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Authors: Dipineto, Ludovico, Manna, Laura, Baiano, Antonio, Gala, Marianna, Fioretti, Alessandro, et al.

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Presence of *Leishmania infantum* in Red Foxes (*Vulpes vulpes*) in Southern Italy

Ludovico Dipineto,[1] Laura Manna,[2] Antonio Baiano,[1] Marianna Gala,[1] Alessandro Fioretti,[1] Angelo E. Gravino,[1] and Lucia F. Menna[1,3] [1] Dipartimento di Patologia e Sanità Animale, Università di Napoli Federico II, via Delpino 1, 80137, Napoli, Italy; [2] Dipartimento di Scienze Cliniche Veterinarie, Università di Napoli Federico II, via Delpino 1, 80137, Napoli, Italy; [3] Corresponding author (email: menna@unina.it)

ABSTRACT: Skin, lymph node (popliteal), and bone marrow samples were collected from 50 red foxes (*Vulpes vulpes*) from May 2004 to May 2005 in southern Italy. Samples were tested for *Leishmania infantum* by polymerase chain reaction (PCR). The parasite was detected by PCR from 20 of 50 (40%) fox carcasses. All 20 positive cases were PCR-positive from lymph node and bone marrow samples, whereas 17 of 20 positive cases were PCR-positive from skin samples. Infection status was not related to age or sex. This is the first report of leishmaniasis in red foxes in Italy based on PCR results, and these results reinforce the assumption that this wild canid can serve as a reservoir for *Leishmania*.

Key words: Italy, *Leishmania infantum*, PCR, red fox, *Vulpes vulpes*.

Canine leishmaniasis has been continuously reported in Italy since the beginning of 1900s and has spread throughout many central and southern regions (Zaffaroni et al., 1999). In addition to dogs, wild canids, such as jackals (*Canis aureus*) and red fox (*Vulpes vulpes*), are potential feral reservoirs for *Leishmania infantum* (Baneth et al., 1998). *Leishmania* has been reported from red fox from France, Portugal, and Italy (Rioux et al., 1968; Bettini et al., 1980; Abranches et al., 1984; Mancianti et al., 1994), and using molecular detection methods, Criado-Fornelio et al. (2000) reported a 74% prevalence of *L. infantum* in wild red foxes in Guadalajara (central Spain).

Because no data on the prevalence of leishmaniasis in foxes were available for southern Italy and considering the possible role of these canids in increasing transmission rates of parasites to dogs and humans, we used molecular-based diagnostics to determine the presence of *Leishmania infantum* in the red foxes living in a *Leishmania* enzootic area of Italy.

Red fox carcasses ($n=50$) were sampled from the western side of the Campania region (southern Italy) between the Province of Napoli and the Province of Caserta. The corners of the collection area were located at 40°54′06″–40°46′38″N, 14°02′45″–4°09′42″E and 41°09′13″–40°53′57″N, 13°54′27″–14°00′32″E, respectively. Carcasses were collected from May 2004 to May 2005. The majority ($n=42$) of these animals died due to vehicular accidents, whereas the remaining died from sarcoptic mange ($n=3$), poaching ($n=3$), and unknown causes ($n=2$), possibly a gastroenteric infection. There were 31 male (18 mature and 13 immature) and 19 female (11 mature and eight immature) foxes.

Skin, lymph node (popliteal), and bone marrow samples were collected at necropsy. Skin samples, weighing approximately 30 mg, consisted of skin biopsies collected from the dorso-lateral thorax area by 4-mm punch biopsy. Popliteal lymph node and bone marrow samples were obtained by using a thin biopsy needle. Samples were stored at −80 C before DNA extraction. DNA was extracted from fox tissues by QIAamp Tissue Kit (Qiagen, Santa Clarita, California, USA), according to the manufacturer's protocol. The polymerase chain reaction (PCR) assay was performed as previously described by Manna et al. (2004).

Primers consisted of a pair of oligonucleotides described by Rodgers et al. (1990) (5′-dGTGGGGGAGGGGCGTTCT-3′[13a] and 5′-dATTTTACACCAACCCCCAGTT-3 [13b]), which were obtained from a commercial source (Celbio S.p.a., Milan, Italy). The PCR amplification was

TABLE 1. Results (number and percentage of positive samples) obtained by polymerase chain reaction (PCR) for *Leishmania infantum* performed on lymph node, bone marrow, and skin collected from 50 red fox carcasses.

Sex, age, and number of foxes analyzed	No. and % positive for *Leishmania* DNA by PCR	Detection of *Leishmania* DNA by PCR in tissues		
		Lymph node	Bone marrow	Skin
Male, young (n=13)	4/13 (31%)	4/13 (31%)	4/13 (31%)	4/13 (31%)
Male, mature (n=18)	9/18 (50%)	9/18 (50%)	9/18 (50%)	7/18 (39%)
Female, young (n=8)	4/8 (50%)	4/8 (50%)	4/8 (50%)	3/8 (38%)
Female mature (n=11)	3/11 (27%)	3/11 (27%)	3/11 (27%)	3/11 (27%)
Total (n=50)	20/50 (40%)	20/50 (40%)	20/50 (40%)	17/50 (34%)

carried out using a DNA thermal cycler (Perkin-Elmer, Warrington, UK) using the following conditions: 94 C for 1 min and 30 cycles at 94 C for 1 min, 60 C for 1 min, and 72 C for 1 min, followed by a 5-min extension period at 72 C. Amplified products were analyzed using electrophoreses on 2.5% agarose gel containing 0.1 μg/ml ethidium bromide (Sigma-Aldrich, St. Louis, Missouri, USA) in Tris-Borate-EDTA buffer. A 50 base pair DNA ladder (Promega, Madison, Wisconsin, USA) was used as a molecular weight marker. Amplification products on gels were visualized under ultraviolet light with a transilluminator and recorded on Polaroid 667 film.

