

Phlebotominae sand flies associated with a tegumentary leishmaniasis outbreak, Tucumán Province, Argentina

Flebotomíneos associados com surto de leishmaniose tegumentar, na Província de Tucumán, Argentina

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ABSTRACT

The distribution of sand flies and cases of tegumentary leishmaniasis in the area surrounding JB Alberdi City, and the proximities of Catamarca province were studied, after an increase of reported cases from JB Alberdi, Tucumán province, in 2003. Of 14 confirmed cases, 57% were females and 57% were less than 15 years old, suggesting peridomestic transmission. However, 86% of them lived close to the Marapa river forest gallery and related wooded areas. Over 1,013 sand flies were collected; *Lutzomyia neivai* (Pinto, 1926) was prevalent at all the sites (92.3%), while *Lutzomyia migonei* (França, 1920) (6.7%) and *Lu. cortelezzi* (Brèthes, 1923) (1%) were also found. The spatial distribution of *Lu. neivai* overlapped that of the cases, with higher abundance in microfocal hot spots close to the river in stable vegetated habitats or modified habitats with shadow and animal blood sources. The cumulative outcome of anthropic, ecological and climatic factors could have contributed to the onset of the outbreak.

Key-words: Tegumentary leishmaniasis. Phlebotominae. *Lutzomyia*. Argentina.

RESUMO

Após um aumento nos casos notificados na Cidade de JB Alberdi, Província de Tucumán, no ano de 2003, foram estudados a distribuição de flebotomíneos e casos de leishmaniose tegumentar nos arredores de JB Alberdi, e na área próxima à Província de Catamarca. De 14 casos confirmados, 57% foram mulheres e 57% tinham menos de 15 anos de idade, sugerindo transmissão peridomiciliar. Contudo, 86% dos casos residiam perto da galeria florestal do rio Marapa e perto de ilhas de vegetação residual. De 1.013 flebotomíneos coletados, *Lutzomyia neivai* (Pinto, 1926) foi a espécie dominante em todos os sítios (92,3%). Também, se capturaram *Lutzomyia migonei* (França, 1920) (6,7%) e *Lutzomyia cortelezzi* (Brèthes, 1923) (1%). A distribuição espacial de *Lu. neivai* se sobrepõe com a dos casos com alta abundância em pontos quentes microfocais próximos ao rio, em habitats estáveis com vegetação, ou habitats modificados com sombras e fonte de sangue animal. O efeito acumulativo de fatores antrópicos, ecológicos e climáticos pode ter contribuído à geração do surto epidêmico.

Palavras-chaves: Leishmaniose tegumentar. Flebotomíneos. *Lutzomyia*. Argentina.

The first domestic focus of tegumentary leishmaniasis (TL) in Tucumán province was reported in 1949 by Romaña & Ábalos¹⁹, involving dogs, humans and *Lutzomyia* (*Evandromyia*) *cortelezzi* (Brèthes, 1923) near the residences, but most of the TL cases were scattered in the region up to 1986. Since then, in the southern *Yungas* montane foothills of the Tucumán-Catamarca

area, several tegumentary leishmaniasis peridomestic outbreaks have taken place^{12,21}.

Lutzomyia (*Nyssomyia*) *neivai* (Pinto, 1926) (*Lutzomyia intermedia sensu lato*), *Lutzomyia cortelezzi*, *Lutzomyia migonei* (França, 1920) and *Brumptomyia pinto* (Costa Lima, 1932) had been previously reported in Tucumán by 1947². Collections

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Recebido para publicação em 13/4/2005

Aceito em 15/5/2006

after 1990 reported *Lutzomyia shannoni* (Dyar, 1929), besides the previously cited species (except *Br. pintoi*)¹², and *Lu. neivai* naturally infected with *Leishmania (Viannia) braziliensis* Vianna, 1911⁵. In Catamarca, only a single record of both *Lu. cortelezzii* and *Lu. migonei* was registered, from the village of Tapso¹⁹.

