Asymptomatic infection in family contacts of patients with human visceral leishmaniasis in Três Lagoas, Mato Grosso do Sul State, Brazil

Infecção assintomática em contactantes de pacientes com leishmaniose visceral humana em Três Lagoas, Mato Grosso do Sul, Brasil

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Abstract

The Brazilian city of Três Lagoas, Mato Grosso do Sul State, has experienced an urban outbreak of visceral leishmaniasis since 2000. In 2002, due to the increase in the number of cases, 46 families with cases of visceral leishmaniasis were studied to verify the prevalence of asymptomatic infection in household contacts. Indirect immunofluorescence and ELISA showed a 36.4% positive infection rate. There were no cases of symptomatic disease among these contacts. There was no statistically significant difference in gender or age. Median age was 21 years, and the 10-19-year age bracket was the most heavily affected (23%). As for family characteristics, no differences were observed in schooling or family income; most families (58.7%) owned their homes, which were built of masonry (97.8%) and had adequate infrastructure. All the families reported what were probably phlebotomine sand flies in the peridomicile. In conclusion, asymptomatic visceral leishmaniasis infection is frequent and occurs in both males and females, regardless of age.

Visceral Leishmaniasis; Communicable Diseases; Disease Outbreaks; Infection

Introduction

Visceral leishmaniasis shows a worldwide distribution, affecting 65 countries [1]. The disease is transmitted through the bite of insects from genera *Phlebotomus* and *Lutzomyia* in the Old and New Worlds, respectively [2]. There are more than 30 vector species, with *Lu. longipalpis* playing an outstanding role in the Americas [3] and *Phlebotomus* spp. in the Old World [4]. In the late 1990s, *Lu. cruzi* was incriminated as a vector in the municipality (county) of Corumbá, Mato Grosso do Sul State, Brazil [5].

Lu. longipalpis is spreading in Brazil, with widespread distribution in four regions: the North, Northeast, Southeast, and Midwest. It was initially a sylvatic species and is currently adapted to the urban environment in the peridomicile and intradomicile and in outbuildings with domestic animals [6,7].

In the Americas, visceral leishmaniasis is caused by *Leishmania (Leishmania) chagasi,* an obligatory intracellular protozoan. The disease is endemic in Brazil in more than 19 States, constituting an important public health problem, especially in Ceará, Bahia, Piauí, Maranhão, Rio Grande do Norte, Minas Gerais, and recently Mato Grosso do Sul and Tocantins [8,9,10].

Various control measures have been used, like vector control, culling of infected dogs with or without clinical manifestations of the disease, and early diagnosis and treatment of human pa-

tients to minimize severity and reduce case-fatality. However, these measures have not proven effective, and new studies are under way with the aim of reducing the number of individuals at risk of acquiring the disease [9,11].

Visceral leishmaniasis can be classified in three clinical forms, based on the hosts' clinical and laboratory characteristics: asymptomatic, or infection without clinical manifestations of the disease; oligosymptomatic or sub-clinical; and classical. The classical form of the disease is manifested by fever, weight loss, hepatosplenomegaly, and pancytopenia, and when not treated adequately can evolve to death [12,13].

It is not known exactly why some cases of visceral leishmaniasis course as asymptomatic infection while others evolve to clinical illness. Host factors may determine this susceptibility, including nutritional status (e.g., vitamin A deficiency), host immune response, age, migrations, and co-infections such as with HIV [13,14,15,16,17].

Two studies in Brazil showed ratios of 18:1 and 11:1, respectively, between cases of infection without clinical manifestations and cases of classical disease [18,19].

Asymptomatic infection is the most frequent clinical form and is normally associated with the presence of a case of visceral leishmaniasis in the family or vicinity, suggesting exposure to the same risk factors, including risk of infection between family members [15,20].

Based on the above, the current study aimed to estimate the percentage rate of asymptomatic infection in family contacts of visceral leishmaniasis patients and the epidemiological conditioning factors for acquiring the infection in Três Lagoas, a municipality (county) in Mato Grosso do Sul State, Brazil, where the disease has spread recently, with autochthonous cases confirmed in October 2000, and where it has expanded rapidly and is difficult to control [21]. Importantly, no autochthonous cases of tegumentary leishmaniasis have been reported in the same municipality of Três Lagoas.

