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Epidemiological aspects of cutaneous leishmaniasis in the Iguazú falls area of Argentina

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A B S T R A C T

Over the last three decades the incidence of American cutaneous leishmaniasis (ACL) has increased sharply in Argentina and throughout the world. In the Iguazú Falls area, on the border between Brazil and Paraguay, the incidence of human ACL has risen since 2004. Most of the 36 cases of human ACL reported until 2005 have involved males over 15 years old (75%) infected during deforestation to establish individual farms. Captures carried out in primary forest, periurban areas, and deforested land sites yielded 18,438 sand flies belonging to 13 species; the most prevalent species were *Lutzomyia (Nyssomyia) whitmani* (87.4%) and *Lutzomyia (Mygonemyia.) migonei* (7.6%). Cluster analysis was used to group traps according to species and abundance of sand flies. The group of traps located in recently deforested places, in pig and chicken dwellings of houses where ACL cases had been reported in the past, and at one house with an active ACL case, had the highest abundance of *Lu. whitmani* and *Lu. whitmani* + *Lu. migonei* as well as the highest ratio of *Lu. whitmani/Lu. migonei*. *Leishmania* sp. infections, both in *Lu. whitmani*, in *Lu. quinquefer*, and in smears from human cases were detected by DNA kinetoplast amplification using a generic PCR protocol. The risk of ACL outbreak in the Iguazú Falls area is still associated with economic and leisure activities in primary-secondary forest, including deforestation, rural settlements, fishing, hunting, and ecotourism. In addition, the risk of periurban transmission seems likely, and this is discussed within the framework of surveillance and prevention strategies.

1. Introduction

The incidence of American cutaneous leishmaniasis (ACL) has increased steadily since the early 1980s, and it is spreading to new epidemiological scenarios. Man-made "ecological chaos" – from war, migration, unorganized peri-urbanization, deforestation, local climate change, HIV pandemics, and adaptation to the peridomicilary environment of permissive vectors – has been proposed as the main driver of this disease emergence (Desjeux, 2001; Campbell-Lendrum et al., 2001; Ashford, 2007; Shaw, 2007). Thus, any focus in a new eco-epidemiological scenario near populated cities should be characterized to assess epidemic trends.

In Argentina, the incidence of ACL due to *Leishmania braziliensis* has increased in peridomestic environments during the last two decades, and *Lutzomyia neivai* has been implicated as the main vector (Córdoba-Lanús et al., 2006; Salomón et al., 2006). The province of Misiones is located in northeastern Argentina in the subtropical area of Amazonian domain, and it is almost completely surrounded by rivers. Coniferous woods have replaced most of the native trees in the forest in the Iguazú Falls area (Iguazú National Park, Urugua-í Provincial Reserve) on the Border between Argentina, Brazil and Paraguay. Close to this area, 129 cases of ACL were reported in 1998 from a peri-urban neighborhood of Puerto Esperanza Village (incidence 4.2%), although only nine cases of ACL had been reported for the whole province from 1977 to 1997 (Salomón et al., 2001, 2002). Another 20 cases were reported during 2003–2004, clustered in time and space among deforestation-related workers with activities around the Urugua-í artificial lake area (incidence 5%), 15 km away from the previous focus (Salomón et al., 2006). Puerto Esper-

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anza outbreak was associated with peridomestic transmission and *Lu. neivai* abundance, whereas Urugua-i cases were associated with activities at the edge of the forest and *Lutzomyia whitmani* presence.

Since 2004, the rate of reported ACL cases from Puerto Iguazú has been increasing. Puerto Iguazú City is located at the trinational border and has a resident population of 31,000, and it has many more non-residents due to commercial and tourist activities. More than a million tourists from all over the world visit Iguazú Falls and the National Park every year. Therefore, in this ACL transmission scenario, large populations may be at risk, and there is also a lack of knowledge of the eco-epidemiology of emergent foci.

The aim of this work is to describe this new scenario for ACL transmission in the southernmost area of the Amazon region from entomological and parasitological perspectives. The results will contribute to a better understanding of the epidemic shift of ACL in America from sylvatic to periurban environments, thereby providing the basis for appropriate control measures.

2. Materials and methods

2.1. Study area and human cases

The study area was the department of the northern area of Iguazú in the province of Misiones, Argentina (25° 36′ S, 54° 35′ W). The area includes Puerto Iguazú City, the National Park, and the Provincial Natural Reserve. The city of Puerto Iguazú is surrounded by the border with Paraguay and Brazil on the shore of the Paraná-Iguazú rivers at altitudes ranging of 140–240 m above sea level. The phytogeographic area is classified as Paranaense Forest: a subtropical humid forest from the Amazonian domain (Cabrera, 1971). The area of primary-secondary forest known as “2000 hectáreas” (2K), which lies south of the city, west of Iguazú Falls National Park, and north of the Urugua-í Provincial Park, has experienced the following human interventions: (a) since 1950, wood extraction by the army; (b) in 2001–2002, marginal deforestation and human settlement, mainly in a narrow area bordering the road; (c) since 2003 and peaking in 2004, intense deforestation in patches of the remaining area by newly settled, individual farmers (Fig. 1).

