Title: Intradomiciliary and peridomiciliary captures of sand flies (Diptera: Psychodidae) in the leishmaniasis endemic area of Chapare province, Tropic of Cochabamba, Bolivia.

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Abstract

In South America, cutaneous leishmaniasis is the most frequent clinical form of leishmaniasis. Bolivia is one of the countries with higher incidence, with 33 cases per 100,000 individuals, and the disease is endemic in 70% of the territory. In the last

decade, the number of cases has increased, the age range has expanded, affecting children under 5 years old, and a similar frequency between men and women is found. An entomological study with CDC light traps was conducted in three localities (Chipiriri, Santa Elena and Pedro Domingo Murillo) of the municipality of Villa Tunari, one of the main towns in the Chapare province (Department of Cochabamba, Bolivia). A total of 16 specimens belonging to 6 species of the genus *Lutzomyia* were captured: *Lu. aragaoi*, *Lu. andersoni*, *Lu. antunesi*, *Lu. shawi*, *Lu. yuilli yuilli* and *Lu. auraensis*. Our results showed the presence of two incriminated vectors of leishmaniasis in an urbanized area and in the intradomicile. More entomological studies are required in the Chapare province to confirm the role of vector sand flies, the intradomiciliary transmission of the disease and the presence of autochthonous cases of cutaneous leishmaniasis.

Keywords: Sand flies, *Lutzomyia*, intradomiciliary, peridomiciliary, CDC light traps, Bolivia

1 Introduction

Leishmaniasis is a parasitic disease affecting man and other mammals. After malaria, leishmaniasis is the most important vector-borne disease in terms of the number of people affected. Globally, 350 million people are at risk of infection in 98 countries around the world (WHO, 2010). In South America, cutaneous leishmaniasis (CL) is the most frequent clinical form, with less presence of cases of mucosal leishmaniasis (ML) and in a minor extent of visceral leishmaniasis (VL) (Rojas et al., 2009). Bolivia is one of the countries with higher incidence of CL in the area, with 33 cases per 100,000 individuals (García et al., 2009). In the last decade, the number of cases has increased up to 2,000 new cases per year in the country (Alvar et al, 2012), where the disease is endemic in 70% of the territory, mainly in the provinces of La Paz, Beni and Pando (Herrera, 2013). *Leishmania (Viannia) braziliensis* is the most prevalent species causing CL in Bolivia (85% of the cases). The other species involved are *L. (Leishmania) amazonensis, L. (V.) lainsoni and L. (V.) guyanensis* (García et al., 2009).

Transmission to humans and other mammalian species is through the bite of phlebotomine sand flies (Diptera: Psychodidae) (Lainson, 1988). The distribution of leishmaniasis is closely related to the distribution of vector species. From 86 sand fly species present in Bolivia, only few are incriminated as vectors of leishmaniasis: *Lutzomyia carrerai*, *Lu. llanosmartinsi*, *Lu. yucumensis*, *Lu. nuneztovari*, *Lu. shawi* and *Lu. longipalpis* (Bustamante et al., 2012; García et al., 2009).

The epidemiology and clinical features of the disease are highly variable due to the interaction of many factors dependent on the parasite, vector, vertebrate host and the environment (Bailey et al., 2007). In Cochabamba Department of Bolivia, leishmaniasis was a typically sylvatic disease affecting rural communities, basically males in working age (21 to 30 years old) that enter to the forest to work (traditional pattern) (García et

al., 2011). Changes on transmission pattern were noticed in the Chapare province of Bolivia (García et al, 2007), where 65.3% of the 1,400 cases of leishmaniasis detected between 2002 and 2010 in the Department of Cochabamba were reported (García et al., 2011). In the last decade, a new and uncommon event occurred in the area: the age range has expanded affecting children and a similar frequency between men and women is found (García et al., 2007; Rojas et al., 2009). Current data available in the municipality of Villa Tunari, in the period 2009-2013, are: 330 cases in men and 162 cases in women, from these 21 cases in children < 5 years (13 women, 8 men) (Vidal, Lozano and Torrico, data not published). In the area, entomological studies were conducted in the Isiboro-Secure national park of Villa Tunari municipality (Bustamante et al., 2012; García et al., 2007), where the risk of transmission is the highest of the Cochabamba Department tropical region. The objective of the present work was to obtain data on the sand flies present outside the national park and in the surroundings of more urbanized areas of this region.

