

ORIGINAL ARTICLE

RISK FACTORS ASSOCIATED WITH AMERICAN CUTANEOUS LEISHMANIASIS IN AN ENDEMIC AREA OF BRAZIL

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SUMMARY

Brazil is among the top five countries worldwide regarding the number of cases of leishmaniasis, which are present in all of the regions of the country. The northeastern region continues to have higher numbers of cases every year and in the state of *Pernambuco*, 34% of the municipalities are endemic for this disease. The diversity of vectors, reservoirs and etiological agents, in association with socioeconomic and environmental conditions, gives rise to factors that can modify the behavior of American cutaneous leishmaniasis. Consequently, the aim of the present study was to determine the risk factors associated with American cutaneous leishmaniasis in the municipality of *Timbaúba*, Brazil. A case-control study was conducted. A validated questionnaire was used for data collection. The study included 58 cases and 174 controls, and they were serologically diagnosed at the *Oswaldo Cruz* Foundation (FIOCRUZ). Our results showed that some factors were associated with American cutaneous leishmaniasis: biological (gender), economic (work activity, hours spent away from home and water supply) and peridomestic (presence of animals). In our study, the associations of these variables with leishmaniasis were linked to precarious housing conditions and poverty, which are parameters that can be managed in order to prevent the disease in this region.

KEYWORDS: Cutaneous leishmaniasis; Risk factors; Case-control study.

INTRODUCTION

Leishmaniasis is considered a neglected disease and threatens about 350 million people worldwide. In Brazil, it is present in all Brazilian states, and some municipalities in the state of *Pernambuco* are endemic for this disease^{1,2}.

The municipality of *Timbaúba*, which is located in the northern “Zona da Mata” region of *Pernambuco*, is considered an endemic area of American cutaneous leishmaniasis (ACL)². In *Timbaúba*, during the years 2012-2014, there were 58 cases of ACL and the occurrence rate was 54.7 cases per 100,000 inhabitants, according to the Brazilian Information System for Notifiable Diseases (SINAN)³. According to Brito *et al.*⁴ the occurrence rate of ACL cases in the entire state of *Pernambuco* is 5.22 per 100,000 inhabitants. Thus, *Timbaúba* is one of the municipalities with the highest number of ACL cases in the state.

Some social and environmental factors, such as the prevalence of ACL, rapid urbanization and population concentration, may modify the disease cycle. This may lead to a situation of disease migration from rural

to urban areas, in combination with geolocation of sandflies compatible with *Leishmania* spp. strains in these areas^{5,6}.

In Brazil, few studies have comprehensively analyzed risk factors for ACL in regions or cities. Pedrosa & Ximenes⁷ identified risk factors for ACL in the state of *Alagoas*, Brazil, in a case-control study. Regarding domestic risk factors, they found a strong association of ACL with the group of individuals reporting four or more years of education. Considering peridomestic factors, they found an association of ACL with people living in houses located less than 200 meters from woods or forests.

Nunes *et al.*⁸, also in the state of *Alagoas*, described an epidemiological profile of patients with leishmaniasis who were treated in a referral hospital. They found that the presence of ACL was associated with low socioeconomic status, rural workers, housewives and students. According to these authors, the risk of acquiring ACL is mainly related to parameters present in domestic areas, and low socioeconomic status is an aggravating factor. Apart from this, one of these factors is based on the socioeconomic performance of the region’s population, which may vary over time and according to the space.

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Epidemiological studies to identify risk factors for ACL may provide guidance for control measures within the community, thereby making preventive actions more effective. Thus, an investigation that takes into consideration risk factors for ACL is important to increase the understanding of its dynamics, therefore attaining control strategies of greater efficiency in the municipality of *Timbaúba, Pernambuco*, Brazil.

MATERIAL AND METHODS

Area of study

The municipality of *Timbaúba, Pernambuco*, is located in the northern region of the “*Zona da Mata*”, at latitude 07° 30’ 19” South and longitude 35° 19’ 06” West. It has 53,825 inhabitants, among whom 48.1% are men and 51.9% are women. Its area is around 293 km² and the main economic activity is agriculture, especially sugarcane production⁹.

