

## Studies on the feeding habits of *Lutzomyia (N.) intermedia* (Diptera, Psychodidae), vector of cutaneous leishmaniasis in Brazil

Estudos sobre os hábitos alimentares de *Lutzomyia (N.) intermedia* (Diptera, Psychodidae), vetor de leishmaniose cutânea no Brasil

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### Abstract

*The precipitin test was applied to identify the blood meal sources of Lutzomyia intermedia, collected in two distinct habitats: inside houses and in the peridomicile, in Mesquita, Rio de Janeiro State, Brazil, a transmission area of Leishmania (V.) braziliensis. The following antisera were tested: human, avian, equine, rodent, and opossum. From a total of 370 females analyzed, 128 specimens from the domicile and 59 from the peridomicile reacted with specific antisera. The anthropophily of L. intermedia was confirmed in both habitats; likewise, the feeding of this sand fly species on domestic animals, observed in previous entomological surveys, was confirmed by the strong reactivity with avian, canine, and equine antisera. However, feeding on rodents, mammals frequently found inside and around houses, represents further evidence related to the vector competence of L. intermedia, since synanthropic and sylvatic rodents have been considered a putative reservoir of L. (V.) braziliensis.*

*Insect Vectors; Psychodidae; Leishmaniasis*

### Introduction

In the State of Rio de Janeiro, Brazil, *Lutzomyia intermedia* is considered the principal vector of cutaneous leishmaniasis, caused by *Leishmania (V.) braziliensis* <sup>1,2,3,4</sup>. The vector competence of *L. intermedia* is suggested by its distribution and frequency in areas with the occurrence of human cases of cutaneous leishmaniasis, its particularly high density in both the intradomicile and peridomicile, its anthropophily, and the fact that it has been found naturally infected with *Leishmania* sp. of the Periplarian Section (sub-genus *Viannia*) <sup>5</sup>. It is noteworthy that even though it is considered a highly anthropophilic species, it is heavily attracted to dogs and equines, as suggested by domestic reservoirs of *L. (V.) braziliensis* <sup>4,6</sup>. Studies conducted in cutaneous leishmaniasis transmission areas in the State of Rio de Janeiro in the late 1990s demonstrated the presence of *L. intermedia* including in remnants of the Atlantic Forest located 800 meters from households with human and canine cases of cutaneous leishmaniasis <sup>7</sup>.

Accurate knowledge of the blood meal source for vectors of human pathogens provides information on the preference for hosts under natural conditions. The intensity of anthropophily is one of the essential factors in the evaluation of vector capacity, while data on the attraction to other hosts can shed light on the association

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between potential vectors and natural reservoirs of pathogens.

Current knowledge on the feeding habits of *L. intermedia* is based on data obtained from captures using human bait or in animals, using a Castro aspirator or CDC light traps<sup>2,3,4,7</sup>. Therefore, in order to better define the feeding sources for females of this important American cutaneous leishmaniasis vector in Southeast Brazil, the precipitin test was applied to specimens collected in the domiciliary environment in an endemic area in the State of Rio de Janeiro.

## Materials and methods

### Sand fly collection

Specimens were collected in a community located 600 meters above sea level in the municipality of Mesquita (22°45'37" S; 42°26'52" N), Rio de Janeiro State. The area was described previously by Rangel et al.<sup>4</sup>. Captures (a total of 17) were performed in 1999 and 2000 in the interior of dwellings where human (a total of 23 human cases were registered in the locality during this period) and canine cases of cutaneous leishmaniasis had been reported, as well as in the peridomiciliary environment (up to 10 meters from the dwelling), where domestic, synanthropic, and sylvatic animals circulate. A Castro aspirator (one capturer) and CDC-type light traps (three) were used from 18:00 to 20:00 and from 18:00 to 6:00, respectively. The sand flies were transported alive to the laboratory at the Instituto Oswaldo Cruz, and the engorged females were killed by immersion in liquid nitrogen.

