Lutzomyia longipalpis naturally infected by *Leishmania (L.) chagasi* in Várzea Grande, Mato Grosso State, Brazil, an area of intense transmission of visceral leishmaniasis

Lutzomyia longipalpis naturalmente infectado por *Leishmania (L.) chagasi* em Várzea Grande, Mato Grosso, Brasil, uma área de transmissão intensa de leishmaniose visceral

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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.*

Psychodidae; Insect Vectors; Leishmaniasis

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 [1]. In 2003, that municipality was considered an area of intense transmission by the Department for Epidemiological Surveillance in the Brazilian Ministry of Health [2].

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 (15º32'30"S, 56º17'18"W) is located in the state of Mato Grosso, near to its capital, Cuiabá [3].

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 [4] 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 [4,5], as well as on the preva-

lence and incidence of human and canine cases of AVL.

Pools containing ten *Lu. longipalpis* females each were prepared for DNA isolation [6]. 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 (cacophony) [7,8].

The pool samples were also amplified with specific primers for *Leishmania* spp. [9]. 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 [10].

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 [11].

Results

The efficacy of DNA extraction was confirmed by the presence of a 220bp fragment in every pool of *Lu. longipalpis* DNA (Figure 1). The amplification product for *Leishmania* spp. (120bp) was detected in 3 out of 42 pools tested (Figure 2). 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 *Apa*LI and *Hae*III digestion, respectively (Figure 3).

Discussion

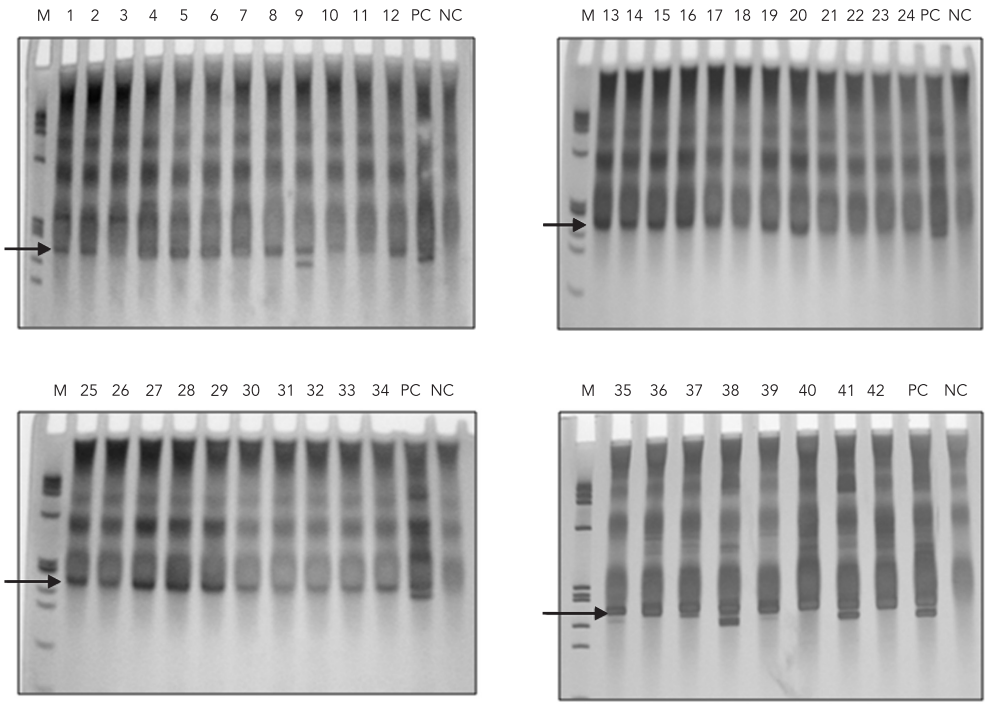
One of the main disadvantages of dissection, besides low sensitivity, is the assumption that all motile flagellates in the sand fly guts are *Leishmania* parasites. Among molecular methodologies used in the detection of *Leishmania*, the PCR has been widely reported in the literature for many purposes, 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 (Table 1). 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 [1,3]. The last species was shown to be widely distributed in Mato Grosso [12,13] but it was not captured in Várzea Grande.

Figure 1

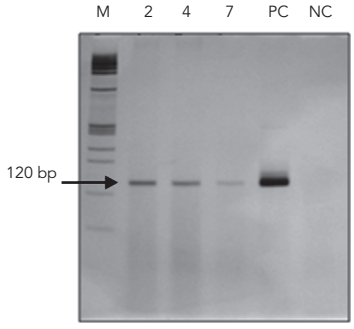
PAGE of PCR-amplified DNA of *Lu. longipalpis*.



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

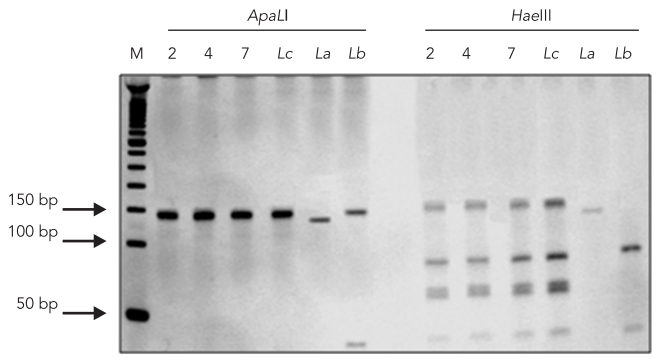
PAGE of PCR-amplified DNA of *Leishmania*-infected *Lu. longipalpis* using specific primers for *Leishmania* spp.



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

Figure 3

PAGE of RFLP products after digestion with *ApaL*I or *Hae*III.



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.

Table 1

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

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

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

Resumo

A leishmaniose visceral americana (LVA) é causada por parasitos 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 peridomésticas 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 detectado 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.

Acknowledgments

To the technicians from the Entomology Laboratory at the Mato Grosso State Health Department and those from the Leishmaniasis Laboratory at the René Rachou Research Center/Fiocruz. To Fiocruz and to the Mato Grosso State Health Department for partial financial support.

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Submitted on 11/Mar/2010
Final version resubmitted on 08/Jun/2010
Approved on14/Jun/2010