DOI: 10.7589/JWD-D-21-00027

Journal of Wildlife Diseases, 58(1), 2022, pp. 198–204

Detection of Anti–*Leishmania infantum* Antibodies in Wild European and American Mink (*Mustela lutreola* and *Neovison vison*) from Northern Spain, 2014–20

Jacobo Giner,[1,2] Sergio Villanueva-Saz,[1,2,3,9] Antonio Fernández,[1,2,3] María Asunción Gómez,[4] Madis Podra,[4] Patricia Lizarraga,[5] Delia Lacasta,[1,3] Héctor Ruiz,[1] María del Carmen Aranda,[6] María de los Ángeles Jimenez,[7] Raquel Hernández,[8] Andrés Yzuel,[2] and Maite Verde[1,2,3] [1]Department of Animal Pathology, Veterinary Faculty, University of Zaragoza, Calle Miguel Servet, 177, 50013 Zaragoza, Spain; [2]Clinical Immunology Laboratory, Veterinary Faculty, University of Zaragoza, Calle Miguel Servet, 177, 50013 Zaragoza, Spain; [3]Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), Calle Miguel Servet, 177, 50013 Zaragoza, Spain; [4]Tragsatec, Tragsatec, Division of Environmental Services, Julian Camarillo 6A-4A Planta, Sector B, 28037 Madrid, Spain; [5]Centro de Recuperación de Fauna de Martioda, Martioda Entitatea, 3, 01191 Martioda, Alava, Spain; [6]Fundación para la Investigación en etología y biodiversidad, Casarrubios del Monte, 45950 Toledo, Spain; [7]Departamento Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Av. Puerta de Hierro, s/n, 28040 Madrid, Spain; [8]Técnico externo del Gobierno de Aragón, Calle Miguel Servet, 177, 50013 Zaragoza, Spain; [9]Corresponding author (email: svs@unizar.es)

ABSTRACT: The European mink (*Mustela lutreola*) is listed as a critically endangered species because of ongoing population reduction from habitat degradation and the effects of introduced species, such as American mink (*Neovison vison*). This small, fragmented population becomes vulnerable to many other threats, including diseases. Leishmaniosis is a zoonotic disease caused by the protozoan parasite *Leishmania infantum* found in the Mediterranean area, which affects many mammals, including wild small mammals. Furthermore, clinical disease caused by *L. infantum* has recently been described in other mustelids. To assess the exposure to *Leishmania* sp. infection in mink species in northern Spain, blood samples from 139 feral American mink and 42 native European mink from north Spain were evaluated for *Leishmania* sp. infection using enzyme-linked immunosorbent assays against *Leishmania* spp. antibodies, with 52.4% of American mink and 45.3% of European mink being found seropositive. This finding raises questions regarding how the disease may affect these species and the potential repercussions for conservation efforts. Despite a high seroprevalence being observed in wild mink of both species in this study, association with clinical or pathologic signs of disease has yet to be elucidated.

Key words: Leishmania infantum, serologic survey, wild minks.

Leishmaniosis, caused by *Leishmania infantum*, is a vector-borne, zoonotic disease endemic in southern Europe, which is spreading to northern regions (Pennisi et al. 2015). This parasite is transmitted under natural conditions by female phlebotomine sand flies during blood feeding. In Spain, dogs (*Canis familiaris*) are considered to be the main reservoir for *L. infantum*. However, the role of other potential reservoirs for this parasite, such as wild small mammals, is being investigated (Alcover et al. 2020). Detection of the parasite infection in wild carnivores in Spain has been shown, suggesting the existence of a sylvatic cycle of the *L. infantum* independent of dogs (Sobrino et al. 2008). A recent study detected a seroprevalence of 20% among 200 farmed American mink (*Neovison vison*) without any skin or visceral lesions. Nevertheless, seropositivity was associated with poor body condition (Tsakmakidis et al. 2019).

Recently, the first clinical cases of leishmaniosis in mustelids were published in a domestic ferret (*Mustela putorius furo*), and a captive Eurasian otter (*Lutra lutra*). The ferret had a papular lesion in the right pinna (Giner et al. 2020), and the otter had bilateral epistaxis, plus signs of anorexia, apathy, and weight loss (Cantos-Barreda et al. 2020).

