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Low infectiousness of a wildlife host of *Leishmania infantum*: the crab-eating fox is not important for transmission

O. COURTENAY[1,2]*, R. J. QUINNELL[1,3], L. M. GARCEZ[4] *and* C. DYE[1,5]

[1] *Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK*
[2] *Ecology and Epidemiology Group, Department of Biological Sciences, University of Warwick, Coventry, UK*
[3] *School of Biology, University of Leeds, Leeds, UK*
[4] *Instituto Evandro Chagas, Belém, Pará, Brazil*
[5] *Communicable Diseases, World Health Organization, Geneva, Switzerland*

SUMMARY

The epidemiological role of the crab-eating fox *Cerdocyon thous* in the transmission of *Leishmania infantum* is assessed in a longitudinal study in Amazon Brazil. A total of 37 wild-caught foxes were immunologically and clinically monitored, and 26 foxes exposed to laboratory colonies of the sandfly vector *Lutzomyia longipalpis*, over a 15-month period. In total 78 % (29/37) of foxes were seropositive for anti-*Leishmania* IgG on at least 1 occasion, and 38 % (8/37) had infections confirmed by PCR and/or by culture. Point prevalences were 74 % (serology), 15 % (PCR), and 26 % (culture). No signs of progressive disease were observed. None of the foxes were infectious to the 1469 sandflies dissected from 44 feeds. A conservative estimate of the possible contribution of foxes to transmission was 9 % compared to 91 % by sympatric domestic dogs. These results show that crab-eating fox populations do not maintain a transmission cycle independently of domestic dogs. The implication is that they are unlikely to introduce the parasite into *Leishmania*-free dog populations.

Key words: *Leishmania infantum*, infectiousness, *Cerdocyon thous*, Brazil, fox.

INTRODUCTION

Zoonotic visceral leishmaniasis (ZVL) is an important vector-borne disease of humans and domestic dogs, caused by *Leishmania infantum* (= *L. chagasi*, Mauricio *et al.* 1999). The principal reservoir ('source host') of ZVL is the domestic dog; however, a number of wild animal species may also represent important reservoirs (Ashford, 2000). In Latin America, the crab-eating fox *Cerdocyon thous* has long been considered a potential source of human infection (Deane & Deane, 1955). Foxes show a high prevalence (up to 42 %) of parasitologically confirmed infection (Deane & Deane, 1955; Silveira *et al.* 1982; Lainson *et al.* 1990; Courtenay *et al.* 1994), and have high contact rates with peridomestic *Lu. longipalpis* (the principal sandfly vector), and infected dogs (Courtenay, Quinnell & Chalmers, 2001). However, the significance of crab-eating foxes as a ZVL reservoir will depend on their ability to transmit infection to sandflies successfully, rather than their infection rate, and on the likelihood that they can (re)introduce the pathogen into

uninfected dog populations. Here we address 3 fundamental questions (i) what is the prevalence of infection and disease in a wild fox population, (ii) what proportion of infected foxes are infectious to *Lu. longipalpis*, and (iii) what are the relative contributions of foxes and domestic dogs to transmission. Data were obtained from a longitudinal study of a free-ranging crab-eating fox population in a highly endemic region of Amazon Brazil.

MATERIALS AND METHODS

Study design

Fieldwork was conducted in the municipality of Salvaterra, Marajó island, Pará, Brazil (48° 03′ W, 00° 46′ S). The study area, spatial ecology, and epidemiology of ZVL in the sympatric fox and dog populations have been described (Courtenay *et al.* 1994, 2001, 2002; Quinnell, Dye & Shaw, 1992; Quinnell *et al.* 1997, 2001; Macdonald & Courtenay, 1996).