During 2003, an outbreak of TL was reported in JB Alberdi, Tucumán province. In this study the distribution of sand flies and cases in the JB Alberdi area, and the proximities of Catamarca province were studied the same year as the outbreak. The results are discussed in the framework of the time-space risk distribution and the regional epidemiological trends, in order to contribute to the design of appropriate surveillance and control strategies.

MATERIAL AND METHODS

Study area. Sand fly collections were performed in JB Alberdi county, Tucumán province, and Ali Jilan and Bañado de Ovanta villages in Catamarca province (Table 1). The region belongs to the anthropic modified foothills of the *Yungas* subtropical montane forest, in its southernmost region. Outside the natural reserve of the Escaba dam (36km from JB Alberdi, 27° 67' S, 65° 77' W) the native vegetation remains in patches, mainly alongside the rivers and streams. Transitional vegetation with the eastern xerophytic forests is also present, but most of the land is highly degraded due to intensive agricultural cultivation (sugar cane, soybean, wheat, tobacco)^{3,6}. Precipitation

in the area is characterized by a monsoonal regime, with 60-90% of the total annual precipitation concentrated in the summer and early fall months (November-April)²⁸.

Cases. Files of cases were investigated from the Concepción Hospital records and the Epidemiological Surveillance System of Tucumán province. The cases confirmed by parasitological diagnosis were visited, the houses georeferenced and the gender, age and date of ulcer appearance confirmed. The transmission scenario in Catamarca province was confirmed with local physicians.

Sand flies. Collections were performed with mini CDC light traps²⁷ at 18 sites in Tucumán, sites 1-10 (Figure 1, Table 1) and Catamarca provinces, Ali Jilan sites 11-14 and Bañado de Ovanta sites 15-17 (Table 1). In Tucumán, the traps were placed overnight on two consecutive nights, and in Catamarca province, captures occurred on one night only, between October 28th and November 1st, 2003. The minimal temperatures during collections days were 14-19°C and the maximal, 27-35°C. The sites were associated with recent TL human cases, except Bañado de Ovanta (Catamarca), which was related to cases reported in 1999. The sites were rated according to their habitat suitability for sand flies with an empirical scale ranged from 1 to 4, which it was obtained by adding the following dichotomous attributes (presence 1, absence 0): a) river or stream at a distance $\leq 200\text{m}$; b) surrounding land of 5m width, surface shadowed by the canopy $\geq 70\%$; c) patch of trees of at least 15m in width and 5m in length or dense patch of sugar cane of 2m in diameter; d) a resting place for domestic animal

Table 1 - Phlebotominae captured by site with mini light trap, Tucumán (sites 1-10) and Catamarca (site 11-14 Ali Jilan, 15-17 Bañado de Ovanta) provinces, Argentina, 2003. Habitat rate, coordinates, abundance and relative abundance (%) by species, *Lutzomyia neivai* relative capture, and female proportion (captures > 10 individuals) (F%).

Habitat rate	Site	Coordinates	<i>Lu. neivai</i>	<i>Lu. migonei</i>	<i>Lu. cortelezzii</i>	Relative capture (%)	F%
4	1	27° 38' 13" S 65° 39' 23" W	339 (92.6)	24 (6.6)	3 (0.8)	100.0	54.6
4	2	27° 37' 59" S 65° 39' 14" W	333 (93.0)	21 (5.9)	4 (1.1)	98.2	47.4
3	3	27° 37' 14" S 65° 41' 29" W	165 (89.7)	18 (9.8)	1 (0.5)	48.7	57.6
2	4	27° 37' 43" S 65° 40' 31" W	53 (100.0)	0	0	15.6	41.5
2	5	27° 37' 22" S, 65° 36' 21" O	21 (87.5)	2 (8.3)	1 (4.2)	6.2	52.3
2	6	27° 37' 22" S 65° 36' 00" W	7 (77.8)	1 (11.1)	1 (11.1)	2.1	
1	9	27° 33' 15" S 65° 37' 05" W	5 (100.0)	0	0	1.5	
0	7	27° 35' 28" S 65° 36' 00" W	0	0	0	0	
0	8	27° 35' 48" S 65° 37' 24" W	1 (100.0)	0	0	0.3	
0	10	27° 32' 35" S 65° 36' 54" W	1 (100.0)	0	0	0.3	
2	11	28° 08' -10' S	10	2	0		
	14	65° 29' -30' W	(83.3)	(16.7)			
0	15	29° 03' -05' S	0	0	0		
	17	65° 18' -19' W					

(pigs, goats, horses, chickens) or minimally disturbed vegetation of 50m². All captured sand flies were dry stored until identification using the keys of Young and Duncan³¹, with modifications by Marcondes¹³.

