Phlebotominae (Diptera: Psycodidae) fauna in the Chaco region and Cutaneous Leishmaniasis transmission patterns in Argentina

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In Argentina, the incidence of American Cutaneous Leishmaniasis (ACL) has shown a steady increase over the last few decades. In the Chaco biogeographical region, specifically, several outbreaks of ACL were recently reported in addition to the usual time-space scattering of ACL cases. However, little is known about the sandfly composition in the eastern, humid Chaco (HC) region or the western, dry Chaco (DC) region. Therefore, phlebotomine captures were performed throughout this region and an analysis of the distribution of reported ACL cases was conducted in order to assess the vector diversity in ACL endemic and epidemic scenarios in the Chaco region. The results support the hypothesis of two distinct patterns: (1) the DC, where Lutzomyia migonei *was the most prevalent species, had isolated ACL cases and a zoonotic cycle; (2) the HC, where* Lutzomyia neivai *was the most prevalent species, had an increase in ACL incidence and outbreaks and an anthropozoonotic cycle. The epidemic risk in the Chaco region may be associated with the current climate trends, landscape modification, connection with other ACL foci, and* Lu. neivai *predominance and abundance. Therefore, changes in sandfly population diversity and density in the Chaco region are an indicator of emergent epidemic risk in sentinel capture sites.*

Key words: American Cutaneous Leishmaniasis - Chaco - Argentina - *Lutzomyia migonei* - *Lutzomyia neivai*

During the past few decades, Cutaneous Leishmaniasis incidence has increased globally at an alarming rate as a result of concomitant changes in micro-climate, land-use, migration and urbanization patterns, as well as other factors (Mott et al. 1990, Desjeux 2001, Campbell-Lendrum et al. 2001, Shaw 2007). Simultaneously, the "Gran Chaco" region has been attracting the attention of multilateral research efforts and funding agencies focused on health and environmental conservation, including organizations such as the Pan-American Health Organization/World Health Organization, Canada's International Development Research Center and the World Wildlife Fund (World Wide Fund for Nature).

The "Gran Chaco" region is a biogeographical area with threatened endemic flora and fauna that extends over more than 1,000,000 km^2 of Paraguay, Bolivia and northeastern Argentina (Morello & Adamoli 1968, Cabrera 1976, Zuleta & Bolkovic 1994, Trucco Aleman 2007). In Argentina, above the 31ºS, the dry Chaco (DC) gently slopes from the Andean foothills eastwards towards the humid Chaco (HC), which is delimited on the east by the Parana River.

An increase in rain precipitation (Minetti & Vargas 1997, Villalba et al. 1998) during the XX century has led to severe fragmentation of the Argentinean Chaco landscape due to the overgrazing of cattle, overexploitation of timber stocks, agricultural burns and, recently, the planting of transgenic soybean cultures (Adamoli et al. 1990, Bucher & Huszar 1999). These environmental changes have been accompanied by intense migration-urbanization, as well as population impoverishment. More than 30% (31.6%) of the Argentinian Chacos' homes lack basic necessities (INDEC 2001).

American Cutaneous Leishmaniasis (ACL) cases in the Chaco region were found to be scattered both temporally and geographically. Outbreaks were reported that related to the deforestation of "hot spots" from the 1934 "Chaco war" (González & Oliveira y Silva 1939, González & Arce Queirolo 1955, Miranda et al. 1999). During the last 15 years, reports of ACL incidence and periurban outbreaks have increased (Salomón et al. 2001, Yadón et al. 2001, 2003). The endemic dispersion or epidemic concentration of different ACL transmission patterns was hypothesized to be associated with different phlebotominae diversity/abundance in the different landscapes (Salomon et al. 2006b, c).

