

## Tegumentary leishmaniasis outbreak in Bella Vista City, Corrientes, Argentina during 2003

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*Bella Vista City, Corrientes, Argentina, reported an epidemic outbreak of tegumentary leishmaniasis during 2003. The mean age of the 31 cases was  $25.0 \pm 13.7$  years old, with a sex ratio male:female 1.8, and without mucosal involvement. They clustered in two contiguous neighbourhoods, 96% in the periurban border and 4% in the peripheral outskirts. The transmission peak was estimated to have occurred during April 2003. Four species (3608 sand flies) were captured in nine sites: *Lutzomyia neivai* (90.1%), *Lu. pessoai* (8.9%), *Lu. migonei* (0.8%), and *Brumptomyia avellari* (0.2%). The outskirts/rural capture ratio of *Lu. neivai* was up to 3, and the outskirts/periurban up to 200. Therefore, the 'urban' transmission in this southernmost known focus is still an ecotone-border associated risk. The changes in human distribution or activities, patches of the secondary vegetation, periurban streams, rainfall of the previous year, and river period floods could all contribute to 'urban' outbreaks in the region. Tegumentary leishmaniasis risk should be assessed for any project that involves changes in land use throughout an endemic area.*

Key words: leishmaniasis - *Lutzomyia neivai* - urbanization - ecoepidemiology - Argentina

Tegumentary leishmaniasis (TL) is endemic in area of Northern Argentina that includes nine political provinces and three bio-regions: the 'Yungas' and 'Paranaense' subtropical forests, and the xeric 'Chaco'. Although TL was identified in Argentina almost a century ago, the first recorded outbreak took place in 1984-1987 in the 'Yungas' region, and this was found to be due to *Leishmania braziliensis* (Sosa Estani et al. 2000, Segura et al. 2000). After this outbreak, the relative increased incidence of TL both children and females suggested a transmission shift from the forest to peridomestic habitats (Sosa Estani et al. 2001, Sosa Estani & Salomon 2002). In addition, since the early 1980s the re-emergence of TL has been reported for many countries in the region, and urban transmission was proposed by many other investigators (Mott et al. 1990, Desjeux 2001, Campbell-Lendrum et al. 2001, Bejarano et al. 2002, Leonardo & Rebêlo 2004, Lemos & Lima 2005).

*Lutzomyia intermedia sensu lato*, currently termed *Lu. neivai* (Marcondes 1996), is widely distributed in the province of Corrientes. This species of *Lutzomyia* was first recorded in Corrientes City in 1926 and again in 1997-2000 (Bejarano & Duret 1950, Borda et al. 2002), it was also reported in Santo Tomé, Colonia Pellegrini, and Apipé Grande (San Antonio) during 1951 (Duret 1952), in Santa Tecla, Ituzaingó, Villa Olivari and Ita-Ibaté (Spinelli et al.

1999, Salomón et al. 2002) during 1993-1998, and in Bella Vista in 1998 (Borda et al. 1998b). Other phlebotomine species also found in the province of Corrientes include *Lu. cortelezzii*, *Lu. migonei*, *Lu. pessoai*, *Lu. shannoni*, *Lu. fischeri*, *Brumptomyia guimaraesi*, and *Br. avellari*, the former four species were recorded in Corrientes City (Borda et al. 2002), the last seven were recorded in Santa Tecla and the last species was found in Ituzaingó (Spinelli et al. 1999, Salomón et al. 2002) (Fig. 1)

The average incidence of TL in the province of Corrientes, in the 'Paranaense' northeastern region was 2.2, 6.8, and 11.7 cases/year for the whole province in the periods 1955-1974, 1975-1994, and 1995-2004 respectively, according to the records of the National Surveillance System and the Dermatologic Dispensary of Corrientes.