Additional data sources. The population numerator for the computed incidence rate was taken from the National Institute of Statistics and Census¹¹. Weather data were provided by Servicio Meteorologico Nacional, Fuerza Aerea Argentina, from the station at San Miguel de Tucumán (26° 50' S, 65° 12' W), whose records are representative of rainfall patterns throughout the region¹⁰.

Data analysis. The Fisher's exact test and χ^2 test were used for bivariate analysis. All statistical tests were considered significant at $P < 0.01$.

RESULTS

Tucumán province reported 33 cases of TL during 2003, 14 of them clustered around JB Alberdi village, with over 23 suspected cases in the area. Patient age in these cases ranged from two to 54 years old, 8/14 were less than 15 years old and 8/14 were females. Their spatial distribution shows two cases of residents in an urban area (JB Alberdi and Villa Belgrano) and the remaining 12 (85.7%) related to the river Marapa, four of them within a radius of 0.5km, and 13 within a radius of 2km (Figure 1). Taking into account the population of the two villages involved (18,348) the urban incidence during 2003 was 0.01%, while the rural incidence was 0.12%, if the remaining population of JB Alberdi county (9,858)

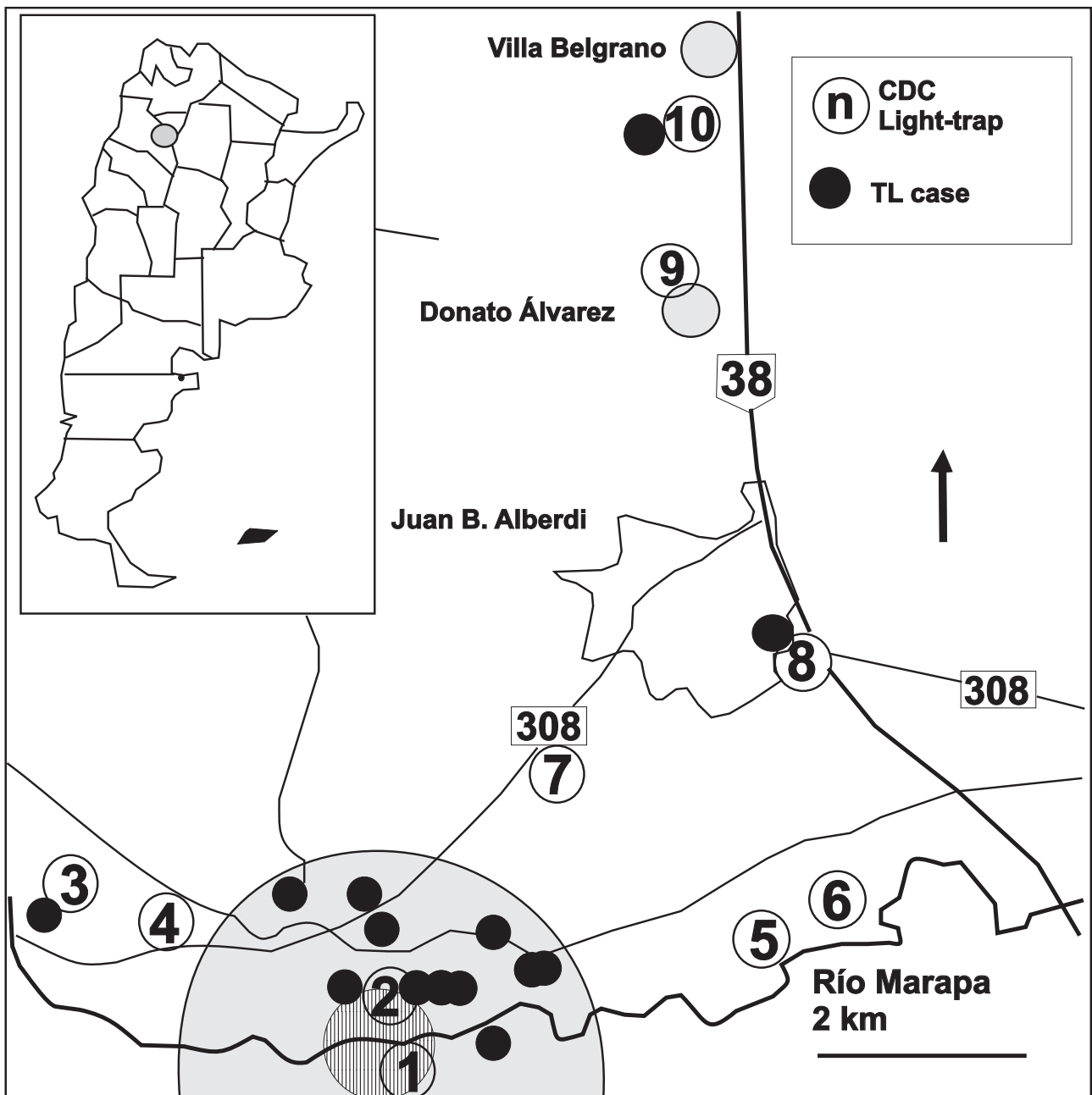


Figure 1 - Map of the Tucumán study area showing the location of cases (black dots), the phlebotomine capture sites (numerated according to Table 1), and the areas of 0.5 and 2km where the cases and larger population of phlebotomine were clustered. In the right corner map of Argentina with the Tucumán-Catamarca study area encircled.

