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Assessment of an optimized dog-culling program in the dynamics of canine *Leishmania* transmission

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Abstract

In Brazil, zoonotic visceral leishmaniasis (ZVL) control programs based on the mass elimination of seropositive dogs have failed to reduce the number of leishmaniasis cases. However, these programs have been done under sub-optimal conditions. We studied a cohort of dogs in an urban area in Brazil to determine, whether a dog-culling program optimized with: (i) replacement of a relatively low-sensitivity indirect immune-fluorescent test on blood eluate by a more sensitive enzyme-linked immunosorbent assay on serum blood samples; (ii) shortening of the time interval from serodiagnosis to removal of dogs; (iii) screening a high proportion of the dog population could reduce the incidence of canine *Leishmania* infection (CLI). The study ran from December 1997 to July 2000, with four follow-up assessments performed at approximately 8-month intervals. All dogs seropositive for anti-*Leishmania* antibodies were promptly eliminated. A large number of new dogs immigrated to the study area throughout the study period. They comprised 43.8–49.8% of the cohort at each follow-up assessment, and upto 15% of them already had *Leishmania* infection. Overall, 42 news cases of CLI were identified, for a crude incidence rate of 11.8 cases per 100 dog-years (95% CI 8.6–15.6). In the first, second, third and fourth follow-up assessments the incidence rates were 8.2 (95% CI 3.0–17.9), 12.2 (95% CI 6.3–21.2), 16.4 (95% CI 8.5–28.6) and 13.6

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(95% CI 7.1–23.8), respectively. There was no statistically significant change in these rates throughout the study period. Our results suggest that dog-culling programs do not reduce the incidence of CLI, even with an optimized intervention. Possible reasons for this failure include: currently available serologic methods lack sufficient sensitivity and/or specificity to accurately identify all infected dogs warranting removal in order to prevent *Leishmania* transmission; destroyed dogs are immediately replaced by susceptible puppies, and quite often, by already infected dogs; and other reservoirs may be involved in maintaining canine infection. Further efforts on ZVL control should be directed to developing new strategies or to testing control methods already in place with properly designed trials.

Keywords: Incidence; Canine visceral leishmaniasis; *Leishmania* infection; Control; Prevention; Cohort study

1. Introduction

Canine visceral leishmaniasis (CVL) was first documented in Tunisia by Nicolle and Comte (1908). In Latin America, CVL is caused by infection with *Leishmania chagasi* (Maurício et al., 1999) and is usually transmitted by the sandfly *Lutzomyia longipalpis*. In Brazil, the prevalence of canine infection in endemic regions ranges from 1 to 36%, and reaches upto 67% in certain areas (Coutinho et al., 1985; Paranhos-Silva et al., 1996; França-Silva et al., 2003).

Domestic dogs are believed to be the main vertebrate reservoir for zoonotic visceral leishmaniasis (ZVL), a major public health problem in tropical America. Over the past 5 years, the Brazilian Ministry of Health reported a national annual average of 3500 cases, and ZVL is acknowledged as an important emerging disease. In developed areas such as the Mediterranean, it is both a medical and a veterinary problem, whereas in developing areas it is primarily a medical problem (Tesh, 1995).

Control programs in Brazil have focused on the mass elimination of seropositive dogs. However, Brazilian National Health data for past decades show that widespread culling of seropositive dogs does not reduce the number of human cases (Vieira and Coelho, 1998). This has prompted a reassessment of the dog control policy in Brazil (Costa and Vieira, 2001). Although, some studies suggest that dog elimination control programs are ineffective (Dietze et al., 1997; Courtenay et al., 2002), it is questionable, whether limitations of the intervention methods used in these studies, such as the use of low-sensitivity screening assays and delays in removing seropositive dogs, might have been responsible for the apparent failure of this control strategy. In order to contribute to solving this controversy, we conducted a prospective cohort study to determine the impact of an optimized dog-culling program on the incidence of canine *Leishmania* infection (CLI).

2. Methods

2.1. Study area

The study site is located in the city of Jequié, Bahia, Brazil, an endemic area for CVL, with a population of 1,47,202 inhabitants (Instituto Brasileiro de Geografia e Estatística, 2001). We selected a borough (São Judas Tadeu) with 376 households and 1873 inhabitants

to perform the study, because its CLI prevalence was high (31%) and its isolated location on the periphery of the city made boundaries easy to demarcate (Paranhos-Silva et al., 1996; Moreira et al., 2003).