Bella Vista City, on the Paraná river shore, according to the Dermatologic Dispensary had two TL cases in 1988, one in 1991, and six in 1998. However during 2003 an outbreak took place in the City, the first recorded for the province, with suspected urban transmission. It was the southernmost known epidemic focus, and one of the scarce epidemics located in the eastern region of Argentina. Thus, this study was conducted in order to define the distribution of vectors and cases, and the risk factors for TL infection in this area. The results characterize the risk distribution of TL infection in time and space, and are discussed in the frame of the TL urbanization hypotheses and the appropriate strategies of surveillance and control.

### MATERIALS AND METHODS

*Study area - Bella Vista:* Bella Vista City (28°31'LS, 59°02'LV), Bella Vista department, province of Corrientes,

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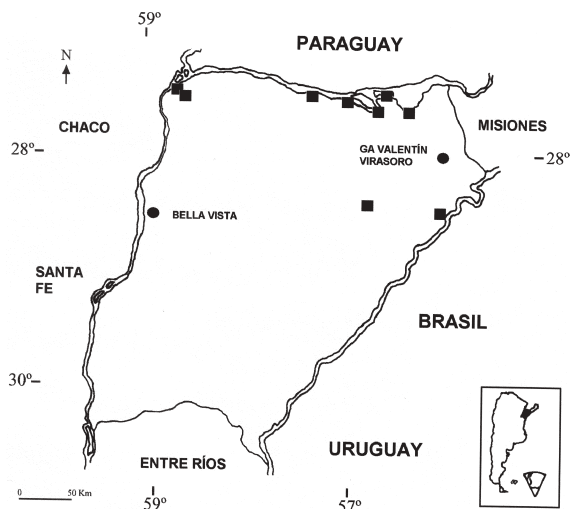


Fig. 1: province of Corrientes, records of phlebotomine in previous reports (squares), Bella Vista where the captures of this study were performed, and GA Valentín Virasoro with captures reported in the discussion (dots).

140 km south of Corrientes City. The city is located 32 m above the Parana river and 66 m above sea level, in front of the Ramsar site 'Jaakanigás'. The climate is subtropical-tropical without a dry season, although 60% of the precipitation falls in summer and autumn. The annual variation of temperature is small with a mean temperature of 20°C. Bella Vista is close to the isohyetal line of 1100 mm, and the relative humidity throughout the year was found to be an average of 71%. The area belongs to the bioregion 'Chaco oriental', in transition to the 'Paranaense' region (Cabrera 1976). Still water pools are extensively dispersed and occasionally discharge water into the Parana river. The river shore alternates between gallery forests, pioneer woods in overflowed banks, *Butia yatay* palm forests, and seasonally flooded 'carrizales' (Carnevali 1994). The 'carrizales' are dense herbaceous patches of *Panicum grumosum* and *P. rivulare* up to 5 m height, that keep the soil saturated with water all the year round. This study was performed in 'Florida', a periurban neighbourhood of Bella Vista on the border of the city, and in 'Epam', a contiguous peripheral neighbourhood with lower density of houses, outside the urban edge (Fig. 2).

**Epidemiological studies** - Descriptive and matched case-control studies were conducted, the matched case-control studies was designed to define risk factors to have TL in the area with epicentre in Bella Vista. **TL case definition:** a person with skin ulcerations consistent with TL and positive parasite confirmation performed on a smear of the scrapings. **Control definition:** a person without skin ulcerations or scars consistent with TL. Acceptable age differences between cases and controls were  $\pm 3$  years for cases aged  $\geq 10$  years,  $\pm 2$  years for cases aged 6-9 years,  $\pm 1$  year for cases aged 1-5 years, and the cases of  $< 12$  months old were paired with  $< 12$  months old con-

trols. Two controls were randomly selected among the city blocks or area surrounding the residence of each case (two TL cases had only one control). After the informed consent, both cases and controls were examined by a physician, and a questionnaire was performed asking about regular habits, work and spare time activities, household and peridomestic features, wild and domestic animals occurrence, and surrounding landscape description. The probable date of infection was assumed to be 21 days prior to the self-reported beginning of the skin ulceration (Sosa Estani & Salomon 2002). Fisher's exact or  $\chi^2$  tests were performed on proportional data, the difference between means by normal distribution with ANOVA test, and Wilcoxon test in the non-parametric analyses. Barlett's test was used to analyse the homogeneity of the data to be contrasted. Statistical significance was assumed with a value of  $P < 0.05$ . The Odds Ratio (OR) was taken as a risk estimator with a confidence interval of 95% level of significance. The variables related with habits and household factors (domestic and peridomestic) were ordered and analysed with EpiInfo software for Windows (3.2.2. DCD version)

