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Experimental transmission of *Leishmania* (*Leishmania*) *amazonensis* to immunosuppressed mice through the bite of *Lutzomyia longipalpis* (Diptera: Psychodidae) results in cutaneous leishmaniasis

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ABSTRACT

Lutzomyia longipalpis is the natural vector of *Leishmania (Leishmania) infantum*, but it is also permissive for several *Leishmania* species that are related to cutaneous leishmaniasis (CL). Maranhao State (Northeast of Brazil) is endemic for CL and has the highest number of cases of diffuse cutaneous leishmaniasis (DCL) in the country. It is a rare disease associated with a defective immune response mainly caused by *L. (L.) amazonensis*. Additionally, the number of immunosuppressed patients infected with the etiologic agents of CL has increased, including regions in which the main vectors of CL are rare. Therefore, we investigated whether *Lu. longipalpis* is able to transmit *L. (L.) amazonensis* to uninfected and immunosuppressed mice, resulting in CL. For that, 291 sand flies took an initial blood meal in mice infected with *L. (L.) amazonensis.* Of these, 17 underwent a second feeding on uninfected and immunosuppressed mice (of which 58.8% were also positive for *Leishmania* according to data on the dissection of the intestine). After 27 days of infection, these mice exhibited leishmaniotic lesions. The occurrence of parasites on the animal's skin was confirmed by limiting dilution and immunohistopathological analyses. Parasite DNA was also detected in paw lesions and inguinal lymph nodes. DNA sequencing confirmed the *Leishmania* species in insects and mice. The results confirmed the ability of *Lu. longipalpis* to become infected and experimentally transmit *L. (L.) amazonensis* to immunosuppressed rodents, resulting in leishmaniotic lesions. Our data open perspectives for the potential role of *Lu. longipalpis* in the epidemiology of urban cutaneous leishmaniasis, especially in immunosuppressed patients.

KEYWORDS: Cutaneous leishmaniasis. Experimental infection. Host-parasite interaction. Permissive vector. Vector l competence.

INTRODUCTION

Lutzomyia longipalpis (Diptera, Psychodidae) is the most relevant vector of *Leishmania (Leishmania) infantum* (Kinetoplastida, Trypanosomatidae), the etiological agent of visceral leishmaniasis (VL) in the New World[1]. Nevertheless, natural infection[2,3] and experimental infection studies[4,5] have highlighted the ability of *Lu. longipalpis* to sustain the development of other species causing cutaneous leishmaniasis (CL).

In the American continent, CL is caused by several species of *Leishmania*, among which *L. (Viannia) braziliensis* and *L. (L.) amazonensis* are the most prevalent in Brazil[6]. In the Northeast of the country, Maranhao State is endemic

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to CL and has the highest number of diffuse cutaneous leishmaniasis (DCL), a rare disease associated with a defective immune response mainly caused by *L. (L.) amazonensis*, whose vector is *Bichromomyia flaviscutellata*[7]. Moreover, other studies have described the high prevalence of *Leishmania* spp. infections in immunosuppressed people[8,9], including HIV/CL[10] and HIV/DCL[11] coinfections in Maranhao State.

Studies have described the spread of CL in urban environments[12,13] in which *Lu. longipalpis* feeds on several domestic and synanthropic animals[2,14] , showing a higher adaptive capacity to seasonal variations than other sand flies species[15]. Additionally, studies have reported the circulation of several species of *Leishmania,* including *L. (L.) amazonensis,* concomitantly with *Lu. longipalpis* in Brazil[2,3]. These facts have generated the hypothesis that *Lu. longipalpis* may be infected and be able to transmit *L. (L.) amazonensis*, mainly in areas where its natural vectors are rare[12-17].

Despite this permissiveness, there are no studies regarding the ability of *Lu. longipalpis* to experimentally transmit *L. (L.) amazonensis* to mice, in the same way it was investigated for *L. (V.) braziliensis*[18], *L. (L.) mexicana*[19] and *L. (L.) major*[20]. Therefore, taking into account the occurrence of CL in immunocompromised patients, our study aimed to test the hypothesis that *Lu. longipalpis* has the capacity to transmit *L. (L.) amazonensis* to immunosuppressed rodents, resulting in cutaneous lesions.