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However, little is currently known about the sandfly fauna during the Chaco's epidemic and inter-epidemic periods. In this paper, we present unpublished findings from sandfly captures performed at different sites within the Argentinean Chaco since 2000. The captures were related to ACL human incidence and have been compared to the phlebotomine diversity/abundance of known epidemic scenarios. The results of these findings allow for the assessment of current and potential epidemic scenarios of ACL in the Chaco region. This paper, therefore, will facilitate the proper allocation of surveillance resources and contribute to future discussions about ACL emergence and environmental and climate changes.

MATERIALS AND METHODS

Area description - The Argentinean Chaco is divided by the isohyetal line of 900 mm into the DC and the HC. The HC is an eastern corridor with a maximum width of 200 km, between 58°-60°W (Fig. 1). In the western DC, the rainfall averages between 500-800 mm/year (commensurate with the continental dry pattern), while in the HC, the rainfall reaches 1,200 mm/year (commensurate with the Atlantic Humid pattern). The climate is subtropical, with a dry season that begins in June, persists through September and peaks in July. The rainy season begins in November, persists through March and peaks in spring and fall, with intensive short storms (UNNE 1987). The average annual temperature ranges from 28°C-14°C, with a broad day/night amplitude (i.e., the temperature can rise 15°C in a couple of hours) and summer/winter amplitude (0°C-45°C). The DC contains the "Heath Pole" of South America (Prohaska 1959) and a xeric landscape. On the other hand, the HC's main rivers (Parana and Paraguay) are lined by gallery forests alternating with grassed and flooded wetlands. The landscape is a mosaic that moves from the eastern savannas and grasslands to the western thorn forests and the open woodlands populated by Quebracho/Algarrobo and Quebracho/Palo Santo trees (*Aspidosperma, Bulnesia, Schinopsis, Prosopis*) and thorny shrubs and cacti (Morello & Adamoli 1968, Cabrera 1976). This region boasts many endemic animals including the Chacoan Pecary (*Catagonus wagneri*) and it claims the highest known diversity of Armadillos (Zuleta & Bolkovic 1994).

Phlebotomine captures - Adult phlebotomine sandflies were captured using CDC minilight traps (Sudia & Chamberlain 1962) operated from 19h-9h over two nights at each site in the provinces of Santiago del Estero, Salta, Formosa and Chaco (coordinates, localities and dates are detailed in Table I and sites are reported in Fig. 1). Adult phlebotomine sandflies were also captured during November 2007 using a Malaise trap from 9h-21h after dusk, once in Córdoba, 10 km south to Icho Cruz, in Las Jarillas, a leisure point on the river shore (31°32'02''S, 64°33'01''W). All captured sandflies were dry-stored until they were identified according to the characteristics published by Young and Duncan (1994) and Andrade Filho et al. (2003). In Monte Alto, Chaco,

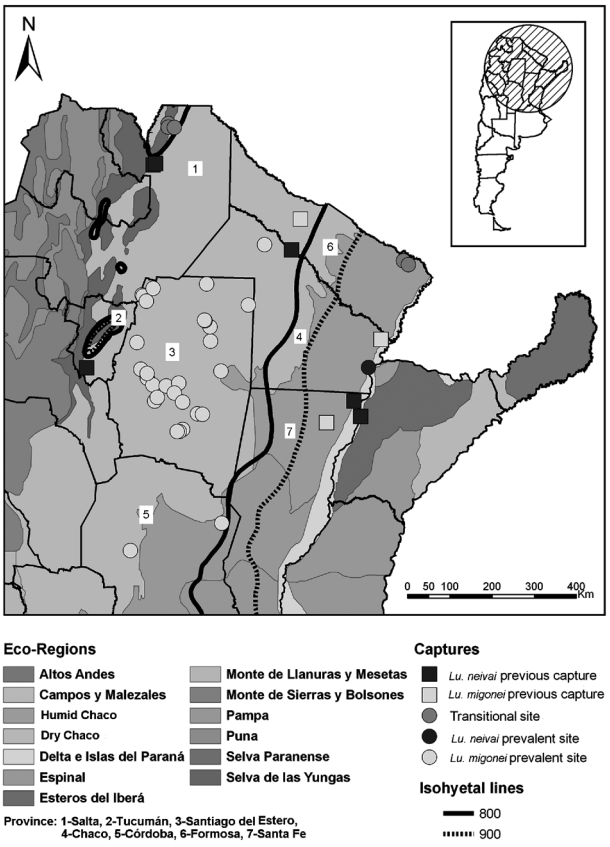

Fig. 1: phlebotomine capture site locations in Chaco region, Argentina, during 2000-2007, along with previous captures cited in the discussion, according to the species prevalence diversity pattern.