**Entomological study - Bella Vista:** light minitraps CDC like were settled from 20.00 to 8.00 h in nine sites simultaneously for three consecutive nights, between October 14th and 17th 2003 (minimal temperature 19°C). Sites (Fig. 2) - A: Epam peripheral neighbourhood (PN); A.1: PN extradomestic: 28°29'13"LS, 59°01'57" LW, 70 m from the house with a case, in secondary vegetation-banana trees ecotone, 1 km from Florida neighbourhood; A.2: PN peridomestic: 5 m from the house; A.3: PN shore:

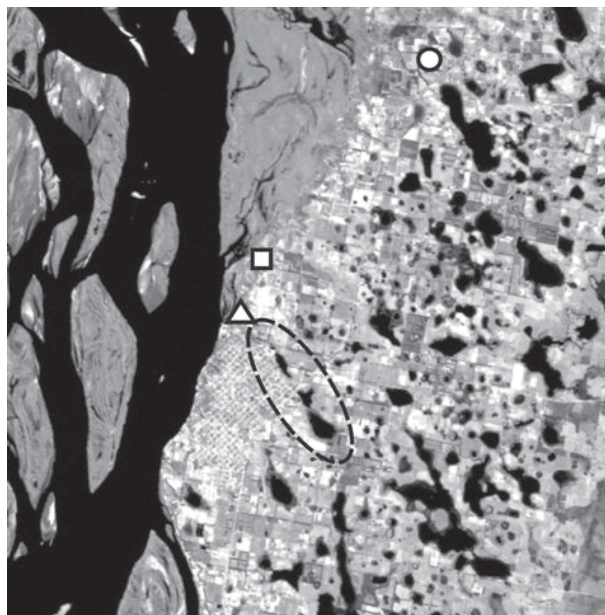


Fig. 2: Landsat 7 TM image from 4/8/2003, Bella Vista, province of Corrientes. Phlebotomine capture rural station (circle), 'Epam' peripheral neighbourhood ecotone site (square), 'Florida' periruban marginal station (triangle). The dot-lined ellipse includes the natural still water pools that drain on the water stream in the edge of the periruban neighbourhood.

28°29'21"LS, 59°02'07" LW, 'carrizal' in clay alluvial land used after the seasonal overflowing of the river to make bricks (work related risk); B: Florida periurban neighbourhood (UN) in the house of cases; B.1: UN peridomestic 1: 28°29'42"LS, 59°02'19" LW, family farm contiguous to Epam neighbourhood; B.2: UN peridomestic 2: 28°29'44"LS, 59°02'21" LW, on the shore of a water stream; B.3: UN peridomestic 3: 28°21'50"LS, 59°02'12" LW, on an vegetated alley; C: R: 28°27'02"LS, 59°00'03" LW, rural house with cases during 1998, 6 km from Florida; C.1: R extradomestic: secondary vegetation 100 m from the house; C.2: R peridomestic: 15 m from the house in a chicken dwelling; D: Farm, 28°29'45"LS, 59°02'25" LW, 'Alto del gallero' extensive area of fruit trees mainly citrus and strawberry cultures (risk site according to the community perception). All the phlebotomine were kept dry until they were treated with phenol-lactic acid, and identified according to the keys of Young and Duncan (1994), and Marcondes modifications (1996). Fisher's exact test or  $\chi^2$  statistical tests were performed, and the results were assumed to be significant when they had associated probabilities of  $P < 0.05$ .

