**Abstract**

The American visceral leishmaniasis (AVL) is caused by parasites belonging to the genus *Leishmania* (Trypanosomatidae) and is transmitted to humans through the bite of certain species of infected phlebotomine sand flies. In this study, we investigated the natural infection ratio of *Lutzomyia longipalpis*, the main vector species of AVL in Brazil, in Várzea Grande, Mato Grosso State. Between July 2004 and June 2006, phlebotomine sand flies were captured in peridomestic areas using CDC light-traps. Four hundred and twenty (420) specimens of *Lu. longipalpis* were captured. 42 pools, containing 10 specimens of *Lu. longipalpis* each, were used for genomic DNA extraction and PCR (polymerase chain reaction) amplification. *Leishmania* spp. DNA was detected in three out of the 42 pools tested, resulting in a minimal infection ratio of 0.71%. Restriction fragment length polymorphism (RFLP) analysis indicated that *Leishmania* (*L.*) chagasi was the infective agent in the positive pools.

**Introduction**

American visceral leishmaniasis (AVL) is a public health problem in Brazil. In Várzea Grande, Mato Grosso State, a total of 138 human cases of AVL were reported between 1998 and 2005. In 2003, that municipality was considered an area of intense transmission by the Department for Epidemiological Surveillance in the Brazilian Ministry of Health. In this context, we carried out the present study in order to determine the natural ratio of *Leishmania*-infected *Lutzomyia longipalpis* and the infecting *Leishmania* species in that area.

**Materials and methods**

Várzea Grande (15°32’30"S, 56°17’18"W) is located in the state of Mato Grosso, near to its capital, Cuiabá. Phlebotomine captures were carried out for two years during three consecutive days per month, from July 2004 to June 2006. CDC light traps were mounted in peridomestic areas in five houses across three districts of intense AVL transmission (São Matheus and Eldorado – two residences each – and Parque Sabiá – one residence) totaling five traps per day. The selection of areas and residences was based on previous entomological data, as well as on the preva-
Pools containing ten Lu. longipalpis females each were prepared for DNA isolation. In order to confirm the extraction of phlebotomine sand fly DNA, these pool samples were amplified by polymerase chain reaction (PCR) in the presence of primers for a constitutive Lutzomyia gene (ca-cophony).

The pool samples were also amplified with specific primers for Leishmania spp. In every PCR reaction set, both negative (no DNA) and positive controls (kDNA purified from Leishmania (V.) braziliensis) were included. Product analysis was performed by PAGE (polyacrylamide gel electrophoresis).

Since each pool sample comprised ten Lu. longipalpis females, the minimal infection rate was calculated as the number of positive pools times 100 divided by the total number of specimens tested.

Positive PCR samples were submitted to PCR-RFLP (restriction fragment length polymorphism), aimed to distinguish among the infecting parasite species according to a published protocol.

Results

The efficacy of DNA extraction was confirmed by the presence of a 220bp fragment in every pool of Lu. longipalpis DNA. The amplification product for Leishmania spp. (120bp) was detected in 3 out of 42 pools tested. Minimal infection rate was calculated as 0.71% that corresponds to, at least, three infected females among a total of 420 individuals. RFLP analysis of those Lu. longipalpis pools indicated L. (L.) chagasi as the infecting agent with typical gel profiles: a single 120bp and 120, 80, 60 and 40bp fragments after ApaLI and HaeIII digestion, respectively.

Discussion

One of the main disadvantages of dissection, besides low sensitivity, is the assumption that all motile flagellates in the sand fly guts are Leishmania parasites. Among molecular methodologies used in the detection of Leishmania, the PCR has been widely reported in the literature for many purposes, including the assessment of infection ratios in both experimentally and naturally infected phlebotomine sand flies. PCR-positive results, however, may be due to the presence of fragments of Leishmania DNA from non-established infections or blood meals from unsusceptible animals, besides live promastigotes. Although neither dissection nor PCR positive results alone can incriminate a given species as an AVL vector, PCR is a particularly effective screening tool in epidemiological surveys due to its high sensitivity and speed. Any positive result may be regarded as additional evidence for the involvement of a certain species in transmission.

