Spatial and Temporal Patterns of Phlebotomine Sand Flies (Diptera: Psychodidae) in a Cutaneous Leishmaniasis Focus in Northern Argentina

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ABSTRACT Phlebotomine sand flies (Diptera: Psychodidae) were captured in an area of Argentina endemic for American cutaneous leishmaniasis (ACL). A total of 44,944 flies were collected during a 130-wk interepidemic period from 1990 through 1993. These sand flies included *Lutzomyia neivai* (Pinto) (97.8%), *Lutzomyia migonei* (França) (1.2%), *Lutzomyia cortelezzii* (Brèthes) (0.8%), *Lutzomyia shannoni* (Dyar) (0.1%), and *Lutzomyia punctigeniculata* (Floch and Abonnenc) (0.1%). *Lutzomyia neivai* was more abundant in secondary forests and peridomestic environments associated with human cases than in primary forest or xeric thorn scrub areas. Time series analyses of species densities suggested a bimodal or trimodal annual pattern related to rainfall peaks, a 5-wk reproductive cycle, and peridomestic local populations that were located adjacent to secondary forests. In general, sand fly abundance was correlated with the rainfall of the previous year. *Lutzomyia neivai* spatial distributions were consistent with ACL incidence patterns during the study and in the recent outbreaks in Argentina. However, *Lu. migonei* also may be involved in peridomestic transmission. Our results suggest that there is a need for improved, long-term surveillance of sand flies and ACL cases, as well as development of effective intervention strategies.

KEY WORDS *Lutzomyia*, American Cutaneous Leishmaniasis, Argentina, vector ecology, seasonal abundance.

AMERICAN CUTANEOUS LEISHMANIASIS (ACL) in Argentina was initially described in 1915 (Bernasconi 1928); however the first well-documented outbreak occurred during 1984–1987 in northeastern Salta province (Sosa Estani et al. 2000). Sand flies of the genus *Lutzomyia* (Diptera: Psychodidae) transmit *Leishmania* parasites in the New World, and during this epidemic *Leishmania (Viannia) braziliensis* Vianna was identified as the causative agent (Grimaldi et al. 1989, Segura et al. 2000). During this outbreak, incidence was similar in both sexes and involved all age groups, an epidemiological pattern that differs from former disease risk that typically involves men working in forests (Sosa Estani et al. 2001). After the outbreak in the 1980s, several foci appeared throughout the endemic area (Salomon et al. 2001b, 2002). Incidental sand fly captures of presumed vectors were recorded in the area before 1947 (Bejarano and Duret 1950), but since then no records were published until the 1990s (Salomon et al. 1995).

The current study represents the first systematic evaluation of the spatial and seasonal abundance of phlebotomine sand flies in Argentina, and was undertaken in the area of Salta province where the 1984–1987 outbreak occurred. Sand fly species abundance was evaluated over time in relation to landscape characteristics, distance to human dwellings, and seasonal weather variation. Results are considered in the framework of epidemic forecasting models and the feasibility of proposed control strategies.

Materials and Methods

Study Area. Adult sand flies were captured in Shannon traps placed in the municipalities of Pichanal, Embarcacion, and Mosconi (22° 30′ to 24° 10′ S, 63° 10′ to 64° 25′ W), in Salta province (Fig. 1). This area is ≈80 km south of the Bolivia border and west of the Andean foothills at altitudes ranging from 250 to 450 m. The phytogeographic area is classified as subtropical humid forest in the east, grading to xeric forests in the west (Cabrera 1971).