Weather data were provided by the experimental agriculture station INTA Bella Vista, the river height data by the local navy station of the 'Prefectura Naval Argentina' at Bella Vista Port, and the satellite images by the aerospace national agency CONAE. Six Landsat 7 ETM images were used from the study area (Path/Row 226/80), from April 2001, January, March, May, and December 2002, and April 2003. The images were georeferenced using the satellite ephemerides and the Nearest-Neighbour Method. The areas covered with water were estimated based on band 5 (mid-infrared), the pixels with values of the digital numbers (DN) between 0 and 22 were assumed as covered with water.

RESULTS

There were found 31 cases in Bella Vista during October 2003 according to the case definition. The age distribution and localization of the lesions are shown in Table I. The male:female ratio was 1.8 but the age distribution did not differ significantly between genders. All the cases had skin lesions, whereas no mucosal involvement was observed. The median period of ulcer evolution up to the diagnosis was 90.0 days, range 15-240 days. All the cases healed after the standard chemotherapy (pentavalent antimonials 20 mg/kg/day). The residence of the cases was 96% in the periurban neighbourhood Florida, and the remaining 4% in the peripheric and contiguous neighbourhood Epam. The time distribution of cases, according to the probable date of infection (Fig. 3), suggests a transmission peak in April 2003, ending in July 2003. Twenty five cases were included in the case-control study, 6 of them were under 15 years old. The case distribution by age, lesion location, and occupation is shown in Table I.

Seventy-three questionnaires were obtained (25 cases and 48 controls). The sex distribution and mean age between cases and controls did not differ significantly. The frequency of exposure among cases and controls was significantly different in relation to questions about "to work in the Carrizal", and "to have a house-water stream < 500 m". However, only "to have cats in the house" was a protective factor according to the paired analysis (Table II).

In Bella Vista 3608 phlebotomine of four species were captured: *Lu. neivai* (90.1%), *Lu. pessoai* (8.9%), *Lu. migonei* (0.8%), and *Br. avellari* (0.2%) (Table III). The proportion of species different from *Lu. neivai* captured in the outskirts habitats (periurban and peripheral) was 0.2 % while in the rural sites it was 26.7%, mainly *Lu. pessoai* (24.3%). The *Lu. neivai* captured either of each

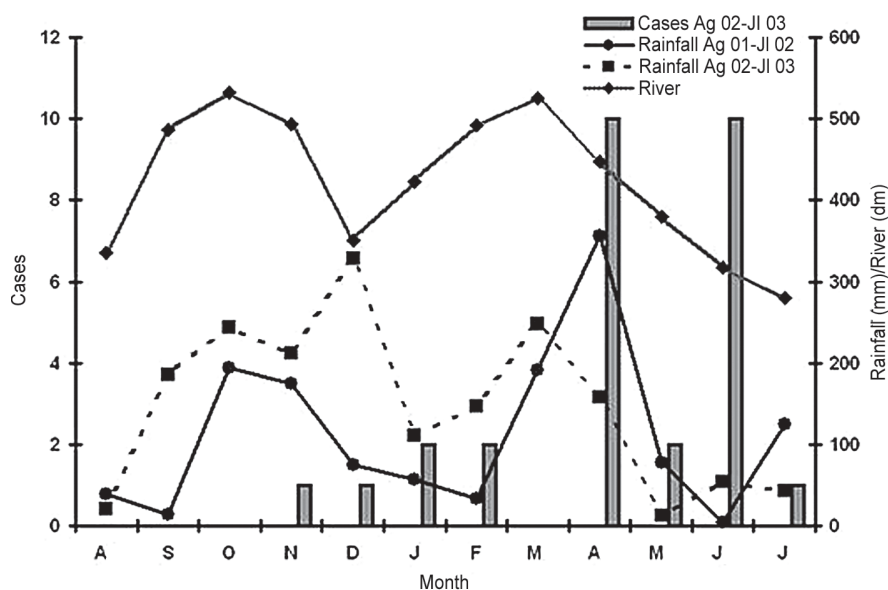


Fig. 3: cases of tegumentary leishmaniasis in Bella Vista, province of Corrientes by month according to the probable date of infection from August 2002 to July 2002. Bella Vista cumulative rainfall records (mm) by month from August 2001 to July 2002, and August 2002 to July 2003. Maximum height of the river (m), Bella Vista Port, from August 2002 to July 2003.



