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## Infectiousness in a Cohort of Brazilian Dogs: Why Culling Fails to Control Visceral Leishmaniasis in Areas of High Transmission

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The elimination of seropositive dogs in Brazil has been used to control zoonotic visceral leishmaniasis but with little success. To elucidate the reasons for this, the infectiousness of 50 sentinel dogs exposed to natural *Leishmania chagasi* infection was assessed through time by xenodiagnosis with the sandfly vector, *Lutzomyia longipalpis*. Eighteen (43%) of 42 infected dogs became infectious after a median of 333 days in the field (105 days after seroconversion). Seven highly infectious dogs (17%) accounted for >80% of sandfly infections. There were positive correlations between infectiousness and anti-*Leishmania* immunoglobulin G, parasite detection by polymerase chain reaction, and clinical disease (logistic regression,  $r^2 = 0.08-0.18$ ). The sensitivity of enzyme-linked immunosorbent assay to detect currently infectious dogs was high (96%) but lower in the latent period (<63%), and specificity was low (24%). Mathematical modeling suggests that culling programs fail because of high incidence of infection and infectiousness, the insensitivity of the diagnostic test to detect infectious dogs, and time delays between diagnosis and culling.

In Latin America, zoonotic visceral leishmaniasis (ZVL) is caused by infection with *Leishmania chagasi* (*Leishmania infantum* [1]) and is usually transmitted by the sandfly *Lutzomyia longipalpis*. ZVL is a major public health problem, particularly in Brazil, where 2000–3000 cases are reported per year [2]. Domestic dogs are the principal reservoir hosts, and control programs in Brazil focus on the mass elimination of seropositive dogs and residual insecticide spraying. However, Brazilian national health records show that widespread culling (i.e., the elimination of 176,000 seropositive dogs during 1990–1997) has not been associated with a reduction in the number of human cases [2]. It has thus been suggested that there is a need to reassess dog control policy in Brazil [3–5], although this conclusion has been disputed [6]. There have been no replicated, controlled trials of current dog culling methods, and the 3 published field trials have used more sensitive diagnostic techniques and more efficient dog removal regimes than those used in practice by public health services. Two trials did show some reduction, but not elimination, of canine [7] or canine and hu-

man infection [8], whereas the third trial did not reduce either human or canine infection rates [9].

There are a number of possible reasons for the apparent failure of dog culling programs to control ZVL. Any effect of dog culling may have been masked by local or temporal variation in disease incidence [6]. However, theory shows that dog culling is a relatively ineffective control method, requiring a high proportion of dogs to be killed to achieve a marked reduction in disease transmission [4]. The effort required will depend on a number of population parameters, in particular the basic reproduction number ( $R_0$ ) of the infection, the latent period before dogs become infectious, and the proportion of dogs that become infectious [4]. The effectiveness of culling will depend on accurate diagnosis, and available serologic tests may not reliably identify all infectious dogs, particularly those dogs in the latent period between infection and the development of infectiousness. Moreover, there is a long interval between serodiagnosis and culling [7], which will further reduce the effective sensitivity of diagnosis. Current culling programs aim to remove all infected dogs, rather than only infectious dogs. If a proportion of infected dogs never become infectious, their removal may be counterproductive, because they may be replaced by susceptible dogs that do become infectious. Thus, the specificity of serodiagnosis to identify infectious dogs may be important. Finally, although neither asymptomatic humans nor crab-eating foxes (*Cerdocyon thous*) appear to be important for transmission, it is possible that other hosts, such as opossums (*Didelphis* species), have an epidemiologic role [10–12].

A better understanding of the population dynamics of infectiousness in dogs is required to improve dog culling strategies

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or to develop novel control programs. To date, published studies of infectiousness in ZVL have been cross-sectional and/or limited to a few dogs [13–21]. Here, we describe a longitudinal study of infectiousness in a cohort of 50 dogs exposed to natural *L. chagasi* infection in Amazon Brazil. The aims of the study were to determine the proportion of infected dogs that became infectious and the extent of variation in infectiousness between dogs; the latent period between infection and infectiousness; the relationship between infectiousness and immunologic, parasitologic, and clinical parameters; and the sensitivity of diagnostic tests for the identification of infectious and noninfectious dogs. Finally, we sought to use these parameter estimates in mathematical models to simulate the effect of dog culling.

