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Epidemiological aspects of American cutaneous leishmaniasis and phlebotomine sandfly population, in the municipality of Monte Negro, State of Rondônia, Brazil

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ABSTRACT

Introduction: This work was carried out on the purpose of identifying the species of phlebotomine sandflies in the municipality of Monte Negro, state of Rondonia, Brazil, that may have been transmitting the American cutaneous leishmaniasis (ACL), and concisely describe epidemiological aspects of disease. **Methods**: The epidemiologic and socioeconomical indicators were obtained from government institutions and the local Municipal Secretary of Health. Phlebotomine sandflies were captured using CDC light traps between July 2006 to July 2008. The total of 1,240 of female sandflies were examined by PCR method directed to k-DNA. **Results**: There has been a significant decrease in the incidence of ACL of about 50% over the last ten years in the municipality. A total of 1,935 specimens of 53 sandfly species were captured, three of the genus *Brumptomyia* genus and 50 of the genus *Lutzomyia.* The predominant species was *Lutzomyia acanthopharynx*, *Lutzomyia whitmani*, *Lutzomyia geniculata* and *Lutzomyia davisi.* None were positive for *Leishmania* sp. **Conclusions:** Four sandflies species were found in the State of Rondonia for the first time: *Brumptomyia brumpti*, *Lutzomyia tarapacaensis*, *Lutzomyia melloi* and *Lutzomyia lenti.* The presence of *Lutzomyia longipalpis,* was also captured. Socioeconomical improvement of Brazilian economy and the increase of environmental surveillance in the last 15 years collaborated in the decrease of people exposed to vectors, reducing the incidence of ACL.

Keywords: Phlebotomine sandflies. Epidemiology. Leishmaniasis. Amazon.

INTRODUCTION

In the Americas, *Leishmania* is transmitted by the females of some species of phlebotomine sandflies of the genus *Lutzomyia*. According to Rangel & Lainson[1], over than 400 *Lutzomyia* species have been found in Brazil, 122 found in the Amazonian region, out of which 25 were found to have an anthropophlic behavior.

One-hundred and six (106) species of *Lutzomyia* and three species of *Brumptomyia* were recorded in the State of Rondônia[2-4]. According to Shaw et al.[5], the most prevalent *Leishmania* species found in humans in the State of Rondônia are *Leishmania* (*Viannia*) *braziliensis, L.* (*V.*) *lainsoni* and *L.* (*V.*) *guyanensis*. In 2010, 922 occurrences of American cutaneous leishmaniasis (ACL) were recorded in the State of Rondônia[6], and such an incidence has been gradually decreasing over the last 10 years. Analysis of the ACL transmission circumstances in each area is complex due to the diversity of species of the etiologic agents, vectors and reservoirs involved in the transmission cycle, which has been a challenge for the healthcare system regarding both the diagnosis and treatment due to the broad spectrum of lesions, operational difficulties for diagnosis and chemotherapy toxicity.

One of the challenges regarding the ACL control and diagnosis is the lack of knowledge about the epidemiologic conditions, specially the study on the distribution of the phlebotomine sandflies population, the frequency and the characterization of its species, although some studies have already been carried out on the occurrence of phlebotomine sandflies and ACL in the State of Rondônia[3,4,7,8].

Nevertheless, some areas remain unexplored while others have been in geo-economic transformation process, and required further study in order to identify the phlebotomine species that account for the occurrence of ACL. Such study was carried out with forest sections nearby the dwellings around the rural area of the municipality of Monte Negro in Rondônia, an ACL endemic region.

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METHODS

Study area and population

The study area is situated in the Brazilian Western Amazon, in the municipality of Monte Negro **(Figure 1)**, located at 250km southwest of the City of Porto Velho (capitol), central region of the State of Rondônia, between 10º15'S and 63º17'W. The weather is characterized by two well marked seasons, one dry and the other humid, with a mean annual precipitation up to 2,020mm, and mean temperature of 25.8ºC[9]. A population of 14,204 inhabitants occupying an area of 1,413.4km^2 has been estimated out of which 60% live in the rural area. Its economy is based on agriculture (coffee, corn, and rice), cattle farming (beef and dairy cattle), and wood harvest exploitation and processing[10].

Epidemiologic data collection

The data were obtained from the Ministry of Health of Brazil[11] for the period between 1990 and 2010 for Brazil, Rondônia and Monte Negro, and the data regarding the nine autochthonous cases in Monte Negro were reported by the Secretary of Health of Monte Negro in 2006.

