Factors associated with asymptomatic infection in family members and neighbors of patients with visceral leishmaniasis

Fatores associados com a infecção assintomática em familiares e vizinhos de pacientes com leishmaniose visceral

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Abstract

This study analyzed factors associated with asymptomatic Leishmania chagasi *infection in family members and neighbors of patients with visceral leishmaniasis (VL). A cross-sectional study was conducted with 361 individuals in the counties comprising Ilha de São Luís (São Luís Island) in Maranhão State, Brazil. The Montenegro skin test (MST) and serology by enzyme-linked immunosorbent assay (ELISA) were performed to detect infection. Odds ratios (OR) and prevalence ratios (PR) and their 95% confidence intervals (95%CI) were estimated by multilevel logistic regression and Poisson regression, respectively. The rates of positive reactions were 71.3% for MST and 9.7% for ELISA. The variable associated with infection according to MST was living in the same household as the index case (PR = 1.36; 95%CI: 1.03-1.78). According to ELISA, the statistically significant variables were: living in the county of Raposa (OR = 3.56; 95%CI: 1.24-10.19) and living in the same household as the index case (OR = 2.70; 95%CI: 1.19-6.08). Family members of individuals with LV are at increased risk of infection. Priority control measures should target these families.*

Visceral Leishmaniasis; Infection; Asymptomatic Infections

Introduction

Visceral leishmaniasis (VL) is a zoonosis with major public health relevance due to its urbanization, geographic expansion, and high case-fatality in untreated patients. In recent decades, Brazil has undergone intense changes in the VL transmission pattern, and a disease that was traditionally reported in the poor urban areas of the Northeast began to expand to large and medium-sized cities [1,2]. Conventional methods used thus far to control the disease have proven ineffective for detaining its expansion. The increasing spread of VL cases and the occurrence of new cases in previously unaffected areas point to the need for more effective control programs [3,4].

Epidemiological studies in endemic VL areas have demonstrated that a large contingent of individuals is infected with *Leishmania (Leishmania) chagasi* (sin. *L. infantum*), but with no clinical manifestations of the disease. An estimated 7.5% of individuals less than 15 years of age are infected by *L. chagasi* each year in endemic areas of Northeast Brazil, and 12% to 20% of infected persons evolve to the clinically manifest disease [5,6,7,8,9,10].

Asymptomatic infection is the most common clinical form in endemic areas and is normally associated with the presence of a case of classic VL in the family or neighborhood, suggesting that these individuals are subject to similar risk of infection,

since they have similar socioeconomic and environmental conditions and habits [10,11,12].

Various factors have been associated with predisposition to the development of classic VL, including: age less than five years, malnutrition, decreased lymphocyte proliferation, low production of interferon gamma, and HIV infection [13,14]. As for factors associated with asymptomatic infection, the findings vary according to the different types of study. In Moreno et al. [15] the factors were: household's familiarity with the vector, lack of garbage collection, garbage not removed or burned, raising pet birds, and proximity to eroded areas. Nascimento et al. [8] observed an association between asymptomatic VL and classic VL in the family, type of water supply, and insecticide spraying. According to Caldas et al. [7], *L. chagasi* infection was associated with the child's age (≥ 2 years), residence in the Vila Nova neighborhood, and report of family members with a history of VL. Information on predisposing factors for asymptomatic infection is relevant, considering the transition to endemic VL in metropolitan areas of Brazil.

The counties (municipalities) comprising the São Luís Island, Maranhão State, were among the first in Brazil where VL became an urban endemic, in the 1980s [16]. In these counties, foci of the disease are located in periurban areas that were occupied in association with deforestation. The housing is precarious, with deficient garbage collection and sanitation. In some areas, many of the residents suffer from low socioeconomic conditions. Contact with domestic animals is commonplace, resulting in the accumulation of organic matter that produces favorable conditions for LV transmission.

Various studies in the last decade have aimed to elucidate the epidemiology of asymptomatic *L. chagasi* infection in the counties forming São Luís Island [6,7,8,17].

The current study thus proposed to analyze factors associated with *L. chagasi* infection, identified by the Montenegro skin test (MST) and enzyme-linked immunosorbent assay (ELISA) in family members and neighbors of individuals with VL.

Material and methods

Study design

This was a cross-sectional analytical epidemiological study from October 2007 to March 2008 with family members and neighbors of VL patients. All patients were less than 16 years old.