**Materials and Methods**

*Study design.* Field work was conducted in the municipality of Salvaterra, Marajó Island, Pará, Brazil. The study area and design are described in detail elsewhere [22, 23]; there is a high incidence of canine infection ( $8.66 \times 10^{-3}/\text{day}$ ) [23]. In brief, 126 uninfected, sentinel dogs were placed in the field in 8 cohorts and were exposed to natural disease transmission. Each cohort was sampled for 4–13 sampling rounds between April 1993 and July 1995 at a mean interval of 67.3 days. To investigate infectiousness to the sandfly vector, 50 of the 126 study dogs were exposed to female laboratory-bred *L. longipalpis* on a mean of 3.5 occasions (feeds) per dog (range, 1–12 feeds). The mean age of these dogs at the start of the study was 4.1 months; 15 dogs were male, and 34 were female (1 not sexed).

*Sandfly colonies.* First- and second-generation laboratory-bred *L. longipalpis* adults were used for xenodiagnosis from 2 colonies established and replenished with sandflies caught in the study site. A smaller number of 80th-generation adults from a colony established with flies from Santarém, Pará, were also used. *L. longipalpis* from Marajó and Santarém are very similar genetically

and have the same pheromone type [24, 25]. Colonies were maintained, as described elsewhere [26].

*Xenodiagnosis.* Dogs were placed into individual cages ( $0.75 \times 0.75 \times 2$  m) sheathed in sandfly-proof netting. For each feed, a mean of 75.6 (SE, 4.1; range, 8–160) unfed female sandflies (2–3 days after emergence) and an approximately equal number of male sandflies were introduced into the cage and were allowed to feed overnight (1900–0700 h). Visibly engorged flies were then placed into Barraud cages or tubed individually, with sugar solution provided ad libitum. Four to 5 days after the blood meal, flies were dissected and examined for visible promastigotes in the gut under  $\times 100$  magnification. A total of 6002 female sandflies were dissected from 173 feeds, with a mean of 34 flies per feed (SE, 1.9; range, 1–96 flies) surviving to dissection. On the day after xenodiagnosis, blood and bone marrow samples were obtained, as described elsewhere [23].

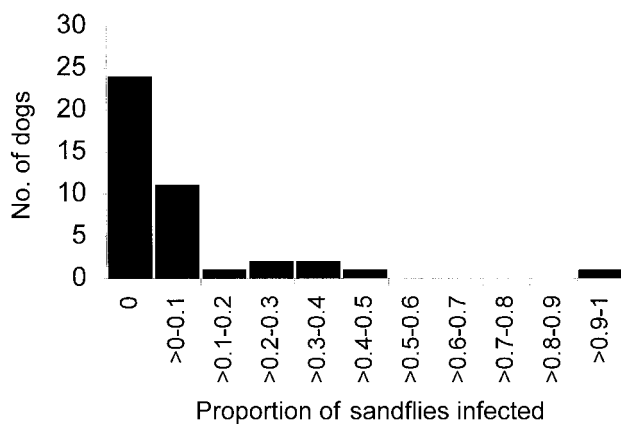
*Clinical examination and polymerase chain reaction (PCR).* Dogs were examined for 6 signs of canine ZVL: alopecia, dermatitis, chancres, conjunctivitis, onychogryphosis (excessive nail growth), and lymphadenopathy (enlarged popliteal lymph nodes), and each was scored on a semiquantitative scale from 0 (absent) to 3 (severe). Scores were added to give an overall clinical score. Dogs that developed severe disease were killed with sodium pentobarbital. For parasitologic diagnosis, DNA was extracted from bone marrow samples and was amplified by PCR, as described elsewhere [27].