TABLE I

Age distribution, localization of lesions, and occupation of cases with tegumentary leishmaniasis. Bella Vista, province of Corrientes, Argentina, 2003

|   | N (%)     |
|---|-----------|
| Age group                                   | 31 (100)  |
| 1-9 years old                               | 2 (6.5)   |
| 10-19 years old                             | 9 (29.0)  |
| 20-29 years old                             | 11 (35.5) |
| 30-39 years old                             | 4 (12.9)  |
| 40-49 years old                             | 2 (6.5)   |
| 50-59 years old                             | 3 (9.7)   |
| Localization of lesions                     | 31 (100)  |
| Legs and feet                               | 13 (41.9) |
| Arms and hands                              | 8 (25.8)  |
| Trunk                                       | 3 (9.7)   |
| Head/neck                                   | 3 (9.7)   |
| Multiple lesions                            | 4 (12.9)  |
| Occupation                                  | 19 (100)  |
| Brick-maker                                 | 4 (21.1)  |
| Farmer                                      | 2 (10.5)  |
| House-wife                                  | 3 (15.8)  |
| Handicrafting                               | 2 (10.5)  |
| Without occupation                          | 2 (10.5)  |
| Others (mason, traveler, greengrocer, etc.) | 5 (26.3)  |
| Without data                                | 1 (5.3)   |

Age distribution and localization of lesions total N = 31 reported cases; occupation total N = 19 cases older than 14 years old included in the case-control study.

sex or both together in the ecotone of the peripheral neighbourhood were 2-3 times that of the rural site, and up to 200 times that of the periurban traps. The proportion of females was similar between peridomestic and extradomestic captures of the same capture station, but

differed significantly among stations, decreasing as the human intervention in the environment increased: rural > peripheral neighbourhood > periurban neighbourhood. The proportion of *Lu. neivai* gravid females captured was similar in the three peridomestic sites, but the difference of the ratio extra/peridomestic was significant between stations from 0.35 in the peripheral neighbourhood to 3.51 in the rural landscape ( $P < 0.05$ ) (Table III).

The monthly data of mean and minimal temperature, sunshine, and relative humidity of the years 2001, 2002, 2003, and 1950-1990 average did not differ significantly (data not shown). However, the 2002 rainfall showed a different pattern mainly during April (average 1950-1990: 139.5 mm, 2001: 136.2 mm, 2002: 355.7 mm, 2003: 158.0 mm), one year before the peak of cases according to the probable date of infection (Fig. 3). The height of the Parana river at Bella Vista Port during 2003 was significantly higher than the records of 2002, with a peak in March (maximum/minimum 2002: 3.45/2.80 m, 2003: 5.25/4.36 m) (Fig. 3). The average between the maximum-minimum height difference of the river from January to April was also higher in 2003 ( $1.13 \pm 0.18$  m) than in 2002 ( $0.66 \pm 0.04$  m).

Based on the satellite imagery, the area covered by water in close proximity to the cases of Florida neighbourhood (Fig. 2) was computed during the overflowing of the water pools. The flooded area showed an increasing trend between the 4/18/2001, 5/23/2002, and 4/8/2003 serial images of 359, 383, and 416 km<sup>2</sup> estimated respectively. However during the intermediate dates of 1/15/2002 and 12/17/2002 the areas were 287 and 320 km<sup>2</sup>, so the annual range was 96 km<sup>2</sup> in both years. In the satellite images no area of deforestation was observed that correlated with the TL epidemic outbreak.

