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BRIEF COMMUNICATION

NATURAL INFECTION OF PHLEBOTOMINES (Diptera: Psychodidae) IN A VISCERAL-LEISHMANIASIS FOCUS IN MATO GROSSO DO SUL, BRAZIL

João Cezar do NASCIMENTO(1,2), Byanca Regina de PAIVA(4,6), Rosely dos Santos MALAFRONTE(4), Wedson Desidério FERNANDES(2,5) & Eunice Aparecida Bianchi GALATI(3)

SUMMARY

The main purpose of this study was to investigate natural infection by *Leishmania* in phlebotomine females in a visceral-leishmaniasis focus in Antonio João county in Mato Grosso do Sul State, Brazil. Between June and October 2003, the digestive tracts of 81 females captured in Aldeia Campestre, Aldeia Marangatu and Povoado Campestre were dissected. The females were separated by species, location, area and date of capture into 13 groups and kept in ethanol 70%. To identify the *Leishmania* species using the PCR technique, amplifications of the ribosomal-DNA (rDNA) and mini-exon genes were analyzed. Of the 81 specimens, 77 (95%) were *Lutzomyia longipalpis,* making this the most common species; only one specimen of each of the species *Brumptomyia avellari*, *Evandromyia cortelezzii*, *Evandromyia lenti* and *Nyssomyia whitmani* was found. Trypanosomatids were identified in eight of the nine groups of *Lutzomyia longipalpis* (10.39%) one group from Aldeia Campestre, one from Aldeia Marangatu and six from Povoado Campestre; of the eight groups, one from Aldeia Marangatu and another, with promastigotes forms also confirmed by dissection (1.23%) from Povoado Campestre, were identified by PCR as *Leishmania chagasi* (2.6%). The other groups gave negative results. These findings indicate that there is a high risk of leishmaniasis transmission in this area.

KEYWORDS: *Lutzomyia longipalpis; Leishmania chagasi;* Phlebotomines; Natural infection.

INTRODUCTION

The estimated rate of natural infection of a particular species by an infectious agent is an important parameter which must be taken into account when measuring a species' vectorial capacity[9]. Estimates of the infection rate in phlebotomines based on dissection of the insects are frequently carried out. Infection rates obtained by this method range from 0 to 9%[1,9,11,19] but are usually in the region of 0.2%[9,10,14,17,18].

The use of molecular techniques has meant that more accurate data for the infection rates of protozoan flagellates in phlebotomines are available, as in theory only one *Leishmania* parasite can be detected in each phlebotomine. This fact was not confirmed, however, when specimens collected in the field were used in DNA extraction[5].

Lutzomyia longipalpis (Lutz & Neiva, 1912) was identified for the first time as a possible vector of American visceral leishmaniasis (AVL) when CHAGAS[2] (1936) observed that it was the most commonly-found hematophagous insect in the domicile and peridomicile areas of the dwelling where the first case of the disease was reported, in Sergipe State. CHAGAS *et al.*[3] (1938) identified this phlebotomine in an area where AVL was present in Pará State, reinforcing the suspicion, later confirmed by DEANE & DEANE[4] (1954) in Ceará State, that it acts as a vector for the disease. Since then, the presence of *Lu. longipalpis* has been associated with AVL over a large area of the American continent, from Mexico to Argentina[7].

The vectorial competence of *Lu. longipalpis* regarding the etiologic agent of AVL, *Leishmania* (*L.*) *chagasi*, CUNHA & CHAGAS, 1937, was shown by experimental infection of the phlebotomines with the parasite and transmission of the disease to hamsters[14], and by the discovery of naturally-infected *Lu. longipalpis* phlebotomines, which subsequently transmitted the disease to hamsters[13].

The aim of this study was to identify natural infection of phlebotomines by flagellate forms of *Leishmania* in a visceral-leishmaniasis focus in indigenous areas of Antônio João county in Mato Grosso do Sul State. This was done by dissecting female phlebotomines and also submitting them to polymerase chain reaction (PCR) for molecular identification of the parasite.

(1) Regional Entomology Laboratory, State Department of Health/SES-MS, Dourados, Mato Grosso do Sul, Brazil.
(2) Masters Program in Entomology and Biodiversity Conservation, Federal University of Mato Grosso do Sul, Dourados, Mato Grosso do Sul, Brazil.
(3) Epidemiology Department, Faculty of Public Health, University of São Paulo, São Paulo, SP, Brazil.
(4) São Paulo Institute of Tropical Medicine, University of São Paulo, São Paulo, SP, Brazil.
(5) Biological Sciences Department, Dourados Campus, Federal University of Mato Grosso do Sul, Dourados, Mato Grosso do Sul, Brazil.
(6) Institute of Biomedical Sciences, Parasitology Department, University of São Paulo, São Paulo, SP, Brazil.
Correspondence to: Eunice Galati, Av. Dr. Arnaldo 715, 01246-904 São Paulo, SP, Brasil. E-mail: egalati@usp.br

MATERIAL AND METHODS

Characteristics of the area studied: The study was carried out in Aldeia Campestre, Aldeia Marangatu and Povoado Campestre, indigenous areas located in Antônio João county (22º11'28" SL and 55º56'51" WL), in Mato Grosso do Sul State. The county, including the Campestre district, covers 1,143.76 km^2 and has 7,408 inhabitants. Agriculture and livestock raising, particularly cattle raising, constitute the main economic activity[12].

