

Tegumentary leishmaniasis and sand flies in a border area between Argentina and Bolivia

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Background: Some sand flies are of medical importance because they are vectors of *Leishmania* parasites that are responsible for leishmaniasis. The aim of this study was to make a retrospective epidemiological analysis of tegumentary leishmaniasis (TL), to identify *Leishmania* spp. from patient isolates and to describe the diversity of sand flies from a border area between Bolivia and Argentina.

Methods: TL cases included in the study were diagnosed in an endemic area of the north of Argentina from 1985 to 2017. The parasites isolated were characterized by the cytochrome B method. Sand flies were captured with Centers for Disease Control traps in Aguas Blancas and Media Luna-Algarrobito localities.

Results: A total of 118 cases of TL were analysed. Eight isolates were characterized as *Leishmania (Viannia) braziliensis*. A total of 1291 sand flies were captured, including *Nyssomyia neivai*, *Cortelezzii* complex, *Evandromyia sallesi*, *Migonemyia migonei* and *Micropygomyia quinquefer*. Within the area, sand flies were found in the backyards of houses.

Conclusions: In this region there exists the possibility of peridomestic transmission of TL in the neighbourhoods peripheral to the urban area and in rural environments as well as the risk of transmission to travellers that pass through the customs offices.

Keywords: Argentina, Bolivia, *Leishmania (Viannia) braziliensis*, sand flies, tegumentary leishmaniasis, travellers

Introduction

The leishmaniasis are a group of protozoan diseases caused by parasites of the genus *Leishmania* transmitted by dipterous insects commonly known as sand flies (Diptera: Psychodidae: Phlebotominae). Currently the leishmaniasis are endemic in 98 countries worldwide with 2 million new cases per year. Further, 350 million people are at risk of infection.¹ In addition, these protozoan diseases rank among the 10 most important medical problems that affect international travelers.² The spectrum of clinical manifestations includes visceral leishmaniasis (VL) and tegumentary leishmaniasis (TL). The latter form includes simple

or multiple cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and diffuse cutaneous leishmaniasis (DCL).³

In northern Argentina, *Leishmania (Viannia) braziliensis* is the most frequently isolated species among patients with TL,^{4–6} although rare cases attributed to *Leishmania (Viannia) guyanensis*, *Leishmania (Viannia) panamensis*^{7,8} and *Leishmania (Leishmania) amazonensis* have been confirmed.⁹ Visceral leishmaniasis due to *Leishmania (Leishmania) infantum* has also been reported.^{10,11} Also, Salta province reported the highest number of Argentinian CL cases (n=69) in 2009, with an incidence of <1 per 10 000 inhabitants.¹² In Bolivia, in addition to the parasite species referred to above, *Leishmania (Viannia)*

lainsoni has been described as a causal agent of TL.¹³ In Tarija, between 33 and 37 CL cases were reported, with an incidence of <1 per 10 000 inhabitants in 2008.¹² It has been suggested that the transmission cycle of these *Leishmania* species is zoonotic.¹⁴

In the northern provinces of Argentina, nine sand fly species have been reported.^{11,15–17} Most studies conducted in Bolivia have focused on the central, eastern and northern regions of that country, but information about sand fly species involved in the transmission and other epidemiological aspects of leishmaniasis in the southern province of Tarija, bordering on Argentina, is incomplete.^{13,18–20}

Migration offices located on the border of Bolivia and Argentina, receive hundreds of people from different places around the world who cross the frontier every day. These travellers frequently visit areas located within the ecoregion of Yungas in Bolivia, which overlaps the areas in Argentina that exhibit the highest incidence rates of cases and where the vector has been reported. This shows the higher risk of transmission of leishmaniasis in this border area.^{5,21–23}

Thus the aim of the present study was to describe the epidemiological situation of CL and its temporal variation in an urban area (Aguas Blancas) and rural communities (Media Luna-Algarrobito) in Argentina on the Bolivian frontier. We have also described the spatial distribution and diversity of sand flies in the area. These contributions seek to delimit the areas of high transmission risk of CL and VL. Considering this is a border with immigration and customs offices of both countries, with an international bridge that connects the cities of Aguas Blancas (Argentina) and Bermejo (Bolivia) over the Bermejo River, the movement of tourists and international trade in both directions increases the exposure risk to all those using this border point, highlighting the need for improved surveillance with the ultimate purpose of improving and promoting preventive measures against leishmaniasis for the local population, travellers and temporary visitors.

Materials and methods

Study area

The study was performed in the north of Salta province, Argentina, in a border area with Bolivia (Figure 1). The study area, which is part of the Yungas ecoregion, includes the following localities: Aguas Blancas (22°44'0"S, 64°22'0" W), an urban area of 2395 inhabitants of the Oran department separated by the Bermejo River from the city of Bermejo, department of Tarija, Bolivia, and the rural areas of Media Luna (22°41'28.58"S, 64°15'1.73"W) and Algarrobito (22°36'46.59"S, 64°14'1.54"W), with a total of 98 inhabitants, that belongs to the General José de San Martín department.