## DISCUSSION

In Bella Vista, province of Corrientes, during August-October 2003, 31 cases of TL were recorded. The physical

TABLE II

Risk and protective factors associated with tegumentary leishmaniasis. Bella Vista, province of Corrientes, Argentina, 2003

| Habits                              | Exposed/Total | % exposed | P    | ORm (CI 95%)   | P     |
|-------------------------------------|---------------|-----------|------|----------------|-------|
| To work in the carrizal             |               |           |      |                |       |
| Cases                               | 12/24         | 50.0      |      |                |       |
| Controls                            | 10/44         | 22.7      | 0.02 | 2.0 (0.5-11.4) | 0.22  |
| Household factors                   |               |           |      |                |       |
| To have cats in the house           |               |           |      |                |       |
| Cases                               | 7/25          | 28.0      |      |                |       |
| Controls                            | 20/45         | 44.4      | 0.27 | 0.25 (0.1-0.7) | 0.003 |
| Peridomestic factors-distance       |               |           |      |                |       |
| House-water stream < 500 m          |               |           |      |                |       |
| Cases                               | 11/25         | 44.4      |      |                |       |
| Controls                            | 9/47          | 19.1      | 0.04 | 1.9 (0.5-10.8) | 0.25  |
| House-secondary forest < 500        |               |           |      |                |       |
| Cases                               | 19/24         | 71.2      |      |                |       |
| Controls                            | 27/45         | 60.0      | 0.17 | 2.2 (0.4-21.1) | 0.25  |
| House-high secondary forest < 500 m |               |           |      |                |       |
| Cases                               | 15/25         | 60.0      |      |                |       |
| Controls                            | 20/48         | 41.7      | 0.21 | 1.2 (0.5-5.7)  | 0.30  |

P: P value; ORm: matched Odds Ratio; CI: confidence interval.

TABLE III  
Phlebotomine captures with CDC light minitrap, Bella Vista, province of Corrientes, Argentina, October 14 to 17 2003

| Site                          | <i>Lu. neivai</i> | <i>Lu. migonei</i> | <i>Lu. pessoai</i> | <i>Br. avellari</i> | Phlebotomine<br>/night $\pm$ SD | <i>Lu. neivai</i><br>female % | <i>Lu. neivai</i><br>gravid % |
|-------------------------------|-------------------|--------------------|--------------------|---------------------|---------------------------------|-------------------------------|-------------------------------|
| Periph. neigh. extradomestic  | 1583              | 1                  | 1                  | 0                   | 636.0 $\pm$ 88.7                | 56.1 <sup>a</sup>             | 1.4                           |
| Periph. neigh. peridomestic   | 233               | 1                  | 0                  | 0                   | 78.0 $\pm$ 20.7                 | 65.2 <sup>a</sup>             | 3.9                           |
| Periph. neigh. shore          | 300               | 0                  | 0                  | 0                   | 100.0 $\pm$ 27.2                | 61.6 <sup>a</sup>             | 1.6                           |
| Periurb. neigh. peridomestic1 | 158               | 0                  | 0                  | 0                   | 52.7 $\pm$ 9.2                  | 44.3 <sup>b</sup>             | 5.7                           |
| Periurb. neigh. peridomestic2 | 8                 | 0                  | 0                  | 1                   | 2.7 $\pm$ 0.6                   | *                             | *                             |
| Periurb. neigh. peridomestic3 | 4                 | 0                  | 0                  | 0                   | 1.3 $\pm$ 1.1                   | *                             | *                             |
| Rural extradomestic           | 432               | 4                  | 76                 | 6                   | 172.7 $\pm$ 55.9                | 72.2 <sup>c</sup>             | 14.4                          |
| Rural peridomestic            | 534               | 21                 | 245                | 0                   | 266.7 $\pm$ 68.6                | 86.1 <sup>c</sup>             | 4.1                           |
| Farm                          | 0                 | 0                  | 0                  | 0                   | 0                               |                               |                               |
| Total                         | 3252              | 27                 | 322                | 7                   |                                 |                               |                               |