The European mink (*Mustela lutreola*) belongs to the Mustelidae family (Carnivora) and is classified as a critically endangered species, according to the International Union for Conservation of Nature Red List (Maran et al. 2016). During the 20th century, numbers of European mink declined, and the range of distribution has been reduced to a few fragmented populations; today, this

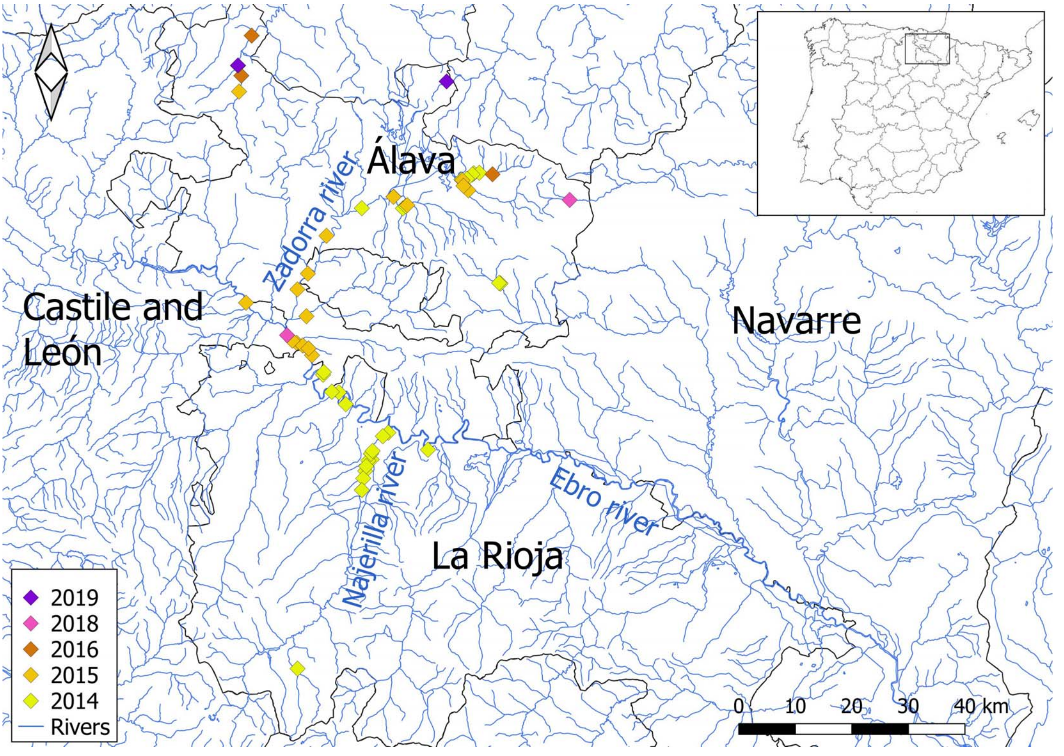

FIGURE 1. Location of *Leishmania*-seropositive American minks detected by enzyme-linked immunosorbent assay, from 137 individuals from the Cantabrian and Ebro basins, northern Spain, 2014–20. The main map shows the region of Castile and León; see inset map of Spain for the area depicted.

species faces extinction (Amstislavsky et al. 2008). Several causes have been put forward to explain the disappearance of the species in different time periods. Overhunting was the most critical cause during the first half of the 20th century; at present, climate change, destruction of habitat, and the presence of the introduced American mink in the same region in which European mink reside aggravate the situation and often make it irreversible (Frankham 2003).

Our study aimed to determine the prevalence of natural infection with *L. infantum* in wild European and American mink in northern Spain using an in-house enzyme-linked immunosorbent assay. The results would help ascertain the degree of exposure to the parasite in both mink species (native and introduced) in their two distribution areas in northern Spain: the Ebro basin, with a semiarid climate with dry, hot summers and cold winters; and the Cantabrian basin, characterized by mild winters and warm summers.

From 2014 to 2020, a total of 181 animals (139 American mink and 42 European mink) were examined. For each animal, information that included geographic coordinates, river basin, sex, and body scoring was obtained. Blood samples from native European mink were obtained from various sources: population surveys of the European mink in the Spanish distribution areas; periodic mink population controls in river drainages; campaigns to capture founders for the European mink breeding program in Spain; and accidental trapping during culling campaigns of feral American mink. Samples from feral American mink were collected during population control operations conducted by several governmental authorities and performed by rangers and biologists acting as trappers. This survey was included under the LIFE project, approved by the European Commission for

Table 1. Summary of *Leishmania* seropositivity based on enzyme-linked immunosorbent assay of American mink (*Neovison vison*) and European mink (*Mustela lutreola*) from the Cantabrian and Ebro basins, northern Spain, 2014-2020.