The clinical files of the ACL cases diagnosed during 2004–2005 at the SAMIC Referential Hospital of Iguazú city were checked to compute statistics on sex, age, probable site of infection, and probable date of infection.

2.2. Sand fly collections

Adult sand flies were captured with minilight traps (Sudia and Chamberlain, 1962) operated from 17:00 to 09:00 h. The traps were placed at a height of 1.5 m and were shadowed by the canopy. Collections were performed at each site three times between 26 October and 12 November 2005. The traps were located at 14 different capture stations (Fig. 1), and 29 capture sites including different habitats of the domiciliary capture stations (Table 1). The capture stations H1 and H3 were houses where individuals that had ACL in July and August 2005 respectively lived; H2 was a house where an active ACL case was reported. All captured sand flies were stored dry (except those used for molecular research on parasites, which were kept at −20 °C) and identified using the keys of Young and Duncan (1994), with modifications by Marcondes (1996).

2.3. Sand fly abundance and diversity analysis

Cluster analyses were performed to differentiate sites according to the species composition and abundance. The matrix included the 3-day cumulative abundance of 10 species of phlebotomine caught at the 26 sites with sand flies. The three species of *Brumtomyia* were combined as *Brumptomyia* spp. because the females were morphologically indistinguishable; the F1, H7, and P2 sites with no sand flies were excluded. The Bray-Curtis quantitative dissimilarity coefficient was computed (relative distance from 0 for a pair of sites with identical taxonomic composition and abundance to 1) (Bray and Curtis, 1957), the group average was used as a group linkage method (Infostat, 2007^{TM}), and the 0.5 distance was used as a criterion for building the cluster. One-way ANOVA was used to test differences between groups for three variables: (a) *Lu. whitmani* + *Lu. migonei* abundance (*Lw* + *Lmg*), (b) *Lu. whitmani*/*Lu. migonei* rate (*Lw*/*Lmg*), and (c) *Lu. whitmani* abundance (*Lw*). Data were transformed by *ln* prior to statistical analysis in order to comply with the assumptions of normality and homocedasticity. When significant differences were found, means were compared using an *a posteriori* Tukey test (significance at $p \leq 0.05$) (Zar, 1996). In addition, a diversity index ($D = 1 - \Sigma pi^2$) was computed for all sites, *pi* is the proportion of sand flies of the species *i* in each capture site *p* (Krebs, 1978).

2.4. Molecular detection of parasites

2.4.1. Sand fly sample

From the original capture of sand flies, a random sample of 138 females and 10 males as a negative control for PCR was selected for molecular detection of the *Leishmania* sp. parasite. Females were pooled according the species and capture site in groups varying from 10 (*Lu. whitmani* × 12 pools), to 8 (*Lu. pessoai* × 1), 5 (*Lu quinquefer* × 1), 5 (*Lu. migonei* × 1) individuals, besides the pool of 10 males of *Lu. whitmani*. DNA extraction was performed by a protocol involving proteinase K digestion, phenol purification, and ethanol precipitation (Pita-Pereira et al., 2005). DNA was frozen at −20 °C until use.

2.4.2. Clinical samples

Samples were obtained from three patients with clinical symptoms; positive smears were taken as dermal scrapings from the border of the ulcers with a sterile wooden toothpick. The samples were taken at the SAMIC Hospital, Puerto Iguazú on 8–21 August 2005; the ulcers selected for scraping had been developing for 3–5 months. The probable site of transmission was used to select entomological trapping sites within 2K. Informed consent was obtained from all participants, and all procedures were in accordance with the Helsinki Declaration. The samples were stored in 200 μl of TE buffer. DNA extraction was performed by the boiling protocol, which involves heating the samples for 10 min then freezing them at −20 °C until use (Belli et al., 1998).

2.4.3. Analysis by polymerase chain reaction (PCR)

The PCR was performed as described by Belli et al. (1998). The genus–specific primers (13A, 13B) amplified a 120 bp product which target in the conserved block (CSB) of the DNA kinetoplast present in most species of the *Leishmania* genus. *L. braziliensis* HOM/BR75M2903 and the *L. amazonensis* IFLA/BR67/PH8 strains were employed as PCR controls. These strains were provided by the National Institute of Parasitology “Dr. Mario Fatala Chabén” (Buenos Aires, Argentina).

3. Results

During 2004–2005, 36 cases of ACL were reported by the referral Hospital of Puerto Iguazú; 27 (75%) were males, and 9 (25%) were younger than 15 years old. The geographic site where transmission was self-reported to occur was the 2K area for 31 (86%) cases, and another 4 (11%) were due to work related to primary forest and leisure activities, including the National Park,