Phlebotomine cumulative captures of three consecutive nights by species. Periph. neigh.: Epam peripheral neighbourhood; Periurb. neigh.: Florida periurban neighbourhood; *Lu. neivai* female (%): female proportion of *Lu. neivai* (%); *Lu. neivai* Gravid % \*: proportion of gravids among females of *Lu. neivai* (%) capture with less than 20 individuals; <sup>a,b,c,d,e</sup>: each letter differed significantly from the other with a  $P < 0.05$

and epidemiological pattern was the same as in other foci of the country where the parasite characterized was *Leishmania (V.) braziliensis* (Sosa Estani & Salomón 2002), although *L. (V.) guyanensis* was reported previously in a single isolated from other site of the province of Corrientes (Marco et al. 2005). This is the southernmost known TL outbreak in Argentina up to now and the first confirmed in the province of Corrientes. The National Surveillance System reported 98 cases for Corrientes in 1982 and 253 cases in 1984, but these figures are assumed as typing errors because the reference center of Corrientes, the Dermatologic Dispensary, recorded only two cases in 1982 and no cases in 1984, and the local university center, Cenpetrop, reported that before 1988 only one or two TL cases were reported each year in the province (Borda et al. 1998a).

The entomological survey captured 3608 phlebotomine and, 90% of these were identified as *Lu. neivai*. This species has adapted to the human modified environments and has been incriminated as the vector during epidemics of *L. (V.) braziliensis* in several Argentinean foci (Salomón 2002, Córdoba Lanús et al. 2006), while the vector during inter-epidemic zoonotic cycles could be *Lu. migonei* o *Lu. shannoni* as suggested for other areas (Alexander & Maroli 2003, Chavez & Añez 2004). Phlebotomine captures during the first season of activity of sand flies after the outbreak, was assumed to be representative of the relative abundance distribution during *Leishmania* transmission, because there were not reported or observed major environmental changes since then up to the study. The greatest species diversity was found in the rural areas with less human intervention, and the highest *Lu. neivai* abundance in the peripheral ecotone, distribution described in the peridomestic transmission scenarios of Argentina (Salomon et al. 2001a,b, Salomon et al. 2006b), and other foci of the region where *Lu. intermedia* (from

the same species complex as *Lu. neivai*) was the incriminated vector (Kawa & Sabroza 2002, Lemos & Lima 2005, Dos Santos 2005, Massafera et al. 2005). The relative proportion of females and gravid females suggest breeding sites in the rural secondary vegetation, and potential colonization of peridomestic habitats in surrounding vegetation patches. In the province of Salta, located in Northwestern Argentina, this kind of rural/periurban and extra/peridomestic distribution was associated with a meta-population pattern (Salomon et al. 2004), where a peridomestic sand fly population could be re-colonized from an extradomestic source population after a focal insecticide treatment, or the peridomestic population may lead to the extinction by natural causes. The *Lu. neivai* abundance and the proportion of gravid females (potentially infective) could also be thought as risk indicators for increased of TL transmission. The spatial distribution of these indicators in Bella Vista shows a risk gradient associated with the stability, proximity, and size of the vegetation patches. Therefore, the risk is higher in the peripheral ecotone and lower in the periurban sites, which is consistent with the risk factor "to have high vegetation (secondary forest) close to the house" observed in the questionnaires.

However, in order to define the risk distribution in addition to vector abundance, the social use of the space should also be taken into account (Kawa & Sabroza 2002). Most of the cases reside in the Florida neighbourhood, the area with the highest human density among the areas sampled in search of vectors. On the other hand, at least 50% of the cases were located in the 'carrizal'-peripheral neighbourhood during the hours of vector activity making clay bricks, walking to and from the farm for the evening-night chores (selection, packaging, track loading), or recollecting 'carrizal' herbs (frost prevention and handicrafts). These abundance-exposure results indicate

the need to be aware of any changes in the land use and its real estate value in TL endemic areas. The land related changes could increase the human population in sites with high vector density, or push the vector populations to crowded neighbourhoods. In Bella Vista, during the three years prior to the outbreak, abandoned cultures of citrus trees close to the periurban Florida were deforested to be reactivated or reconverted in farms, or to build the peripheral neighbourhood Epam in front of the 'carrizal'.

