



# Lutzomyia whitmani is the main vector of American Cutaneous Leishmaniasis in the Brazilian Federal District and the most prevalent species in residential areas of the Administrative Region of Sobradinho\*

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DOI: <http://dx.doi.org/10.1590/abd1806-4841.20142589>

**Abstract:** Although cases of cutaneous Leishmaniasis have been reported in Brasilia - DF, its mode of transmission is still unknown. Center of Disease Control traps (CDC trap) placed around Sobradinho, a periurban area in the Brazilian Federal District, were able to capture a sample of phlebotomines composed of 89% *Lutzomyia whitmani*, 7% *Lu. bacula*, and 3% *Lu. davisii* specimens. Being of 77% of these specimens were captured in peridomiciliary. PCR analyses showed that the specimens were negative for *Leishmania* DNA. However, the high prevalence of *Lu. Whitmani* in the studied region suggests that it may be the main vector for the transmission of Leishmaniasis in peridomiciliary areas in the studied region.

Keywords: Leishmania; Leishmaniasis, cutaneous; Polymerase chain reaction

The etiological agents of American Tegumentary Leishmaniasis (ATL) consist of different species of *Leishmanias*, who transmit the disease to humans through phlebotomine bites (*Diptera: Psychodidae: Phlebotominae*), which generally affect the skin and mucosal membranes.

By 2003, autochthonous cases had been confirmed in all 19 Brazilian states which registered cases of ATL since 1980.<sup>1</sup>

Phlebotomine infections are usually detected through the dissection and examination of the digestive tubes of *Leishmania* specimens. The parasites identified are then extracted and isolated in cultures or animal tissues. However, this method has a low sensitivity and is slow to provide results.<sup>2</sup>

Polymerase chain reaction (PCR) techniques have also proved to be sensitive in detecting parasite RNA or DNA in humans, canines and phlebotomines.<sup>3</sup> In the latter, *Leishmania* infections have been successfully diagnosed by standard PCR techniques, using

initiators developed from mini-exons in tandem repeats specific to the *L. Viannia* subgenus.<sup>4</sup> In an endemic region in Brazil, the infection rate observed in a countryside area was 3.9%.<sup>4</sup>

The presence of isolated cases and outbreaks of ALT<sup>5</sup> provides strong evidence of autochthonous transmission in the Brazilian federal district (DF).

The goal of the present study was to survey phlebotomine species, their ecotopes and importance in the transmission of ALT.

The study was conducted in two houses and a farm, located in the communities of Fercal and Boa Vista, in the Administrative Region of Sobradinho, Brasilia/DF, between March 2009 and March 2010.

Three to five *Center for Disease Control* (CDC) light traps were placed in open areas, peridomiciliary locations (chicken coops, pigsties and corrals) and inside households (bedroom and living room) for three nights a month.

Received on 06.03.2013.

Approved by the Advisory Board and accepted for publication on 08.05.2013.

\* This study was conducted at the Dermatromycology Laboratory of the University of Brasilia(UnB) - Brasilia (DF), Brasil.

Conflict of interest: None

Financial Support: Partial FAP-DF (193000332/2007), CNPq (478575/2008-4) and Coordination for the Improvement of Higher Education Personnel (Capes).

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The captured insects were preserved in ice in styrofoam containers and transported to the Dermatromycology Laboratory of the University of Brasilia (UnB). After specimens were separated, female spermatechae were dissected, classified and identified according to established criteria<sup>6</sup> and divided into pools with five to ten individuals from the same species, separated by date and location of collection. Cells were suspended in 42 DNA extraction tubes containing a lysis buffer composed of 200 mM NaCl, 100 mM TrisHCL (pH 8.5), 5mM EDTA and 0.2% SDS. The tubes were incubated at 56 C° overnight with 12.5 mg/ml K proteinase.<sup>4</sup>

The amplification of 120 bp fragments common to all species of Leishmania was conducted using 150 (sense) 5' GGG(G/T)AGGGGCGTTCT(C/G)CGAA-3' and 152 (antisense) 5' (C/G)(C/G)(C/G)(A/T)CTAT(A/T)TTACAC-CAACCC-3' primers. The amplification products were analyzed by gel electrophoresis in 1.5% agarose gel with ethidium bromide staining, and visualized under UV light in a Vilber Lourmat transilluminator, produced in Torcy, France. The DNA of *L. (V.) braziliensis* (MHOM/BR/1975/M2903) was used as a positive control, and ultrapure water was used as a negative control.

The quantity of the extracted DNA was measured using a Nano-drop spectrophotometer at 220 to 750nm. All reactions were performed in duplicate.

Eighty seven (17%) of the 513 specimens captured were male, while 426(83%) were female. *L. whitmani* (89%) specimens were found in all locations, while *L. bacula* (7%) specimens were detected in Boa Vista and Fercal, and *L. davisi* (3%) and *L. termitophila* (1%) were only found in Boa Vista (Table 1). Fifty-seven percent of specimens were captured in peri-residential areas, while 23% were gathered from open areas and 20% were captured inside households.

*L. whitmani* and *L. davisi* were most prevalent in May and, especially, in September.

PCR results were negative for *Leishmania* DNA. (Figure 1).

