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Cohort study on canine emigration and *leishmania* infection in an endemic area for american visceral leishmaniasis. Implications for the disease control

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Abstract

American visceral leishmaniasis is a main public health matter in Brazil. Since dogs have been incriminated as the main urban reservoir of AVL agent *Leishmania chagasi*, a cohort study aimed at understanding the dynamics of the canine infection was carried out in Jequié—an endemic community in the Northeast of Brazil. The inhabited urban and periurban areas of Jequié were divided into 140 clusters of 0.25 km^2. All 1681 dogs domiciled in 34 randomly selected clusters were screened for *Leishmania* antibodies in an enzyme-linked immunosorbent assay. After the seropositive dogs were painlessly eliminated, a cohort of 1286 seronegative dogs was followed up for 18 months, yielding a total of 1739.7 dog-years. The overall incidence of *Leishmania* infection, as assessed by the detection of *Leishmania* antibodies in blood samples collected every six months, was 6.55 cases/100 dog-years (95% confidence interval; CI 6.04–7.26). Two subsets of clusters, with 0.70 and 1.35 relative risks of infection, were identified. The annual emigration rate was 2.26 cases/100 dog-years (95% CI 1.86–2.66). The implications of these findings for the control of American visceral leishmaniasis are discussed.

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1. Introduction

American visceral leishmaniasis (AVL), caused by *Leishmania chagasi*, is endemic in many areas of Latin America (Grimaldi and Tesh, 1993). In Brazil, both the increasing number of reported cases (Marzoch et al., 1994) and the appearance of new foci (Jeronimo et al., 1994) are causes of great concern.

It has been proposed that sand flies, infected through feeding on foxes near human dwellings, subsequently transmit *L. chagasi* to dogs or human beings (Deane and Deane, 1955; Lainson, 1989). A peri-domestic/domestic cycle would be originated, in which dogs are the main reservoir of the parasite (Deane and Deane, 1955). Based on the assumption that AVL can be eradicated by the interruption of the peri-domestic/domestic cycle, the Brazilian government implemented an AVL control program relying mainly on the elimination of dogs seropositive for *Leishmania* antibodies. The program has not, however, checked the spreading of AVL in Jequié, an endemic focus in the Northeast of Brazil (Nascimento et al., unpublished data). A better understanding of the dynamics of canine leishmaniasis may contribute to the evaluation of existing and/or the development of more effective control strategies.

In this paper, the incidence of canine leishmaniasis in Jequié as well as the emigration rate of the canine population were investigated. The obtained data permit us a more critical vision on the current control strategy used in the Northeast of Brazil and highlight one of the factors contributing for the ineffectiveness of such strategy.

2. Materials and methods

2.1. Study area

The Jequié municipality, with a surface area of 3113 km^2, is situated at 13°52′ S and 40°4′ W, 112 km from the Atlantic Ocean and 216 m above sea level (Fig. 1, inset). It is a region of semi-arid tropical climate, with an annual average temperature of 24°C and a rainfall of 50 cm. The population in 1991 was 144 572 inhabitants, 79% of whom living in urban and periurban areas, where AVL is endemic (Oliveira-dos-Santos et al., 1993) and dogs are found infected with *L. chagasi* in a high prevalence (23.5% of dogs with circulating *Leishmania* antibodies in 1993) (Paranhos-Silva et al., 1996). These areas were divided into 140 clusters of 0.25 km^2 (Fig. 1).

2.2. Cohort and statistical analysis

A single-stage cluster sampling technique was used for selecting a sample from the dog population by a single random method (Cochran, 1977). The clusters were of unequal size in terms of canine population. In each of the 34 selected cluster, venous blood samples were collected from all domiciled dogs, with informed consent of the animal owners. The initial cohort was composed of 1681 dogs aged 6 months or older, included in a previous seroprevalence and parasitological study (Paranhos-Silva et al., 1996). All seropositive dogs were painlessly eliminated, following guidelines of the Brazilian National Foundation of Health, and the remaining 1286 dogs were followed up. Blood samples were collected at 6, 12 and 18 months, in order to estimate the incidence of the infection by the detection of *Leishmania* antibodies, and all dogs that seroconverted were killed. A dog was considered to be under risk of infection from the time of the first examination until three months before seroconversion was documented, or until the last time it was bled (in case of an unplanned interruption of follow up or death), or during 18 months for seronegative animals at the end of the study. The canine emigration rate in each selected cluster was determined, based on the number of dogs moving out of each cluster during the study period.