Most of the area is covered with primitive vegetation on hill slopes and at the bottom of valleys. The area consists mainly of scrubland on a smoothly rolling terrain and is classified as Semideciduous Latifoliate Xeromorphic Tropical Woodland[6]. The underlying structures consist of various sedimentary rocks in the Furnas, Ponta Grossa, Aquidauana and Botucatu formations, with some granite in the southeast. There are two topographical levels with raised undulations, separated by an abrupt escarpment, one of which faces the river Apa, of the Paraguay basin, and the other tributaries of the Paraná river[12].

Methodology for capturing phlebotomines and identifying leishmania parasites: The phlebotomines were captured using automatic luminous CDC traps installed once every month from 6 pm to 6 am in the domicile and peridomicile areas of the dwellings in Aldeia Campestre, Aldeia Marangatu and Povoado Campestre in Antônio João county in Mato Grosso do Sul State, between June and October 2003. The phlebotomines were analyzed in the Dourados Regional Entomology Laboratory, of the Mato Grosso do Sul State Department of Health (SES-MS).

The nomenclature adopted for the phlebotomines follows that set out by GALATI[8] (2003). The females were dissected in a saline solution and examined under a bacteriological microscope (at 400x) to identify the phlebotomine species and the promastigote forms in their digestive tracts. After examination, the insects, or parts of them, were immersed in ethanol 70% in Eppendorf tubes. The number of females per tube varied as they were grouped according to species, location, area and date of capture. The tubes were sent to the Institute of Tropical Medicine at the University of São Paulo to investigate the presence of, and identify, the parasite by means of molecular techniques.

DNA was extracted from each group of insects according to the protocol described by OSKAM *et al.*[15], with minor modifications (1996). After DNA extraction, two PCR methodologies were used. The first (the initial screening) identified trypanosomatids by means of primers that amplify ribosomal DNA genes (rDNA)[20]. The second methodology, which used primers that were complementary to the mini-exon sequences, enabled the *Leishmania*[16] species among the trypanosomatids to be identified. The amplification products were analyzed in a 1.5% agarose gel.

RESULTS AND DISCUSSION

Eighty-one female phlebotomines belonging to the following species were dissected: *Lutzomyia (Lu.) longipalpis* (77), *Nyssomyia whitmani* (1) (Antunes & Coutinho, 1939), *Evandromyia (Barrettomyia) cortelezzii* (1) (Brèthes, 1923), *Evandromyia (Aldamyia) lenti* (1) (Mangabeira, 1938) and *Brumptomyia avellari* (1) (Costa Lima, 1932) (Table 1).

Phlebotomine females were separated into 13 groups for purposes of dissecation and PCR analysis. Of those groups, a general infection rate of 1.23% was observed under optical microscope observation. One sample (1.29%) from groups of *Lu. longipalpis*, was captured in the domicile area of Povoado Campestre (July/2003).

Since phlebotomines are hosts of non pathogenic *Trypanosomas*,

Table 1

Number of phlebotomines dissected, by species, month, location and area where were captured: Aldeia Campestre (Al. Cam.), Aldeia Marangatu (Ald. Mar), Povoado Campestre (Pov. Cam.) in Antônio João county in Mato Grosso do Sul State (June to October 2003)

Group number	Month	Species	No. of ♀	Location			Area		PCR
				Ald. Cam	Ald. Mar	Pov.Cam	Dom	Perid	
01	June	*Lutzomyia longipalpis*	11	-	-	X		X	+
02	June	*Nyssomyia whitmani*	1	X			X		-
03	June	*Lutzomyia longipalpis*	1	X			X		-
04	June	*Evandromyia cortelezzii*	1			X	X		-
05	July	*Evandromyia lenti*	1	X				X	-
06	July	*Brumptomyia avellari*	1		X		X		-
07	July	*Lutzomyia longipalpis*	3	X				X	+
08	July	*Lutzomyia longipalpis*	12			X		X	+
09*	July	*Lutzomyia longipalpis*	1			X	X		*L.(L.) chagasi*
10	August	*Lutzomyia longipalpis*	3		X			X	*L.(L.) chagasi*
11**	August	*Lutzomyia longipalpis*	33			X		X	+
12	August	*Lutzomyia longipalpis*	2			X		X	+
13	October	*Lutzomyia longipalpis*	11			X		X	+
Total		5 species	81	4	2	7	5	8	

Dom. = domiciles; Perid. = peridomiciles; PCR = Polymerase Chain Reaction; + = positive for Trypanosomatids; * Group with promastigote forms of *Leishmania* sp. detected by dissection; ** Group 11 was subdivided into two equal groups (11a and 11b) because of the larger number of phlebotomines in it.

Endotrypanum and *Crithidia* species, that are similar and can be confused in microscopic identification, they were submitted to initial screening with rDNA primers. All but one group of *Lu. longipalpis* were positive for trypanosomatids and two of them were positive for *Leishmania (L.) chagasi*.