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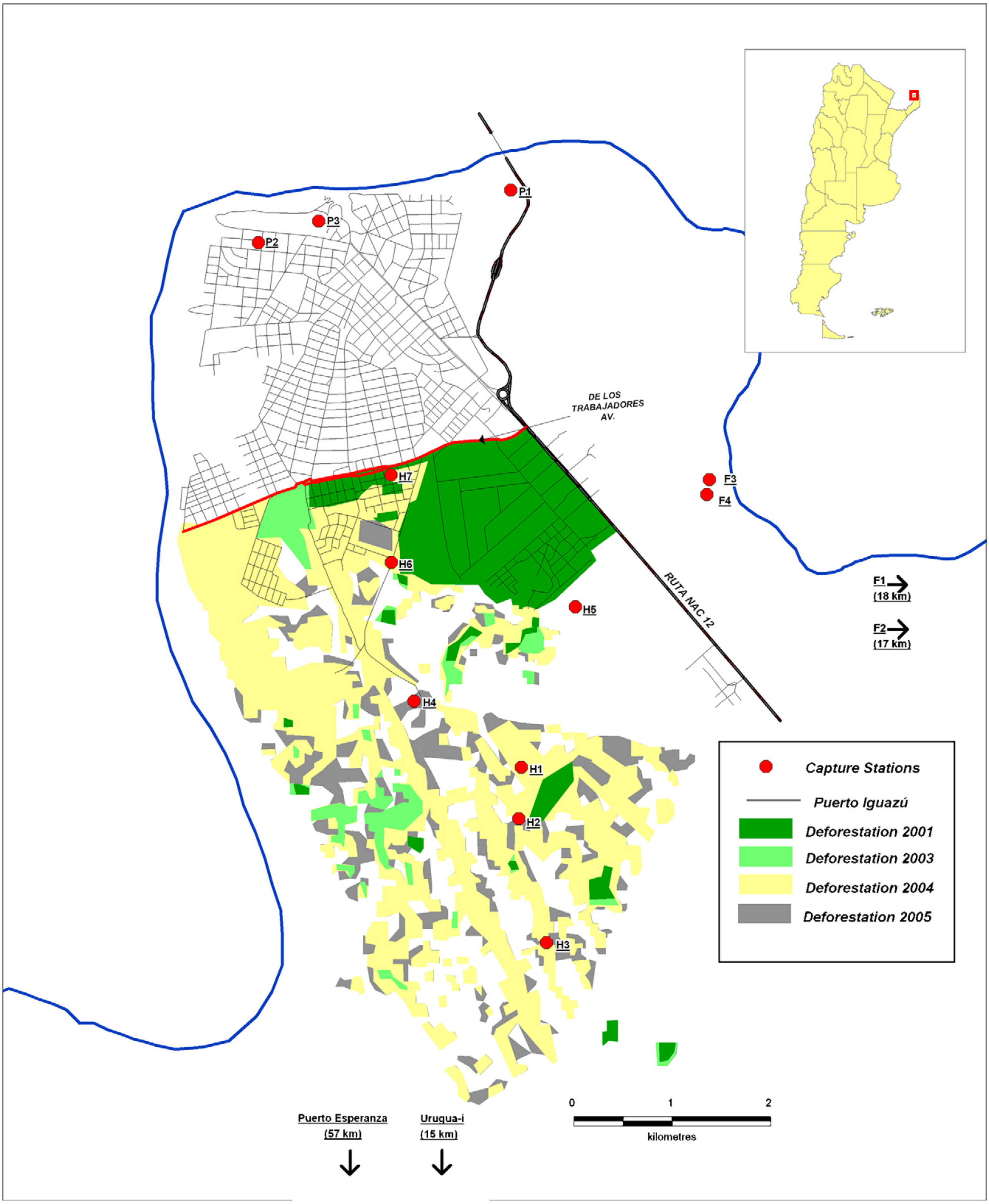

Fig. 1. Puerto Iguazú and surroundings, Misiones province, Argentina. Images show the sand fly capture sites in 2005 (F1 and F2 in the western part of Iguazú Falls National Park were excluded), and deforestation in the "2000 hectáreas" areas between 2001 and 2005. Capture sites coordinates: F1-25° 40′ 34″ S, 54° 26′ 46″ W, F2-25° 41′ 03″ S, 54° 26′ 50″ W, F3-25° 37′ 03″ W, 54° 32′ 25″, F4-25° 37′ 08″ W, 54° 32′ 26″ W, P1-25° 35′ 27″ S, 54° 33′ 37″ W, P2-25° 35′ 44″ S, 54° 35′ 09″ W, P3-25° 35′ 37″ S, 54° 34′ 47″ W, H1 25° 38′ 38″ S, 54° 33′ 34″ W, H2 25° 38′ 55″ S, 54° 33′ 35″ W, H3 25° 39′ 36″ S, 54° 33′ 25″, H4 25° 38′ 16″ S, 54° 34′ 13″ W, H5 25° 37′ 45″ S, 54° 33′ 14″), and H6 25° 37′ 30″ S, 54° 34′ 21″ W, H7 25° 37′ 01″ W, 54° 34′ 06″ W.

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Table 1
Cumulative captures and diversity index of Phlebotominae sand flies carried out between 26 October and 12 November 2005, in Iguazú, Argentina. Captures lasted three nights each and were done with CDC-minilight traps. Results are tabulated by species and site.