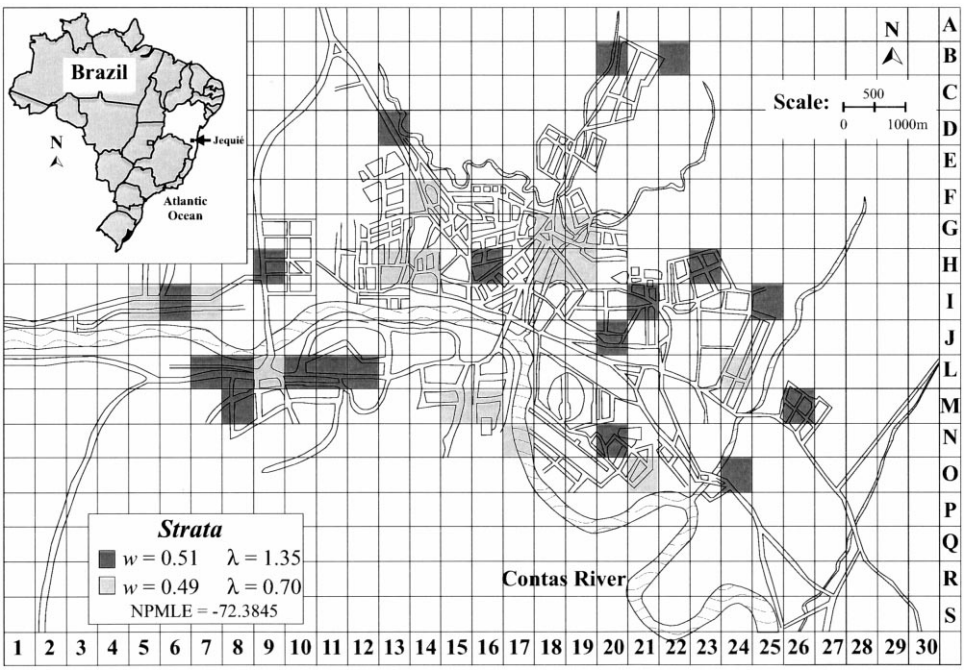

Fig. 1. Spatial distribution of canine *Leishmania* infection in Jequié, Bahia, Brazil. Data from a cohort domiciled among 34 sample clusters. Stratification was based on a mixture method within an empirical Bayes framework. The subpopulation relative risk is represented by λ. Inset: localisation of Jequié in Brazil.

The following formula was used for calculation of the incidence i, namely the unbiased estimate of I (incidence of *Leishmania* infection in the dog population under risk):

$$i = \sum_{\alpha=1}^{c} n_{i\alpha} / \sum_{\alpha=1}^{c} n_{\alpha},$$

where $n_{i\alpha}$ is the number of new cases and the n_{α} dog-years in the α^{Th} sample cluster, and c the number of sample clusters. Its variance was calculated in accordance with Cochran (1977) and used in calculations of confidence intervals (CI). Gender differences in incidence were analysed by the Cox–Mantel's test for survival data analysis (Lee et al., 1975).

A standardised incidence ratio SIR_{α}, denoting the relative risk of *Leishmania* infection in relation to living in each cluster, was defined as:

$$\text{SIR}_{\alpha} = \lambda_{\alpha} = O_{\alpha}/E_{\alpha}, \quad \textit{with} \quad E_{\alpha} = i.n_{\alpha},$$

where $O_{\alpha} = n_{i\alpha}$ are the observed cases and E_{α} the expected cases in the αth sample cluster. A cluster stratification was carried out using a mixture model with the nonparametric empirical Bayes' approach (Schlattmann et al., 1996).

The migration pattern was analysed using Tiago de Oliveira's statistic for heterogeneity (Poisson overdispersion) (Böhning, 1994). The spatial analysis and cluster stratification were modelled using DismapWin (Schlattmann, 1996). This is represented in Fig. 1, where strata's weight (w) and nonparametric maximum likelihood estimator (NPMLE) are given alongside the SIR.

The mean interruption of follow up in the clusters was 4.4% (95% CI 3.28–5.52). The overall annual mortality rate, excluding dogs sacrificed due to *Leishmania* infection, was 1.70 deaths/100 dog-years (95% CI 1.35–2.04), without overdispersion among clusters ($P = 0.95$, Tiago de Oliveira's statistic). In the studied community, there was no reliable source to figure out the dogs' age, what makes impossible a clear picture about the canine mortality pattern. Undoubtedly, the measured rate, much lower than one could expected, was influenced by the deliberated killing of seropositive dogs as well as by the emigration and interruption of follow-up.

2.3. Serologic assay

An ELISA has been described and validated elsewhere (Paranhos-Silva et al., 1996), and was carried out using a soluble extract of *L. chagasi* promastigotes, sera of dogs diluted 1:400 and a goat anti-dog IgG-peroxidase conjugate (Sigma, St. Louis, MO). Positive and negative control sera were included in every assay. Values above the mean of the results obtained with sera from 102 healthy dogs (from a non-endemic area) plus three standard deviations were considered positive. All sera were tested in duplicates and those yielding positive results were re-tested at least once.