Study population

The study population includes cases diagnosed with TL seen at the Instituto de Investigaciones de Enfermedades Tropicales (IIET), Universidad Nacional de Salta, Argentina. The inclusion criteria were patients from Aguas Blancas, Media Luna-Algarrobito or Bolivia diagnosed between 1986 and 2017, with one or more

ulcers compatible with CL or MCL and a positive result for microscopic examination of smears and/or Montenegro skin test (MST).²⁴ The whole database was de-identified. The project was evaluated and approved by the Bioethics Committee of the National University of Salta.

The demographic data for Aguas Blancas for the years 1991, 2001 and 2010 were arranged by age and sex and the census radius that includes areas of Media Luna-Algarrobito was obtained from the Dirección de Estadísticas y Censos de la Provincia de Salta. Linear regression was applied in order to estimate the population sizes of the years for which census data were not available.

Diagnostic methods

The diagnosis of TL was performed at IIET by a combination of methods executed in parallel as previously described. Briefly, *Leishmania* amastigotes were microscopically identified in smears of dermal scrapings, the MST was applied and analysis of clinical features of the patients was considered.⁸ Diagnosis by microscopy was undertaken, semi-quantitatively classifying each smear according to an increasing amastigote count: += 1–10 parasites/1000 fields; ++=1–10 parasites/100 fields; +++ ≥10 parasites/10 fields and –=no amastigotes/1000 fields. The MST was applied by intradermally injecting 0.1 mL of leishmanin (40 µg of protein/mL) into the forearm for evaluating the induration size after 48 h. Leishmanin was locally prepared with a soluble extract of promastigotes of *L. (V.) braziliensis*, obtained in culture from a patient of our region (strain MHOM/AR/03/OLO1) as described elsewhere.²⁴ Indurations were considered positive if the diameter was ≥5 mm. In a previous study by our group, MST performance was analysed retrospectively, highlighting its high sensitivity of >98%, which is in agreement with previous studies demonstrating a sensitivity of 97.4% and a specificity of 93.9%.^{24,25}

Molecular identification of *Leishmania* parasites

A volume between 0.3 and 0.5 mL was aspirated from the lesion edges with a syringe with 0.5 mL of sterile proline balanced salt solution (PBSS) containing 100 U/mL penicillin and 50 µg/mL streptomycin. The material aspirated was inoculated into Difco blood agar (USMARU) medium containing 20% of defibrinated rabbit blood.⁴ Positive samples were characterized by cytochrome *b* gene (*cytb*) sequencing, slightly modified from Locatelli et al.⁶ Briefly, classic FTA filter paper card fragments of 2 mm diameter containing culture samples were washed three times for 5 min with FTA purification reagent (Whatman BioScience, Boston, MA, USA) and twice in TE⁻¹ buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and then used as a template for the first polymerase chain reaction (PCR). *Leishmania* spp. were identified by a nested PCR performed with the primers for *cytb* (modified from Kato et al.²⁶), L.cyt-ASm (5'-GCGGAGAGRARAG AAAAGGC-3') and L.cyt-ARm (5'-TGCTAAAARCCAATCATAAAT ACTATA-3'), with an initial denaturation at 94°C for 15 min and subsequently 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1.5 min and extension at 72°C for 1 min, and a final extension phase at 72°C for 7 min. From the first PCR

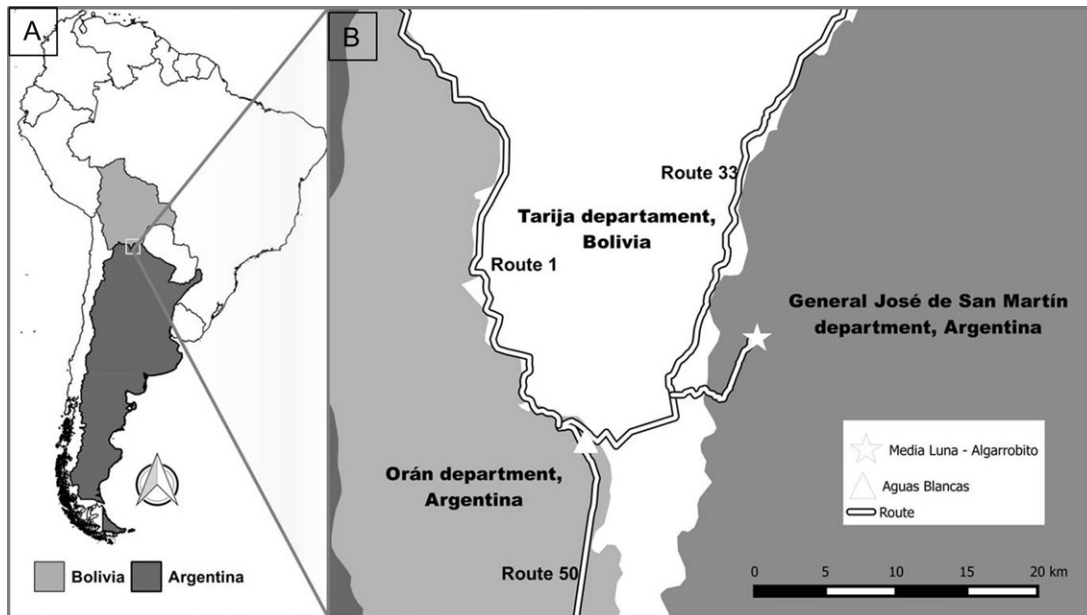


Figure 1. (A) Location of the study area in Latin America. (B) Different sites studied in Oran and General José de San Martín departments in northern Salta (Argentina) and the routes that converge at the Customs office.

