

Eclectic feeding behavior of *Lutzomyia* (*Nyssomyia*) *intermedia* (Diptera, Psychodidae, Phlebotominae) in the transmission area of American cutaneous leishmaniasis, State of Paraná, Brazil

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ABSTRACT

Introduction: The blood meal source of sandflies provides valuable information about the vector/host interaction and allows for an understanding of American cutaneous leishmaniasis (ACL) transmission mechanisms. The aim of this study was to identify the blood meal sources of *Lutzomyia* (*Nyssomyia*) *intermedia* in an endemic area of leishmaniasis in Brazil's State of Paraná using a precipitin test. **Methods:** Sandflies were collected in the rural locality of Epitácio Pessoa within the City of Adrianópolis, State of Paraná, in southern Brazil. A total of 864 female sandflies were captured, and 862 (99.8%) were identified as *L. intermedia* species. However, two unidentified specimens were considered to be part of the genus *Lutzomyia*. **Results:** Among the females examined, 396 specimens presented reactions to a certain type of tested antiserum, and most (67.9%) reacted to the simple type. These sandflies fed mainly on the blood of birds, opossums, and rodents, but specimens that fed on the blood of humans, dogs, horses, cattle, and cats were also found. Among the cross-reactions found (32.1%), bird/rodent, bird/opossum, bird/dog, bird/human, and horse/dog cross-reactions were the most common. **Conclusions:** These results demonstrate a tendency in the eclectic feeding behavior of *L. intermedia* and support its potential role as a vector for ACL in the study area.

Keywords: American cutaneous leishmaniasis. *Lutzomyia* (*Nyssomyia*) *intermedia*. Feeding behavior. Precipitin test.

INTRODUCTION

Over the last 20 years, the number of leishmaniasis cases in Brazil has increased¹. In the southern region of the country, State of Paraná accounts for 94.9% of American cutaneous leishmaniasis (ACL) cases² that have been described since the early nineteenth century. The first report in the literature on six cases in the City of Adrianópolis was described by Miranda³, but only after 1980 was this city considered to be an endemic area⁴. In one of the other endemic areas, also located in the Ribeira River Valley, this disease has been known since the beginning of the century; in the last two decades alone, 339 cases were reported⁵.

The search to identify natural vertebrate reservoirs of *Leishmania* has been one of the most interesting subjects related to the transmission of leishmaniasis around the world⁶. The primary reservoirs of *Leishmania* are wild animals, and particularly rodents and canids. In the Americas, more than 40 species of mammals can harbor *Leishmania*, but few species have been shown to play an important role in the natural

transmission cycle of the parasite. However, domestic and synanthropic animals have assumed an important role in the maintenance of this parasite in transmission areas⁷.

Investigations into the feeding habits of sandflies have great ecological and epidemiological significance because these studies enable the correct identification of potential mammalian reservoirs and the vector's blood feeding preferences. In addition, these studies help to reveal the natural transmission cycle in a given area and allow us to develop strategies for disease control⁸⁻¹⁰.

Investigations into the feeding habits of sandflies have been performed using precipitin tests^{10,11}. This test is an important tool for understanding vector/host interaction and ACL transmission mechanisms. Other techniques have been performed to identify the blood meal sources of arthropod vectors, including ELISA¹² and several methods involving PCR, among which prepronociceptin (PNOC) gene amplification^{8,13}, restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR), and cytochrome b (cytb) gene amplification and sequencing^{9,14,15} are best known.

The aim of the present study was to identify the blood meal sources of *Lutzomyia* (*Nyssomyia*) *intermedia* in an endemic ACL area in State of Paraná in southern Brazil using the precipitin test.

In addition, the role of mammals as possible reservoirs of *Leishmania* is discussed. Despite the importance of the State of Paraná in the case of ACL, the precipitin test to determine the sandfly's blood meal sources studies haven't been published yet in this region.

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METHODS

Study area

Insects were collected in the City of Adrianópolis in the rural locality of Epitácio Pessoa (**Figure 1**) (24°47'31" S and 48°59'28" W), approximately 280m above sea level. This city is

located in the northeast region of State of Paraná, on the border with State of São Paulo. The city occupies 1,349km², with a population of 6,376 inhabitants and a population density of 4.7 inhabitants per km². The vegetation is composed of the Atlantic Forest, and the region possesses a warm, tropical climate, with high temperatures in the summer and mild temperatures in the autumn and the winter and high rainfall year round¹⁶.



FIGURE 1 - Map of the City of Adrianópolis, State of Paraná, Brazil, showing the study area.