### Screening and identification of sand flies

The specimens were screened in a cold chamber (cryolizer), and the *L. intermedia* specimens (engorged females) were individualized in Eppendorf tubes and stored at -15°C. Before initiating the precipitin test, the head of each female was separated and diaphanized using 20.0% potash. Species confirmation was performed by visualization of the cibarium under a stereoscopic microscope.

### Precipitin test

Identification of the blood ingested by the sand flies targeted the host group using the precip-

itin method, according to Templis & Lofy<sup>8</sup>. The reaction consisted of adding 0.2ml of 0.85% sodium chloride solution to each Eppendorf tube, and then macerating the female sand fly. The tubes containing the females were stored under refrigeration ( $\pm 18^{\circ}\text{C}$ ) for 24 hours. Subsequently the material was centrifuged at 2,000rpm for 10 minutes. Previously lyophilized antiserum samples (Sigma Immuno Chemicals) were reconstituted with 2.0ml of bidistilled water. Capillary tubes containing equal parts of sand fly eluate and the test antisera were kept at room temperature for 2 hours, after which the agglutination reactions were read. The choice of antisera was based on prior knowledge of the mammals commonly found in the area where the captures were performed. Thus, the specimens collected in the intradomicile were tested against human, rodent, canine, and avian antisera, while those captured in the peridomicile were tested against human, equine, opossum, avian, and rodent antisera.

## Results

Of the 240 *L. intermedia* females captured in the intradomicile, only 128 (53.3%) had intestinal content that was reactive to the precipitin test. Among the reactive specimens that displayed strong reactivity (well-defined, opaque white clumps) to only one blood source, more than one-third (39.8%) reacted to rodent antiserum, followed by avian (23.7%), canine (20.4%), and human (16%) antisera, respectively (Figure 1).

A few sand flies reacted to more than one blood source: three of six reacted to canine/rodent antisera. Of the specimens that showed weak reactivity (pale clumps), 34.0% reacted to human antiserum, followed by rodent (25.7%), avian (22.9%), and canine (17.5%) antisera, respectively.

From the peridomiciliary environment, the intestinal content of 130 sand flies was tested, and 45.4% of the samples were reactive. Considering only strong signs of reactivity, the rodent and avian antisera predominated with 26.5% each, followed by human and equine antisera, with 23.5% each (Figure 2). For the samples with weak reactivity, the most frequent reactions were to human antiserum (32.0%), followed by rodent and avian (24.0% each) and equine (20.0%) antisera. There was no reactivity to opossum antiserum, and no double reactivity was observed.

Figure 1

Percentage of *Lutzomyia intermedia*-positive females collected inside houses, for different antisera. Municipality of Mesquita, Rio de Janeiro State, Brazil.

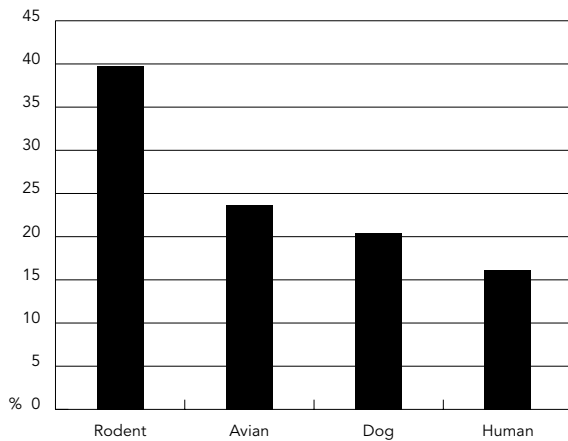
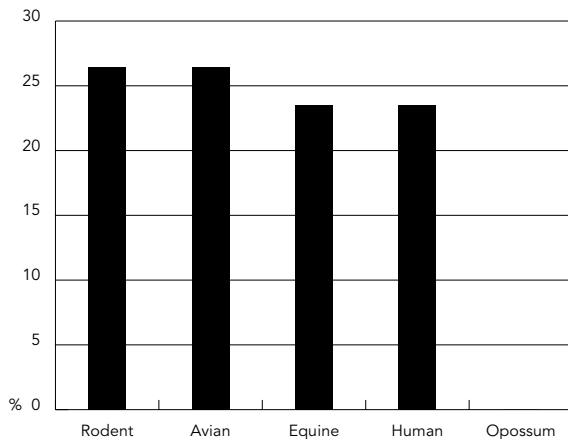


