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Major Article

Blood feeding patterns of *Nyssomyia intermedia* and *Nyssomyia neivai* (Diptera, Psychodidae) in a cutaneous leishmaniasis endemic area of the Ribeira Valley, State of São Paulo, Brazil

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ABSTRACT

Introduction: The aim of this study was to identify the blood feeding sources of *Nyssomyia intermedia* (*Ny. intermedia*) and *Nyssomyia neivai (Ny. neivai)*, which are *Leishmania* vectors and the predominant sandfly species in the Ribeira Valley, State of São Paulo, Brazil, an endemic area for cutaneous leishmaniasis. **Methods:** Specimens were captured monthly between February 2001 and December 2003 on a smallholding and a small farm situated in the Serra district in the Iporanga municipality. The blood meals of 988 engorged females were tested using the avidin-biotin immunoenzymatic enzyme-linked immunosorbent assay (ELISA). Seven blood meal sources were investigated: human, dog, chicken, bovine, pig, horse and rat. **Results:** The results showed that among the females that fed on one or more blood sources, the respective percentages for *Ny. intermedia* and *Ny. neivai,* respectively, were as follows: human (23% and 36.8%), pig (47.4% and 26.4%), chicken (25.7% and 36.8%) and dog (3.9% and 0%), and the differences in the blood sources between the two species were statistically significant (p = 0.043). **Conclusions:** Both species had predominant reactivity for one or two blood sources, and few showed reactivity indicating three or four sources. Many different combinations were observed among the females that showed reactivity for more than one source, which indicated their opportunistic habits and eclecticism regarding anthropic environmental conditions.

Keywords: Phlebotomine. Sandfly. *Nyssomyia intermedia. Nyssomyia neivai.* Blood feeding sources. American cutaneous leishmaniasis.

INTRODUCTION

The subfamily Phlebotominae is an insect group that has worldwide distribution. It comprises approximately 500 species[1] whose females feed on a wide range of vertebrates, including mammals, birds, amphibians and reptiles in the Americas.

Many sandfly species have the ability to transmit pathogens to animals, some of which are reservoirs for cutaneous and visceral leishmaniasis agents[2,3].

The Ribeira Valley region has one of the highest incidences of cutaneous leishmaniasis cases in the State of São Paulo [4,5]. This region had a total of 1,174 cutaneous leishmaniasis cases registered between 1998 and 2006 and one of the highest average annual incidence coefficients in the State of São Paulo (47.71/100,000 inhabitants). The Serra district is situated in the Iporanga municipality, which has an incidence coefficient of 152.9/100,000 inhabitants, one of the highest in the Ribeira Valley region[4].

Understanding sandfly behavior and its implications for transmitting *Leishmania* in this area involves studying their blood feeding habits, as this information is relevant when estimating the degree of host-vector contact.

Highly specific and sensitive methods of detecting blood sources are necessary to identify the host-feeding patterns of blood-sucking insects. The use of the biotin-avidin enzyme-linked immunosorbent assay (ELISA) method allows the detection of small volumes of blood from single or various sources[6,7-9]. The aim of the present study was to report on the feeding patterns of *Nyssomyia intermedia* (*Ny. intermedia*) (Lutz & Neiva, 1912) and *Nyssomyia neivai (Ny. neivai)* (Pinto, 1926), which are the predominant sandfly species among the 19 species found in the Serra district[4,10] and the suspected transmission vectors of cutaneous leishmaniasis agents[11].

METHODS

Study area and sandfly sampling

The Serra district (24º 33' 19.7'S, 48º 40' 42"W, 198m above sea level [ASL]) is situated on the banks of the Betari river, a

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tributary of the Ribeira river in the municipality of Iporanga, an area containing hostels for tourists who visit the Parque Estadual do Alto Ribeira (PETAR), which is situated in the speleological province of the Ribeira Valley in the Serra de Paranapiacaba in the south of the State of São Paulo within the Atlantic forest domain.

Two areas on the left bank of the Betari river were sampled: the JRA smallholding (24º 33" 03.96"S, 48º 40' 33.83"W, 185m ASL) and the AG small farm (24º 33' 01.14"S, 48º 40' 16.33" W, 202m ASL), which are approximately 520m from one another.

The JRA smallholding contains a dwelling in which a family of four and a dog lived and a peridomicile with a pigsty, a chicken-house and a goose-shelter. The AG small farm contained two dwellings, one of which was temporarily inhabited by a family (two people). The other dwelling is permanently inhabited by one person, with a hen house, two pigsties, horses and cows in the proximity of the two houses.