*Immunologic assays.* Lymphoproliferative responses to *L. chagasi* crude antigen were measured as a stimulation index, as described elsewhere [27], with a stimulation index of  $>5$  considered positive. IgG and IgG subclass responses to *L. chagasi* crude antigen were measured by ELISA [27, 28], and results were expressed as arbitrary units per milliliter calculated from a reference serum sample. The date of seroconversion was determined from changes in antibody levels through time, as described elsewhere [23].

*Definition of infection.* Dogs were considered to be infected if they tested positive by parasite culture, PCR, or serology [27]. Because the date of infection was unknown, the date of patent infection (i.e., the first date when a dog tested positive by parasite culture, PCR, or serology) was used in all analyses; the true date of infection would have been earlier, because there was a significant prepatent period [23].

*Statistical analysis.* Infectiousness was analyzed as a binary variable (infectious or not) or, when indicated, as the proportion of sandflies infected. Data were analyzed by univariate and multivariate logistic regression. In multivariate analysis, the minimum significant model was achieved by backward elimination of non-significant explanatory variables ( $P > .05$ ). Linear and nonlinear variation in infectiousness with time were examined by fitting a model including time and time<sup>2</sup>.  $r^2$  values were calculated as the proportion of total deviance explained by each variable. When nonindependent repeat samples from the same dogs were analyzed, general estimating time series equations with robust SEs were used to control for autocorrelation. All analysis was done with Stata software, version 6.0 [29].

*Sensitivity and specificity of diagnostic techniques.* We assessed the sensitivity of serologic, parasitologic, and clinical parameters for the detection of latently infectious dogs (in the period between patent infection and infectiousness), infectious dogs (all samples



**Figure 1.** Variation in infectiousness of dogs infected with *Leishmania chagasi*. The frequency distribution of proportion of sandflies (*Lutzomyia longipalpis*) infected (total no. positive/total no. dissected) by 42 infected dogs.

after the development of infectiousness), and infected but never-infectious dogs. All postinfection samples from each dog were included. Two definitions of seropositivity were used, one on the basis of our longitudinal seroconversion data and one on the basis of a typical cutoff of mean + 3 SD of uninfected dogs (4006 U/mL) [23]. Dogs with a clinical score  $\geq 7$  (polysymptomatic) were considered to be positive by clinical examination.

**Simulation of dog culling.** Following [4], we divided the total number of dogs ( $D$ ) into ever-infectious dogs (that become infectious) and never-infectious dogs. Ever-infectious dogs can be uninfected ( $S$ ), latent (infected but not infectious;  $L$ ), or infectious ( $I$ ); never-infectious dogs are either uninfected ( $R$ ) or infected ( $Q$ ). Changes in the numbers of these dogs through time are given by the following formulas:

$$\frac{dS}{dt} = \alpha\beta D - \frac{CIS}{D} - \delta S,$$

$$\frac{dL}{dt} = \frac{CIS}{D} - (\sigma + \delta)L,$$

$$\frac{dI}{dt} = \sigma L - \delta I,$$

$$\frac{dR}{dt} = (1 - \alpha)\beta D - \frac{CIR}{D} - \delta R,$$

and

$$\frac{dQ}{dt} = \frac{CIR}{D} - \delta Q,$$

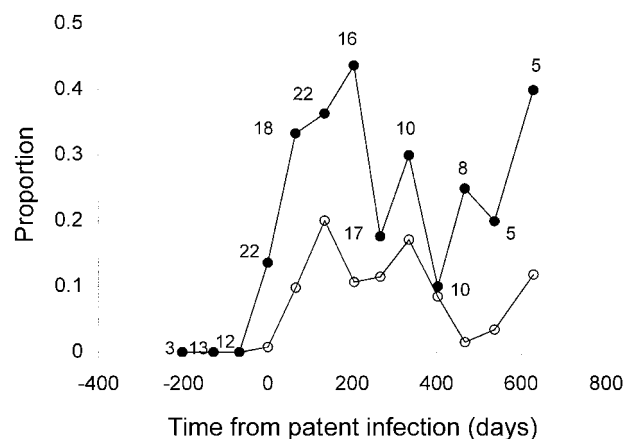
where  $\alpha$  is the proportion of dogs born susceptible,  $\beta$  is the dog birth rate,  $C$  is vectorial capacity,  $\delta$  is the dog death rate, and  $\sigma$  is the rate at which latent dogs become infectious. For simulation of the precontrol situation, difference equations based on equations 1–5 were used with a time interval of 1 day and the simulation run until equilibrium. The effect of annual diagnosis and pulse culling of dogs was incorporated by removing proportions  $p_L$ ,  $p_I$ , and  $p_Q$  of the latent, infectious, and never-infectious infected dogs each year, where  $p$  is the proportion of that class of dogs that can be detected by a given diagnostic test. The total dog population was kept constant by replacing culled dogs with uninfected dogs. The effect of a time delay between diagnosis and culling of infected dogs was simulated by splitting  $L$ ,  $I$ , and  $Q$  dogs into 2 groups after diagnosis, detected and undetected, according to the sensitivity of the test. Thereafter, until culling, dog status was allowed to change as usual, with the exception that no dogs could enter the detected groups. Parameter estimates were  $\alpha = 0.43$  (this study),  $\beta = \delta = 0.0011$  per day in [30],  $\sigma = 0.0050$  per day (this study). A value of  $C$  was chosen to produce the observed average time to infection of 115 days before culling [23]. The basic reproduction number ( $R_0$ ) was thus 8.9; this is higher than our previous estimate [23], which used a less accurate estimate of dog mortality rate. Simulations considered 2 diagnostic tests, a high-sensitivity test such as ELISA ( $p_L = 0.625$ ,  $p_I = 0.964$ , and  $p_Q = 0.758$ ) and a low-sensitivity test, such as indirect immunofluorescent antibody test with filter paper eluates, with a sensitivity of 46% of ELISA ( $p_L = 0.288$ ,  $p_I = 0.443$ , and  $p_Q = 0.349$ ) [7], and 2 time delays, 0

and 120 days, between detection and culling. Simulations were done in Microsoft Excel.

## Results

**Variation in infectiousness to sandflies between dogs.** Among 50 dogs, 43 became infected with *L. chagasi* during the study, of which 36 were positive by parasitology (PCR or parasite culture) and serology, 3 by parasitology, and 4 by serology. Twenty-four dogs underwent xenodiagnosis (40 feeds) when apparently uninfected, of which 17 were tested before patent infection and 7 never showed evidence of infection. None of 1322 sandflies were infected during these 40 feeds. Of the 43 dogs that became infected, 42 underwent xenodiagnosis at or after the development of patent infection; of these, 18 (42.9%) were infectious on at least 1 occasion after infection. The proportion of infected sandflies was 501 (10.7%) of 4680 in all 133 feeds and 501 (27.5%) of 1822 in the 36 infectious feeds only. There were significant differences between dogs in the proportion of sandflies infected ( $\tau = 3.37$ ;  $P < .01$ ), and only 7 (17%) of the 42 dogs transmitted infection to >10% of the total number of sandflies that fed on them (figure 1).

**Variation in infectiousness through time.** The proportion of infectious dogs increased from 0 before patent infection to a mean of 26.8% (95% confidence interval [CI], 17.5–38.6) after patent infection, with no significant variation through time from patent infection in infected dogs (figure 2; table 1). The mean proportion of flies infected (by all dogs that underwent xenodiagnosis) reached a peak of 20% at 135 days after patent infection and declined significantly thereafter (slope vs. time = .0062,  $P = .028$ ; slope vs. time<sup>2</sup> = -.000011,  $P = .012$ ). No dog was infectious before seroconversion; infectious dogs became infectious a median of 105 days (95% CI, 59–144 days) after seroconversion, 135 days (95% CI, 61–186 days) after patent infection, and 333 days (95% CI, 255–400 days) after the dogs were placed in the



**Figure 2.** Variation in proportion of infectious dogs (●) and proportion of sandflies infected (○) with time since patent infection. Nos. of dogs examined by xenodiagnosis at each time point are shown.

