,2 - 93,2
Total	89	100,0	

n: number of participants
*CI: Confidence Interval

All participants reported the presence of frequent garbage collection, in which 72.2% (65/90) said it was daily or at least three times a week and 27.8% (25/90) less than three times a week. As for the basic sanitation conditions, 66.3% (59/89) of the participants affirmed the sewage system as the destination of their waste, 32.6% (29/89) reported a septic tank, and only 1.1% (1/89) had open sewage (Table 3).

Piped water from the municipality's distribution network was reported by 100% (90/90) of the participants. The presence of vegetation was observed near the residence of 50% (45/90) of the participants. In addition, 14.4% (13/90) of the participants live near rivers or streams, 33.7% (30/90) have open sewers near their homes, and 47.8% (43/90) reported the presence of vacant land (Table 3).

Table 3. Distribution of participants according to service and infrastructure of streets and environmental factors of endemic areas for VL - Petrolina (PE), 2019.

VARIABLES	N	%	CI (95%)
Garbage collection			
Yes, daily, or more than 3x a week	65	72,2	61,9 - 80,6
Yes, less than 3x a week	25	27,8	19,4 - 38,1
Total	90	100,0	
Destination given to the garbage			
Sewage network	59	66,3	55,7 - 75,5
Septic tank	29	32,6	23,5 - 43,2
Open sky	1	1,1	0,2 - 7,8
Total	89	100,0	
Origin of the water used			
Piped	90	100,0	- -
Other	0	0,0	- -
Presence of forest			
Yes	45	50,0	39,6 - 60,4
No	45	50,0	39,6 - 60,4
Presence of river or stream			
Yes	13	14,4	8,5 - 23,5
No	77	85,6	76,5 - 91,5

Presence of open sewage			
Yes	30	33,7	24,5 - 44,3
No	59	66,3	55,7 - 75,5
Presence of vacant lots			
Yes	43	47,8	37,5 - 58,2
No	47	52,2	41,8 - 62,5

n: number of participants
*CI: Confidence Interval

The most prevalent animal species in the household environment was the canine with 28.9% (26/90), and in the peridomestic environment the feline in 73.3% (66/90) of the participants. Gallinaceous animals were mentioned in both environments, but with a higher proportion in the peridomicile, being present in the residence of 14.4% (13/90) of the participants (Table 4).

Table 4. Distribution of participants according to the presence of animals in the intra and peridomicile of endemic areas - Petrolina (PE), 2019.

ANIMAL PRESENCE	N	%	Average	CI (95%)
Intradomicile				
Puppy				
Yes	26	28,9	1,4	1,2 - 1,6
No	64	71,1		
Total	90	100,0		
Cat				
Yes	12	13,5	3,2	1,9 - 4,4
No	77	86,5		
Total	89	100,0		
Chicken				
Yes	2	2,3	15	- -
No	87	97,8		
Total	89	100,0		
Other				
Yes	8	8,9	2	0,9 - 3,1
No	82	91,1		
Total	90	100,0		
Intradomicile				
Puppy				
Yes	62	68,9	2,0	1,2 - 2,7
No	28	31,1		
Cat				
Yes	66	73,3	2,1	1,5 - 2,7
No	24	26,7		
Chicken				
Yes	13	14,4	2,2	0,6 - 3,7
No	77	85,6		
Other				
Yes	3	3,3	1	- -
No	87	96,7		
Total	**90**	**100,0**		

n: number of participants
*CI: Confidence Interval

DISCUSSION

The individual who showed symptoms of the disease was considered infected with VL in the oligosymptomatic or classic form of the disease. The individual considered asymptomatic was notified to Sinan for clinical and laboratory investigation, and subsequent conclusion of the case, since all localities in this study have their endemicity known and proven, according to the VL Surveillance and Control Manual of the Ministry of Health (MOH). In addition, the MH considers an individual as VL suspect when he/she presents the association of fever with hepatosplenomegaly (identified by palpation or imaging exam), in addition to coming from an endemic area[1].