was equally exposed, although actually less than 20% of this figure live close to the Marapa river. Diagnosis took place between March and September, however the appearance of ulcers occurred in April for 8 cases, June for 2, and a single case in October 2002, March, July and August 2003 (Figure 2). The cumulative rainfall during the season 2001-2002 (November-October) was significantly higher than that of 2002-2003 (Figure 2), while the temperature records did not differ between these periods.

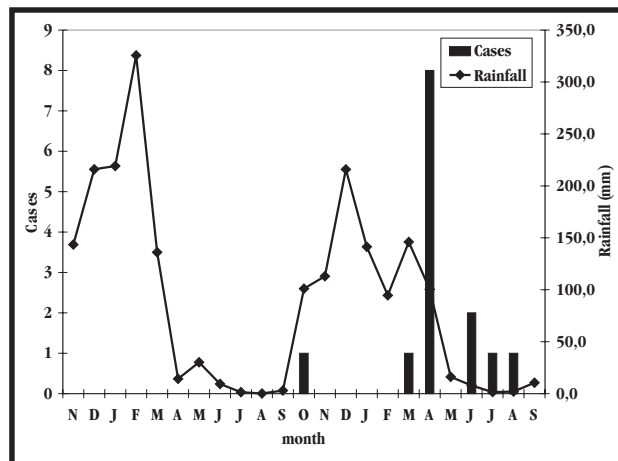


Figure 2 - Cumulative rainfall (mm) and tegumentary leishmaniasis cases by month since November 2001 to September 2003, JB Alberdi, Tucumán, Argentina.

Captures produced a total of 1,013 sand flies of three species: *Lu. neivai* 92.3%, *Lu. migonei* 6.7%, and *Lu. cortelezzii* 1% (Table 1). The prevalent species at all the sites was *Lu. neivai*, which was also the only species found in peridomestic degraded habitats far away from the river (sites 8, 9, 10 Figure 1 and Table 1) or in small patches of vegetation less than 10m wide (site 4). On the other hand, all three species together were found only in habitats close to the Marapa river, with dense vegetation coverage or patches with relatively less anthropic degradation. *Lu. neivai* abundance increased as the *habitat rate* scale increased (CR, Table 1).

The species proportion did not differ significantly among sites where the three species were found together and traps collected more than 10 insects; *Lu. neivai* relative abundance ranged from 87.5% to 93% of the whole capture. The female proportion of *Lu. neivai* did not differ either, despite the fact that one of the two sites with highest captures was in a large undisturbed vegetated area and the other was close to the house of three cases with several pig dwellings.

