

PERIDOMESTIC RISK FACTORS FOR CANINE LEISHMANIASIS IN URBAN DWELLINGS: NEW FINDINGS FROM A PROSPECTIVE STUDY IN BRAZIL

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Abstract. Despite the wealth of information on the prevalence and correlates of canine *Leishmania* infection (CLI), data on its incidence are still sparse, and little is known regarding risk factors for CLI. We studied a cohort of dogs in an urban area in Brazil to determine whether incidence varied with age, breed, and environmental characteristics. The mean follow-up was 1.5 years, and the crude incidence rate was 11.8 cases/100 dog-years (95% confidence interval [CI] = 8.6–15.6). In the multivariate analysis, short fur was the strongest predictor of CLI (relative risk [RR] = 9.4). In addition, our data indicate that raising pigs (RR = 4.1), chickens (RR = 3.3), or other livestock (RR = 2.6) significantly increased the risk of CLI. Thus, suggesting control measures directed towards modifying the environmental factors favoring contact between vectors, reservoirs, and susceptible humans, such as proximity to pigpens or hen houses. Furthermore, conventional control programs of insecticidal spraying of human dwellings should also apply insecticide in and around animal sheds.

INTRODUCTION

In Latin America, canine visceral leishmaniasis (CVL) is caused by infection with *Leishmania chagasi* and is usually transmitted by the bite of the phlebotomine sand fly *Lutzomyia longipalpis*.¹ In several Brazilian cities, CVL has become a serious health problem, and in localities where the disease is endemic, domestic dogs represent the main reservoir host for *Leishmania* infection in humans and other dogs.^{2–5} The prevalence of canine *Leishmania* infection (CLI) has been shown to vary from 1% to 36% in endemic areas in Brazil.^{2–4,6} In Jequié in northeastern Brazil, Paranhos-Silva and others⁷ have reported the average incidence of CVL to be 6.5 cases/100 dog-years, although the incidence varied markedly among town clusters. Several putative risk factors for CVL have been reported, including age, male sex, breed, fur length, outdoor lifestyle, and rural environment.^{4,8–10} The proximity of hen houses has also been acknowledged as a possible environmental risk for CVL, but the role chickens play in the transmission of *L. chagasi* has not been completely explained.^{11,12} Other domestic and wild mammals have been incriminated as either a meal source for the sand fly *Lu. longipalpis*, or as a possible reservoir.^{13–17} In a study in Colombia, cows and pigs were the preferred hosts of the phlebotomine.¹⁸ Although many studies have been published on possible risk factors for leishmaniasis infection in dogs living in areas endemic for CVL, the majority of them refer to prevalence data.^{4,8,9,19} Notwithstanding valuable to assess the burden of infection in a population, estimation of prevalence should not be used to measure infection risk. To determine risk, it is necessary to obtain incidence rates, which include only new events over a period of time. The identification of true determinants of *Leishmania* infection in dogs is crucial for designing and planning adequate prevention strategies in the canine population, and could lead ultimately to improved and successful control programs of zoonotic visceral leishmaniasis (ZVL). In this report, we estimated the incidence of *Leishmania* infection in a cohort of dogs, and determined whether incidence varied with age, sex, breed, and other animal and environmental characteristics.

METHODS

Study area. Jequié city is an endemic area for CVL. It is situated at 13°52'S and 40°4'W, 112 km from the Atlantic Ocean and 216 meters above sea level. The town has a surface area of 3,113 km². The population in 2000 was 147,202 inhabitants, with 88.5% living in the urban area and 11.5% in the rural zone. It is a region with a semi-arid tropical climate, with an annual average temperature of 24°C and rainfall of 500 mm yearly. The natural predominant vegetation consists of small deciduous trees, shrubs, cactus, and grasses. However, some rural areas of the municipality are covered by tropical rain forest or secondary woodland. We selected a borough (São Judas Tadeu) with 376 households and 1,873 inhabitants to perform the study because the prevalence of CLI there was greater than the average in other areas in Jequié (31.0% versus 23.5%, respectively³), and due to its isolated location in the periphery of the city with boundaries easy to demarcate.