Subjects and methods

A cross-sectional epidemiological study was conducted from January 1 to July 30, 2002, in the urban area of the municipality of Três Lagoas, where visceral leishmaniasis was already epidemic.

Description of the study area

The city of Três Lagoas (20.75º S; 51.67º W), with an area of 10,207km^2, is located on a plain in the eastern region of the State of Mato Grosso do Sul, bordering on the State of São Paulo and 324km from the State capital, Campo Grande. Total population is 79,059, with a population density of 7.73 inhabitants/km^2. The main source of income is cattle-raising, and recent incipient industrial growth has led to local population growth [22].

The climate is hot, humid, and tropical, with the rainy season in the summer and dry season in the winter. Total annual precipitation varies from 900mm to 1,400mm. The yearly quarter with the heaviest rainfall is November, December, and January. The predominant plant cover is uniform, with clear fields, savannah, and evergreen forest [22].

Study population

The study included 46 of the 60 families with patients with clinical manifestations of visceral leishmaniasis from January to July 2002, confirmed by the Três Lagoas Municipal Health Department.

A questionnaire was applied to family members of visceral leishmaniasis patients, including personal identification data, symptoms, and epidemiological characteristics such as: type of dwelling, occupational conditions, general household characteristics, proximity to forests, presence of animals and probable phlebotomine sand flies in the domicile and peridomicile, sewage and waste disposal, family income, schooling, and time/frequency spent by children outdoors. As for questions on the possible presence of sand flies in the domicile or peridomicile, subjects were asked to describe the insect, and the answer was only considered positive when the description matched this vector, with the following principal parameters (small insect, from 1mm to 3mm, light color, covered with bristles, low-flying).

Confirmed cases of visceral leishmaniasis were based on clinical manifestations of the parasitosis, associated with observation of amastigote forms in Giemsa-stained bone marrow smears or serology with indirect immunofluorescence (IIF) with a titer of ≥ 1:80.

Families were excluded if they refused to sign the informed consent, changed addresses, or were not located.

The study was approved by the Institutional Review Board of the Federal University in Mato Grosso do Sul.

Sero-epidemiological survey

Blood samples were drawn from 220 family members and analyzed with IIF and enzyme-linked immunosorbent assay (ELISA).

IIF was performed with the Biomanguinhos kit (Biomanguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil) according to the manufacturer's instructions, with control and test sera at dilutions of 1:40 and 1:80, adding the anti-IgG/fluorescein conjugate at 1:100. ELISA was performed at the Cell Ultrastructure and Biology Laboratory of the Oswaldo Cruz Institute, Oswaldo Cruz Foundation, in Rio de Janeiro, Brazil.

Cases of asymptomatic infection were defined as those with IIF serology titer ≥ 1:80 and/or ELISA with a titer greater than 0.305 (cutoff: 0.278).

Statistical analysis

Data were stored in Excel 2007 (Microsoft Corp., USA) and tabulated in BioEstat 4.0 (Sociedade Civil Mamirauá, Manaus, Brazil). Categorical variables were analyzed with the χ^2 test with Yates correction, with significance set at $p < 0.05$. The other variables were analyzed with the Student t, ANOVA, Mann-Whitney, and Kruskal-Wallis tests, according to normality and number of variables.

Results

All the interviewees were asymptomatic contacts of visceral leishmaniasis cases. The serological methods showed a 36.4% positive infection rate (Table 1). These asymptomatic individuals were followed up for six months and remained symptom-free.

The number of contacts per household varied from 2 to 12 persons, with a median of 5 individuals per household. Median age was 21 years.

No statistically significant difference was observed in distribution by gender or age bracket (Table 1).

As for the social and epidemiological characteristics of the families, there was a higher (but statistically non-significant) infection rate in families whose head-of-household had complete primary or incomplete secondary schooling and family income of 3 to 5 times the minimum wage (Table 2).

The majority of families (58.7%) owned their homes, most of which were built of masonry (97.8%), with ceramic roof tiles, running water, and public garbage collection (97.8%), while 95.7% had septic tanks and 100% had electricity. Some homes had vegetation in the domicile (34.8%) and 26% were located close to the forest.