The captures made in Catamarca province are not quantitatively comparable with those of Tucumán province, because the former occurred on a single stormy night. However, *Lu. neivai* and *Lu. migonei* were found in Ali Jilan, where TL cases were reported during 2003.

DISCUSSION

Cases of TL were clustered in the JB Alberdi area in both time and space during 2003. The main TL transmission period took place during the first half of the fall of 2003. The age and

gender distribution of the cases suggests peridomestic transmission. The spatial distribution of cases shows a strong risk association with the Marapa river forest gallery, probably in scattered *hot spots*. The urban cases may be due to behavioral risk related to the river or to a periurban transmission of low probability, even during high transmission periods.

The TL cases reported from Tucumán province to the National System of Epidemiological Surveillance (SINAVE), show four periods with more than 15 cases/year since 1970: 1986-1988 (125 cases), 1991-1992 (88 cases), 1995-1997 (81 cases), 2003-2004 (83 cases). Although transmission seems to be distributed along the SW-NE axis of the foothills of *Yungas montane* forest since the first reports^{19 29}, for at least the two last epidemic waves the cases were concentrated in the southern region from April to June, where cases were also present during inter-epidemic years (data from Tucumán Epidemiological Surveillance System). Catamarca province, with a smaller area of transmission and less populated than the Tucumán area, also showed a peak in the period 1986-1988 (135 cases) and 5-7 cases/year in 1990-1992, 1999 and 2003 (SINAVE). Thus, leishmaniasis is endemic in the region between 27° and 29°S, but parasite circulation increased with epidemic outbreaks during the last decade in this area of transitional vegetation and intensive agriculture land use.

Three out of the five recorded species for the province were collected in the present work. *Brumptomyia pinto* was reported in the 1940's for the last time², and a single individual of *Lu. shannoni* was collected between 2001-2003 in the studied area¹². In contrast, *Lu. cortelezzii* was reported for the first time in the province since 1943, although in the captures related to the JB Alberdi TL outbreak, *Lu. neivai* was the most abundant species and the only species found in highly disturbed habitats, such as periurban vegetation.

Lutzomyia neivai has a great capacity for adaptation to human modified environments, it was incriminated as a vector of *L. (V.) braziliensis* in several TL foci in Argentina^{21 22}, and it was found naturally infected in Tucumán in the study area near site 1⁵. *Lu. migonei*, was also present in the sites close to cases; it has been found naturally infected in Brazil¹, where it was implicated as vector¹⁷. Thus, *Lu. migonei* might play a role as a secondary vector, it could be required to sustain the zoonotic cycle during interepidemic periods, or it could be a *hinge* species between the zoonotic cycle and human transmission⁴.

The spatial distribution of sand flies indicates that the highest abundance was found in the forest gallery and adjacent forested places of the Marapa river, associated with the size, density, stability and contiguity of the patches of secondary forest. The risk of an effective human-Phlebotominae contact decreases consistently from the river to the periurban area and nearby villages. This risk could also be increased by a peak of sand flies due to the rainfall pattern and/or to the reduction of insectivores.

In relation to the rainfall pattern in northwestern Argentina, *Lu. neivai* showed a positive association between an above average rainfall peak, or difference between years, and sand fly abundance

during the fall of the following year, the season of highest risk²³. The time relationship between the rainfall and the cases in this outbreak, was consistent both with the lag between the precipitation and the time of appearance of cases during the fall (Figure 2). In the study area, forest fires also showed an association of a year lag with unusual rainfall peaks, when young trees (fuels) invade shrublands under conditions of increased moisture availability¹⁰, a scenario that might also enlarge the sand fly breeding area.