2 Materials and methods

2.1 Area of study and data collection

The study was conducted in the municipality of Villa Tunari, one of the main towns in the Chapare province (Department of Cochabamba, Bolivia). The population of Villa Tunari municipality is about of 71,000 inhabitants (88% from rural areas) (INE 2014, data of 2012; http://censosbolivia.ine.gob.bo/). The climate is tropical humid with an average annual temperature of 24º C and an average annual relative humidity of 81%. The town of Villa Tunari and its environs consist of valley rain forests between 200-400 m above the sea level (a.s.l.) that surrounds the area's main waterway, the Chapare River. Specifically, the study was performed in three localities of an area with a high incidence outside the Isiboro-Secure national park (730 cases / 100.000 inhabitants)

(Vidal, Lozano and Torrico, data not published): Chipiriri, Santa Elena (Chipiriri district) and Pedro Domingo Murillo (Villa 14 de Septiembre district), near to Villa Tunari town, and with similar environmental conditions (Figure 1).

Householders of every house were informed in advance and received and signed an informed consent to participate in the study. It also included consent for making photographs of sampling sites and surrounding areas.

The GPS Test mobile application was used to record the geographical coordinates. Data were entered into an Access database (Microsoft).

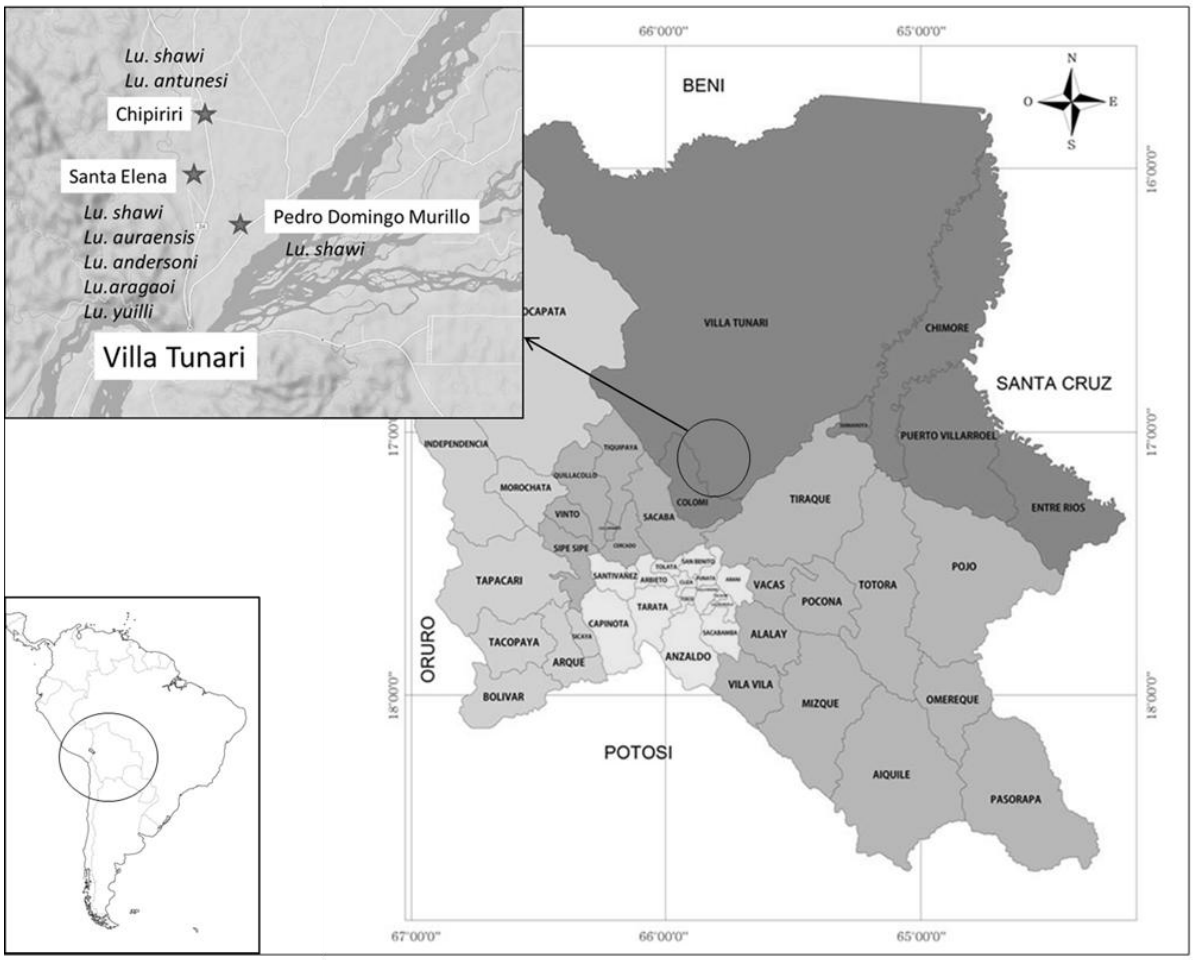

Figure 1. Area of study of the entomological survey of sand flies in Villa Tunari municipality (Chapare province, Department of Cochabamba, Bolivia) and species captured.

2.2 Study of sand flies