solution, 1 μ l was used as a template for a second PCR by using the primers L.cyt-S (5'-GGTGTAGGTTTGTAGTYTAGG-3') and L.cyt-Rm (5'-CTACAATAAACAAATCATAATATRCAATT-3'), performed for 10 min at 94°C for initial denaturation, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and a final extension at 72°C for 1 min, concluding with a polymerization step at 72°C for 7 min. The TaKaRa Ex Taq DNA polymerase hot start version (Takara-Bio, Shiga, Japan) was used in both PCRs. The PCR products were visualized on 1% agarose gels, then purified and quantified using a NanoDrop-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The amplicons were sequenced using a BigDye terminator cycle sequencing kit, version 1.1 (Applied Biosystems, Foster City, CA, USA), purified in Sephadex G-50 spin columns (Amersham Biosciences, Piscataway, NJ, USA) and analysed on an Applied Biosystems Hitachi 3130 Genetic Analyzer automated sequencer, employing the primers L.cyt-S and L.cyt-Rm. The obtained chromatograms were edited on Chromas Lite version 2.6.5 (Technelysium, South Brisbane, QLD, Australia; <https://technelysium.com.au/wp/chromas/>) and aligned by MEGA7 software (<https://www.megasoftware.net>).²⁷ Data were analysed performing a neighbour joining tree with 1000 bootstrap replications using MEGA7 software and compared with the Argentinean strains of *L. (V.) braziliensis* genotypes AB1 and AB2 (gene bank ID for AB1 genotype: MH841941 and for AB2 genotype: MH841942). In addition, The polymorphism-specific PCR (PS-PCR) technique was applied on a sample with negative amplification for *cytb* sequencing (Table 1).⁸

Entomological study

Sand flies were captured with Centers for Disease Control light minitraps (John W. Hock Company, Gainesville, FL, USA) located at nine sites in Aguas Blancas and 16 sites in Media Luna-

Table 1. Characterization of parasites isolated from TL patients by age and sex in Aguas Blancas (Salta, Argentina) and Bermejo (Bolivia)

Location	Sex	Age (y)	<i>cytb</i> genotype	Clinical form
Bermejo, Bolivia	M	34	AB2 ^a	CL
Bermejo, Bolivia	M	60	ND	CL
Bermejo, Bolivia	M	24	AB1 ^b	MCL
Aguas Blancas, Argentina	M	9	AB1 ^b	CL
Aguas Blancas, Argentina	M	40	AB1 ^b	CL
Aguas Blancas, Argentina	F	13	AB1 ^b	CL
Aguas Blancas, Argentina	M	54	AB1 ^b	CL
Aguas Blancas, Argentina	F	18	AB1 ^b	CL

ND: not determined by *cytb* sequencing. *Leishmania (V.) braziliensis* was identified by PS-PCR.

^aSequence identical to gene bank accession number MH841942.

^bSequence identical to gene bank accession number MH841941.

Algarrobito from 19:00 to 09:00 the following day. The studied sites' coordinates were registered with eTrex Vista GPS (Garmin, Olathe, KS, USA). In Aguas Blancas, samplings were performed during a total of six nights: three nights in November 2013 and another three nights in February 2015. In Media Luna-Algarrobito the samplings were performed at nine sites in October 2011 (one night of capture) and August 2016 at nine sites (two nights of capture). Only two sites were coincident for both samplings in Media Luna-Algarrobito.

The morphological analysis for identification of sand fly species consisted of the identification of the following structures of taxonomic value: cibarium and spermathecae (females) and cibarium and external genitalia (males).^{28,29} Females of *Evandromyia sallesi* and *Evandromyia cortelezii* cannot be differentiated by their morphological characteristics so they were classified as *Cortelezii* complex.

Statistical analysis

The TL prevalence was calculated according to age, sex and residence of patients and analysed by means of the χ^2 test. The same test was used to compare the frequency of TL cases by age and sex of patients from Bolivia (test of goodness-of-fit adjustment). We used generalized linear models (GLMs) (Poisson link function) to compare the mean sand fly abundance (night-trap) by geographic area, species, sex and month of capture. The sand fly abundance from the edge and the central sites of the city (Aguas Blancas) were also compared with the same method. All analyses with p-values <0.05 for a level of significance of 95% were considered statistically significant. For all statistical analyses, Infostat software version 2016 (Infostat v 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. <http://www.infostat.com.ar>) was used. QGIS 2.10.1 (<http://www.qgis.org/es/site/>) was used for the drawing of the maps.

Results

A total of 118 patients who met the case definition for this study were included in the analysis. Most patients were residents of Bolivia (56.78%), whereas 26.27% were from Aguas Blancas and 16.95% from Media Luna-Algarrobito. Regarding the smears, 93 patients were positive, 16 were negative and 9 were not tested. In contrast, by MST, 100 patients were positive, 11 were negative and 7 were not tested. A total of 102 suspected cases of TL were positive according to their smears and/or MST, of which 75 were positive for both tests, 11 were positive only by smears and 16 were positive only by MST.