Site	Lw	Lmg	Lp	Lf	Lsh	Lmi	Lq	Lmo	Ln	Lc	Ba	Bp	Bg	BH	DI
F1															
F2						3		6				3			0.6250
F3										2					0.0000
F4	3		1											1	0.5600
H1h	9	3													0.3750
H1p	1,022	39	32	2											0.1268
H1e1	5													1	0.2778
H1e2														1	0.0000
H2h	2,133	41	64	13							2				0.1025
H2hu	29	5													0.2509
H2p	845	35	27	3		1		1	4		3			3	0.1577
H2e	1,139	13	47	4	5	7					7		2	18	0.1569
H3h	138	6		3	3										0.1512
H3hu	12														0.0000
H3p	2,635	215	119		13	2		1					1	2	0.2155
H3pu	2,246	820	174	26	32				3						0.4724
H3c	4,756	185	161	10	2						3			3	0.1348
H3e	3										1		1	10	0.3200
H4p	317	18	15	4				1			4	1	1	6	0.2486
H5b	778	14	15	14				1						1	0.1055
H5e1	18														0.0000
H5e2	18	9	6							1	2			2	0.6828
H5e3		2													0.0000
H5e4	1	2		1											0.6250
H6	3														0.0000
H7															
P1							11				1				0.1528
P2															
P3							1								0.0000
Total	16,110	1407	661	80	55	13	12	10	7	3	23	4	5	48	

F1 and F2 refer to Iguazú Falls National Park; F3 and F4, to Santa María sanctuary; P1, to the ParaguayArgentina bridge; P2, to the hotel shore; and P3, to the port. Within the area denominated "2000 hectareas," H1, H2, and H3 refer to houses where previous ACL cases had been reported; H4, to a camping area; H5, to "Guira Oga"; H6 and H7, to new neighbors. Letter indicate the following: h, inside the house; p, pigsty; c, chicken house; e, forest nearest edge; hu and pu, traps under the house and pigsty (palaphites). H5b is a biotery where mice and chicks are raised to feed carnivore birds. Lw: *Lu. whitmani*, Lmg: *Lu. migonei*, Lp: *Lu. pessoai*, Lf: *Lu. fischeri*, Lsh: *Lu. shannoni*, Lmi: *Lu. misionensis*, Lq: *Lu. quinquefer*, Lmo: *Lu. monticola*, Ln: *Lu. neivai*, Lc: *Lu. cortelezzii*, Ba: *Br. avellari*, Bp: *Br. pintoi*, Bg: *Br guimaraesi*. BH: *Brumptomyia* females (those of different species were morphologically indistinguishable), DI: Diversity index.

military exercises, and hunting. The cutaneous lesion was first observed by the patients between April and July of 2004 in 21 (78%) of the 27 cases for which the data were reported. The satellite images show intense deforestation during this period (Fig. 1).

Sand fly captures produced a total of 18,438 individuals belonging to 13 species: *Lu. (Nyssomyia) whitmani* (87.4% of flies collected), *Lu. (Mygonemyia) migonei* (7.6%), *Lu. (Pintomyia) pessoai* (3.6%), *Lu. (Pintomyia) fischeri, Lu. (Psathromyia) shannoni* (0.4%), *Lu. (Pintomyia) misionensis, Lu. (Micropygomyia) quinquefer, Lu. (Pintomyia) monticola, Lu. (Nyssomyia) neivai, and Lu. (Evandromyia) cortelezzii* (<0.1%), *Brumptomyia avellari, Br. pintoi, and Br. guimaraesi* (0.4%) (Table 1).

Cluster analysis grouped the collections of the traps located in 2K into several groups. The first group comprised traps in peridomestic environments – pig and chicken dwellings – of houses where ACL cases had been reported in the past, traps in the house and the forest edge at the site with an active ACL case (H2h, H2e), and traps outside a facility to breed chicken and mice within Güirá Oga Center for the reintroduction of native carnivorous birds (H5b) (Fig. 2). This group has the highest abundance of *Lu. whitmani* and *Lu. whitmani* + *Lu. migonei*, as well as the highest ratio of *Lu whitmani/Lu. migonei* (Table 2). The closest cluster to the former group includes the remaining pigpen in the camping area without permanent human inhabitants (H4p), and the house more distant from the road and closest to the primary forest main patch (H3h). Group 1 has significantly more total sand flies than group 2, and a ratio *Lw/Lmg* two times higher, although without significant difference (Table 2). The third main group (Group 4 in Table 2) involves traps at the house with a previous ACL case (H1h), traps located under the houses, and forest edge traps, but all the abundance parameters were significantly lower than in the former groups. The traps in the surroundings of Puerto Iguazú City were clustered because only *Lu. quinquefer* were caught (P1-3) but these results were not included in the cluster analysis to avoid distortions due to such a distinctive group. Capture sites in the recent periurban neighborhoods settled in the 2K border showed either no sand flies in places deforested in 2001 (H7) or scarce sand flies in the one with ongoing building (H6) (Table 1). Some traps in places with little landscape modification did not capture *Lu. whitmani* (F2, F3 and H1e2) or captured less *Lu whitmani*, than *Brumptomyia* sp. (H3e), so there were isolated by the cluster analysis (Fig. 2).

The trap under the pigsty closest to the primary forest (H3pu) had an exceptionally high abundance of *Lu. migonei* – in fact the abundance was four times greater than over the pigsty (H3p) – and H3pu abundance was not computed for the Lw/Lmg ratio of the group 4. If this trap was excluded, the remaining seven sites of group 1 showed diversity indexes ranging from 0.10 to 0.21, while 16 out of the other 18 grouped sites had diversity indexes below or above these values, clustering again the group 1 by this index (Table 1).