MATERIALS AND METHODS

Sand flies, parasite and mice

Specimens of *Lu. longipalpis* were reared in the laboratory, free from *Leishmania* infection, as previously described[21]. Twelve hours before the experiments, the feeding of female sand flies was suspended to increase the probability of the insects feeding on the mice infected by *L. (L.) amazonensis* (IFLA/BR/1968/PH8 strain). Promastigotes were grown in Schneider medium (Sigma, St Louis, MO, USA), supplemented with 1% antibiotic (Penicillin 100 µL/mL and streptomycin 50 µL/mL) and 20% inactivated bovine fetal serum (GIBCO), and kept in an incubator at 27 °C.

Balb/c mice (*Mus musculus,* 25 g) were obtained from the Vivarium of the University of Campinas, Sao Paulo, Brazil, under pathogen-free conditions. The animals were kept in polycarbonate boxes under controlled conditions: temperature 21 ± 2 °C and an alternating light-dark cycle of 12 h. This study was approved by the Ethics Committee on Animal Experimentation/UFMA (23115.006003/2017-11).

Experimental infection of mice for the 1st blood meal

Eight male Balb/c mice, aged 15 weeks, were experimentally infected in the right hind paw by inoculation with 1 x 10^7 promastigotes of *L. (L.) amazonensis* in the stationary phase, as previously described[22]. On the 41st day after infection, mice were anesthetized (300 mg/kg of ketamine and 60 mg/kg of xylazine) to feed the female sand flies (three to four days of age) emerging from the insect colony. The animals were placed in nylon and glass-lined cages, with metal frames (30 × 30 × 30 cm), placed in a biochemical oxygen demand (BOD) oven, in the dark, at a temperature of 27 ºC and a relative humidity of 80%.

Immunosuppression of mice for the 2nd blood meal

Four uninfected male Balb/c mice, aged 35 weeks and weighing 28 g, had their susceptibility to infection increased by the administration of a single dose of cyclophosphamide (50 mg/kg/mouse) intraperitoneally[23]. After 48 h, the animals were anesthetized and subjected to the exposure to sand fly bites following the same protocol described above.

Experimental design

Firstly, infection experiments (called ExpA) corresponding to the infection of *Lu. longipalpis* by *L. (L.) amazonensis* were carried out by the biting of experimentally infected mice (1st blood meal). The tests were carried out according to the number of available females emerging from the insect colony, totaling 485 females with three to four days of life, from the F1, F2, and F3 generations. Eight infected Balb/c mice were placed inside a cage to serve as a food source for sand flies, so that two animals were exposed to the same group of sand flies, sequentially, for 60 min each. Each group (27 to 65 females/animal) had a total of 120 min available to feed, totaling 12 blood meal attempts (groups I–XII). Supplementary Figure S1A shows the experimental insect infection scheme.

The transmission experiment (called ExpB) refers to the transmission of *L. (L.) amazonensis* to an uninfected mouse through the bite of *Lu. longipalpis* from ExpA during the insects' second blood meal (Supplementary Figure S1B). ExpB was performed 5 to 10 days after ExpA by offering an immunosuppressed and infection-free animal as a food source for insects, for 60 min. Among the insects fed on four animals during 12 attempts, two survived for at least two months after the feeding. The two surviving animals were named Animal A and Animal B.

All engorged females from the first or second feeding were investigated for the presence of the parasite by midgut dissection and *Leishmania* DNA detection. The experimental infection rate was considered as the number of positive females for *Leishmania* in relation to the total number of engorged females. Supplementary Table S1 displays all groups used in each assay and the rates of engorged females, experimental infection rates and animal outcomes after being a food source for sand flies.

Dissection of engorged *Lutzomyia longipalpis*

All females (N = 17) that underwent the second blood meal were dissected to assess the presence or absence of *L. (L.) amazonensis* promastigotes in the intestine. From 6-10 days after the second meal, females were anesthetized for 5 min at –20 ºC and individually dissected, exposing the intestine using drops of 0.9% NaCL to observe the presence of promastigote forms under an optical microscope.

