Hourly activity of *Lutzomyia neivai* in the endemic zone of cutaneous leishmaniasis in Tucumán, Argentina: preliminary results

Ana Denise Fuenzalida[1,3]/+, María Gabriela Quintana[1,3], Oscar Daniel Salomón[2,3], Mercedes Sara Lizarralde de Grosso[1]

[1]Instituto Superior de Entomología Dr Abraham Willink, Universidad Nacional de Tucumán, Miguel Lillo 205, 4000 Tucumán, Argentina
[2]Instituto Nacional de Medicina Tropical, Puerto Iguazú, Argentina
[3]Red de Investigación de las Leishmaniasis en Argentina, Resistencia, Argentina

In the present work, the hourly activity of Lutzomyia neivai *was studied in the southern part of the province of Tucumán, Argentina, in an area of transmission of cutaneous leishmaniasis during two months of higher activity. In addition, the variables that influenced the abundance of* Lu. neivai *were evaluated. A total of 1,146 individuals belonging to* Lu. neivai *(97%) and* Lutzomyia migonei *(3%) were captured. The hourly activity of* Lu. neivai *was mainly nocturnal, with a bimodal pattern in both months. In January, the variable that most influenced the abundance of* Lu. neivai *was the temperature, whereas in April, that variable was humidity. These results may contribute to the design of anti-vectorial control measures at a micro-focal scale.*

Key words: hourly activity - *Lutzomyia neivai* - Argentina

The reported incidence of cutaneous leishmaniasis (CL) in Argentina has increased in the last two decades, despite no changes in the quality of the surveillance, and this trend shows that the epidemic outbreaks will continue to increase in intensity, frequency and distribution due, to a large extent, to climatic trends, anthropic modifications and unplanned peri-urbanisation, all of which increase the number of people exposed to CL (Sosa-Estani & Salomón 2002, Salomón et al. 2006a).

In Argentina, the endemic area of CL includes nine provinces in the north, including four ecological regions: the subtropical forest of the northwest (Yungas) and its associated foothill area, the subtropical forest of the northeast (Paranaense) and the forest with the Parana and Uruguay basins, and between both basins the dry and humid Chaco (Sosa-Estani & Salomón 2002).

A total of 28 species of Phlebotominae, distributed in three genera, have been recorded to date in Argentina. These species include 23 species belonging to *Lutzomyia*, four to *Brumptomyia* (França & Parrot 1921) and one to *Oligodontomyia* (Galati 1995). The species incriminated as vectors of *Leishmania (Viannia) braziliensis* associated with CL and mucosal leishmaniasis in Argentina are *Lutzomyia neivai* (Pinto 1926), *Lutzomyia whitmani* (Antunes & Coutinho 1939), *Lutzomyia migonei* (França 1920) and *Lutzomyia pessoai* (Coutinho & Barretto 1940). *Lu. neivai* and *Lu. whitmani* have been found to be naturally infected with *Leishmania* (Córdoba-Lanús et al. 2006, Salomón et al. 2009).

Financial support: PFIP (TU13/25 2006)
+ Corresponding author: fuenzalidadenise@gmail.com
Received 7 February 2011
Accepted 6 June 2011

In the province of Tucumán, the first Phlebotominae were captured in 1926 in the city of Concepción by Shannon. Until the 1950s, three species had been recorded: *Lu. neivai*, *Lu. migonei* and *Lutzomyia cortelezzii* (Bretes 1923). These species were found in a different location in the province, mainly in the foothill area, with vegetation corresponding to transition between Chaco Serrano from the phytogeographic region of Chaco and Yungas, with an annual accumulated rainfall between 600-800 mm and average temperatures between 25-27ºC. *Lutzomyia shannoni* (Dyar 1929) has also been reported to be present in this area (Córdoba-Lanús & Salomón 2002).

In the 1980s, the average number of CL reported cases per year in Argentina ranged between 40-80 cases (Cedillos & Walton 1988). Subsequently, according to the National System of Epidemiological Vigilance, four periods of outbreak were recorded in Tucumán: 1986-1988 (125 cases), 1991-1992 (88 cases), 1995-1997 (81 cases) and 2003-2004 (88 cases) (Salomón et al. 2006b).

In this work, our aim was to determine the hourly activity of *Lu. neivai* in the peridomestic environment based on the relative abundance and to identify the meteorological variables that modulate the above-mentioned activity in an area of transmission of CL in Tucumán in the months with higher *Lu. neivai* activity. These results will contribute to the design of anti-vectorial control strategies by providing an assessment of potential outbreaks by reporting the hours of the greatest abundance and, therefore, the sites/hours of the greatest risk of effective human-vector contact, and by exploring the predictive capacity of meteorological variables on the activity of the vectors.

In Tucumán, the department of Monteros (27º13'25.1"S 65º36'4.6"W) was selected among the 15 departments with CL cases and entomological antecedents because the last main outbreak took place there in 2004 (Salomón et al. 2006b) and because the monthly dynamics of Phlebotominae was investigated in this area for two consecutive years at different sites within the department and entomological screening captures were performed. The two houses with the highest number of captures were selected. These houses were located within the borders of La Florida Provincial Nature Reserve (Fig. 1). The area belongs to the phytogeographical province of the Yungas (400-700 m above sea level). The selected houses were 260 m apart and had homogeneous peridomestic environments, with primary vegetation surrounded by fields of sugar cane. The houses are referred to as site 1 and site 2.