Among the cases from Bolivia, 9 (13%) were MCL and there were peaks in 1997 and 1998 (Figure 2A). The frequency of males (68%, $p < 0.05$) was significantly higher than that observed for females. The age range with the greatest number of CL cases was from 15 to 64 years old ($p < 0.0001$).

In Aguas Blancas, 31 cases of CL have been diagnosed since 1998. Between 1998 and 2014, the prevalence per year was $\leq 0.16\%$. In 2015 a peak of cases was registered, with an observed prevalence of 0.46%, which was followed by a decrease to 0.17% (Figure 2B). The observed prevalence in males was higher than in females (2.45% and 0.84%, respectively; $p < 0.05$). The prevalence by age was 0.95% for children <15 years and 1.82% for adults, and no statistically significant differences were observed between them ($p = 0.176$).

In Media Luna-Algarrobito, TL cases have been diagnosed since 1997. The prevalence per year did not exceed 3.09%, except in 2002 when the registered prevalence was 14.25% (Figure 2C). Of the 20 cases diagnosed in these rural areas, 1 was diagnosed as MCL. Among adults, the male prevalence

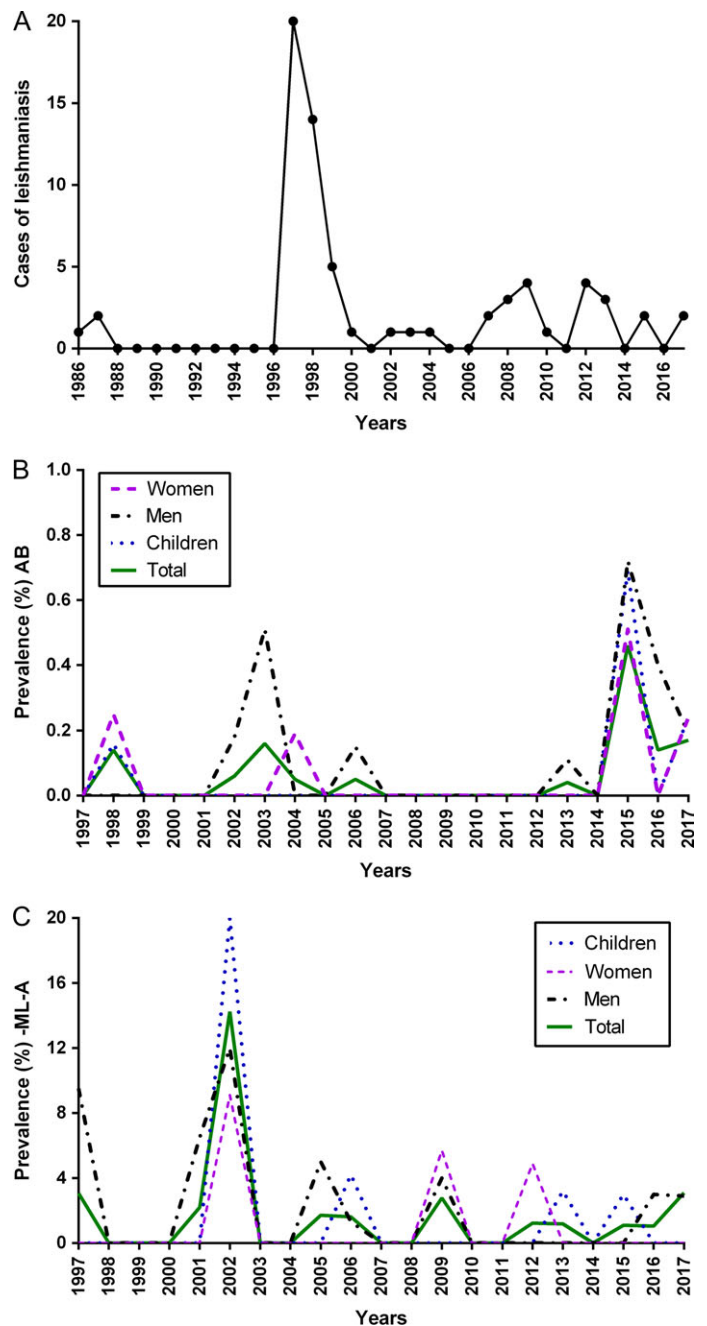


Figure 2. (A) Number of cases of leishmaniasis from Bolivian patients diagnosed at the IIET and reported per year from 1986 to 2017. (B) Prevalence of TL in Aguas Blancas per year from 1997 to 2017. (C) Prevalence of TL in Media Luna-Algarrobito per year from 1997 to 2017.

(38.89%) was significantly higher than that observed in women (17.24%, $p < 0.05$). The prevalence by age group was 26.92% for children, while for adults it was 30.77%, with no significant statistical differences ($p = 0.738$).

For the comparison of overall prevalence of TL between the two study areas, only the cases between 1997 and 2017 were considered, because it is in that period that TL cases were registered in both study areas. In Aguas Blancas, an overall prevalence

of 1.62% was observed, significantly lower than in Media Luna-Algarrobito, where the prevalence was 29.23% ($p < 0.0001$). In subgroup analysis, this difference in prevalence continued to be significant when analysed by sex and age.

Three parasites from Bolivia and five from Aguas Blancas were successfully isolated and identified as *L. (V.) braziliensis*. Six of these parasites corresponded to the Ab-1 sequence of the *cytb* gene and one was isolated to the Ab-2 sequence (Table 1).