captures were performed twice a month from 2001-2003. We computed the geometric mean by month of *Lutzomia neivai* per night as well as the precipitation and maximal and minimal temperature recorded daily in Resistencia, 8 km from the capture site (Facultad de Ingenieria, UNNE Resistencia, database), for the entire capture period. Sandflies reported in previous papers from neighboring ecologic regions are presented for comparative purposes, with references and sites reported (Fig. 1).

Statistical analysis - Multiple regression analysis was applied in order to examine the relationship between the geometric means of sandfly abundance and environmental variables (predictors) (InfoStat 2007^{TM}). The independent variables applied in the analysis included precipitation (pp; mm), evapotranspiration (et; mm piche) and maximum temperature ($t_{max;}$ ºC). The Pearson simple correlation coefficient was used to determine which variables were included in the multiple lineal regression model, such that minimum temperature (t_{min}) was excluded from the analysis ($r_{tmax\text{-}tmin} = 0.95$; $p < 0.001$). The media and ranges of included variables (11 samples) included precipitation [56.9 (4.6-202.1)], maximum temperature [27.60 (21.31-32.34)] and evapotranspiration [71.42 (15.17-101.42)]. Outliers were detected graphically by residuals of sandfly abundance and data

TABLE I

Phlebotomine caught with CDC traps by site and species in the Chaco region, Argentina, 2000-2007

Province	Locality	Month/year	Coordenates (S, W)	*Ln*	*Lm*	*Lc*	*Lq*	*Ls*	*Lp*	*Br*
Salta	Piquirenda	10/2005	22º23'38", 63º47'45"	3						
Salta	Tartagal Km 6	10/2005	22º28'04", 63º47'26"	58	13					1
Salta	Tartagal Km 18	10/2005	22º28'14", 63º39'19"	27	24	3				1
Tucumán	7 de Abril	11/2004	26º17'13'', 64º30'09''	1	33	3				
St Estero	La Fragua	11/2004	26º03'45'', 64º20'45''		25	4				
St Estero	El Mojón	11/2004	26º05'15'', 64º19'20''		3	1				
St Estero	Nueva Esperanza	11/2004	26º11'54'', 64º14'53''	12	78	10				
St Estero	Los Quiroga	11/2004	27º39'29'', 64º21'14''			1				
St Estero	Camino de la Costa	11/2004	27º50'51'', 64º11'41''	1	8	2				
St Estero	Gral Pinto	11/2004	27º59'21'', 64º03'31''			1				1
St Estero	Pozo Hondo	11/2004	27º05'13'', 64º27'29''		3					
St Estero	La Dormida	11/2004	28º19'55'', 64º00'31''		2	3				
St Estero	Robles	4/2008	27º55'25'', 64º05'13''		4					
St Estero	Brea Pozo	4/2008	28º15'09'', 63º56'55''		27	4				
St Estero	Tintina	4/2008	27º01'43'', 62º42'07''		19	2				
St Estero	Donadeu	4/2008	26º43'14'', 62º43'28''		28	10[a]				
St Estero	Campo Gallo	4/2008	26º35'19'', 62º50'45''		10	2				
St Estero	Huachana	4/2008	26º34'42'', 62º51'40''		15					
St Estero	Monte Quemado	4/2008	25º48'26'', 62º49'54''		5					
St Estero	Taboada	4/2008	28º00'26'', 63º44'23''		2	1				
St Estero	Suncho Corral	4/2008	27º56'26'', 63º26'21''		1555	64				
St Estero	Quimili	4/2008	27º39'36'', 62º26'09''		10	2				
St Estero	Pampa Guanacos	4/2008	26º13'50'', 61º49'44''		1					
St Estero	Garza	4/2008	28º09'13'', 63º32'29''		22	3				
St Estero	Lugones	4/2008	28º20'32'', 63º20'06''		1	3				
St Estero	Col Dora1	4/2008	28º35'15'', 62º58'15''		1	1				
St Estero	Col Dora2	4/2008	28º35'46'', 62º51'00''		3					
St Estero	Yacu Human	4/2008	28º55'31'', 63º19'19''		2					
St Estero	Río Dulce	4/2008	28º58'26'', 63º24' 06''		4					
St Estero	Los Telares	4/2008	28º58'47'', 63º27'04''		22	5				
St Estero	La Banda (62 sites)	11/2007								
		4/2008	27º44'-45', 64º12'-15'	2	115	14				
Chaco	Nueva Pompeya	10/2006								
		9/2007	24º55'57", 61º30'01"	2	1333	157	20		46	
Chaco	Monte alto	9/2001								
		10/2003	27º26'42", 58º54'51"	3365	40	15		19		5
Córdoba	Altos de Chipión	11/2004								
		5/2005	30º54'19'', 62º18'11''		23					
Formosa	Laguna Blanca	10/2007	25º08'26", 58º14'26"	29	1					
Formosa	Lagun Naineck	10/2007	25º12'53", 58º07'12"	8						1