The presence of seropositive cases with absence of symptoms of the classic form of the disease was expected in all the locations studied, since these are endemic regions, and the frequency of asymptomatic individuals is estimated to be between 40 and 60% of the seropositive individuals[1]. The low prevalence identified in this study may be associated with the small number of individuals analyzed in each location, or even the fact that children aged 0 to 12 years were not included. According to a study by Dos Santos Marques et al.[18], asymptomatic children were identified in three areas of Belo Horizonte, Minas Gerais, aged 3 months to 10 years, with prevalence ranging from 29% to 34%. Cavalcante et al.[19] also identified a high prevalence in asymptomatic patients in the municipality of Raposa, located in the state of Maranhão, with a total prevalence of 19.8%, 53% in those older than 15 years and 19% in those younger than 5 years.

The method used in this study for laboratory diagnosis was IFT, which, because it is indirect, has limitations. False positives can be identified, since antibodies may remain present after clinical cure of previously affected individuals, besides the possibility of cross-reactions with other diseases such as trypanosomiasis, leprosy, malaria, schistosomiasis, tuberculosis, and other leishmaniases[20]. The previously mentioned studies[18,19] that evaluated infection in asymptomatic patients also used an indirect diagnostic method, the Enzyme-Linked Immunosorbent Assay (ELISA). In Ethiopia, Abbasi et al.[21] found a prevalence of asymptomatic patients of around 17% in more than 4,000 people examined in an endemic region, using the Polymerase Chain Reaction (PCR) method for diagnosis. For Dourado et al.[20] none of these methods presents 100% sensitivity and specificity and points out that the gold standard is the identification of amastigotes of the protozoan parasite in bone marrow or spleen puncture samples.

The results of the present study corroborate other studies, in which among the people most affected by VL are males and people of mixed race/colour[12, 19, 22, 23, 24]. According to D'Oliveira et al.[14], the presence of human cases in the family or neighbours is associated with new cases of the disease in clinical or asymptomatic forms, suggesting that individuals in these regions are exposed to the same risk factors.

Because it is considered a neglected disease by the WHO, it is expected to be more frequent in socially vulnerable regions, with little or no infrastructure services, low education level, and low income. Socioeconomic and environmental conditions and lifestyle habits can significantly influence the epidemiology of the disease in endemic areas, affecting human settlements of low socioeconomic status, under precarious housing situations, and urban infrastructure[19]. In a study by Costa et al.[25], we evaluated the impact of urban services on the occurrence of VL in Teresina, Piauí state, and obtained significant associations between some aspects, such as inadequate sewage system and irregular garbage collection. The presence of garbage accumulation and inadequate sanitation may benefit the vector adaptation to the peri-urban environment[26] since they need moisture and organic matter to survive in the environment.

The presence of animals in intra and peri-domiciliary environments also favours the maintenance of the sand fly in the urban environment because, besides serving as a blood source for females, they provide a humid environment with organic matter. The dog, as the main VL reservoir in urban areas, is considered a determining factor for the occurrence of the disease, since canine infection precedes human infection. Borges et al.[27] found the presence of dogs in 64.6% of households with confirmed human cases, attributing to dog owners a 2.2-fold increase in the risk of contracting VL when compared to individuals who do not own a dog.

In addition to dogs, other animals have been identified as VL reservoirs in the urban environment. Costa et al.[28] analyzed 200 cats from endemic areas in the municipality of Araçatuba, state of São Paulo, and found a prevalence of infection of 14.5%. However, Tanure et al.[29] found a predilection of the female Lutzomyia longipalpis for the blood of chickens in environments with the presence of other animals such as dogs and cats.

CONCLUSION

The identification of an asymptomatic individual reinforces the importance of expanding human prevalence studies in endemic areas. However, it was possible to identify a possible case with clinical manifestation, which, although an evaluation by medical specialists is necessary, the data from this study indicate strong evidence that it is a case of chronic VL that requires proper care.

Visceral leishmaniasis is considered a public health problem because of its magnitude and clinical, biological, and epidemiological complexity. Many studies show that the degradation of the natural environment contributes to the good adaptation of the sand fly that causes the disease in the urban environment. However, the actions to control the disease are restricted to the chemical control of the vector, even though the various socioeconomic and environmental factors that condition the transmission of the disease are known.

ACKNOWLEDGMENTS

To the VIII Regional Health Management (GERES), Pernambuco State Health Secretariat (SES-PE), and Municipal Health Secretariat (SMS) of Petrolina. To CNPq for the MCH research productivity scholarship (314019/2021-9). To Instituto de Tecnologia em Imunobiológicos da Fundação Oswaldo Cruz (Bio-Manguinhos/FIOCRUZ) for the Indirect Immunofluorescence Kits.

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