**Table 1.** Relationships between probability of being infectious and immunologic, clinical, or parasitologic parameters and age or sex of infected dogs.

Variable	n	Odds ratio	P	r <sup>2</sup>
Sex	133	0.914	NS	—
Age, months	133	1.004	NS	—
Time, days <sup>a</sup>	133	1.002	NS	—
Time <sup>2</sup> , days <sup>a</sup>	133	1.000	NS	—
PCR	96	5.494	.039	0.123
Clinical score	126	1.139	.0095	0.083
Anti- <i>Leishmania</i> <sup>b</sup>				
IgG	133	3.080	.0002	0.183
IgG1	104	2.808	.0019	0.145
IgG2	100	4.453	NS	—
IgG3	104	2.559	.0008	0.100
IgG4	104	1.975	.015	0.080
Proliferative response	99	0.776	NS	—

NOTE. n, no. of sandfly feeds; NS, not significant ( $P > .05$ ); PCR, polymerase chain reaction.

<sup>a</sup> Time since patent infection.

<sup>b</sup> Log-transformed.

study site. Previously, we have estimated that seroconversion occurs on average 94 days after infection [23], giving a latent period between infection and infectiousness of  $94 + 105 = 199$  days.

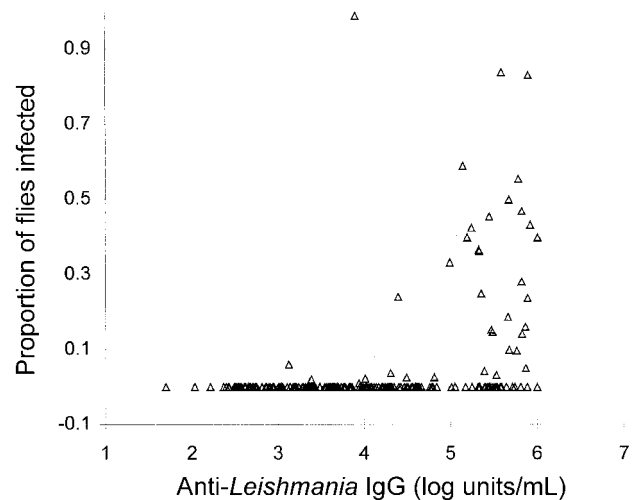
*Correlates of infectiousness in infected dogs.* The probability that an infected dog was infectious significantly increased with the strength of its anti-*Leishmania* IgG response and its total clinical score and was higher for PCR-positive dogs (table 1). No association was observed between the probability of infectiousness and sex, age, time since patent infection, or cellular immune response. The strongest correlation was with anti-*Leishmania* IgG, which explained 18.3% of the total variation in infectiousness: Infected dogs with  $<5.0$  log units of IgG were infectious in 10 (11.8%) of 85 feeds, compared with 26 (54.2%) of 48 feeds on dogs with titers  $\geq 5.0$  log units. There were similar relationships with all IgG subclasses, although the relationship with IgG2 was not significant ( $P = .07$ ; table 1). Two variables were significant in multivariate analysis, anti-*Leishmania* IgG and PCR, which together explained 30.3% of the variation in infectiousness in infected dogs. There was a trend toward increased mortality when infectious, although this was not significant (odds ratio, 2.39; 95% CI, 0.75–7.56;  $P = .14$ ). When infectiousness was analyzed as the proportion of flies infected, rather than the probability of a dog being infectious, there were significant positive relationships in infected dogs with anti-*Leishmania* IgG (slope = 1.233;  $P < .0001$ ; figure 3) and PCR status (slope = 3.806;  $P < .0001$ ) but not clinical score ( $P > .10$ ).