Sampling

Thirty-seven foxes were captured in 5 capture rounds between April 1994 and July 1995 (Table 1), and anaesthetized as described previously

* Corresponding author: Ecology and Epidemiology Group, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK. Tel: +44 24 7652 4550. Fax: +44 24 7652 4619. E-mail: orin. courtenay@warwick.ac.uk

Table 1. *Sampling regime of foxes, (A) the number of animals caught and sampled per capture round, and (B) the frequency of samples obtained per animal*

(A)

Capture round	Mid-sample date	Trapping period (days)	Mean inter-round interval (days)	Number of foxes sampled	Number of foxes experimentally exposed to *Lu. longipalpis*
1	29 April 1994	33		9	2
2	17 June 1994	23	49	12	7
3	15 November 1994	25	151	22	16
4	16 March 1995	15	121	21	11
5	26 July 1995	11	132	10	8
Totals				37	26

(B)

Frequency of samples	Number of foxes examined	
	Serology*, parasitology, clinical	Xenodiagnosis
1	13	12
2	14	10
3	7	4
4	3	0
Total	74	44

* 1 Serum sample lost prior to testing.

(Courtenay *et al.* 1994, 2002). At the field station in the local town of Salvaterra, 20 ml of blood were taken from the jugular vein. Bone marrow was aspirated from the iliac crest with a 16×25 mm Klima needle (Veterinary Instruments, Newcastle) into a 20 ml syringe containing 0·5 % EDTA, and divided between tubes for PCR, 4 sterile Difco blood-agar slopes, 2 Syrian hamsters, and 1–4 thin smears. Foxes were sampled on 1–4 occasions (only once per round, at first capture) at a mean interval of 4·3 months (s.e. 0·29, range: 1·3–8·9 months), producing a total of 74 serological, parasitological and clinical samples (1 blood sample was lost prior to testing) (Table 1). Two of the foxes had serological (IFAT) and clinical records from a previous study (Courtenay *et al.* 1994). To investigate infectiousness to the sandfly vector, 26 of the foxes were exposed to female laboratory-bred *Lu. longipalpis* on the day of capture, in 1–3 feeds at a mean interval of 5 months (s.e. 0·50, range: 3·5–9·0). This gave a total of 44 xenodiagnostic feeds (Table 1). The entire sampling procedure took approximately 1·5 h to complete; no injuries were incurred. All foxes were weighed, measured, and ears permanently marked with tattoo before being replaced in the trap of capture and released once fully awake. Fox ages were known from observed birth dates (Macdonald & Courtenay, 1996), or estimated to the nearest year on the basis of toothwear by comparison of dental material of known age collected previously from the study site (Courtenay *et al.* 1996). The median age of the fox

population at first capture was 9 months (range: 2–104 months); 23 foxes were male, and 14 were female.

Xenodiagnosis

Laboratory-bred colonies of *Lu. longipalpis* were used for xenodiagnosis, as described elsewhere (Courtenay *et al.* 2002). In 28 of the 44 feeds, sedated foxes were placed in an individual wire cage measuring $0·3$ m $\times 0·3$ m $\times 1$ m, sheathed in sandfly proof netting. In the other 16 feeds, only the animal's head was exposed by placing it in a smaller gauze cage ($0·2$ m $\times 0·2$ m $\times 0·2$ m). In each feed, an average of 92 (s.e. 6·5) 2- to 3-day-old adult female *Lu. longipalpis*, and an approximately equal number of males, were introduced into the cage and allowed to feed for approximately 1 h in darkness (under black hessian). This proved sufficient time for all female flies to obtain a bloodmeal. Blood-fed flies were then maintained in the laboratory and examined 4–5 days after feeding as previously described (Courtenay *et al.* 2002). An average of 34 (s.e. 2·9) flies per feed survived to dissection. Selection of foxes for sandfly feeds depended on the availability of adult sandflies at the time that foxes were sampled.

Immunology

IgG responses to *L. infantum* crude antigen were measured by ELISA as described (Quinnell *et al.* 1997) using a rabbit anti-dog IgG peroxidase

conjugate (Sigma). Results were expressed as arbitrary units of specific IgG per ml of serum, calculated from a standard curve (a highly positive dog serum) titrated on each plate. This reference serum was assigned an arbitrary number of units/ml equal to its end-point titre (Quinnell *et al.* 1997).

