

Leishmania in an Ecotourism Area in Rio de Janeiro: Still a Concern for Public Health?

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Abstract

We evaluated some eco-epidemiological characteristics of the sand fly fauna in an ecotourism area in the Atlantic Forest located in Rio de Janeiro, south-eastern Brazil. During a period of one year, sandflies were collected in three different locations, where the sampled residences were located, respectively, one inside the forest, the other two, respectively at the edge of the forest and the other in a more urbanized area. These three types of ecotopes were evaluated: home, peridomicile and kennel. Four hundred and fifty-six sandflies were collected and six species belonging to five genera were identified: *Migonemyia migonei*, *Lutzomyia longipalpis*, *Nyssomyia intermedia*, *Evandromyia sallesi*, *Evandromyia edwardsi* and *Brumptomyia wedge*. The two most abundant species collected were *M. migonei* and *L. longipalpis*, contributing 70% and 18% respectively, totaling 88% of the individuals collected. The results suggested that modifications of the natural environment due to anthropic action probably resulted in changes in the composition of the sand fly population. At point (3), where spraying occurred irregularly, even representing a degraded environment, only one species was captured, *M. migonei*. Differently at points (1) and (2), areas located respectively in the interior and on the edge of the Atlantic Forest, a greater number of sand fly species was observed. However, after a few years, anthropic actions ceased, followed by the implementation of reforestation projects and currently the landscape is very different, showing considerable forest recovery. For this reason, ecotourism activities are increasing in the area, creating potentially dangerous conditions caused by the exposure of greater numbers of people and dogs to insect vectors. Therefore, the implementation of environmental education projects is essential. However, we suggest that the use of warning signs to be placed at the entrances to the main traffic routes, alerting tourists to the risk of infection and indicating protective measures, would be very useful.

Keywords

Eco-Epidemiological, Leishmaniasis, *Leishmania infantum*, *Lutzomyia* sp, Sandflies

1. Introduction

Leishmaniasis is a neglected tropical diseases (NCDs), being considered an important public health problem. It is present in 98 countries and territories, with more than 350 million people at risk [1]. About 1.3 million new cases are estimated each year, with more than 90% of visceral leishmaniasis cases divided into six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan, and 1 million for the cutaneous form, mainly in Afghanistan, Algeria, Brazil, Colombia, Iran, Pakistan, Peru, Saudi Arabia, Syria and Tunisia and mucocutaneous especially in Brazil, Peru and Bolivia [1].

In Brazil, specifically in the State of Rio de Janeiro, visceral leishmaniasis occurs in Barra de Guaratiba, Grumari and Ilha de Guaratiba [2]-[4]. The first autochthonous human case was recorded in 1977, in Serra do Barata, Realengo. Subsequently, other human and canine cases of the disease occurred in new locations in the West Zone, such as Bangu, Campo Grande and Jacarepaguá [5].

Sandflies are the vector insects of *Leishmania*, belonging to the Psychodidae family, order Diptera and suborder Nematocera, being divided into six subfamilies, Bruchomyiinae, Trichomyiinae, Horaiellinae, Psychodinae, Sycoracinae and Phlebotominae [6].

The subfamily Phlebotominae is divided into five genera [7] of which two occur in the Old World and three take place in the New World.

In the Americas, Brazil is the country with the highest annual incidences of both American Visceral Leishmaniasis (AVL) and American Tegumentary Leishmaniasis (ATL) [8]. They are prevalent in Brazilian territory, where their notification is mandatory [9].

The expansion of these diseases in the country shows a change in their epidemiological profiles, moving from rural to peri-urban areas, with human cases recorded even in capitals such as Campo Grande [10], Belo Horizonte [11] and Rio de Janeiro [4].

The principal vector of the *American Tegumentary Leishmaniasis* in Rio de Janeiro State is *L. (N.) intermedia*, with *L. migonei* being considered a secondary vector [12]-[14]. Other potential vector species have been registered in endemic areas of the state, such as *L. (N.) whitmani*, *L. (N.) flaviscutellata*, *L. (Pintomyia) fischeri* and *L. (P.) pessoai* [12] [13] [15]-[17].

