

Sandflies (Diptera: Psychodidae) associated with opossum nests at urban sites in southeastern Brazil: a risk factor for urban and periurban zoonotic *Leishmania* transmission?

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Sandflies associated with opossum nests are reported for the first time in the yards of residences located in the urban area of the municipality of Monte Mor, situated in the metropolitan region of Campinas, state of São Paulo, Brazil. Eleven specimens of Evandromyia cortezezzii and one of Evandromyia lenti were captured in two Didelphis albiventris nests. Ev. cortezezzii is considered a secondary vector species for the transmission of Leishmania (Vianina) braziliensis and Leishmania (Leishmania) infantum in the Neotropics. This association may contribute to the introduction, establishment and maintenance of urban and periurban zoonotic transmission outbreaks of Leishmania and should therefore be investigated further.

Key words: reservoir - leishmaniasis - opossum

Opossums (*Didelphis* sp.) are synanthropic wild small mammals with nocturnal and solitary habits and are commonly found associated with human dwellings due to their search for food and shelter.

Two species of opossum are found in the state of São Paulo (SP): *Didelphis aurita* (the black-eared opossum), originally an inhabitant of the Atlantic forest and Araucaria forest biomes, and *Didelphis albiventris* (the white-eared opossum), a species more commonly found in urban centres and in areas of the *Caatinga* and *Cerrado* (Brazilian savannah) natural vegetation (Cerqueira & Tribe 2007).

Leishmania spp, the causative agents of American cutaneous leishmaniasis (ACL) and American visceral leishmaniasis (AVL), have been identified in naturally infected *Didelphis* sp. specimens in urban and periurban areas of various localities in Brazil (Arias & Naiff 1981, Sherlock et al. 1984, Cabrera et al. 2003, Carreira et al. 2012, Humberg et al. 2012), including SP (Yoshida et al. 1985). There was 91.6% infection prevalence among 112 animals examined in the municipality of Bauru, SP, which is an endemic area for AVL (Santiago et al. 2007).

Sandflies are biological vectors of *Leishmania*. Some species of sandfly are naturally attracted to (Adler et al. 2003) and feed on opossum blood as well as on the blood of human beings and their domestic animals (Dias et al. 2003, Oliveira-Pereira et al. 2008). They are able

to transmit *Leishmania* among these various vertebrate species, connecting wild zoonotic cycles of leishmaniasis with periurban and urban cycles.

This study reports on the presence of sandflies in natural shelters of opossums of the species *D. albiventris* found in residences in the urban environment of the municipality of Monte Mor, in the metropolitan area of Campinas, SP, during the routine activities of the Municipal Service for the Control of Zoonoses and Vectors, as the result of requests from the townspeople.

A residence in the district of Nova Alvorada [22°56'06"S 47°15'02"W, 579 m above sea level (a.s.l.)] was inspected on 16 September 2010. The animal was found inside a disused wood-burning stove situated on the covered porch of the dwelling. The presence of sandflies was perceived by chance when the animal and the leaves lining its nest were being handled. Three insects were captured on that same day and another eight were captured on the following day.

Another dwelling in the suburb of Paulista (22°55'58"S 47°16'16"W, 595 m a.s.l.) was inspected on 22 October 2012. A female opossum was found with eight of her cubs among a heap of bricks under the open sky in the yard of the house. A single sandfly specimen was found when the place was inspected during the removal of the marsupials. In both cases, the females had built their nests from bits of plastic, leaves and/or dry twigs in the yard of the dwellings to protect their cubs that were in one case still in their mother's pouch and in another case outside of it.

The insects were collected manually with the aid of a test tube containing cotton wool soaked in ether or in wide-mouthed plastic bottles. The insects were killed by freezing, clarified, stained, mounted in resin between a coverslip and slide and identified according to Galati (2003).

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The properties on which the opossums were found and the sandflies were captured, although located in an established urban area and bordering paved streets, were located on the edges of neighbourhoods with empty plots, pastures and nearby wooded areas. The second house was located next to a house that had a small chicken coop, from which the opossum was reported to have stolen eggs.

In the former residence, seven males and three females of *Evandromyia cortelezzii* and a female of *Evandromyia lenti* were captured. In the latter residence, one female of *Ev. cortelezzi* was obtained.

Ev. cortelezzii was reported for the first time in an urban area in association with opossum nests in two human dwellings approximately 2 km and two years apart.

Although the number of captured *Ev. cortelezzii* specimens was small, this species is rarely abundant in entomological surveys of sandflies in Brazilian leishmaniasis transmission areas (Oliveira et al. 2003, Souza et al. 2003), but is present in urban areas (Colla-Jacques et al. 2010), and is occasionally the only sandfly species found in this type of locality (Carvalho et al. 2005).

Although it is not considered an important species in the epidemiology of leishmaniasis in Brazil, *Ev. cortelezzii* has aroused scientific interest by virtue of its having been found naturally infected by *Leishmania infantum chagasi* in an endemic area for ACL in Argentina (Rosa et al. 2012), as in that country it is considered to be the probable secondary vector species both for AVL and ACL (MS 2010).

A survey of previous entomological surveys conducted in the studied area revealed that no other specimens of *Ev. cortelezzii* or *Ev. lenti* have been identified among collected sandflies. *Nyssomyia neivai* was the most abundant species captured in the municipality of Monte Mor, followed by *Nyssomyia whitmani*, *Migonemyia migonei* and *Pintomyia pessoai* (Cutolo et al. 2009), all of which are species associated with the transmission of ACL. *Ny. neivai* was also the most abundant species collected in chicken coops, pig pens and dog shelters in a study of human and canine ACL in a rural area of this same municipality, which contained dogs infected with *Leishmania braziliensis*. Opossums were the most common wild animals captured in the studied area from 2009-2012, with 58 specimens collected in urban and rural areas of the municipality. Of those, 55 were *D. albiventris* and three were *D. aurita*. One of the *D. albiventris* specimens was found to have *Leishmania* sp. DNA by means of polymerase chain reaction performed on a biopsied ear skin sample (unpublished data).

The area of AVL incidence in canine and human hosts is undergoing expansion and urbanisation in SP. Although a high infection prevalence (91.6%) has been found in *Didelphis* sp. in the municipality of Bauru (Santiago et al. 2007), the role that wild animals potentially play as *L. infantum chagasi* reservoirs is still poorly understood in the various Brazilian municipalities that have reported transmission.

The association between sandflies and opossums in urban areas may contribute to the introduction of *Leishmania* spp as well as to the establishment and maintenance

of their foci of transmission. The present study is the first report of *Ev. cortelezzii* and *Ev. lenti* captured in association with *D. albiventris* burrows in an urban area that demonstrates the affinity of these insects with this host species. The role that these sandfly-*Didelphis* sp. associations may play in the epidemiology of ACL and AVL prompts further investigation.

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