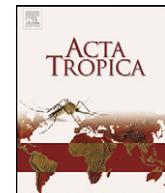




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Short communication

Natural infection of *cortelezzii* complex (Diptera: Psychodidae: Phlebotominae) with *Leishmania braziliensis* in Chaco, ArgentinaJuan Rosa^{a,*}, Daniela Pita Pereira^b, Reginaldo Peçanha Brazil^c, José Dilermando Andrade Filho^{d,1}, Oscar Salomón^{e,2}, Enrique Szelag^{a,3}^a Área Entomología, Instituto de Medicina Regional, Universidad Nacional del Nordeste, Av. Las Heras 727, (CP 3500) Resistencia, Chaco, Argentina^b Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, FIOCRUZ, Pavilhão Leônidas Deane – sala 209, Avenida Brasil 4365, CP 926, Manguinhos, 21045-900, Rio de Janeiro, RJ, Brazil^c Laboratório de Bioquímica e Fisiologia de Insetos, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil 4365, CP 926, Manguinhos, 21045-900, Rio de Janeiro, RJ, Brazil^d Centro de Referência Nacional e Internacional para Flebotomíneos, Centro de Pesquisas René Rachou/Fundação Oswaldo Cruz, Avenida Augusto de Lima, 1715, Barro Preto, 30.190-002, Belo Horizonte, Minas Gerais, Brazil^e Instituto Nacional de Medicina Tropical, Neuquén y Jujuy s/n, (CP 3370) Puerto Iguazu, Misiones, Argentina

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ABSTRACT

In Argentina, American Cutaneous Leishmaniasis (ACL) extends up to 29°S in the phytogeographic regions of the Yungas (west), Chaco (center) and Paranaense (east). Since the Phlebotominae vectors of this disease in the western Chaco (dry Chaco) are unknown, in the present work, we studied the natural infection in Phlebotominae by PCR-ERFLP and Dot blot in order to incriminate these organisms as potential vectors. Captures with CDC-type traps were performed monthly in the domicile, the peridomicile and the forest in the Municipio Misión Nueva Pompeya, Chaco, Argentina, in two sites with human cases of ACL: Los Pozos (24°54'S, 61°22'W) and Fortín Arenales (24°58'S, 61°21'W), from November 2006 to December 2007. A total of 1702 Phlebotominae were captured: *Mygonemyia migonei* (83.8%), *cortelezzii* complex (11.1%), *Mycopigomyia peresi* (3.3%), *Mycopigomyia quinquefer* (1.2%), *Pintomyia torresi* (0.2%) and *Nyssomyia neivai* (0.2%). Although no significant differences were found in species diversity, there were significant differences in abundance between both sites studied. A total of 80 phlebotomine females were analyzed: 50 of the *cortelezzii* complex and 30 *My. migonei*. No intestinal flagellates were observed by light microscopy. Two pools of 10 individuals of the *cortelezzii* complex of the peridomicile and forest of Fortín Arenales were reactive by PCR and Dot blot for *Leishmania (Viannia) braziliensis*. In Argentina, *Evandromyia cortelezzii* has been incriminated as a likely vector of ACL because of its abundance in areas of sporadic outbreaks. In the present work, *Ev. cortelezzii* females were found naturally infected, thus reinforcing the hypothesis that the members of the *cortelezzii* complex act as vectors of the disease.

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In Argentina, leishmaniasis is distributed up to 29°S in nine provinces within the phytogeographic regions of the Yungas, Chaco and Paranaense (Salomón et al., 2001a). The parasite most frequently isolated from outbreaks of American Cutaneous Leishmaniasis (ACL), from both human patients and vectors, has been found to be *Leishmania (Viannia) braziliensis* (Segura et al., 2000; Córdoba Lanús et al., 2005). *Leishmania (Leishmania) amazonensis* and *Leishmania (Viannia) guyanensis* have also been described by Frank et al. (2000) and Marco et al. (2005) as causative agents of

ACL cases and *Leishmania infantum* has been reported by Salomón et al. (2008a) as the causative agent of Visceral Leishmaniasis (VL) after the first autochthonous human case in 2006.