Leishmania (*L.*) *amazonensis* DNA confirmation by polymerase chain reaction and sequencing

Engorged females (N = 291) and samples of the mice used in the transmission test (lesion of the paw, spleen, liver and inguinal lymph node) had DNA extracted for the detection of *Leishmania* DNA by amplifying a 300 to 350 base pair (bp) fragment of the *ITS-1* gene, using the primers LITSR (5'-CTGGATCATTTTCCGATG-3') and L5.8S (5'-TGATACCACTTATCGCACTT-3') according to a previous study[24]. Amplified DNA was purified using ExoSAP-IT PCR Product Cleanup Reagent; (Thermo Fisher Scientific™, Waltham, USA), and the DNA sequencing was performed by the company ACT Gene Analises Moleculares Ltda. The sequences were deposited on the GenBank platform of the National Center for Biotechnological Information, under the access numbers available in Supplementary Tables S2 and S3. The sequences obtained were aligned with *ITS-1* sequences, and the phylogeny reconstruction was performed following previous studies[11,25].

Determination of parasitic load

To confirm that *L. (L.) amazonensis* was transmitted via *Lu. longipalpis*, mice were examined weekly for their general physical condition (shedding of hair and changes in skin condition) in addition to the appearance of skin lesions. When there were clinical signs suggestive of infection, the mouse was euthanized by anesthetic overdose (60 mg/kg of xylazine and 300 mg/kg of ketamine), and spleen, liver, lymph nodes and skin lesions of the paw and snout were collected for the investigation of the presence of the parasite.

The parasitic loads of the spleen, liver, inguinal lymph nodeand foot injuries were determined using the limiting dilution method[26]. This assay was performed in duplicate and incubated at 27 ºC. After 24, 48 and 72 h, the samples from each well were analyzed and defined as positive or negative, depending on the presence or absence of promastigotes in each well. The total number of parasites/samples was calculated as previously described[27].

Histopathological and immunohistochemistry analysis

Fragments of spleen, liver and skin lesion of the left paw were collected, fixed in 10% formaldehyde for 24 h, later processed and embedded in pure paraffin. Subsequently, 5-µm sections were prepared and the slides were stained using hematoxylin and eosin and Giemsa techniques. Histological sections were observed under a light microscope (40 X magnification).

The immunohistochemical analysis was performed to identify amastigotes in the skin, spleenand liver, as previously described[28]. Briefly, serum from a dog naturally infected by *L. (L.) amazonensis* was used as a primary antibody, and a polyclonal goat anti-mouse was used as a secondary antibody. The immunohistochemistry assay was stained with 3 3'-diaminobenzidine, and tissue samples were counterstained with Harris' hematoxylin. The immunohistochemistry analysis was considered positive when amastigotes were visualized stained in brown color. In this manner, samples were scored as positive or negative. We used spleen samples from a non-infected mouse and samples with amastigotes identified by histology as the negative and the positive control, respectively.

The proportion of blood-feeding and mortality of *Lutzomyia longipalpis*

We calculated the proportion of females who performed blood feeding (PBF) from the first blood meal (mouse infected by *Leishmania* inoculation) and the second blood meal (naive mouse). The mortality proportion (PM) was calculated as the number of engorged females killed after the first blood meal in relation to the total number of females that were engorged, dead, or survived[14]. The differences between the first and second feeding proportions were analyzed using the non-parametric Mann–Whitney test ($p < 0.05$).

RESULTS

Proportion of blood-feeding and mortality of *Lutzomyia longipalpis*

We used 485 female sand flies in the infection tests distributed in groups I-XII, which were exposed to feed on eight mice infected with *L. (L.) amazonensis* (ExpA). Of these, 291 (0.60) took the first blood meal, of which 146 (50%) survived and were exposed to the second feeding in the uninfected animal (ExpB), with a blood meal proportion of 0.12 (17), and a significant difference in the proportion of first blood meal (p = 0.0001) (ExpB) (Table 1). The female sand flies were preferably fed on the mice's snouts and feet; however, feeding was sporadically observed on the animal's tail, earsand eyelids.