River	River basin	No. mink	Year (n)[a]	No. seropositive mink	Serology classification (no.)	Sex seropositive (no.)	Year seropositive (n)
American mink							
Alegria	Ebro	1	2014	0			
Aramayona	Cantabrian	2	2019 (2)	1	Low (1)	Female	2019 (1)
Ayuda	Ebro	5	2014 (1) 2015 (4)	1	Low (1)	Male	2015 (1)
Barrundia	Ebro	13	2014 (10) 2015 (2) 2016 (1)	7	Low (7)	Male (3) Female (4)	2014 (6) 2016 (1)
Bayas	Ebro	1	2014	0			
Berron	Ebro	6	2014 (6)	3	Low (3)	Female (3)	2014 (3)
Ebro	Ebro	28	2014 (11) 2015 (15) 2016 (2) 2018 (1)	14	Low (13) High (1)	Female (7) Male (7)	2014 (6) 2015 (7) 2018 (1)
Ega	Ebro	2	2014 (1) 2015 (1)	1	Low (1)	Female (1)	2014 (1)
Errekabarri	Ebro	1	2015 (1)	1	Low (1)	Female (1)	2015 (1)
Izoria	Cantabrian	7	2015 (7)	0			
Najerilla	Ebro	16	2014 (16)	11	Low (11)	Female (5) Male (6)	2014 (11)
Nervion	Cantabrian	16	2014 (3) 2015 (6) 2016 (4) 2017 (1) 2018 (1) 2019 (1)	7	Low (7) Moderate (1)	Female (2) Male (5)	2015 (4) 2016 (2) 2019 (1)
Salburua	Ebro	3	2014 (3)	2	Low (2)	Female (1) Male (1)	2014 (2)
Urbion	Ebro	1	2014 (1)	1	Low (1)	Female (1)	2014 (1)
Yalde	Ebro	1	2014 (1)	1	Low (1)	Male (1)	2014 (1)
Zadorra	Ebro	34	2014 (12) 2015 (19) 2016 (3)	12	Low (12)	Female (6) Male (12)	2014 (5) 2015 (7)
Zirautza	Ebro	1	2018 (1)	1	Low (1)	Male (1)	2019 (1)
European mink							
Alegría	Ebro	2	2014 (1) 2019 (1)	1	Low (1)	Female (1)	2019 (1)
Alhama	Ebro	1	2017 (1)				
Arroy	Ebro	2	2020 (2)	2	Low (2)	Female (2)	2020 (2)
Bayas	Ebro	2	2016 (2)	2	Low (2)	Male (2)	2016 (2)
Cidacos	Ebro	3	2017 (1) 2019 (2)	0			
Ea	Ebro	1	2019 (1)	0			
Ebro	Ebro	7	2015 (1) 2016 (1) 2017 (2) 2020 (3)	6	Low (6)	Female (1) Male (5)	2015 (1) 2017 (2) 2020 (3)

Table 1. Continued.

River	River basin	No. mink	Year (n)[a]	No. seropositive mink	Serology classification (no.)	Sex seropositive (no.)	Year seropositive (n)
Ega	Ebro	5	2015 (1) 2016 (1) 2017 (2) 2020 (1)	3	Low (3)	Female (2) Male (1)	2015 (1) 2017 (1) 2020 (1)
Iregua	Ebro	1	2017 (1)	1	Low (1)	Female (1)	2017 (1)
Laguna de los dos Reinos	Ebro	1	2019 (1)	1	Low (1)	Male (1)	2019 (1)
Leza	Ebro	2	2017 (2)	2	Low (2)	Female (2)	2017 (2)
Najerilla	Ebro	3	2018 (1) 2019 (1) 2020 (1)	2	Low (2)	Female (1) Male (1)	2018 (1) 2020 (1)
Oja	Ebro	2	2014 (2)	0			
Salburúa	Ebro	1	2014 (1)	0			
Tiron	Ebro	2	2014 (2)	1	Low (1)	Female (1)	2014 (1)
Zadorra	Ebro	3	2014 (1) 2017 (1) 2018 (1)	0			
Zirauntza	Ebro	4	2016 (2) 2018 (1) 2020 (1)	1	Low (1)	Female (1)	2016 (1)

[a] n = number of mink tested in a given year.