Of the 27 species of Phlebotominae described in Argentina, *Nyssomyia neivai* (Yungas region), *Nyssomyia whitmani* and *Mycopigomyia quinquefer* (Paranaense region) have been found naturally infected with *L. (V.) braziliensis*, whereas *Lutzomyia longipalpis* (Paranaense region) has been found naturally infected with *L. infantum* (Córdoba Lanús et al., 2006; Salomón et al., 2006). In the phytogeographic region of the humid Chaco, *Ny. neivai* is the most abundant, followed by *Mygonemyia migonei*, similar to that observed in the regions of the Yungas and Paranaense. In the dry Chaco region, however, the dominant species are *My. migonei*, *Evandromyia cortelezzii* and *Evandromyia sallesi*, whereas *Ny. neivai* is less abundant or absent (Salomón et al., 2008b, 2011; Rosa et al., 2010). Given the lack of information on natural infection of sand flies and the continuous incidence of new cases of ACL in the west

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phytogeographic region of the province of Chaco and the low abundance of vectors incriminated in other regions, the purpose of this study was to demonstrate the natural infection of sand flies with *Leishmania* and typify the parasite species with the aim to better understand their involvement as vectors.

Study area and sampling site. The study was made from November 2006 to December 2007 in the Municipio Misión Nueva Pompeya ($24^{\circ}55'S$, $61^{\circ}35'W$) in the dry phytogeographic region of Western Chaco, Chaco, Argentina. Two sites were selected: Fortín Arenales ($24^{\circ}58'S$, $61^{\circ}21'W$), because it presented a human case of active ACL, and Los Pozos ($24^{\circ}54'S$, $61^{\circ}22'W$), because it is a historic site of transmission (Fig. 1). Two dwellings were chosen at each site (Stations 1 and 2). The ecotones are quebracho (*Schinopsis* and *Aspidosperma*) and palo santo forests (*Bulnesia*), as well as cardonales and vinalares (*Prosopis ruscifolia*). The two sites showed evidence of deforestation and human impact on the forest.

Capture of Phlebotominae. Sand flies were caught with CDC-like light traps in the domicile (inside the house), in the peridomicile (hen-houses, lamb-pens or cattle-sheds, goats and horses) up to 10 m from the house, and in the forest, up to 110 m from the house. The traps were left for at least 12 h (19:00 to 07:00 h), monthly.

The live specimens trapped were separated into containers suitable for transport to the laboratory for studies of natural infection with *Leishmania* and taxonomic identification highlighting both *Ev. sallesi* and *Ev. cortelezzii* females were identified as belonging to the *cortelezzii* complex, because these sand flies are indistinguishable by morphological characteristics (Galati, 2003).

Natural infection. Dissection. Female sand flies were dissected individually to observe intestinal flagellates by light microscopy, and then identified and pooled in groups of 10 specimens of the same species for DNA extraction and PCR technique.

DNA extraction and multiplex PCR-Dot blot hybridization. The insects (pools of unfed males and females) were subjected to molecular analysis for *Leishmania* infection. The pools were stored at $-20^{\circ}C$ until DNA extraction. DNA was extracted as previously described by Pita Pereira et al. (2005) and rigorous procedures were assumed to control potential contamination, e.g. we included negative control groups (male sand flies) in the DNA extraction step and decontaminated instruments and working areas with diluted chloride solution and ultraviolet light. The multiplex PCR was designed to simultaneously amplify the cacophony gene IVS6 region in sand flies of the neotropical genus *Lutzomyia* (as an internal control for