2.2. Study design

In the baseline assessment (December 1997), we conducted a population-based canine survey of all domiciled dogs, aged 6 months 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 study ran from December 1997 to July 2000, during which time four follow-up assessments were performed, at approximately 8-month intervals. This was a dynamic cohort since, at each follow-up survey, new dogs that had immigrated to the study area were tested for *Leishmania* infection, and if seronegative, included in the cohort. Otherwise, these dogs and those in the cohort that seroconverted were killed within 14 days of the survey.

2.3. Data and blood collection

Information on age, sex, breed, and other characteristics was gathered using a standardized questionnaire, administered to the consenting owners of each animal by trained and certified interviewers. All data were collected at study entry and updated at each follow-up assessment. Blood samples were collected by venipuncture from all animals available at each survey.

2.4. Serology

An enzyme-linked immunosorbent assay (ELISA), which had been previously validated and described elsewhere (Paranhos-Silva et al., 1996), was used to determine the presence of anti-*Leishmania* antibodies. Positive and negative control sera were included in every assay. All sera were tested in duplicate and those yielding positive results were retested at least once.

2.5. 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 (dogs with only one assessment did not contribute any time to the total of dog-years). The overall incidence rate as well as the specific rates for each follow-up interval were estimated with respective 95% confidence intervals (CI).

3. Results

The mean 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

Table 1
Selected characteristics of dogs in the study cohort, Jequié, Bahia, 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%)
Gender			
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%)

the whole cohort, in the analysis sample (comprised by dogs with at least two assessments), 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 the distribution of age, gender, and breed. Yet, 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 average lost to follow-up rate of the four follow-up surveys was 35.8%, ranging from 29.8 to 47.9% (Table 2). Of the 202 dogs excluded, 129 (63.9%) moved out of the area, 35 (17.3%) died, 1 (0.5%) had owner's consent withdrawn, and on 37 (18.3%) there was no information available. A large number of new dogs immigrated to the study area throughout the study period. They comprised 43.8–49.8% of the cohort at each follow-up assessment

Table 2
Population dynamics in a cohort of dogs in Jequié, Brazil, 1997–2000

Assessment	Number of dogs in the cohort	Proportion (%) of dogs lost to follow-up	Proportion (%) of immigrant dogs	Seropositivity (%) among immigrant dogs
Baseline (December 1997)	181	–	–	–
Follow-up 1 (July 1998)	223	29.8 (54/181)	49.8 (111/223)	13.5 (15/111)
Follow-up 2 (March 1999)	231	34.1 (74/217)	43.7 (101/231)	12.9 (13/101)
Follow-up 3 (November 1999)	195	47.9 (106/219)	49.2 (96/195)	14.6 (14/96)
Follow-up 4 (July 2000)	239	31.7 (58/183)	49.8 (119/239)	4.2 (5/119)

Table 3
Incidence density rates of canine *Leishmania* infection in a cohort of dogs in Jequié, Brazil, 1997–2000

Assessment	Number of dogs in the cohort	Number of incident cases	Dog-years	Incidence/100 dog-years (95% CI)
Baseline (December 1997)	181	–	–	–
Follow-up 1 (July 1998)	223	6	72.8	8.2 (3.0–17.9)
Follow-up 2 (March 1999)	231	12	98.7	12.2 (6.3–21.2)
Follow-up 3 (Novemer 1999)	195	12	73.3	16.4 (8.5–28.6)
Follow-up 4 (July 2000)	239	12	88.0	13.6 (7.1–23.8)

(Table 2). Upto 15% of the dogs immigrating to the study site were already seropositive for *Leishmania* antibodies.

The average follow-up time 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 specific incidence rates for each follow-up interval are shown in Table 3. Although, the incidence rate of CLI in the first follow-up interval seemed smaller than the rates in the following assessments, there was no statistically significant change in the incidence of CLI throughout the study period.