Simultaneous captures were performed in both houses in the peridomestic environments for 24 h for seven consecutive days in January and April. Two multi-samplings adapters for CDC-like mini light traps were used [M&G similar to Collection Bottle Traps, John W. Hock Company (johnwhock.com)]. The hour of identification of each capture corresponds to the hour before the listed time (captures labelled 00:00 midnight belong to the trap activity from 11:00-12:00 am). The temperature (ºC) and the relative humidity (RH%) (percentage of saturation) were also recorded using an Onset-Hobo-Data Logger (model U12).

Lu. neivai adults were identified with dichotomous keys (Young & Duncan 1994, Andrade Filho et al. 2003).

To determine whether there is a relation of dependence between the abundance of *Lu. neivai* and "month", "site" or "day", a bivariate analysis was performed using the Chi-square test. The results were considered significant at $p \leq 0.05$. To quantify the magnitude of the association, Cramer's V coefficient was used. To investigate the association between the meteorological variables and the hourly activity, a stepwise multiple regression analysis was performed. The raw data were processed using the following formula to standardise data and ensure that the relationship was linear: $\log_2(\text{count}) + 1$.

A total of 1,146 individuals were captured belonging to two species, *Lu. neivai* and *Lu. migonei*. The dominant species was *Lu. neivai* (97%) with an overall female:male ratio (F:M) of 0.6 (Table I).

Lu. neivai is the principal species incriminated in the transmission of CL in northwest Argentina (Salomón et al. 2002, 2006a, b) and these insects have been found naturally infected with *L. (V) braziliensis* in Yánima, department of Alberdi, Tucumán (Córdoba-Lanús et al. 2006), 43 km away from the study area studied here.

The relative abundance of *Lu. neivai* was similar to that reported previously in Tucumán (Córdoba-Lanús & Salomón 2002). There is a strong association between deforestation and the risk of transmission in the region as the result of the "border effect"; even small modifications in the landscape have led to an increase in *Lu. neivai* abundance (Salomón et al. 2006a, Quintana et al. 2010).

The bivariate analyses showed significant differences in the abundances of *Lu. neivai* (Table I). Although both sites were homogeneous, the captures in site 2 were more stable between months, probably due to the fact that there were more feeding sources (pigs, poultry, horses and humans) and more potential sites to rest and breed, as has been described in similar scenarios in Tucumán and Salta (Salomón et al. 2006a, b, 2008, Quintana et al. 2010).

TABLE I

Relative abundance of Phlebotominae by sites and species, department of Monteros, Tucumán, Argentina

Capture	Sites	*Lutzomyia neivai*	*Lutzomyia migonei*	F:M
January[a]	Site 1[c]	68[g]	31[k]	0.44[m]
	Site 2[d]	212[h]	29[k]	0.57[m]
April[b]	Site 1[e]	476[i]	7[l]	0.75[n]
	Site 2[f]	315[j]	8[l]	0.78[n]
Total	-	1,071	75	0.6

a-n: each letter differed significantly from the other with $p < 0.05$; F:M: *Lu. neivai* female/male ratio.

It should be highlighted that *Lu. neivai* was prevalent in both months and that its abundance was threefold greater during April, the period of the year with the highest risk of transmission (Córdoba-Lanús & Salomón 2002, Salomón et al. 2006a, b).

The F:M of *Lu. neivai* was significantly different, but the Cramer's V coefficient was weak, suggesting a low association between the variables. Therefore, the difference is not representative and has no biological interpretation.

The hourly activity of *Lu. neivai* at the micro-focal level showed a bimodal pattern in both months, but was more marked in April. In both months, the greater activity of insects was continuous between 9:00 pm-06:00 am, like the patterns found in Venezuela for *Lutzomyia pseudolongipalpis* (Felinciangeli et al. 2004). In January, the hour of the greatest abundance was 00:00 midnight (22.88ºC, 87.58 RH%) (Fig. 2A), while in April the hour of the greatest abundance was 03:00 am (16.20ºC, 95.33 RH%) (Fig. 2B). These results are important in the context of control programs, as the main risk of human-vector contact in the autumn would be indoors due to the human activity/hour/place and the vector abundance/hour patterns. Therefore, intra-domicile insecticide spraying could be effective if the endophily/endophagy of the vectors is confirmed, as in other foci (Rangel & Lainson 2009). On the other hand, to avoid the risk associated with human activities in the peridomestic environments during the summer, the control program should focus on personal protection.

The meteorological variables recorded at the capture site were significantly associated with the abundance of *Lu. neivai*. Although these variables are correlated, the importance of each of them to explain the pattern of activity by hour was different according to the month of capture. The captures in January showed that the principal factor that affected the abundance of *Lu. neivai* was the temperature (Fig. 2A). In the model, the predictive variable included was temperature, which explained 74% of the abundance of *Lu. neivai*, with a negative association; the greatest number of captures was obtained at a mean of temperature 22ºC (Table II). In April, the predictive variable that affected the abundance of *Lu. neivai* was humidity (Fig. 2B), which explained 30% of the abundance of *Lu. neivai*, with a positive association, with the highest captures obtained at 95 RH% (Table II).