Figure 2

Percentage of *Lutzomyia intermedia*-positive females collected in the peridomicile, for different antisera. Municipality of Mesquita, Rio de Janeiro State, Brazil.



## Discussion

For better epidemiological knowledge of diseases in which the pathogens are transmitted by insects, it is essential to understand key aspects of vector ecology and behavior, especially feeding habits. Feeding behavior can define the degree of sand fly anthropophily, in addition to attraction to the parasite's natural reser-

voirs, two essential requirements in order for a species to be classified as transmitting diseases to humans<sup>9</sup>.

Application of the precipitin test to studies on sand fly biology helped demonstrate the attraction to possible leishmania hosts, in addition to more precisely evaluating the species' anthropophily. Several studies already performed in this direction observed in Panama<sup>10</sup> that *L. shannoni* displayed a strong attraction to rodents and edentates, in addition to confirming its low anthropophily, while *L. ylephiletor* was only attracted to edentates; in addition, bat antiserum was associated with *L. vespertilionis*. Since the intestinal content of *L. trapidoi* reacted to different antisera, the authors considered this species an effective disease vector. In the central Amazon region, specimens of the *L. shannoni* group were found to have fed mostly on sloth<sup>11</sup>; this may be epidemiologically relevant (even though this specific sand fly is not considered a leishmaniasis vector), since sloth have been incriminated as reservoirs for *L. (V.) guyanensis* in Panama. A study in Colombia<sup>12</sup>, an endemic area for visceral leishmaniasis, suggested that *L. longipalpis* displays low anthropophily but is attracted to dogs, and based on this information the authors discuss the local epidemiology. A study in Peru<sup>13</sup>, showed the eclectic feeding habits of *L. peruensis* and *L. verrucarum*, although the former was more anthropophilic. A recently study on the stomach content of *L. longipalpis* with the precipitin test provided information backing the hypothesis on the transmission of visceral leishmaniasis in an anthropic environment in the municipality of Raposa, Maranhão State, Brazil<sup>14</sup>.

*L. intermedia* is considered a sand fly with variable feeding behavior in terms of its blood source; its dispersion in varied environments ranging from the interior of dwellings to remnants of the Atlantic Forest places it in association with both domestic and sylvatic animals. This may occur due to this sand fly's behavior plasticity, since it adapts easily to environmental alterations, making it virtually predominant in the new epidemiological profile of cutaneous leishmaniasis<sup>15</sup>, contrary to other sand flies, which have begun to appear less frequently<sup>16</sup>. Rangel et al.<sup>3,4</sup> observed this sand fly biting domestic animals (especially dogs) in the peridomicile.

Based on our studies, *L. intermedia* may be feeding spontaneously on any of the animals whose antisera were tested in this analysis, i.e., humans, dogs, horses, rodents, and birds, but not opossums. The variety of the blood content

clearly reinforces the sand fly's eclectic feeding habits in terms of food sources and corroborates the data on this vector's activity in the peridomestic environment and its association with domestic reservoirs<sup>4</sup>. Although we found a small percentage of specimens that reacted to more than one antiserum, this suggests the habit of "tasting" before actually performing the blood meal. The fact that *L. intermedia* appears to be so eclectic in its food sources may explain the low percentage of reactivity to human antiserum. Still, this sand fly's anthropophily was confirmed in both the domiciliary and peridomestic environments, thus reinforcing its important role as a vector for *L. (V.) braziliensis* in Southeast Brazil<sup>2,3,4,17</sup>.