a: two *Lutzomyia cortelezzii* males and two *Lutzomyia sallesi* males (the males in the other sites were all *L. cortelezzii*); *Br: Brumptomyia spp.; Lc: Lutzomyia cortelezzii* complex *(Lu. cortelezzii-Lu. sallesi); Lm: Lutzomyia migonei; Ln: Lutzomyia neivai; Lp: Lutzomyia peresi; Lq: Lutzomyia quinquefer; Ls: Lutzomyia shannoni.*

collected in November were considerer outliers. The fulfillment of independence, homoscedasticity and normality in the multiple linear regression model was tested. The best combination of predictors was considered to be the one that had all terms in the equation below a significant level ($p < 0.05$) and the highest determination coefficient (R^2). No validation was performed for the selected model.

Human ACL cases - The average and standard deviation of the cases reported by the Central Laboratory of Chaco province and the Direction of Epidemiology of Santiago del Estero province were computed and classified as either the DC or HC, according to the reporting department (intra-province political districts). Cases reported from the Formosa province were not included, as each department has a dry and a humid area. The population denominator was taken as the middle period point according to the 2001 census (INDEC 2001).

RESULTS

The phlebotomine caught by species in the Chaco region and the captures sites are shown in Table I and Fig 1. In the Monte Alto capture site located in the HC near the Parana river, *Lu. neivai* is abundant and in greater

numbers than the remaining species. *Lutzomyia migonei* is prevalent in many sites of the DC in the provinces of Santiago del Estero, Tucumán and Chaco. In Córdoba, however, *Lu. migonei* is the sole species found and the first phlebotomine recorded for the province. The fly was captured in Icho Cruz using a Malaise trap. In Los Quiroga and General Pinto, the only species that was captured was *Lutzomyia cortelezzii-sallesi* (females indistinguishable) and *Brumptomyia* spp. (females indistinguishable), although both are zoophilic and lack a proven association with ACL transmission. The collections made in the highly modified environments of the Formosa region (at an industrial chicken farm in Laguna Blanca), and the Yungas-Chaco transitional ecotones of Salta showed few sandflies, with *Lu. neivai* prevalent, or almost equal in abundance to *Lu. migonei* in the more typical Chacoan landscape (Tartagal Km 18).

