Detection of *Leishmania Infantum* in red foxes (*Vulpes vulpes*) in Central Greece

STELIOS KARAYIANNIS[1], PANTELIS NTAIS[2], IPPOKRATIS MESSARITAKIS[2], NIKOLAOS TSIRIGOTAKIS[2], EMMANOUIL DOKIANAKIS[2] *and* MARIA ANTONIOU[2]*

[1] *Stelios Karagiannis, Ktiniatriki Epe, Vasilikon 25, Lamia, Greece*
[2] *Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, Faculty of Medicine, University of Crete, Greece*

(*Received 12 June 2015; revised 31 July 2015; accepted 10 August 2015; first published online 24 September 2015*)

SUMMARY

This is the first record of *Leishmania* detection in foxes in Greece. Spleen, lymph nodes, bone marrow and blood samples were collected from 47 red foxes (*Vulpes vulpes*) found dead or captured, narcotized and freed after bleeding, from November 2009 to 2011, in Fthiotida prefecture, central Greece. This is an endemic for canine leishmaniasis area with several human visceral leishmaniasis cases. The samples were tested for *Leishmania infantum* and *Leishmania tropica* by molecular methods (polymerase chain reaction (PCR) and restriction fragment length polymorphism) and serology (indirect immunofluorescent antibody test; when blood samples were available). *Leishmania infantum* DNA was detected in 28 animals (59·5%). PCR positivity was related to animal age, sex, weight, characteristics of the area trapped, presence of leishmaniasis symptoms and presence of endo- and ecto-parasites. The results were related to dog seropositivity obtained earlier in the area. The findings support the hypothesis that this wild canid may serve as a reservoir for *Leishmania* in areas where the sandfly vectors are found. In the prefectures of Larisa and Magnisia, adjacent to Fthiotida, *Phlebotomus perfiliewi and Phlebotomus tobbi* (known vectors of *L. infantum*) have been reported.

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

INTRODUCTION

Red foxes, *Vulpes vulpes* belong to the family Canidae and are abundant in many parts of Greece, including Fthiotida prefecture. Foxes may play a role in the transmission of *Leishmania infantum* (causative agent of visceral leishmaniasis (VL) in humans and canine leishmaniasis (CanL)), via sandfly vectors, yet this has not been demonstrated with xenodiagnosis studies. *Leishmania infantum* has been reported in red foxes in France, Portugal, Italy, Serbia and Spain (Rioux *et al*. 1968; Bettini *et al*. 1980; Abranches *et al*. 1984; Mancianti *et al*. 1994; Criado-Fornelio *et al*. 2000; Cirović *et al*. 2014); and in Brazil (the synonym of *L. infantum* in the New World, *Leishmania chagasi*) (Courtenay *et al*. 1994); but also wolves (*Canis lupus*) were found infected with *L. infantum* in Croatia (Beck *et al*. 2008), Portugal and Spain (Sastre *et al*. 2008; Sobrino *et al*. 2008) as well as golden jackals (*Canis aureus*) in Israel (Hervás *et al*. 1996), Iraq (Sukker, 1984) and Kazakhstan (Musabekov *et al*. 1997). The causative agent of cutaneous leishmaniasis (CL), *Leishmania tropica*, although considered an anthroponotic infection, has been sporadically reported from domestic dogs in Iran and Morocco (Mohebali *et al*. 2005; Rhajaoui *et al*. 2007) and described in red foxes, golden jackals and the rock hyrax (*Procavia capensis*) in Israel (Talmi-Frank *et al*. 2010), Jordan and the Palestinian Authority (Jacobson *et al*. 2003; Talmi-Frank *et al*. 2010).

In Greece, two *Leishmania* species are known, *L. infantum* causing VL and CanL in 41/54 prefectures (in the majority of which it is endemic) and *L. tropica* causing CL in Crete and the Ionian islands (Ntais *et al*. 2013). In Fthiotida prefecture, an average of 5 VL cases per year is reported and 58·8% of the dogs were found seropositive, whilst culture of the parasite showed *L. infantum* (Ntais *et al*. 2013). *Phlebotomus perfiliewi* and *Phlebotomus tobbi*, known sandfly vectors of *L. infantum*, have been described in the area (Ivović *et al*. 2007; Xanthopoulou *et al*. 2011). Leishmaniasis prevalence data on foxes are not available for Greece. The survey was conducted in the prefecture of Fthiotida, central Greece, where 47 foxes were made available from local veterinarians. After necropsy, biological material was examined for *Leishmania* presence so that the possible role of these canids in the epidemiological chain of the parasite could be evaluated.

