Incipient colonisation of *Lutzomyia longipalpis* in the city of Resistencia, province of Chaco, Argentina (2010-2012)

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Lutzomyia longipalpis *was recorded for the first time in Argentina in 2004, in the province of Formosa. In the following years, the vector spread to the south and west in the country and was recorded in the province of Chaco in 2010. From November 2010-May 2012, captures of Phlebotominae were made in the city of Resistencia and its surroundings, to monitor the spread and possible colonisation of* Lu. longipalpis *in the province of Chaco. In this monitoring,* Lu. longipalpis *was absent in urban sampling sites and its presence was restricted to Barrio de los Pescadores. This suggests that the incipient colonisation observed in 2010 was not followed by continuous installation of vector populations and expansion of their spatial distribution as in other urban centres of Argentina.*

Key words: visceral leishmaniasis - sandflies - Phlebotominae

In Argentina, *Lutzomyia longipalpis*, the vector of *Leishmania infantum* (syn. *Leishmania chagasi*), was recorded for the first time in 1951 and again in 2001 in the rural areas of the province of Misiones (Salomón et al. 2001). Despite regular and intensive captures of Phlebotominae at various places in the country since 1998, it was not until 2004 that *Lu. longipalpis* was recorded in the city of Clorinda (province of Formosa). It was found in peridomestic environments in urban areas, represented by populations focally abundant and persistent in time. Simultaneously, an ongoing outbreak of urban visceral leishmaniasis (VL) was occurring on the opposite bank of the Pilcomayo River, in the city of Asunción (Paraguay) (Salomón & Orellano 2005). In the following years, the vector spread to the centre, south and west of the country and was recorded in the provinces of Misiones (2006), Corrientes (2008-2009), Chaco, Entre Ríos (2010) and Salta (2013) (Salomón et al. 2008c, 2009, 2011a, b, Bravo 2013).

Until 2006 in Argentina, only a few isolated cases of VL had been recorded and these were in the 1980s and restricted to rural scenarios of the Chaco region (Salomón et al. 2001). In 2006, the first human case of autochthonous urban VL due to *L. infantum* was recorded in Posadas, Misiones, in conjunction with the presence of infected dogs and a wide distribution of the vector in the urban area (Salomón et al. 2008c). Simultaneously, a human VL focus in the city of La Banda, province of Santiago del Estero, suggested that the existence of sporadic transmission of *L. infantum* associated with the putative vector *Migonemyia migonei*, in the Chaco bioregion (Salomón et al. 2010). In 2009, there was active transmission of VL in accordance with the continuous presence of *Lu. longipalpis*, dogs infected with *L. infantum* and one autochthonous human case in the city of Corrientes (Gould et al. 2013).

The aim of this work was to contribute to the development of an entomological surveillance strategy in the city of Resistencia to monitor the spread and possible colonisation of *Lu. longipalpis* in an area receptive to VL transmission. The choice of Resistencia and its surroundings on the west bank of the Paraná River was based both on *Lu. longipalpis* records in the city from 2010s (Salomón et al. 2011b) and on its proximity to Corrientes, located on the eastern, opposite shore of the river.

Resistencia (27º27'S 58º59'W), located in the Chaco bioregion (Burkart et al. 1999), is characterised by a warm climate with no dry season and a rainfall of approximately 1,300 mm per year. It is located in the department of San Fernando, which hosts 390,874 inhabitants, concentrated in Resistencia, Barranqueras, Fontana and Puerto Vilelas. Its proximity to Corrientes forms one of the largest urban centres of northeastern Argentina (B in Figure), totalling nearly 750,000 inhabitants distributed throughout more than 220,000 homes (INDEC 2010).

For this urban entomological surveillance of the VL vector, sampling was based on a stratified systematic sampling method. The city was initially separated into four main strata: north, south, east and west, divided by its main avenues (A in Figure).

From November 2010-May 2012, each main stratum was sampled once every season, except in winter (because, according to previous experience in the region, capture in this season is low to null) (Salomón et al. 2008b, Rosa et al. 2010). Due to logistical constraints, only two strata per season were sampled (north and east in one season and south and west the next season, until every stratum was sampled in all seasons).