Captures at Suncho Corral were performed simultaneously in a pigpen and an adjoining stable, 323 sandflies were collected in the former and 1,296 close to the horses. *Lu. migonei* was significantly more abundant in the stable than in the pigsty (1,259/296), but neither *Lu. cortelezzii* abundance (27/37) nor the sex ratio females/males (*Lu. migonei* 0.6-0.7, *Lu. cortelezzii* 2.8-2.0) in both sites were significantly different.

Lu. neivai isolated in the HC showed a bimodal annual pattern with an activity season that spikes from March-April (autumn), with its highest peak in November (spring) (Fig. 2). During the drier and colder winter, only a few flies were captured. The *Lu. neivai* adult populations abruptly recovered during the rainy season and subsequently decreased during the months of lower precipitation or higher temperatures, until the fall when the population progressively increased.

The best combination of climatic predictors of sandfly abundance includes precipitation as a polynomial of the second degree: ln sandflies (t_{max}, et, pp) = -118.73 + 8.84 t_{max} - 0.55 et - 1.48 pp + 0.01 pp^2. The regression coefficients with the corresponding 95% confidence intervals (CI 95%), p-values and R^2 of the best model obtained are shown in Table II.

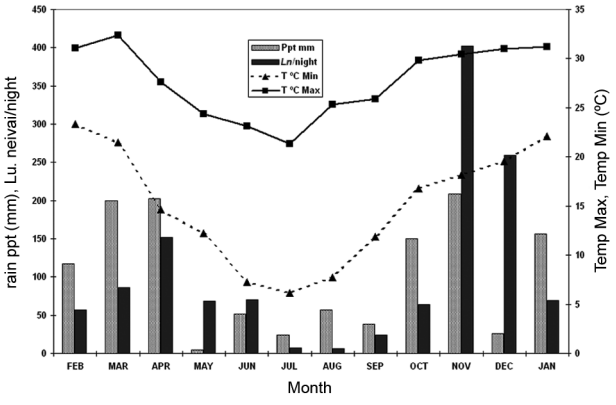

Fig. 2: *Lutzomyia neivai*/night (*Ln*/night) abundance in Monte Alto, precipitation (ppt mm) and maximal (TºC Max) and minimal (TºC Min) temperature in Resistencia, geometric mean averaged by month, Chaco, Argentina, 2001-2.

TABLE II

Regression coefficients with the corresponding 95% confidence intervals (CI), p-values and R^2 of the best model obtained

Variable	Coefficients	CI 95%	p-value
Maximum temperature	8.84	5.45/12.24	0.00007
Evapotranspiration	-0.55	0.98/-0.13	0.0179
Precipitation	-1.48	-2.21/-0.74	0.0027
Precipitation^2	0.01	0.0041/0.01	0.0014
Constant	-118.73	-204.08/-33.37	0.0144
R^2	0.92		

The cumulative 244 ACL cases reported in the province of Chaco from 1994-2005 were produced by 23 of its 25 departments. This includes the departments with the highest rates in each region; the cases of ACL reported in the humid region were 10.7 (± 4.8) cases/10,000 inhabitants (Libertad, Bermejo, 1st Mayo, Libertador General San Martín), and the cases reported in the dry region were 5.2 (± 1.5) cases/10,000 inhabitants (9th Julio, Almirante Brown, Gral. Güemes, Independencia).

In Santiago del Estero there were 111 cumulative cases of Leishmaniasis between 1996-2005, although 28 were secondary muco-cutaneous cases and seven were cutaneous with more than a year of evolution according to documentation. The remaining 77 ACL cases were reported from 14 of 27 departments. The departments along the Northern border of Salta and Chaco were among the four departments with the highest rates (Pellegini, 20.5/10,000 inhabitants, and Copos, 3.7/10,000). However, in both departments, the majority of the cases were in the hyperendemic area of Salta or Catamarca during the infection period. Furthermore, the Southern contiguous departments reported few cases (Alberdi, 1.2/10,000, and Jimenez, 0 cases). However, San Martín and Loreto, South of the capital in the Rio Dulce basin, had 6.6 and 2.3 cases/10,000, respectively. These cases included at least five familiar clusters with two to six individuals.