Study area

São Luís Island is located on the northern seacoast of Maranhão State, Brazil, on the Gulf of Maranhão, and has an area of 905km^2. The island consists of the counties of Paço do Lumiar, Raposa, São José de Ribamar, and São Luís, where 1,111 VL cases were reported in the last 10 years, representing 14.75% of all cases reported in the State.

Study sample

The study used a convenience sample consisting of family members of all patients with a diagnosis of VL reported to the Maranhão State Health Secretariat through the Information System on Notifiable Diseases (SINAN) in the first semester of 2007, plus residents of a neighboring household within a 200-meter radius. The selection criterion for the household within a 200-meter radius was justified by the fact that sand flies have short and low flights, in a skipping pattern no greater than 200 meters [18]. The following order was used to select the neighboring household: first choice, the household to the right, second choice to the left, third in front, and fourth in back.

Logistics

The selected households were visited by previously trained members of the research team, and all the residents were invited to participate in the study. Participants answered an individual interview with questions on demographic and epidemiological characteristics, and underwent clinical examination. After the research team filled the participants' answers into the interview form, they drew biological samples (peripheral blood) from the subjects to test for anti-*Leishmania* serum antibodies and applied the MST for detection of *L. chagasi* infection.

MST was performed with antigen produced from *L. chagasi* promastigotes killed by ultrasonication and standardized in 40μgN/mL according to the technique developed by Melo et al. [19]. An inoculum of 0.1mL was injected on the anterior surface of the subject's right forearm. The diameter of the skin induration was measured 48 or 72 hours later, marking the border of the induration with a ballpoint pen. The reaction was considered positive when the antigen inoculation site showed a skin induration ≥ 5mm.

To detect anti-*Leishmania* IgG antibodies by ELISA, a 5 to 8mL peripheral blood sample was drawn using a 10mL syringe and 21-guage scalp or 25x7 needle. The blood was stored in tubes without anticoagulant and kept under refrigera-

tion until transportation to the Center of Tropical Pathology at the Federal University in Maranhão (NPAT-UFMA), where the samples were centrifuged and processed. The serum was kept in aliquots in properly identified polypropylene tubes, dated, and later sent to the Gonçalo Muniz Research Center at the Oswaldo Cruz Foundation (Salvador, Brazil) to conduct the tests according to the technique by Badaró et al. [14]. For transportation, the material was stored in styrofoam containers with dry ice and maintained hermetically sealed. The reaction was considered positive when the level of absorbency was ≥ 0.05, representing the mean plus two standard deviations in the absorbencies of 20 sera from healthy individuals unexposed to *Leishmania*.

Variables

The explanatory variables were: sex, age (< 15 and ≥ 15 years), self-reported or parent-reported skin color (white, brown, or black), time of residence in the household (< 6 months, 7-12 months, or > 12 months), and type of participant (family member or neighbor of index case). The second-level variables were: county of residence (São Luís, São José de Ribamar, Paço do Lumiar, or Raposa), area of residence (urban versus rural), family income (< 2 times the minimum wage versus > 2 times the minimum wage), type of housing (tile/brick/cement or straw/wattle/packed earth), water source (public running water, artesian well, or non-artesian well/water hole), sewage disposal (sewage system, septic tank, open-air pit, or ditch/woods/other), garbage disposal (public garbage collection, empty lot, or burned/buried), household spraying (yes/no), dog in the household (yes/no), and pigsty (yes/no), chicken coop (yes/no), and sand fly breeding sites in the peridomicile (yes/no).

Statistical analysis

Statistical analyses used Stata, version 11.0 (Stata Corp., College Station, USA). Evaluation of factors associated with *L. chagasi* infection identified a hierarchical structure with two levels of analysis: (1) family members and neighbors and (2) households. The response variable, presence of asymptomatic infection, can vary as a function of the explanatory variables, which were measured at the first level (demographic characteristics) and second level (socioeconomic, epidemiological, and environmental characteristics).

The multilevel modeling began with univariate analysis, using estimation of the unadjusted prevalence ratios or odds ratios and 95% confidence intervals (95%CI). The model with ELISA as the response variable used logistic regression, since occurrence of the event was 9.7%. When the response variable was MST, since occurrence of the event was high (71.3%), Poisson regression was used with robust adjustment of variance, since estimation of odds ratios for high-prevalence events can lead to false-positives [20]. Next, multivariate analysis was performed with multilevel Poisson regression, where the outcome variable was MST, and multilevel logistic regression, where the outcome variable was ELISA. Independent variables with p-value less than 0.20 were included in the final model. Selection of the variables used the stepwise method, with backward elimination of the variables. Variables with significance less than or equal to 0.05 were kept in the final model. The models were adjusted using the maximum likelihood ratio test.