Environmental, economic and demographic indicators were obtained from government institutions, namely Brazil's National Institute for Space Research (INPE), Institute for Applied Economic Research (IPEA) and Brazilian Institute of Geography and Statistics (IBGE).

Phlebotomine sandfly capture

The phlebotomine sandflies were captured between July 2006 to July 2008 in residual woods located nearly 30 to 50m from the dwellings in nine human cases of ACL out of a total of 20 cases reported within the period to the Secretary of Health of Monte Negro **(Figure 1)**. Three visits were made at each site (27 visits in the total). As there were used 2 or 3 Centers for Disease Control (CDC) light traps from 6AM to 6PM per capture, a total of 81 captures were realized. The phlebotomine sandflies were identified according to the entomologic keys of Young & Duncan[12].

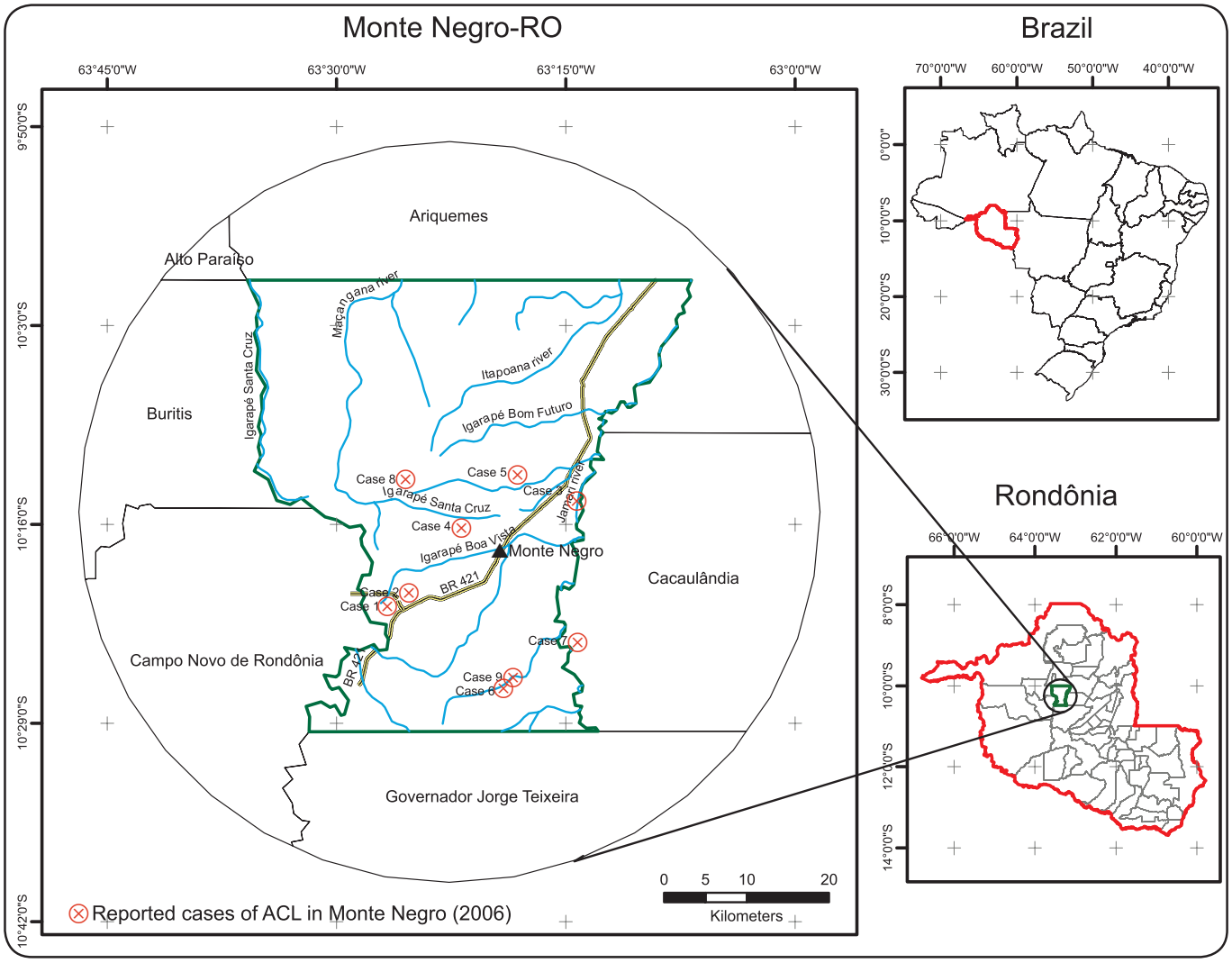

FIGURE 1 - Map of Brazil highlighting the State of Rondônia, Brazil, and the localization of the municipality of Monte Negro, in the West Amazon and the nine cases of American cutaneous leishmaniasis (ACL) registered during the study in 2006.

RO: State of Rondônia.

Extraction of DNA from captured females of phlebotomine sandflies

Female sandflies were initially sorted by species, and date and place of capture, and were then grouped into 2 to 20 specimens in micro test tubes for deoxyribonucleic acid (DNA) extraction based on the method adapted by Mukhopadhyay et al.[13]. Twenty microliters of buffer was added in the micro test tubes containing groups up to 10 specimens, and 35µL of buffer in the tubes containing over 10 specimens. They were heated for 10min up to 95°C in water bath (bain-marie), and then the insects were macerated by using a homogenizer, added with buffer STE (NaCl 0.1M, Tris-HCl 10mM pH 8.0 e EDTA. 1mM) until completing a volume of 50µL. Next, they were again heated in water bath (37ºC) up to 95°C for 10min, and then centrifuged at 12,000rpm for 2min. About 20µL of supernatant (DNA) was then transferred to another tube. After the extraction they were stored at 20º C for polymerized chain reaction (PCR).

Detection of natural infection phlebotomine sandfly by *Leishmania* sp.