MATERIALS AND METHODS

Study area

The study area involved the region in and around 11 villages in the West Fthiotida prefecture, endemic

* Corresponding author. University of Crete, Medical School, Voutes 710 03, Heraklion, Crete, Greece. E-mail: antoniou@med.uoc.gr

doi:10.1017/S0031182015001158

for leishmaniasis (VL and CanL). The villages were: Ano Kallithea (co-ordinates: 38.8786415, 22.106884100000002), Kato Kallithea (38.90217680000001, 22.159112100000016), Ag. Sostis (38.8957468, 22.164228099999946), Fteri (38.9004674, 22.047290799999928), Sperchiada (38.9075721, 22.127325400000018), Makrakomi (38.9424649, 22.115891199999965), Archanio (38.9727357, 22.167467100000067), Mesopotamia (38.92429449999999, 22.166226199999983), Grammeni (38.9649864, 22.19606909999993), Syka (38.8816247, 22.197596100000055), Mendenitsa (38.7514889, 22.6180944) (Fig. 1).

The area is a protected valley, of the Sperchios River, providing water for wild life, with trees and bushes offering an all year round coverage of the terrain; at 125–840 m altitude.

Animal sampling

Animal samples ($n = 47$) were collected over a 3-year period, November 2009 to 2011, from areas where red foxes, *V. vulpes* (Linnaeus, 1758), are abundant. Animal carcasses were provided mainly by hunters. The animals had died due to vehicular accidents ($n = 17$), poaching ($n = 16$) or were found dead possibly due to disease ($n = 12$). In two cases animals were captured in trapping cages, narcotized and freed after bleeding. The veterinarian examined them for ectoparasites (fleas after combing the animal); identified the foxes using morphological characters; sexed; weighed and classified them as 'adults', 'juveniles' and 'possibly old' based on body size (taking into account differences due to sex) and the condition of the animal's teeth. Also, foxes were characterized as 'possibly old' if they had white hair on their face. They were examined for signs of classical CanL symptoms (low weight for their age, sex and species; dermatitis furfuracea; skin lesions; lymph node swelling; periophthalmic alopecia; onychogryphosis; splenomegaly) and were classified into three categories accordingly: 'not in good health' (with at least three CanL symptoms), 'in moderate health' (with two CanL symptoms), 'in good health' (no apparent clinical signs). Biological samples: blood, spleen and lymph nodes (mandibular and popliteal) were obtained at necropsy from each animal, when possible, and stored at −80 °C in plastic tubes for safety until testing. The organs of the animals as well as their intestine contains were examined for parasites and parasite eggs. All relevant data for each animal were recorded by the veterinarian in a questionnaire.

Serological testing

Red fox sera were tested serologically using anti-dog, anti-IgG (immunoglobulin G) antibodies, by indirect immunofluorescent antibody test (IFAT, *Leishmania* SPOT IF, BioMerieux France). A series of 2-fold serum dilutions, starting from 1/40 were performed and a cut-off titer of ⩾1/160 was regarded positive since all foxes lived in *Leishmania* endemic areas.

Polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP)

DNA was extracted from all biological samples available from each fox using DNeasy blood and tissue kit (QIAGEN, EU) following the manufacturer's instructions. *Leishmania* molecular screening was performed by targeting a repetitive sequence (Minodier *et al*. 1997) using the modifications of Christodoulou *et al*. (2012) as well as amplifying the *Leishmania* ITS1 region followed by a *Hae*III restriction endonuclease digestion of the positive PCR products (Schönian *et al*. 2003) in order to identify which parasite species had infected the animal. All samples were tested in duplicates. Positive controls were used in all PCR assays which consisted of DNA extracted from *L. infantum* (Nicolle, 1908) (MCAN/GR/2009/GD70) and *L. tropica* (Wright, 1903) (MCAN/GR/2009/GD52) which had derived from Greek dogs and typed by enzyme electrophoresis (Ntais *et al*. 2013). Negative controls included samples from dogs born and lived in non-endemic areas in Crete (at >1000 m altitude), that had been tested serologically, by PCR and culture and proved negative.