Table 1
Incidence of canine *Leishmania* infection in clusters of the town of Jequié, State of Bahia, Brazil.

Sample cluster	No. of dogs	No. of seropositive dogs	Dog-years	Annual incidence
5-I	17	1	24.0	4.2[a]
6-I	23	2	30.8	6.5
7-I	37	2	54.0	3.7
7-L	37	3	46.8	6.4
8-L	18	2	24.0	8.3
8-M	5	2	2.5	80.0
9-H	17	2	22.5	8.9
9-L	18	1	26.3	3.8
10-L	30	7	37.8	18.5
11-L	40	4	54.8	7.3
12-L	8	1	10.5	9.5
13-D	18	1	16.0	6.2
13-H	72	1	104.3	1.0
14-F	126	10	158.0	6.3
14-H	73	5	107.3	4.7
15-M	48	3	68.0	4.4
16-H	44	4	58.0	6.9
16-M	73	5	103.3	4.8
17-N	47	2	60.7	3.3
18-G	37	1	54.0	1.8
18-H	37	3	49.3	6.1
19-G	56	2	81.3	2.5
19-H	60	3	86.0	3.5
20-B	2	1	2.8	36.4
20-J	118	15	158.8	9.4
20-N	55	8	69.0	11.6
21-I	69	9	94.8	9.5
21-O	30	1	43.5	2.3
22-B	12	2	15.0	13.3
23-H	19	3	24.8	12.1
24-L	10	0	15.0	0.0
24-O	16	4	20.5	19.5
25-I	12	3	13.5	22.2
26-M	2	1	1.8	57.1
Total	1286	114	1739.7	6.55

[a] No. of cases/100 dog-years.

3. Results

3.1. Emigration rate

The cohort of 1286 seronegative dogs (701 males, 581 females and four unknown) summed up to 1739.7 dog-years of observation (Table 1). Dog emigration occurred in 15 out of 34 studied clusters, with an overall rate of 2.26 cases/100 dog-years (95% CI 1.86–2.66), without heterogeneity among clusters ($P = 1.00$, Tiago de Oliveira's statistic) (Fig. 2).

3.2. Incidence

The overall annual incidence of *Leishmania* infection was 6.55 cases/100 dog-

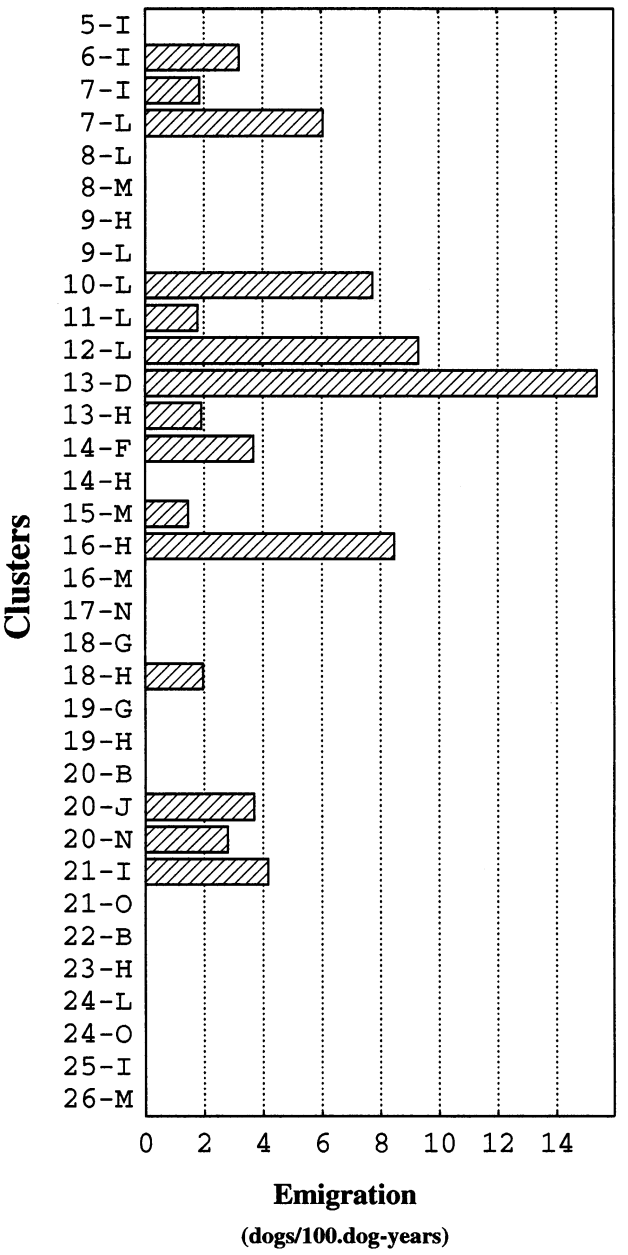

Fig. 2. Emigration rate of dogs in the town of Jequié, Bahia, Brazil.