*Sensitivity and specificity of diagnostic techniques.* The sensitivity of seropositivity to detect currently infectious dogs was 96.4%–100% but was much lower in the latent period (62.5%–75.0%; table 2). Serology also detected a high proportion of non-infectious dogs (75.8%–80.6%), that is, had a low specificity for infectious dogs (19.4%–24.2%). PCR was generally less sensitive than serology, whereas a high clinical score had very low sensitivity but was the most specific test for infectious dogs (91.2%). Use of a combination of tests did not improve test performance.

*Simulation of the effect of dog culling.* The simulations showed that use of a low-sensitivity test with a 120-day interval between diagnosis and culling, comparable to current control programs, results in little reduction in the proportion of infectious dogs, from 0.31 before control to 0.15–0.25 afterward (figure 4, top). Use of a high-sensitivity test with a 120-day interval resulted in a reduction to 0.04–0.16, whereas a low-sensitivity test with no time delay resulted in a reduction to 0.13–0.23 (figure 4, bottom). Only a high-sensitivity test with no time delay resulted in a sustained reduction of the proportion of infectious dogs to near zero.

**Discussion**

Assessment of the likely effectiveness of control strategies for ZVL requires an understanding of the population dynamics of infectiousness in dogs. Here, we use data from a field study of infectiousness of canine ZVL to estimate the length of the latent period between infection and infectiousness and the proportion of dogs that become infectious and to simulate the effect of dog culling. Because the incidence of infection is high, and infectiousness develops on average 6 months after infection, effective control through culling requires a very high proportion of infectious dogs to be removed each year, and thus the use of a highly sensitive diagnostic test without a long delay between detection and culling (figure 4, bottom). These results suggest 2 reasons why culling programs have failed to reduce significantly the incidence of canine and human ZVL. First, mass-screening surveys usually have used the indirect immunofluorescent antibody test on filter paper eluates to detect infected dogs, a test that is known to be insensitive [7]. Second, there is typically a delay between detection of seropositive dogs



**Figure 3.** Relationship between proportion of sandflies infected by dog during xenodiagnosis and its anti-*Leishmania* IgG level (log units/mL) for 173 feeds on 50 dogs.

**Table 2.** Sensitivity and specificity of diagnostic techniques to detect infectious dogs in latent period, currently infectious dogs, and never-infectious infected dogs.

Test	Latent period	Infectious period	Noninfectious dogs
ELISA			
Longitudinal cutoff	75.0 (12/16)	100.0 (55/55)	80.6 (50/62)
Cutoff, 4006 U/mL	62.5 (10/16)	96.4 (53/55)	75.8 (47/62)
PCR positive	81.8 (9/11)	75.0 (33/44)	53.7 (22/41)
Clinical condition <sup>a</sup>	0 (0/14)	34.5 (19/55)	8.8 (5/57)

NOTE. Data are % (no. infectious/total no. of times dogs were exposed to *Lutzomyia longipalpis* [feeds]) at time of ELISA, polymerase chain reaction (PCR), or clinical examination. Analyses include samples from infected dogs only.