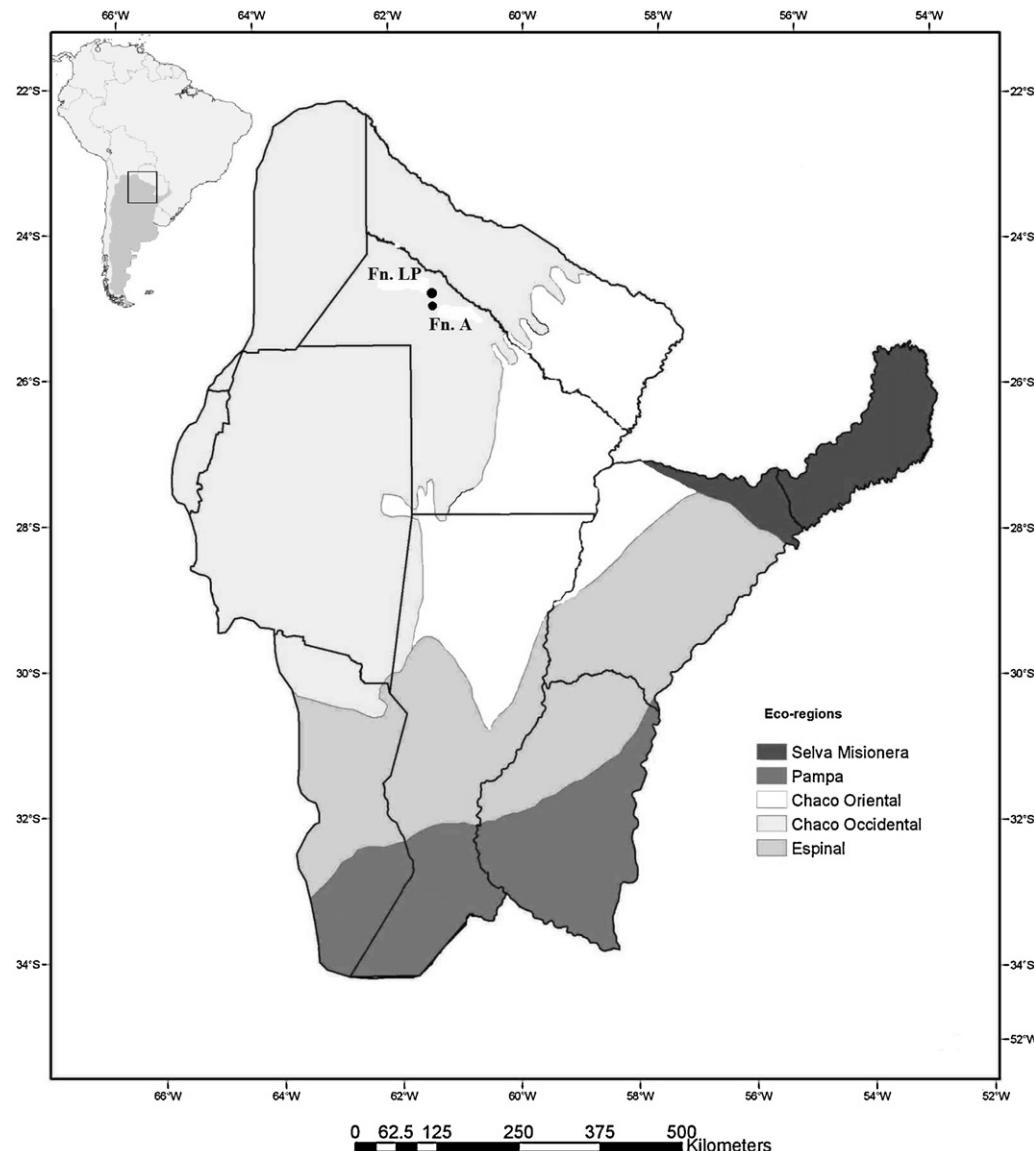


Fig. 1. Study area and its location in South America.

the polymerase enzyme activity), and the conserved kinetoplast DNA minicircle region from *Leishmania* spp. (Pita Pereira et al., 2005). The amplified products were further subjected to Dot blot hybridization using a *L. (V.) braziliensis*-specific biotinylated probe.