Sand Fly Collections. The capture sites were categorized as primary forest, secondary forest, xeric woodland, and rural or periurban (more than one dwelling per 50 m^2). Fourteen sites with recent ACL

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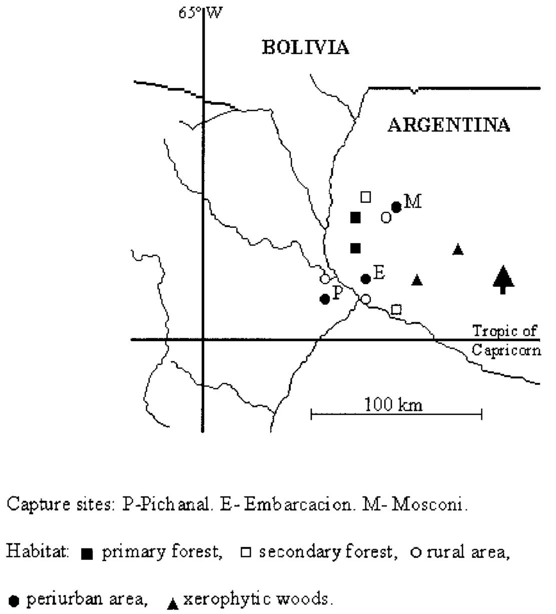

Fig. 1. Map of study areas showing the three regions Embarcacion (E), Mosconi (M), and Pichanal (P). Samples at these sites were taken from primary forest, secondary forest, xeric woodlands, perirurban areas, and rural areas.

cases were sampled in the periurban and rural areas (Fig. 1). Sand flies were captured on house walls (termed "domestic" sites), 10–50 m from houses within vegetation (peridomestic), and at 200–400 m (extradomestic). Captures were made at each site three times between October and November to detect environmental associations and to obtain peridomestic abundances. Other samples were made at one rural and one periurban site in each municipality once a wk (year 1), or once every 2 wk (year 2) for a 130-wk period beginning in October. The capture device was a modified Shannon trap (1.5 × 2.0 m white screen) operated from 2000 hours to 2300 hours (September to May), or from 1900 hours to 2200 hours (June to August). Domestic captures were added to the schedule at the Pichanal and Embarcacion rural sites during year 2. In addition to the screening captures, horse bait (two times) and CDC light trap (four times) collections were made 200 m from the Shannon trap captures.

Sample Processing. All captured sand flies were stored dry or in 70% ethanol until identification was made (Young and Duncan 1994, Marcondes 1996). An aliquot of females was immersed in phosphate-buffered saline (pH 7.4, 10% dimethyl sulfoxide) and stored in liquid nitrogen until dissection for parasites search were undertaken.

Weather Data. Daily weather data through the study period were provided by Cargill S.A. of the Orán Experimental Station, located between Pichanal and Embarcacion villages. The weather data were correlated with sand fly density time series.

Data Analysis. Fisher test and χ^2 tests were used for bivariate analysis unless otherwise stated. All statistical tests were considered significant at $P < 0.01$. Sand fly abundances were estimated by William's geometric mean (excluding null captures), when the number of null captures between data sets did not differ significantly. Time series statistics were computed by the SYSTAT software package (Wilkinson et al. 1992) and lowess smoothing was used with a tension parameter of $F = 0.15$ (Cleveland 1979). The stationary series (first-order transformed) excluded odd week data from year 1 so that the collection intervals were equally distributed. The natural logarithm (Ln) and logarithm (capture +1) (Log) of each interval point were computed, the latter to include the null captures. Autoregressive integrated moving average (ARIMA) models, and multivariate general linear stepwise models ($P = 0.15$ including covariances) were tested (Wilkinson et al. 1992, Morrison et al. 1995).

Results

Captures for comparison between different degree of human environment modification produced a total of 7,084 sand flies of five species: *Lu. neivai* (Pinto), *Lu. migonei* (França), *Lu. cortelezii* (Brèthes), *Lu. shannoni* (Dyar), and *Lu. punctigeniculata* (Floch and Abonnenc). The mean relative abundance of *Lu. neivai* was higher in secondary forests or peridomestic habitats than in primary forests (Table 1). However, at one secondary forest site the collections were similar to those obtained at the nearest rural peridomestic site located in a goat pen adjacent to a bedroom (396–410 phlebotomines/h).