The amplification for the genus *Leishmania* was based on the PCR method directed to the conserved region of the *kinetoplast* DNA (*kDNA)* minicircles among all the species of *Leishmania* sp., and performed with primers 5'-GGG(GT) AGGGGCGTTCT(G/C)CGAA-3' and 5'-(G/C)(G/C)(G/C) (A/T)CTAT(A/T)TTACACCAACCCC3'[14]; the product obtained was 120pb. The total volume was 50µL divided into 4µL of the DNA of the samples and 46µL of a mixture compounded by 37.25µL of sterile milli-Q water, 5µL of 10X taq buffer, 1.5µL of $MgCl_2$, 1µL of dNTPs (10 mM), 0.75µL of primer 1 (10µM), 0.75µL of primer 2 (10µM) and 0.5 of Taq polymerase (5U)[15].

The amplification was processed by an automatic thermal cycler using the following cycle: initial denaturation at 95ºC for 5min, followed by 40 repetitions of denaturation at 94ºC for 30 seconds, annealing at 55ºC for 30sec, and extension at 72ºC for 1min and 30sec. The final extension was at 72ºC for 10min.

The products amplified by PCR were analyzed by agarose gel electrophoresis 2% stained in ethidium bromide solution and examined by exposure to ultraviolet light (UV). For negative control, male phlebotomine sandfly DNA was used, while for positive control the sandfly DNA was used added to 200µL of a culture of *Leishmania amazonensis* strain IFLA/BR/67/PH8.

RESULTS

One thousand nine hundred and thirty five phlebotomine sandflies were captured: 695 males (36%) and 1,240 females (64%) **(Table1** and **Table2)**. A total of 53 species were identified: three of the genus *Brumptomyia* (32 samples - 1.6%) and 50 of the genus *Lutzomyia* (1,903 samples - 98.4%). The specimens of the genus *Lutzomyia* were sorted into the following subgenus: *L.* (*Evandromyia*) (15), *L.* (*Lutzomyia*) (23), *L.* (*Micropygomyia*) (5), *L.* (*Nyssomyia*) (321), *L.* (*Pressatia*) (8), *Psathyromyia* (60), *L.* (*Psychodopygus*) (379), *L.* (*Sciopemyia*) (68), *L.* (*Trichophoromyia*) (76), *L.* (*Viannamyia*) (14); and into the following groups **(Table 2)**: Aragaoi Group (9), Migonei Group (98), Oswaldoi Group (103), Saulensis Group (44), Verrucarum Group (29) and more 651 samples of a ungrouped species (*L. acanthopharynx*).