Population and study design

A case-control study was conducted. A validated questionnaire was used for data collection. We recruited cases among autochthonous people in the municipality of *Timbaúba, Pernambuco*, that underwent laboratory confirmation of infection, whether or not skin lesions and/or mucous characteristics were present, and who have been living in *Timbaúba* for more than one year. Laboratory confirmation was obtained using the immunofluorescence technique (IFA) (Biomanguinhos® leishmaniasis kit). Titers of 1:40 from the reactions were considered to be positive, in accordance with the definitions of the Brazilian Ministry of Health². The controls were defined as individuals who have been living in *Timbaúba, Pernambuco*, for more than one year, who did not present any injury or scar suggestive of ACL and whose IFA tests were non-reactive for leishmaniasis.

The controls were matched by age with the cases. The cases were classified as young (0 to 18 years old) or adult (> 18 years old). For each young case, three young controls were recruited. For each adult case, three adult controls were recruited. In addition, the cases were matched with controls according to the neighborhood so that, for each case, three individuals from the three nearest houses were recruited as controls.

All the participants in our sample were informed on the aims of the study and after acceptance, they read and signed the free and informed consent statement. All confirmed cases were reported to the epidemiological surveillance agency.

The sample size calculation assumed an α error of 5% and a β error of 20%, corresponding to a power of 80%. It is possible that, for some variables, a confidence interval of smaller amplitude could have been obtained with a larger sample size, so that some associations would have become statistically significant. On the other hand, the chance of finding a non-genuine association was lower. For the magnitude of the difference in the exposure frequency, we used the study by Sosa-Estani *et al.* as our reference¹⁰.

The individuals who participated in the study were predominantly from rural areas, so that only 19% of the cases were from urban areas. The cases were screened through an active search independently of the area where these individuals were living.

We found 60 individuals who were suspected cases. Of these, two were excluded because their IFA results were negative. Thus, 58 cases remained in the study.

To compose the control group, 179 individuals were interviewed and blood samples were collected. However, five were excluded because reactive results were obtained by IFA. Thus, 174 controls remained in the study.

Hence, this study was conducted with a total of 232 individuals, i.e. 58 cases and 174 controls, thus maintaining a ratio of 1:3. This ratio was used to ensure reliability of the data and avoid distortions that could be produced either through a random or through a systematic error.

Data collection

For each case and control, a structured and undisguised questionnaire that had previously been validated by Pedrosa & Ximenes⁷ was applied. Information was gathered at the homes of the individuals selected. The questionnaire was answered by the individuals themselves or by their guardians if they were under the age of 18 years. The variables related to housing and environmental conditions were checked by the researcher.

Regarding the possibility of cases underreporting, the researcher had a direct contact with healthcare workers, nurses and coordinators from the municipal Health Department of *Timbaúba*. It could be seen that notification of almost all of the ACL cases was provided. Even if detection failures could have occurred, the selection bias would be minimal.

Regarding information bias, it is possible that for some variables, the person who spoke with the participants may have provided wrong information. To minimize this type of error, the aims of the research were carefully explained. The subjects were provided with information about where the researcher came from and where the data would go. Concerning confounding factors, a multivariate analysis was conducted in order to adjust each of the factors in accordance with the others.

For data processing, a database using the Epi Info™ 7.0 software (Centers for Disease Control and Prevention, Atlanta, USA), was created. Double data entry was used in order to correct possible typing errors. Statistical analysis was applied.

