



Article/Artigo

Risk factors for *Leishmania chagasi* infection in an endemic area in Raposa, State of Maranhão, Brazil

Fatores associados à infecção por *Leishmania chagasi* em uma área endêmica em Raposa, Estado do Maranhão, Brasil

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ABSTRACT

Introduction: Infection with *Leishmania chagasi* is the most common clinical presentation for visceral leishmaniasis in endemic areas. The municipality of Raposa is an endemic area in State of Maranhão, Brazil, and have had registration cases of visceral leishmaniasis disease. For this reason, a cross-sectional study was conducted to evaluate the risk factors for infection with *L. chagasi* detected by Montenegro skin test. **Methods:** The sample comprised 96% of the inhabitants of the villages of Maresia, Pantoja, and Marisol located in the municipality of Raposa, corresponding to 1,359 subjects. Data were collected using a questionnaire. Univariate and multivariate logistic regression models were applied to evaluate the association between the variables studied and infection of *L. chagasi*. **Results:** The variables associated with infection upon nonadjusted analysis were a straw roof, mud walls, floors of beaten earth, presence of sand flies inside or outside of the dwelling, and bathing outdoors. Adjusted analysis showed that the presence of sand flies inside/outside the dwelling was a risk factor, and age younger than 10 years was a protective factor against asymptomatic infection. **Conclusions:** The results highlight the extent to which precarious living conditions of the population strengthen the epidemiological chain of visceral leishmaniasis.

Keywords: Visceral leishmaniasis. Kalazar. *Leishmania chagasi*. Montenegro skin test. Risk factors.

RESUMO

Introdução: A infecção por *Leishmania chagasi* é a apresentação clínica mais comum de leishmaniose visceral em áreas endêmicas. O município de Raposa é área endêmica no Estado do Maranhão, tendo registrado casos da doença. Por isso, realizou-se um estudo transversal, com o objetivo de estudar os fatores de risco para infecção por *L. chagasi* detectada pelo teste intradérmico de Montenegro. **Métodos:** O estudo envolveu 96% dos moradores das localidades de Maresia, Pantoja e Marisol do município da Raposa, totalizando 1.359 indivíduos. O levantamento dos dados foi realizado utilizando um questionário. Para verificar a associação entre as variáveis estudadas e a infecção por *L. chagasi*, foram utilizados os modelos de regressão logística uni e multivariada. **Resultados:** Na análise não ajustada, as variáveis associadas à infecção foram: cobertura da casa de palha, paredes de taipa, piso de chão batido, a presença de flebotomíneos dentro ou fora do domicílio e o local do banho fora de casa. Na análise ajustada, a presença de flebotomíneos dentro ou fora do domicílio foi considerada fator de risco e a idade menor que 10 anos revelou-se como fator de proteção para a infecção assintomática. **Conclusões:** Evidenciou-se também, o quanto a precariedade das condições de vida da população contribuiu para o fortalecimento da cadeia epidemiológica da doença.

Palavras-chaves: Leishmaniose visceral. Calazar. *Leishmania chagasi*. Intradermorreação de Montenegro. Fatores de risco.

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Received in 23/11/2010

Accepted in 05/05/2011

INTRODUCTION

Visceral leishmaniasis is a disease of great concern for public health because of its high incidence and lethality, especially among untreated patients and malnourished children. Visceral leishmaniasis shows a wide geographical distribution and is found in Asia, Europe, the Middle East, Africa, and the Americas. In the Americas, the disease is also called american visceral leishmaniasis (AVL) or neotropical kala-azar^{1,2}.

In Latin America, AVL has been described in at least 12 countries, with 90% of cases occurring in Brazil. The disease is found in 20 states, affecting four of the five regions of the country. The highest incidence is observed in the northeast, accounting for approximately 45% of all cases, followed by the Southeast, North, and mid-west regions^{1,3,4}. The annual average number of cases in Brazil is 3,500. The incidence rate of the disease has reached 20.4 cases/100,000 inhabitants in some places of the Northeastern States such as Piauí, Maranhão, and Bahia. According to official reports, mortality rates can reach 10% in some places^{1,5}.