Leishmania infantum was detected in 20 (40%) of 50 foxes examined (Table 1). The presence of the parasite was similar in male (13/31, 42%) and female (7/19, 37%) as well as mature (12/29, 42%) and young (8/21, 38%) foxes (Table 1). Of the 20 positive animals, 17 tested positive by PCR on all biologic samples; skin samples were negative for three of the 20 positive animals (Table 1).

The role of foxes in the epidemiology of canine and human leishmaniasis cannot be evaluated solely on the basis of this study. However, the prevalence of leishmaniasis detected in this study indicates that a considerable proportion of the fox population living in the southern Italy is infected. Peridomestic transmission from foxes to people and/or dogs via sand flies can take place either when wild canids enter towns to forage for food or when the household dogs range in the extensive noncultivated areas surrounding the towns.

Comparative data on the prevalence of leishmaniasis in foxes in Europe is limited (Mancianti et al., 1994; Criado-Fornelio et al., 2000), and only two studies are available from Italy. Using indirect immunofluorescence assay, enzyme-linked immunosorbent assay, and microscopy for detection of *Leishmania*, Mancianti et al. (1994) and Bettini et al. (1980) reported that 18% (9/50) and 6% (1/16) of foxes were infected, respectively. Furthermore, Gramiccia et al. (1982) demonstrated an enzymatic variant from a fox using enzyme typing by starch gel electrophoretic techniques. These reported prevalence rates for leishmaniasis are lower than those reported in the present study and can be explained by the lower sensitivity associated with the diagnostic procedures used in the previous studies.

To our knowledge, this is the first molecular-based report of leishmaniasis in foxes in Italy, and our results reinforce the assumption that this wild canid can be considered an important reservoir for this parasite. In addition, the majority of foxes that were subject to necropsy were in a healthy body condition, and can be considered, at least theoretically, as subclinical carriers.

LITERATURE CITED

ABRANCHES, P., F. M. CONCEICAO-SILVA, AND M. C. D. SILVA PEREIRA. 1984. Kala-azar in Portugal. V. The sylvatic cycle in the enzootic focus of

Arrabida. Journal of Tropical Medicine and Hygiene 87: 197–200.

BANETH, G., G. DANK, E. KEREN-KORNBLATT, E. SEKELES, I. ADINI, C. L. EISENBERGER, L. F. SCHNUR, R. KING, AND C. L. JAFFE. 1998. Emergence of visceral leishmaniasis in central Israel. American Journal of Tropical Medicine and Hygiene 59: 722–725.

BETTINI, S., E. POZIO, AND L. GRADONI. 1980. Leishmaniasis in Tuscany (Italy): (II) *Leishmania* from wild Bodentia and Carnivora in a human and canine leishmaniasis focus. Transactions of the Royal Society of Tropical Medicine and Hygiene 74: 77–83.

CRIADO-FORNELIO, A., L. GUTIERREZ-GARCIA, F. RODRIGUEZ-CAABEIRO, E. REUS-GARCIA, M. A. ROLDAN-SORIANO, AND M. A. DIAZ-SANCHEZ. 2000. A parasitological survey of wild red foxes (*Vulpes vulpes*) from the province of Guadalajara, Spain. Veterinary Parasitology 92: 245–251.

GRAMICCIA, M., R. MAAZOUN, G. LANOTTE, J. A. RIOUX, S. LE BLANCQ, D. A. EVANS, W. PETERS, S. BETTINI, L. GRADONI, AND E. POZIO. 1982. Enzymatic typing of 11 strains of *Leishmania* isolated in mainland Italy from the visceral murine, canine and vulpine forms. Demonstration of an enzymatic variant in the fox (*Vulpes vulpes*) and the dog. Annales de Parasitologie Humaine et Comparee 57: 527–531.

MANCIANTI, F., W. MIGNONE, AND F. GALESTRI. 1994. Serologic survey for leishmaniasis in free-living red foxes (*Vulpes vulpes*) in Italy. Journal of Wildlife Diseases 30: 454–456.

MANNA, L., F. VITALE, S. REALE, S. CARACAPPA, L. M. PAVONE, R. D. MORTE, G. CRINGOLI, N. STAIANO, AND A. E. GRAVINO. 2004. Comparison of different tissue sampling for PCR-based diagnosis and follow-up of canine visceral leishmaniosis. Veterinary Parasitology 125: 251–262.

RIOUX, J. A., J. L. ALBARET, R. HOUIN, J. P. DEDET, AND G. LANOTTE. 1968. Ecologie des leishmanioses dans le sud de la France. 2. Les reservoirs selvatiques. Infestation spontanee de renard (*Vulpes vulpes* L.). Annales de Parasitologie Humaine et Comparee 43: 421–428.

RODGERS, M. R., S. J. POPPER, AND D. F. WIRTH. 1990. Amplification of kinetoplast DNA as a tool in the detection and diagnosis of *Leishmania*. Experimental Parasitology 71: 267–275.

ZAFFARONI, E., L. RUBAUDO, P. LANFRANCHI, AND W. MIGNONE. 1999. Epidemiological patterns of canine leishmaniosis in western Liguria (Italy). Veterinary Parasitology 81: 11–19.

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