Parasitology/PCR

DNA extracted from bone-marrow biopsies was amplified in 2 PCRs with primers specific for minicircle DNA (AJS31/DBY, Scrimgeour *et al.* 1998) or ribosomal DNA (R221/R332, Van Eys *et al.* 1992), Southern blotted and hybridized with digoxygenin-labelled internal probes, as previously described (Quinnell *et al.* 2001). Samples which were positive with both primer sets were considered as positives; samples positive with only one primer set were excluded from analysis. Parasitological examination of *in vivo* (hamster) and *in vitro* cultures and smears was by standard techniques (Quinnell *et al.* 1997).

Clinical examination

Foxes were examined for 6 signs of canine ZVL: alopecia, dermatitis, chancres, conjunctivitis, onychogryphosis (excessive nail growth) and lymphadenopathy (enlarged popliteal, prescapular or premaxillar lymph nodes). Each symptom was scored on a semi-quantitative scale from 0 (absent) to 3 (severe).

Analysis

Since an intrinsic cut-off of seropositivity for foxes was not known (there was no bimodality in the frequency distribution of fox IgG titres, nor were negative control sera available), we used the cut-off titre of 2253 units/ml calculated for the sympatric dog population (Quinnell *et al.* 1997).

Linear and non-linear variation in prevalence with host age was analysed by fitting a full logistic regression model including age and age[2]; the age[2] term was removed from the model if not significant. Prevalence was analysed as a binomial variable using general estimating time series equations with robust standard errors to control for autocorrelation due to the non-independence of repeat samples from the same foxes (StataCorp, 1999). All analysis was performed in Stata 6.0 (StataCorp, 1999). Estimates of incidence, λ, and recovery, ρ, rates were calculated (i) from changes in longitudinal IgG titre, defining seroconversion as ≥ 4-fold change from minimum to maximum titre (Quinnell *et al.* 1997), and (ii) from the maximum likelihood fit of an incidence-recovery model to the age-seroprevalence data (Courtenay *et al.* 1994). Infectiousness was assessed as the pro-

portion of sandflies infected, with the upper 95% CL for zero proportions calculated as $-\ln(0.05)/N$, where N is the number of fed sandflies dissected.

Multi-host transmission model

To quantify the relative contribution of foxes and dogs to transmission, we use the vector-borne disease models of Ross-Macdonald (Macdonald, 1957) and Garrett-Jones (1964) which estimate the basic reproductive number R_0, defined as the number of secondary cases which arise following the introduction of a single case into a fully susceptible host population,

$$R_0 = \left(\frac{e^{-\mu t}}{\mu} \right) \times \left(\frac{ma^2 b}{r} \right), \tag{1}$$

which to incorporate multiple hosts (Rogers, 1988), in this case for foxes (f) and dogs (d), is expanded to

$$R_0 = \left(\frac{e^{-\mu t}}{\mu} \right) \times \left[\left(\frac{m_f a_f^2 b_f}{r_f} \right) + \left(\frac{m_d a_d^2 b_d}{r_d} \right) \right], \tag{2}$$

where the longevity factor of the sandfly vector $e^{-\mu t}/\mu$, is determined by t the extrinsic incubation period of the parasite in the infected sandfly, and μ the daily mortality rate of sandfly; m is the number of flies per host; a is the daily biting rate of individual female sandflies on the host species; b is the probability of a sandfly acquiring infection from the host (one component of vectorial competence); and r is the daily recovery rate of the host from the infectious state, or $1/r$ the average duration of host infectiousness in days.