A spread of AVL to periurban and urban areas has been observed over recent decades. Factors that contributed to the expansion of the disease include transformation of the environment through deforestation and the migration of man from rural areas to the periphery of towns where he lives under precarious conditions of housing and sanitation^{1,6,7}.

In Latin America, *Leishmania chagasi* is the species that is most commonly isolated from patients with AVL^{8,9}. The most important reservoirs of the parasite in Brazil are dogs and foxes. Transmission occurs indirectly through the bite of the hematophagous sand fly, *Lutzomyia longipalpis*, whose habitat is the domestic and peridomestic environment where the female feeds on the blood of dogs, humans, other mammals, and birds^{4,10-12}. Infection with *L. chagasi* is characterized by a broad spectrum of clinical manifestations ranging from the asymptomatic form

to discrete (oligosymptomatic), moderate, and severe manifestations that, if not treated, can lead to the death of the patient^{12,13}. Inapparent or asymptomatic infection is defined as the presence of infection in the absence of clinical manifestations and commonly occurs in patients from endemic areas with epidemiological and immunological evidence of infection^{14,15}. According to Caldas et al.¹⁴, epidemiological surveys conducted in AVL-endemic areas have shown that a portion of the subjects present evidence of infection with *L. chagasi* in the absence of a history of clinical manifestations, with the observation of high rates of individuals with positive serology (28%) or a positive Montenegro skin test (MST) (19%). Of these, about 20% show clinical manifestations and develop the classical form of the disease, and the remaining ones progress to the oligosymptomatic form or cure^{1,16}.

In Brazil, some individual infections developed by AVL have been observed, and higher rates of infection are monitored in some States such as Bahia (18:1)¹⁷, Ceará (11:1)¹⁸, and Maranhão (28:1)¹⁴. This rate was 5:1 in a study conducted in Kenya². Therefore, the objective of the present study was to investigate the risk factors for asymptomatic infection with *L. chagasi* in a population from an AVL-endemic urban area.

METHODS

A cross-sectional study was conducted between August 2006 and August 2007 in the municipality of Raposa located in the Ilha de São Luis, Northern region of the State of Maranhão. The municipality is organized into towns and villages, the most important being Alto do Farol, Araçagi, Curupu, Vila Pirâmide, Vila Nova, Bom Viver, Maresia, Pantoja, and Marisol^{12,19}. The present study was conducted in the last three villages.

Maresia, Pantoja, and Marisol are the result of an invasion that occurred approximately 4 years earlier. The economic activity of the residents is based on fishing and handicraft production¹⁴. The epidemiology of AVL in these villages was responsible for 20% of the AVL cases registered in the municipality in 2003 and 34% in 2004¹². This place was chosen for the study because it is a recent endemic area of AVL²⁰.

The sample comprised 96% of the residents of Maresia, Pantoja, and Marisol who met the following inclusion criteria: living in one of the three villages for more than 6 months and no past or present history of AVL.

The study was designed and conducted in two phases: In the first phase, a population census was performed; in the second phase, a questionnaire was used for the collection of demographic, socioeconomic, and epidemiological data. In addition to the use of questionnaire, the subjects were submitted to physical examination and the Montenegro skin reaction for the detection of infection with *Leishmania* sp.

The antigen was prepared by the Gonçalves Moniz Research Center/FIOCRUZ, Salvador, BA, from promastigote forms of *L. amazonensis* (MHOMBr-88-BA-125). An induration diameter of ≥ 5 mm was defined as a positive reaction.

The data were analyzed with the Epi-Info 3.3.2 program of the World Health Organization. The variables were classified into two or more categories, with the first being considered the baseline category the one associated with the lowest risk. For analysis of the association between the variables studied and infection with *L. chagasi*, the relative risk (RR) and its respective 95% confidence interval adjusted or not for the prevalence of infection detected by the MST were calculated. Adjusted analysis, including all variables that presented a p value ≤ 0.20 , was performed by logistic regression using the STATA 9.0 program.