Statistical analysis and mapping of the results

The χ^2 test with SPSS v. 22 was applied to examine statistical significance of *Leishmania* positivity of the animal, by PCR, and: gender, sampling year, health status, presence of ecto- or/and endo-parasites, characteristics of the environment the animals lived; each factor was tested separately. In all cases a P value <0·05 was considered statistically significant.

RESULTS

A total of 47 foxes were examined using blood, spleen and/or lymph nodes according to availability: 28 males and 19 females. The majority, 31 animals, were 'adults', 9 were 'juveniles' and 7 were 'possibly old'. Five animals were classified as not in good health, 25 as in moderate health and 17 in good health (Table 1). Of these animals, 28 were PCR positive for *Leishmania* (59·5%): 23 spleens, 2 lymph nodes, 2 bone marrow, 1 blood (both from blood sample with and without the anticoagulant ethylenediaminetetraacetic acid, EDTA); 18 of the 28 males (64·3%) and 10 of the 19 females (52·6%).

Three out of the 9 sera tested had IgG antibodies against *Leishmania*; since foxes and dogs may not react the same way to dog reagents, *Leishmania*

https://doi.org/10.1017/S0031182015001158 Published online by Cambridge University Press

Fig. 1. The geographical distribution of the red foxes tested for the presence of *Leishmania*. The position of the villages in the Sperchios valley, in the vicinity of which the animals were found, is shown, indicating the number of polymerase chain reaction positive animals over the number of animals tested.

Table 1. Health status and results of 47 red foxes from Fthiotida prefecture, central Greece, by polymerase chain reaction (PCR) and indirect immunofluorescent antibody test (IFAT) performed in different tissues for *Leishmania infantum*

47 Red foxes: test results and health status		PCR (±) 28/19	PCR (+) IFAT (–) 25	PCR (–) IFAT(+) 0	PCR (+) IFAT (+) 3
Not in good health					
No. of individuals	5	3/2	2	–	1
Males/females	3/2				
Age	5 'adults'				
In moderate health					
No. of individuals	25	17/8	15	–	2
Males/females	13/12				
Age	18 'adults' 4 'juveniles' 3 'possibly old'				
In good health					
No. of individuals	17	8/9	8	–	–
Males/females	12/5				
Age	8 'adults' 5 'juveniles' 4 'possibly old'				

positive animals were considered only if they were PCR positive. Nevertheless, no PCR negative animal presented IgG antibodies against the parasite. Two of the seropositive animals were in moderate health and hosted other parasites as well, (ecto- and endo-parasites) whilst the third animal was not in good health. PCR-RFLP analysis revealed the presence of *L. infantum* in the PCR positive animals. The geographical distribution of the animals tested and the PCR results are shown in Fig. 1.

The χ^2 test showed significantly higher number of PCR positive animals living in <300 m altitude compared with animals living in higher altitudes ($\chi^2 = 8{\cdot}87$; $P = 0{\cdot}003$). There was no statistical

https://doi.org/10.1017/S0031182015001158 Published online by Cambridge University Press

relationship between gender, age and the presence of ecto- or endo-parasites with PCR positivity, nor the health status of the animal.

Three different ectoparasite spp and 6 endoparasites were found in PCR positive and PCR negative for *Leishmania* animals. Ectoparasites: fleas in 12 animals; *Sarcoptes scabiei* in 3; *Demodex* sp. in 1. Endoparasites: *Dirofilaria immitis* in the heart and/or lungs in 8 animals; *Trichuris vulpis* in 4; *Toxascaris leonina* in 3; *Ancylostoma caninum* in 2; *Taenia* sp. in 2; *Dipylidium caninum* in 1.