The capture of phlebotomines on the JRA smallholding were performed monthly from February 2001 to December 2003 in the hen house, in the pigsty and on the porch of the house (with modified automatic light traps) and from May 2001 to June 2002 in the peridomicile (with modified black and white Shannon traps), as described by Galati[4,12].

The captures on the AG small farm were performed monthly from March 2001 to December 2003 using modified automatic light traps in a hen house, a pigsty and on the porch of one house[4].

During a dispersion study, sandflies were also obtained from two other locations: a peridomicile situated 180m from the AG small farm and a cave located 2km from the farm. The captures were performed monthly on eight consecutive days from August to December 2003 using electric manual aspirators in the morning and at night in resting places (i.e., the nest of a dog's litter, on rocks and tree trunks, and in pigsties, hen houses, birds' nests, rat burrows, banana groves and cattle pens), automatic light traps in peridomiciles, domestic animal shelters, the forest, the forest's edge, pastures, banana groves, caves, or Shannon traps in a peridomiciliary area. A more detailed description is given in Galati[10].

Sample collection and processing

Engorged female specimens were captured, conditioned in refrigerated Petri dishes and transported to the Laboratory of Public Health Entomology/Phlebotominae of the Epidemiology Department, Public Health School of the São Paulo University.

Parts of the abdomen and the thorax of each engorged female were placed in a microtube and stored at -20◦C until the ELISA test could be performed. The species were identified by the head and genitalia as described by Andrade Filho[13], who distinguished them based on the number of rings, the width of both: the base of the terminal knob and the apex of the individual sperm ducts situated at the junction of the spermathecae, and the length of the individual and common ducts. The generic name abbreviations follow Marcondes[14].

Blood meal analysis

The hosts of the blood-fed phlebotomines were identified using a biotin-avidin ELISA in accordance with Marassá[7,8]. The feeding behavior was investigated using host-specific anti-human, anti-chicken, anti-dog, anti-rat, anti-horse, anti-pig and anti-bovine sera.

Statistical analysis

For the statistical analysis, Fisher's exact test was used to compare the differences between the blood sources of the two sandfly species.

RESULTS

The species that were identified in the blood ingested phlebotomines captured on the JRA smallholding and the AG small farm and their respective percentages, as determined by the biotin-avidin ELISA, are listed in **Tables 1, 2** and **3**.

On the JRA smallholding, 141 engorged females were captured, 83.7% of which were reactive **(Table 1)**. Among those insects that fed on one or more blood sources, the respective percentages for *Ny. intermedia* and *Ny. neivai,* respectively, were human (23% and 36.8%), pig (47.4% and 26.4%), chicken (25.7% and 36.8%) and dog (3.9% and 0%), and the difference in the blood source frequency between the two species was statistically significant ($p = 0.043$).

For the specimens captured on the porch, no statistically significant difference with regard to the blood source ($p = 0.345$) was found between the two species, and the proportions of those insects that fed on the respective sources were as follows: human (41.7%), pig (25%), chicken (26.2%) and dog (7.1%) for both sandfly species combined.

On the AG small farm, of the 596 engorged females captured with CDC and investigated, 78.2% were reactive. No statistically significant differences between the two species with regard to blood source were found ($p = 0.591$). The blood sources identified in the two species together were as follows: pig (60.3%), chicken (22.5%), human (10.5%), horse (4.9%), bovine (1.4%) and dog (0.4%) **(Table 2)**.

No statistically significant difference between the blood sources found in the *Ny. intermedia* and *Ny. neivai* that were captured on the porch was observed ($p = 0.400$); the frequencies of the blood sources that were found in these two species were as follows: pig (46%), human (20.6%), chicken (16.9%), horse (11.7%), bovine (3.6%) and dog (1.2%).

The number of sources and their respective percentages for the *Ny. Intermedia* that were identified on the AG small farm and JRA smallholding, respectively, were one (65.4% and 46.7%), two (26.3% and 41.3%), three (7.3% and 12%) and four (1% and 0%), and the differences between the two sites were statistically significant ($p = 0.006$), However, for *Ny. neivai*, the respective figures for the AG small farm and JRA smallholding were one (59% and 53.8%), two (33.8% and 34.6%), three (6.5% and 11.6%) and four (0.7% and 0%), indicating no significant differences ($p = 0.654$).