The endemic stability of the parasite in each host population R_{0x} is estimated from the proportional contribution (pR_0) of each host$_x$ to total R_0 where:

$$pR_{0x} = \left(\frac{m_x a_x^2 b_x}{r_x} \right) \bigg/ \left[\left(\frac{m_f a_f^2 b_f}{r_f} \right) + \left(\frac{m_d a_d^2 b_d}{r_d} \right) \right] \tag{3}$$

and

$$R_{0x} = pR_{0x} \times R_0. \tag{4}$$

Parameter estimates

Values of b were measured for foxes (this study) and domestic dogs (Courtenay *et al.* 2002) by xenodiagnosis; the average probability of an infected dog generating infection in a sandfly was $b_d = 0.107$; a conservative estimate of infectiousness in infected foxes is calculated as the upper 95% CL of mean infectiousness, i.e. $b_f = -\ln(0.05)/N$ where N is the total number of sandflies dissected. Sandfly host preference for, and daily biting rates on, dogs and foxes (ma^2) are not known but are set as equal, as suggested by similarities in incidence rates in the two hosts (see Discussion section). Dogs in the study site

take on average $T = 333$ days to become infectious (Courtenay *et al.* 2002), and have a life-expectancy (L) of 905 days (Courtenay, 1998), so assuming infectiousness in dogs persists for life, $r_d = 1/(L - T) = 0.00175$ per day. Neither the duration of infectiousness nor latent period in foxes is known, but conservative estimates are gained from assuming foxes and dogs to be equal. The average life-expectancy of infected Marajó foxes is $L = 1143$ days (Courtenay *et al.* 1994; Courtenay, 1998), which gives $r_f = 1/(L - T) = 0.00123$ per day. A value of $R_0 = 8.9$ was calculated previously for Marajó (Courtenay *et al.* 2002).

RESULTS

Prevalence of infection

The prevalence of infection in all samples was 74.0% (54/73) by serology, 15.2% (10/66) by PCR and 25.8% (8/31) by *in vivo* or *in vitro* parasite culture. Point prevalences per sampling round were 59–89% (serology), 0–27% (PCR), and 0–50% (parasite culture). The cumulative prevalence of infection in foxes was 78.4% (29/37) by serology, 22.9% (8/35) by PCR and 38.1% (8/21) by parasite culture. Seroprevalence increased with fox age to a plateau at unity, without subsequent decline (age: slope $b = 0.149$, $P = 0.088$, Fig. 1). The proportion of PCR positives increased to 41.7% in the 25–84 month age class, but thereafter declined sharply with age (age: $b = 0.148$, $P = 0.038$; age²: $b = -0.0016$, $P = 0.015$). The proportion of parasite positives increased to a peak of 50% in the same age class, and declined at a similar rate as PCR in older age-classes (age: $b = 0.168$, $P = 0.0011$; age²: $b = -0.022$, $P < 0.001$. There were no significant differences in the prevalence of infection between sexes.

Incidence and recovery

Repeat serum samples were obtained from 23 foxes, of which 8 were seronegative at first sample. There was evidence for seroconversion in 4 of these 8 foxes, with increases from minimum to maximum IgG level of $> 4 \times$ (range: $4.95 \times - 220 \times$), giving a mean incidence of $\lambda = 0.120$/month. No other fox showed a change in antibody level of this magnitude (mean $1.47 \times$, range: $0.66 \times - 2.71 \times$, $n = 19$), and there was no evidence for serorecovery: none of the 16 foxes with > 2253 units/ml subsequently fell below this cut-off (i.e. $\rho = 0$). Of the 2 study foxes that had been sampled during a previous study, one had been shown to be seropositive (IFAT titre 80–320), and was still seropositive (ELISA 10295–12004 units/ml) during the current study 58 months after the first sample. The incidence–recovery model fitted to the age–seroprevalence data gave similar values of $\lambda = 0.100$/month (95% CL 0.070–0.129), and $\rho = 0$.