A total of 1291 sand flies were collected: 374 in Aguas Blancas and 917 in Media Luna-Algarrobito. The last locality presented a higher average of sand flies per night-trap ($n=5.26$), whereas Aguas Blancas was fewest ($n=1.45$), with statistically significant differences ($p < 0.05$). The average number of sand flies for Aguas Blancas was 1.67 *Nyssomyia neivai* per night-trap, 2 *Cortelezzii* complex, 1.72 *Ev. sallesi* and 0.58 *Migonemyia migonei*. The average for *Mg. migonei* showed statistical differences with the other species ($p < 0.05$). The mean of *Micropygomyia quinquefer* was not calculated because this species was found in only one sampled site. The average number of sand flies for Media Luna-Algarrobito was 19.06 *Ny. neivai* per night-trap, 1.47 *Mg. migonei*, 0.06 *Cortelezzii* complex and 0.11 *Ev. sallesi*. *Nyssomyia neivai* ($p < 0.001$) and *Mg. migonei* ($p < 0.05$) showed significant differences with the other species, while *Cortelezzii* complex and *Ev. sallesi* did not ($p > 0.05$). Overall, 1.9% of the captured sand flies could not be identified at the species level due to taxonomic structure losses during processing.

When we compared female means we found that in Aguas Blancas there were no significant differences between species (*Ny. neivai*: 1.77; *Mg. migonei*: 0.98; *Cortelezzii* complex: 0.39). Meanwhile, in Media Luna-Algarrobito, *Ny. neivai* showed a higher average (15.62; $p < 0.0001$) than *Mg. migonei* (0.45) and *Cortelezzii* complex (0.06) and these last two species showed no significant differences between them ($p > 0.05$).

In Aguas Blancas, sand flies were captured at eight of the nine sites, with the highest frequency of captures occurring on the edges of the city ($p < 0.0001$) (Figure 3). *Nyssomyia neivai* was present at six of nine sampling sites, but it was not captured at the sites in the centre of the city (sites 1, 7 and 8). *Cortelezzii* complex and *Ev. sallesi* were captured at seven sampling sites (2, 3, 4, 5, 6, 7 and 9) and *Mg. migonei* at eight sampling sites. Table 2 shows the abundance of sand flies per night-trap by sex and site. Male sand flies were found at statistically higher averages than females ($p < 0.0001$).

In Aguas Blancas, the total abundance of sand flies captured during November 2013 was higher than that in February 2015 ($p < 0.0001$). Regarding the abundance of *Ny. neivai* by sampling month, a lower abundance was observed in November 2013 (1.44 sand flies per night-trap) compared with February 2015 (1.89 sand flies per night-trap) without statistically significant differences ($p > 0.05$). However, *Cortelezzii* complex (including *Ev. sallesi*) showed a greater abundance in the first sampling (7.50 sand flies per night-trap) than the second (0.60, $p < 0.0001$). The average number of *Mg. migonei* also demonstrated significant

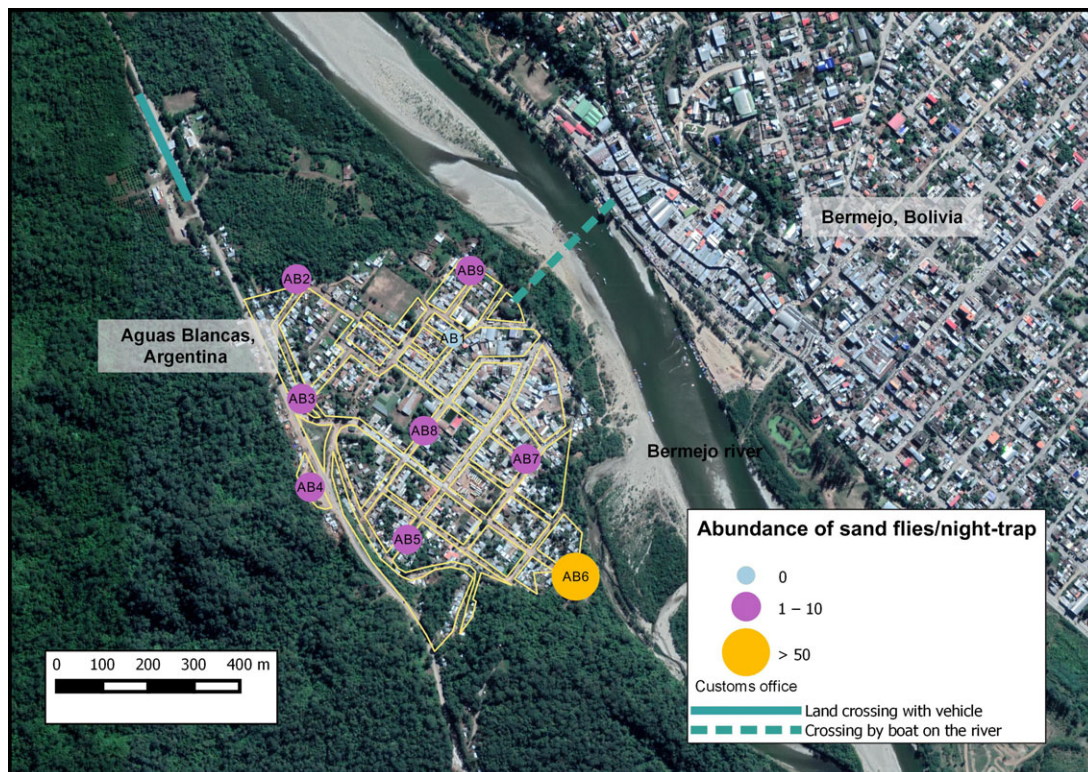


Figure 3. Abundance of sand flies by site on the day of capture in Aguas Blancas. The continuous line represents the Customs office and the place where vehicles line up to cross from one country to another on route 50, which is surrounded by primary vegetation. The dashed line represents the circuit that people use to cross by boat from one country to another. The Customs office is located on the Argentina side (next to site AB9) and is surrounded by wild vegetation.