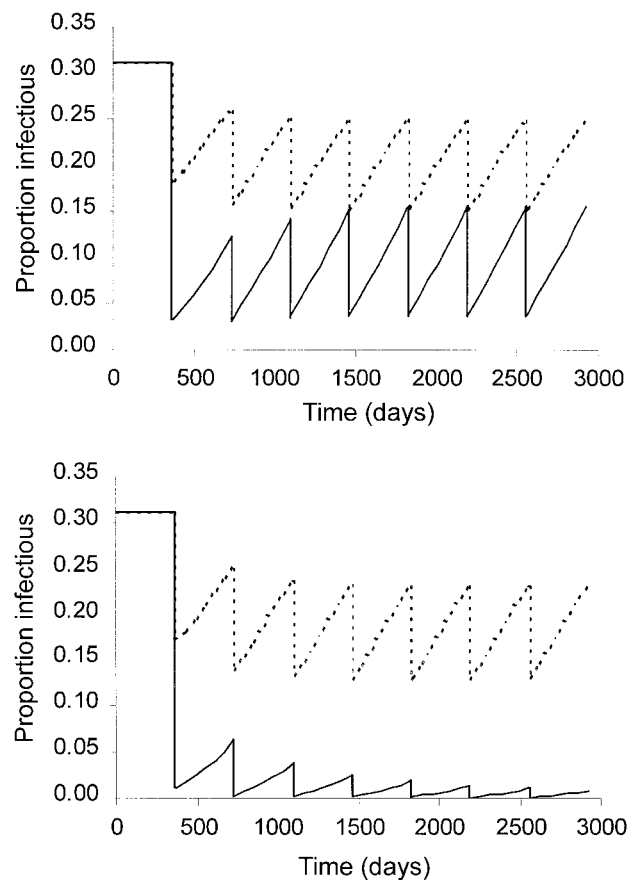
<sup>a</sup> Polysymptomatic dogs (defined as total clinical scores >7).

and culling of 80–180 days [2, 7]. Any such delay will have the result of further reducing the effective sensitivity of the diagnostic test. Our results support those of a recent field trial, in which dog seroprevalence was reduced by 27% after a single cull within 7 days of diagnosis by ELISA, compared with reductions of only 9% with an 80-day interval after diagnosis by indirect immunofluorescent antibody testing [7]. When the incidence of canine infection is high, effective dog control will thus require both a highly sensitive and rapid diagnostic test that can be carried out in the field. Here, we show that ELISA is a sensitive test for currently infectious dogs but is less sensitive for dogs in the latent period. The true sensitivity in the latent period will be lower than estimated, because the precise date of infection of study dogs was unknown and thus infected dogs could not be detected early in infection. PCR has been reported to be as sensitive as serology to detect infectious dogs when done on lymph node aspirates [21] but here proved to be less sensitive than serology. In contrast to ELISA and PCR, immunochromatographic dipstick tests for human visceral leishmaniasis are easily used in the field [31], but their sensitivity and specificity for dogs (particularly infectious dogs) requires further investigation [32]. Assessment of clinical condition is straightforward in the field but unfortunately very insensitive.

Previous models have shown that dog culling is likely to be much less efficient for ZVL control than interventions directed at the sandfly vector [4, 33]. These models, and the simulations presented here, assume that culled dogs will be rapidly replaced with young susceptible dogs, although this assumption has been criticized [6]. Unfortunately, the effect of dog culling on dog population dynamics has not yet been reported. However, there is evidence that dogs that die of natural causes are indeed rapidly replaced, and dog populations in our study site are stable, despite high mortality rates [30]. Moreover, the assumption of a rapid replacement rate has little effect on the results of our simulations, because the proportion of infectious dogs is not strongly dependent on dog population size. The replacement of culled dogs has another implication for culling programs: if culling could be targeted at infectious (rather than infected) dogs, the effectiveness of culling may be increased, because the

proportion of never-infectious dogs in the population may increase. Our data show that only a percentage (43%) of infected dogs become infectious. However, control targeted at these dogs would require a specific diagnostic test for infectious dogs, whereas our results show that serology did not distinguish infectious from never-infectious dogs (specificity <25%), because both classes of dog seroconverted after infection. A much smaller proportion of dogs (7 [17%]/42) became highly infectious. If sandflies bite all dogs equally, these highly infectious dogs would be expected to be responsible for 88% of all transmission. Similar levels of aggregation in parasite transmission have been reported for a variety of vectorborne and sexually transmitted disease pathogens [34] and indicate the importance of high coverage rates for any control measure directed at dogs, to ensure that the highly infectious dogs are included.