Phlebotomine fauna. A total of 1702 Phlebotominae were captured: *My. migonei*: 83.7% (22.9% females; 60.8% males); *cortelezzii* complex: 11.2% (7% females – not identified by similar morphology; 4.2% males); *Ev. cortelezzii*: 0.63%; *Ev. sallesi*: 3.57%; *Mycopsgomyia peresi*: 3.2% (2.5% females; 0.7% males); *My. quinquefer*: 1.1% (0.7% females; 0.4% males), *Pintomyia torresi*: 0.2% (0.2% females; 0% males) and *Ny. neivai*: 0.2% (0% females; 0.2% males).

Natural infection. A total of 80 phlebotomine females were dissected and eight pools were prepared according to species and site: *cortelezzii* complex: 50 females (Los Pozos: one pool; Fortín Arenales: four pools) and *My. migonei*: 30 females (Los Pozos: two pools; Fortín Arenales: one pool). No intestinal flagellates were observed by optical microscopy in the dissected females. Two pools of females of the *cortelezzii* complex from Fortín Arenales, which presented a human case with active ACL, were positive for 120 bp for the PCR reaction and Dot blot hybridization for *L. (V.) braziliensis*.

In Argentina, *My. Migonei*, which has been incriminated in the zoonotic cycle of transmission of ACL, is the most abundant species in the phytogeographic region of dry Chaco and the second most abundant in the phytogeographic area of the wet Chaco after *Ny. neivai* (Salomón et al., 2008b). In Brazil, Azevedo et al. (1990) recorded *My. migonei* naturally infected with *L. (V.) braziliensis* but it was incriminated in the peridomestic transmission cycle of this parasite some years ago by Rangel et al. (1986). *Ny. neivai*, however, was the last species in abundance (three specimens) captured in the forest of Los Pozos and in the domicile of Fortín Arenales. This species has been reported to be associated with endemic-epidemic foci of ACL in areas of the wet Chaco, Yungas and Paranaense (Salomón et al., 2001b, 2006). Although it has been found naturally infected with *L. (V.) braziliensis* in the province of Tucumán (Córdoba Lanús et al., 2006). In wet areas of the province of Chaco, it was the most abundant species associated with peridomicile and forest, and was the least in the region of study (Rosa et al., 2010). *My. quinquefer* has been found naturally infected with *Leishmania* sp. in the Paranaense region in Iguazú (Misiones), associated with the domicile and forest (Salomón et al., 2009). A similar association has been described in Brazil (Andrade Filho et al., 2001; Galati et al., 2006; Brazil et al., 2006). The presence of *My. migonei* in environments close to human dwellings would incriminate it as a probable vector of ACL in areas of active transmission and/or ACL antecedents in Argentina. The *cortelezzii* complex is formed by four species, *Ev. cortelezzii*, *Ev. sallesi*, *Evandromyia corumbaensis* and *Evandromyia spelunca* (Carvalho et al., 2011). The *Ev. cortelezzii* and *Ev. sallesi* were found naturally infected with *Le. chagasi* in Minas Gerais, Brazil (Carvalho et al., 2008; Saraiva et al., 2009). The *cortelezzii* complex also has been found naturally infected by *Leishmania braziliensis* in Belo Horizonte, Minas Gerais State, Brazil (Saraiva et al., 2010). In Argentina, however, it has been incriminated as a probable vector of ACL only on the basis of its distribution (Romaña et al., 1949). The results of this study confirm the infection of females of the *cortelezzii* complex with *L. (V.) braziliensis* in an area of active transmission of ACL, thus reinforcing the hypothesis that the members of the *cortelezzii* complex act as vectors of the disease.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.actatropica.2012.04.008>.