Table 2. Abundance of sand flies by sex per site per night-trap in Aguas Blancas and Media Luna-Algarrobito

Site	Cortelezzii complex		<i>Ev. sallesi</i>		<i>Mg. migonei</i>		<i>Ny. neivai</i>		<i>Mi. quinquefer</i>		<i>Lutzomyia</i> spp.		Total per site
	F	M	F	M	F	M	F	M	F	F			
AB1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AB2	2.00	2.40	1.00	2.00	0.60	0.60	0.00	0.00	0.00	0.00	0.00	0.00	8.60
AB3	1.33	0.67	0.17	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	2.83
AB4	0.17	0.50	0.00	0.17	0.33	2.17	0.00	0.00	0.00	0.00	0.00	0.00	3.33
AB5	0.33	0.00	0.00	0.50	0.17	1.17	0.00	0.00	0.00	0.00	0.33	0.00	2.50
AB6	11.40	8.40	3.20	3.00	8.40	16.20	0.00	0.00	0.40	0.80	0.00	0.00	51.80
AB7	0.50	0.17	0.17	0.17	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	1.17
AB8	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17
AB9	0.33	0.83	0.00	0.33	0.00	0.33	0.17	0.00	0.00	0.00	0.00	0.00	2.00
ML-A1 ^a	0.33	0.67	2.67	15.33	63.67	126.00	0.00	0.00	1.67	3.00	0.00	0.00	213.33
ML-A2 ^c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ML-A3 ^c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ML-A4 ^b	0.00	0.00	0.00	0.00	20.00	7.50	0.00	0.00	0.00	0.00	0.00	0.00	27.50
ML-A5 ^c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
ML-A6 ^c	0.00	0.00	0.00	0.00	3.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	4.00
ML-A7 ^c	0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.37	0.37	0.00	0.00	1.11
ML-A8 ^a	0.00	0.00	0.00	0.00	35.67	12.67	0.00	0.00	0.33	0.00	0.00	0.00	48.67
ML-A9 ^c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
ML-A10 ^b	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
ML-A11 ^b	0.00	0.00	0.00	0.00	5.50	4.00	0.00	0.00	0.00	0.00	0.00	0.00	9.50
ML-A12 ^b	0.00	0.00	0.00	0.00	0.50	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50
ML-A13 ^b	0.00	0.00	0.00	0.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.00
ML-A14 ^b	0.00	0.00	0.00	0.00	6.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	6.50
ML-A15 ^c	0.00	0.00	0.00	0.00	6.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	8.00
ML-A16 ^c	0.00	0.00	0.00	0.00	2.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00
Total AB	16.07	12.97	4.53	6.33	9.83	20.80	0.17	0.17	0.40	1.13	0.00	0.00	72.40
Total ML-A	0.33	0.67	2.67	15.33	151.33	157.54	0.00	0.00	2.37	3.37	0.00	0.00	333.61

AB: Aguas Blancas; ML-A: Media Luna-Algarrobito; F: female; M: male.

^aThree nights of sampling.

^bTwo nights of sampling.

^cOne night of sampling.

variation between sampling months (November 0.94, February 0.22; $p < 0.01$).

In Media Luna-Algarrobito, sand flies were captured at 14 of the 16 sampling sites. Sampling site 1 had the highest abundance of sand flies per night-trap ($n=213.33$) (Figure 4), with *Ny. neivai* being the predominant species ($n=189.66$), followed by site 8 ($n=48.33$). *Nyssomyia neivai* was captured at all the positive sampling sites. Cortelezzii complex, *Ev. sallesi* and *Mg. migonei* were present only at site 1. Table 2 shows the abundance of sand fly species per night-trap by sex and site. Regarding the abundance of *Ny. neivai* by sampling month, a greater abundance was observed in October 2011 (37.06 sand flies per night-trap) compared with August 2016 (7.09 sand flies per night-trap), with statistically significant differences ($p < 0.0001$). The abundance of *Mg. migonei* was higher in October 2011

(3.79 sand flies per night-trap) than August 2016, when only one specimen was captured at site 1.

Of the total sand flies for both study areas, the highest mean was observed in October 2011, followed by August 2016, November 2013 and February 2015, with statistically significant differences between all months ($p < 0.0001$) (Table 3). This observation coincides with the comparison of mean female sand flies by month and study area.