Ethical considerations

The study was approved by the Ethics Committee of the University Hospital, Federal University of Maranhão (protocol 33104-444/2006). The wish and decision of the participant or responsible person were respected throughout the study. All subjects signed a free informed consent form.

TABLE 1 - Nonadjusted analysis of the association between infection with *Leishmania (Leishmania) chagasi* detected by the Montenegro skin test and demographic and socioeconomic variables, Raposa, State of Maranhão, Brazil, 2007.

Variables	Positive MST		Negative MST		RR (CI)	P
	n	%	n	%		
Gender						
female	706	83.5	140	16.5	1	
male	422	82.7	91	17.3	0.99 (0.94-1.04)	0.7632
Age (years)						
0 to 10	426	77.3	125	22.7	1	
>10	702	86.9	106	13.1	0.89 (0.84-0.94)	<0.0001
Place of residence						
pantoja	67	80.7	16	19.3	1	
maresia	956	82.5	203	17.5	1.02 (0.92-1.14)	0.7965
marisol	105	89.7	12	10.3	1.11 (0.98-1.26)	0.1085
Length of stay in the area (years)						
<2	282	80.6	68	19.4	1	
≥ 2	846	83.8	163	16.2	1.04 (0.98-1.10)	0.1859
Last origin						
other municipalities	222	85.7	37	14.3	1	
São Luis	129	87.8	18	12.2	1.02 (0.95-1.11)	0.6696
Raposa	777	81.5	176	18.5	1.08 (1.01-1.15)	0.0842
Roof						
tile	844	81.8	188	18.2	1	
straw	284	86.9	43	13.1	1.06 (1.01-1.12)	0.0412
Wall material						
brick	484	79.5	125	20.5	1	
mud	535	85.5	91	14.5	1.08 (1.02-1.13)	0.0070
straw	109	87.9	15	12.1	1.11 (1.02-1.19)	0.0402
Household income						
>2 minimum wages	90	8.0	28	12.1	1	
0 to 2 minimum wages	1038	92.0	203	87.9	1.10 (0.99-1.22)	0.0562
Garbage destination						
public collection/burned/buried	918	82.0	201	18.0	1	
open air disposal	210	87.5	30	12.5	1.07 (1.01-1.13)	0.0512
Total	1,128		231			

MST: Montenegro skin test; RR: relative risk; CI: confidence interval.

RESULTS

The census performed in the villages of Maresia, Pantoja, and Marisol, municipality of Raposa, MA, revealed a population of 1,417 inhabitants; of these, 1,359 fulfilled the inclusion criteria and accepted the request to be part of the study population. Analysis of the demographic data showed that the age group of 0 to 10 years was associated with *L. chagasi* infection, which was a protective factor for infection (Table 1). For the type of housing, the variables associated with infection were a straw roof (RR=1.06, p=0.0412), mud wall (RR=1.08, p=0.007), straw wall (RR=1.11, p=0.040), and floor of beaten earth (RR=1.07, p=0.007) (Table 1). Household income (0.0562) and disposal of garbage (0.0512) in the open air showed a borderline significant p value, indicating a possible association with *L. chagasi* infection (Table 1).

Regarding the other variables, insecticide spraying, presence of animals in the domestic or peridomestic area and life habits, the presence of a pig pen close to the dwelling, presence of sand flies, and bathing outdoors (backyard) were associated with *L. chagasi* infection (Table 2).

Variables that continued to be associated with infection upon the adjusted analysis were age of 0-10 years as a protective factor and the presence of sand flies inside and outside the dwelling (Table 3).

TABLE 2 - Nonadjusted analysis of the association between infection with *Leishmania (Leishmania) chagasi* detected by the Montenegro skin test and epidemiological variables, Raposa, State of Maranhão, Brazil, 2007.