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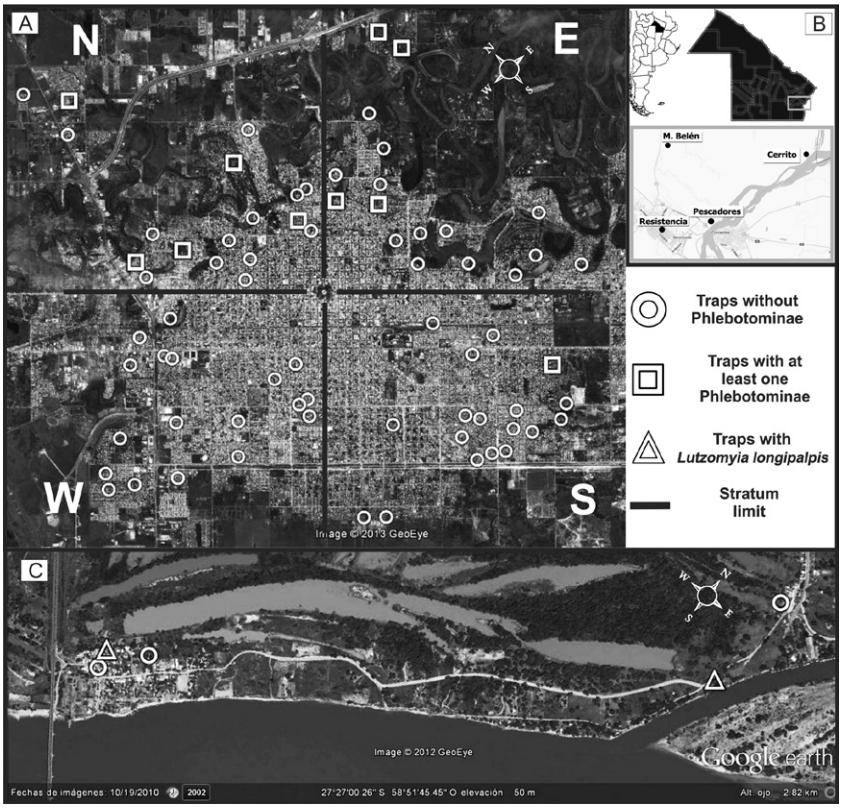

Satellite image showing the result of the study area during the period November 2010-May 2012, Chaco, Argentina. A: city of Resistencia; B: Phlebotominae capture site locations in Chaco, Argentina; C: Barrio de los Pescadores. Images taken from Google Earth, v.4.0.2416 (beta) (earth.google.es).

TABLE

Phlebotominae (females/males) sampled by sites and species in Chaco, Argentina, November 2010-May 2012

Sites	TTr/Tr+	*Lutzomyia longipalpis*	*Migonemyia migonei*	*Nysomyia neivai*	Cortelezzii complex	Total
Resistencia	72/10	0/0	1/0	3/11	5/5[a]	9/16
Margarita Belén	6/2	0/0	3/2	1/0	2/0	6/2
Barrio de Pescadores	5/2	0/5	0/0	0/0	0/0	0/5
Isla del Cerrito	2/0	0/0	0/0	0/0	0/0	0/0
Total	85/14	0/5	4/2	4/11	7/5	15/23

a: males of *Evandromyia sallesi*; TTr/Tr+: number of total traps/number of traps with at least one Phlebotominae.

The samples were selected by listing and numbering the neighbourhoods of each main stratum. Subsequently, six neighbourhoods were randomly selected in each stratum in each season (12 neighbourhoods/traps per season) using a random number table.

In each neighbourhood, the house considered the "worst case epidemiological scenario" was selected as the sample site. At each sample site, insects were captured by light CDC traps during a single night, from the evening of one day to the morning of the next day (from 05.00 pm-09.00 am). The traps were located at 1.5 m, above the animal shelter (a kennel, a henhouse etc.) in the courtyard of the house. If the weather conditions were not optimal for the capture (rainfall, strong winds), traps were reinstalled the following night.

Urban centres near Resistencia were also selected as sentinel sites to monitor any spread of the vector from Resistencia. These included the villages of Margarita Belén (27º14-16'S 58º59-57'W), located next to National Road Nº 11, a point of connection with the province of Formosa, Isla del Cerrito (27º17-18'S 58º37-36'W), a city bordering both Paraguay and the province of Corrientes

(with active transmission of VL), and Barrio de los Pescadores (27º27-26'S 58º52-51'W), in the municipality of Colonia Benítez, an urban area separated from Corrientes city by the Paraná River (B in Figure). Two traps were installed in Isla del Cerrito, 10 in Margarita Belén and five in Barrio de los Pescadores, which was decided based on the number of inhabitants, geographical area and epidemiological interest.

The Phlebotominae caught in each trap were separated from other insects by using a stereoscopic microscope. They were then preserved in alcohol 70º F for at least 48 h and then clarified and mounted for identification. Species were identified under a microscope according to Galati' keys (2003) and genus abbreviations following Marcondes (2007). Due to the sympatry of *Evandromyia cortelezzii* and *Evandromyia sallesi* in the region (Rosa et al. 2012) and the impossibility of differentiating females of these species by morphological characteristics, they are cited in this text as cortelezzii complex.

During the study period, 85 traps, 14 of which caught at least one Phlebotominae, were installed, obtaining a total of 38 Phlebotominae distributed in three locations. The species recorded were *Lu. longipalpis, Mg. migonei, Nyssomyia neivai, Ev. cortelezzii* and *Ev. sallesi* (Table).

Margarita Belén had the highest rate of specimens caught per trap [1.33 Phlebotominae per trap (Phl/Tr)], followed by Barrio de los Pescadores (1 Phl/Tr) and Resistencia (0.35 Phl/Tr), whereas no Phlebotominae was recorded in Isla del Cerrito. It is important to note the presence of *Lu. longipalpis* in Barrio de los Pescadores (C in Figure).