Xenodiagnosis

Xenodiagnosis was performed on 26 foxes at a median age of 24 months (range: 5–114 months) with 12, 10 and 4 individuals exposed on 1, 2 and 3 occasions each (Table 1). None of the 1469 sandflies from 44 feeding trials were infected on dissection. Of these, 1228 flies were dissected from 37 feeds on 21 infected foxes, of which 1157 flies (35 feeds) were fed on 20 seropositive foxes, and 390 sandflies (10 feeds) were fed on 8 foxes with current infection confirmed by PCR or parasite culture. The 95% CL for the proportion of sandflies infected were thus 0–0.0024 (infected foxes), 0–0.0026 (seropositive foxes) and 0–0.0077 (PCR- or culture-positive foxes).

Clinical signs

Only 1 fox (an 18-month-old male) showed any symptoms of canine ZVL, including slight popliteal lymph node enlargement, extra nail growth on one rear foot, and a small patch of dermatitis on one pinna (clinical scores of 1 in each case) on first examination. *Demodex* or *Sarcoptes* mites (the aetiological agents of mange) were not detected by microscopy of ear scrapes. On recapture 4 months later, the skin and nail conditions had spontaneously cured, though slight lymphadenopathy (prescapular and popliteal) was still detected. There was no significant weight change between captures. This fox had the highest antibody level of all sampled foxes (217624 units/ml) at initial capture; at recapture, the antibody level was similar (207666 units/ml), and parasites were isolated by culture. Xenodiagnosis (dissection of 26 and 49 fed *Lu. longipalpis*) was negative on both occasions.

Partitioning R_0

The upper 95% CL of the proportion of sandflies infected by infected foxes was $b_f = 0.0024$, and the proportion of sandflies infected by infected dogs was $b_d = 0.107$ (Courtenay *et al.* 2002). The proportion of total transmission due to foxes calculated using equation 3 is $pR_{0f} = 0.031$ corresponding to $R_{0f} = R_0 \times pR_{0f} = 8.9 \times 0.031 = 0.28$, and $pR_{0d} = 1 - 0.031 = 0.969$ giving $R_{0d} = 8.6$. The value of R_{0f} is below the threshold ($R_0 = 1$) for endemic persistence. These data were also used to estimate the critical level of infectiousness in foxes b_{fcrit}, necessary to maintain a transmission cycle independent of infectious dogs giving $b_{fcrit} = (b_d \times r_f)/((R_0 - 1) \times r_d) = 0.0096$ which is $3.9 \times$ higher than the observed upper 95% CL for infectiousness of foxes. Equivalent calculations based on data from only those foxes with parasitologically confirmed infections gave values of $b_f = 0.0077$, $pR_{0f} = 0.092$, and $R_{0f} = 0.82$.
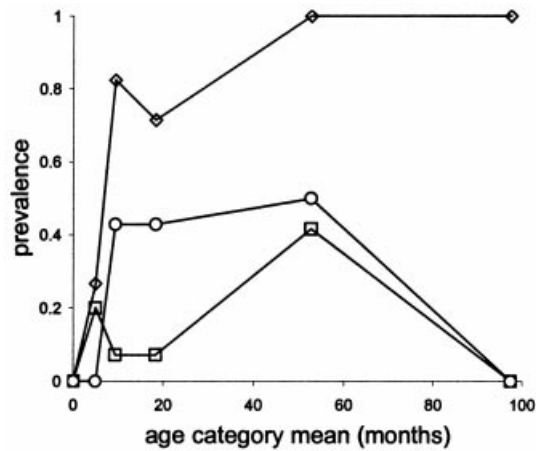
Fig. 1. The age-prevalence of *Leishmania infantum* infection in crab-eating foxes. Prevalence was assessed by serology (◇), PCR (□) or *in vitro/in vivo* culture (○). Values are shown for mean ages of age-class: 0–6, 7–12, 13–24, 25–84, and 85–114 months.