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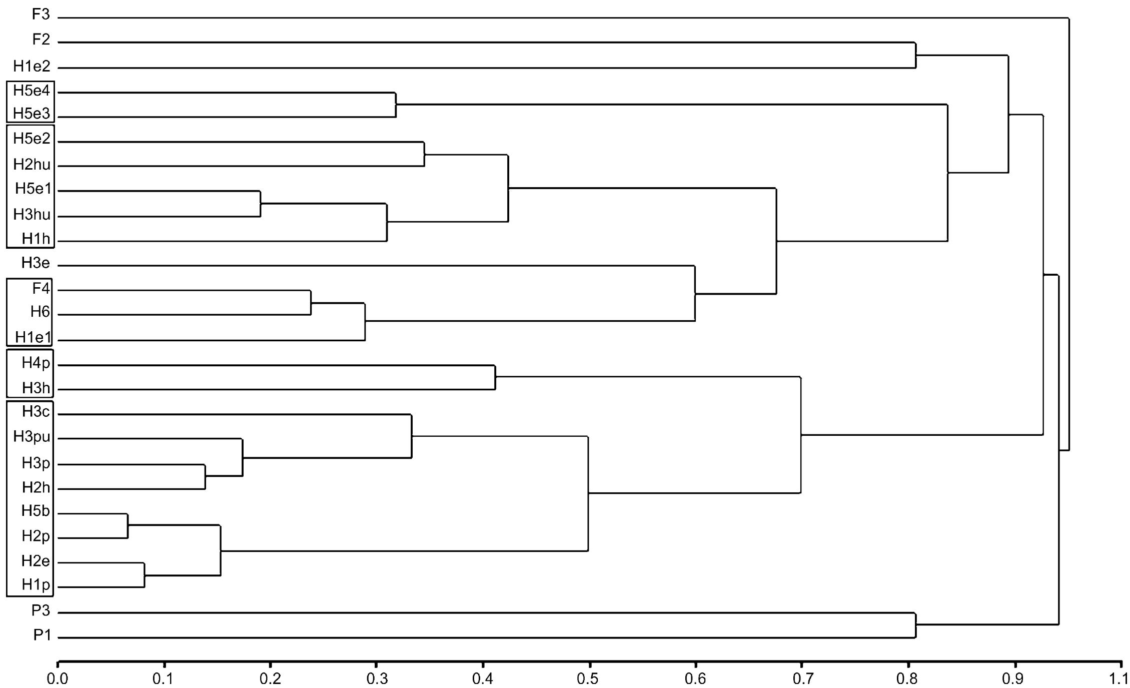

Fig. 2. Clustering analysis of phlebotomine sand fly cumulative captures (three nights), in Iguazú, Argentina, between 26 October and 12 November 2005. Clusters are framed by squares. Sites with no sand flies were excluded, the *Brumptomyia* species was added, and *Lu. quinquefer* captures were not computed. Distance measure: Bray-Curtis index (0.5 was used as the criterion for building groups). Group linkage method: group average.

All three human samples were positive for *Leishmania* sp. by PCR. Four of 16 *Lutzomyia* sp. pools employed for molecular analysis gave positive PCR results. Three pools corresponded to *Lu. whitmani* collected in H1p, H2e, and H3p (group 1); the fourth was *Lu. quinquefer* from P1. The negative ones were samples of *Lu whitmani* from H2e (3 pools), H2h (2 pools), H3c (2 pools), and one pool from H1p, H3p, *Lu migonei* H2e, *Lu pessoai* H1p, and *Lu whitmani* males H1p.

4. Discussion

The results presented here indicate that the ACL cases clustered in time and space in the Iguazú Falls area during 2004–2005 were associated mainly with *L. braziliensis* transmission by *Lu. whitmani*, which was related to the activities of settlers who breed chickens and pigs in recently deforested patches in the area known as "2000 hectáreas." An ongoing qualitative assessment of social exposure to risk showed *Leishmania* infection during poaching in natural reserves and fishing in the Urugua-í artificial pond. In the years leading up to 2004, several cases were also associated with *Lu. whitmani* and deforestation in areas other than the "2000 hectáreas" study area around the Urugua-í Provincial Reserve (Salomón et al., 2006).

The relative abundance of *Lu. whitmani* and *Lu. migonei* was significantly higher in the animal dwellings close to the ecotone of recent deforested patches. The sand fly traps at these sites clustered as a single group, and these were also the only source of infected *Lu. whitmani* and the primary source of ACL human cases. *Lu. whitmani* and *Lu. migonei* have already been reported in captures performed with light and Shannon traps in other ACL foci; they are prevalent in homes (Loiola et al., 2007; Missawa and Maciel, 2007), they are strongly attracted by hens close to residual forests (Lonardoni et al., 2006; Teodoro et al., 2007), and they are opportunistic feeders in domestic environments (Muniz et al., 2006). Both species have been incriminated as *L. braziliensis* vectors (Rangel and Lainson, 2003), and *Lu. migonei* has been suggested to link the zoonotic and anthropozoonotic cycles (Chaves and Añez, 2004). From the perspective of disease control, it is noteworthy *Lu. whitmani* and *Lu migonei* captures in the space under the pigpen, a potential resting or breeding site for sand flies. *Lu. migonei* relative abundance is higher below than above the pigpen, this species was usually found close related to domestic animals (Rangel and Lainson, 2003; Salomón et al., 2006). Neither *Lu. migonei* or *Lu. pessoai* were found to be infected in the pools tested in this study, but *Lu. quinquefer* collected from the periurban surroundings of Puerto Iguazú and the international border area were infected.