Shannon traps captured 15 times more *Lu. neivai* than did CDC light traps, and the female:male sex ratio in the former was three times higher. In one xeric woodland site, however, five specimens of *Lu. neivai* were obtained solely in light traps. Horse bait and simultaneous Shannon trap collections were similar (157–174 phlebotomines/h), however the *Lu. migonei* relative abundance was higher by 3% to 25% (Table 1).

No *Leishmania* sp. parasites were found in any of the 3,548 females examined, which included 3,341 *Lu. neivai*, 108 *Lu. cortelezzii*, 94 *Lu. migonei*, and 5 *Lu. punctigeniculata*.

One rural and one periurban station in each of the Pichanal (P), Embarcacion (E), and Mosconi (M) study areas were selected for the 130-wk collections because these stations generally had elevated daily capture rates and ACL antecedents. Shannon traps were standardized to operate from 2000 hours to 2300 hours because no significant differences were noted between *Lu. neivai* females/h during 2000 hours to 2300 hours and 2300 hours to 0600 hours collections during two overnight captures ($n = 2,944$, Wilcoxon two-tailed test, $\alpha = 0.1$).

During the 130-wk collection period, 37,178 phlebotomines were trapped (Table 1). The relative species abundances were similar among the domestic, peridomestic and extradomestic sites. The exception occurred where *Lu. shannoni* and *Lu. punctigeniculata*

Table 1. *Lutzomyia* sand flies captured by habitat and trap method in Salta Province, Argentina

Habitat	Mean *Lutzomyia*/h (species relative abundance %)					Total
	Lu. neivai	*Lu. migonei*	*Lu. cortelezzi*	*Lu. shannoni*	*Lu. punct.*[a]	
Primary forest[b]						
site a	—	8.4 (23.1)	28.0 (76.9)	—	—	36.4 (100)
site b	0.5 (2.6)	3.0 (15.89)	15.5 (81.6)	—	—	19 (100)
Secondary forest[b]						
site a	21.3 (100)	—	—	—	—	21.3 (100)
site b	348.3 (96.8)	—	0.3 (0.1)	11.3 (3.1)	—	359.9 (100)
Rural[c]	405.5 (99.7)	1.4 (0.3)	—	—	—	406.9 (100)
Periurban[c]	30.3 (83)	1.0 (2.7)	5.2 (14.3)	—	—	36.5 (100)
Xeric Woodland	—	—	—	—	—	0
Trap Type	Captured (species relative abundance %)					Total
	Lu. neivai	*Lu. migonei*	*Lu. cortelezzi*	*Lu. shannoni*	*Lu. punct*[a]	
Shannon[d]	6,686 (94.4)	194 (2.7)	169 (2.4)	27 (0.4)	8 (0.1)	7,084 (100)
Horse	237 (75.2)	78 (24.8)	—	—	—	315 (100)
Light	364 (99.2)	2 (0.5)	1 (0.3)	—	—	367 (100)
Shannon[e]	36,651 (98.6)	245 (0.6)	208 (0.6)	35 (0.1)	39 (0.1)	37,178 (100)
Total	43,938 (97.8)	519 (1.2)	378 (0.8)	62 (0.1)	47 (0.1)	44,944 (100)

[a] *Lu. punctigeniculata.*
[b] Site a more modified by human activities than site b.
[c] Peridomestic sites with high, homogeneous captures.
[d] Wk 1–7.
[e] Wk 8–130.

were identified together with the lowest *Lu. neivai* abundance at station M. *Lutzomyia neivai* was the prevalent species at all sites. *Lutzomyia migonei* abundance in rural stations near a secondary forest was 1.9–2.8 times higher in peridomestic than extradomestic samples.