In Brazil, the main vector of *Leishmania infantum* is *L. longipalpis*, which has already been found in several studies in the State of Rio de Janeiro [4] [12] [18]. This species is highly adapted to environments degraded by human action and has an eclectic diet, from humans to domestic animals [19] [20].

Given the recognized importance of studying leishmaniasis due to its re-emerging nature, in addition to still being an important public health problem, it is essential to identify and map the main vector species of the parasites, as well as the characteristics and potential changes in their habitats.

Thus, the main objective of the present study was to determine the occurrence, distribution and density of the main species of the sandfly fauna in Barra de Guaratiba according to the different ecotopes (domicile, peridomicile and kennel).

The use of geographic information systems (GIS) and remote sensing (RS) techniques was used to identify the occurrence of areas with higher and lower densities of sandflies, as well as analyze changes in forest cover, which potentially influenced epidemiological conditions.

2. Material and Methods

2.1. Study Area

Barra de Guaratiba is a coastal neighborhood in the West Zone of the city of Rio de Janeiro, Brazil, which comprises the slopes of the Pedra Branca Massif.

It is located between the coordinates “43°32'44” and “43°33'55” east longitude and 23°00'44” and 23°04'35” south latitude, with a territorial area of 13,950.12 ha and a forested area of 0.27% of the total area in 2001 [21].

The climate is humid tropical, with annual precipitation around 1400 mm, average relative humidity of 75% and average annual temperature of 27.5°C.

The place has an area of Atlantic Forest that has suffered for some years with actions of environmental degradation, due to disorderly urban occupation, but after the implementation of reforestation projects, the area currently shows a significant recovery of the Atlantic Forest.

On the coast, the region has a part of mangrove that mixes with a restinga environment [22].

The local population usually reports the presence of marsupials, rodents, armadillos and small primates in the forest area and close to homes. The neighborhoods of Vargem Grande, Recreio dos Bandeirantes and Grumari border the neighborhood of Guaratiba. Barra de Guaratiba is considered an area of the city of Rio de Janeiro where LAV is endemic. The presence of *Lutzomyia longipalpis* in the peridomestic environment indicates the possibility of the emergence of new human cases in this area [23].

2.2. Sand Flies Captures

CDC traps were placed in areas that we observed in a previous study as hotspots for leishmania transmission, where several cases of canine visceral leishmaniasis were detected [22].

The collections of sandflies were carried out in three different places where the residences were located, one within a remaining strip of forest and the other two on the periphery of it. They are located respectively on the following waypoints.

House1 - 23° 3'44.70"S and 43° 33'41.28"O

House 2 - 23° 3'55.44"S and 43° 33'35.67"O

House 3 - 23° 4'2.72"S and 43° 33'55.10"O

House (1): The kennel was in good condition, covered by a roof and with a cement floor. It was located very close to the house, about two meters. House (1) was close to the Forest, but not inserted in it, surrounded by other houses built very close. In this area the spraying was irregular.

Houses (2 and 3): the kennel of the two houses was approximately 10 m away from them, they were very precarious, with a lot of organic matter and banana leaves, dark and damp. In House (2) a case of human visceral leishmaniasis had already been reported. The two houses were very simple and almost located within the forest. There was no spraying in that area.

Phlebotomine sand flies were collected using CDC light traps. The collections were carried out every 15 days on the three points from September 2001 to September 2002.

The traps were installed at 50 - 70 cm above ground level and placed in the residences in three types of ecotopes: domicile, peridomicile and kennel, with six traps per domicile, totaling 18 traps. They were exposed between 5:00 pm on the first day and removed between 7:00 am on the following day. After collection, the specimens were stored in 70% alcohol for later assembly and identification, according to Gallati, 2003 [24].

Data were organized in spreadsheets and descriptive analyzes were recorded in Microsoft Excel and Spearman's correlation test was applied to the parameters studied.