The high percentage (83%) of female specimens captured is in agreement with other findings in the literature.<sup>7</sup> The high availability of blood in chicken coops, pigsties and corrals (all of which were assessed in the present study) may have been especially attractive to females seeking to lay eggs.

The presence of *L. whitmani* and *L. davisi* among the captured specimens is worth noting, given their well-established role in the transmission of ATL. The former is found in all five regions of Brazil, as it is an

TABLE 1: Total number captured and percent female phlebotomines per species per area assessed.

Species	Boa Vista		Fercal				Total	%
	Boa Vista	%	House 1	%	House 2	%		
<i>L. whitmani</i>	342	90.2	16	4.2	21	5.6	379	100
<i>L. termitophila</i>	5	100	-	-	-	-	5	100
<i>L. bacula</i>	28	96.6	1	3.4	-	-	29	100
<i>L. davisi</i>	13	100	-	-	-	-	13	100



FIGURE 1: Polymerase chain reactions for the Leishmania genus conducted in female phlebotomines. The letter "M" indicates molecular weight markers. The letter "C+" indicates positive controls (*L. (V.) braziliensis* DNA). Lines: 1 to 8 female *L. whitmani* captured in the Administrative Region of Sobradinho, in Boa Vista and Fercal, Federal District, Brazil. The letter "B" indicates negative controls (ultrapure water)

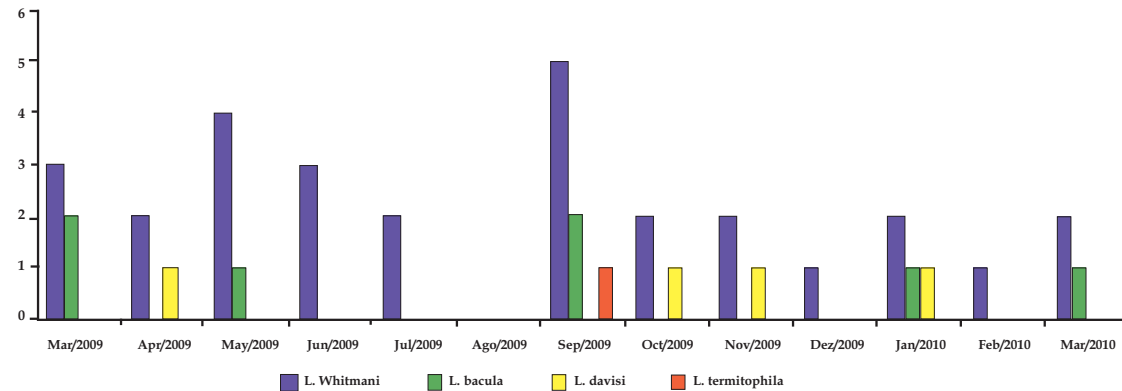
opportunistic species with a widely varying diet which allows it to adjust easily to urban environments. Its predominance among the captured specimens was to be expected, since previous studies have suggested that it may be the major vector for *L. (V.) braziliensis*, the most prevalent species in the DF.

*L. bacula*, which is mostly found in Northern, Central-Western and Southern Brazil, has not yet been implicated in the transmission of ATL.<sup>8</sup>

Natural infection findings suggest that *L. davisi* may be a potential vector,<sup>9</sup> and have some involvement in the transmission of ATL by *L. (V.) braziliensis* and *L. (V.) naiffi*, in Northern, Northeastern and Central-western regions of Brazil.<sup>9</sup>

At the time of writing, specimens of *L. termitophila* have been located in Northern, Northeastern,

Central-Western and Southeast Brazil, with one study reporting the presence of the species in the DF. Its ecology and biology have not been well investigated, probably due to a lack of evidence implicating it in the transmission of *Leishmania*. However, its presence in affected areas, especially near households and other known vectors of leishmaniasis make it a relevant topic of study.



GRAPH 1: Monthly distribution of phlebotomines captured between March 2009 and March 2010, in the Administrative Region of Sobradinho, Federal District, Brasil

Although the specimens were negative for *Leishmania* DNA, the presence of the disease in the areas assessed has been confirmed by the reports of outbreaks and of isolated cases, seen at the University Hospital of Brasilia-UnB and the Brazilian Notifiable Diseases Information System (SINAN).

Most of the *L. whitmani* and *L. davisi* specimens were captured in September and March, in decreasing order. These months correspond to the beginning and end of the rainy season, respectively (Graph 1). However, the month during which the greatest number of individuals was captured corresponds to that during which the lowest air humidity indices are reported in Brasilia (10 to 13%).

As the presence of *Leishmania* in phlebotomine vectors in the DF could not be confirmed, it is important to investigate the possibility of disease transmission by non-phlebotomine insects such as ticks.<sup>10</sup>

It is suggested that future studies capture a larger number of specimens using a higher quantity of traps spread out over a wider range of locations. □

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How to cite this article: Barreto MB, Carneiro AL, Torres FAG, Sampaio RNR. *Lutzomyia whitmani* is the main vector of American Cutaneous Leishmaniasis in the District Federal and predominates in peridomestic Region Administrative of Sobradinho. *An Bras Dermatol.* 2014;89(2):372-4.