DISCUSSION

This is the first field study of infectiousness of crab-eating foxes infected with *L. infantum*. The results clearly show that infected foxes transmit infection at most very infrequently to the sandfly vector *Lu. longipalpis*. None of 390 sandflies fed on 8 foxes with confirmed infection, and none of 1228 sandflies fed on 21 seropositive and/or parasite positive foxes, became infected. The upper 95% CL for the proportion of infected sandflies with these sample sizes is 0·77% or 0·24% respectively. In contrast, 10·7% of sandflies fed on sympatric infected dogs became infected (Courtenay *et al.* 2002). The fox and dog infectiousness studies were carried out concurrently, using the same sandfly colonies and diagnostic techniques, though with some technical differences: foxes were anaesthetized, and in a proportion of feeds only their heads were exposed. In contrast, dogs were not anaesthetized and sandflies had access to the entire body in all feeds. The site of feeding is unlikely to account for the observed difference in infection rates, since *Lu. longipalpis* has been shown to acquire infection more readily when feeding on the ears compared to the abdomen of infectious dogs (Travi *et al.* 2001). It is also unlikely that the anaesthetic interfered with transmission since the mean proportion of fox-fed flies that survived to dissection (0·43, 95% CL 0·36–0·51) was similar to that of dog-fed flies (0·47, 95% CL 0·43–0·51), and infection of a high proportion of sandflies exposed to anaesthetized dogs has been reported (Travi *et al.* 2001; Molina *et al.* 1994; Killick-Kendrick *et al.* 1994; Alvar *et al.* 1994).

The low infectiousness of wild-caught crab-eating foxes is likely to be associated with their lack of symptomatic disease. Two recent studies have clearly shown that dogs with clinical ZVL are more infectious to *Lu. longipalpis* than asymptomatic infected dogs (Courtenay *et al.* 2002; Travi *et al.* 2001), though this relationship is not apparent in European studies (Molina *et al.* 1994; Alvar *et al.* 1994; Guarga *et al.* 2000). The only wild-caught fox to have been xenodiagnosed prior to the current study was one from NE Brazil which had advanced signs of ZVL and infected 10/10 *Lu. longipalpis* fed on it (Deane & Deane, 1954) (see Courtenay *et al.* (1996) for nomenclature of that specimen). The only animal to present any clinical signs in this study had recovered spontaneously by the time of the next sample. Similarly, none of the seropositive ($n = 24$) nor parasite positive ($n = 14$) crab-eating foxes examined to date in the Amazon region have shown symptomatic infection (Lainson, Shaw & Lins, 1969; Lainson & Shaw, 1971; Silveira *et al.* 1982; Lainson *et al.* 1987, 1990; Courtenay *et al.* 1994). One caveat with studies of wild animals is that sick animals may behave differently, and thus be difficult to trap. Our previous behavioural observations of this population using radio-telemetry and night vision equipment did not reveal any sick foxes nor significant variations in spatial behaviour between ecologically matched animals with positive *versus* negative (IFAT) antibody titres (Courtenay *et al.* 1994; Macdonald & Courtenay, 1996). Contrary to the results here, the only other fox to be examined for infectiousness prior to this study was an experimentally infected seropositive but asymptomatic captive animal which infected 7/22 *Lu. longipalpis* fed on it (Lainson *et al.* 1990).

In contrast to the pronounced inter-specific differences in infectiousness, the prevalence and incidence of *L. infantum* infection in foxes were similar to those of sympatric dogs. The prevalence of infection in foxes was 74% (serology), 15% (PCR), and 26% (parasite culture). Using the same methods, the prevalence of infection in samples from 126 sentinel dogs was 48% (serology), 42% (PCR), and 19% (parasite culture) (Quinnell *et al.* 1997, 2001), and incidence rates were 0·10–0·12/month in foxes compared to the 0·11–0·26/month in dogs (Quinnell *et al.* 1997; Courtenay, 1998). Previous studies of foxes on Marajó have reported prevalences of 42% (parasitology) and 52% (serology) (Silveira *et al.* 1982; Lainson *et al.* 1990; Courtenay *et al.* 1994). Foxes are thus commonly infected, but very rarely infectious or symptomatic. The decline in the proportion of parasite-positive foxes with age suggests that infected foxes successfully clear parasites, though this was not accompanied by serorecovery nor a decline in antibody level; indeed, 1 fox found to be seropositive during a previous study was still seropositive 4·8 years later. The fox serology data must be interpreted cautiously, as we use the cut-off calculated for the sympatric dog population (Quinnell *et al.* 1997). The true cut-off is likely to differ between the two species, because of inter-