Among the 53 species captured, some were reported in the State of Rondônia for the first time: *B. brumpti* (0.4), *L. tarapacaensis* (0.7%), *L. melloi* (0.05%) and *L. lenti* (0.05%). The most abundant species found in this survey were *L. acanthopharynx* (651 samples - 33.6% out of the total collected), *L. whitmani* (180 samples - 9.3%), *L. geniculata* (155 samples - 8%) and *L. davisi* (151 samples - 7.8%), which represent 58.7% of the total.

Other phlebotomine sandfly species, of ACL vectors were captured in Monte Negro such as *L. flaviscutellata, L. umbiquitalis*, *L. complexa* and *L. carrerai*. *L. longipalpis*, the visceral leishmaniasis (VL) vector in Brazil, was also captured.

For natural infection analysis, a PCR was performed with all females phlebotomine sandflies collected. However, no sample has been found to be positive for *Leishmania* sp.

Since 1990, SINAN[6] has been reporting a gradual decrease by 53% in the ACL incidence in Monte Negro, a decrease of 43% in Rondônia, while, in Brazil, it has been noticed a decrease rate of about 31%. The reduction of the number of cases after 1990, as shown in **Figure 2**, reported by the Brazilian Ministry of Health sets a consistent decrement pattern.

Nine human cases were followed up in this study, all them from the rural zone, and the most affected group was represented by men (98%) between 15 and 50 years old, who lived in little settlements around the urban area of Monte Negro.

According to National Institute for Space Research (INPE)[16], between 2004 and 2010 there was a reduction in deflorestation, from 27,772km^2/year to 7,646km^2/year.

The economic results also must be looked upon. Between 2004 and 2009 there was an increase by 18% in the number of families benefitted

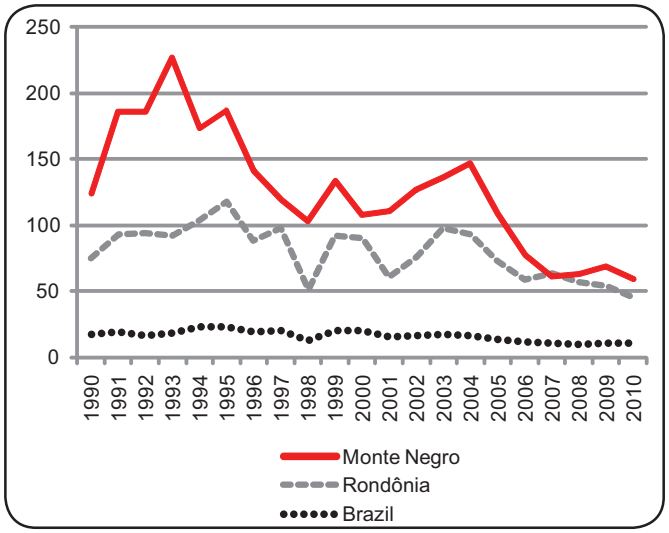

FIGURE 2 - Incidence of American cutaneous leishmaniasis in the municipality of Monte Negro, State of Rondônia, Brazil, between 1990 and 2010 (occurrences per 100 thousand inhabitants).

Source: *Sistema de Informação de Agravos de Notificação* (SINAN), 2011.

TABLE 1 - Phlebotomine sandfly population of the genus Brumptomyia and subgenera Lutzomyia was captured using CDC light traps in Monte Negro, State of Rondônia, Brazil, between July 2006 to July 2008.