Variables	Positive MST		Negative MST		RR (CI)	P
	n	%	n	%		
Insecticide spraying						
no	894	83.2	180	16.8	1	
yes	234	82.1	51	17.9	1.01 (0.95-1.08)	0.7152
Animal in domestic area						
none	310	82.2	67	17.8	1	
dog	341	83.2	69	16.8	1.01 (0.95-1.08)	0.7986
Animal in peridomestic area						
none	191	80.3	47	19.7	1	
dog	699	82.8	145	17.2	1.03 (0.95-1.10)	0.5702
dog and opossum	131	85.1	23	14.9	1.06 (0.97-1.16)	0.2800
Pig pen in peridomestic area						
no	900	84.1	170	15.9	1	
yes	228	78.9	61	21.1	0.94 (0.88-1.00)	0.0446
Chicken coop in peridomestic area						
no	556	84.9	99	15.1	1	
yes	572	81.3	132	18.8	0.96 (0.91-1.00)	0.0871
Presence of sand flies						
no	584	80.9	138	19.1	1	
yes	544	85.4	93	14.6	1.06 (1.01-1.11)	0.0324
Use of a mosquito net						
no	772	84.1	146	15.9	1	
yes	356	80.7	85	19.3	1.04 (0.99-1.10)	0.1411
Use of repellents						
yes	133	83.6	26	16.4	1	
no	995	82.9	205	17.1	0.99 (0.92-1.07)	0.9058
Place of bathing						
indoors	214	78.1	60	21.9	1	
outdoors	914	84.2	171	15.8	1.08 (1.01-1.15)	0.0199
Total	1,128		231			

MST: Montenegro skin test; RR: relative risk; CI: confidence interval.

TABLE 3 - Adjusted analysis of the association between infection with *Leishmania (Leishmania) chagasi* detected by the Montenegro skin test and demographic, socioeconomic, and epidemiological variables, Raposa, State of Maranhão, Brazil, 2007.

Variables	RR (95% CI)	p value
Age (0 to 10 years)	0.49 (0.33-0.73)	0.001
Household income (<2 minimum wages)	1.08 (0.97-1.20)	0.142
Living in Marisol	1.11 (0.98-1.26)	0.093
Living in Maresia	1.07 (0.98-1.10)	0.220
Time of residence >2 years	1.04 (0.98-1.10)	0.161
Straw roof	1.01 (0.95-1.07)	0.633
Mud wall	1.02 (0.95-1.09)	0.469
Straw wall	1.06 (0.98-1.15)	0.119
Bathing outdoors	1.03 (0.95-1.11)	0.445
Chicken coop in the peridomestic area	0.95 (0.90-1.00)	0.062
Pig pen in the peridomestic area	1.08 (0.67-1.73)	0.749
Presence of sand flies	1.06 (1.01-1.11)	0.014
Not using a mosquito net	0.99 (0.94-1.05)	0.973

RR: relative risk; CI: confidence interval.

DISCUSSION

Visceral leishmaniasis is endemic in Ilha de São Luis since the 1980s⁵, and an increased rate of migration of individuals from rural to urban areas and from other states has been observed during this period. These people settled in overcrowded and inadequate settlements constructed at the periphery of Ilha de São Luis (consisting of four municipalities), generally in recently inhabited and occupied areas characterized by deforestation and burning^{21,22}. These conditions offer an excellent habitat for *Lu. Longipalpis*, and the density of this species can reach elevated levels inside dwellings and animal shelters. This sociogeographic profile applies to many of the residents of Marisol, Pantoja, and Maresia.

In the present study, the proportion of infection detected by the MST was more frequent among individuals older than 10 years. A higher incidence of infection in the population older than 10 years was also reported by Moreno et al²³. About 30% of adults from endemic areas without a history of the disease present a strong positive skin test. This finding might be explained by a longer time

of exposure to the parasite, conferring higher resistance against the disease; a positive MST indicates that one individual is not sick and that his cellular immunity is satisfactory at that time^{23,24}.