specific differences in immune responsiveness or test performance. However, 3 observations suggest that the use of the dog cut-off of 2253 units/ml is reasonable. First, the 4 foxes judged to seroconvert had maximum initial antibody levels of 1208 units/ml (and no fox with an initial antibody level above this cut-off showed $> 2.7 \times$ increase in antibody level). Second, the maximum antibody level in the 8 youngest foxes (< 6 months old), which are likely to be seronegative, was 1845 units/ml. Finally, 3 of 18 (17%) samples from foxes classed as seronegative were positive by PCR, a very similar proportion to that observed in seronegative dogs (15%) (Quinnell et al. 2001).

The results of this study indicate that Marajó crab-eating foxes represent a 'sink' host for L. infantum, and are not an important source of (re)-infection for either humans or dogs. Even with a highly conservative estimate of fox infectiousness (from parasitologically confirmed infections only), the contribution of foxes to transmission by canids was at most 9% compared to at least 91% by dogs, and the basic case reproduction number for foxes (R_{0f}) was estimated as at most 0.82, below the threshold condition ($R_0 = 1$) for pathogen persistence. These calculations assume that the quantity $m \times a^2$ is not greater for foxes than dogs, where m is the number of flies per host, and a is the daily biting rate per female sandfly. This assumption is supported by our observations that (i) sandflies occur at much higher densities inside than outside villages, thus dogs spend much longer in areas of high fly density than foxes, and flies are equally willing to bite foxes and dogs in the laboratory; (ii) the incidence of infection, which depends on $m \times a$, is not greater in foxes than dogs. Our results suggest that all, or nearly all, fox infections result from transmission from other species, such as domestic dogs. Foxes probably become infected in the peridomestic environment, in which they are known to spend significant periods of time (Courtenay et al. 2001), and where Lu. longipalpis occurs at high densities in animal pens (Quinnell & Dye, 1994; Kelly, Mustafa & Dye, 1996). In contrast, Lu. longipalpis has not been found at fox sleeping sites or surrounding areas of savanna habitats, though small numbers are present in residual gallery forest (Lainson et al. 1990). This scenario is consistent with parasite typing data, which show no differences between 4 fox and 4 dog isolates from Brazil (Mauricio et al. 1999, 2001).

These results suggest that control measures in Brazil should continue to be targeted at peridomestic transmission from domestic dogs; the probability that the crab-eating fox could be responsible for human infection during dog control, or could introduce or reintroduce the parasite into uninfected dog populations, is negligible. The geographical range of the crab-eating fox extends from Venezuela to Argentina (Courtenay & Maffei, 2002), and whilst it is possible that there is geographical variation in ZVL susceptibility, few foxes have been examined outside Amazon Brazil (Courtenay, 1998). Opossums (Didelphis spp.) have also been implicated as a potential reservoir host, as L. infantum has been isolated from 2% of D. albiventris in NE Brazil (Sherlock et al. 1984; Sherlock, 1996), and 23–32% of D. marsupialis in Colombia (Corredor et al. 1989a, b; Travi et al. 1994). Both species have been shown to be able to infect Lu. longipalpis (Sherlock, 1996; Travi et al. 1998), but their relative importance in transmission has not been studied.

In conclusion, the current study demonstrates the importance of comparative studies of infectiousness in endemic populations. We show that crab-eating fox populations are not an important source of L. infantum and, modifying an earlier hypothesis (Lainson et al. 1990), we suggest that foxes acquire infection in the peridomestic, rather than sylvatic, environment, where it 'spills-over' from domestic dogs. We predict therefore that successful infection control in domestic dogs will result in reduced infection rates in sympatric wildlife populations. A field trial in NW Iran is currently underway to test this hypothesis.

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