Experimental infection of sand flies by *Leishmania* (*L.*) *amazonensis*

Of the 291 females of *Lu. longipalpis* that took the first blood meal in a mouse infected with *L. (L.) amazonensis*, we identified *Leishmania* DNA in 153 insects (infection rate = 52.6%; Table 2, Figure 1A). Additionally, 21 females were dissected after the blood meal, of which ten (47.6%) had flagellate forms of the parasite in their intestines (Table 2).

Transmission of *Leishmania* (*L.*) *amazonensis* to mice by *Lutzomyia longipalpis*

For this analysis, we considered only the groups IX, XI and XII, whose females had made the first blood meal (N = 74), of which seven were able to perform the second blood meal on two uninfected mice that survived for at least, two months after the bites (called animal A and animal B). We observed promastigotes in 4/7 of these sand flies.

Animal A: Eight days after the first blood-feeding, 22 surviving insects were subjected to a second blood meal in animal A. Of the 22 females, only two insects performed the second feeding in this mouse (Table 3), one of which was positive for the presence of *Leishmania* promastigotes. Nevertheless, after 96 days of observation of the animal, there were no clinical signs of disease. This animal was euthanized, and the limiting dilution test revealed no promastigote forms, indicating no transmission of the parasite to Animal A.

Animal B: This animal was subjected twice, at different times, to the second blood-feeding of sand flies (in groups XI and XII):

In group XI, after seven days of the first blood-feeding, 36 females were subjected to a second blood meal in animal B, and only three females (8%) were able to perform the second feeding, of which 2/3 were later confirmed as positive by intestine dissection and polymerase chain reaction (PCR) (Table 3).

Table 1 - Proportions of engorged females and proportion of mortality of *Lutzomyia longipalpis* females that performed blood feeding on mice, according to the first (ExpA) and the second (ExpB) blood meals.

Group	ExpA: first blood meal					ExpB: second blood meal		
	TF	EF	PBF	TFD	PM	TF	EF	PBF
I	38	11	0.29	7	0.64	4	3	0.75
II	41	9	0.22	6	0.67	3	1	0.33
III	29	6	0.21	0	-	6	1	0.17
IV	37	11	0.30	5	0.45	6	0	-
V	65	34	0.52	23	0.68	11	0	-
VI	50	28	0.56	11	0.39	17	0	-
VII	57	40	0.70	26	0.65	14	5	0.36
VIII	32	31	0.97	20	0.65	11	0	-
IX	30	27	0.90	5	0.19	22	2	0.09
X	27	19	0.70	19	1.00	0	0	-
XI	51	48	0.94	12	0.25	36	3	0.08
XII	28	27	0.96	11	0.41	16	2	0.13
Total	485	291	0.60	145	0.50	146	17	0.12
Mean (SD)			0.61 (0.27)		0.54 (0.21)			0.25 (0.22)

TF = Total females exposed to feed on mice; EF = Engorged females; PBF = Proportion of blood-feeding; TFD = Total Females dead after the first blood meal; PM = Proportion of mortality of the first blood meal; SD = Standard deviation.

Table 2 - Experimental infection rate and number of dissected *Lutzomyia longipalpis* females exposed to the first blood meal (ExpA) on mice infected with *Leishmania (Leishmania) amazonensis.*

Group	Engorged females	*Intestine dissection (+)	**PCR	#Infection rate (%)
I	11	3 (3)	6	54.5
II	9	1 (0)	7	77.8
III	6	1 (1)	4	66.7
IV	11	0	11	100.0
V	34	2 (0)	11	32.4
VI	28	0	23	82.1
VII	40	5 (2)	19	47.5
VIII	31	1 (1)	4	12.9
IX	27	2 (1)	20	74.0
X	19	0	10	52.6
XI	48	3 (2)	20	42.0
XII	27	3 (1)	18	52.0
Total	291	21 (10)	153	52.6

*Number of females, whose intestine was dissected to investigate the presence of *Leishmania*; (+)-Number of females, in which the parasite was observed in the intestine; **PCR: positive Polymerase Chain Reaction for *Leishmania*; # Calculated through the number of females positive for *Leishmania* DNA in relation to the number of engorged females.