On the AG small farm, 251 engorged females were captured by aspiration in their resting places, and 80.9% were reactive **(Table 3)**. No statistically significant difference was found among the blood sources observed for either sand fly species ($p = 0.583$). The distribution of blood sources for the two species together was as follows: chicken (40%), pig (30%), human (21%), horse (1%) and bovine (8%).

TABLE 1 - The blood meal distribution identified between January 2001 and December 2003 (using automatic CDC light traps) by source and sandfly species in the JRA smallholding, Serra district in Iporanga municipality according to the collection site.

	Nyssomyia intermedia								*Nyssomyia neivai*									
Sandfly species site	pigsty		henhouse		porch		subtotal		pigsty		henhouse		porch		subtotal		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Source																		
human	1	1.5	2	25.0	7	21.8	10	9.3	-	-	-	-	9	42.8	9	26.5	19	13.5
chicken	1	1.5	-	-	-	-	1	0.9	1	12.5	1	20.0	-	-	2	5.9	3	2.1
pig	28	41.8	-	-	4	12.5	32	30.0	-	-	-	-	2	9.5	2	5.9	34	24.1
dog	-	-	-	-	-	-	-	-	-	-	-	-	1	4.8	1	2.9	1	0.7
human/chicken	-	-	-	-	3	9.4	3	2.8	-	-	-	-	2	9.5	2	5.9	5	3.5
human/pig	5	7.5	-	-	3	9.4	8	7.5	-	-	-	-	-	-	-	-	8	5.6
human/dog	-	-	-	-	3	9.4	3	2.8	-	-	-	-	-	-	-	-	3	2.1
human/chicken/pig	5	7.5	-	-	5	15.6	10	9.3	1	12.5	-	-	1	4.8	2	5.9	12	8.5
human/chicken/dog	-	-	-	-	1	3.1	1	0.9	-	-	-	-	1	4.8	1	2.9	2	1.4
chicken/pig	15	22.3	4	50.0	3	9.4	22	20.6	3	37.5	-	-	3	14.2	6	17.7	28	19.9
chicken/dog	-	-	-	-	2	6.3	2	1.9	-	-	-	-	1	4.8	1	2.9	3	2.1
reagent total	55	82.1	6	75.0	31	96.9	92	86.0	5	62.5	1	20.0	20	95.2	26	76.5	118	83.7
no reagent	12	17.9	2	25.0	1	3.1	15	14.0	3	37.5	4	80.0	1	4.8	8	23.5	23	16.3
total	67	100.0	8	100.0	32	100.0	107	100.0	8	100.0	5	100.0	21	100.0	34	100.0	141	100.0
Sources (n)																		
one	30	54.5	2	33.3	11	35.5	43	46.7	1	20.0	1	100.0	12	60.0	14	53.8	57	48.3
two	20	36.4	4	66.7	14	45.2	38	41.3	3	60.0	-	-	6	30.0	9	34.6	47	39.8
three	5	9.1	-	-	6	19.3	11	12.0	1	20.0	-	-	2	10.0	3	11.6	14	11.9
total	55	100.0	6	100.0	31	100.0	92	100.0	5	100.0	1	100.0	20	100.0	26	100.0	118	100.0
Host sources																		
human	11	12.9	2	20.0	22	38.6	35	23.0	1	10.0	-	-	13	48.2	14	36.8	49	25.8
pig	53	62.4	4	40.0	15	26.3	72	47.4	4	40.0	-	-	6	22.2	10	26.4	82	43.2
chicken	21	24.7	4	40.0	14	24.6	39	25.7	5	50.0	1	100.0	8	29.6	14	36.8	53	27.9
dog	-	-	-	-	6	10.5	6	3.9		-	-	-	-	-	-	-	6	3.1
total	85	100.0	10	100.0	57	100.0	152	100.0	10	100.0	1	100.0	27	100.0	38	100.0	190	100.0

CDC: Centers for Disease Control.

For the *Ny. intermedia* and *Ny. neivai* captured by aspiration in the peridomicile, no statistically significant difference was observed ($p = 0.649$), and the blood source frequencies were as follows: chicken (38%), human (33%), pig (13%), bovine (13%) and horse (3%).

DISCUSSION

Knowledge of the sandfly feeding pattern is relevant to understanding the host animals that are involved in the leishmaniasis transmission cycle and can also help to identify the conditions that favor increases in the population density of vectors, particularly in the anthropic environment (e.g., the increased risk of infection among humans and domestic and synanthropic animals).