There was no difference between *Lu. neivai* abundance in each of the three consecutive sampling hours at stations P and E throughout the study period, nor at station M for the major collection period during weeks 87–94 (Fig. 2). Consequently, the *Lu. neivai* capture rates/h/d data were amenable to average and geometric mean computations. Samples at the rural E and P stations were both adjacent to secondary forest and rivers (E:P abundance ratio = 4.8:1). Captures at periurban sites were only noticeable at station P (periurban:rural abundance ratio = 1.2), but an almost continuous 2-km length, 50-m width residual forest

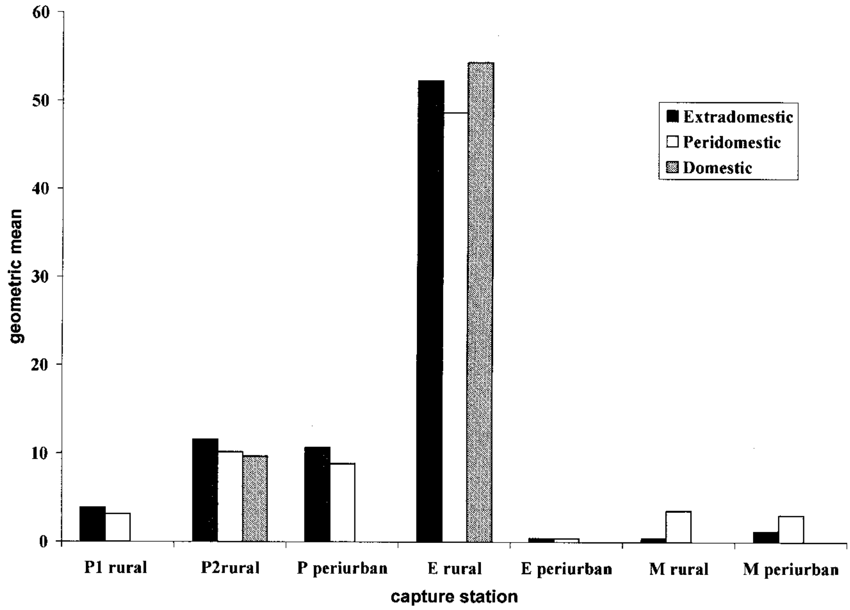

Fig. 2. Geometric mean of *Lutzomyia neivai* collected per hour in Salta province, Argentina at Pichanal (P), Embarcacion (E), and Mosconi (M) rural and periurban stations, in extradomestic, peridomestic, and domestic settings during weeks 60–107 (year 2). P1 rural *L. neivai* were collected during weeks 8–55 (year 1), P2 rural were collected during weeks 60–107 (year 2).

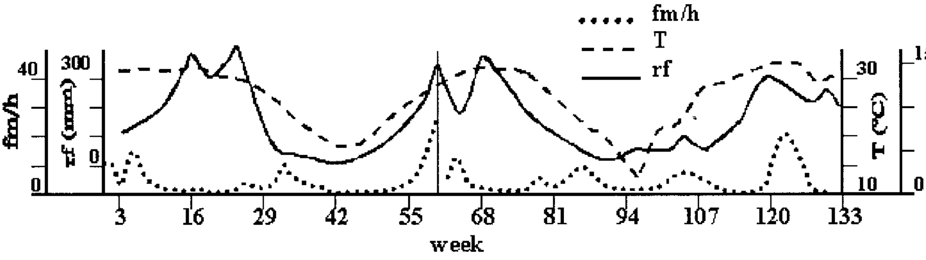

Fig. 3. 'Lowess' smoothed series of females *Lutzomyia neivai* captured per hour (fm/h) in Salta, Argentina at Pichanal rural peridomestic site. Weekly (left, year 1) or every other week (right, year 2) collections. Rainfall (rf) and mean temperature (T) records during the same period.

separated the secondary forest and the periurban station. Relatively few captures at periurban stations E and M were recorded in these drier areas that lacked nearby rivers or streams. The proportion of captures with no sand flies compared by year, station, or site did not differ significantly, except at periurban M. However, the geometric mean abundance of *Lu. neivai* was three to five times higher in year 2 than in year 1 (P rural, Fig. 2).