4. Discussion

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%) and in western Liguria (11.2%), both in Italy (Gradoni et al., 1988; Zaffaroni et al., 1999), but it was higher than previously reported estimates in the same area (6.6 cases/100 dog-years) (Paranhos-Silva et al., 1998), and elsewhere in Brazil (6.4 cases/100 dog-years) (França-Silva et al., 2003). Despite strict adherence to a well-defined protocol to screen and eliminate seropositive dogs promptly, the rates of *Leishmania* infection have not decreased throughout the study period, but instead remained stable. Thus, our findings do not support the removal of serologically positive dogs as a strategy to reduce the incidence of CLI, and consequently, argue against using this measure in ZVL control programs. Some earlier studies have also questioned the effectiveness of killing infected dogs (Alencar et al., 1974; Dye, 1996; Dietze et al., 1997; Courtenay et al., 2002). Nevertheless, Ashford et al. (1998) found that culling dogs was partially successful, and Palatnik-de-Sousa et al. (2001) reported that the removal of seropositive dogs led to a significant reduction of the incidence of canine and human *Leishmania* infection. However, even when such conclusions are drawn from controlled trials, the methodological shortcomings in the study design, analysis or in the conduction of the interventions invariably impair the interpretation and validity of these data.

Numerous obstacles exist that prevent dog-culling programs from achieving good disease control. Limitations including long intervals between serodiagnosis and dog removal, lower than ideal sensitivity and/or specificity of currently available screening tests, and owner's unwillingness to give up seropositive dogs are difficult to overcome, further complicating these interventions. Moreover, a pivotal reason for the failure of dog-culling to control

CVL is the lack of a reliable test to identify infectious rather than infected dogs. This is an important distinction because a proportion of infected dogs might never become infectious, thus their removal would be actually counterproductive, since they may be replaced by susceptible dogs that do become infectious. In addition, other reservoirs may be involved in maintaining canine infections (Gradoni et al., 1983; Corredor et al., 1989), and interventions aimed at an individual species may be less effective when multiple reservoirs are involved in transmission.

We found a high turn-over of dogs in the study area, where destroyed dogs were immediately replaced by susceptible puppies. Furthermore, a fraction of the dogs immigrating to the study site was already infected. This combination would negatively affect the efforts aimed at reducing the prevalence of CLI in the dog population and might have also played an important role in the failure of the dog-culling program. Indeed, in this scenario, modeling theory indicates that a high proportion of infectious dogs should be killed in order to produce a marked reduction in disease transmission (Dye, 1996). It might be the case, that such an effective performance cannot be accomplished even with an optimized intervention such as the one described herein. Thus, this advises against recommending a dog-culling intervention as a nation-wide ZVL control strategy, since such programs are too laborious and therefore, likely to be less thorough and effective when applied in a large-scale.

4.1. Methodological merits and limitations

The main strength of this study was its prospective design that allowed for proper calculation of CLI incidence rates at short time intervals. In addition, the study area was surveyed thoroughly and we achieved a high coverage of the dog population. The losses to follow-up were mainly due to emigration from the study area and are likely to be non-differential, thus it is improbable that selection bias might have distorted our results. Also, all seroconverted dogs were killed promptly, avoiding long intervals between serodiagnosis and dog removal.

The main limitation was that we could not establish a control site. Ethical considerations precluded that we surveyed dogs for anti-*Leishmania* antibodies in a control area without removing the seropositive animals. The inclusion of a control group would have supplied the expected rate of CVL transmission in the absence of the intervention. Nonetheless, the lack of a comparison group should not compromise the interpretation of our findings, since the infection rates remained stable throughout the study suggesting that the intervention has not prevented *Leishmania* transmission in the study area.

4.2. Conclusions and public health implications

We conclude that dog-culling programs do not reduce the incidence of CLI, even when the intervention is optimized with: (i) replacement of a relatively low-sensitivity indirect immune-fluorescent test on blood eluate by a more sensitive ELISA test on serum blood samples; (ii) shortening of the time interval from serodiagnosis to removal of dogs; (iii) screening a high proportion of the dog population. Possible reasons for this failure include: currently available serologic methods lack sufficient sensitivity and/or specificity to accurately identify all infected dogs warranting removal in order to prevent *Leishmania* transmission; destroyed dogs are immediately replaced by susceptible puppies, and quite

often, by already infected dogs; and other reservoirs may be involved in maintaining canine infection. Further efforts on ZVL control should be directed towards developing new strategies, such as a canine or human vaccine against *Leishmania*, and towards conducting properly designed ZVL control trials to evaluate the effectiveness of other available control approaches, such as residual insecticide spraying or the use of insecticidal dog collars. The fundamental role of human susceptibility to leishmaniasis, with focus on the alleviation of malnutrition, also remains to be investigated. Of utmost importance, cost-effectiveness studies should always be performed before broad control measures are recommended and adopted nationwide.

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