DISCUSSION

The role of red foxes in the epidemiology of canine and human leishmaniasis cannot be evaluated on the basis of this study. However, the results indicate that a considerable proportion of the red fox population in this area (59·5%) shows a high prevalence of infection/exposure. If this wild canid plays the role of reservoir host it may help in increasing the transmission rates of *Leishmania* to dogs and humans and its geographical dispersion. This is facilitated by the fact that red foxes are known to dwell in great distances (Dolev, 2006), according to the availability of resources, and that competent sandfly vectors are present in the area. The fact that dog densities are much higher than fox densities would make dogs more important hosts than wild canids. Nevertheless, quantitative xenodiagnosis studies are required in order to clarify the role of the red fox in *Leishmania's* epidemiological chain.

The natural ecosystem of the red foxes in the study is the Sperchios Valley consisting of agricultural land, forests and mountains. Mountain Oiti, an animal reserve south of these villages offers protection and cover to the animals (Fig. 1). The red foxes, due to the destruction of their habitats, visit inhabited areas during the night to search for food, seen in the gardens of houses where, in most cases, dogs live. Transmission, therefore, via sand fly vectors, may take place either when wild canids enter towns or when household dogs range in areas surrounding towns.

Asymptomatic *L. infantum* infections seem to be common in dogs and other canids (Courtenay *et al.* 1994). However, intense cutaneous parasitism in dogs and foxes allows sand flies to become infected when feeding on the skin (Deane and Deane, 1954). In Brazil, laboratory experiments showed that the crab eating fox (*Cerdocyon thous*) was infectious to sandflies, even in the absence of clinical leishmaniasis due to *L. chagasi* (Lainson *et al.* 1987, 1990), a situation that allows the parasite to circulate unnoticed. In the animals studied, at least three classical CanL symptoms were observed in 5 animals, 3 of which were PCR positive, and 2 classical CanL symptoms in 25 animals, 17 of which were PCR positive. That is, 20/28 PCR positive for *Leishmania* red foxes appeared symptomatic. This high percentage is unusual and may be related to other factors, such as availability of food and other diseases; the fact that the clinical signs recorded are not strictly specific to *L. infantum* should be noted. Interestingly, 27 of the 47 animals studied had ecto- and/or endo-parasites indicating unfavourable health conditions in the area; the presence of other parasites was not statistically associated with *Leishmania* PCR positivity.

Comparative data on the prevalence of leishmaniasis in foxes in Europe is limited. Using IFAT, enzyme-linked immunosorbent assay and microscopy for detection of *Leishmania*, Mancianti *et al.* (1994) and Bettini *et al.* (1980) reported 18% (9/50) and 6% (1/16) of the foxes tested, respectively, to be positive. These prevalence rates, however, which are lower than those reported in the present study, can be explained by the higher sensitivity of the PCR method. In similar studies in Italy, the parasite was detected by PCR from 40% of fox carcasses by Dipineto *et al.* (2007) and 52·2% by Verin *et al.* (2010). Interestingly, Gramiccia *et al.* (1982) demonstrated an enzymatic variant from a fox using enzyme typing by starch gel electrophoretic techniques.

Leishmania tropica has been reported in the spleens of infected asymptomatic wild canid species (foxes and jackals) which implies that these animals may also be infected and may play the host for this parasite (Talmi-Frank *et al.* 2010). The involvement of wild canids in the sylvatic life of *L. tropica* may be important in Greece and red foxes from the Ionian islands Kerkyra, Leukada and Kefalonia must be examined for the presence of *L. infantum* and *L. tropica* since both species are found in humans and dogs and proven vectors of *L. infantum* (*P. perfiliewi, P. tobbi, Phlebotomus neglectus*) and *L. tropica* (*Phlebotomus similis*) have been reported in the area (Ntais *et al.* 2013).

ACKNOWLEDGEMENTS

We thank the veterinarians who provided fox samples.

FINANCIAL SUPPORT

This work was funded by the EU grant FP7-261504 EDENext and is catalogued by the EDENext Steering Committee as EDENext364 (http://www.edenext.eu). The contents of this publication are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission.

REFERENCES

Abranches, P., Conceiçao-Silva, F. M. and Silva-Pereira, M. C. (1984). Kala-azar in Portugal. V. The sylvatic cycle in the enzootic endemic focus of Arrabida. *American Journal of Tropical Medicine and Hygiene* **87**, 197–200.

https://doi.org/10.1017/S0031182015001158 Published online by Cambridge University Press

Beck, A., Beck, R., Kusak, J., Gudan, A., Martinkovic, F., Artukovic, B., Hohsteter, M., Huber, D., Marinculic, A. and Grabarevic, Z. (2008). A case of visceral leishmaniosis in a gray wolf (*Canis lupus*) from Croatia. *Journal of Wildlife Diseases* **44**, 451–456.