The entomological survey was carried out in urbanized areas (villages or hamlets with a higher concentration of housing in sylvatic or forested regions). CDC light traps were placed in 36 inhabited houses from September to December 2014 at altitudes ranging from 196 to 309 m a.s.l. The houses were selected following the next criteria: (i) A case of human leishmaniasis occurred in the past in the house or in the neighbouring houses and (ii) The inhabitants slept in the house regularly. Two CDC light traps were placed once at each house: one in peridomiciliary and another in intradomiciliary environments. Peridomicile: outdoors, less than 10m away from the house, where people often perform their activities. Intradomicile: indoors, preferably in the bedroom. The traps were set at sunset and left in operation all night (from 6 p.m. to 6 a.m.). The bags containing the sand flies were placed in a freezer for 10 minutes minimum in order to reduce their activity. The sand flies were recovered, collected in vials correctly labelled containing 70% alcohol and stored until their morphological identification. Specimens were mounted on Hoyer's medium and identified following the keys of Young and Duncan (1994).

3 Results

Sand flies were captured in 9 houses (Table 1). A total of 16 specimens belonging to 6 species of the genus *Lutzomyia* were captured (11 females and 5 males): *Lu. aragaoi*, *Lu. andersoni*, *Lu. antunesi*, *Lu. shawi*, *Lu. yuilli yuilli* and *Lu. auraensis*. All species were captured isolated with the exception of *Lu. andersoni* and *Lu. yuilli yuilli* (CDC

16). *Lu. andersoni*, *Lu. aragaoi*, *Lu. antunesi* and *Lu. auraensis* were captured in only one house, whilst *Lu. shawi* and *Lu. yuilli yuilli* in 2 and 5 houses, respectively. In one of the houses two traps were placed at different periods (CDC 8 and CDC 15, Table 1) resulting in the capture of one different species at each time, *Lu. yuilli yuilli* and *Lu. auraensis*. A total of eight specimens were captured in the intradomicile, with the same number in the peridomicile (Table 2). At least one specimen was found in the intradomicile for each species, with the exception of *Lu. aragaoi* and *Lu. auraensis*.