The remaining *Lu. longipalpis* females that were positive for trypanosomatids were captured in the peridomicile area; one in Aldeia Campestre and five in Povoado Campestre. The females infected by *L (L.). chagasi* came from Povoado Campestre and Aldeia Marangatu and were captured in domicile and peridomicile areas, respectively.

The rates for natural infection of *Lu. longipalpis* observed using both PCR techniques may be underestimated, as some of the groups consisted of various specimens. The values found therefore reflect the minimum rate (MR = No. of positive groups X 100/ total number of species) observed for this phlebotomine, namely, 10.39% for trypanosomatids and 2.60% for *L.* (*L.*) *chagasi*. The groups of numbers (2) *N. whitmani*, (3) *Lu. longipalpis*, (4) *E. cortelezzii*, (5) *E. lenti* and (6) *B. avellari* were negative (Table 1 and Fig. 1).

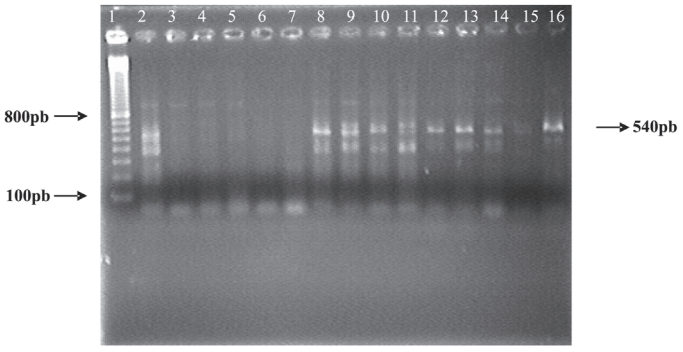

Fig. 1. - Electrophoretic profile in 1.5% agarose gel of the amplification product of the ribosomal DNA region of phlebotomine populations sampled in Antônio João county in Mato Grosso do Sul State (June-October/2003). MW marker = 100bp ladder (Pharmacia 100bp ladder), 2 to 15 = insect groups (1 to 13), 16 = positive control (*Leishmania (L.) amazonensis).* ***Lanes 12 and 13 correspond to groups 11a and 11b.**

The sensitivity of the PCR technique in detecting trypanosomatids was 8.05 times greater than that of the dissection technique. The former technique was around twice as effective as the latter in identifying leishmanias.

In spite of the extremely small sample size, *Lu. longipalpis* infected by *L. (L.) chagasi* was still found in the domicile area. This result, together with the natural infection rate in the peridomicile area, demonstrates the high risk of infection for humans and canines as compared with the rate of 0.39% found by SANTOS *et al.*[19] (1998) for *Lutzomyia cruzi* (Mangabeira, 1938) in the Corumbá and Ladário region, also in Mato Grosso do Sul and considered hyperendemic for AVL.

In summary, in this study we were able to detect the presence of trypanosomatids and to identify the agent of leishmaniasis, *Leishmania (L.) chagasi*, in natural conditions in the digestive tracts of *Lu. longipalpis* females in Aldeia Campestre, Aldeia Marangatu and Povoado Campestre in Antônio Jõao county in Mato Grosso do Sul State. The PCR techniques used were found to be substantially more effective than the dissection technique in detecting natural infection in phlebotomines.

RESUMO

Infecção natural de flebotomíneos (Diptera: Psychodidae) em foco de leishmaniose visceral no Mato Grosso do Sul, Brasil

Com o objetivo de investigar a infecção natural por *Leishmania* em fêmeas de flebotomíneos, em um foco de leishmaniose visceral, no município de Antônio João, Estado de Mato Grosso do Sul, no período de junho a outubro de 2003, dissecou-se o trato digestivo de 81 fêmeas de cinco espécies de flebotomíneos capturadas em três localidades: Aldeia Campestre, Aldeia Marangatu e Povoado Campestre. Após dissecção estas foram divididas em 13 grupos monoespecíficos e armazenadas em etanol 70%. Para identificação das espécies de *Leishmania* pela técnica de PCR, esses grupos foram analisados por meio da amplificação dos genes de DNA ribossômico e mini-exon. Das fêmeas analisadas, *Lutzomyia longipalpis* foi a espécie mais freqüente com 95% (77/81) dos espécimes e apenas um exemplar das demais espécies, *Brumptomyia avellari*, *Evandromyia cortelezzii*, *Evandromyia lenti* e *Nyssomyia whitmani*, foi encontrado. Tripanosomatídeos foram identificados em oito dos nove grupos de *L. longipalpis* (10,39%), sendo um da Aldeia Campestre, seis do Povoado Campestre e um da Aldeia Mangaratu. Desses, dois (2,6%) foram identificados, por PCR, como *Leishmania chagasi* sendo um proveniente da Aldeia Mangaratu e outro, que em dissecção apresentou formas promastigotas (1,23%), proveniente de Povoado Campestre. Os demais grupos foram negativos. Esses resultados apontam para um alto risco de transmissão de leishmaniose na área.

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