Discussion

The demographic phenomena of migration and short-stay dispersion are important from an epidemiological point of view and are of interest for national and international public health

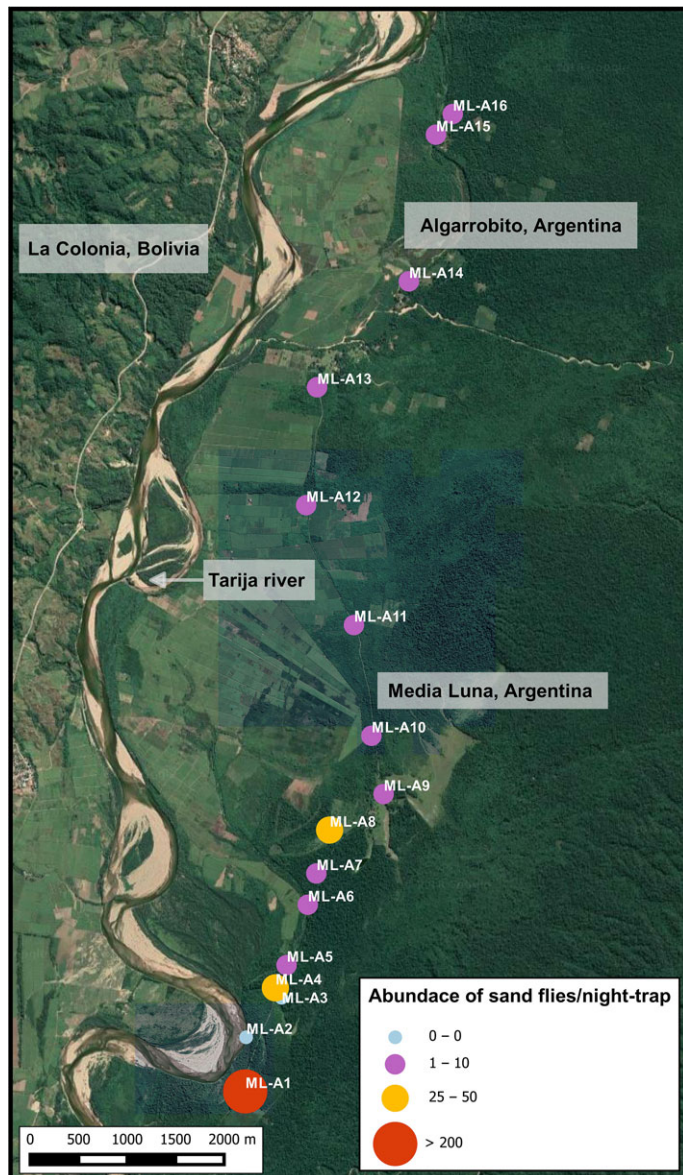


Figure 4. Abundance of sand flies by site on the day of capture in Media Luna-Algarrobito. The landscape combines small crops with wild vegetation. The ML-A1 site corresponds to a house with a barn with abundant organic matter in the soil.

systems. Travellers that come from countries where leishmaniasis is not endemic have been infected by *Leishmania* parasites after traveling to countries such as Bolivia, Brazil and Argentina.^{2,30} These studies suggest that international borders can provide favourable scenarios for the transmission and dissemination of various diseases due to the large number and great concentration of susceptible people that may be exposed to the vectors.

We detected genotypes AB1 and AB2 of *L. (V.) braziliensis* in CL cases from both sides of the Argentina–Bolivia border. This is in agreement with previous studies carried out in the Orán department, Salta province, where the same *L. (V.) braziliensis* genotypes were isolated and characterized from ulcers of patients

Table 3. Average of sand flies captured per sampling month in Media Luna-Algarrobito and Aguas Blancas

Month	Study area	Mean	SE
October 2011	Media Luna-Algarrobito	13.95	0.58
August 2016	Media Luna-Algarrobito	2.36	0.19
November 2013	Aguas Blancas	1.46	0.14
February 2015	Aguas Blancas	0.6	0.09

All means had a significant difference ($p < 0.05$). SE: Standard error.

with CL and MCL.^{4,6} However, there are no published studies reporting which *Leishmania* species circulate in areas of the Tarija department (Bolivia), which borders on Argentina. Although cases of CL caused by *L. (L.) amazonensis* were detected in Argentina,⁹ no DCL cases or its vectors *Bichromomyia flaviscutellata* or *Lutzomyia nuneztovari anglesi* have been reported, suggesting that those cases were imported rather than locally acquired. In addition, regarding VL, which is endemic in some departments of central and northern Bolivia, *Mg. migonei* and *Mi. quinquefer* have been associated with *L. (L.) infantum* transmission, which has been found in this bordering area. This situation implies the need for surveillance in order to prevent outbreaks or the establishment of VL in the region.^{11,31} Also, other risk factors, such as the international transport of dogs, could play an important role in the establishment of VL in the region, and especially in northwest Argentina.

Seasonal labour-related activities in agriculture and lumber production are the main reasons for migrants from Bolivia to move to small cities of northern Argentina. This situation is reflected in the fact that most of the Bolivian patients that were diagnosed with TL were men of working age. We suggest that many of these cases were infected in rural, wild or deforested areas in Orán (Argentina),^{5,21,32} although we cannot rule out the possibility that some patients might have been infected in their country of origin. In addition, the highest number of registered Bolivian patients in the period of our study coincides with the outbreak that occurred in 1997–1998 (Figure 2A) north of Salta province, related with deforestation activities.^{12,32,33} In the Tarija department, the presence of *Ny. neivai* and an outbreak of CL in Bermejo have been reported.^{19,20} Dujardin et al.¹⁸ reported *Mi. quinquefer* in Tarija and we also identified this species in the study areas, suggesting the existence of an ecological continuity for these sand fly species.