Studies have demonstrated differences in the age group affected between asymptomatic *L. chagasi* infection and AVL disease, with the observation of a lower proportion of asymptomatic infection in children younger than 10 years. However, this age group is more affected by the disease^{14,18}. The higher susceptibility of children of this age to the clinical form of the disease is probably due to higher rates of nutritional deficiencies and consequent lower immunological resistance^{5,18,24}.

Roof, wall, and floor materials of the dwelling were risk factors for infection with *L. chagasi*. The type of housing, with mud construction and straw roofs, facilitates the entry of the vector through holes and other openings^{4,21}. Borges²⁵ observed that plastered walls offer more protection for residents against AVL than a non-plastered dwelling.

Nascimento et al.⁵ showed that environments of low socioeconomic level are prone to the occurrence of AVL, affecting human settlements characterized by precarious housing conditions. In the study of Silva et al.⁷ including patients from Ilha de São Luis with a diagnosis of visceral leishmaniasis, the dwellings visited were mainly made of mud (68%) and had a straw roof (48%) and floor of beaten earth (66%), which were in agreement with the present findings.

The variable, household income, showed borderline significance ($p=0.0562$), with a substantial number of subjects infected with *L. chagasi* earning less than two minimum wages (92%). This result agrees with the findings of Caldas et al.²¹ who also studied a sample from the municipality of Raposa, MA, and observed that most children were from families earning less than two minimum wages. The mean monthly household income was 1.7 minimum wages (US\$315,112), demonstrating the conditions of poverty of this population.

The destination of household waste is an important variable for the epidemiology of AVL, as household waste serves as a potential breeding site for the vectors. This was demonstrated by Borges²⁵ who observed an increase in the chance of contracting visceral leishmaniasis among residents of areas where no system of garbage collection is available. In the present study, waste destination also showed borderline significance ($p=0.0512$) similar to the type of housing.

Although the dog is an important element in the epidemiological chain of AVL, no significant association was observed between the presence of dogs in domestic or peridomestic areas and infection with *L. chagasi*. Nascimento et al.⁵ and Caldas et al.²¹ also found no association between the presence of dogs in domestic/peridomestic areas and *L. chagasi* infection; thus, this variable was not considered a risk factor.

The presence of sand flies inside and outside the dwelling was a risk factor for infection with *L. chagasi*. Similar results were reported by Caldas et al.²¹ who studied *L. chagasi* infection in children from the same municipality and observed that the chance of infection was almost two times higher among children living in houses with sand flies compared to those living in houses without these insects. Another risk factor was bathing outdoors. Generally, people take a bath outdoors.

Lu. longipalpis easily adapts to the peridomestic area and variable temperatures and can be found inside dwellings and in shelters of domestic animals¹. According to Rebelo et al.²⁶, the rapid adaptation of this vector to peridomestic environments in rural areas and even in peripheral neighborhoods of large urban center is due to the progressive modification of the primitive vegetation at these sites.

According to Cebino Neto²⁷, the neighborhoods with predominant vegetation covering that passed through a messy occupation, followed by an increase population density, have registered the highest incidence rates of visceral leishmaniasis. Trees and plantations around the houses have been treated as a significant factor of Kala azar. According to Ranjan et al.²⁸, some trees are considered source of fructose for Phlebotomine sandflies and also produce dark and damp environments around the peripheral neighborhood, the ideal places of shelter for the mosquito.

Knowledge about the risk factors for infection with *L. chagasi* is the first step to the elaboration of more effective control strategies. Collective health measures designed to improve the overall living conditions of this population need to be implemented.

ACKNOWLEDGMENTS

We thank the Municipal Secretariat of Health of Raposa, Maranhão for the participation of its community health agents in the active identification of the subjects for the study. We are also indebted to the communities of the villages of Maresia, Pantoja, and Marisol that, in a gesture of selflessness, agreed to participate in the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

Fundação de Amparo a Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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