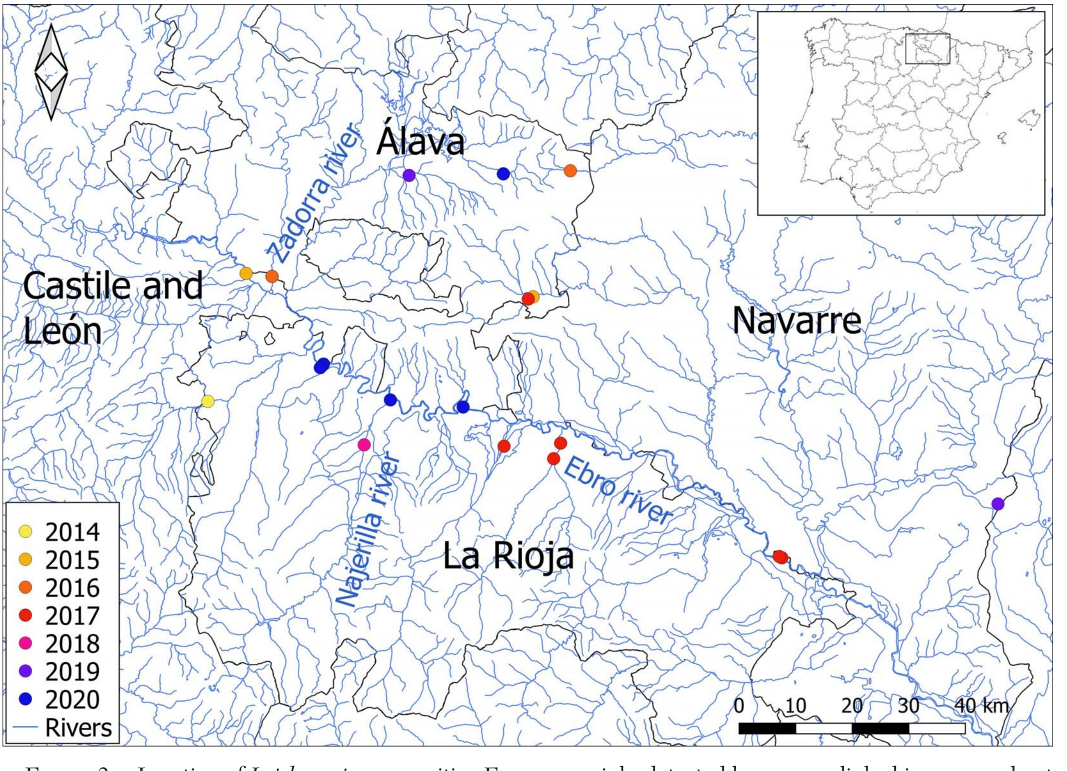

Figure 2. Location of *Leishmania*-seropositive European minks detected by enzyme-linked immunosorbent assay, from 42 individuals from the Cantabrian and Ebro basins, northern Spain, 2014–20. The main map shows the region of Castile and León; see inset map of Spain for the area depicted.

TABLE 2. Seroprevalence of *Leishmania infantum* in American mink (*Neovison vison*) and European mink (*Mustela lutreola*) from the Cantabrian and Ebro basins, northern Spain, 2014–20 by gender, species, and habitat.

	European mink[a]			American mink		
	22/42			63/137		
Animals (Seropositive animals/total)	*n*	%	95% CI	*n*	%	95% CI
Gender						
Male	10	23.8	(13.5–38.5)	31	22.6	(16.4–30.3)
Female	12	28.6	(17.2–43.6)	32	23.4	(17.1–31.1)
River basin						
Ebro	22	52.4	(37.7–66.6)	55	40.1	(32.3–48.5)
Cantabrian	0	0	NA	8	5.8	(3.0–11.1)

[a] *n* = number seropositive; 95% CI = 95% confidence internal; NA = not available.

the conservation of the European mink (00NAT/E/7299, 00NAT/E/7335, and 00NAT/E/7331). The care and use of animals were carried out according to the Spanish Policy for Animal Protection (RD 53/2013), which meets European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

A total of 139 American mink were included (72 females and 67 males), whereas 42 European mink were evaluated (24 females and 18 males). These animals came from various riverbanks in northern Spain. The total number of samples processed in the sampling period (2014–20) ranged from 1 to 67 in each year. All animals in this study were apparently healthy and presented an ideal condition (3/5) using a body scoring system based on a five-point scale (Rouvinen-Watt and Armstrong 2002). For that scale, animals in an ideal condition have the following characteristics: the mink has a slender neck and a straight body shape, there is a slight amount of subcutaneous body fat, and the shoulder, hip bones, and the ribs can be easily felt.