Nyssomyia intermedia s. lat. has been implicated as a vector of the cutaneous leishmaniasis agent in the southeastern and southern regions of Brazil[15,16]. Now, *Ny. neivai* is again considered to be a distinct species from *Ny. intermedia*[17], and *Ny. intermedia* has been found to be more closely associated

TABLE 2 - Blood meal distribution identified by source and sandfly species according to the collection site at the AG small farm, Serra district, Iporanga municipality, between March 2002 and December 2003.

	Nyssomyia intermedia								*Nyssomyia neivai*									
Sandfly species site	forest + forest edge		porch		pgsty/ henhouse		subtotal		forest + forest edge		porch		pgsty/ henhouse		subtotal		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Source																		
human	3	10.3	9	5.2	-	-	12	2.7	-	-	3	5.7	-	-	3	1.9	15	2.5
bovine	-	-	3	1.7	-	-	3	0.7	-	-	-	-	-	-	-	-	3	0.5
chicken	-	-	2	1.1	14	6.0	16	3.7	-	-	1	1.9	4	4.0	5	3.1	21	3.5
horse	-	-	5	2.9	-	-	5	1.2	-	-	4	7.5	-	-	4	2.5	9	1.5
pig	3	10.3	37	21.3	138	59.5	178	40.9	-	-	14	26.5	56	56.7	70	43.5	248	41.6
human/chicken	1	3.5	3	1.7	-	-	4	0.9	-	-	-	-		-	-	-	4	0.7
human/horse	1	3.5	1	0.6	-	-	2	0.5	-	-	1	1.9	-	-	1	0.6	3	0.5
human/pig	1	3.5	9	5.2	-	-	10	2.3	1	11.1	4	7.5	-	-	5	3.1	15	2.5
human/bovine	-	-	1	0.6	-	-	1	0.2	-	-	-	-	-	-	-	-	1	0.2
chicken/bovine	-	-	2	1.1	-	-	2	0.5	-	-	-	-	-	-	-	-	2	0.3
chicken/pig	3	10.3	13	7.5	43	18.6	59	13.6	1	11.1	2	3.8	34	34.3	37	23.0	96	16.1
horse/pig	1	3.5	7	4.0	-	-	8	1.8	-	-	4	7.5	-	-	4	2.5	12	2.0
human/bovine/pig	-	-	1	0.6	-	-	1	0.2	1	11.1	-	-	-	-	1	0.6	2	0.3
human/chicken/pig	9	31.0	9	5.2	-	-	18	4.1	1	11.1	4	7.5	-	-	5	3.1	23	3.9
human/horse/pig	1	3.5	2	1.1	-	-	3	0.7	1	11.1	-	-	-	-	1	0.6	4	0.7
chicken/dog/pig	-	-	2	1.1	-	-	2	0.5	-	-	-	-	-	-	-	-	2	0.3
chicken/horse/pig	-	-	-	-	-	-	-	-	-	-	1	1.9	-	-	1	0.6	1	0.2
pig/horse/bovine	-	-	-	-	-	-	-	-	-	-	1	1.9	-	-	1	0.6	1	0.2
human/bovine/horse/pig	-	-	-	-	-	-	-	-	-	-	1	1.9	-	-	1	0.6	1	0.2
human/chicken/horse/pig	-	-	2	1.1	-	-	2	0.5	-	-	-	-	-	-	-	-	2	0.3
human/chicken/dog/pig	-	-	1	0.6	-	-	1	0.2	-	-	-	-	-		-	-	1	0.2
subtotal	23	79.4	109	62.6	195	84.1	327	75.2	5	55.5	40	75.5	94	95.0	139	86.3	466	78.2
no reactive	6	20.6	65	37.4	37	15.9	108	24.8	4	44.5	13	24.5	5	5.0	22	13.7	130	21.8
total	29	100.0	174	100.0	232	100.0	435	100.0	9	100.0	53	100.0	99	100.0	161	100.0	596	100.0
Reagent																		
human	16	32.0	38	20.9	-	-	54	11.5	4	31.0	13	19.7	-	-	17	8.2	71	10.5
pig	18	36.0	83	45.6	181	76.0	282	60.0	5	38.0	31	47.0	90	70.3	126	60.9	408	60.3
chicken	13	26.0	34	18.7	57	24.0	104	22.1	2	15.0	8	12.1	38	29.7	48	23.2	152	22.5
horse	3	6.0	17	9.3	-	-	20	4.3	1	8.0	12	18.2	-	-	13	6.3	33	4.9
cattle	-	-	7	3.8	-	-	7	1.5	1	8.0	2	3.0	-	-	3	1.4	10	1.4
dog	-	-	3	1.7	-	-	3	0.6	-	-	-	-	-	-	-	-	3	0.4
total	50	100.0	182	100.0	238	100.0	470	100.0	13	100.0	66	100.0	128	100.0	207	100.0	677	100.0

TABLE 3 - Blood meal distribution identified by source and sandfly species according to the collection site at the AG small farm, Serra district in Iporanga municipality (captured with aspirators in resting places) between August and December 2003.