Capture methods

Three consecutive samplings (one per week) were performed in January 2013 using CDC light traps running continuously from 6 pm to 6 am. The traps were installed on two properties in the following ecotypes: household (kitchens), peridomicile (yards and animal shelters), and forest. The captured insects were frozen on dry ice while still in the field to interrupt the digestive process and were transported in appropriate containers to the Laboratory of Molecular Parasitology at the Federal University of Paraná (UFPR).

Insect identification

Females were screened under a stereomicroscope and dissected for specific identification with a taxonomic key according to Young and Duncan¹⁷. After identification, each female was stored in microtubes of 0.6µl that were kept at room temperature.

Precipitin test

The precipitin test was conducted at the Entomology Department of Oswaldo Cruz Institute/FIOCRUZ in the City of Rio de Janeiro. The digestive tract and the stomach contents of the specimens were removed and triturated in a 0.85% saline solution. This mixture was left for 12h at a temperature of approximately 4°C and then centrifuged for 5min at 1,500rpm. The supernatant was exposed to the antisera of chickens, pigs, dogs, cats, horses, cattle, opossums, armadillos, rodents, and humans¹⁸.

RESULTS

Of the 3,357 sandflies collected during the study period, 864 were females. Of these females, 862 (99.8%) belonged to the species *L. intermedia*, and two specimens were identified as belonging to other species of the genus *Lutzomyia*. During

the samplings, 744 (86.1%) females were captured in the peridomicile; 71 (8.2%) were captured in the household; and 49 (5.7%) were captured in the forest.

Of the 862 females analyzed, all showed intestinal content suggestive of blood. In the precipitin test, 396 (45.8%) reacted to a certain type of tested antiserum, and the majority reacted to the simple type (67.9%), meaning that they had fed on only one host. The other 32.1% presented cross-reactions with two types of varied combinations of antisera. A total of 468 (54.2%) females presented no reaction to the tested antisera. In addition, 32.8% of the specimens that experienced a simple reaction were found to have fed on birds, whereas the other specimens had fed on the following animals: opossums (9.6%), rodents (7.9%), humans (5.6%), horses (4.6%), dogs (4.6%), cattle (2%), and cats (1%) (**Table 1**).

The blood-fed females that were found to have fed on more than one host presented the following combinations: bird and rodent (5.8%), bird and opossum (4.8%), bird and dog (2.3%), bird and human (2.3%), horse and dog (2.3%), and rodent and opossum (2%) (**Table 2**).

DISCUSSION

Studies on the feeding behaviors of vector insects are essential for understanding the epidemiology of parasites because these studies may define the degree of anthropophily and attraction to natural reservoirs, which are two requirements for labeling a species as a vector.

In the present study, the species *L. intermedia* was found in high densities in the following ecotypes: peridomicile (86.1% of captured specimens), household (8.2% of captured specimens), and forest (5.7% of captured specimens). According to previous studies¹⁹, this species is highly anthropophilic and predominantly lives within households and peridomiciliary environments. Studies performed in the Ribeira River Valley in

TABLE 1 - Simple reactions to the different types of antisera verified by the precipitin test.

Antisera	Ecotype							
	household		peridomicile		forest		Total	
	n	%	n	%	n	%	n	%
Bird	12	3.0	116	29.3	2	0.5	130	32.8
Opossum	1	0.2	36	9.1	1	0.2	38	9.6
Rodent	1	0.2	27	6.8	3	0.8	31	7.9
Human	1	0.2	17	4.3	4	1.0	22	5.6
Horse	1	0.2	16	4.0	1	0.2	18	4.5
Dog	1	0.2	17	4.3	-	-	18	4.5
Cattle	-	-	8	2.0	-	-	8	2.0
Cat	-	-	3	0.8	1	0.2	4	1.0
Total	17	4.3	240	60.6	12	3.0	269	67.9

TABLE 2 - Cross-reactions to the different types of antisera verified by the precipitin test.