TABLE II

Summary of stepwise regression

Dependent variable (log)	Multiple R	Multiple R-square	R-square change	F	p level
Temperature (January)	0.86044	0.740357	0.740357	62.73185	0
Relative humidity (April)	0.547501	0.299758	0.299758	9.417689	0.00562

F: the F test of Snedecor; R: regression coefficient.

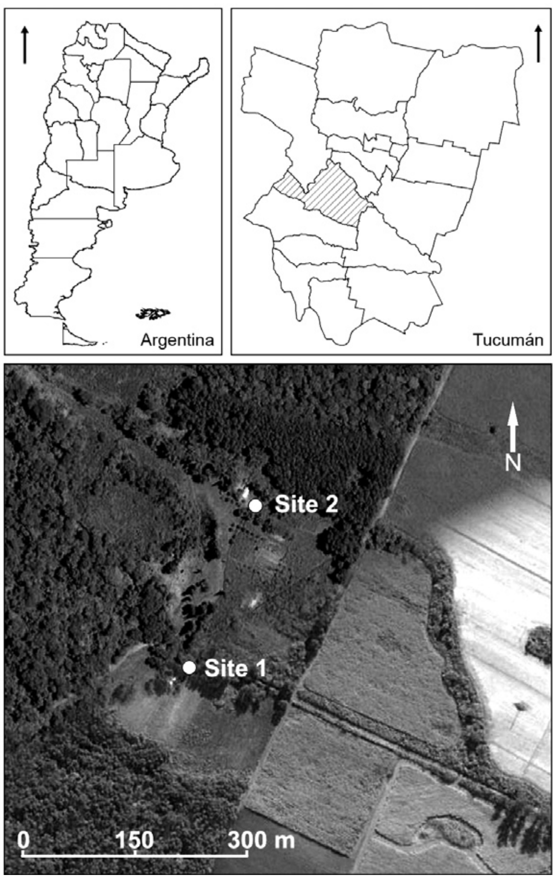

Fig. 1: map of Argentina with Tucumán highlight, the two sampling stations (site 1 and 2), which bounding the Provincial Reservoir La Florida, department Monteros, Tucumán, are indicated with a dot below. Image from Google Earth version 4.0.2416 (beta) (earth.google.es).

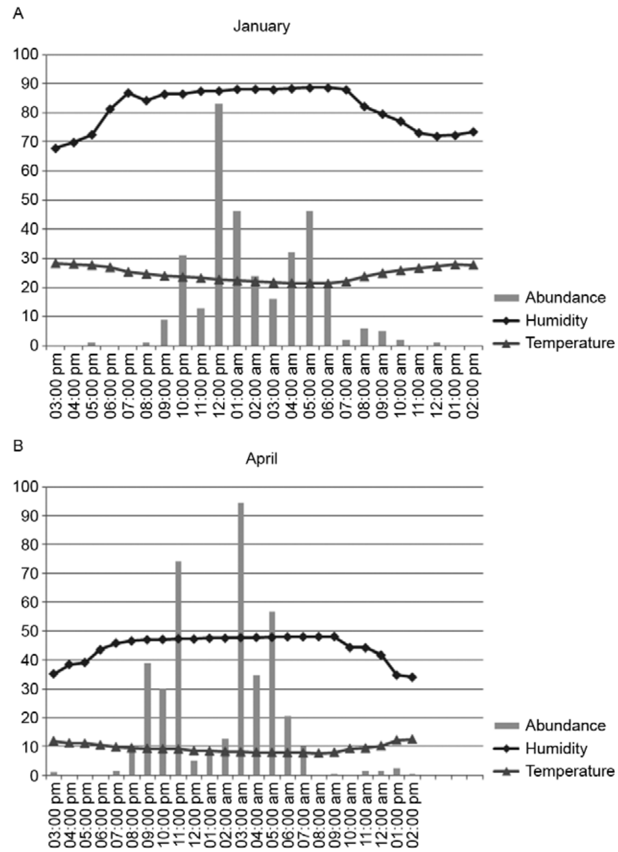

Fig. 2: abundance of *Lutzomyia neivai* and temperature and humidity variations by hour in January (A) and April (B).

In conclusion, in Yungas, *Lu. neivai*, the dominant species in peridomestic areas and the edges of modified areas, has a bimodal pattern of activity in the two seasons of higher abundance. In summer, greater activity was recorded in the first half of the night, when the human population remains in peridomestic environments, whereas in autumn, when the risk of transmission is higher than in summer, the peak of vector activity took place when the human population was indoors, where insecticide-based strategies would be effective. The modulating effects on phlebotomine activity observed in response to the temperature in the summer and in response to the humidity to a lesser extent in the autumn suggest that a predictive model of *Lu. neivai* hourly activity by season is feasible and could incorporate other variables, such as wind speed and light intensity.

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