Visceral leishmaniasis in border areas: clustered distribution of phlebotomine sand flies in Clorinda, Argentina

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Three years after the first report of Lutzomyia longipalpis *in Clorinda, Argentina, a border city near Asunción, Paraguay, the city was surveyed again.* Lu. longipalpis *was found clustered in the same neighbourhoods in 2007 as in 2004, even though the scattered distribution of canine visceral leishmaniasis was more related to the traffic of dogs through the border.*

Key words: visceral leishmaniasis - *Lutzomyia longipalpis* - Argentina

During the last few decades, visceral leishmaniasis (VL) has become a serious public health threat due to its geographical spread, association with HIV and resistance to therapeutic drugs. In South America, recent reports have shown the dispersion of *Leishmania infantum chagasi* to urban environments in the south with the dog (*Canis familiaris*) as its main reservoir and *Lutzomyia longipalpis* as its main vector (Gontijo & Melo 2004, Lainson & Rangel 2005).

In Argentina, 14 cases of human VL were reported from 1925-1989 in the cutaneous leishmaniasis endemic area. However, up to that time, the only record of *Lu. longipalpis* was at the northeastern border and it was not associated with cases of VL (Salomón et al. 2001). Since the year 2000, due to the *L. i. chagasi*-associated VL outbreaks that occurred in Mato Grosso do Sul (MS), Brazil and Paraguay (Cousiño 2006, Oliveira et al. 2006, Mestre & Fontes 2007), close to the northern Argentinean border, vector surveillance has intensified. In December 2004, *Lu. longipalpis* was found in Clorinda, Argentina, 40 km from Asunción, Paraguay (Salomón & Orellano 2005). During 2006, the first autochthonous focus of *L. i. chagasi* transmission associated with human and canine VL and *Lu. longipalpis* captures was reported in Posadas, Argentina (Salomón et al. 2008b). In October 2007, three years after the first report of *Lu. longipalpis* in Clorinda, the city and surroundings were surveyed again to describe the current dispersion of vectors in this area, which experiences intense movement of humans and dogs to and from an active VL focus through the international border with Paraguay.

Clorinda (25º17'LS 57º43'LW, 62 masl), which has 47,000 inhabitants, is located at the northeastern border of the province of Formosa on the right bank of the Pilcomayo River 10 m away from its junction with the Para-

guay River (Figure). Three pedestrian bridges and many eventual fords link Clorinda to Nananawa in Paraguay, which is 40 km from Asunción, Paraguay's capital city and 115 km away from the capital of the province of Formosa by highway. The Clorinda area belongs to the eastern Chaco Region (Cabrera 1976), which has a subtropical wet climate, Köeppen type A2Aw, mean temperature of 23ºC (-2-43ºC) and an average annual rainfall of 900-1000 mm, with rain occurring throughout the whole year and the driest period running from June-August (Salomón et al. 2008a).

Phlebotominae sand flies were caught with mini-light CDC traps operating overnight from 19:00-9:00 h from October 18-October 31, 2007. Clorinda-Puerto Pilcomayo was divided into 400 x 400 m areas and at least one site was sampled in each area, with the "worst scenario" being the environment more prone to having sand flies (Figure). The traps were located in the shade 1.5 m above the ground during two consecutive nights. At each sampled site, the households were asked about dogs diagnosed with or having died from *L. i. chagasi* infections in the domicile. Additional captures were performed in Laguna Blanca and Laguna Naineck (Figure). The sand flies were kept dry until were macerated in lacto-phenol for identification following the key of Young and Duncan (1994) or as described by Andrade Filho et al. (2003) for *Lutzomyia neivai* (2003).

At 18 out of the 140 sampled sites, 106 sand flies belonging to five species were collected: *Lu. longipalpis* (50.5% of the total sand flies), *Lu. neivai* (43.9%), *Lutzomyia cortelezzii* (2.8%), *Lutzomyia migonei* (0.9%) and *Brumptomyia guimaraesi* (1.4%) (Table). *Lu. longipalpis* was found in 10 areas of Clorinda and Puerto Pilcomayo where it represented 80.6% of the captured sand flies, with a male prevalence of 75.9%. The vector density was low by site (1-4 phlebotomine sand flies/trap), except in Puerto Pilcomayo in the locations called "kilometro 9" and "kilometro 10" (12-28 phlebotomine sand flies/trap) (Table).