Study design. In the baseline assessment (December 1997), we conducted a population-based canine survey of all domiciled dogs six months of age or older living in the study area. All seropositive dogs were painlessly eliminated, following guidelines of the Brazilian National Foundation of Health, and the remaining seronegative dogs were included in our initial cohort for follow-up. The results from the baseline analysis have been described elsewhere.²⁰ The study ran from December 1997 to July 2000, during which time four follow-up assessments were performed at approximately eight-month intervals. Since this was a dynamic cohort, at each follow-up survey new dogs that had immigrated to the study area were tested for *Leishmania* infection, and if seronegative, were included in the cohort. Otherwise, these dogs and those in the cohort that seroconverted were killed. Four hundred forty-seven dogs entered the cohort. The average lost to follow-up rate of the four follow-up surveys was 35.8%, and overall 202 (45.2%) dogs were not followed-up. Thus, the final analysis sample was comprised of 245 (54.8%) dogs. Of the 202 excluded dogs, 129 (63.9%) moved out of the area, 35 (17.3%) died, 1 (0.5%) had an owner who withdrew consent, and 37 (18.3%) had no information available.

Data and blood collection. Information on age, sex, breed, and other characteristics was gathered using a standardized questionnaire administered to consenting owners of each animal by trained and certified interviewers. The breeds were identified according to the American Kennel Club (New York, NY), which recognizes 147 breeds of dogs. Animals were arbitrarily defined as having short fur if their hair length was ≤ 3.0 cm, and long fur if otherwise. We also assessed the presence of henhouses, pigpens, or other livestock (bovine, swine, caprine, equine, or asinine) in the dwelling area (household adjacency). Degree of confinement was defined as "limited to the backyard" when they remained in the backyard most of the time to always or "free to roam" otherwise. All data were collected at study entry and updated at each follow-up assessment. Blood samples were collected by venipuncture from all animals at each survey.

Serology. We used an enzyme-linked immunosorbent assay to determine the presence of antibodies to *Leishmania*. This test has been previously validated and described elsewhere.³ Briefly, microtiter plate wells were coated with a soluble extract of *L. chagasi* promastigotes, sera of dogs were diluted 1:400, and a 1:5000 dilution of a goat anti-dog IgG peroxidase conjugate (Sigma Chemical Co., St. Louis, MO) was used. Positive and negative control sera were included in every assay. Values greater than the mean plus three standard deviations values of the results obtained from 102 healthy dogs were considered positive. All sera were tested in duplicate and those yielding positive results were retested at least once. In addition, all samples were tested blindly and data were analyzed without linkage to dog characteristics.

Statistical analysis. Incidence density rates for CLI were calculated as the number of new infections (defined as seroconversions at follow-up) divided by the number of dog-years of follow-up. Dog-years were defined as the number of years between the initial and the last assessment for each dog at risk for *Leishmania* infection. The crude incidence rate and respective 95% confidence intervals (CIs) were estimated, as well as incidence rates stratified by age, sex, breed, fur length,

degree of confinement, and absence or presence of livestock in the household backyard at study entry. Age-adjusted estimates of the relative risk (RR) of CLI by categories of the variables studied were computed using Cox regression.²¹ In addition to the age-adjusted models, full multivariate models were also fitted, and then non-significant ($P > 0.1$) variables were eliminated in a stepwise backward elimination algorithm, least significant first, to determine the final model.

RESULTS

The average age of the 447 dogs at study entry was 1.7 years (range = 0.5–14). The cohort was largely comprised of young, short-fur mongrel dogs. Selected characteristics of dogs in the whole cohort, in the analysis sample, and in the lost to follow-up group are shown in Table 1. This allows for comparison of variable distributions from cohort entry to follow-up, and assessment of how attrition and exclusion of selected groups might have affected the sample remaining in the cohort. Dogs in the analysis sample were similar to those lost to follow-up with regard to distribution of age, sex, and breed. However, animals lost to follow-up were more likely to have long fur and to be raised confined in the backyard than dogs in the analysis sample (Table 1).