Remote Sensing (RS) and Geographic Information System (GIS) techniques were applied and the area was mapped using Geko 301 GPS to obtain latitude, longitude and altitude. Data on climate, vegetation, and geology, among others, were obtained from the City Hall of Rio de Janeiro and INPE. A buffer was created represented by 200 m radius circles based on the average flight distance of sand flies [25]. ArcGIS software was used for data analysis.

3. Results

In the present study, 456 sandflies belonging to the genera *Lutzomyia* (France, 1924) and *Brumptomyia* (France & Parrot, 1921) were collected and analyzed, distributed in 6 species: *Migonemyia migonei* 318/456, *Lutzomyia longipalpis* 84/456, *Nyssomyia intermedia* 6/456, *Evandromyia cortelezii sallesi* 2/456, *Evandromyia (Barrettomyia) edwardsi* 5/ 456 and *Brumptomyia cunhai* 41/456. The most abundant sand fly species were *M. migonei* and *L. longipalpis*, respectively 70% and 18.4%, representing 88.4% of all specimens collected (Table 1).

Among the specimens collected, 76.4% were males and 23.6% were females (Table 2), with a male/female sex ratio of 3.2:1.0, *Migonemyia migonei* was the species that presented the greatest variation, among the number of males (54%) and females. (16%), with a male/female sex ratio of 3.4:1.0.

Table 1. Species of sandflies captured in Barra de Guaratiba, RJ, Brazil, using CDC light traps, from September 2001 to September 2002.

Sandflies species	Total	%
<i>Migonemyia migonei</i>	318	70
<i>Lutzomyia longipalpis</i>	84	18.4
<i>Brumptomyia cunhai</i>	41	8.9
<i>Nyssomyia intermedia</i>	6	1.3
<i>Evandromyia edwardsi</i>	5	1.0
<i>Evandromyia sallesi</i>	2	0.4
Total	456	100

Table 2. Species collected and proportion between males and females.

Species collected	Males	Females	Total
<i>Brumptomyia cunhai</i>	41	0	41
<i>Evandromyia edwardsi</i>	2	3	5
<i>Lutzomyia longipalpis</i>	56	28	84
<i>Evandromyia sallesi</i>	0	2	2
<i>Migonemyia migonei</i>	246	72	318
<i>Nyssomyia intermedia</i>	3	3	6
Total	348	108	456

During the study period, the vector density was higher in the rainy season between December and April, corresponding to 65% of the collected specimens. The month of February presented the highest percentage of captured specimens, 39% (Table 3).

Table 3. Seasonal variation of sand fly species collected in Barra de Guaratiba, RJ, from September 2001 to September 2002.

Species	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Total
<i>L. longipalpis</i>	3	12	17	0	0	21	7	6	18	0	0	0	0	84
<i>M. migonei</i>	19	23	44	0	0	151	70	8	3	0	0	0	0	318
<i>N. intermedia</i>	1	0	0	0	0	4	1	0	0	0	0	0	0	6
<i>E. sallesi</i>	0	0	0	0	0	2	0	0	0	0	0	0	0	2
<i>E. edwardsi</i>	0	0	1	0	0	0	4	0	0	0	0	0	0	5
<i>B. cunhai</i>	5	34	1	0	0	0	1	0	0	0	0	0	0	41
Total	28	69	63	0	0	178	83	14	21	0	0	0	0	456

At points (2) and (3), a greater number of specimens were collected (mainly of *M. migonei* and *L. longipalpis*) besides a greater variety of species, 88% (402/456). Of the sandflies collected at these points, 18.7% (84/447) were composed of *L. longipalpis* (Table 4).

Table 4. List of species collected according to capture locations.

Species collected	House 1	House 2	House 3	Total
<i>Migonemyia migonei</i>	9	227	82	318
<i>Lutzomyia longipalpis</i>	0	72	12	84
<i>Nyssomyia intermedia</i>	0	4	2	6
<i>Evandromyia sallesi</i>	0	2	0	2
<i>Evandromyia edwardsi</i>	0	4	1	5
<i>Brumptomyia cunhai</i>	0	41	0	41
Total	9	350	97	456

Regarding the three ecotopes, domicile, peridomicile and kennel (in the three residences) it was possible to observe that of the six species collected during the study, five of them were found in the kennel, making up 74.3% of the total specimens collected (Table 5). The most abundant species in the three ecotopes were *M. migonei* (70%), followed by *L. longipalpis* (18.4%) (Table 5). At point 1, only the species *M. migonei* was found in the kennel and domicile (Table 4).