Bettini, S., Pozio, E. and Gradoni, L. (1980). Leishmaniasis in Tuscany (Italy): (II) *Leishmania* form wild Rodentia and Carnivora in a human and canine leishmaniasis focus. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 77–83.

Christodoulou, V., Antoniou, M., Ntais, P., Messaritakis, I., Ivovic, V., Dedet, J. P., Pratlong, F., Dvorak, V. and Tselentis, Y. (2012). Re-emergence of visceral and cutaneous leishmaniasis in the Greek Island of Crete. *Vector-Borne and Zoonotic Diseases* **12**, 214–222.

Cirović, D., Chochlakis, D., Tomanović, S., Sukara, R., Penezić, A., Tselentis, Y. and Psaroulaki, A. (2014). Presence of *Leishmania* and *Brucella* species in the golden jackal *Canis aureus* in Serbia. *BioMed Research International* **2014**, 728516.

Courtenay, O., Macdonald, D. W., Lainson, R., Shaw, J. J. and Dye, C. (1994). Epidemiology of canine leishmaniasis: a comparative serological study of dogs and foxes in Amazon Brazil. *Parasitology* **109**, 273–279.

Criado-Fornelio, A., Gutierrez-Garcia, L., Rodriguez-Caabeiro, F., Reus-Garcia, E., Roldan-Soriano, M. A. and Diaz-Sanchez, M. A. (2000). A parasitological survey of wild red foxes (*Vulpes vulpes*) from the province of Guadalajara, Spain. *Veterinary Parasitology* **92**, 245–251.

Deane, M. P. and Deane, L. M. (1954). Infecção experimental do Phlebotomus longipalpis em raposa (*Lycolopex vetulus*), naturalmente parasitada pela *Leishmania donovani*. *O Hospital* **46**, 651–653.

Dipineto, L., Manna, L., Baiano, A., Gala, M., Fioretti, A., Gravino, A. E. and Menna, L. F. (2007). Presence of *Leishmania infantum* in Red Foxes (*Vulpes vulpes*) in Southern Italy. *Journal of Wildlife Diseases* **43**, 518–520.

Dolev, A. (2006). Modeling the spatial dynamics of rabies in canid vectors using a realistic landscape: a tool for optimizing the spatial scattering of oral rabies vaccination. Dissertation. The Ben-Gurion University of the Negev, Beer Sheba, Israel.

Gramiccia, M., Maazoun, R., Lanotte, G., Rioux, J. A., le Blancq, S., Evans, D. A., Peters, W., Bettini, S., Gradoni, L. and Pozio, E. (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]. [Article in French]. *Annales De Parasitologie Humaine Et Comparee* **57**, 527–531.

Hervás, J., Méndez, A., Carrasco, L. and Gómez-Villamandos, J. C. (1996). Pathological study of visceral leishmaniasis in a jackal (*Canis aureus*). *Veterinary Record* **139**, 293–295.

Ivovic, V., Patakakis, M., Tselentis, Y. and Chaniotis, B. (2007). Faunistic study of sandflies in Greece. *Medical and Veterinary Entomology* **21**, 121–124.

Jacobson, R. L., Eisenberger, C. L., Svobodova, M., Baneth, G., Sztern, J., Carvalho, J., Nasereddin, A., El Fari, M., Shalom, U., Volf, P., Votypka, J., Dedet, J. P., Pratlong, F., Schonian, G., Schnur, L. F., Jaffe, C. L. and Warburg, A. (2003). Outbreak of cutaneous leishmaniasis in northern Israel. *Journal of Infectious Diseases* **188**, 1065–1073.

Lainson, R., Shaw, J. J., Silveira, F. T. and Braga, R. R. (1987). American visceral leishmaniasis: on the origin of *Leishmania* (*Leishmania*) *chagasi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 517.