Ethical issues

The study was approved by the Institutional Review Board of the Presidente Dutra University Hospital at UFMA, case number 33104-1295/2006. All participants signed a free and informed consent form, and for subjects less than 18 years of age the form was signed by a parent or legal guardian.

Results

The study population consisted of 361 individuals, of whom 207 (57.3%) lived in households with a case of symptomatic VL and 154 (42.7%) were neighbors. The participants were distributed in 99 households, of which 50 had reported cases of classic VL and 49 were neighboring households.

As for asymptomatic *L. chagasi* infection, the proportion of positive individuals was 71.3% for MST and 9.7% for ELISA. Two participants were not located in time to measure the MST skin induration, so they were excluded from the analyses that considered the reaction to the skin test. The analyses also excluded seven male individuals and two females, of whom four were family members and five were neighbors, who were absent from the households for more than a week. The proportion of losses was thus 2.4%.

Table 1 shows the unadjusted analysis of factors associated with asymptomatic *L. chagasi* infection, considering individual characteristics. MST reaction was associated with age ≥ 15 years (p = 0.200), time of residence in the neighborhood from 7 to 12 months (p = 0.192), and being a family member of a VL index case (p = 0.006). For ELISA, the statistically significant variables were: 7 to 12 months living in the neighborhood

Table 1

Unadjusted analysis of demographic variables and asymptomatic *Leishmania chagasi* infection detected by Montenegro skin test (MST) and enzyme-linked immunosorbent assay (ELISA). São Luís Island, Maranhão State, Brazil, 2012.

Variables	MST (n = 359) *				PR (95%CI)	p-value	ELISA (n = 361)				OR (95%CI)	p-value
	Positive		Negative				Positive		Negative			
	n	%	n	%			n	%	n	%		
Sex												
Female	145	70.0	62	30.0			21	10.0	187	90.0		
Male	111	73.0	41	27.0	0.95 (0.74-1.22)	0.852	14	9.1	16	90.9	1.11 (0.54-2.27)	0.764
Age (years)												
< 15	124	65.9	64	34.1			21	11.2	168	88.8		
≥ 15	132	77.2	39	22.8	1.17 (0.91-1.49)	**0.200**	14	8.2	158	91.8	1.41 (0.69-2.87)	0.342
Skin color												
White	47	79.6	12	20.4			7	11.9	52	88.1		
Brown/Black	209	69.6	91	30.4	1.14 (0.83-1.56)	0.407	28	9.3	274	90.7	1.31 (0.54-3.17)	0.539
Time residing in area (months)												
≤ 6	32	71.1	13	28.9			1	2.2	45	97.8		
7-12	24	50.0	24	50.0	0.70 (0.41-1.19)	**0.192**	6	12.5	42	87.5	6.42 (0.74-55.65)	**0.091**
> 12	200	75.2	66	24.8	1.05 (0.72-1.53)	0.770	28	10.5	239	89.5	5.27 (0.69-39.73)	**0.107**
Type of participant												
Neighbor	88	57.1	66	42.9			9	5.9	145	94.1		
Household member	168	81.9	37	18.1	1.43 (1.10-1.85)	**0.006**	26	12.6	181	87.4	2.31 (1.05-5.09)	**0.037**

95%CI: 95% confidence interval; OR: odds ratio; PR: prevalence ratio.

* In two participants it was not possible to perform the MST reading.

Factors associated with MST were identified by multilevel Poisson regression and those with ELISA by multilevel logistic regression.

(p = 0.091), more than 12 months living in the neighborhood (p = 0.107), and family member of the index case (p = 0.037).

Table 2 shows the unadjusted analysis of factors associated with asymptomatic *L. chagasi* infection based on contextual or second-level characteristics. Living in the county of Paço do Lumiar (p = 0.006) and living in the rural area (p = 0.102) were associated with the infection as detected by MST. Positive ELISA was associated with living in the county of Raposa (p = 0.025), in houses made of straw or wattle with packed earth floors (p = 0.066), and sand fly breeding sites in the peridomicile (p = 0.200).

The variable associated with *L. chagasi* infection detected by MST after adjusting the model was living in a household with a reported VL case (p = 0.026) (Table 3). Based on ELISA, after adjustment, the following variables were statistically significant: living in the county of Raposa (p = 0.018) and living in the same household as an index case (p = 0.016) (Table 4).