The mean follow-up was 1.5 years (range = 0.6–2.6). Overall, 42 new cases of CLI were identified in 357.5 dog-years of follow-up, for a crude incidence rate of 11.8 cases per 100 dog-years (95% CI = 8.6–15.6). The risk of CLI seemed to increase after the dog's first year of life, but it remained stable thereafter (Table 2). The crude incidence of CLI was higher for dogs with short fur (RR = 4.7, 95% CI = 2.4–9.0), and among dogs living in a household with a pigpen in the backyard (RR = 3.3, 95% CI = 1.1–10.2). Sex, breed, degree of confinement, and presence of henhouses in the backyard were not significant predictors of incident CLI at the bivariate level. The interaction between dog's degree of confinement and presence of livestock in the backyard was also not statistically significant.

TABLE 1
Selected characteristics of dogs in the study cohort, Jequié, Brazil, 1997–2000

Characteristics	Cohort (n = 447)	Analysis sample (n = 245)	Lost to follow-up (n = 202)
Age (years)			
≤ 1	256 (57.3%)	127 (51.9%)	129 (63.9%)
2	85 (19.0%)	55 (22.4%)	30 (14.8%)
3	54 (12.1%)	35 (14.3%)	19 (9.4%)
≥ 4	52 (11.6%)	28 (11.4%)	24 (11.9%)
Sex			
Male	237 (52.8%)	135 (55.1%)	102 (50.5%)
Female	210 (47.2%)	110 (44.9%)	100 (49.5%)
Breed			
Mixed	404 (90.4%)	225 (91.9%)	179 (88.6%)
Pure	43 (9.7%)	20 (8.2%)	23 (11.4%)
Fur length			
Long	41 (9.2%)	14 (5.7%)	27 (13.4%)
Short	406 (90.8%)	231 (94.3%)	175 (86.6%)
Degree of confinement			
Limited to the backyard	178 (39.8%)	78 (31.8%)	100 (49.5%)
Free to roam	269 (60.2%)	167 (68.2%)	102 (50.5%)
Presence of livestock in the backyard			
Chickens	98 (21.9%)	46 (18.8%)	52 (25.7%)
Pigs	13 (2.9%)	6 (2.4%)	7 (3.5%)
Others	17 (3.8%)	8 (3.3%)	9 (4.5%)

TABLE 2

Crude and age-adjusted relative risks for *Leishmania* infection in 245 dogs according to selected characteristics, Jequié, Brazil, 1997–2000

Characteristics	Crude relative risk (95% confidence interval)	Age-adjusted relative risk (95% confidence interval)
Age (years)		
≤1	1 (referent)	
2	1.6 (0.8–3.4)	
3	1.8 (0.8–4.3)	
≥4	1.4 (0.5–3.6)	
Sex		
Female	1 (referent)	1 (referent)
Male	1.2 (0.7–2.2)	1.1 (0.6–2.1)
Breed		
Mixed	1 (referent)	1 (referent)
Pure	1.5 (0.5–4.6)	1.5 (0.5–4.9)
Fur length		
Long	1 (referent)	1 (referent)
Short	4.7 (2.4–9.0)*	6.4 (3.2–12.8)*
Degree of confinement		
Limited to the backyard	1 (referent)	1 (referent)
Free to roam	0.7 (0.4–1.3)	1.6 (0.8–3.0)
Presence of livestock		
Chickens		
No	1 (referent)	1 (referent)
Yes	1.5 (0.7–3.1)	1.6 (0.7–3.6)
Pigs		
No	1 (referent)	1 (referent)
Yes	3.3 (1.1–10.2)†	4.4 (1.3–14.8)‡
Others		
No	1 (referent)	1 (referent)
Yes	1.9 (0.8–4.3)	1.9 (0.8–4.6)

* $P < 10^{-3}$.

† $P = 0.03$.

‡ $P = 0.01$.

We then examined the age-adjusted associations between baseline potential risk factors and incident CLI. The adjusted RR of CLI for dogs with short fur compared with those with long fur was even higher than in the crude analysis (RR = 6.4, 95% CI = 3.2–12.8). The same was true for dogs living in a household with a pigpen in the backyard (RR = 4.4, 95% CI = 1.3–14.8) (Table 2).

