Polish Journal of Veterinary Sciences Vol. 22, No. 4 (2019), 781–784

DOI 10.24425/pjvs.2019.131408

Short communication

Carriage of pathogenic *Leptospira* in carnivores at the wild/domestic interface

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Abstract

The carriage of pathogenic *Leptospira* was investigated by PCR in 51 wild carnivores, 20 domestic dogs with outdoor access, and 27 free-roaming domestic cats sampled in periurban Barcelona (NE Spain). Overall prevalence was 7.7%, with DNA confirmed in 3/30 common genets (*Genetta genetta*) (serovars Icterohaemorraghiae and Sejröe), 1/9 red foxes (*Vulpes vulpes*) (Canicola) and 2/27 cats (Icterohaemorraghiae). Though most of the dogs were vaccinated against *Leptospira*, DNA of the serovar Canicola was detected in the urine of 25% of the vaccinated animals, and the serovar Icterohaemorraghiae in one non-vaccinated dog.

Key words: domestic/wildlife interface, leptospirosis, spirochetes, surveillance

Introduction

Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira* (Shieh et al. 1999). The disease has a worldwide distribution and affects humans, domestic animals and wildlife. Human infection usually occurs through indirect contact with water or soil contaminated by the urine of infected animals (Shieh et al. 1999). There has been a recent increase in western countries in human exposure through recreational activities such as hiking, while occupational exposure is decreasing (Monahan et al. 2009). Leptospires exploit some mammal species such as rodents as reservoir hosts by establishing chronic infections in the renal tubules of the kidneys ("carrier phase") that can persist for months. The host can subsequently contaminate the environment with *Leptospira* upon urination. Carnivores may become infected either when preying on rodents or indirectly via contaminated water (Shieh et al. 1999). The aim of the present study was to identify the species and serovars of pathogenic leptospires shed by carnivores (wild and domestic) in a large conurbation in Spain.

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Table 1. Sample size, observed prevalence, and *Leptospira* serovar identification in carnivore samples (kidney or urine) surveyed in the Barcelona metropolitan area (Spain). Prevalence and confidence intervals were calculated when sample size ≥25.

Host	*n*	Positive	Prevalence	95% C.I.	Serovars*
Common genet *Genetta genetta*	30	3	10.0%	2.7-26.3	1 Ic, 1 Se, 1 un
Stone marten *Martes foina*	8	0	-	-	-
Eurasian badger *Meles meles*	2	0	-	-	-
Lesser weasel *Mustela nivalis*	2	0	-	-	-
Red fox *Vulpes vulpes*	9	1	-	-	1 Ca
Overall wild	51	4	7.8%	3.9 -18.5	
Free roaming domestic cat	27	2	7.4%	2.0-23.3	2 Ic
Domestic dogs (unvaccinated)	4	1	-	-	1 Ic
Domestic dogs (vaccinated)	16	4	-	-	4 Ca
Overall (excluding vaccinated)	82	7	8.5%	4.1-16.9	-

* Ic: Icterohaemorrhagiae, Ca: Canicola, Se: Sejroë, un: undetermined.

Materials and Methods

Ninety-eight individuals were sampled between 2011 and 2013 in two Natural Parks which encroach into the Barcelona Metropolitan Area or in residential areas adjacent to them: Collserola (41º26′ N, 2º08′ E) and Sant Llorenç del Munt (41º38′ N, 2º01′ E). Detailed information about the study areas can be found in Millán et al. (2016). Twenty-four wild carnivores were trapped and anesthetized with a combination of ketamine (Imalgene©; Merial, Lyon, France) and medetomidine (Domtor©; Salud Animal-Pfizer, Madrid, Spain) and reversed with atipamezole (Antisedan©; Salud Animal-Pfizer, Madrid, Spain). Urine was obtained by applying pressure to the animal's abdomen, kept in sterile tubes, and frozen at -20°C. Twenty-seven carcasses of road-killed wild carnivores were collected in Collserola in the period 1999-2013, necropsied, and a piece of kidney collected for analysis. Twenty domestic dogs with outdoor access and 27 free-roaming domestic cats were sampled for urine in the residential areas after oral consent of the owners without the use of anesthesia. A questionnaire was completed by the owners, including information about the vaccination status of the animals. Immunization records were requested to confirm the information given by the owners. According to that information, 16 dogs (80%) had been vaccinated against *Leptospira* Canicola and Icterohaemorrhagiae. Urine and kidney samples were analyzed by PCR using pathogenic leptospires-specificprimers LipL32/270F (5′-CGCTGAAAT GGGAGTTCGTATGATT-3′) and LipL32/6692R (5′-CCAACAGATGCAACGAAAGATCCTTT-3) with previously reported protocols and conditions (Millán et al. 2014, 2018). Positive DNA reactions were later subjected to a second PCR in order to differentiate *Leptospira* serovars, detecting the oligonucleotide i-Repl as a target of the amplification. PCR was performed using the primers Rep1 (5′-AGCGGGTATGACTCCGC-3′) and iRep1 (5′ -GCGGACTCATACCCGCT- 3′). This protocol allows species and serovars of epidemiologic relevance, such as Bataviae, Canicola, Icterohemorrhagiae, Pomona, Pyrogenes and Autumnalis, among others belonging to *L. interrogans*, Javanica and Ballum (belonging to *L. borgpetersenii*), and Grippotyphosa (belonging to *L. kirschneri*) to be differentiated (Millán et al. 2018).