years (95% CI 6.04–7.26), with $S_i^2 = 9.85 \times 10^{-6}$ (Table 1). The incidence for males (6.1 cases/100 dog-years; 95% CI 5.41–6.79) and for females (7.1 cases/100 dog-years; 95% CI 6.23–7.97) did not differ significantly ($P = 0.47$, Cox–Mantel test). Incidence was rather different through the town clusters, ranging from 0 to 80 cases/100 dog-years. The *SIR* stratification using the mixture method revealed a two-component pattern, composed of stratum 1 with relative risk $\lambda_1 = 1.35$ and $w_1 = 0.51$ (high risk) and stratum 2 with $\lambda_2 = 0.70$ and $w_2 = 0.49$ (low risk) (Fig. 1). When the prevalence (previous to the follow-up period) was included as a covariate no change was noted in the model, speaking against a direct relationship between that variable and *SIR*. Only one out of the eight clusters localised in downtown Jequié (clusters 14-F, 18-G, 19-G, 13-H, 14-H, 16-H, 18-H and 19-H, Fig. 1) was of high risk (high-/low-risk cluster ratio of 0.14), contrasting to the finding of 18 of these clusters among the 26 peripheral clusters (high-/low-risk cluster ratio of 2.25).

4. Discussion

The progressive urbanisation of AVL in Brazil during the last decade has been associated with human migrations to the periphery of large cities, where overcrowding and poor sanitation create an excellent habitat for insect vectors (Lainson, 1989). In these periurban and urban areas, dogs seem to be the major reservoir of the parasite (Deane and Deane, 1955), and their migration could indeed be responsible for the origin of new foci. The finding of a relatively high canine emigration rate in Jequié (2.3 cases/100 dog-years), described herein, is indicative of the extent to which infected canine reservoirs of the disease may be moving to non-endemic areas. The emigration was in some cases interurban and resulted from selling or gift, i.e. the dogs were not escorted by their owners (data not shown). The possibility that this may result in new AVL foci should be the subject of future investigations.

The incidence of the infection varied markedly among town clusters, suggesting a spatial heterogeneity in its transmission. The finding of a lower proportion of high-risk clusters in downtown Jequié in relation to the periphery could indicate possible differences in the transmission rates of infection in the two areas. This could not be ascribed to a relatively low of the canine population in low-risk areas, since quite the opposite was observed: mean densities of 253 and of 120 dogs/km^2 were found in central and peripheral clusters, respectively. An entomological survey covering the central and peripheral areas should ascertain whether the observed differences in relative risks could be attributed to differences in sand fly densities.

The control measures recommended by the Brazilian National Health Foundation require the screening for *Leishmania* antibodies of all dogs domiciled in areas within an 1 km radius of houses where human cases were reported, followed by sacrifice of the seropositive dogs. The present results, however, clearly show the distribution of canine infection throughout Jequié, including in places where human cases have not been reported (data not shown). Therefore, the strategy being used

is neglecting an unknown, but potentially large, number of infected domiciled dogs, which could serve as reservoir for the reintroduction of the infection into areas previously freed from *Leishmania*-antibody positive dogs. This is made more likely by the fact that dogs in Jequié are seldom confined: they often circulate freely throughout the neighbourhoods (Paranhos-Silva et al., unpublished data). The situation is further complicated by the existence of an unknown number of homeless dogs, the majority of which (around 70%) seropositive for *Leishmania* antibodies (Paranhos-Silva et al., unpublished data). These facts may easily explain the reoccurrence, described herein, of canine infection in 33 out of the 34 studied clusters, resulting in an overall incidence of 6.55 cases/100 dog-years, despite the elimination of all domiciled seropositive dogs previous to the study as well as those that seroconverted. Indeed, in spite of the elimination of around 15% of the canine population in Jequié by the governmental AVL control program, referred to above, in the last 5 years (Brazilian National Health Foundation, Jequié Office, 1996), the number of reported human AVL cases in Jequié has increased steadily for the last 4 years, without any changes in notification procedures: 8, 25, 123 and 142 cases in 1993, 1994, 1995 and 1996, respectively (Nascimento et al., unpublished data). The inefficacy, as a method for controlling AVL, of the simple elimination of infected dogs has also been recently suggested with the use of a mathematical model (Dye, 1996).

Further investigations in Jequié and in other AVL foci, with vector and vertebrate reservoir surveys, will be necessary to fully unveil the dynamics of *L. chagasi* transmission. This should be fundamental for the designing of new control programs and of future vaccine trials.

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