References

- Andrade Filho, J.D., Lima da Silva, A.C., Falcão, A.L., 2001. Phlebotomine sand flies in the state of Piauí, Brazil (Diptera: Psychodidae: Phlebotominae). Memorias do Instituto Oswaldo Cruz 96, 1085–1087.
- Azevedo, A.C.R., Rangel, E., Queiroz, R.G., 1990. *Lutzomyia migonei* (França, 1920) naturally infected with perypylarian flagellates in Baturité, a focus of cutaneous leishmaniasis in Ceará State, Brazil. Memorias do Instituto Oswaldo Cruz 85, 479.
- Brazil, R.P., Passos, W.L., Fuzari, A.A., Falcão, A.L., Andrade Filho, J.D., 2006. The peridomestic sand fly fauna (Diptera: Psychodidae) in areas of cutaneous leishmaniasis in Além Paraíba, Minas Gerais, Brazil. Journal of Vector Ecology 31, 418–420.
- Carvalho, G.M., Andrade Filho, J.D., Falcao, A.L., Rocha Lima, A.C., Gontijo, C.M., 2008. Naturally infected *Lutzomyia* sand flies in a Leishmania-endemic area of Brazil. Vector Borne and Zoonotic Diseases 8, 407–414.
- Carvalho, G.M.L., Brazil, R.P., Sanguinette, C.C., Andrade Filho, J.D., 2011. Description of *Evandromyia spelunca*, a new phlebotomine species of the *cortelezzii* complex, from a cave in Minas Gerais State, Brazil (Diptera: Psychodidae: Phlebotominae). Parasites & Vectors 4, 158.
- Córdoba Lanús, E., Piñero, J.E., González, A.C., Valladares, B., de Grosso, M.L., Salomón, O.D., 2005. Detection of *Leishmania braziliensis* in human paraffin-embedded tissues from Tucumán, Argentina by polymerase chain reaction. Memorias do Instituto Oswaldo Cruz 100, 187–192.
- Córdoba Lanús, E., Lizarralde de Grosso, M., Piñero, J.E., Valladares, B., Salomón, O.D., 2006. Natural infection of *Lutzomyia neivai* with *Leishmania* spp. in northwestern Argentina. Acta Tropica 98, 1–5.
- Frank, F.M., Fernández, M.M., Caffaro, C.E., Cajal, P., Zoclo, V., Taranto, N., Malchiodi, E.L., 2000. Caracterización de la infección por *Leishmania* spp. en el Chaco Salteño: respuesta humorar, infección doble con *T. cruzi* y especies de *Leishmania* involucradas. Medicina (Buenos Aires) (Supl. III), 86–87.
- Galati, E.A.B., 2003. Morfologia, terminología de adultos e identificação dos táxons da América. In: Rangel, E.F., Lainson, R. (Eds.), Flebotomíneos do Brasil. Fiocruz, Rio de Janeiro, pp. 53–175.
- Galati, E.A.B., Nunes, V.L.B., Boggiani, P.C., Dorval, M.E.C., Cristaldo, G., Rocha, H.C., Oshiro, E.T., Damasceno Junior, G.A., 2006. Phlebotomines (Diptera: Psychodidae) in forested areas of the Serra da Bodoquena, state of Mato Grosso do Sul, Brazil. Memorias do Instituto Oswaldo Cruz 10, 175–193.
- Marco, J.D., Barroso, P.A., Calvopina, M., Kumazawa, H., Furuya, M., Korenaga, M., Cajal, S.P., Rea, M., Borda, C.E., Basombrio, M.A., Taranto, M.M., Hashiguchi, Y., 2005. Species assignation of *Leishmania* from human and canine american tegumentary leishmaniasis cases by multilocus enzyme electrophoresis in North Argentina. The American Journal of Tropical Medicine and Hygiene 72, 606–611.
- Pita Pereira, D., Alves, G.R., Souza, M.B., Brazil, R.P., Bertho, A.L., Figueiredo Barbosa, A., 2005. Identification of naturally infected *Lutzomyia intermedia* and *Lutzomyia migonei* with *Leishmania (Viannia) braziliensis* in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridisation assay. Transactions of the Royal Society of Tropical Medicine and Hygiene 99, 905–913.
- Rangel, E.F., Souza, N.A., Wermelinger, E.D., Azevedo, A.C., Barboza, A.F., Andrade, C.E., 1986. Flebotomos de Vargem Grande, Foco de leishmaniose tegumentar no estado do Rio de Janeiro. Memorias do Instituto Oswaldo Cruz 81, 347–349.
- Romaña, C., Najera, L., Conejos, L., Abalos, J.W., 1949. Leishmaniosis tegumentaria en perros de Tucumán. II. Focos domésticos de leishmaniosis. An. Inst. Medicina Regional. 2, 282–292.
- Rosa, J.R., Salomón, O.D., Andrade Filho, J.D., Carvalho, G.M.L., Szelag, E.A., Stein, M., Tapia, E.S., Brazil, R.P., 2010. Distribution of sandflies (Diptera: Psychodidae) in the province of Chaco, Argentina. Neotropical Entomology 39, 303–305.
- Salomón, O.D., Sosa Estani, S., Monzani, A.S., Studer, C., 2001a. Brote epidémico de leishmanioses tegumentaria en Puerto Esperanza, Provincia de Misiones, 1998. Medicina (Buenos Aires) 61, 385–390.
- Salomón, D.O., Bogado de Pascual, M., Molinari, M.L., Verri, V., 2001b. Study of a cutaneous leishmaniasis outbreak in General Vedia, Province of Chaco, 1996. Revista do Instituto de Medicina Tropical de São Paulo 43, 99–104.
- Salomón, O.D., Sosa Estani, S., Ramos, K., Orellano, P.W., Sanguesa, G., Fernández, G., Sinagra, A., Rapascioli, G., 2006. Tegumentary leishmaniasis outbreak in Bella Vista city, Corrientes, Argentina during 2003. Memorias do Instituto Oswaldo Cruz 101, 767–774.
- Salomón, O.D., Sinagra, A., Nevot, M.C., Barberian, G., Paulin, P., Estevez, J.O., Riarte, A., Estevez, J., 2008a. First visceral leishmaniasis focus in Argentina. Memorias do Instituto Oswaldo Cruz 103, 109–111.
- Salomón, O.D., Rosa, J.R., Stein, M., Quintana, M.G., Fernández, M.S., Visentín, A., Spinelli, G., Bogado de Pascual, M., Molinari, M., Morán, M., Valdez, D., Romero, B., 2008b. Phlebotominae (Diptera: Psychodidae) fauna in the Chaco region and cutaneous leishmaniasis transmission patterns in Argentina. Memorias do Instituto Oswaldo Cruz 103, 578–584.
- Salomón, O.D., Acardi, S.A., Liotta, D.J., Fernández, M.S., Lestani, E., López, D., Mastrángelo, A.V., Figueiroa, M., Fattore, G., 2009. Epidemiological aspects of cutaneous leishmaniasis in the Iguazú falls area of Argentina. Acta Tropica 109, 5–11.

