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Abstract. To identify *Lutzomyia* (Nyssomyia) neivai naturally infected by *Leishmania* a multiplex polymerase chain reaction (PCR) was used for the analysis of 450 specimens (270 females, 180 males) collected in an endemic periurban area of cutaneous leishmaniasis in Porto Alegre, Brazil. Insects were grouped into pools of 10 and positive results were achieved in 3/27 *Lu. (N.) neivai* female pools. Infection by *L. (Viannia) braziliensis* was confirmed after hybridizing PCR products with a subgenus–specific biotinylated probe. Considering the detection of three positive pools with at least one infected insect in each, an infection rate of 1.11% was estimated. Our results associated with epidemiologic data suggest a potential ability of *Lu. (N.) neivai* in transmitting *L. braziliensis* in Porto Alegre, where the first notifications of autochthonous cutaneous leishmaniasis in humans occurred in 2002, with an increase in the number of cases in recent years possibly as a consequence of deforestation and agricultural activities in the area.

In Brazil, from 2003 to 2007 the Ministry of Health recorded 181,117 human cases of American cutaneous leishmaniasis (ACL) (Alves W, unpublished data). It has been reported that an increase of ACL incidence in all Brazilian states with dissemination of the disease to the periurban areas of some state capitals, such as Manaus, Rio de Janeiro, and Belo Horizonte, thus constituting a serious health problem.1,2 There are few studies directed to the sand fly fauna in Rio Grande do Sul State,3,4 mainly because of the inexistence of reports on autochthonous ACL cases in the last decades. More recently, in accordance with the Secretariat of Health of Rio Grande do Sul, the epidemiologic situation has changed. From 2002 to 2008, 20 confirmed autochthonous human cases resulting from *Leishmania* (Viannia) braziliensis were reported in the periphery of the city of Porto Alegre, in areas of residual vegetation close to creeks. The region presents rural zone characteristics, such as animal husbandry and agricultural activities in the proximity to residences. In previous entomologic studies, *Lutzomyia* (Nyssomyia) neivai (Pinto) was the most frequent species found in the area and was considered a potential ACL vector.5

In the present study, a multiplex polymerase chain reaction (PCR) assay associated to non-isotopic hybridization was used to evaluate the occurrence of *Lu. (N.) neivai* naturally infected by *Leishmania* parasites in an urban area of tourism activity located in Porto Alegre, where human ACL cases were recently described. Sand flies were collected monthly from October 2006 to May 2007 with Centers for Disease Control and Prevention (CDC) light traps in 10 monitoring stations distributed inside houses, in the peridomicile close to domestic animal shelters and in the forest. A sample constituted of 450 *Lu. (N.) neivai* phlebotomines (270 females, 180 males) was sent to the laboratory for PCR examination after taxonomic identification according to Young and Duncan6 for genus and subgenus, and Marcondes7 and Andrade-Filho and others8 for the species level taking into account the morphologic characteristics of males and females. The insects were grouped into pools of 10 specimens and submitted to molecular analysis for *Leishmania* infection. The multiplex PCR9,10 was designed to simultaneously amplify the cacophony gene IVS6 region in sand flies of the neotropical genus *Lutzomyia* (as an internal control for the polymerase enzyme activity), and the conserved kinetoplast DNA minicircle region from *Leishmania* spp. The amplified products were further submitted to dot blot hybridization using a *L. (Viannia)*-specific biotinylated probe.9

The PCR assay showed positive results in 3 out of 27 female pools analyzed, and hybridization confirmed the infection with parasites from the subgenus Viannia (Figure 1). Considering the occurrence of at least one infected insect in each pool of 10 phlebotomines, we found that the minimal infection rate for *Lu. (N.) neivai* was 1.11%. The PCR approach was highly sensitive and able to reveal on agarose gel, a 120 bp fragment from *Leishmania* kDNA minicircles in all three positive sand fly pools before the hybridization step. All samples analyzed yielded a 220 bp amplified product corresponding to a constitutive gene (cacophony) from *Lutzomyia* spp., thus confirming the integrity of the insect DNA preparations and the absence of eventual PCR inhibitors (Figure 1).

In Brazil, ACL resulting from *L. (V.) braziliensis* has been reported in all states and involves a diversity of sand fly species, such as *Lu. (Psychodopygus) wellcomei* (Fralha, Shaw, & Lainson, 1971); *Lu. (P.) complexa* (Mangabeira, 1941); *Lu. (N.) whitmani* (Antunes & Coutinho, 1939); *Lu. Migonei* (França, 1920), and *Lu. (N.) intermedia* (Lutz & Neiva, 1912), based on evidences regarding their anthropophily, natural infection by *Leishmania* parasites, and spatial distribution in accordance with human cases transmission sites.11

Marcondes and others12 discussed the epidemiologic role of *Lu. (N.) neivai* in the transmission of *L. (V.) braziliensis* in
some aspects concerning the vectorial competence of this sand fly species were studied in Argentina\textsuperscript{13} in a transmission area of ACL, where it was reported a high \textit{Lu. (N.) neivai} density associated with human cases in the domiciliary environment, and in the secondary and primary forest. Studies on \textit{Lu. (N.) neivai} in southeastern Brazil brought more insights about the biologic characteristics of this species and suggested that dispersion from the forest to peridomical areas could be important for the transmission of ACL inside houses.\textsuperscript{14} In 2006, Córdoba-Lanús and others\textsuperscript{15} reported for the first time natural infection of \textit{Lu. (N.) neivai} with \textit{Leishmania} from an area in Argentina where \textit{Leishmania (Viannia)} was the main circulating parasite. More recently, also in Argentina, in an area considered as hyperendemic for ACL, the transmission was associated to \textit{Lu. (N.) neivai} in periurban vegetation and ecotone modified regions.\textsuperscript{16}

Taking into account the first reports of ACL in Porto Alegre, south Brazil, studies concerning the sand fly fauna were performed. Captures carried out inside houses and in peridomical areas, with the occurrence of human ACL cases, revealed the predominance of \textit{Lu. (N.) neivai} (94.9\%) without the identification of \textit{Lu. (N.) intermedia} (Azevedo A and others, unpublished data). Epidemiologic data from the Secretariat of Health of Porto Alegre\textsuperscript{3} suggested \textit{Lu. (N.) neivai} as the main vector of ACL, because of its anthropophilic characteristic and spatial and seasonal abundance in anthropic environments. This information associated with the evidence of natural infection by \textit{Leishmania} indicate the importance of \textit{Lu. (N.) neivai} in the epidemiologic cycle of ACL in Porto Alegre, and suggests this species as another \textit{L. (V.) braziliensis} vector in the southeast and south regions of Brazil.

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