4 Discussion

The period of capture corresponds with the one with highest captures recorded in previous studies in the area (Bustamante et al., 2012; García et al., 2007), but the present study includes more urbanized areas instead of forested ones. Three localities were chosen taking into account the presence of the disease and the acceptance and access to the communities: Chipiriri, Santa Elena and Pedro Domingo Murillo. Chipiriri was one of the locations with a higher incidence of leishmaniasis in the area between 2009 and 2013 (Vidal, Lozano and Torrico, data not published). All the households selected for the study had presented one case of leishmaniasis in the past, either in the house itself or in the neighbourhood, and shared very similar ecological characteristics but differences in the collected specimens are observed.

All the six species captured were previously found in Bolivia among the 86 recorded in the country (Duncan & Young, 1994), although *Lu. andersoni* and *Lu. auraensis* are new findings in the Chapare region as they were not previously captured in the Isiboro-Secure national park despite the highest number of sand flies captured (4,463 and 945 specimens in 2000 and 2007, respectively) (Bustamante et al., 2012; García et al., 2007). In the case of *Lu. auraensis*, this species was previously captured in the nearest province of Carrasco (Bermúdez et al., 1993).

In the present study, the number of captured sand flies is low (16 specimens), as it was in an aforementioned study carried out also with CDC traps in the Isiboro-Secure park of the Chapare province (Bustamante et al., 2012). CDC traps were used in this preliminary study, as they are more useful for capturing inside houses, but additional studies, including a greater number of sites of capture, trapping in different periods of the year as well as using Shannon traps (de Souza et al., 2004) are required to define the characteristics of the phlebotomine fauna in urbanized and sylvatic areas of the region. None of the females presented blood in their abdomen neither eggs. Even if people did activities in the intra- and peridomicile during sunlight, when sand flies are expected to be more active, this result is not surprising because the few specimens caught as well as the traps were placed only for one night in every house. Three out of the four species captured inside houses are considered antropophilic (*Lu. yuilli yuilli*, *Lu. shawi* and *Lu. antunesi*) (Le Pont et al., 1990), as it is suspected for one of the species found only in the peri-domicile (*Lu. auraensis*) (Valdivia et al., 2012). In former studies these species were found naturally infected with flagellates, by dissection or molecular techniques, in Bolivia (Bermúdez et al., 1993; García et al., 2007) and in other countries of South America (Brazil, Colombia and Peru) (Lainson et al., 1976; Ryan et al., 1984, 1987; Santamaría et al., 2006; Vásquez Trujillo et al., 2013; Valdivia et al., 2012). In the case of *Lu. shawi* in Bolivia and *Lu. auraensis* in Peru the *Leishmania* species was identified (*L.(V.) braziliensis*, *L. (V.) guyanensis* and *L. (V.) lainsoni*) (García et al., 2007; Valdivia et al., 2012). In the Chapare province *Lu. yuilli yuilli* was not considered the suspected vector in the Isiboro-Secure natural park because of its lower abundance, and no studies on *Leishmania* parasitism were conducted with this species (García et al., 2007). In the present study, females of *Lu. shawi* and *Lu. yuilli yuilli* were found inside houses and, although a low number of specimens was captured, the number of *Lu. yuilli*