Although the capture rate of *Lu. cortelezii* did not differ significantly throughout the study, *Lu. shannoni* and *Lu. punctigeniculata* were collected only during the first 52-wk period and *Lu. migonei* was more abundant during that period as well (Mann-Whitney $P = 0.02$).

Lutzomyia neivai abundance was elevated during autumn (weeks 23–37 and 75–90), with a single spring peak (weeks 1–7), or a double spring and summer peaks (weeks 64–67, 101–106, 116–121) (Fig. 3). The bimodal distribution appeared to become trimodal depending on rainfall (single long rainfall period versus distinct spring and summer peaks). The peridomestic-domestic and extradomestic captures followed similar trends, although the former usually shifted to later dates and with lower abundance. The autumn peaks had a higher proportion of females with eggs (20–50%) than spring-summer peaks (0–10%).

Fourier periodograms of *Lu. neivai* time series identified four peak sets ($\text{magnitude}^2 > 0.4$): the main one at weeks 5–6 (0.31–0.40-wk cycles) and additional peaks at weeks 2, 8, and 14. Autocorrelation and partial autocorrelation plots also showed significant associations with lag times of 2, 4–6, 8–12, and 14–16 wk; whereas the correlation coefficients rose from 0.29 to 0.46–0.45–0.65 only when transformed data series from weeks 12–38 and 72–91 consistent captures were considered. Cross-correlations between peridomestic and extradomestic logarithmic series displayed significant associations at the week of capture, at weeks 10–12, and at week 20. The series for females only showed about the same pattern as that for the total capture.

First-order difference logarithmic series fit the ARIMA model with significant parameters, but with an important error. The more parsimonious models had an autoregression and a moving average parameter, ARIMA (1,1) (LnE and Log P, Table 2), or a single moving average parameter, ARIMA (0,1) (LnP and LogE, Table 2). In the ARIMA (1,1) series, the autoregression parameter had a larger standard error (confidence interval close to 0), and the correlation between both parameters was high (Table 2).

Lutomyia neivai abundance and weather variables showed significant cross correlation lag times (coefficient 0.35–0.45) grouped in four time sets: 1) rainfall with weeks 40–52 abundance; 2) relative humidity and temperature (mean, maximum, minimum and thermal amplitude) with weeks 16–20; 3) weeks 6–8; and 4) the weeks within the month of capture. The cross-correlation ARIMA residuals, i.e., weather differenced series, also showed significant associations with rainfall (46 wk previous) and thermal amplitude (26 wk previous). Pearson's correlation coefficient between ARIMA residuals and rainfall differenced

Table 2. Summary of ARIMA models fit to 128-wk time series

Series	MSE	Parameter	Estimate	ASE	CI (95%)	AC
LnE	1.30	AR	0.472	0.148	0.176–0.768	0.65
		MA	0.932	0.069	0.794–1.070	
LnP	1.28	MA	0.797	0.086	0.625–0.969	—
LogE	5.45	MA	0.757	0.159	0.438–1.075	—
LogP	3.80	AR	0.441	0.126	0.189–0.693	0.40
		MA	0.941	0.037	0.867–1.015	
Log (E + P)	4.45	MA	0.795	0.088	0.619–0.971	—
LogE residuals = $(0.18 \times TA_{-26}) + (0.067 \times Rf_{-46})^a$ $r^2 = 0.25$ F test, $P < 0.001$						

Differenced logarithm *Lutzomyia neivai*/h (Ln), and logarithm_2 (*L. neivai*/h + 1) (Lg), at extradomestic (E) and peridomestic (P) rural Pichanal sites, and LgE residuals multiple regression with climatic variables. MSE, mean standard error; ASE, absolute standard error; CI, confidence interval; AC, asymptotic co-relation; AR, autoregressive; MA, moving average.