In relation to insectivore abundance, until 2001 a stable population of the bat *Tadarida brasiliensis* (Saint-Hilaire, 1824) inhabited the Escaba Dam structure. It was estimated to be $10\text{-}12 \times 10^6$ individuals, which ate 7,000kg/insects/day (Bárquez R: unpublished report cited in⁷). During July and November 2002 the bats were relocated from inside the dam, although the law protected these populations¹⁸. The current population, after relocation was estimated to be $0.45\text{-}0.90 \times 10^6$ individuals (Mosa S, Regidor H, Núñez F: unpublished report cited in⁹). *T. brasiliensis* eats mostly moths and other insects between 5 and 9mm long³⁰, so phlebotomine are probably excluded from their diet. However, the bat may be a significant blood source for sand flies^{14 20}, thus the relocation and radical reduction in the *Tadarida* populations could have impacted the abundance and dispersion of phlebotomine. Furthermore, *Lu. neivai* abundance in time and space is consistent with a metapopulation distribution²³, so the Escaba reserve and the gallery forest of the dam basin, including the Marapa river, may constitute a source population.

In Catamarca, there was a single registration of *Lu. cortelezzii* and *Lu. migonei* from 1943¹⁹, therefore this is the first report of *Lu. neivai* and *Lu. migonei* in the active TL transmission area of this province (cases 2002-2003). No sand flies were collected in the locations that reported TL cases in 1999 (Bañado de Ovanta), but the place has been intensively deforested and the land eroded by droughts since the transmission period.

The TL outbreaks took place due to the cumulative effects of anthropic, ecological and climatological variables. The southern region of Tucumán province was endemic for TL transmission, and *Lu. neivai*, a vector of *L. (V.) braziliensis*, was concentrated in patches of trees, although it can colonize peridomestic habitats. During the fall of 2003, *Lu. neivai* populations close to JB Alberdi city might have been unusually high due to the 2002-2003 rainfall pattern and the 2002 insectivore depletion. Human activities associated with Marapa river *hot spots* from dusk to dawn, including subsistence, religious pilgrimage and recreation, after the summer rains, increased the risk of transmission. Reservoir-parasite distribution might also have been modified by the climatological and ecological disturbances.

From a regional point of view, the frequency and intensity of tegumentary outbreaks has increased in Argentina since 1970. The increase in precipitation during the three last decades of the 20th century has been also unprecedented for the past 200 years, with a *jump* (variation 26%) around 1956^{15 28}. This period may be close to being reverted, as long periods of excess or deficit in precipitations might have recurrence intervals every 54-65 years¹⁷. On the other hand,

the species diversity is the same along the 700km of the *Yungas* from the Bolivian border, despite the high local endemicity and the north-south discontinuity³. Thus, species such as *Lu. neivai* and *Lu. migonei*, adapted to human modified habitats, could colonize new areas from long term refugees to drier vegetated environments, as the rainfall increases. Furthermore, agricultural practices maintain vegetal corridors to prevent fire, wind and to protect stream banks, thus concentrating vectors and potential reservoirs without predator control, and creating *hot spots*^{25 26}. Focal deforestation for citrus culture in Tucumán grew during the 1980s and 1990s in the transitional forests along the western foothills. In JB Alberdi county, 2,590 hectares were deforested from 1990 to 2002, and the potential remaining area for deforestation is less than 300 hectares²⁴.

In conclusion, TL endemic transmission and epidemic episodes will continue in the southern *Yungas* region and the transitional area to the west. The outbreaks may involve urban population and large areas, however the main source of transmission seems to be spatially restricted to small foci related to the vector and eventual reservoir abundance. This space and time (fall) risk concentration should be taken into account with regard to surveillance and interventional strategies, both for vector monitoring and control, and for recommendations regarding the avoidance of risk behaviors.

ACKNOWLEDGEMENTS

The authors are grateful to the National Coordination of Vector Control team, Dr M. Zaidenberg, Mr J. Zárate (Tucumán delegation) and especially Mr R. Moya, for the operational support and technical collaboration. The authors also thank the teams of JB Alberdi Hospital, Drs A. Tilca, R. Villafañe, P.H.C agents A. Martínez, R. Fenoglio, S. Fenoglio; JB Alberdi Municipality Lic. M. Victoria; Concepción Hospital Drs A.M. Cadiñano, D. Agu, L. Grellet de Canseco, M. Lazarte; Catamarca P.H.C. Ayosa.

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