Ten out of the 72 traps installed in Resistencia captured Phlebotominae, predominantly in the northern and eastern areas of the city (A in Figure). *Ny. neivai, Mg. migonei* and cortelezzii complex specimens were captured. The cortelezzii complex specimens were represented by four males of *Ev. sallesi* and one of *Ev. cortelezzii*. Phlebotominae were recorded in the three seasons studied, with a predominance of Phlebotominae in the summer months (6/10 traps with Phlebotominae), followed by spring (3/10) and fall (1/10).

Lu. longipalpis was absent in Resistencia's urban sampling sites, even where it had been reported for the first time in 2010 (Salomón et al. 2011b). Its presence was restricted to Barrio de los Pescadores, the area closest to Corrientes.

The greatest Phlebotominae abundance per trap was found in Margarita Belén, followed by Barrio de los Pescadores and then Resistencia. This difference could be explained by the degree of urbanisation of each site. Rural areas have close contact with forest remnants and the farm animals, which favours the development of Phlebotominae populations, whereas urban environments show a more limited number of sites for vector development and these are usually restricted to peripheral areas of the city (Quintana et al. 2012). However, given the low abundance observed in the captures, this trend should be confirmed through continuous monitoring. In turn, it cannot be ruled out that the absence of Phlebotominae in Isla del Cerrito is due to the climatic conditions (minimum temperature 9ºC).

In Resistencia, all traps with at least one Phlebotominae were located among the "worst case scenario" sample sites or near habitats with favourable conditions for insects: the presence of chickens and/or dogs, dirt ground, shady trees and proximity to ponds or creeks. These variables have been found associated with the urban presence of Phlebotominae in other locations in Argentina, by providing potential sites of shelter, food and/or breeding sites to Phlebotominae (Santini et al. 2012). In turn, the high relative abundance found in the northern and eastern strata of Resistencia could be favoured by the creeks and ponds caused by the Negro River, although these preliminary results should be confirmed through continuous and simultaneous monitoring across strata.

The seasonal result showed higher Phlebotominae catches in the summer, followed by autumn and spring, which is consistent with previous studies of seasonal variation and association with climatic variables in rural areas of the province (Salomón et al. 2008b, Rosa et al. 2010). Therefore, for a restricted surveillance, captures in summer and spring should be prioritised. Studies show that captured species are also the most diverse in rural areas in the Chaco region, except for *Lu. longipalpis,* which, in recent studies in Argentina, has had an almost exclusively urban presence (Salomón et al. 2008a, b, Rosa et al. 2010).

Ny. neivai, Mg. migonei and cortelezzii complex have been implicated in the transmission of cutaneous leishmaniasis in the rural area of the biogeographic provinces of eastern and western Chaco. *Ny. neivai* and cortelezzii complex have been found naturally infected with *Leishmania braziliensis* in Argentina (Lanús et al. 2006, Rosa et al. 2012) and the former is considered an epidemic vector of cutaneous leishmaniasis (Quintana et al. 2012). The presence of *Mg. migonei* is highlighted, as this species is a probable vector of *L. infantum* in sporadic transmission scenarios in the Chaco region (Salomón et al. 2010) and a species whose natural infection with *L. infantum* has been described in a VL focus in Brazil, without the presence of *Lu. longipalpis* (de Carvalho et al. 2010).

The finding of *Lu. longipalpis* in the urban area of Resistencia in April 2010 suggests an active dispersal of the VL vector within Argentina in a southwestern direction (Salomón et al. 2011a, b). The absence of *Lu. longipalpis* during subsequent systematic monitoring could be due to weather conditions (very low winter minimum temperature 2010) that could lead to a diapause (Rajinder et al. 2010, Carvalho et al. 2011) or environmental conditions, which limited continuous colonisation or spread to other sites in the city. The persistence of the VL vector in Barrio de los Pescadores can be explained by a continuous colonisation from the city of Corrientes (the source population) across the Paraná River or to habitat conditions or climate buffering due to the same river. Further studies will be required to assess the quality of the Paraná River as a physical barrier for sandfly dispersion, as large rivers in Brazil have shown to be (Coutinho-Abreu et al. 2008, Saraiva et al. 2012). Therefore, surveillance of human and canine VL should be prioritised in Barrio de los Pescadores because of the presence of the vector and the continuous flow of dogs between Corrientes and Chaco.

Although human and canine VL are mandatory reportable diseases, up to now there are no recent records in the province of Chaco, so the need to strengthen the diagnostic capabilities is also emphasised.

In conclusion, this preliminary monitoring of *Lu. longipalpis* in Resistencia showed that the incipient colonisation of the VL vector observed in 2010 with captures of low relative abundance was not followed by continuous installation of vector populations and expansion of their spatial distribution as in other urban centres of Argentina (Santini et al. 2012). Determining the potential ability of *Lu. longipalpis* to spread in this biogeographic zone after a mild winter and colonisation of larger urban areas from continuous peripheral foci (Barrio de los Pescadores) requires an entomological surveillance strategy and appropriate search for canine infection, not only to understand the dispersal dynamics of the VL vector, but also to monitor the risk of active transmission of VL in the Chaco region by both primary and secondary vectors as *Mg. migonei* (Rosa et al. 2010, Salomón et al. 2010).

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