The questionnaire was structured with questions relating the following variables: gender, age, education (years), number of people living in the house, number of people living there who were working, per-capita income, location of work activity (rural or urban), average number of hours spent away from home, leisure activities undertaken in forest area (hours), sleeping at work site (number of nights), any time spent hunting, any time spent fishing, frequency of evening walks in the countryside, household chores performed outdoors, time spent gathering firewood, time spent carrying water to the house from an outside source to the house, sleeping with mosquito nets, sleeping in the outer area of the house, use of insecticide (per month), use of repellent (per month), sleeping with windows opened, household location (rural or urban), screens on windows and doors, wall construction material, roofing material, number of rooms, number of people per bedroom, water supply, presence of bedroom, electricity inside the house, presence of gas stove, distance of woodpile from the house (meters), distance of river from the

house (meters), distance of crops from the house (meters), distance of neighbor's house from home (meters), presence of animals inside the house, presence of animals in the peridomestic area (outside the house).

The questionnaire was applied to all the participants of the study and none of them refused to answer it.

Data analysis

For data analysis we used the Epi Info™ 7.0 software (Centers for Disease Control and Prevention, Atlanta, USA). The association between ACL and each potential risk factor was studied. A univariate conditional logistic regression was used for the final models.

The univariate logistic regression was used to obtain odds ratios (OR) and their respective 95% confidence intervals (95% CI), and the *p* values of the likelihood ratio tests. Variables that presented an association with ACL in the univariate model with *p* < 0.1 were tested in the multivariate model. Those that showed *p* < 0.05 in the final model were retained.

Ethical considerations

The authors declare that this research was approved by the Ethics Committee of the Federal University of Pernambuco (*Universidade Federal de Pernambuco*) under the protocol CAAE: 02720312.5.0000.5208, and

that it has followed the ethical principles of the Helsinki Declaration of 1983.

RESULTS

Regarding the variable “age distribution”, there was a higher concentration of cases (74.14%) in the activeadult group (> 18 years old) than in either the young group (0-18 years old; 25.86%).

Regarding the variables relating biological and social factors in the multivariate analysis, the best model showed association of the variables “*per capita* income” and “gender” with ACL, more precisely, of male individuals with *per-capita* income greater than R\$ 50.00 (fifty *reais* or the equivalent of approximately 15 American dollars) (Table 1).

In the second multivariate analysis, variables relating “labor and leisure activities” showed the association of “average number of hours away from home” with “work activity” (Table 2).

In the third multivariate analysis, this time with the group of variables related to peridomestic areas, the best model associated “presence of animals in the household” with “presence of animals in the peridomestic area” (Table 3).

The final multivariate analysis model performed for this study

Table 1

Multivariate analysis of variables related to biological and social factors associated with ACL in the city of *Timbaúba*, Pernambuco, Brazil, 2012-2013

Variables	Cases		Controls		Adjusted OR ^a	CI ^b 95%	<i>p</i>
	N°	%	N°	%			
Gender							
Male	36	62.07	38	21.84	5.98	3.11-11.48	< 0.001
Female	22	37.93	136	78.16	1		
Per-capita income							
≤ R\$ 50.00	04	6.90	35	20.11	0.27	0.08-0.85	0.02
> R\$ 50.00	54	93.10	139	79.89	1		

^aOR = Odds Ratio; ^bCI = Confidence Interval. R\$- *reais* (the Brazilian currency).

Table 2

Multivariate analysis of variables related to labor and leisure activities associated with the ACL in the city of *Timbaúba*, Pernambuco, Brazil, 2012-2013

Variables	Cases		Controls		Adjusted OR ^a	CI ^b 95%	<i>p</i>
	N°	%	N°	%			
Hours spent away							
> 4	18	31.03	20	11.49	3.92	1.84-8.35	<0.001
0 – 4	40	68.97	154	88.51	1		
Work activity							
Rural	38	65.52	79	45.40	2.57	1.34-4.91	<0.001
Urban	20	34.48	95	54.60	1		

^aOR = Odds Ratio; ^bCI = Confidence Interval.