Nevertheless, literature data have shown that the infection ratios of Leishmania in phlebotomine vectors are usually low, even in endemic areas. Average values remain below 3%, hardly reaching 10% in a few cases, when assessed either by dissection or PCR-based methods for Leishmania DNA detection. Therefore, the minimal infection rate of 0.71% determined for Várzea Grande is in accordance with other literature data for Latin America.

Due to the prevalence of cutaneous leishmaniasis (CL) in Mato Grosso, L. (V.) braziliensis and L. (V.) amazonensis, two etiological agents for CL in Latin America, were also included as references in RFLP. However, the infecting Leishmania in the Lu. longipalpis positive pools from Várzea Grande was unequivocally identified as L. (L.) chagasi. Although Lu. longipalpis is the main vector of VL in Brazil, Lu. cruzi was also suggested as such. The last species was shown to be widely distributed in Mato Grosso but it was not captured in Várzea Grande.
Figure 1

PAGE of PCR-amplified DNA of *Lu. longipalpis*.

Note: the arrow points to the amplification product (220bp) of the constitutive Lutzomyia gene. M: PhiX174RF DNA/HaeIII size marker; NC: negative control (no DNA); PC: positive control (DNA from laboratory-reared *Lutzomyia longipalpis*).

Figure 2

PAGE of PCR-amplified DNA of Leishmania-infected *Lu. longipalpis* using specific primers for Leishmania spp.

Note: the test groups are identified on top. M: PhiX174RF DNA/HaeIII size marker; NC: negative control; PC: positive control.
Table 1

Natural infection ratios of different phlebotomine sand fly species by *Leishmania* spp. in Latin American localities.

<table>
<thead>
<tr>
<th>Country, state and locality</th>
<th>Specimens (n)</th>
<th>Technique</th>
<th>Infection rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tucumán and Salta</td>
<td>440</td>
<td>PCR</td>
<td>9.1</td>
<td>Córdoba-Lanús et al. 14</td>
</tr>
<tr>
<td>Brazil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahia, Corte de Pedra</td>
<td>4,027</td>
<td>PCR</td>
<td>0.4</td>
<td>Miranda et al. 15</td>
</tr>
<tr>
<td>Maranhão, Buriticupu</td>
<td>1,100</td>
<td>PCR</td>
<td>0.4</td>
<td>Oliveira-Pereira et al. 16</td>
</tr>
<tr>
<td>Mato Grosso do Sul, Antônio João</td>
<td>81</td>
<td>Dissection</td>
<td>1.2</td>
<td>Paiva et al. 10</td>
</tr>
<tr>
<td>Mato Grosso do Sul, Antônio João</td>
<td>81</td>
<td>PCR</td>
<td>3.9</td>
<td>Paiva et al. 10</td>
</tr>
<tr>
<td>Mato Grosso do Sul, Campo Grande</td>
<td>203</td>
<td>PCR</td>
<td>1.9</td>
<td>Silva et al. 17</td>
</tr>
<tr>
<td>Mato Grosso do Sul, Corquinho</td>
<td>613</td>
<td>Dissection</td>
<td>0.2</td>
<td>Galati et al. 18</td>
</tr>
<tr>
<td>Mato Grosso, Várzea Grande</td>
<td>420</td>
<td>PCR</td>
<td>0.7</td>
<td>Present study</td>
</tr>
<tr>
<td>Minas Gerais, Belo Horizonte</td>
<td>398</td>
<td>PCR</td>
<td>0.0</td>
<td>Souza et al. 19</td>
</tr>
<tr>
<td>Minas Gerais, Santa Luzia</td>
<td>211</td>
<td>PCR</td>
<td>0.9</td>
<td>Carvalho et al. 20</td>
</tr>
<tr>
<td>Piauí, Teresina</td>
<td>1,832</td>
<td>Dissection</td>
<td>1.1</td>
<td>Silva et al. 21</td>
</tr>
<tr>
<td>Rio de Janeiro, Rio de Janeiro</td>
<td>400</td>
<td>PCR</td>
<td>2.0</td>
<td>De Pita-Pereira et al. 8</td>
</tr>
<tr>
<td>Rio Grande do Sul, Derrubadas</td>
<td>920</td>
<td>PCR</td>
<td>0.3</td>
<td>Silva &amp; Grunewald 22</td>
</tr>
<tr>
<td>Colombia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boyacá, Oanche and Pauna</td>
<td>-</td>
<td>PCR</td>
<td>0.5-1.6</td>
<td>Santamaría et al. 23</td>
</tr>
<tr>
<td>Santander, Piedecuesta</td>
<td>7,391</td>
<td>PCR</td>
<td>1.9</td>
<td>Flórez et al. 24</td>
</tr>
<tr>
<td>Mexico</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campeche, La Libertad</td>
<td>1,288</td>
<td>Dissection</td>
<td>2.8</td>
<td>Rebollar-Téllez et al. 25</td>
</tr>
<tr>
<td>Venezuela</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucre, Paria</td>
<td>549</td>
<td>PCR</td>
<td>1.3</td>
<td>Jorquera et al. 26</td>
</tr>
<tr>
<td>Puerto Cabello, Urama</td>
<td>65</td>
<td>PCR</td>
<td>7.7</td>
<td>Rodríguez et al. 27</td>
</tr>
<tr>
<td>Táchira, Independencia</td>
<td>1,633</td>
<td>Dissection</td>
<td>11.6</td>
<td>Rodríguez et al. 27</td>
</tr>
</tbody>
</table>

