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Detection of *Leishmania* in Red Foxes (*Vulpes vulpes*) from Southeastern France Using Real-time Quantitative PCR

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ABSTRACT: The role of red foxes in the natural cycle of *Leishmania* infection is not well known. In the Var area, southeastern France, from 2006 to 2012, we conducted a longitudinal epidemiologic survey of foxes using quantitative PCR. Among 92 red foxes screened, prevalence of *Leishmania infantum* infection was 9%. Red foxes may be considered a bioindicator of parasite circulation in this biotope.

Key words: Fox, France, *Leishmania infantum*, leishmaniosis, predictive sentinel, real-time PCR, *Vulpes vulpes*.

Zoonotic leishmaniosis caused by *Leishmania infantum* is endemic on the French Riviera. Domestic dogs (*Canis lupus familiaris*) are considered the main reservoir of the parasite (Aoun et al. 2009). The potential role of wild carnivores, including Canidae, as sentinels or reservoirs of *L. infantum* is debated. The red fox (*Vulpes vulpes*) is a wild canid found in most all areas of semiwooded Eurasia, North Africa, Australia, and North America. In the Cevennes region of southeastern France in the 1960s, natural *Leishmania* infection of foxes was demonstrated using bone marrow culture (Rioux et al. 1968). To complete these data, we conducted a longitudinal epidemiologic survey using real-time quantitative PCR to determine the geographic distribution and prevalence of *L. infantum* in red foxes in the department of Var, southeastern France.

In the north of the Var region, the large military camp of Canjuers surrounding the village of Comps-sur-Artuby (43°684.756′S, 6°454.811′E) is in a rural area at an elevation of 800–1,000 m. The 35,000-ha camp is a natural reserve for wild flora and fauna. Wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) and red foxes are the main large wild mammals breeding in the reserve. These animals can play a role in the sylvatic cycle of human parasitic diseases. A military hunting society is authorized to regulate the population sizes of all three species during the hunting season. From 2006 to 2012, we collected samples of spleen (and other organs when possible) from 92 hunter-killed red foxes, (90 from the military camp of Canjuers and two from the city of Hyères). The samples were stored at −20 C until testing.

DNA extraction was performed on pellets of nucleated cells using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). For maximum yield, we performed overnight breakdown with proteinase K in animal tissue lysis buffer before purification. DNA was eluted in 100 μL of distilled water and stored at −80 C. We used a real-time quantitative PCR for the detection and quantification of *L. infantum* DNA as previously described (Mary et al. 2004; Mary et al. 2006; Aoun et al. 2009). Assays were performed in 25-μL final volume using 1 μL of sample DNA. A standard curve was established from *Leishmania* DNA extracted from 5×10^6 parasites: 1 μL of serial dilutions, ranging from 50,000 to 0.0001 parasites/μL was introduced into reaction tubes. TaqMan® chemistry allowed a two-step temperature (94 C and 55 C) cycling over 45 cycles. Quantification of host-nucleated cells was performed using a quantitative PCR targeting the albumin gene and a plasmidic DNA as standard. This technique used primers hybridizing to conserved regions of the albumin gene (ggctgactgctgtgcaaaaca and aagtaaggatgtcttctggc). Parasite loads were expressed as the number of *L. infantum*

TABLE 1. Results of PCR for *Leishmania infantum* in red foxes (*Vulpes vulpes*) of Var, France.[a]

Location	Year	Fox No.	Sex	Age	*Leishmania*/10^6 nucleate cells		
					Spleen	Liver	Kidney
Canjuers military camp	2006	1	M	Y	6	0	0
	2008	2	M	Y	0	7	0
		3	M	Y	0.03	0	0
		4	M	Y	0.3	0	0
	2009	5	F	A	1	NE	NE
	2012	6	M	A	4	NE	NE
Hyères	2006	7	M	Y	1.3	0	0
		8	F	Y	12,000	10	0

[a] M = male; F = female; Y = young; A = adult; NE = not evaluated.

per 1 million host cells, taking into account the concentration and dilution during extraction and amplification.