[a] TA_{-26} = differenced thermal amplitude shifted by 26 wk. Rf_{-46}—differenced rainfall shifted by 46 wk.

series (shifted 46 wk) was $r^2 = 0.13$ ($P = 0.03$), the coefficient with the thermal amplitude series (shifted 26 wk) was also $r^2 = 0.13$, ($P < 0.01$), the coefficient of the best fitted multiple regression was $r^2 = 0.25$ with no significant covariance (Table 2).

Discussion

Lutzomyia neivai, Lu. migonei, Lu. cortelezii, Lu. shannoni, and *Lu. punctigeniculata* were identified in an epidemic area of ACL in Salta province, Argentina, during an interepidemic period (Sosa Estani et al. 2000, Salomon et al. 2001a). *Lutzomyia neivai* was relatively more abundant in modified rather than in less-disturbed environments, in humid (near rivers) than in xeric secondary forests, and it was the prevalent species in peridomestic habitats. Previously, this species was identified as *Lu. intermedia* (Lutz & Neiva) before the distinction between *Lu. intermedia s. s.* and *Lu. neivai* was made (Marcondes 1996).

Lutzomyia neivai was first recorded in Salta in 1988 after an outbreak caused by *Le. (V.) braziliensis* (Salomon et al. 1995). This phlebotomine was probably present in Salta before this date, but in low abundance because it had been reported in adjacent Argentinian provinces and in Brazil ACL foci up to 1950 (Castro 1959, Tolezano 1994); *Lutzomyia neivai* has recently been found in low abundances in residual forests (Souza et al. 2001). The ACL reemergence in Salta occurred after intense deforestation that was initiated in the late 1970s. The main outbreaks (1985–1987, 1997–1998) had a peridomestic transmission pattern, with similar human incidence by sex and age (Salomon et al. 2001a, Sosa Estani et al. 2001). The ACL reemergence after deforestation with peridomestic epidemics and high *Lu. intermedia s.l.* peridomestic abundance (60%–100%) also was reported from *Le. (V.) braziliensis* foci in Paraguay (Hashiguchi et al. 1992), and in the Brazilian states of Bahia (Pereira and Hoch 1990), Rio de Janeiro (Rangel et al. 1990), São Paulo (Tolezano 1994), and Parana (Teodoro and Kuhl 1997). During the study period, ACL rates (Sosa Estani et al. 2000) and *Lu. neivai* densities had similar trends, Embarcacion:Pichanal ACL incidence ratio was two and *Lu. neivai* abundance ratio was five, year 1: year 2 human infection incidence was two and *Lu. neivai* abundance was three to five, whereas none of the other species increased in abundance in year 2. *Leishmania* parasites were not isolated from the Salta province phlebotomines in our study; however, the dissected females came from a population peak with a high proportion of nullipars during an endemic period when infection rates are usually low.

In contrast, *Lu. migonei* has been found naturally infected with *Le. (V.) braziliensis* in Ceará, Brazil (Azevedo et al. 1990), and was incriminated in a peridomestic transmission cycle involving dogs and horses (Rangel et al. 1986, Yoshida et al. 1990). In Salta, as in Brazil (Aguiar et al. 1987, Rangel et al. 1990), the relative abundance of *Lu. migonei* increased more than 10-fold when horse bait was paired with Shannon trap and human bait. Therefore, the relative role of *Lu. migonei* in *Leishmania* transmission may be related to animal management (e.g., nightly corraling near human habitation). When goat or pig enclosures are adjacent to human sleeping quarters, the domestic captures of *Lu. neivai* increase, and can equal the extradomestic capture (e.g., E rural).

ACL prevalence in site M was relatively high (1.1/1,000 rural, 1.9/1,000 periurban) (Sosa Estani et al. 2000) but the phlebotomine captures were low in rural and periurban environments. The risk factors related to primary forest (i.e., hunting) was the highest among the three municipalities (Sosa Estani et al. 2001); consequently, the capture sites in our study and the sites of actual transmission may not coincide. Furthermore, the outbreak to the north of Mosconi (Tartagal) that occurred four months after the collections described herein was associated with heavy deforestation activity (Salomon et al. 2001c).