In contrast, large numbers of people from different places in Argentina, Bolivia and other countries arrive daily in Aguas Blancas by bus and private transport. This constant geographic funnel (Figures 1B and 3) could generate an important scenario for exposing numerous susceptible people to leishmaniasis. Thus it is possible that a certain number of cases reported in other nearby cities of Salta province (Orán or Tartagal), in other provinces of Argentina and Bolivia and in other countries might have occurred by virtue of exposure to vectors in this border area.³⁰

Considering the impact on the local population, the presence of *Ny. neivai*, *Mg. migonei* and the Cortezii complex in the

neighbourhoods of Aguas Blancas may be an indication of the risk for domestic or peridomestic transmission in this area (Figure 3). This is consistent with the increasing urbanization and domiciliation of leishmaniasis vectors.^{23,34,35} It is important to note that most of the cases that contributed to the prevalence peaks of 2015 and 2017 in Aguas Blancas came from a new settlement located on the southern edge of the city near site AB6 (Table 2, Figures 2B and 3).

All the captured sand fly species from Aguas Blancas and Media Luna-Algarrobito are suspected of transmitting some species of the genus *Leishmania*. *Nyssomyia neivai*, *Mg. migonei* and *Cortelezzii* complex were found to be naturally infected by different species of *Leishmania* in several areas of Argentina and other Latin America countries. In addition, the anthropophilia of these sand fly species has also been described.³⁶⁻⁴¹

Previous studies suggest that in northern Argentina, the possible periods of the year with the highest risk of transmission are summer-autumn and spring-summer, with peaks of bimodal or trimodal abundances, all associated with temperatures and precipitation with a particular temporal lag.¹⁷ In agreement with this study, in Media Luna-Algarrobito we found greater sand fly abundance in October compared with August. Likewise for Aguas Blancas, the abundance in November was significantly greater than in February, which results in a seasonal variation of the transmission risk that must be considered when planning preventive and control measures.

We also observed interannual peaks of TL prevalence in Aguas Blancas and Media Luna-Algarrobito at intervals of 3-4 y (Figures 2B and C). This observation has been reported in other studies showing cycles in the incidence of leishmaniasis of 3-4 y associated with changes in temperature and El Niño Southern Oscillation indices.⁴² In Aguas Blancas, between 2007 and 2012 there were no cases of TL reported. This could be related to vector control activities by the local public health system for malaria control (Figure 2B).

The higher prevalence of CL in Media Luna-Algarrobito than in Aguas Blancas can be explained by the locations of the houses, which combines family agriculture with moderately preserved and dense wild vegetation.⁴³ Sand flies were more abundant in this rural area than in Aguas Blancas. Sampling sites 1 and 8 exhibited the greatest sand fly abundance (Figure 4). These insects can easily access the peridomiliary (and the intradomiliary) environment in almost all the houses.²¹ Therefore the risk of transmission of leishmaniasis will depend on the distance from the house to an infected wild reservoir. In fact, the absence of significant differences by age group may suggest home and/or peridomiliary exposure in Aguas Blancas and Media Luna-Algarrobito.^{21-23,35}

The under or overestimation of population sizes by linear regression represents a limitation of the present study. However, we consider that its purpose in seeking to demonstrate the occurrence of leishmaniasis cases and its maintenance over time does not affect our conclusions. Regarding entomological surveys, it is necessary to carry out prospective studies in order to confirm that less frequent sand fly species may be present in the study area but were undetected due to the limited capture effort.

Epidemiological patterns of transmission may be involved in the ecological scenarios of this border area between Bolivia and Argentina and might include peridomestic transmission in the

rural area as well as in the peri-urban area (Figures 3 and 4). Currently, prevention and control programs for leishmaniasis do not cover these scenarios, a fact that should be re-evaluated. For example, the use of mosquito nets and the implementation of entomological monitoring and fumigation over areas of risk, as well as the weeding of peridomiliary areas might have a positive impact in the current epidemiological situation. Many of the cases of patients from Bolivia may be due to agricultural, logging and/or recreational activities (hunting, fishing, etc.). Prevention policies should include health education on leishmaniasis in schools, the use of digital media to educate individuals on personal protection measures and measures to avoid exposing workers. The geographic funnel at border crossings can represent an important risk factor for travellers while they wait to complete their immigration and customs procedures (at times for hours) in areas that serve as a refuge for sand flies (Figure 3).

Conclusions

The majority of sand fly species reported in our study are suspected of transmitting parasites that cause leishmaniasis. The abundance of sand flies is higher in the rural area. Also, in the urban area, the highest number of sand flies is found at the margins of the city of Aguas Blancas. *Nyssomyia neivai* is the most prevalent species, being present in all the sites where sand flies were caught. In addition, *L. (V.) braziliensis* is endemic in the south of Bolivia and in the north of Argentina. With control measures being insufficient if carried out just by a single country at a border area, we propose further studies in border areas to provide the knowledge necessary to determine integrated international prevention measures.

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