Genus	Subgenera	Species	Males	Females	Total	Percentage
Brumptomyia		*B. brumpti**	9	-	9	0.46
		B. pentacantha	3	-	3	0.15
		B. sp.	-	20	20	1.03
Lutzomyia	(*Evandromyia*)	*L. tarapacaensis**	2	11	13	0.70
		L. monstruosa	0	2	2	0.10
	(*Lutzomyia*)	*L. evangelistai*	-	3	3	0.15
		L. longipalpis	6	-	6	0.31
		L. sherlocki	2	12	14	0.72
	(*Micropygomyia*)	*L. micropyga*	3	2	5	0.25
	(*Nyssomyia*)	*L. antunesi*	24	71	95	4.90
		L. flaviscutellata	16	26	42	2.20
		L. shawi	-	3	3	0.15
		L. umbratilis	1	-	1	0.05
		L. whitmani	60	120	180	9.30
	(*Pressatia*)	*L. triacantha*	4	4	8	0.41
	(*Psathyromyia*)	*L. campbelli*	1	1	2	0.10
		L. dendrophyla	8	13	21	1.08
		L. lutziana	16	16	32	1.65
		L. punctigeniculata	1	-	1	0.05
		L. shannoni	3	1	4	0.21
	(*Psychodopygus*)	*L. amazonensis*	-	5	5	0.26
		*L. carrerai **	1	1	2	0.10
		L. claustrei	2	9	11	0.60
		L. complexa	2	3	5	0.25
		L. davisi	51	100	151	7.78
		L. geniculata	26	129	155	8.01
		*L. hirsuta **	16	26	42	2.20
		L. lainsoni	1	7	8	0.41
	(*Sciopemyia*)	*L. servulolimai*	8	20	28	1.44
		L. sordellii	13	27	40	2.06
	(*Trichophoromyia*)	*L. auraensis*	22	-	22	1.13
		L. clitella	9	-	9	0.46
		*L. melloi**	1	-	1	0.05
		L. octavioi	5	-	5	0.25
		L. ubiquitalis	8	3	11	0.60
		L. sp.	-	28	28	1.44
	(*Viannamyia*)	*L. furcata*	6	8	14	0.72
Total			**334**	**709**	**1,043**	**54.0**

***B:** Brumptomyia*; ***L:** Lutzomyia;* **CDC:** Centers for Disease Control. *The first time for Rondônia; **Taxons renamed as species by Carvalho GML et al. Mem Inst Oswaldo Cruz 2006; 10:129-136. The relative abundance of each species collected in the study area expressed as a percentage of the total of 1935 of sandflies collected.

TABLE 2 - Sand flies groups of **Lutzomyia** *captured in Monte Negro, State of Rondônia, Brazil, from July 2006 to July 2008 with CDC light traps.*

Genus	Group	Species	Males	Females	Total	Percentage
Lutzomyia	Aragaoi	*L. abunaensis*	2	-	2	0.10
		L. aragaoi	3	1	4	0.20
		L. barretoi barretoi	2	-	2	0.10
		L. coutinhoi	1	-	1	0.05
	Migonei	*L. bacula*	23	52	75	3.87
		*L. lenti**	1	-	1	0.05
		L. termitophila	0	5	5	0.25
		L. walkeri	6	10	16	0.82
		L. williamsi	1	-	1	0.05
	Oswaldoi	*L. goiana*	60	43	103	5.32
		L. wilsoni	2	-	2	0.10
	Verrucarum	*L. nevesi*	6	12	18	0.93
		L. serrana	6	5	11	0.60
	Ungrouped species	*L. acanthopharynx*	248	403	651	33.64
Total			**361**	**531**	**892**	**46.0**

L: *Lutzomyia;* **CDC:** Centers for Disease Control. *The first time for Rondônia; **Taxons renamed as species by Carvalho GML et al. Mem Inst Oswaldo Cruz 2006; 10:129-136. The relative abundance of each species collected in the study area expressed as a percentage of the total of 1935 of sandflies collected.

by the "Family Allowance Program"(*Bolsa-Família*) in the North Region, and the increase in the formal employment rate in the main Brazilian metropolitan areas by 24.4% between 2002 and 2010 (Source: www.ipeadata.gov.br on Dec 28, 2011)[17]. On the other hand, the migratory tendency to the North Region, resulted in a negative balance of less 35,159 people between 2004 and 2009[10,17].

DISCUSSION

From all 53 species captured, four were new records for the State of Rondônia: *B. brumpti*, *L. tarapacaensis*, *L. melloi* and *L. lenti*. The surveys carried out in the State of Rondônia[2-4], and the new reports of phlebotomine species made by this work totalize 110 species of *Lutzomyia* and 4 *Brumptomyia* for Rondônia. In the present study *L. acanthopharynx*, *L. whitmani*, *L. geniculata* and *L. davisi* together represented 58.7% of all the sandflies caught. According to Gil et al.[3,4], the species *L. davisi* and *L. whitmani* were the most abundant, and were the vectors closely associated with ACL transmission around the region, along with other predominant species such as *L. umbratilis*, *L. llanosmartinsi*, *L. dendrophyla, L. nevesi*, *P. carrerai* and *P. hirsutus*.