A total of 82.2% of the families reported that the children played outdoors in the late afternoon/dusk. Some 56% of the households had dogs, while other domestic animals included cats (19.5%), horses (13%), and chickens (13%) (Table 2). Among the infected contacts there was a statistically significant difference between those that had pet dogs (60.2%) and those without dogs in the household (39.8%) ($p = 0.003$), as well as in the households that had a stove, TV set, and refrigerator and those with 4 to 6 rooms (Table 2).

The accumulation of garbage in the peridomicile was not a risk factor for infection, which was more frequent in the households without accumulated garbage (Table 2).

The presence of insects suggestive of sand flies was reported in all the households.

The mean number of contacts per household was similar in relation to the various epidemiological and social variables, except for a statistically significant difference as to home ownership,

Table 1

Distribution by age bracket, gender, and seropositive rate for family contacts of visceral leishmaniasis contacts in the municipality of Três Lagoas, Mato Grosso do Sul State, Brazil, 2002.

Age bracket (years)	Examined		Seropositive		Females (n = 115)		Males (n = 105)	
	N	%	n	%	Positive	Negative	Positive	Negative
≤ 4	31	14.1	12	38.7	6	14	6	5
5 to 9	22	10.0	8	36.4	6	7	2	7
10 to 19	51	23.2	26	50.9	11	10	15	15
20 to 29	38	17.2	11	28.9	6	13	5	14
30 to 59	62	28.2	18	29.0	12	22	6	22
≥ 60	16	7.3	5	31.2	3	5	2	6
Total	220	100.0	80	36.4	44	71	36	69

Table 2

Epidemiological and social characteristics of 46 family contacts of visceral leishmaniasis cases in the municipality of Três Lagoas, Mato Grosso do Sul State, Brazil, 2002.

Characteristics	Households (number of contacts)	Positive for visceral leishmaniasis infection (%)	p	Mean contacts per household	Standard deviation	p
Dog(s) in household						
Present	26 (128)	60.2	0.003	4.9	2.13	0.431 *
Absent	20 (92)	39.8		4.6	2.25	
Accumulated garbage						
Absent	30 (143)	67.5	0.000	4.8	1.57	0.320 **
Peridomicile	16 (77)	32.5		4.4	1.31	
Monthly family income (number of times minimum wage)						
1 to 2	23 (111)	30.2	0.431	5.0	1.80	0.445 ***
3 to 5	11 (45)	37.7		4.2	1.08	
> 5	10 (50)	28.0		5.0	2.67	
Unknown	4 (14)	04.1		-	-	
Number of rooms						
2 to 3	4 (16)	6.3	0.000	4.0	1.15	0.210 ***
4 to 6	28 (123)	65.0		4.4	1.62	
> 7	14 (81)	28.7		5.8	2.87	
Household assets						
Stove, refrigerator, or TV	7(36)	17.5	0.000	5.14	1.77	0.187 ***
Stove, TV, refrigerator	36 (162)	76.3		4.5	1.97	
Stove, TV, refrigerator, stereo	3 (22)	6.2		7.3	4.04	
Schooling (head of family)						
Incomplete primary	21 (92)	35.0	0.080	4.4	1.32	0.080 ***
Complete primary plus incomplete secondary	9 (55)	37.5		6.1	2.57	
Secondary and university	5 (27)	15.0		5.4	1.67	
Not reported	11 (46)	-		4.2	-	
Home ownership						
Own	27 (148)	61.3	0.000	5.5	2.12	X between 1 and 3 = 0.01 #
On loan	8 (34)	20.0		5.5	2.14	
Rented	11 (38)	18.7		3.7	0.90	

* Mann-Whitney test;

** Student t test;

*** Kruskal-Wallis test;

ANOVA.

with a prevalence of families that owned their own homes as opposed to renting (Table 2).

Discussion

The urbanization of visceral leishmaniasis has motivated discussion and implementation of control measures throughout Brazil, but the results have been limited and largely ineffective [23]. The disease is currently spreading unchecked, mainly in urban areas in the Southeast and Midwest [24].