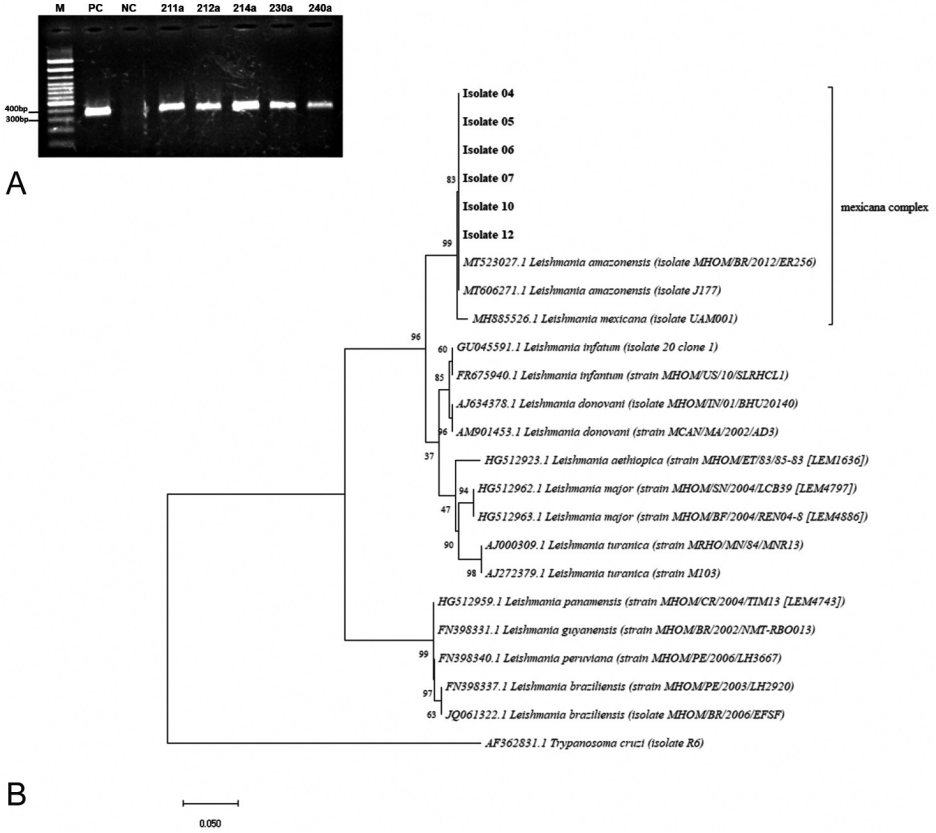

Figure 1 - Confirmation of *Leishmania amazonensis* DNA in *Lutzomyia longipalpis* fed on infected mice **(A)**: **1% a**garose gel showing amplification products of 300-350 bp generated by primers from *Leishmania ITS1 sequence* . M = 100 pb marker; PC: positive control; NC: negative control; 211a - 240a: positive samples from sand flies infected with *Leishmania (Leishmania) amazonensis*. (**B**) Evolutionary scenario of *ITS-1* gene inferred by the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The branch lengths are in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Sequences from our study are highlighted in bold (isolates 04 to 07 refer to samples of sand flies infected with *L. (L.) amazonensis*; isolate 10 refers to the sample of the strain isolated from the lesion of animal B and isolate 12 refers to the sample of the strain isolated from culture, the same as the positive control in Figure 1A). Brackets indicate sequences from the Mexican complex,including *Leishmania amazonensis* and *Leishmania mexicana*. *Trypanosoma cruzi* is the outgroup.

Table 3 - Number of females of *Lutzomyia longipalpis* that underwent the second blood meal in two uninfected and immunosuppressed mice (A and B) and the total of females diagnosed as positive for *Leishmania (Leishmania) amazonensis* through direct observation of parasites by intestinal dissection and DNA detection by Polymerase Chain Reaction (PCR).

Group/animal	Incubation*	Females exposed to the 2nd blood meal	Engorged females	Females infected with *Leishmania*	
				Intestine dissection	PCR
IX/ Animal A	8	22	2	1	1
XI/ Animal B	7	36	3	2	2
XII/ Animal B	6	16	2	1	1
Total	-	74	7	4	4

*Number of days after the 1st blood meal.