Table 2
Characterization of groups identified by the cluster analysis in Fig. 1 according to *Lu. whitmani* abundance *(Lw)*, *Lu. whitmani* + *Lu. migonei* abundance (*Lw* + *Lmg*), and *Lu. whitman/Lu. migonei* rate (*Lw/Lmg*) by site.

Group	Sites	*Lw* (mean; S.D.)	*Lw* + *Lmg* (mean; S.D.)	*Lw*/*Lmg* ratio (mean; S.D.)
1	H1p, H2p, H3p, H3pu, H3c, H2h, H2e, H5b	1944.25; 1340.43^{A}	2114.50; 1451.51^{A}	40.5; 26.04^{A}
2	H3h, H4p	227.50;126.57^{B}	239.50; 135.06^{B}	20.31; 3.18^{A}
3	H6, H1e, F4	3.67; 1.15^{D}	3.16; 1,15^{D}	
4	H1h, H3hu, H5e1, H2hu, H5e2	17.20; 7.66^{C}	20.60; 9.69^{C}	3.60; 1.97^{B}
5	H5e3, H5e4		2.50; 0.71^{D}	

^{AD} Different letters show significant differences ($p \leq 0.05$). See Table 1 for explanation of the sites. Nongrouping sites and sites with no sand flies were excluded. The results from H3pu were excluded because of the exceptionally high relative proportion of *Lu. migonei*.

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Lu. neivai is usually the incriminated ACL vector in Argentina (Salomón et al., 2006). This report, therefore, provides the first evidence of an ACL outbreak in the country with *Lu whitmani* as the suspected vector. It is reasonable to suspect *Lu whitmani* because of its prevalence, spatial distribution, and natural infection with *Leishmania* sp. *Lu. whitmani* was recorded in French Guyana, Peru, Paraguay, Argentina, and 26 states of Brazil, and it is incriminated as the vector of *L. braziliensis* in sylvatic Amazonia cycles as well as in peridomestic ones (Rangel and Lainson, 2003; Leonardo and Rebêlo, 2004; Oliveira-Pereira et al., 2006; da Costa et al., 2007).

The domiciliation of *Lu. whitmani* is an event that could conceivably happen across the entire range of its distribution as a result of local evolutionary pressures. Though geographically distant populations of *Lu. whitmani* show different habitat preferences, degrees of adaptation to human environments, anthropophily, and endophagy, these populations show a continuum of inter-breeding mitochondrial lineages, and peridomestic populations conserve sylvatic haplotypes at least two decades after deforestation (Ready et al., 1998; Campbell-Lendrum et al., 1999a,b, 2000; Ishikawa et al., 1999). Further, introgression between highly domesticated *Lu. intermedia* and both *Lu. neivai* and *Lu. whitmani* have been observed (Marcondes et al., 1997; Mazzoni et al., 2006).

The captures in deforested peridomestic habitats (Hp) were significantly higher than those in the respective forest edges (except H2p-H2e with an active ACL case), and those from forested capture stations (F). Abundant sandfly populations in areas that have been recently deforested could lead to increased vector-human contact or vector adaptation to the peridomciliary environment (Walsh et al., 1993; Campbell-Lendrum et al., 2001; Rotureau et al., 2006; Ashford, 2007). With regard to Puerto Iguazú, the increased investment in forestry together with the protection of large properties as federal, county, or private natural reserves reduced the land available for urban spread. All these issues came together to make “2000 Hectáreas” a coveted large landed estate. Although human intervention in this area started with wood extraction around 1950, deforestation to settle periurban houses began in 2001, and increased sharply during 2003–2004 with patch deforestation by individual farmers. This process has been reinforced in recent decades by land overvaluation because of its attractiveness to tourist/eco-tourist operators and forestry related activities (Mastrangelo, 2006).

In conclusion, ACL in the Iguazú Falls area is still associated with primary-secondary forest: new settlers, deforestation workers, native Americans living within the residual forest, builders of tourist dwellings, park rangers, military personnel, and local and non-resident people who engage in economic or leisure activities. In this scenario, prevention should focus on individual protection and small-scale short-term physical or chemical barriers. Any development project in the area that involves deforestation or man-made alteration of the environment should be monitored for ACL risk.

In addition to the immediate risk of ACL infection following deforestation (Ashford, 2007) or accidental contact with forest “hot spots,” there is potential risk of ACL periurbanization due to *Lu. whitmani* peridomestic distribution or *Lu. neivai*–*Lu. quinquefer* peridomestic outbursts in an area where *L. braziliensis* already circulates (Shaw, 2007). Thus, regular trapping of sand flies at sentinel sites should be used to monitor periurban transmission. Further, as the risk of transmission of leishmaniasis varies according to the disease and risk perception, animal management, and settlement strategies, the cultural perspective of the medical anthropology and other disciplines should be incorporated to ACL operational control researches.