* Variable numbers of different species were tested and infection rates remained within the specified range.

Note: the test groups are identified on top.
Positive controls: Lc – *Leishmania (Leishmania) chagasi* (MHOM/BR/74/PP/75); La – *Leishmania (Leishmania) amazonensis* (IPLA/BR/67/PH8); Lb – *Leishmania (Viannia) braziliensis* (MHOM/BR/75/M2930); M: 50 bp DNA ladder.

Figure 3

PAGE of RFLP products after digestion with ApaI or HaeIII.
Resumo

A leishmaniose visceral americana (LVA) é causada por parasitas pertencentes ao gênero *Leishmania* (Trypanosomatidae) e transmitida ao homem através da picada de certas espécies de flebotomíneos, previamente infectados. Neste trabalho, investigamos o índice de infecção natural de *Lutzomyia longipalpis*, principal vetor da LVA no Brasil, em Várzea Grande, Estado do Mato Grosso. De julho de 2004 a junho de 2006, foram feitas capturas de flebotomíneos em áreas peri-domésticas utilizando armadilhas de luz CDC. Foram capturadas 420 espécimens de *Lu. longipalpis*. Quarenta e dois grupos, formados por 10 espécimens de *Lu. longipalpis* cada um, foram submetidos à extração de DNA genômico e amplificação por PCR (reação em cadeia da polimerase). DNA de *Leishmania* spp. foi detected em 3 dos 42 grupos testados, resultando em um índice mínimo de infecção de 0,71%. A análise de polimorfismos de fragmentos de restrição (RFLP) indicou *Leishmania (L.) chagasi* como a espécie infectante nos grupos positivos.

*Psychodidae; Insetos Vetores; Leishmaniose*

Contributors

N. A. Missawa carried out the field captures, laboratory experiments and literature review, and prepared the first version of the article. E. M. Michalsky participated in the planning and execution of the laboratory experiments, as well as in data interpretation, discussion and critical review of the article. C. L. Fortes-Dias contributed in the analysis and interpretation of the data, literature review, discussion and critical review of the article. E. S. Dias planned and supervised the field captures and laboratory experiments, participated in data interpretation, literature review, discussion and critical review of the manuscript.

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