Lutzomyia neivai in Salta province was periodically abundant during spring to fall, with discontinuous captures (bi or trimodal) as a result of the rainfall peaks, and inactive during winter, with few or no captures. This is consistent with other longitudinal studies (Gomes and Galati 1987, Rangel et al. 1990). A quiescent preimaginal stage was proposed for *Lu. longipalpis* (Morrison et al. 1995), but adult survival within microenvironmental forested patches can not be discounted (Salomon et al. 2001c). The autumnal peak showed a higher proportion of females with eggs than that observed during the spring-summer peaks. Therefore, the relative risk of transmission should be higher during the fall. The computed population cycle period of 5 wk (Fourier, Autocorrelation) was similar to the time needed for larval development (24–49 d) in experimental studies (Rangel et al. 1985). The peridomestic:extradomestic series cross-correlation suggested a possible recolonization phenomenon with a 5 -wk periodicity (positive 10-wk association).

Despite the life cycle periodicity, the ARIMA model indicated that *Lu. neivai* abundance variations were explained mainly by variables from the environment (MA parameter). Biologically intrinsic cyclic variables (AR parameter) may have been expressed during favorable seasons (postrain summer to fall populations). *Lutzomyia neivai* abundance showed positive, long lag time associations with the previous year's rainfall (new potential breeding sites), and it also showed a shorter lag time association with temperature and relative humidity up to 20 wk earlier (related to individual metabolic-activity). Outbreaks may be generated in this way by unusual rainy periods (El Niño phenomena) followed by years of moderate temperature and rainfall (Salomon et al. 2002).

Our data indicate sufficiently clear trends such that surveillance strategies might be designed based on time series analysis and modeling for ACL in Argentina. This approach requires that phlebotomine capture data be obtained at critical locations (gallery forest close to villages with ACL case records), reliable weather data be available, and remote sensing observations be reviewed in near-real time (e.g., to detect extent of flooding and deforestation episodes). With such information, community-based programs can focus control measures on zones with a high risk of transmission during expected phlebotomine population density peaks. These measures may include the regulation of fishing and deforestation, recommendations on animal and water management, and education programs concerning protective clothing and bednet usage during peak seasons. Anti-vector recommendations might include regular insecticide spraying on resting sites, or spraying before model forecasted epidemic periods. The intervention, according to our time series analyses, should be applied at least twice a year (seasonal pattern), with 1-mo intervals (5-wk population cycle), and after the rainfall summer peaks (high reproductive potential). However, even with these precautions, the peridomestic control will reduce the risk of transmission only for a short period, because the extradomestic population may recolonize local peridomestic habitats. Indeed, a spatial barrier both chemical and physical may prove necessary in foci with human settlements near forests to avoid peridomestic recolonizations (Salomon et al. 2001a, 2001b).

The present longitudinal study supported the hypothesis of peridomestic ACL transmission in Salta. *Lutzomyia neivai* was the suspected vector of *Le. (V.) braziliensis* in peridomestic habitats, although *Lu. migonei* and *Lu. cortelezzii* also may have a role in the transmission. *Lutzomyia neivai* abundance was associated with river basins and nearby gallery forests, as well as with domestic animal aggregations. Peridomestic environments continuous or contiguous to *Lu. neivai* favorable habitats may have high phlebotomine abundance despite of the rural or periruban location. *Lutzomyia neivai* abundance was also correlated with the previous year's rainfall, with population peaks in the fall and one or two in the spring-summer period. *Lutzomyia neivai* metapopulation has a stable secondary forest population that may colonize the domestic-peridomestic environments with local smaller populations. The spatial and seasonal relationships described here were consistent with the timing and landscape characteristic of recent outbreaks of ACL in Argentina (Salomon et al. 2001a, 2001b, 2001c, 2002); hence, validated entomological models should be developed to forecast epidemics and design surveillance strategies.

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