Results of the full multivariate model analysis are shown in Table 3. Short fur was the strongest predictor of incident CLI in the final model. The presence of a pigpen in the backyard remained significantly associated with increased risk of CLI after controlling for other baseline variables. In addition, the presence of a henhouse or other livestock in the backyard was also found to significantly increase the risk of CLI in the multivariate model (Table 3). Again, the interaction of dog's degree of confinement and presence of animal sheds in the backyard did not reach statistical significance, and was dropped from the final model.

DISCUSSION

Despite the wealth of information on the prevalence and correlates of CLI, data on the incidence of CLI are still sparse, and little is known regarding risk factors for CLI. The overall incidence rate of CLI in our population (11.8 cases/100 dog-years) was similar to the annual incidence found on the Isle of Elba (12.4%)²² and in western Liguria (11.2%),¹⁰ both in Italy, but it was higher than previously reported estimates in the same area (6.6 cases/100 dog-years),⁷ and elsewhere in Brazil (6.4 cases/100 dog-years).⁴

Our data suggest that there is an increased risk of *Leishmania* infection after the dog's first year of life, but failed to demonstrate any further increment thereafter. The frequency of CLI has been shown to increase with age in previous cross-sectional studies.^{2,3,8,23} However, in these studies, increasing age-specific prevalence may have resulted from the cumulative sum of rather constant age-specific incidence, and the very nature of their design precludes inferences based on incidence rates. Consistent with our findings, Fisa and others²⁴ have reported that CLI incidence rates among dogs in Spain remained constant in all age groups.

Short fur was the strongest predictor of CLI in our study in both age-adjusted and multivariate analyses. In a study in southern Spain, dogs belonging to long-haired breeds showed a significantly lower seroprevalence for antibodies to *Leishmania* (20.9%) than those belonging to short-haired breeds (37.6%).²⁵ Similarly, Sideris and others²⁶ have also reported lower rates of infection in long-furred dogs (36.7%) than in short-furred animals (60.8%) during a survey in the Athens Basin in Greece. In Brazil, we have previously described a 1.4 fold increase in CLI prevalence among short-haired dogs in a cross-sectional study of CLI correlates.²⁰ França-Silva and others⁴ have also reported similar findings at another endemic area in Brazil. The apparent protection conferred to dogs with long fur may be due to the interference of their heavy fur on the sand fly's ability to land and bite. Conversely dogs with shorter or less fur would be more vulnerable to sand flies bite. Other potential factors such as CO₂ and heat irradiation may also be involved.

As described herein, dogs living in households with pigpens in the yards were at significantly greater risk for *Leishmania* infection. Pigpens are important blood-feeding, mating, and oviposition sites for *Lu. longipalpis*, as indicated by previous studies collecting recently emerged sand flies²⁷ or adult females with follicular sacs.²⁸ Moreover, in a study using emergence traps to locate larval microhabitats of *Lu. longipalpis*, 73.3% of the teneral *Lu. longipalpis* captured were from the soil at the edges of pigpens. In addition, the proportion of pigpen sites positive for *Lu. longipalpis* (62.5%) was signifi-

TABLE 3

Multivariate analysis of risk factors for *Leishmania* infection in 245 dogs in Jequié, Bahia, Brazil, 1997–2000

Characteristics	Relative risk (95% confidence interval)
Age (years)	
≤1	1 (referent)
≥2	1.8 (0.9–3.5)
Fur length	
Long	1 (referent)
Short	9.4 (4.3–20.7)*
Presence of livestock	
Chickens	
No	1 (referent)
Yes	3.3 (1.4–7.7)†
Pigs	
No	1 (referent)
Yes	4.1 (1.2–13.8)‡
Others	
No	1 (referent)
Yes	2.6 (1.1–6.6)‡

* $P < 10^{-4}$.

† $P < 10^{-2}$.

‡ $P < 0.05$.

cantly higher than other resting sites (10.5%).²⁷ In another study of the feeding pattern of sand flies, calculation of forage ratios for several vertebrates indicated that pigs were second only to cows as preferred hosts for *Lu. Longipalpis*.¹⁸ Although the attraction of pigs for *Lu. longipalpis* is quite evident, the role they may play in *Leishmania* transmission is unclear. Brazil and others¹⁶ have reported detection of numerous intracellular amastigote forms in a slide from a skin lesion biopsy of a pig, but they failed to isolate and identify the microorganism.