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Salomón, O.D., Rosa, J.R., Fabiani, M., San Miguel, S.R., Szelag, E.A., Nepote, M., Parras, M.A., 2011. Distribución de *Lutzomyia longipalpis* en el Chaco, Argentina, 2010. Medicina (Buenos Aires) 71, 225–230.

Saraiva, L., Carvalho, G.M., Gontijo, C.M., Quaresma, P.F., Lima, A.C., Falcão, A.L., Andrade Filho, J.D., 2009. Natural infection of *Lutzomyia neivai* and *Lutzomyia sallesi* (Diptera: Psychodidae) by *Leishmania infantum chagasi* in Brazil. Journal of Medical Entomology 46, 1159–1163.

Saraiva, L., Andrade Filho, J.D., Silva, S.O., Andrade, A.S.R., Melo, M.N., 2010. The molecular detection of different *Leishmania* species within sand flies from a cutaneous and visceral leishmaniasis sympatric area in Southeastern Brazil. Memorias do Instituto Oswaldo Cruz 105, 1033–1039.

Segura, E.L., Piquin, A.L., Cuba Cuba, C.A., Abramo Orrego, L., McMahon-Pratt, D., Montamat, E.E., Momen, H., Grimaldi Jr., G., 2000. Molecular and biologic characterization of *Leishmania* parasites implicated in an epidemic outbreak in Northwestern Argentina. Parasitology Research 86, 504–508.