The point prevalence of infectious dogs in our population (27%) was very similar to that in a Colombian study [21] but was low, compared with the 75%–92% reported in most pre-



**Figure 4.** Simulation of effect of annual culling on proportion of infectious dogs in population. Simulations assumed that high sensitivity (solid line) or low sensitivity (broken line) serologic test was used to identify infected dogs. Seropositive dogs were then culled after 120-day interval between serodiagnosis and culling (top) or with no interval between serodiagnosis and culling (bottom).

vious European and Latin American studies [13, 15, 17, 19]. The proportion of flies infected by infectious dogs (28%) was comparable to the 8%–29% reported for *L. longipalpis* [13, 17, 21] but was lower than the 37%–70% reported for *Phlebotomus perniciosus* in southern Europe [15, 18–20]. Differences in vector competence, both within and between vector species, may account for some of this variability. There have been no previous estimates of the latent period between infection and infectiousness. In the current study, no dogs became infectious before infection was detectable by serology, parasite culture, or PCR, although a proportion of dogs (14%) were infectious at the same time that infection was detected by these methods. This suggests that parasite dissemination in the skin occurs with or later than parasite multiplication in the bone marrow and antibody production. Although sandflies can be infected by feeding at the site of experimental inoculation in the prepatent period [16], our results suggest that this is not epidemiologically significant. The median latent period was estimated as 135 days after patent infection and 199 days after the actual day of infection. These figures probably are overestimates, because the onset of infectiousness in some dogs may have been missed because of the relatively long and irregular intervals between xenodiagnoses on individual dogs. This is supported by the data presented in figure 2, which suggest that the median time to infectiousness was, in fact, <67 days after patent infection.

The factors that determine whether a dog becomes infectious after infection are unknown. A negative correlation between infectiousness and the percentage of circulating CD4<sup>+</sup> cells has been shown in dogs [20] and in patients with visceral leishmaniasis and human immunodeficiency virus coinfection [35], which suggests that suppression of cellular immune responses may be associated with increased infectiousness. However, we did not find a significant negative correlation between infectiousness and the strength of the specific lymphoproliferative response. Infectiousness appears to correlate with parasite load, because both the probability of infectiousness and the proportion of flies infected were strongly positively correlated with anti-*Leishmania* antibody levels and PCR positivity. Surprisingly, an association between infectiousness and indirect immunofluorescent antibody titer was not found in European dogs followed before and after chemotherapy [14, 18]. The relationship between infectiousness and clinical status of infected dogs also varies between studies. Here, the probability of a dog being infectious was correlated with clinical score: asymptomatic infected dogs were infectious in only 21.3% (16/75) of feeds, compared with 62.5% (15/24) of feeds on polysymptomatic dogs. Similarly, on the basis of combined data from southern European studies, 60% (6/10) of asymptomatic dogs but 100% (15/15) of polysymptomatic dogs were infectious to *P. perniciosus* [14, 15, 18, 19]. However, there was no relationship between the proportion of flies infected and clinical score in this study, and other studies have not shown any association between infectiousness and clinical condition and suggested that

asymptomatic dogs may be an important source of infection [15, 19]. Our longitudinal data allow a more detailed consideration of the role of asymptomatic dogs in transmission and show that the majority of infectious asymptomatic dogs (9 [75%] of 12) were in fact presymptomatic, developing symptoms after becoming infectious. Only 2 of 9 dogs that clearly remained asymptomatic were infectious, thus, presymptomatic or symptomatic dogs were responsible for the vast majority of sandfly infections (499 [99.6%] of 501).

In summary, this is the first large-scale study of infectiousness in dogs exposed to natural *Leishmania* infection. The results suggest that the observed failure of dog culling to control ZVL results from the high incidence of infection and infectiousness in areas of endemicity, lack of sensitivity of diagnostic tests, particularly indirect immunofluorescent antibody testing on filter paper eluates, and time delays between diagnosis and culling, which further reduce effective test sensitivity. Future effort in ZVL control may be better invested in vaccine development, vector control (such as residual insecticide spraying), or the use of insecticidal dog collars [36–38].

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