Species blood site	*Nyssomyia intermedia*						*Nyssomyia neivai*						Total	
	peridomicile		rock walls on forest edge		total		peridomicile		rockwalls on forest edge		Total			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Source														
human	14	14.3	-	-	14	9.7	6	17.6	-	-	6	10.3	20	9.9
chicken	16	16.4	-	-	16	11.0	6	17.6	-	-	6	10.3	22	10.8
cattle	13	13.3	-	-	13	9.0	2	6.0	-	-	2	3.5	15	73
pig	8	8.2	9	19.1	17	11.7	2	5.0	7	29.1	9	15.5	26	12.8
horse	1	1.0	-	-	1	0.7	1	2.9	-	-	1	1.7	2	1.0
human/chicken	13	13.3	-	-	13	9.0	6	17.6	-	-	6	10.3	19	9.4
human/bovine	6	6.1	-	-	6	4.1	1	2.9	-	-	1	1.7	7	3.4
human/pig	2	2.0	-	-	2	1.4	1	2.9	1	4.2	2	3.5	4	2.0
human/horse	1	1.0	-	-	1	0.7	1	2.9	-	-	1	1.7	2	1.0
human/chicken/ bovine	2	2.0	-	-	2	1.4	2	6.0	-	-	2	3.5	4	2.0
human/chicken/pig	6	6.1	2	4.3	8	5.5	3	8.8	-	-	3	5.2	11	5.4
chicken/pig	15	15.3	36	76.6	51	35.1	3	8.8	16	66.7	19	32.8	70	34.5
chicken/horse	1	1.0	-	-	1	0.7	-	-	-	-	-	-	1	0.5
subtotal	98	100.0	47	100.0	145	100.0	34	100.0	24	100.0	58	100.0	203	100.0
no reacting	26	21.0	10	17.5	36	20.0	10	22.7	2	7.7	12	17.1	48	19.1
total	124	100.0	57	100.0	181	100.0	44	100.0	26	100.0	70	100.0	251	100.0
Sources (n)														
single source	52	53.1	9	19.1	61	42.1	17	50.0	7	29.2	24	45.3	85	41.9
two sources	38	38.8	36	76.6	74	51.0	12	35.3	17	70.8	29	54.7	103	50.7
three sources	8	8.1	2	4.3	10	6.9	5	14.7	-	-	-	-	15	7.4
total	98	100.0	47	100.0	145	100.0	34	100.0	24	100.0	53	100.0	203	100.0
Host sources														
human	44	32.1	2	2.3	46	20.6	20	35.7	1	2.4	21	21.6	67	20.9
pig	16	11.7	47	54	63	28.1	9	16.1	24	58.5	33	34	96	29.9
chicken	53	38.7	38	43.7	91	40.6	20	35.7	16	39.1	36	37.1	127	39.6
horse	3	2.2	-	-	3	1.3	2	3.6	-	-	2	2.1	5	1.6
bovine	21	15.3	-	-	21	9.4	5	8.9	-	-	5	5.2	26	8.1
total	137	100.0	87	100.0	224	100.0	56	100.0	41	100.0	97	100.0	321	100.0

with the areas of Atlantic forest located in the coastal States of Espírito Santo, Rio de Janeiro and São Paulo, whereas *Ny. neivai* is more common in the whole Southern region and westward from the Atlantic plateau in the State of São Paulo. However, these species are sympatric in the Ribeira Valley region of São Paulo[13,18,19] where the present study was performed[4].

Although studies that focus on the feeding habits of *Ny. intermedia*[20] and *Ny. neivai*[21,22] have been performed, the present research analyzed their feeding pattern in a sympatric area.

In the peridomicile of the JRA smallholding, *Ny. neivai* was more attracted to humans and chickens, corroborating the observations of Casanova[21]. The *Ny. intermedia* and *Ny. neivai* found on the porch of the smallholding presented a distinct pattern of blood source preference compared with those found on the AG small farm. On the JRA smallholding, both species took more human blood, which could be attributed to the greater number of people who permanently reside in the dwelling and to the greater diversity in blood meal combinations, which may be the result of the smallholding's proximity to the residual forest, hen house and pigsty, thereby providing the opportunity for and ease of contact with humans.