Table 5. List of species collected according to the three different ecotopes: domicile, peridomicile and kennel.

Species collected	Domicile	Peridomicile	Kennel	Total
<i>Migonemyia migonei</i>	28	66	224	318
<i>Lutzomyia longipalpis</i>	4	7	73	84
<i>Nyssomyia intermedia</i>	0	2	4	6
<i>Evandromyia sallesi</i>	0	0	2	2
<i>Evandromyia edwardsi</i>	3	2	0	5
<i>Brumptomyia cunhai</i>	3	2	36	41
Total	38	79	339	456

4. Discussion

The percentage of males captured in four of the six species collected was higher than females. It probably occurs because females, in addition to lights, are also probably more attracted to host stimuli [26].

Taking into account *L. longipalpis* is considered the most important vector species of *Leishmania infantum* in New World. The higher proportion of males captured probably occurred also due to some factors such as, the use of light traps to collect sandflies; traps close to breeding sites, which favors the majority of males that are born before females; sexual behavior of males that form mating aggregates [27].

These results corroborate with other studies performed in other regions of Brazil [28]-[30].

Regarding seasonality, a greater number of individuals were captured during the rainy periods. According to Forattini, 1973 [6], environments that are more humid favor the appearance of adult forms [27]. Similar results were found in other studies carried out in Mato Grosso do Sul, Maranhão and Ceará [27] [29]-[32].

The population increase of sandflies observed during and after the rainy season is an extremely important aspect and it must be considered for the control of the adult forms of the vector.

In fact, the Visceral Leishmaniasis Surveillance and Control Manual, guides the accomplishment of two annual cycles of insecticide application with an interval of three to four months, being able to carry out the first cycle at the beginning of the rainy season, preferably at the month of September and the second in January of the following year [9].

Data related to sandflies in the three collection points suggest that changes in the natural environment due to human action resulted in alteration in the composition of the sandfly population.

At point (1), where the environment suffered the greatest anthropic effect and spraying occurred, although with irregular frequency, only one species was captured, *M. migonei*. This suggests that, like *L. longipalpis*, under certain conditions this species may also develop an ability to survive anthropic environments.

In contrast, in the other two locations, one within the forest remnant and the other closer to the forest, species diversity was greater.

Making a temporal analysis of the eco-epidemiological aspects related to the vectors of *Leishmania* in Barra de Guaratiba.

Since the first reports, around 1995, when it was defined as an endemic area for American visceral leishmaniasis, at which point the Brazilian Agency for Infectious Disease Control (FNS), an entity of the Ministry of Health, carried out serological research on canines in this location and the seropositive dogs were eliminated.

In addition, spraying with pyrethroid insecticide was carried out biannually in homes and yards, but even so canine seroprevalence remained at 25% and eight autochthonous human cases were reported during 1995-1996.

Souza *et al.* 2003 [33], in work carried out from March 1994 to February 1995 in the Barra de Guaratiba neighborhood. They observed that *L. longipalpis* was the most frequent species, representing 76.2% of the total, followed respectively

by *M. migonei* with 12, 3%, *N. intermedia* with 7.4%, *Micropygomyia schreiberi* with 2.7% and *L. sallesi*, *L. edwardsi* in addition to *Lutzomyia (Pintomyia) fischeri* with less than one percent [33].

In a subsequent survey, also lasting one year (1997-1998) and in Barra de Guaratiba, Cabrera *et al.* 2003 [23], observed a lower relative percentage in relation to the other species of the most important vector species of *L. infantum*. Here *L. longipalpis* was only the fifth most common species, representing just 6.4% of the total number of sandflies captured.

