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

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. The disease is transmitted through the bite of insects from genera Phlebotomus and Lutzomyia in the Old and New Worlds, respectively. There are more than 30 vector species, with Lu. longipalpis playing an outstanding role in the Americas and Phlebotomus spp. in the Old World. In the late 1990s, Lu. cruzi was incriminated as a vector in the municipality (county) of Corumbá, Mato Grosso do Sul State, Brazil.

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. 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. 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-
patients to minimize severity and reduce case-fa
tality. 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, hepatospleno-
megaly, 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 defi-
ciency), 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 clas-
sical 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 leishman-
iasis patients and the epidemiological condi-
tioning 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 rap-
idly and is difficult to control 21. Importantly, no
autochthonous cases of tegumentary leishmani-
asis have been reported in the same municipal-
ity 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 epidi-
emic.

**Description of the study area**

The city of Três Lagoas (20.75° S; 51.67° W), with
an area of 10,207km², 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 pop-
ulation is 79,059, with a population density of
7.73 inhabitants/km². 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, Decem-
ber, and January. The predominant plant cover
is uniform, with clear fields, savannah, and ever-
green 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, con-
firmed by the Três Lagoas Municipal Health De-
partment.

A questionnaire was applied to family mem-
ers of visceral leishmaniasis patients, including
personal identification data, symptoms, and epi-
demiological characteristics such as: type of dwell-
ing, 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/fre-
cuency spent by children outdoors. As for ques-
tions on the possible presence of sand flies in the
domicile or peridomicile, subjects were asked to
describe the insect, and the answer was only con-
sidered positive when the description matched
this vector, with the following principal param-
eters (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 para-
sitosis, 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 mem-
ers 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 χ² 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

<table>
<thead>
<tr>
<th>Age bracket (years)</th>
<th>Examined</th>
<th>Seropositive</th>
<th>Females (n = 115)</th>
<th>Males (n = 105)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>≤ 4</td>
<td>31</td>
<td>14.1</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>5 to 9</td>
<td>22</td>
<td>10.0</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>10 to 19</td>
<td>51</td>
<td>23.2</td>
<td>26</td>
<td>11</td>
</tr>
<tr>
<td>20 to 29</td>
<td>38</td>
<td>17.2</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>30 to 59</td>
<td>62</td>
<td>28.2</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>≥ 60</td>
<td>16</td>
<td>7.3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>220</td>
<td>100.0</td>
<td>80</td>
<td>44</td>
</tr>
</tbody>
</table>

**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 χ² 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,
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. The disease is currently spreading unchecked, mainly in urban areas in the Southeast and Midwest.

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-

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

* Mann-Whitney test;
** Student t test;
*** Kruskal-Wallis test;
# ANOVA.
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. Similar results were found in the States of Bahia and Minas Gerais. 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. 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.

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.

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.

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), Sabará (Minas Gerais State, Brazil), and Sudan. These findings further corroborate the observations by Costa & Vieira and Tesh, 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. 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.

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 frequente, ocorrendo em homens e mulheres, independentemente de faixa etária.

Leishmaniose Visceral; Doenças Transmissíveis; Surtos de Doenças; Infeção

Contributors


Acknowledgments

Os autores gostariam de agradecer ao Departamento de Ultrastruutura e Biologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, pela realização dos testes ELISA, ao Departamento Municipal de Saúde e Controle de Vektors de Três Lagoas e ao Departamento de Vigilância de Saúde do Estado de Mato Grosso do Sul pelo apoio logístico e aos dados, e ao Ministério da Ciência e Tecnologia do Brasil (DECIT – MS) pelo apoio financeiro.

References