In Clorinda, in spite of the wider distribution of canine VL, most of the sand flies captured were clustered in the northern peripheral area, other than one trap in the downtown area in a house with a chicken
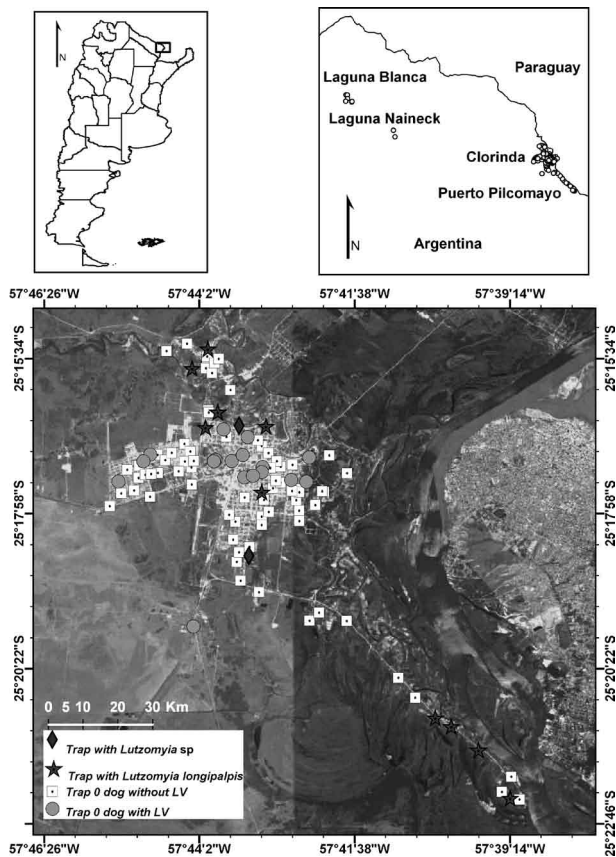
Phlebotomine capture site locations in Clorinda, Puerto Pilcomayo, Laguna Blanca and Laguna Naineck, Formosa, Argentina. Image was taken from Google Earth, version 4.0.2416 (beta) (http://earth.google. es). LV: visceral leishmaniasis.

house, while in Puerto Pilcomayo, *Lu. longipalpis* was found associated with chickens or pigs in houses on the border with Paraguay (Figure). On the other hand, in Laguna Blanca and Laguna Naineck, none of the phlebotomine sand flies trapped was *Lu. longipalpis*, even on a poultry farm where most of the specimens of *Lu. neivai* were found (Table).

Lu. longipalpis spatial distribution in Clorinda-Puerto Pilcomayo in October 2007 clustered in northern and southern outskirt areas, almost in the same sites described in December 2004 when the species was first reported in the province of Formosa (Salomón & Orellano 2005), with no further spread to Laguna Blanca or Laguna Naineck. Similar to our results, high spatial heterogeneity of this vector has also been reported in the southern Brazilian focus of Campo Grande, where 90% of the sand flies captured in the urban environment were *Lu. longipalpis* and 79.4% of the males were associated with peridomestic chicken houses (Araujo e Silva et al. 2007, Oliveira et al. 2008, Silva et al. 2008).

However, since the first report of *Lu. longipalpis* in Clorinda and in spite of its clustered distribution, at least 40 dogs with canine VL due to *L. chagasi* have been reported scattered all over the city. Although these cases could be attributed to intense dog movement across the border with Paraguay where the 2006 canine VL prevalence for Asunción was estimated to be 58% (Cousiño 2006), many of the infected dogs were born in Clorinda and never visited Paraguay, so local transmission cannot be discarded. Therefore, we can assume that the vectorial-related risk is spatially restricted during periods of low transmission (captures were performed after an exceptionally cold winter and before the rainfall season). On the other hand, during periods more suitable for the vectors, *Lu longipalpis* may colonise other neighbourhoods of the city, such as the yard with chickens and dogs in the downtown area that had phlebotomine sand flies. According to this hypothesis, *Lu. longipalpis* may have a metapopulational structure, already proposed for cutaneous leishmaniasis vectors in Argentina, with source populations and extinguishable sink populations (Salomón et al. 2004). If this is the case, a focused antivectorial intervention before the seasonal spread of *Lu. longipalpis* might have a significant impact on preventing both canine VL and its eventual and predictable overspill to human VL. To evaluate this strategy, further investigations are required: a spatially intensive capture to delineate the "islands" of high sand fly abundance, time-longitudinal captures to describe the seasonal dynamics of the vectors and controlled trials to assess insecticide intervention effectiveness (Salomón 2008).