On the AG small farm, the majority of the blood meals identified in the peridomicile and on the porch comprised pig blood. This observation might be attributed to the large number of pigs that were present on this site during the captures between March 2002 and December 2003. The confinement of these animals and their large areas of exposed skin favor the concentration of male and female sand flies for courtship and provide blood sources for the females, as observed with regard to henhouses for *Lutzomyia longipalpis*[23].

The aspirations that were performed on the AG small farm in resting places in the peridomicile and on the rock walls at the forest edge did not reveal any exclusive blood meal preference. The variety of food sources suggests the same eclectic feeding behavior that was observed in the peridomiciliary environment and its association with domestic animal hosts[24].

On the AG small farm, the blood meal analysis also showed a mixture of ingested blood, as demonstrated by the proportion of females that obtained blood from more than one source.

The blood meal analyses demonstrated that *Ny. intermedia* and *Ny. neivai* tended to feed on multiple hosts. The variety of food sources that were identified corroborated their eclectic feeding habits and their opportunistic behavior. However, this eclectic feeding pattern was restricted to domestic animals for the specimens collected in the peridomiciles, on porches, and on the slopes at the forest's edge. This behavior may be associated with the fact that these two species breed in peridomiciliary sites, as observed for *Ny. neivai*[25] (at the time identified as *Lu. intermedia s. lat*.) and *Ny. intermedia*[26].

Some large mammals, such as swine, do not appear to be *Leishmania* reservoirs because there has been only one published report of *Leishmania* amastigotes in a lesion on the ear of a pig living in the peridomicile of a dwelling of a human case of cutaneous leishmaniasis[27] and one Brazilian report of infection caused by *Leishmania infantum*. Although these pigs produced *Leishmania* antibodies, neither the presence of the parasite nor its DNA was detected[28]. Similarly, no such role can be attributed to cattle with regard to *Leishmania donovani*, the agent of visceral leishmaniasis in Bangladesh[29].

However, domestic dogs are frequently reported to be naturally infected with *Leishmania braziliensis*, the agent of cutaneous leishmaniasis with the widest distribution in South America[30-35,36]. Often this scenario occurs in old colonization areas that also have a high prevalence of human infection by this parasite, sometimes with significant association between the occurrence of human cases and the presence of infected dogs in the domicile. Under these circumstances, the dog has been incriminated as the infection source or as the reservoir of the parasite[30-35]. In the same manner, equines, which may have high natural infection rates in some areas, may be an infection source for humans and other animals[30,33]. However, the role of these animals as reservoirs of this parasite has not been well established[37,38].

In the present study, the low rate of dog blood reactivity may not reflect a lack of attraction between the two sandfly species investigated and dogs but instead may reflect the scarcity of this blood source; only one dog was present in one of the households (JRA smallholding), and evidently, a dog entered areas of the AG small farm.

Despite the greater number of females examined, no rat blood was detected; therefore, it seems that the populations of *Ny. intermedia* and *Ny. neivai* in this study area are rarely attracted to rodents, which contrasts with the findings of Afonso[39] for *Ny. intermedia* in the State of Rio de Janeiro.

A portion of the 19.1% of specimens that were non-reactive can be attributed to other animal sources that were not investigated. However, non-reactivity may also have been observed because many females with dark abdomens and increased volumes were actually full of eggs rather than blood. It is also possible that the amount of blood or its state of degradation prevented the identification of its source.

The great availability of avian hosts, which are refractory to the development of this parasite, and some larger mammalian hosts, which are less effective at transmitting the agent of cutaneous leishmaniasis than rodents[38], may dilute the transmission of this parasite in these areas. This scenario may explain the absence of human cases of cutaneous leishmaniasis at these two sites, despite the high density of these vectors observed by Galati[4,10,12].

In conclusion, although the risk of humans contracting cutaneous leishmaniasis in the described scenario is small, the opportunistic feeding behavior of *Ny. intermedia* and *Ny. neivai* suggests that actions should be taken at the local level to mitigate the vulnerability of human populations exposed to these vectors of cutaneous leishmaniasis agents, particularly in the presence of synanthropic mammals that may be reservoirs of this parasite. In any case, the presence of these sandflies in high densities is a source of annoyance to both residents and domestic animals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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