In the present work we performed multiple simultaneous captures to determine the spatial distribution of vector abundance, and so the risk of human-vector effective contact at focus scale, immediately after the ACL transmission peak. However, in order to understand the sand fly population structure and dynamics, longitudinal captures at representative sites are carrying on to propose prevention and control measures based on evidence.

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References

Ashford, R.W., 2007. Disease as a stabilizing factor in the protection of landscape: the Leishmaniases as models. Ecohealth 4, 99–103.
Belli, A., Rodriguez, B., Aviles, H., Harris, E., 1998. Simplified polymerase chain reaction detection of new world *Leishmania* in clinical specimens of cutaneous Leishmaniasis. Am. J. Trop. Med. Hyg. 58, 102–109.
Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349.
Cabrera, A.L., 1971. Fitogeografía de la República Argentina. Bol. Soc. Argent. Bot. 14, 1–50.
Campbell-Lendrum, D.H., Pinto, M.C., Brandão-Filho, S.P., de Souza, A.A., Ready, P.D., Davies, C.R., 1999a. Experimental comparison of anthropophily between geographically dispersed populations of *Lutzomyia whitmani* (Diptera: Psychodidae). Med. Vet. Entomol. 13, 299–309.
Campbell-Lendrum, D.H., Brandão-Filho, S.P., Ready, P.D., Davies, C.R., 1999b. Host and/or site loyalty of *Lutzomyia whitmani* (Diptera: Psychodidae) in Brazil. Med. Vet. Entomol. 13, 209–211.
Campbell-Lendrum, D.H., Brandão-Filho, S.P., Pinto, M.C., Vexenat, A., Ready, P.D., Davies, C.R., 2000. Domesticity of *Lutzomyia whitmani* (Diptera: Psychodidae) populations: field experiments indicate behavioural differences. Bull. Entomol. Res. 90, 41–48.
Campbell-Lendrum, D., Dujardin, J.P., Martínez, E., Feliciangeli, M.D., Pérez, J.E., Silans, L.N., Desjeux, P., 2001. Domestic and peridomestic transmisssion of American cutaneous leishmaniasis: changing epidemiological patterns present new control opportunities. Mem. Inst. Oswaldo Cruz 96, 159–162.
Chaves, L.F., Añez, N., 2004. Species co-occurrence and feeding behavior in sand fly transmission of American cutaneous leishmaniasis in western Venezuela. Acta Trop. 92, 219–224.
Córdoba-Lanús, E., Lizarralde de Grosso, M., Piñero, J.E., Valladares, B., Salomón, O.D., 2006. Natural infection of *Lutzomyia neivai* with *Leishmania* spp. in northwestern Argentina. Acta Trop. 98, 1–5.
da Costa, S.M., Cechinel, M., Bandeira, V., Zannuncio, J.C., Lainson, R., Rangel, E.F., 2007. *Lutzomyia (Nyssomyia) whitmani* s.l. (Antunes & Coutinho 1939) (Diptera: Psychodidae: Phlebotominae): geografical distribution and the epidemiology of American cutaneous leishmaniasis in Brazil. Mem. Inst. Oswaldo Cruz 102, 149–153.
Desjeux, P., 2001. The increase in risk factors for leishmaniasis worldwide. Trans. R. Soc. Trop. Med. Hyg. 95, 239–243.
Ishikawa, E.A., Ready, P.D., de Souza, A.A., Day, J.C., Rangel, E.F., Davies, C.R., Shaw, J.J., 1999. A mitochondrial DNA phylogeny indicates close relationships between populations of *Lutzomyia whitmani* (Diptera: Psychodidae Phlebotominae) from the rain-forest regions of Amazônia and northeast Brazil. Mem. Inst. Oswaldo Cruz 94, 339–345.
Krebs, C.J., 1978. Ecology: The Experimental Analysis of Distribution and Abundance, 2nd ed. Harper & Row Publishers, New York, NY, USA, 678 pp.
Leonardo, F.S., Rebêlo, J.M., 2004. A periurbanização de *Lutzomyia whitmani* em área de foco de leishmaniose cutânea, no estado de Maranhão. Bras. Rev. Soc. Bras. Med. Trop. 37, 282–284.
Loiola, C.F., Silva, D.A., Galati, E.A., 2007. Phlebotomine fauna (Diptera: Psychodidae) and species abundance in an endemic area of American cutaneous leishmaniasis in southeastern Minas Gerais. Bras. Mem. Inst. Oswaldo Cruz 102, 581–585.
Lonardoni, M.V., Silveira, T.G., Alves, W.A., Maia-Elkhoury, A.N., Membrive, U.A., Membrive, N.A., Rodrigues, G., Reis, N., Zanzarini, P.D., Ishikawa, E., Teodoro, U., 2006. Leishmaniose tegumentar americana humana e canina no Município de Mariluz, Estado do Paraná. Bras. Cad. Saúde Pública 22, 2713–2716.
Marcondes, C.B., 1996. Redescription of *Lutzomyia (Nyssomyia) intermedia* (Lutz & Neiva, 1912), and resurrection of *L. neivai* (Pinto, 1926) (Diptera, Psychodidae Phlebotominae). Mem. Inst. Oswaldo Cruz 97, 457–462.