176 *yuilli* was greater than of *Lu. shawi*. *Lu. yuilli yuilli* was also captured in a highest
177 number of houses and throughout the period of study which would suggest its role as a
178 vector in the area.
179 In general terms, it is considered that the number of sand flies is higher in the
180 peridomiciliary habitat than inside the houses, and some authors suggest a greater risk
181 of leishmaniasis transmission in the peridomicile (Bustamante et al., 2012). In our study
182 low captures were obtained in both peridomicile and intradomicile when using the same
183 kind of traps. Females, including the suspected vectors *Lu. shawi* and *Lu. yuilli yuilli*,
184 were also captured in both sites. Our results suggest that transmission of leishmaniasis
185 could occur in the intradomicile environment in more urbanized areas of Chapare
186 province as well as in the peridomiciliary. Apart from differences due to the type of
187 traps, the low number of catches in these more urbanized areas would indicate that the
188 transmission of the disease to the population would occur mainly in the forest or
189 sylvatic environments as mentioned (Bustamante et al., 2012; Rojas et al., 2009).
190 Probably different transmission cycles of the disease could occur on a same focus, as
191 suggested by other authors (Le Pont et al., 1992). The possibility that primary sylvatic
192 foci of transmission passed to humanized secondary foci of transmission exists (Le Pont
193 et al., 1992). Indeed, the intradomiciliary transmission is suspected or has been already
194 confirmed in different parts of South America (Campbell-Lendrum et al., 2001; de
195 Souza et al., 2004). The fact that the transmission cycle could have adapted to the
196 domestic habitat may provide one explanation for the recent increasing trend in human
197 CL in Chapare province (García et al., 2009). Unfortunately, not previous captures and
198 results are available in the area of the study that allows to indicate a change in sand fly
199 abundance and behaviour. Our results showed the presence of two incriminated vectors
200 of leishmaniasis in an urbanized area and specifically in the intradomicile. More

entomological studies are necessary in the Chapare province to confirm the vector species, the intradomiciliary transmission and the presence of autochthonous cases.

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283 Table 1. Characteristics of the positive sampling sites for sand flies.

Date	Locality	Latitude	Longitude	Altitude	Intradomicile		Peridomicile	
					N.	Species (sex)	N.	Species (sex)
04-05/09/2014	Santa Elena	S 16° 56' 35"	W 65° 24' 05"	266	0		1	*Lu. yuilli yuilli* (1F)
06-07/09/2014*	Santa Elena	S 16° 56' 35"	W 65° 24' 06"	279	2	*Lu. yuilli yuilli* (2F)	1	*Lu. yuilli yuilli* (1F)
17-18/10/2014	P. D. Murillo	S 16° 47' 55"	W 65° 25' 38"	240	0		1	*Lu. shawi* (1F)
18-19/10/2014	Chipiriri	S 16° 54' 56"	W 65° 24' 03"	254	1	*Lu. shawi* (1F)	0	
19-20/10/2014*	Santa Elena	S 16° 56' 35"	W 65° 24' 06"	279	0		1	*Lu. auraensis* (1M)
19-20/10/2014	Santa Elena	S 16° 56' 35"	W 65° 24' 05"	269	4	*Lu. yuilli yuilli* (2F; 1M), *Lu. andersoni* (1F)	0	
19-20/10/2014	Santa Elena	S 16° 56' 41"	W 65° 24' 03"	274	0		2	*Lu. yuilli yuilli* (1F; 1M)
09-10/11/2014	Santa Elena	S 16° 56' 46"	W 65° 24' 01"	282	0		1	*Lu. aragaoi* (1M)
05-06/12/2014	Santa Elena	S 16° 56' 36"	W 65° 24' 02"	269	0		1	*Lu. yuilli yuilli* (1F)
06-07/12/2014	Chipiriri	S 16° 54' 49"	W 65° 23' 51"	264	1	*Lu. antunesi* (1M)	0	

284 * Same household. One of the CDC traps placed in September had the light off when recovered. F: female, M: male.

Highlights

In Bolivia cutaneous leishmaniasis is the most frequent clinical form of the disease.

An entomological survey with CDC light traps was conducted in Bolivia.

A total of 16 specimens belonging to 6 species of the genus *Lutzomyia* were captured.

The results showed the presence of two incriminated vectors of leishmaniasis.

The vectors were found in the intradomicile and in an urbanized area.

Graphical abstract

Title: Intradomiciliary and peridomiciliary captures of sand flies (Diptera: Psychodidae) in the leishmaniasis endemic area of Chapare province, Tropic of Cochabamba, Bolivia.

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Summary (25 words)

Entomological survey of sand flies performed in Villa Tunari municipality (Chapare province, Department of Cochabamba, Bolivia) using CDC light traps. The results showed the presence of two incriminated vectors of leishmaniasis disease.