Our data also showed an increased likelihood of *Leishmania* infection in dogs with hen houses or other livestock raised in the yards of their living places. In a previous study in the same area, Rodrigues and others¹¹ have reported that dwellings of persons with visceral leishmaniasis were 4.21 times more likely to have chicken houses in the yard than those whose occupants were unaffected. Similarly, Morillas and others²⁵ showed a higher prevalence of *Leishmania* skin test positivity in villages where most of the houses have stables to keep animals (mainly mules and goats), as compared with villages in which houses rarely have stables. Nevertheless, chickens are unable to sustain infections with *Leishmania*, and the role different mammalian livestock may play in the transmission of the protozoan to other hosts has yet to be established.

Even without acting as reservoirs for *L. chagasi*, the presence of these domestic animals in close proximity to human dwellings may be important in maintaining a large peridomestic vector population. When the sand fly population density turns very high, it becomes more likely that a hungry fly enters a house, and bites dogs or humans. Of note, livestock (such as cattle, pigs, and chickens) are commonly raised in poor urban and periurban Brazilian communities. Many of these livestock are periodically brought to grazing areas for several days and then returned to their sheds in the residences' backyard. Furthermore, urban livestock populations fluctuate significantly depending on periods of trading and slaughter. In this scenario, a large *Lu. longipalpis* population may become abruptly deprived of its preferred hosts, and begin to bite dogs or humans more frequently, offsetting a potential zooprophylactic effect that the presence of these animals might have exerted. The resulting new feeding pattern by *Lu. longipalpis* would increase its vector capacity in this setting, and intensify *Leishmania* transmission to dogs and humans.

It has been proposed that nutrient availability determines the flight range of sand flies.²⁹ Previous studies have shown that *Lu. longipalpis* are capable of moving relatively long distances (up to 500–700 meters) over short periods of time.^{30,31} Despite that, in the context of our study area, an urban setting with randomly spaced animal sheds among human dwellings, it is likely that sand flies change their feeding habits and start biting dogs and men more often, when facing shortage of preferred hosts.

The prospective design of the present study allowed for proper calculation of incidence rates; thus, inferences were based on valid risk estimates. This study was to some extent an intervention study as well as an observational cohort study, since all seroconverting dogs were killed. One may argue this culling could have led to a decrease in incidence through time. However, the rates remained rather stable throughout the study period. The blind assessment of the study outcome pre-

vented observation bias to occur. Lastly, the use of multivariate analysis provided estimates of the effect of putative risk factors studied, net of age, sex, breed, and other confounders.

The main limitation of our study, and the Achilles' heel of cohort studies, was the losses to follow-up. The group of dogs with no follow-up information differed in important characteristics (such as presence of livestock in the backyard) that would put them at a higher risk for CLI than those in the analysis sample. Thus, while the estimate of CLI incidence obtained with the analysis sample is internally valid, it may underestimate the true incidence in the population. Nonetheless, the majority of dogs excluded had moved out of the study area, and emigration was not associated with the variables investigated. Therefore, most of the losses was non-differential and would not have distorted our results in a substantial manner.

The results of this study contribute information to the otherwise limited knowledge on the incidence of CLI. Better understanding of the epidemiology of CLI is crucial to plan effective control strategies in the canine population, and also for prevention of ZVL. Our data indicate that raising pigs, chickens, or other livestock in the adjacency of urban dwellings significantly increases the risk of CLI, suggesting that ZVL control measures should be directed towards modifying the environmental factors favoring contact between vectors, reservoirs, and susceptible humans, such as proximity of domiciles to pigpens or hen houses. Another possible strategy would be to reduce contact of dogs with animal sheds at night when vectors are most active. However, these approaches may not be feasible or may be difficult to implement. Alternatively, conventional control programs of insecticidal spraying of human dwelling should also apply insecticide in and around animal sheds in an attempt to reduce the sand fly population more effectively. Further studies are warranted to determine whether these approaches would improve current prevention/control programs.

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