Antisera	Ecotype							
	household		peridomicile		forest		total	
	n	%	n	%	n	%	n	%
Bird/rodent	1	0.2	21	5.3	1	0.2	23	5.8
Bird/opossum	2	0.5	16	4.0	1	0.2	19	4.8
Bird/dog	1	0.2	7	1.8	1	0.2	9	2.3
Bird/human	3	0.8	5	1.3	1	0.2	9	2.3
Horse/dog	1	0.2	8	2.0	-	-	9	2.3
Rodent/opossum	2	0.5	6	1.5	-	-	8	2.0
Horse/bird	1	0.2	6	1.5	-	-	7	1.8
Bird/cattle	1	0.2	4	1.0	2	0.5	7	1.8
Bird/cat	-	-	6	1.5	-	-	6	1.5
Rodent/dog	1	0.2	5	1.3	-	-	6	1.5
Human/dog	-	-	3	0.8	1	0.2	4	1.0
Horse/human	-	-	3	0.8	-	-	3	0.8
Cattle/cat	-	-	3	0.8	-	-	3	0.8
Horse/cattle	-	-	2	0.5	1	0.2	3	0.8
Rodent/horse	-	-	3	0.8	-	-	3	0.8
Horse/opossum	-	-	2	0.5	-	-	2	0.5
Cat/opossum	-	-	1	0.2	-	-	1	0.2
Cat/dog	-	-	1	0.2	-	-	1	0.2
Rodent/cattle	-	-	1	0.2	-	-	1	0.2
Horse/cat	-	-	1	0.2	-	-	1	0.2
Cattle/dog	-	-	1	0.2	-	-	1	0.2
Rodent/cat	-	-	-	-	1	0.2	1	0.2
Total	13	3.3	105	26.5	9	2.3	127	32.1

Brazil have reported that this species represented nearly 100% of specimens captured and was also found in the household. These results indicate very close contact between the species and domestic animals and humans⁵. Furthermore, several studies have reported natural infection by *L. braziliensis*^{20,21}. The precipitin test revealed that 45.8% of the analyzed insects had simple reactions. The most common hosts were birds, followed by opossums, rodents, and humans. Insects that had cross-reactions fed mostly on bird/rodent and bird/opossum combinations. These results corroborate the findings of previous studies that observed the association of this species with potential reservoirs and activity in the peridomicile^{11,22}. Simple blood meals of females collected in the peridomicile include synanthropic animals, such as opossums and rodents, whereas the samples from the forest included more human blood. Considering that marsupials and rodents have been found to become infected with *Leishmania (Viannia)*^{6,22,23,24} and that the

presence of humans in the forest environment makes infection by this parasite easier, it is possible that *Leishmania* transmission occurs both within the forest and in the peridomestic areas of the location studied.

Certain species, including *Lutzomyia intermedia*, *Lutzomyia (Coromyia) migonei*, and *Lutzomyia (Nyssomyia) whitmani*, feed on various hosts and possess a significant dispersal ability. Feeding preferences influence species dispersal, but certain species are restricted to the areas near their source of blood meals²⁵.

In the current study, the area in question is characterized by a destruction of primary vegetation. The area possesses secondary vegetation remnants, with banana plantations, *Pinus* sp., cassava, corn, and other crops and pastures. This characteristic human activity may change the reservoir host availability and thus influence the feeding habits of sandflies, by leading human contact with the natural cycle of the parasite.

The maximum flight range of female sandflies recorded was 128m²⁶. Galati et al.²⁷ found that the median dispersion distance of *Ny. intermedia* was 109m. In the current sampling, blood meals from peridomicile animals, such as cattle and horses, were found in insects caught within the forest. The households on the properties that were surveyed were less than 200m away from the forest, a finding that shows that sandfly traffic may occur between forest, peridomicile, and household ecotypes. Previous studies in State of Rio de Janeiro reported the same behavior in this species^{21,28}.

Other studies have offered hypotheses that explain ACL cases that have occurred outside of the sandfly dispersal area as an adaptation of the insect and/or reservoirs to the peridomicile. These hypotheses may explain the transit of these animals between forest fragments and households. These forest fragments are part of human habitats, and humans may acquire the parasite in the forest during periods of insect activity (dusk and night)^{29,30}.

Among the analyzed specimens, 54.2% presented a negative result for the precipitin test. This result may indicate the absence of a direct relationship with the antisera used because the tests were performed only with antisera of the animals most commonly found in the study area. Another possibility could be an insufficient amount of blood in the intestinal content.

The current study revealed that *L. intermedia* presented very eclectic feeding behavior in this period of the capture because the specimens captured fed on various domestic and synanthropic animals and on human populations. In total, 32.8% of sandflies fed on birds. The birds may have been chickens because these birds are predominant in the ecotypes studied. However, it is important to note that although chickens cannot sustain a *Leishmania* infection³¹ and are not characterized as a source of infection from *L. intermedia*, these animals are attractive to the vector, a fact that could have epidemiological importance for leishmaniasis.

The blood feeding preferences of *L. intermedia* described herein show that there is a condition for sandfly attraction to the peridomicile: the presence of domestic animals seems to favor the presence of sandflies, especially when the domestic animals are birds (chickens). In addition, the houses in this region are built close to vegetation, which includes forest fragments in which insects and reservoirs (particularly rodents and opossums) live. These conditions, combined with the presence of humans in the forest for several activities, enable the transmission of *Leishmania* in the study area.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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