Table 3
Multivariate analysis of variables related to peridomicile associated with ACL in the city of *Timbaúba*, *Pernambuco*, Brazil, 2012-2013

Variables	Cases		Controls		Adjusted OR ^a	CI ^b 95%	p
	N ^o	%	N ^o	%			
Animals outside the house							
Yes	47	81.03	100	57.47	2.62	1.25-5.51	0.01
No	11	18.97	74	42.53	1		
Animals inside the house							
Yes	47	81.03	104	59.77	2.31	1.09-4.88	0.02
No	11	18.97	70	40.23	1		

^aOR = Odds Ratio; ^bCI = Confidence Interval.

indicated that ACL in *Timbaúba* was associated with “male gender”, “spending more than four hours away from home”, “no water supply at home” and “presence of animals inside the house” (Table 4).

DISCUSSION

In our study, in which we ascertained the risk factors associated with ACL in an endemic area of Brazil, we observed that the distribution of ACL among patients who belonged to the group of adults was 74.14%. Our results corroborate those of other authors, who observed percentages of 39.8% to 56.7% for the same age group^{7,10,11}. ACL is more important in the age group of economically active adults in comparison with visceral leishmaniasis, which affects higher percentages of children¹².

Males of the working age group are most likely to be affected by leishmaniasis since they are the individuals who generally work in rural

areas. This was a significant feature of our study in this municipality. Theoretically, this working environment is closer to sandfly habitats, which would therefore increase their chances of becoming infected⁷.

However, a study conducted in India¹³ found a greater concentration of cases among teenagers, probably due to acquired immunity. This was not observed in our study, but an immunological survey may be necessary to address this issue.

The analysis of biological and social factors in our study revealed that the gender variable was associated with ACL, so that males were at higher risk. This corroborates the results found by Curti *et al.*¹⁴, who conducted a study on the epidemiological aspects of ACL in the northwestern region of the state of *Paraná*, Brazil, in which males accounted for 72.5% of the cases. These results are also in accordance with data from the Ministry of Health reporting that 74% of the cases occur among men². In the Amazon

Table 4
Final Multivariate analysis of risk factors associated with ACL in the city of *Timbaúba*, *Pernambuco*, Brazil, 2012-2013

Variables	Cases		Controls		Adjusted OR ^a	CI ^b 95%	p
	N ^o	%	N ^o	%			
Gender							
Male	36	62.07	38	21.84	4.09	1.96-8.55	<0.001
Female	22	37.93	136	78.16	1		
Hours spent away							
> 4	18	31.03	20	11.49	3.58	1.37-9.35	0.009
0 – 4	40	68.97	154	88.51	1		
Water supply							
Without	21	36.21	119	68.39	5.99	2.75-13.06	<0.001
With	37	63.79	55	31.61	1		
Animals inside the house							
Yes	47	81.03	104	59.77	3.12	1.35-7.17	0.007
No	11	18.97	70	40.23	1		

^aOR = Odds Ratio; ^bCI = Confidence Interval.

region, Soares *et al.*¹⁵ compared two contrasting populations and provided epidemiological evidence that the incidence of ACL may be male-biased even when exposure levels are comparable between genders.

Among the social factors, we observed that in our study the variable “*per-capita* income” showed that individuals with incomes lower than R\$ 50.00 had lower chances of acquiring ACL. Similarly, but using family income data, a study by Pedrosa & Ximenes⁷ showed that family income greater than or equal to the minimum wage constituted a risk regarding the acquisition of ACL.

However, these authors believed that their results relating family income may have been due to information bias because, as occurred in our study, the target population was mainly composed of people living in rural areas, who may not be able inform what their total income was, and may have wrongly reported their total income at the time they answered the questionnaire. Nonetheless, the study in India found comparable data, i.e. that ACL was more incident among the low-income population¹³.

In the analysis of factors related to work and leisure activities, we observed an association between ACL with the variable “average number of hours away from home”. This indicated that the amount of time spent away from home (> 4 h) increased the chances of acquiring ACL. This became more pronounced when the individual’s work activity was in the rural area, because there are higher concentrations of sandflies in rural areas and therefore individuals working in rural areas are more exposed to insect vectors of ACL.