Finally, in order to examine the TL epidemic, weather variables were integrated with the entomological data and social land use data. During 1998, in the same site identified in this study as 'rural' capture station' (28°27'02"LS, 59°00'03" LW) a cluster of five cases of TL was reported and two *Lu. intermedia sensu lato* individuals were captured (Borda et al. 1998b). This report only indicates parasite circulation and the existence of the competent vector at least 5 years before the main epidemic outbreak, but it does not provide any conclusion about the progression of the risk or the phlebotomine abundance. The trend of TL transmission since 1998 was not known because any local based surveillance or diagnostic system was settled in Bella Vista despite the closeness of the cases reported to the city. The actual phlebotomine abundance was not known because the team that reported two sandflies in 1998 captured again in Bella Vista only two *Lu. neivai* (Borda et al. 2005), during the same period of 2003 that the phlebotomine reported here were collected. However, epidemiological local records confirm that an epidemic outbreak of TL took place during the first semester of 2003 in Bella Vista, and that the transmission peaked one year after an extraordinarily high period of rainfall. The rainfall-*Lu. neivai* abundance correlation with a lag of 52 weeks was already reported for the northeastern and northwestern peridomestic foci of Argentina (Salomon et al. 2002, 2004). On the other hand, the river trend and overflow data during the same year of the outbreak could imply an increase in the potential breeding surface of phlebotomine in the 'carrizal' and brick making area. Lastly, the overflow of pools and the draining streams on the edge of the city may cause the migration of wild infected animals to peridomestic periurban habitats. During the phlebotomine captures, many sinanthropic and wild marsupials, edentate and rodents were observed in the backyards of the houses close to the water stream. The inhabitants explained that this 'invasion' happens due to the growing land covered by water from the inland pools. This perception is consistent with the satellite imagery estimates. The high frequency of exposure among cases compare to controls in regards to the factor "to have a stream of water near the house" and the protective factor "to have cats in the house" could be associated with this parasite source related to closeness of potential reservoir to houses and predation on the reservoirs. These are the only risk and protection factors identified among the surveyed factors. Possible confounders or age bias was eliminated by the design, or minimized as the recalling factor (the questionnaires were made at the end of the outbreak).

However, other factors as over-matching (controls were selected in the same block of cases), and the small size of the sample limited the discussion about the risk factors associated with *Leishmania* infection so should be cautiously interpreted.

Phlebotomine captures were also performed with light minitraps CDC like in the northeastern region of province of Corrientes, in a natural reserve 7 km from Gobernador Agrónomo Valentín Virasoro City, close to the Uruguay river basin (28°02'LS, 56°00' LW). The sand fly/night captured there from July 2nd to 4th 2003 were 2.5 *Lu. shannoni*, 1.5 *Lu. neivai*, and 0.5 *Lu. cortelezzii*. Therefore, any generalization of the results of Bella Vista to other scenarios in the area should be assessed previously in each focus (unpublished data)

In conclusion, during the 2003 TL outbreak in Bella Vista, province of Corrientes, the active transmission was possible found in the eastern outskirts of the city and the city border. The risk increased from the periurban neigh-bourhood, Florida, to the marginal ecotone of the Epam neighbourhood. The probability of transmission was associated with the proximity and size of dense patches of secondary vegetation related to human distribution or activities. The potential increase of breeding sites of sand flies, and relocation of vectors and reservoirs due to progressive deforestation, the proximity of the houses to the secondary vegetation, the water streams connected to natural pools cycle, the rainfall of the previous year, and the height of the river during the same year could all have contributed to the outbreak. The confluence of weather, environmental, and cultural factors implies that the epidemic phenomena are not necessarily periodic, and therefore, sporadic human cases should be expected regularly. Furthermore, Bella Vista is located in front of a Ramsar site promoted for ecological and sport fishing tourism, national and international, where populations of *Lu. neivai* have been already reported (Salomón et al. 2006a). Although the results indicate that the urban transmission in the southernmost foci of TL is still a border-green patch ecotone related problem, it also reinforces the recommendations about the settlement of a local-based system of surveillance, diagnosis and treatment of cases, and the entomological surveillance at sentinel sites. Likewise, TL risk should be assessed for any project that involves time or space changes in land use throughout the endemic area, specifically new neighbourhood building projects that are close to city borders.

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