Marcondes, C.B., Day, J.C., Ready, P.D., 1997. Introgression between *Lutzomyia intermedia* and both *Lu. neivai* and *Lu. whitmani*, and their roles as vectors of *Leishmania braziliensis*. Trans. R. Soc. Trop. Med. Hyg. 91, 725–726.

Mastrangelo, A., 2006. Miserias preciosas. Trabajo infantil y género en minería artesanal (Misiones, Argentina). In: CETEM-CNOq (Ed.), Trabalho infantil e gênero na pequenha mineração sul americana. PROSUL, Rio de Janeiro, Brasil, pp. 135–151.

Mazzoni, C.J., Souza, N.A., Andrade-Coelho, C., Kyriacou, C.P., Peixoto, A.A., 2006. Molecular polymorphism, differentiation and introgression in the period gene between *Lutzomyia intermedia* and *Lutzomyia whitmani*. BMC Evol. Biol. 6, 85.

Missawa, N.A., Maciel, G.B., 2007. List of species in the genus Lutzomyia, Franca, 1924 (Psychodidae Phlebotominae) from the State of Mato Grosso. Rev. Soc. Bras. Med. Trop. 40, 11–14.

Muniz, L.H., Rossi, R.M., Neitzke, H.C., Monteiro, W.M., Teodoro, U., 2006. Estudo dos hábitos alimentares de flebotomíneos em área rural no sul do Brasil. Rev. Saude Publica 40, 1087–1093.

Oliveira-Pereira, Y.N., Rebelo, J.M., Moraes, J.L., Pereira, S.R., 2006. Diagnóstico molecular da taxa de infecção natural de flebotomíneos (Psychodidae Lutzomyia) por Leishmania sp na Amazônia maranhense. Rev. Soc. Bras. Med. Trop. 39, 540–543.

Pita-Pereira, D., Alves, C.R., Souza, M.B., Brazil, R.P., Bertho, A.L., Figueiredo, B.A., Britto, C.C., 2005. Identification of naturally infected *Lutzomyia intermedia* and *Lutzomyia migonei* with *Leishmania (Viannia) braziliensis* in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridisation assay. Trans. R. Soc. Trop. Med. Hyg. 99, 905–913.

Rangel, E.F., Lainson, R., 2003. Ecologia das leishmanioses. In: Rangel, E.F., Lainson, R. (Eds.), Flebotomíneos do Brasil. Editora Fiocruz, Rio de Janeiro, pp. 291–310.

Ready, P.D., de Souza, A.A., Rebelo, J.M., Day, J.C., Silveira, F.T., Campbell-Lendrum, D., Davies, C.R., Costa, J.M., 1998. Phylogenetic species and domesticity of *Lutzomyia whitmani* at the southeast boundary of Amazonian Brazil. Trans. R. Soc. Trop. Med. Hyg. 92, 159–160.

Rotureau, B., Joubert, M., Clyti, E., Djossou, F., Carme, B., 2006. Leishmaniasis among gold miners, French Guiana. Emerg. Infect. Dis. [serial on the Internet]. July 2006. Available from http://www.cdc.gov/ncidod/EID/vol12no07/05-1466.htm.

Salomón, O.D., Sosa Estani, S., Monzani, A.S., Studer, C., 2001. Brote epidémico de leishmaniosis tegumentaria en Puerto Esperanza, provincia de Misiones 1998. Medicina (Buenos Aires) 61, 385–390.

Salomón, O.D., Rossi, G.C., Spinelli, G.R., 2002. Ecological Aspects of Phelobotomine (Diptera, Psychodidae) in an Endemic Area of Tegumentary Leishmaniasis in the Northeastern Argentina 1993–1998. Mem. Inst. Oswaldo Cruz 97, 163–168.

Salomón, O.D., Orellano, P.W., Quintana, M.G., Pérez, S., Sosa Estani, S., Acardi, S., Lamfri, M., 2006. Transmisión de la leishmaniasis tegumentaria en Argentina. Medicina (Buenos Aires) 66, 211–219.

Shaw, J., 2007. The leishmaniasis—survival and expansion in a changing world. A mini-review. Mem. Inst. Oswaldo Cruz 102, 541–547.

Sudia, W., Chamberlain, R.W., 1962. Battery operated light trap, an improved model. Mosq. News 22, 126–129.

Teodoro, U., Lonardoni, M.V., Silveira, T.G., Dias, A.C., Abbas, M., Alberton, D., dos Santos, D.R., 2007. Luz e galinhas como fatores de atração de *Nyssomyia whitmani* em ambiente rural, Paraná. Bras. Rev. Saúde Pública 41, 383–388.

Walsh, J.F., Molyneux, D.H., Birley, M.H., 1993. Deforestation: effects on vector-borne disease. Parasitology 106 (Suppl.), S55–75.

Young, D.G., Duncan, M.A., 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies Central and South America (Diptera: Psychodidae). Mem. Am. Entomol. Inst. 54, 1–881.

Zar, J.H., 1996. In: Zar, J.H. (Ed.), Biostatistical Analysis, 3rd ed. Prentice-Hall, Upper Saddle River, New Jersey, NJ, USA, p. 662.

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