Results and Discussion

Leptospiral infection was detected in 8.5% of the animals (Table 1). No significant differences were found in the prevalence of wild carnivores depending on the type of sample (7.4% in urine vs 12.5% in kidney; Fisher´s p= 0.65), and thus, the results are shown pooled in Table 1. No differences were found either between wild and domestic carnivores (7.8% vs 9.7%; Fisher´s p= 1.0) or between wild carnivores and free-roaming cats (7.8% vs 7.4%; Fisher´s p= 1.0).

There are very few articles about *Leptospira* carriage in wild carnivores in Europe. The prevalence observed here is very similar to that observed in carnivores in rural parts of southern Spain (8.5%, Millán et al. 2009) but lower than in riparian carnivores in France (23%, Moinet et al. 2010). This difference may be due to a more frequent contact with spirochetes in animals living in aquatic environments as those studied by Moinet et al. (2010), which are favorable for the survival of the spirochetes. In North America, prevalences ranged from 8% in red fox (*Vulpes vulpes*) to 42% in skunk (*Mephitis mephitis*) by using a combination of immunohistochemistry and PCR (Shearer et al. 2014). Higher rates of exposure have been reported

with serological techniques, e.g. about 30% in red foxes in Poland (Żmudzki et al. 2018) and Croatia (Slavica et al. 2008). Worldwide, the review by Andersen-Ranberg et al. (2016) reported a mean prevalence of exposure between 10-12% in Canidae and Felidae and 35% in Mustelidae.

The serovars most commonly detected were those detected in rodents in the same study area (mainly Icterohaemorraghiae) (Millán et al. 2017). Other studies with wild medium-sized carnivores also reported Icterohemorraghiae as the most prevalent serovar (Millán et al. 2009, Moinet et al. 2010, Scialfa et al. 2013), indicating that carnivores became infected upon ingestion of infected prey. Żmudzki et al. (2018), however, found serovars Poi, Saxkoebing, and Sejröe as the most prevalent in Polish foxes, with only 0.8% of the foxes having antibodies against serovar Icterohemorraghiae. In Croatia, serovars more frequently found in foxes and stone martens (*Martes martes*) were Australis, Sejröe and Icterohemorraghiae (Slavica et al. 2008). Therefore, it seems that regional differences in exposure to different serovars do occur in Europe. The infection of one fox by serovar Canicola is noteworthy. Though this serovars is typical of dogs and can be also found in wolves (*Canis lupus*; Millán et al. 2014), and foxes are also members of the family Canidae, infection has not been reported before in red fox, although Żmudzki et al. (2018) found antibodies against this serovar in a small proportion of foxes.

A proportion of free-roaming cats where shedding leptospires at the time of the survey, which poses an infection risk for their owners. Besides this fact, we believe that free-roaming cats can be useful sentinels of environmental contamination with leptospires due to their feeding and movement behavior. We observed that most dogs were vaccinated against *Leptospira*, a markedly higher proportion than in rural parts of Spain, were only one out of 28 dogs was vaccinated (Millán et al. 2009). However, one quarter of the vaccinated dogs had detectable DNA of *Leptospira* Canicola in urine. Dogs are considered the natural host for serovar Canicola (André-Fontaine 2006). This result may indicate either incorrect vaccine preservation or administration, or a lack of complete immunity after administration. In fact, it has been noted that vaccine protection against carrier state or renal shedding is variable (Greene 2011).

Acknowledgements

The present research complied with the regulations for animal experimentation and welfare issued by the European Union (Directive 86/609/CE). Capture and handling of carnivores was approved by authorities in bioethics under permit CEEAH 1871 (Universitat Autònoma de Barcelona, Spain), and by annual authorizations from Catalonia regional government authorities (permits SF/111/2011, SF/153/2012, and SF/064/2013). This study was funded by project CGL2010-17931. We wish to thank L. Cabañeros, F. Llimona, and S. Cahill (Collserola Natural Park), and J. Torrent and A. Miño (St. Llorenç Natural Park) for facilitating our work in the field; Nuria Moix (Clínica Veterinaria Novavet) and Eulalia Curt (Consultori Veterinari La Floresta) for providing some of the pet samples.

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