In group XII, after six days of the first blood-feeding, a total of 16 females were subjected to a second meal in animal B, and only two (12.5%) were successfully fed, of which 1/2 was later confirmed to be positive by dissection and PCR (Table 3). In total, animal B was bitten by five females, of which three (60%) were infected with *Leishmania*. The animal was observed for 67 days developing a lesion (slight swelling) in the left upper paw on the 27th and 22nd days after exposure to females from groups XI and XII, respectively. Figure 2 shows the progression of the lesion.

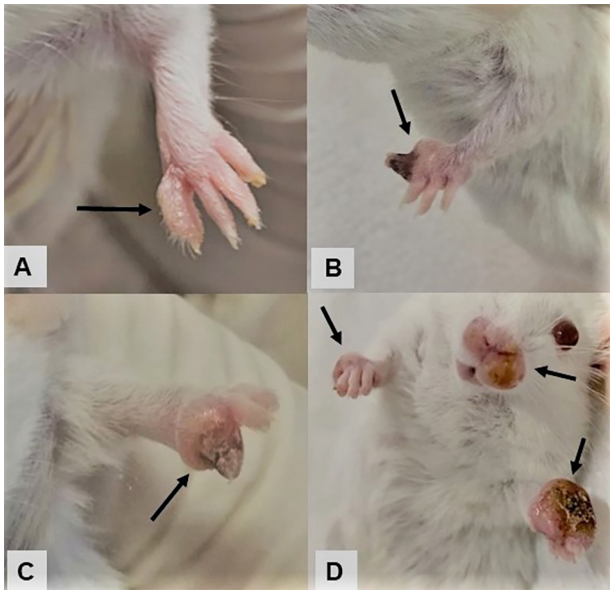

Figure 2 - Progression of the lesion observed in the mouse (animal B) bitten by *Lutzomyia longipalpis* and infected with *Leishmania (Leishmania) amazonensis*. In (A) 27 and 22 days after the bite; (B) 47 and 42 days; (C) 55 and 50 days; (D) 67 and 62 days concerning groups XI and XII, respectively.

Parasitic load and DNA sequencing

Ten weeks after exposure to the bites of infected insects, animal B was euthanized. The limiting dilution test demonstrated the *Leishmania* spread from the paw (location of the insect bite) to the viscera (spleen, liver and inguinal lymph node). The parasitic load observed in the spleen was 4.3×10^4 (0.1 g/mL), 3.0×10^3 (0.3 g)/mL) in the liver, and 8.9×10^3 (0.03 g)/mL in the inguinal node. The lesion on the paw (origin of the insect bite) showed a higher parasitic load of 9.0×10^4 (0.1 g/mL).

To confirm the origin of the lesion as being caused by the bite of *Lu. longipalpis* infected by *L. (L.) amazonensis*, we carried out the DNA sequencing. The result of the NJ analysis (Figure 1B) confirmed the identity of *ITS-1* sequences obtained from the total DNA of infected sand flies that took a second meal in animal B (isolates 4, 5, 6 and 7) from the lesion sample on the paw of animal B (isolate 10), and the sample of the positive control obtained from the cultured strain (isolate 12). All sequences belong to the same clade of the species *L. (L.) amazonensis* in the Mexican complex, indicating that they are the same parasite used in the study.

Histopathology and immunohistochemistry

In the histopathological analysis of the spleen, no morphological changes or the presence of amastigotes were observed in splenic macrophages. When evaluating the spleen samples using the Giemsa staining, no macrophages with *Leishmania* amastigote forms were identified. In the morphological analysis of the liver, we observed a slight degeneration of hepatocytes; there were no macrophages with amastigote forms observed in the morphological analysis. In the skin lesion analysis, we observed an intense neutrophilic inflammatory infiltrate with an area of necrosis and cellular apoptosis. There was an area of multifocal hemorrhage and collagenolysis, in addition to a moderate number of macrophages with *Leishmania* amastigote forms and extracellular amastigote forms in areas of cell degeneration (Figure 3A). We identified amastigote forms using hematoxylin-eosin and Giemsa stainings.