Discussion

In this study, family members of individuals with VL showed increased risk of asymptomatic *L. chagasi* infection. Ranjan et al. [21] showed a similar finding in a study in India, where family members of patients with classic VL showed 1.8 times the odds of being infected when compared to those without cases in the household. Family members and neighbors of patients with a history of classic VL comprise the groups of individuals most susceptible to acquiring the disease, since they have similar epidemiological characteristics to those of the reported case, indicating that they also had high odds of exposure to the parasite.

Although no significant differences were identified between the types of housing among infected versus uninfected individuals, houses built with straw, wattle, and packed earth were associated with positive serology in the univariate analysis. Importantly, households of family members and neighbors, even when located within a given area and sharing similar characteristics, showed variations in the physical structure of the houses and the peridomiciliary area. Some households maintained favorable

Table 2

Unadjusted analysis of socioeconomic, epidemiological, and environmental variables and asymptomatic *Leishmania chagasi* infection detected by Montenegro skin test (MST) and enzyme-linked immunosorbent assay (ELISA). São Luís Island, Maranhão State, Brazil, 2012.

Variables	MST (n = 359) *				PR (95%CI)	p-value	ELISA (n = 361)				OR (95%CI)	p-value
	Positive		Negative				Positive		Negative			
	n	%	n	%			n	%	n	%		
County												
São Luís	153	69.8	66	30.2			17	7.7	203	92.3		
São José de Ribamar	41	57.7	30	42.3	1.12 (0.71-1.75)	0.606	9	12.5	63	87.5	1.70 (0.72-4.01)	0.221
Paço do Lumiar	40	97.5	1	2.5	1.39 (0.98-1.97)	**0.060**	3	7.4	38	92.6	0.94 (0.26-3.37)	0.928
Raposa	22	78.5	6	21.5	0.82 (0.58-1.16)	0.279	9	29.1	22	70.9	3.25 (1.16-9.11)	**0.025**
Area												
Urban	19	50.0	19	50.0			3	5.4	35	94.6		
Rural	237	73.8	84	26.2	0.67 (0.42-1.08)	**0.102**	32	10.0	291	90.0	0.77 (0.22-2.67)	0.692
Family income (minimum wages)												
≥ 2	144	69.9	62	30.1			19	9.2	188	90.8		
< 2	112	73.2	41	26.8	0.88 (0.37-2.10)	0.714	16	10.4	138	89.6	0.87 (0.43-1.75)	0.701
Type of housing												
Tile/Brick/ Cement	145	70.0	62	30.0			15	7.2	193	92.8		
Straw/Wattle/ Packed earth	111	73.0	41	27.0	1.17 (0.49-2.80)	0.713	20	13.1	133	86.9	1.93 (0.95-3.91)	**0.066**
Water source												
Public running water	42	67.7	20	32.3			5	8.1	57	91.9		
Artesian well	113	66.4	57	33.6	1.03 (0.73-1.46)	0.854	16	8.4	176	91.6	1.03 (0.36-2.95)	0.947
Non-artesian well/Water hole	81	75.7	26	24.3	1.11 (0.46-5.75)	0.559	14	13.1	93	86.9	1.71 (0.58-5.01)	0.324
Sewage disposal												
Sewage system	13	61.9	8	38.1			2	9.5	19	90.5		
Septic tank	78	70.3	33	29.7	1.13 (0.63-2.04)	0.673	10	8.9	103	91.1	0.92 (0.18-4.54)	0.921
Open-air pit	124	72.5	47	27.5	1.17 (0.66-2.07)	0.588	19	11.2	152	88.8	1.18 (0.25-5.50)	0.826
Ditch/Woods/ Other	41	73.2	15	26.8	1.18 (0.63-2.20)	0.599	4	7.2	52	92.8	0.73 (0.12-4.31)	0.729
Garbage disposal												
Public garbage collection	33	66.0	17	34.0			5	10.0	45	90.0		
Empty lot	49	69.0	22	31.0	1.04 (0.67-1.62)	0.843	4	5.6	68	94.4	0.52 (0.13-2.07)	0.361
Burned/Buried	174	73.1	64	26.9	1.10 (0.76-1.60)	0.590	26	10.9	213	89.1	1.09 (0.39-3.01)	0.857
Household spraying												
Yes	119	72.1	46	27.9			15	9.1	151	90.9		
No	137	70.6	57	29.4	1.02 (0.79-1.30)	0.867	20	10.3	175	89.7	0.86 (0.42-1.75)	0.696
Dog in household												
No	101	68.2	47	31.8			22	10.4	191	89.6		
Yes	155	73.4	56	26.6	1.07 (0.83-1.38)	0.565	13	8.8	135	91.2	0.87 (0.43-1.75)	0.701
Pigsty in peridomicile												
No	195	76.2	83	23.8			29	10.4	250	89.6		
Yes	61	75.3	20	24.7	1.07 (0.80-1.40)	0.628	6	7.3	76	92.7	0.68 (0.27-1.70)	0.410