In the same study, *N. intermedia* and *M. migonei* were the most frequent, respectively with 39.7% and 30.3%. *Micropygomyia schreiberi* and *Brumptomyia guimaraensi* also preceded *L. longipalpis* with 9.4% and 6.9%. The lowest percentages were *Brumptomyia guimaraensi* 4.5%, *L. quinquefer* 2.2% and *L. (Nyssomyia) whitmani* 0.5% [23].

In relation to our observations on the diversity of sand fly species in Barra de Guaratiba from September 2001 to September 2002.

Taking into account the presence of *Leishmania* vector species, is important to highlight that *L. longipalpis* was the second most abundant species captured with 18.4%. In fact, *M. migonei* was the most abundant with 70%, in addition to other species that presented lower frequency percentages than *L. longipalpis*, such as *Brumptomyia cunhai* with 8.9%, *Nyssomyia intermedia* with 1.3%, *Evandromyia edwardsi* 1.0% and finally *E. sallesi* with 0.4%.

The high percentage of *L. longipalpis* in the area, especially with a high incidence in kennels, showed that the parasite was circulating, at least among dogs, which was confirmed by a high percentage of dogs infected with *L. infantum* in the same locations [22]. In fact, it represented a high risk for the population, as it is known that infection in the canine population precedes human cases of the disease.

Considering the presence of *N. intermedia*, although in a low percentage (1.3%), despite the study site representing an endemic area for visceral leishmaniasis. The potential risk of the emergence of a focus on cutaneous leishmaniasis reinforced the importance of epidemiological surveillance actions in the region, considering that this is one of the most important vector species of cutaneous leishmaniasis.

Souza [33] during 1994-1995, observed a slightly higher percentage of 7.4%. However, later Cabrera [23] during 1997-1998 worryingly observed that this species reached the highest percentage among the others with 39.7%.

Another concerning aspect observed in our study was the presence of *M. migonei* as the most frequent species throughout the year at 70%. In the two previous studies in Barra de Guaratiba, this species came in second place, maintaining high percentages of occurrence, respectively in Souza [33] with 12.3% and Cabrera [23] with 30.3%.

This species represented a high risk of both maintaining the transmission of *L. infantum* and the appearance of infections by *L. braziliensis*. Thus, its presence further increased the risk of cases of visceral but also cutaneous leishmaniasis,

respectively, due to the fact that *M. migonei* has already been described as naturally infected by both species of *Leishmania* [34]-[36].

In relation to the three species that presented only one percent in our study, it is important to highlight that *Evandromyia sallesi* has already been described as naturally infected by *L. infantum*. Thus, it could also be acting as an alternative vector for the causative agent of visceral leishmaniasis [37].

Among the areas selected for the collection, the presence of six species of sand flies was observed: *M. migonei*, *L. longipalpis*, *N.intermedia*, *E. cortelezzii sallesi*, *E. edwardsi* and *B. cunhai*. With the superposition of the mean flight distance of the sandflies and the waypoints of the animals seropositive for *L. infantum*, it was demonstrated that only one seropositive animal was outside the potential area of influence of the vector flight (Silva 2011).

Although previous studies in Barra de Guaratiba were not carried out in exactly the same locations, they suggest that the phlebotomine population appears to have fluctuated during this eight-year period. *L. longipalpis*. For example, initially, there was a high percentage of 76.2% from 1994 to 1995, then it fell to 6.4% in 1997-1998 and finally rose again to 18.4% in 2001-2002.

Another possibility is that the phlebotomine populations were showing a clustered distribution pattern, as has already been observed in other locations [38].

Making a historical analysis of the ecoepidemiological situation in Barra de Guaratiba, since 1995, when the first human cases of visceral leishmaniasis were diagnosed, until now, in our opinion it is very important to observe local environmental changes, to try to predict potential risks for people and animals.

Thus, initially, when the first studies were carried out in 1994-1995, the habitat was very degraded due to human action on the forest and this effect lasted until around 2008-2009, when the first urban studies were carried out (Figure 1).

Until then, although few people were already hiking and camping in the area, putting themselves at risk of infection, it was the local population who presented the first cases of visceral leishmaniasis, resulting in long periods of treatment for infected people, but also in cases of death from the disease.