Both species were captured in single-entry 15×15×60-cm wire-cage traps. Captured European mink were anesthetized intramuscularly with a combination of 5 mg/kg ketamine hydrochloride (Imalgene 1000, Merial, Lyon, France) and 0.10 mg/kg medetomidine hydrochloride (Domtor, Orion Corporation, Espoo, Finland). Atipamezole (Antisedans, Orion) was used for reversal of the medetomidine at five times the medetomidine dose. All European mink were clinically examined and bled by jugular puncture; sex, weight, and body condition score were recorded, and they were marked with subcutaneous, passive transponder tags for identification. After recovery from anesthesia, they were released at their capture locations.

American minks were also anesthetized, and blood samples were collected from the jugular vein or by cardiac puncture. Routine laboratory tests, such as a complete blood cell count and a biochemistry profile were not

TABLE 3. Factors evaluated regarding the presence of anti-*Leishmania* antibodies in American mink (*Neovison vison*) and European mink (*Mustela lutreola*) from the Cantabrian and Ebro basins, northern Spain, 2014–20.[a]

	All minks			American mink		European mink	
	Sex	River basin	Species	Sex	River basin	Sex	River basin
Leishmania seropositivity	0.767	0.132	0.482	0.867	0.183	0.763	Not available

[a] Fisher exact test. Associations with a $P<0.05$ were considered to be statistically significant.

performed. After data collection, and when still under anesthesia, these animals were sacrificed following the welfare legal standards.

An enzyme-linked immunosorbent assay was performed on all sera as described previously, with some modifications using 100 µL of mink sera diluted 1:50 (Giner et al. 2020). Each plate included as a positive control serum from a ferret (*Mustela putorius furo*) from Spain diagnosed with leishmaniosis (Giner et al. 2020) and a negative control serum from a healthy, noninfected ferret. The cutoff was set to 0.200 optical density (OD) units (mean±3 SD values from 40 healthy, indoor ferrets). Sera with an OD≥1.00 were classified as high-positive, those with an OD≥0.60 and <1.00 as moderate-positive, and those with an OD>0.20 and <0.60 as low-positive.

Data were analyzed using SPSS version 22 software (SPSS Inc., Chicago, Illinois, USA). Descriptive analysis of the variables (sex, Ebro basin or Cantabrian basin, and species) was carried out considering the proportion of the qualitative variables. the Fisher exact test and 95% confidence interval (% CI) were used to compare proportions. In all analyses, the significance level was established at $P<0.05$.

Among the American mink, 63/139 were seropositive for *L. infantum* at variable antibody levels, including low-positive (n=61), moderate-positive (0.610 OD value, n=1), and high-positive levels (1.59 OD value, n=1; Fig. 1). We found 44.4% of females (32/72) and 46.3% of males (31/67) were seropositive. In contrast, 22/42 European mink were seropositive for *L. infantum*, all with low antibody levels (Fig. 2); 50.0% of females (12/24) and 55.6% of males (10/18) were seropositive. Real seroprevalence values of 45.3% (95% CI, 34–52.4) and 52.4% (95% CI, 36.4–66.6) of *L. infantum* infection in American and European mink, respectively, were obtained (Tables 1, 2). No significant association ($P>0.05$) was found between seropositivity for anti-*Leishmania* antibodies and the variables studied: river basin, sex, and body score (Table 3).

In Spain, the seroprevalence of canine leishmaniosis differs from one area to another and varies from 3.7% to 34.6%, with the highest prevalence cited for southern and eastern Spain and substantially lower prevalence (3.7–4.4%) in the northern provinces of the Iberian Peninsula (Miró et al. 2012; Montoya et al. 2020).

During the past two decades, many wild mammals have been diagnosed with *Leishmania* infection by serologic and/or molecular methods (Oleaga et al. 2018). In the same way, studies have provided evidence of the wide presence of *L. infantum* infection among wild carnivores in *L. infantum* periendemic northern Spain, with the presence of *Leishmania* in 28% (44/156) of animals in the Basque Country: in 26% of Eurasian badgers (*Meles meles*; n=53), 29% of foxes (*Vulpes vulpes*; n=48), 29% of beech martens (*Martes foina*; n=21), and in 25–50% of less-abundant species, including genets (*Genetta genetta*), wild cats (*Felis silvestris*), pole cats (*Mustela putorius*), weasels (*Mustela nivalis*), and European mink (del Rio et al. 2014). Oleaga et al. (2018) reported a prevalence of 33% for wolves (*Canis lupus*) and an overall prevalence of 40% for all the wild carnivores studied in northwestern Spain, including a prevalence of 70% for the Eurasian otter (*Lutra lutra*), 62% of European pine marten (*Martes martes*), and 67% of beech marten (Oleaga et al. 2018). In Catalonia, a 29.5% prevalence has been detected in wild mammals by *Leishmania* DNA, and specific anti-*Leishmania* antibodies were detected (Alcover et al. 2020).