Corroborating these data, in a study conducted in northern Argentina, Sosa-Estani *et al.*¹⁰ observed that livestock activity increases the risk of ACL because of individuals’ exposure to areas near forests, where there may be breeding sites and residual foci of sandflies. Similarly, Pedrosa & Ximenes⁷ also correlated ACL with rural activity, and according to these authors, proximity to forest areas favored the exposure to the insect vector.

In a study in Libya¹⁶, it was found that being engaged in farm activities was a risk factor, increasing the exposure to ACL infection, as was an occupational requirement to stay outdoors during part of the night.

In the analysis of factors related to domestic matters, the variable “water supply” indicated that households that did not have piped water presented an association with ACL. In these situations, individuals have to fetch water from wells, reservoirs, rivers or other water sources, thus becoming more exposed to the action of sandflies. Like our results, the data from Sosa-Estani *et al.*¹⁰ showed that there was a strong association between the presence of ACL and the habit of fetching water from water sources. For similar reasons, “absence of a bathroom at home”, in our study, was also associated with ACL.

The variables related to domestic matters in our study underscore the precariousness of basic sanitation in rural areas homes. Similarly, the study by Pedrosa & Ximenes⁷ correlated ACL with poor housing conditions. The presence of basic internal structures in the house, such as a bathroom, water pipes and, consequently, a sewage disposal system would benefit rural areas through the reduction of the incidence of ACL and other diseases that result from the absence of these conditions.

In the present study, the analysis of factors related to peridomestic areas revealed, through a multivariate model, that there was an association between ACL and “animal rearing at home and in peridomestic areas”. The univariate model (OR = 0.47; *p* = 0.03) also showed that there was less chance of becoming ill because of ACL in “homes that were at a distance of 100 m or more from woods and forests”.

This was also seen in a retrospective case-control study by Yadon *et al.*¹¹, which was conducted in Santiago Del Estero, Argentina, covering the period between 1990 and 1994. They found that “homes located at distances of less than 150 m from lakes, rivers or water streams, or from forest and cultivated areas,” were associated with a higher risk of ACL. Similarly, Pedrosa & Ximenes⁷ found an association of ACL with “homes at distances of less than 200 m from forest areas”.

According to Ocampo *et al.*¹⁷, in a study in Colombia, ACL transmission by sandflies depends on the presence of dense vegetation near the houses. This corroborates our findings that there was a greater chance of transmission near thick vegetation.

“Animal rearing at home and in peridomestic areas” increases the chances of acquiring ACL. Animal-rearing environments are attractive for sandfly populations, because they increase the number of blood meals options and thus increase the risk of transmission. Other authors have also correlated animal rearing with the risk of acquiring ACL, through factors such as “presence of birds, cats and dogs in the house”^{11,13} and “presence of pigs around the house”¹⁰.

This group of variables (peridomestic factors) correlates the environments that are appropriate for sandflies with higher chances of acquiring ACL. In these situations, simple actions such as building houses distant from forest areas and abstaining from animal rearing close to the house can protect a population from disease transmission.

However, it has been noted that sandflies have entered the urban areas, with reports of occurrences of ACL sandfly vectors, as well as of the disease itself in municipalities that are considered predominantly urban^{18,19}. In *Timbaúba*, occurrences of ACL continue to present rural characteristics, but it seems to be only a matter of time until the migration to urban areas and to cities in northeastern Brazil is observed in parallel with the ACL cases.

In conclusion, based on the final model of our multivariate analysis, the association with ACL is more evident among males, individuals who stay more than four hours away from home, those who do not have water supply at home, and those who have animal rearing inside the house. Deepening the analysis of variable “*per-capita* income” in the final analysis, it was observed that this variable presented a possibility of information bias and the results were inconsistent with the other variables associated with ACL.

Thus, it is possible to recommend the implementation of certain actions to prevent ACL. These include the installation of basic sanitation in houses, especially in rural areas, and avoid animal rearing at home. With regard to animal rearing in peridomestic areas, we suggest that this should be done as far away from the house as possible. Lastly, rural area workers should use individual safety equipment to avoid contact with sandflies.

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