However, today after the gradual but significant recovery of the forest, signs of rudimentary agriculture and even some trails that were initially clearly visible in satellite images, most of them cannot be seen now (Figure 2).

The importance of forest recovery is indisputable, especially for the maintenance of native species, but it is important to highlight that both wild mammals that host *Leishmania* and the insect vectors are part of forest life. The sylvatic cycle of the parasite already occurred long before the arrival of man.

Therefore, it is very likely that with the recovery of the forest the risk of disease transmission is increasing due to the increase in people walking and camping but also due to the recolonization of insect vector species.

Cabrera [23] observed that dogs that lived near the forest represented a 3.49 times greater risk of infection by *L. infantum* when compared to those who live 100 meters or more away from the forest.

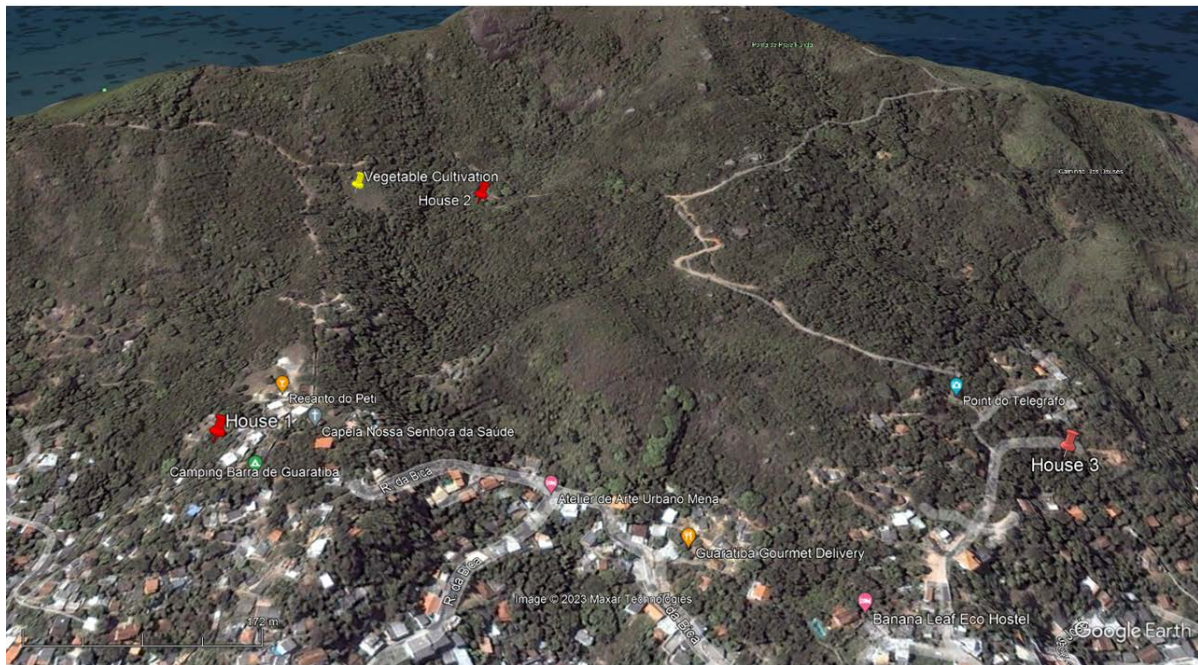


Figure 1. Satellite photograph of the study area in April 2002 showing the approximate location of the 3 houses studied. It is possible to observe that the vegetation was predominantly composed of shrubby trees and the larger trees were scarce and mostly grouped in a few points. Note that most trails are visible. On the right side of house 2 it is still possible to see a square area (yellow mark) where there was some type of vegetable cultivation. At that time there were already ecotourism activities, note the occurrence of a Camping Club close to house 1 (Image Source: Google Earth).