In the immunohistochemical analysis, no amastigote forms of *Leishmania* were observed in the liver and

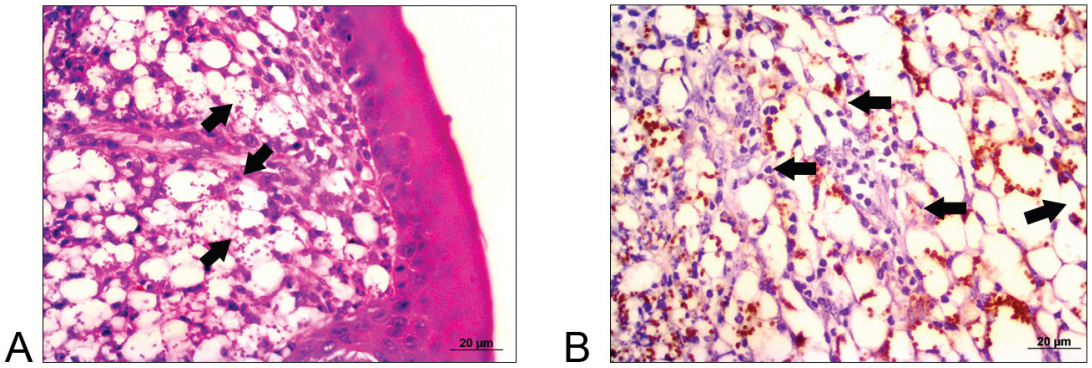

Figure 3 - Visualization of *Leishmania* in the mouse bitten by infected sand flies. **(A)** Hematoxylin and eosin staining of a skin lesion showing intense cell degeneration and several amastigotes (arrows) in the degenerate cell cytoplasm. 40 x magnification. Scale bar: 20 *µm.* **(B)** Skin lesion showing several amastigotes stained in brown color, confirming the *Leishmania* infection origin for this lesion. Immunohistochemistry, Harris' hematoxylin counterstaining, 40 x magnification.

spleen samples. However, numerous forms of *Leishmania* amastigotes were observed in the skin lesion (Figure 3B). Several amastigotes were also observed in regions of degeneration, edema and cell necrosis.

DISCUSSION

In the Americas, from the Southern United States to Northern Argentina, with the exception of Chile, *L. (L.) amazonensis* is transmitted by *B. flaviscutellata*[1]. In Brazil, this vector is rarely found in peridomicile of urban environments[6,15,29], however *L. (L.) amazonensis* is found infecting *Lu. longipalpis* in these areas, where this vector is highly adapted[2]. In this context and considering the increase in the number of patients coinfected with *Leishmania* and HIV, mainly in the Northeast of Brazil[11,30,31], we evaluated the role of the sand fly *Lu. longipalpis* as a competent vector for *L. (L.) amazonensis*. To accomplish this, we carried out an experimental transmission assay of the parasite to an uninfected and immunosuppressed vertebrate mammal.

The Balb/c mouse was previously used as the sole blood-feeding host for female *Lu. longipalpis*, proving to be a very attractive model, with PBF greater than 50%.In natural conditions, these observations may be associated with the sand fly's eclectic eating behavior, displaying no food preference, and their ability to take a blood meal on any available vertebrate animal, including man[2,14]. Despite the PBF rate, more than half of the engorged females died before taking the second feeding. We observed that some females had a distended abdomen due to a large number of eggs. According to some authors, many matured eggs can cause stress and exhaustion, culminating in the death of females[32]. However, despite the high mortality, the surviving females were able to transmit the parasite.

Lu. longipalpis females have also presented high rates of infection by *L. (L.) amazonensis* (53%). This finding demonstrates the high permissiveness of the species, which translates into the ability of this vector to sustain infection by *L. (L.) amazonensis*. This high rate of infection can be explained by the degree of cutaneous parasitism in the mouse because these animals were exposed to insects only after the appearance of leishmaniotic lesions (45 days after infection), increasing the probability of sand flies to become infected[14].