Eight of the 92 red foxes (9%) were positive, six of 90 (7%) from the camp of Canjuers and two of two from Hyères (Table 1). The number of *L. infantum* varied from 0.03 to 12,000 per 10^6 nucleate cells. We found *L. infantum* DNA in spleen (7 of 92) and liver (2 of 38), but not kidney (0 of 23), skin (0 of 14), or blood (0 of 15). Kidney samples were available from six of the seven positive animals, and all were negative. No suspected leishmaniosis lesions were observed at postmortem examination. Both urban foxes of Hyères carried *L. infantum*, but this sample size was too small for a statistical comparison with rural foxes.

Leishmania infantum is the only species of *Leishmania* identified in southern France. Leishmaniosis due to *L. infantum* in red foxes was described in the Cevennes, France, by culturing the parasite (Rioux et al. 1968). In Portugal, the antibody prevalence in red foxes was 6% (4 of 71) using the indirect immunofluorescence test (IFA; Abranches et al. 1984). In Israel, the antibody prevalence using enzyme-linked immunosorbent assay (ELISA) was 5% (1 of 20; Baneth et al. 1998). In Italy, using IFA and ELISA, the antibody prevalence was 18% (9 of 50; Mancianti et al. 1994). PCR testing of spleens of foxes in central Spain showed a prevalence of 74% (50 of 67; Criado-Fornelio et al. 2000). In southern Italy, 40% of foxes (20 of 50) examined had PCR-positive samples from lymph nodes and bone marrow (Dipineto et al. 2007).

Our results confirm the usefulness of PCR for epidemiologic studies. Parasite loads were low, except for one red fox from Hyères. The low parasite load in the red foxes from the Canjuers camp was likely due to the presence of a few copies of kinetoplastic DNA resulting from the destruction of *L. infantum* parasites by phagocytic cells (Mary et al. 2006). This argues for a self-limiting infection rather than acute disease.

Vectors of *Leishmania* are usually more abundant below 800 m (Chamaillé et al. 2010) and abundance of *Phlebotomus perniciosus* is related to altitude and stratification of vegetation. The Canjuers camp ecosystem is not generally favorable for development of sandflies. It is likely that the density of sandflies is low in the Canjuers camp (800–1,000 m) compared with the city of Hyères, located at the sea level. The distribution of sandflies is probably responsible for the low prevalence of *L. infantum* infection among red foxes in the Canjuers camp. Nevertheless, our results highlight the role of red foxes as sentinels for the expansion of leishmaniosis northward.

Hyères is inside the endemic area for leishmaniosis on the French Riviera. In

this endemic area, our observations reinforce the hypothesis that red foxes may serve as a wild reservoir for *Leishmania*, even if domestic dogs remain the major reservoir. Nevertheless, transmission from red foxes to sandflies has not been documented. In the tourist area of the Var department (Gorges du Verdon and Côte d'Azur), the population of red foxes is increasing. Additionally, red foxes tend to adapt to new habitats, especially in the outskirts of cities (Gloor et al. 2001) such as Nice on the French Riviera. Red foxes settle in urban habitats where they find food from human activity and shelter in public gardens, cemeteries, along the slopes of railways, and in residential areas. The presence of red foxes in proximity to humans represents a potential risk for transmission of *L. infantum*. This risk is insidious and insufficiently characterized and all the more relevant because domestic dogs, considered the traditional reservoir for leishmaniosis, are more frequently protected by chemoprophylaxis (insecticide collars) and recently by vaccination. In a previous study, 66.6% of blood samples of military working dogs of Var were PCR-positive for *L. infantum*. These dogs had a low level of parasitemia (Aoun et al. 2009). The role of red foxes in the epidemiologic cycle of leishmaniosis should be further investigated. It would be useful to extend biologic sampling of red foxes to the skin of the animals to determine the parasite load in skin, as has been done for asymptomatic dogs. We also need to isolate and characterize strains of *L. infantum* from red foxes.

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