The species *L. whitmani* and *L. davisi* have proved to be significant vectors of *Leishmania* that cause ACL either in the enzootic cycle and in the peridomestic cycle[3,18]. The prevalence of these vectors suggests the possibility of transmission in the peridomestic environment. The confirmation of the previous studies has associated *L. davisi* with the transmission of *Leishmania* (*Viannia*) *naiffi*[3] and *Leishmania* (*Viannia*) *braziliensis*[18] in the State of Rondônia. Gil et al.[3] complement that the high number of *L. davisi* captured in Shannon-type traps reflects its anthropophilic habit, while Azevedo et al.[19] reports that the high *L. whitmani* density may be associated with the changes in the original vegetation and the better adaptation to new environmental conditions.

Many studies have shown the vectorial potential of *L. whitmani* in the natural anthropic environments regarding the transmission of *Leishmania* (*Viannia*) *braziliensis*, *L.* (*V.) shawi* and *L.* (*V.*) *guyanensis* in some states of Northeast, Southeast, South, West Central and Amazonian region[1,20-23].

In the areas studied other vectors of ACL were identified, such as *L. flaviscutellata,* the main vector of *Leishmania* (*Leishmania) amazonensis*, which has been also found to be parasitized by *Leishmania* (*Viannia*) *guyanensis* in the French Guiana[24]; *L. umbiquitalis*, the vector of *Leishmania* (*Viannia*) *lainsoni* in Brazil[25]; *L. complexa,* species considered to be a *Leishmania* (*Viannia*) *braziliensis* vector[26]; *Leishmania carrerai* is considered to be a *Leishmania* (*Viannia*) *braziliensis* vector according to Grimaldi et al.[27], who has characterized the parasite in phlebotomine sandflies as naturally infected in the Brazilian Amazon.

In addition to the ACL vectors, *L. longipalpis*, the VL vector in Brazil, was collected in the region. Although the incidence of VL in Rondônia has been exclusively associated with allocthonous cases (0.07 per 100 thousand inhabitants)[28], the presence of *L. longipalpis* should be a factor of concern as, in the Amazon region, VL is already autochthonous in the States of Pará, Roraima, and Tocantins[22,29,30] and areas of the Southeast of Brazil, that were not traditional endemic areas of VL, had registered several autochthonous cases over the last ten years[22,30,31]. On the other hand, according to serologic studies

performed in Monte Negro[8], infected dogs and phlebotomine sandflies are in contact as dogs were found to be serologically positive for *Leishmania.*

The knowledge of the phlebotomine sandfly fauna in Rondônia remains still insufficient, while the last surveys regarding the phlebotomine sandfly population has reported new occurrences of its species[3,4].

Although 64% of the phlebotomine sandflies collected were females, the natural infection analysis for PCR was negative for *Leishmania* sp. In endemic leishmaniasis areas, the rates of natural infection by *Leishmania* in phlebotomine sandflies have been historically low[32,33]. Soares et al.[34] explain the low infection rate due to ecologic relations such as the habitat pattern, the intraspecific behavior, the vector feeding cycle, the genetic endowment and immunity of the population of reservoirs of each locality.

The epidemiologic data points to a significant decrease in the incidence a ACL of about 53% and 43% over the last ten years in Monte Negro and Rondônia and 31% in Brazil. The municipality of Monte Negro is an historical area of transmission of ACL that accounted for a incidence of 59 cases/100,000 inhabitants in 2010. The nine humans cases followed up in this study was represented for mens between 15 and 50 years old, which is the most susceptible age group, reported by some authors[35-37]. The nature of the zoonotic transmission becomes evident when it is noticed that 80% of the patients were engaged in activities associated with the permanence in the forest (hunting, fishing, lumbering), although 20% had not such activities, suggesting peridomestic transmission. Murback et al.[38], which suggested that the ACL transmission in man in the productive age bracket are likely to be higher due to the fact that they leave their domiciliary environment for work or leisure, and are then more exposed to the timberland areas.

The economical and environmental surveillance improvement in Brazil in the last 15 years imply in a reduction of the self-employed labor availability for risky activities connected to the likeliness of ACL occurrences such as lumbering, gold digging and clandestine land occupation. Such background may have led to a decrease in the insertion of collectors and hunters inside the woods that has reduced the likeliness of contamination by the ACL vectors, and had a positive impact in the incidence of the disease.

Nevertheless, in spite of the economic advancements, the social development, and better environmental surveillance, ACL appears as a permanent residual focal point in the region.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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