(continues)

Table 2 (continued)

Variables	MST (n = 359) *				PR (95%CI)	p-value	ELISA (n = 361)				OR (95%CI)	p-value
	Positive		Negative				Positive		Negative			
	n	%	n	%			n	%	n	%		
Chicken coop in peridomicile												
No	113	69.7	49	30.3			14	8.7	148	91.3		
Yes	143	72.6	54	27.4	1.04 (0.81-1.33)	0.752	21	10.6	178	89.4	1.03 (0.36-2.95)	0.448
Sand fly breeding sites in peridomicile												
No	52	73.2	19	26.8			10	9.8	92	90.2		
Yes	204	70.8	84	29.2	0.96 (0.71-1.31)	0.830	25	9.7	234	90.3	2.00 (0.68-5.86)	**0.200**

95%CI: 95% confidence interval; OR: odds ratio; PR: prevalence ratio.

* In two participants it was not possible to perform the MST reading.

Factors associated with MST were identified by multilevel Poisson regression and those with ELISA by multilevel logistic regression.

Table 3

Adjusted analysis of type of participant and asymptomatic *Leishmania chagasi* infection detected by Montenegro skin test (MST). São Luís Island, Maranhão State, Brazil, 2012.

Characteristics	PR	95%CI	p-value
Type of participant			0.026
Neighbor	1.00		
Household member	1.36	1.03-1.78	

95%CI: 95% confidence interval; PR: prevalence ratio.

Table 4

Adjusted analysis of county and type of participant and prevalence of asymptomatic *Leishmania chagasi* infection detected by enzyme-linked immunosorbent assay (ELISA). São Luís Island, Maranhão State, Brazil, 2012.

Characteristics	OR	95%CI	p-value
County			
São Luís	1.00		
São José de Ribamar	1.90	0.79-4.54	0.147
Paço do Lumiar	0.73	0.20-2.65	0.636
Raposa	3.56	1.24-10.19	**0.018**
Type of participant			0.016
Neighbor	1.00		
Household member	2.70	1.19-6.08	

95%CI: 95% confidence interval; OR: odds ratio.

conditions for the reproduction and survival of *Lutzomyia (Lutzomyia) longipalpis* in the intra and peridomicile and/or made substantial changes in the area around their houses, while others did not. These different situations within a single area may have been relevant for the increased occurrence of asymptomatic infection in family members of cases.

The findings showed a slight predominance of females, potentially explained by greater difficulty in locating male adults at home, since many were fishermen and were away from home for more than a week. However, losses were low: 1.9% and 0.5% of men and women, respectively. In this study, gender was not a significant variable in the distribution of human infection by *L. chagasi,* confirming findings by Nascimento et al. [8] and Crescente et al. [22].

The statistical association between living in the county of Raposa and positive ELISA can be explained by the fact that this county underwent recent occupation, with haphazard deforestation and individuals living in precarious sanitary, environmental, and housing conditions. Areas where the endemic is recent have shown a higher rate of individuals with a humoral response detected by serology [8,13,23]. This emphasizes the need for other studies in the area to assess the influence of environmental factors on asymptomatic infection, since no association was observed between environmental variables and positive reaction to the tests.

Analyzing the proportion of positive individuals, it is important to highlight that two methods were used to detect asymptomatic infection, with the objective of evaluating participants' cellular and humoral immune responses. The positive rate for infection was considerably higher for MST than for ELISA. According to Barbosa et al. [24], in endemic areas, the high infection rate in individuals with a positive cellular response indicates that the majority of cases present a brief period of humoral response (ELISA+), followed by rapid MST conversion, which remains positive for a more prolonged period. The higher MST rate as compared to ELISA may be explained by re-infections, since persons in endemic areas are permanently exposed to bites by infected sand flies [25,26].