In 1990, Rangel et al.<sup>4</sup> had already described a domestic cycle of cutaneous leishmaniasis transmission in the State of Rio de Janeiro, suggesting dogs and horses as probable domestic reservoirs for *L. (V.) braziliensis*, but without discarding the existence of a primary sylvatic cycle. A recent study<sup>7</sup> in the same region in the State of Rio de Janeiro points to the existence of a primary cycle in remnants of the Atlantic Forest, involving *L. (V.) braziliensis* and *L. intermedia* and suggesting that the sloth *Bradypus variegatus* participates in this chain as the primary reservoir, according to previously observed evidence<sup>18</sup>, based on molecular parasitic detection.

However, studies in the State of Pernambuco (Northeast Brazil) in Atlantic Forest areas proved the importance of synanthropic (*Rattus*

*rattus*) and sylvatic rodents (*Bolomys lasirius*) as reservoirs of *L. (V.) braziliensis* through the isolation and characterization of the parasite in a transmission cycle involving *L. whitmani*<sup>19</sup>. Studies conducted in Serra dos Carajás<sup>20</sup>, in the State of Pará, suggested that the principal reservoir of *L. (V.) braziliensis* would be terrestrial mammals, probably rodents, considering the ground-level flight of the vector in the region, *L. wellcomei*, like *L. intermedia*.

There is a noteworthy and frequent presence of rodents (*R. rattus*) in the area covered by the current study, the results of which clearly proved the attraction of *L. intermedia* to rodents. A preliminary survey conducted in the Pedra Branca Forest Reserve, very close to the study area, showed the occurrence of some small mammals: *Didelphis aurita*, *Metachirus nudicaudatus*, *Micoureus demerarae*, *Gracilinanus agilis*, *Monodelphis americana*, *Akodon cursor*, *Oligoryzomys nigripes*, and *Sphiggurus insidiosus* (D'Andrea PS, personal communication; 2005).

Considering the above-mentioned findings in Pernambuco, and working with the hypothesis that rodents could also be playing an important role as reservoirs for *L. (V.) braziliensis* here in Rio de Janeiro, representing links between the sylvatic and domestic environments, the data presented here are further evidence of the participation by *L. intermedia* in this parasite's transmission chain, thus favoring the transmission in both intradomestic and peridomestic habitats.

## Resumo

Os testes de precipitina foram aplicados na identificação das fontes alimentares de *Lutzomyia intermedia*, de ambientes intra e peri-domiciliar, do Município de Mesquita, Estado do Rio de Janeiro, Brasil, área de transmissão de *Leishmania (V.) braziliensis*. Foram testados os seguintes antígenos: homem, ave, cão, equino, gambá e roedor. De um total de 370 fêmeas analisadas, revelaram reatividade, contra os antígenos testados, 128 espécimes do intra-domicílio e 59 do ambiente peridomiciliar. A antropofilia de *L. intermedia* foi confirmada nos dois ambientes trabalhados; da mesma forma, a alimentação deste flebotômico em ani-

mais domésticos, observada anteriormente em levantamentos entomológicos, foi comprovada pela forte reatividade com antígenos de ave, cão e equino. Entretanto, a alimentação em roedores, animais sinantrópicos no ambiente domiciliar (interior e ao redor das residências), é mais uma forte evidência da competência vetorial de *L. intermedia*, uma vez que roedores sinantrópicos e silvestres são comprovadamente reservatórios de *L. (V.) braziliensis*.

Insetos Vetores; Psychodidae; Leishmaniose

## Contributors

A. C. Gomes performed the precipitin tests and analyzed the resulting data. M. M. S. Afonso conducted the sand fly captures, together with C. R. V. Meneses, and contributed to the insect processing and the precipitin tests and data analysis. E. F. Rangel coordinated the project, conducted captures, processed the insects, and contributed to the data analysis from the precipitin tests.

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