Transmission studies are challenging because of the high mortality rate of insects after the first blood meal, with few specimens remaining to perform the second meal[33]. With a small number of females available for the second blood meal, high infection rates increased the chance of obtaining infected females surviving the first meal. Another factor that implies successful transmission is the state of the vertebrate host's immune system[31]. In this context, immunosuppression of mice may have been a factor that facilitated transmission, however, when the immunosuppressed animal A was subjected once to a second blood meal, it did not show any clinical signs of CL, corroborating the difficulty of transmission, even in immunosuppressed animals.

We confirmed that the same parasite was present in both, insects and mouse lesions through *ITS-1* gene phylogeny tree. Thus, immunosuppression of the uninfected animals was able to demonstrate the competence of the vector *Lu. longipalpis* to transmit *L. (L.) amazonensis* to one of the two immunosuppressed mice. However, more studies using immunosuppressed animals are needed to better understand the relationship between the parasite and the host's weakened immune system.

We highlight a study[34] in which the authors suggested the transmission of *L. (L.) amazonensis* to naïve (and immunocompetent) hamsters by the bite of wild *Lu. longipalpis* naturally infected by *L. (L.) amazonensis*. However, the hamster manifested clinical signs of VL

only after three months, a behavior characteristic of *L. (L.) infantum*. These results differed from the ones of our study, in which the mouse developed skin lesions in less than one month. Moreover, two months after the infected insect bite, the disease has spread to the other paw, snout, and to the animal's internal organs (spleen, liver, and inguinal lymph nodes), the latter less common in CL.

Some studies point to a higher frequency of disseminated cutaneous manifestation in patients with coinfections, including reports of visceralization of the disease in both, humans[35,36] and dogs[17,37]. Other studies have shown that the genetic variation of *L. (L.) amazonensis* strains has been related to the parasite tropism, in addition to causing a variety of clinical manifestations[35,38]. Noteworthy, in the last decade, there have been changes in the profile of CL/AIDS coinfections, a fact justified mainly by the existence of a spatial overlap in the occurrence of the two diseases, with significant impact on the patient's survival, with increased risk of severe and widespread CL[11,36]. The Brazilian Ministry of Health recommends that the health secretaries of the States, including Maranhao, should increase the epidemiological surveillance of the two diseases, AIDS and leishmaniases[39].

Considering that the natural vector of *L. (L.) amazonensis* is primarily wild (*B. flaviscutellata*), we reinforce that it can be replaced by another abundant species in the urban area, in this case, *Lu. longipalpis*, which can behave as a secondary vector for the parasite. Our study corroborates the one by Nogueira *et al*.[4], who demonstrated that *L. (L.) amazonensis* completes its development to the metacyclic forms in *Lu. longipalpis*. Interestingly, in an endemic area for CL in central Colombia, *Lu. longipalpis* was reported but no cases of VL were reported in this area[40]. Another study carried out in a community of Panama in which the environment has undergone urbanization, presenting areas degraded by deforestation, *Lu. longipalpis* was also reported in an area endemic for CL[16]. Other studies have also mentioned rodents[12] and dogs[17] naturally infected by *L. (L.) amazonensis* in endemic areas for VL in Brazil.

In conclusion, our data demonstrated the competence of *Lu. longipalpis* to transmit *L. (L.) amazonensis* to an immunosuppressed mouse, causing leishmaniotic lesions. This finding highlights the epidemiological potential of *Lu. longipalpis* for the occurrence of CL, especially in regions where the proven vectors are rare. We emphasize that both, *Lu. longipalpis* and *L. (L.) amazonensis,* coexist extensively in the Americas, including several regions of Brazil, where patients presenting HIV coinfection, using immunosuppressive drugs, or having comorbidities associated with malnutrition and socialeconomic problems are more vulnerable to the development of leishmaniasis.

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AUTHORS' CONTRIBUTIONS

RCRS, SRFP and JMMR: conceptualization; JMMR and SRFP: project administration and funding acquisition; RCRS, LNPDC, JMSC and CEFA: sample collection and methodology; RCRS, SRFP and JMMR: formal analysis; RCRS, SRFP and JMMR: writing original draft preparation; RCRS and SRFP: writing review and editing. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there have no conflict of interests.

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