The infection rate detected by MST was higher than in other studies in counties on São Luís Island, while ELISA showed a lower infection rate. In a study by Caldas et al. [6] in the county of Raposa, the infection rate in children up to five years of age was 18.6% by MST and 13% by ELISA. In Nascimento et al. [8], individuals under 15 years of age in the county of São José de Ribamar showed a prevalence of 61.7% by MST and 19.7% by ELISA. Oliveira et al. [12], in Três Lagoas, Minas Gerais State, with participants of all ages, showed a 36.4% positive rate by ELISA. The higher infection rate detected by MST in the current study may be explained by the fact that it included family members and neighbors of the index case rather than the general population.

The difference between the positive rate in the current study and the studies cited above may also be explained by some methodological and/or epidemiological differences. The difference in the antigen preparations used in the immunological tests, age differences in the study participants, and the fact that the current study used a convenience sample may have influenced the findings. The comparisons presented here should thus be viewed with caution.

Importantly, the high infection rate detected by MST reveals a large contingent of individuals with cellular immune resistance to *L. chagasi* infection, i.e., the findings indicate that many infected individuals in an endemic area are resistant to the disease [24].

A positive ELISA means antibody production and demonstrates that asymptomatic infection results from humans' permanent exposure to infective bites, whereby the number of exposed or asymptomatic infected individuals greatly exceeds the number of detected cases in some areas [7]. This suggests that repeat infections are common in the endemic areas studied here, leading to development of cellular immunity and resistance to the disease [22].

As with the delayed hypersensitivity test, conclusions based on ELISA should be interpreted with caution, since studies that have simultaneously used molecular and serological tests have shown high false-positive rates with serology [10,27]. According to Moreno et al. [27], serological tests have low absorbency levels in diagnosing asymptomatic individuals as compared to patients with classic VL. These results indicate low levels of circulating anti-*Leishmania* antibodies in asymptomatic individuals. One limitation of this study was its lower external validity, since it used a convenience sample. However, since the main objective was to compare risk factors among family members and neighbors, the essential point was to ensure internal validity.

The high *L. chagasi* infection rate on São Luís Island shows that family members and neighbors of individuals with VL are among the groups most susceptible to acquiring the classic form of the disease. Therefore, they deserve special attention from VL control programs, which should monitor them through the Family Health Strategy in order to ensure early diagnosis and treatment and thus reduce VL case-fatality.

Resumo

Este estudo transversal analisou os fatores associados à infecção assintomática por Leishmania chagasi *em familiares e vizinhos de pacientes com leishmaniose visceral (LV) e foi realizado com 361 indivíduos nos municípios que compõem a Ilha de São Luís, Maranhão, Brasil. Foram realizadas a Intradermorreação de Montenegro (IDRM) e a sorologia por* Enzyme Linked Immunosorbent Assay *(ELISA) para detectar a infecção. Razões de chances (RC) e razões de prevalências (RP) e seus intervalos de 95% de confiança (IC95%) foram estimadas por meio de regressão logística multinível e regressão de Poisson, respectivamente. A proporção de positivos foi de 71,3% para a IDRM e de 9,7% para o ELISA. A variável associada à infecção segundo a IDRM foi residir no domicílio do caso índice (RP = 1,36; IC95%: 1,03-1,78). Pelo ELISA, as variáveis com significância estatística foram: residir no Município de Raposa (RC = 3,56; IC95%: 1,24-10,19) e no domicílio do caso índice (RC = 2,70; IC95%: 1,19-6,08). Familiares de indivíduos com LV têm maior risco de infecção. Medidas de controle devem ser efetivadas e direcionadas prioritariamente a essas famílias.*

Leishmaniose Visceral; Infecção; Infecções Assintomáticas

Contributors

G. S. Moura participated in the data collection and all stages in the production of the article. A. M. Santos contributed to the data's statistical analysis and participated in the elaboration and discussion of the results and writing of the text. D. M. C. Aquino participated in the data collection and writing of the article. A. A. M. Silva participated in the writing and revision of the article. A. J. M. Caldas coordinated the research and participated in all stages in the production of the article.

Acknowledgments

The authors wish to thank the members of the *Epidemiology of Transmissible Diseases* research group for their support during the data collection, the Gonçalo Muniz Research Center/Fiocruz for conducting the serological tests, Prof. Dr. Marco Antônio Barbieri for revision of the article, and FAPEMA.

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Submitted on 06/Dec/2011
Final version resubmitted on 19/Jul/2012
Approved on 30/Aug/2012