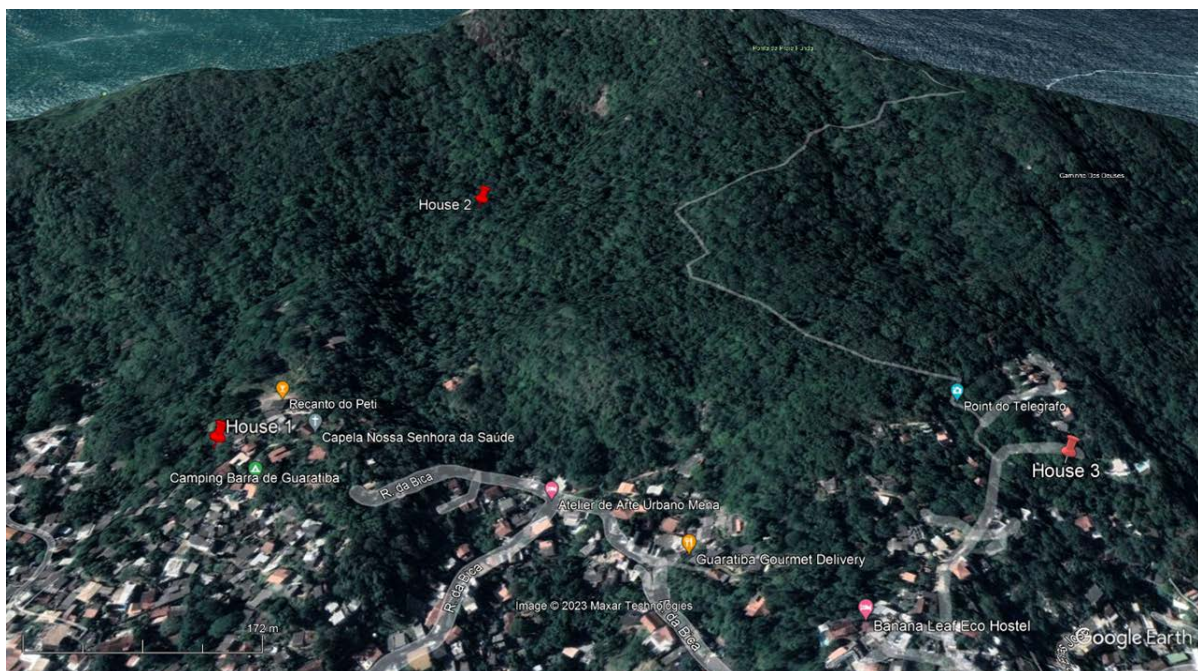


Figure 2. Satellite photograph of the study area in July 2022 showing the approximate location of the 3 studied houses. It is possible to observe that now the vegetation is predominantly made up of larger trees. Note that most of the trails are no longer visible and on the right side of house 2 the vegetable crop no longer exists. With the recovery of the Forest, there was a considerable increase in ecotourism activities. Note that the Camping Club near house 1 still exists (Image Source: Google Earth).

Corroborating this information, in our study it was observed that closer to the forest the diversity of sand fly species was greater than in adjacent degraded areas.

Therefore, it is fundamental to maintain vector control measures such as insecticide spraying, at least at the edge of the forest, to avoid the risk of vectors spreading to populated areas.

Besides, other activities, such as the use of nets impregnated with insecticide, as well as epidemiological surveillance through serological inventories in populations of potential domestic animals hosts, such as dogs and cats.

However, it is very important to highlight, that in these particular conditions, considering areas where ecotourism activities are being developed. It is fundamental to put into practice environmental education projects that aim to inform the population about the transmission of the disease and the risk of contagion, showing effective measures to mitigate vector exposure, and warning people about it, including use repellents and deltamethrin collars on dogs.

We suggest the use of a sign clearly indicating the risk of *Leishmania* infection through sand fly bites, as has already been used in Brazil for Rocky Mountain spotted fever.

Author's Contributions

JCAC—conceived the study, designed the study protocol, analysis and interpretation of these data, drafted the manuscript, and critically revised the manuscript for intellectual content.

RPB—critically revised the manuscript for intellectual content.

AVMS—designed the study protocol, analysis and interpretation of these data.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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