The high occurrence of *L. infantum* in American mink in this study suggests that further studies are needed to develop a deeper knowledge to avoid an added potential risk for European mink. Such studies should include animal monitoring using PCR, xenodiagnostic experiments to confirm that sandflies take blood meals from minks, and traps for the capture of adult *Phlebotomus* spp.

LITERATURE CITED

Alcover MM, Ribas A, Guillén MC, Berenguer D, Tomás-Pérez M, Riera C, Fisa R. 2020. Wild mammals as potential silent reservoirs of *Leishmania infantum* in a Mediterranean area. *Prev Vet Med* 175:104874.

Amstislavsky S, Lindeberg H, Aalto J, Kennedy MW. 2008. Conservation of the European mink (*Mustela lutreola*): Focus on reproduction and reproductive technologies. *Reprod Domest Anim* 43:502–513.

Cantos-Barreda A, Navarro R, Pardo-Marín L, Martínez-Subiela S, Ortega E, Cerón JJ, Tecles F, Escribano D. 2020. Clinical leishmaniosis in a captive Eurasian otter (*Lutra lutra*) in Spain: A case report. *BMC Vet Res* 16:312.

del Río L, Chitimia L, Cubas A, Victoriano I, De la Rúa P, Gerrikagoitia X, Barral M, Muñoz-García CI, Goyena E, García-Martínez D, et al. 2014. Evidence for widespread *Leishmania infantum* infection among wild carnivores in *L. infantum* periendemic northern Spain. *Prev Vet Med* 113:430–435.

Frankham R, 2003. Genetics and conservation biology. *C R Biol* 326 (Suppl 1):S22–S29.

Giner J, Basurco A, Alcover MM, Riera C, Fisa R, López RA, Juan-Sallés C, Verde MT, Fernández A, Yzuel A, et al. 2020. First report on natural infection with *Leishmania infantum* in a domestic ferret (*Mustela putorius furo*) in Spain. *Vet Parasitol Reg Stud Rep* 19:100369.

Maran T, Skumatov D, Gomez A, Põdra M, Abramov AV, Dinets V. 2016. *Mustela lutreola*. In: *The International Union for Conservation of Nature red list of threatened species*. https://www.iucnredlist.org/species/14018/45199861. Accessed February 2021.

Miró G, Checa R, Montoya A, Hernández L, Dado D, Gálvez R. 2012. Current situation of *Leishmania infantum* infection in shelter dogs in northern Spain. *Parasit Vectors* 5:60.

Montoya A, Gálvez R, Checa R, Sarquis J, Plaza A, Barrera JP, Marino V, Miró G. 2020. Latest trends in *L. infantum* infection in dogs in Spain, part II: Current clinical management and control according to a national survey of veterinary practitioners. *Parasit Vectors* 13:205.

Oleaga A, Zanet S, Espí A, Pegoraro de Macedo MR, Gortázar C, Ferroglio E. 2018. *Leishmania* in wolves in northern Spain: A spreading zoonosis evidenced by wildlife sanitary surveillance. *Vet Parasitol* 255:26–31.

Pennisi MG. 2015. Leishmaniosis of companion animals in Europe: An update. *Vet Parasitol* 208:35–47.

Rouvinen-Watt K, Armstrong D, 2002. *Body condition scoring of mink using a five-point scale*. Canadian Centre for Fur Animal Research Nova Scotia Agriculture College, Truro, Nova Scotia, Canada, 1 pp.

Sobrino R, Ferroglio E, Oleaga A, Romano A, Millan J, Revilla M, Arnal MC, Trisciuoglio A, Gortázar C. 2008. Characterization of widespread canine leishmaniasis among wild carnivores from Spain. *Vet Parasitol* 155:198–203.

Tsakmakidis I, Pavlou C, Tamvakis A, Papadopoulos T, Christodoulou V, Angelopoulou K, Dovas CI, Antoniou M, Anastasakis C, Diakou A. 2019. *Leishmania* infection in lagomorphs and minks in Greece. *Vet Parasitol Reg Stud Rep* 16:100279.

Submitted for publication 24 February 2021.
Accepted 29 July 2021.