As in other border cities with similar scenarios of recent introduction of VL, there are some factors that could jeopardise this strategy in Clorinda: (i) the intense traffic of dogs across the border from an active focus of VL (strays, pets, trade of bred dogs); (ii) canine VL underreporting, as animals are more likely to be diagnosed in the areas closer to the focus where the veterinarians have more experience; (iii) movement of dogs inside the city (stray dogs, puppies) and (iv) massive human migration with dogs, as when the road and gas pipeline was built in MS (Correa Antonialli et al. 2007). The first two factors are related to border legislation or law enforcement, the third to responsible pet ownership and the last to health risk assessments of developmental projects.

The recommendations given here about insecticide intervention in vectorial "hot spots" or areas with *Lu. longipalpis* were followed by the authorities. Although this procedure has not been evaluated, in November 2008, of the over 3,661 dogs surveyed in Clorinda, 111 (3.03%) were rK39 positive and 90% of these animals were culled. Additionally, no human cases have been diagnosed or even suspected since 2004 despite clinical surveillance by the local hospital and primary health care agents. During the same period in the foci of MS and Asunción, the figures of canine and human VL rose, although specific environmental characteristics of Clorinda and the surroundings that restrain the vector dispersion cannot be ruled out as an explanation for the decreased incidence of VL.

In conclusion, Clorinda is still at high risk of becoming an intense focus of VL transmission and a source of dispersion to the south along the highly trafficked roads; however, after three years the vector has not colonised

TABLE

Phlebotomine sampled with CDC trap in Clorinda, Puerto Pilcomayo, Laguna Blanca and Laguna Naineck, Formosa, Argentina, from October 18-31 2007 by site, sex and species (only sites with phlebotomine)

Locality	Coordenates (LS, LW)	*Lutzomyia longipalpis*	*Lutzomyia neivai*	*Lutzomyia cortelezzii*	*Lutzomyia migonei*	*Brumptomyia guimaraesi*
Clorinda	25º15'24.0" 57º43'53.7"					1/0
Clorinda	25º15'37.7" 57º43'49.0"			0/1		
Clorinda	25º15'42.4" 57º44'08.6"	1/0				
Clorinda	25º15'24.3" 57º43'54.0"	0/1				
Clorinda	25º16'23.0" 57º43'44.9"	1/2	2/5			
Clorinda	25º16'36.0" 57º43'25.1"		0/2			
Clorinda	25º18'36.9" 57º43'16.1"			0/1		
Clorinda	25º16'36.4" 57º43'00.2"	1/0				
Clorinda	25º16'37.3" 57º43'56.3"	4/0		0/1		
Clorinda	25º17'37.2" 57º43'04.0"	2/0				
Puerto Pilcomayo	25º21'07.0" 57º40'23.1"	9/3				
Puerto Pilcomayo	25º21'15.1" 57º40'08.5"	1/0				
Puerto Pilcomayo	25º21'36.9" 57º39'43.0"	22/6				
Puerto Pilcomayo	25º22'21.5" 57º39'13.8"	0/1				
Laguna Blanca	25º08'01.7" 58º14'19.1"		1/0			
Laguna Blanca	25º08'26.0" 58º14'35.9"		2/27		1/0	
Laguna Naineck	25º12'58.2" 58º07'12.5"		0/8			1/0

the closest villages, where the phlebotomine species are still the usual for the region (Salomón et al. 2008a). The VL canine prevalence is not a good space-time indicator of parasite circulation or transmission intensity in these early VL scenarios close to borders with active VL transmission. However, the spatially clustered distribution of vectors allows for possible anti-vectorial preventive interventions, together with regular surveillance measures of canine VL and human VL, vector dispersion monitoring and international sanitary law enforcement.

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