Asymptomatic infection rates in family contacts were similar in relation to gender, while the clinical disease itself in Três Lagoas during the same period was twice as frequent in males (43 female patients and 106 males). The same was true for age bracket; namely, there was no statistically significant difference in the positive con-

tacts, while among patients the incidence rate in the 0-4-year bracket was 5.9/thousand as compared to 1.8/thousand from 10 to 19 years and over 60 years of age [21]. Similar results were found in the States of Bahia and Minas Gerais [25,26].

The explanation for this difference between visceral leishmaniasis cases with the classical versus asymptomatic forms may lie in the type of host cellular immune response [27]. Another hypothesis is that genetic factors are involved in development of the clinical disease. Recent studies in a mouse model show that these factors may influence the development of the clinical forms of visceral leishmaniasis, although they have not been fully explained [28,29].

The infection rates found in this study are important and worrisome, suggesting that individuals exposed to infection or already infected may be as important as those that develop the disease, due to the risk of their acting as reservoirs for transmitting the parasite [23,30,31,32].

As for the social and epidemiological characteristics of the households studied in Três Lagoas, although masonry homes with adequate infrastructure were widespread in the municipality, the family income and schooling levels were low. Various studies have shown similarities in family income and schooling, but differences in housing infrastructure [18,26,33].

As reported by the subjects, insects suggestive of phlebotomines were present in the peridomicile in all the households, demonstrating the vector's importance in the visceral leishmaniasis transmission chain, as observed in Teresina (Piauí State, Brazil) [34], Sabará (Minas Gerais State, Brazil) [25], and Sudan [12]. These findings further corroborate the observations by Costa & Vieira [11] and Tesh [35], that vector control has been ineffective in reducing the parasite's transmission or avoiding new epidemic areas.

One of the probable causes of the difficulty in controlling the proven visceral leishmaniasis vector *Lu. longipalpis* is that it is frequently found in the anthropic environment and in animal shelters, which serve as a source of food and shelter for the insect [36,37,38]. The presence of children outdoors at dusk, dogs and other domestic animals, and plant cover in the domicile or vicinity, as observed in the study site, can play an important role in the visceral leishmaniasis transmission chain, as already shown in other areas where the disease occurs [33,34,39].

In conclusion, the current study has shown that asymptomatic visceral leishmaniasis infection is frequent, occurring in both males and females and in all age brackets, which does not suggest occupational exposure. Further research is required on potential factors to explain the higher incidence of the clinical disease in men and young children, and on the role that individuals with asymptomatic infection play in the visceral leishmaniasis transmission chain.

Resumo

O Município de Três Lagoas, Mato Grosso do Sul, Brasil, foi alvo de uma epidemia de leishmaniose visceral a partir de 2000. Em 2002, devido ao incremento de casos, estudou-se 46 famílias que apresentavam um caso de doença para verificar-se o percentual de positividade de infecção assintomática por leishmaniose visceral em contactantes. Encontrou-se 36,4% de positividade pelos testes sorológicos Reação de Imunofluorescência Indireta e/ou imunoenzimático ELISA, sem diferença estatisticamente significativa quanto ao sexo e faixa etária. A mediana de idade foi de 21 anos, sendo a faixa etária mais acometida de 10 a 19 anos (23%). Quanto às características familiares não observaram-se diferenças quanto ao nível de instrução e renda familiar; a moradia, em sua maioria, era própria (58,7%), em alvenaria (97,8%), com infra-estrutura adequada. Todas as famílias relataram a presença de provável flebotomíneo no peridomicílio. Conclui-se que a infecção assintomática por leishmaniose visceral é freqüente, ocorrendo em homens e mulheres, independente de faixa etária.

Leishmaniose Visceral; Doenças Transmissíveis; Surtos de Doenças; Infecção

Contributors

A. L. L. Oliveira coordinated the research and – together with R. V. Cunha and M. N. Bóia – participated in all its stages: design, elaboration of the research instrument, data collection, planning and performance of the laboratory tests, and writing of the paper. A. M. M. Paniago, M. A. Sanches, F. H. Paula, and L. G. Pereira participated in the elaboration of the research instruments, fieldwork with clinical and epidemiological evaluation, and data preparation and processing. M. E. C. Dorval, E. T. Oshiro, and C. R. B. Leal collaborated in the planning, laboratory processing of all the samples, and writing of the paper. All the authors participated in the article's final revision.

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