Lainson, R., Dye, C., Shaw, J. J., Macdonald, D., Courtenay, O., Souza, A. A. and Silveira, F. T. (1990). Amazonian visceral leishmaniasis: distribution of the vector *Lutzomyia longipalpis* (Lutz & Neiva) in relation to the fox *Cerdocyon thous* (L.) and the efficiency of this reservoir host as a source of infection. *Memorias do Instituto Oswaldo Cruz* **85**, 135–137.

Mancianti, F., Mignone, W. and Galastri, F. (1994). Serologic survey for leishmaniasis in free-living red foxes (*Vulpes vulpes*) in Italy. *Journal of Wildlife Diseases* **30**, 454–456.

Minodier, P., Piarroux, R., Gambarelli, F., Joblet, C. and Dumon, H. (1997). Rapid identification of causative species in patients with Old World leishmaniasis. *Journal of Clinical Microbiology* **35**, 2551–2555.

Mohebali, M., Hajjaran, H., Hamzavi, Y., Mobedi, I., Arshi, S., Zarei, Z., Akhoundi, B., Naeini, K. M., Avizeh, R. and Fakhar, M. (2005). Epidemiological aspects of canine visceral leishmaniosis in the Islamic Republic of Iran. *Veterinary Parasitology* **129**, 243–251.

Musabekov, K. S., Novak, M. D. and Farisova, O. M. (1997). The jackal, a potential source of leishmaniasis in southern and south-eastern Kazakhstan. *Izvestiya Rossiiskoi Akademii Nauk – Seriya Biologicheskaya* **4**, 86–87.

Ntais, P., Sifaki-Pistola, D., Christodoulou, V., Messaritakis, I., Pratlong, F., Poupalos, G. and Antoniou, M. (2013). Leishmaniases in Greece. *American Journal of Tropical Medicine and Hygiene* **89**, 906–915.

Rhajaoui, M., Nasereddin, A., Fellah, H., Azmi, K., Amarir, F., Al-Jawabreh, A., Ereqat, S., Planer, J. and Abdeen, Z. (2007). New clinicoepidemiologic profile of cutaneous leishmaniasis, Morocco. *Emerging Infectious Diseases* **13**, 1358–1360.

Rioux, J. A., Albaret, J. L., Houin, R., Dedet, J. P. and Lanotte, G. (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.

Sastre, N., Francino, O., Ramirez, O., Ensenat, C., Sanchez, A. and Altet, A. (2008). Detection of *Leishmania infantum* in captive wolves from Southwestern Europe. *Veterinary Parasitology* **158**, 117–120.

Schönian, G., Nasereddin, A., Dinse, N., Schweynoch, C., Schallig, H. D., Presber, W. and Jaffe, C. L. (2003). PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. *Diagnostic Microbiology and Infectious Disease* **47**, 349–358.

Sobrino, R., Ferroglio, E., Oleaga, A., Romano, A., Millan, J., Revilla, M., Arnal, M., Trisciuoglio, M. C. and Gortazar, C. (2008). Characterization of widespread canine leishmaniasis among wild carnivores from Spain. *Veterinary Parasitology* **155**, 198–203.

Sukker, F. (1984). Epidemiology and control of visceral leishmaniasis in Iraq. *Bull End Dis Iraq* **26**, 13–26.

Talmi-Frank, D., Kedem-Vaanunu, N., King, R., Bar-Gal, G. K., Edery, N., Jaffe, C. L. and Baneth, G. (2010). *Leishmania tropica* infection in golden jackals and red foxes, Israel. *Emerging Infectious Diseases* **16**, 1973–1975.

Verin, R., Poli, A., Ariti, G., Nardoni, S., Fanucchi, M. B. and Mancianti, F. (2010). Detection of *Leishmania infantum* DNA in tissues of free-ranging red foxes (*Vulpes vulpes*) in Central Italy. *European Journal of Wildlife Research* **56**, 689–692.

Xanthopoulou, K., Anagnostou, V., Ivović, V., Djurkovic-Djakovic, O., Rogozi, E., Sotiraki, S. and Papa, A. (2011). Distribution of sandflies (Diptera, Psychodidae) in two Ionian Islands and northern Greece. *Vector-Borne and Zoonotic Diseases* **11**, 1591–1594.