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

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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% (8/21) 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; Courténay et al. 1994), and have high contact rates with peridomestic *Lu. longipalpis* (the principal sandfly vector), and infected dogs (Courténay, Quinnell & Chalmers, 2001). However, the significance of crabeating 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 (Courténay et al. 1994, 2001, 2002; Quinnell, Dye & Shaw, 1992; Quinnell et al. 1997, 2001; Macdonald & Courténay, 1996).

Sampling

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

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

(B)

<table>
<thead>
<tr>
<th>Frequency of samples</th>
<th>Number of foxes examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology*, parasitology, clinical</td>
<td>Xenodiagnosis</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
</tr>
</tbody>
</table>

* 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: 13–8.9 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 × 0.3 m × 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 × 0.2 m × 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 digoxigenin-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, chancre, conjunctivitis, onychogryphosis (excessive nail growth) and lymphadenopathy (enlarged popliteal, prescapular or premaxillary 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 (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).

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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 squared; the age 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 proportion 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,

$$R_0 = \left( \frac{e^{at}}{\mu} \right) \times \left( \frac{ma^2b_t}{r} \right),$$

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

$$R_0 = \left( \frac{e^{at}}{\mu} \right) \times \left[ \left( \frac{ma^2b_t}{r_f} \right) + \left( \frac{ma^2b_d}{r_d} \right) \right],$$

where the longevity factor of the sandfly vector $e^{-at}/\mu$, is determined by $t$ the extrinsic incubation period of the parasite in the infected sandfly, and $\mu$ 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 ($pR_{0x}$) of each host, to total $R_0$ where:

$$pR_{0x} = \left( \frac{ma^2b_x}{r_x} \right) / \left[ \left( \frac{ma^2b_t}{r_f} \right) + \left( \frac{ma^2b_d}{r_d} \right) \right]$$

and

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

Parameter estimates

Values of $b$ were measured for foxes (this study) and 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. $b_x = -\ln(0.05)/N$ 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 would have been less likely to be exposed to sandfly bites.
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 Marajo foxes is $L = 1143$ days (Courtenay et al. 1994; Courtenay, 1998), which gives $r_d = 1/(L - T) = 0.00123$ per day. A value of $R_b = 8.9$ was calculated previously for Marajo (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 age to a plateau at about 50 days, 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: $b = 0.148$, $P = 0.038$; age$^2$: $b = -0.0016$, $P = 0.015$). 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: $b = 0.168$, $P = 0.0011$; age$^2$: $b = -0.022$, $P < 0.001$). 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: $+95 \times -220 \times$), giving a mean incidence of $\lambda = 0.120$ per 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 $\lambda = 0.100$ per 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 flies), 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 poplitel) 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.
**Infectiousness of a wildlife host of leishmaniasis**

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 48 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 >27× 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 ($R_0$) 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). Opposums (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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REFERENCES


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413

University of Toronto Press, Canada.

Ecology and reference to the New World. In considerations of the leishmaniases, with particular

and determination

infection of

leishmaniasis in areas of high transmission. Journal of Infectious Diseases (in the Press).


STATACORP (1999). Stata Statistical Software : Release 6.0. Stata Corporation, College Station, TX, USA.