DISCUSSION

The results presented here support the hypothesis that in the Argentinean Chaco two main diversity patterns of phlebotomine captures are observed: (1) *Lu. migonei* as the prevalent species with or without scarce *Lu. neivai* in the DC and (2) *Lu. neivai* as a highly abundant and prevalent species with scarce *Lu. migonei* in the HC (Salomón et al. 2005). Intermediate results with *Lu. neivai* showing increasing prevalence were observed in modified humid periurban sites (Laguna Blanca industrial chicken farm, Laguna Naineck pigsty), or proximal to the Yungas subtropical foothills (gradient from Piquirenda to Tartagal km 6 to Tartagal km 18 had almost an equal abundance of both species). This relative abundance distribution was previously observed in the Formosa province where the *Lu. neivai/Lu. migonei* ratio in the Bermejo river subtropical gallery forest was 18.6 (n = 3,292), in contrast to a ratio of 0.1 (n = 41) in the Chacoan dry landscape near the Pilcomayo river (a similar capture effort with traps was used there). In

the Santa Fe province on the Parana river shore, this ratio was 855 (n = 856), while it was 1.5 (n = 5) in the former Chaco woodlands, which have been deforested since the 1950s (Salomon 2006a, b).

Despite the different capture efforts, the traps in *Lu. migonei* prevalent sites generally had fewer individuals per night than captures in the *Lu. neivai* prevalent sites reported here. A high abundance of *Lu. neivai*, prevalent over *Lu. migonei,* was also reported in the captures related to epidemic ACL outbreaks in modified environments from the subtropical humid regions on both sides of the Argentinean DC. In the Western Yungas foothills, the observed *Lu. neivai/Lu. migonei* ratio was 14.1 (n = 953) in Alberdi, Tucumán, and 96.2 (n = 50,457) in Pichanal/Embarcación, Salta. In the eastern Paranaense region, in Bella Vista, Corrientes, the ratio was 120.4 (n = 3,279) (Salomón et al. 2004, 2006d, e). Furthermore, the sandfly captures made in Argentina prior to 1950 reported *Lu. migonei* and *Lu. cortelezzii* in the Santiago del Estero and Formosa provinces, but *Lu. neivai* (as *Flebotomus intermedius*) was only reported in sites of the humid region of the Chaco province (Castro 1959). *Lu. cortelezzii* historical records should be taken cautiously as the species of the *cortelezzii* series are very similar or sometimes indistinguishable (*Lu. cortelezzi-Lu. sallesi* females) (Galati et al. 1989, Young & Duncan 1994, Galati 2003), and many times sympatric as *Lu. cortelezzii* and *Lu. sallessi* in the captures made at Donadeu, Santiago del Estero reported here. However, these findings represent the first report of Phlebotominae sandflies in the Cordoba province and the southernmost record of *Lu. migonei* in the Central region of Argentina.

Lu. migonei and *Lutzomyia intermedia*, which are closely related to *Lu. neivai*, were incriminated as peridomestic vectors of ACL in foci due to *Leishmania braziliensis* (Azevedo et al. 1990, Pita-Pereira et al. 2005). This parasite was isolated from human ACL cases during ACL outbreaks in Argentina, from ACL cases in Santiago del Estero, and from *Lu. neivai* caught in Tucumán (Cuba-Cuba et al. 1996, Segura et al. 2000, Cordoba-Lanus et al. 2005, 2006). *Lu. neivai* is associated with epidemic outbreaks in highly modified environments, while, *Lu. migonei* is a relatively zoophilic vector, comparatively, which is highly attracted to horses in Argentina and may be involved in the link between the zoonotic and the anthropozoonotic transmission cycles (Chaves & Añez 2004). Many species of vertebrates usually cited as blood sources of phlebotomine are characteristic of the Chaco even edentate, the main source for *Brumptomyia* spp. (Zuleta & Bolcovic 1994).

The recent epidemics reported in the Argentinean Chaco region were located in the humid region or were found to be related to the gallery forests of the rivers from the Yungas and Paranaenese basins, where *Lu. neivai* is the prevalent species. ACL outbreaks were reported in: (1) the General Vedia and Cancha Larga Chaco (Miranda et al. 1999, Salomón et al. 2001); (2) in San Martín, Loreto, Atamisqui and Silipica, Santiago del Estero, with peridomestic transmission enhanced by closeness to the rivers (Yadón et al. 2001, 2003); and (3) in Laishi (Basualdo unpublished observations) and Lomitas, Formosa (Salomón et al. 2006b) on the Bermejo river gallery forest that extends the Yungas landscape inside the DC region (Sennhauser 1991). The annual pattern of *Lu. neivai* in the HC was similar to that which was described for the Yungas region (Salomón et al. 2004): it was bimodal, with the highest peak in spring and another peak in the fall, depending on the precipitation and temperature. The precipitation-temperature dependence of *Lu. neivai* abundance was also observed in the multiple linear regression model. Interestingly, if the sandfly captures in November are excluded, the obtained R^2 is as high as 0.92, suggesting that the climatic variables explain the annual dynamics but not the highest peak. However, it should be noted that the model is limited due to the scarce number of samples and requires further validation. The *Lu. neivai* peak could be related to an autocorrelation component of the sandfly population, or to unsynchronized climatic data (precipitation lag) (Salomón et al. 2004).

In the DC area, where *Lu. migonei* is prevalent, there are annual isolated cases or limited, focused, familiar or "hot spot''-related case clusters, as is typical in zoonotic cycle scenarios. However, there are reports of *Leishmania guyanensis* and *Leishmania amazonensis* infections in the area besides *Le. braziliensis* (Frank et al. 2003, Marco et al. 2005, 2006). Parasite characterization in both Chacoan vectors and human cases is currently an ongoing project.

Therefore, the case reporting distribution of sandfly diversity and dynamics (with the antecedents discussed above) supports the hypothesis of two different ACL transmission patterns: (1) the DC, where *Lu. migonei* was the prevalent species, had isolated ACL cases and a zoonotic cycle, and (2) the HC, where *Lu. neivai* was the prevalent species and in high abundance, had an increase in ACL incidence and outbreaks, and an anthropozoonotic cycle. Furthermore, during the pre-epidemic period prior to the 1950s, *Lu. neivai* was not recorded in the hyperendemic/epidemic area of the Salta province (Castro 1959), while it is highly abundant today (Salomón et al. 2004).

In conclusion, the ACL vector sandfly fauna of the DC region was typically represented by *Lu. migonei* with *Lu. neivai* in lesser abundance or absent. The ACL transmission in this area was consistent with zoonotic cycles, scattered cases, or accidental intrusion within sandfly-infected "hot spot" environments. However, the HC and the gallery forest were related to subtropical humid basins and showed a pattern of *Lu. neivai* prevalence with fewer *Lu. migonei*, similar to the pattern observed in the emergent epidemic scenarios of the subtropical Yungas foothills and the Paranaense woods in peridomestic environments. The *Lu. neivai* annual dynamics in the HC region, which were similar to those of the Yungas-Paranense epidemic region, were dependent on rain and temperature.

There are important public health implications for the relationships between increased prevalence and abundance of *Lu. neivai*, increased ACL incidence and epidemic risk, as well as factors including landscape modification, connections with ACL zoonotic or anthro-

pozoonotic foci and rising humidity. The climate trends observed in the region show a steady increase in rain precipitation that will extend the current agriculture-exploitable area and is enhanced by ENSO activity (Villalba et al. 1998, Minetti et al. 2003, Haylock et al. 2006). Consequently, an increase of ACL emergence can be expected in the Chaco region. The sandfly pattern changes from *Lu. migonei*-prevalent populations to *Lu. neivai*-prevalent/abundant populations can also be used as an indicator for forecasting epidemic risk at sentinel capture sites.

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