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Screening for Leptospira DNA in blood and urine from 30 apparently healthy dogs

Recherche d'ADN de leptospires pathogènes dans le sang et l'urine de 30 chiens apparemment sains☆

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Summary

Asymptomatic carriage of leptospires in dogs has not been extensively studied. A prospective study was conducted on thirty apparently healthy male dogs to screen for Leptospiral DNA in their blood and urine. All dogs were up to date with a bivalent vaccine against [Leptospirosis](#). Microscopic agglutination test (MAT) and real-time PCR analysis in blood and urine were performed for each dog. Six of 30 (20%) dogs had positive PCR results: two from blood samples (all MAT titres ≤ 160) and four from urine samples (one with maximal MAT titres of 2560 and three with all MAT titres ≤ 160). Ten months later, PCR analyses of blood and urine were repeated for these six dogs. Four had negative PCR results from blood and urine. The other two, with positive urine results at the first sampling, had negative PCR results from urine and positive PCR results from blood. In this study, the urinary shedding of leptospires appeared to be transient. As shown previously, serological status was not correlated with PCR results. Low but detectable amounts of Leptospiral DNA in the blood of some apparently healthy dogs raises questions regarding the pathophysiology of Leptospirosis and the diagnostic consequences of high-sensitivity PCR assays.

Résumé

Le portage asymptomatique des leptospires chez le chien est encore mal connu. Une étude prospective sur 30 chiens mâles apparemment sains a recherché la présence

d'ADN de leptospires pathogènes dans leur sang et leur urine. Chaque chien était à jour de ses vaccins contre la leptospirose (vaccins inactivés bivalents sur tous les chiens). Une sérologie par technique de microagglutination (MAT) et une PCR en temps réel sur l'urine et le sang ont été réalisées pour chaque chien. Six chiens sur 30 (20 %) avaient des résultats PCR positifs : deux chiens sur sang (titres MAT ≤ 160 pour ces deux chiens), quatre chiens sur urine (titre MAT maximal de 2560 pour un chien, titres MAT ≤ 160 pour les trois autres). Dix mois plus tard, les analyses PCR ont été répétées pour les six chiens avec un résultat positif. Quatre chiens avaient des résultats négatifs sur sang et urines. Deux chiens, qui avaient initialement une PCR positive sur urines, avaient une PCR positive sur sang et négative sur urines. Dans cette étude, l'excrétion urinaire des leptospires apparaissait discontinue. Comme dans d'autres études, les profils sérologiques n'étaient pas corrélés aux résultats PCR. Trouver dans le sang de chiens apparemment sains des quantités faibles mais détectables d'ADN de leptospires interroge sur la physiopathologie de la leptospirose et les conséquences diagnostiques de tests PCR très sensibles.

Keywords

Dog

Leptospirosis

PCR

Urine

Blood

Mots clés

Chien

Leptospirose

PCR

Urine

Sang

1 INTRODUCTION

[Leptospirosis](#) is a re-emerging worldwide [zoonotic disease](#) caused by spirochetes that can affect many domestic mammals, including dogs. Humans and dogs are both susceptible species and represent incidental hosts who can develop similar severe acute forms of the disease. Many wild rodents are maintenance hosts for leptospires. They chronically shed the spirochetes from their [renal tubules](#) into their urine without expressing any symptoms [\[1\]](#). The [renal](#) carriage of leptospires that is associated with high-intensity, constant and long-term Leptospiuria is characteristic of maintenance

hosts [2]. Dogs are the maintenance host for the Canicola serovar and an incidental host for other serovars [1]. *Canine Leptospirosis* vaccines are reported to provide long-term protection not only against infection but also against the renal carrier state for the vaccine-targeted serogroups [3], [4], [5], [6]. Leptospiuria in incidental hosts is considered a transient, intermittent and low-intensity condition [2], [7]. The asymptomatic carriage of leptospires in canines has not been extensively studied. However, many studies have demonstrated that apparently healthy dogs can have high microscopic agglutination test (MAT) titres for different serogroups, indicating that exposure to *Leptospira* spp. is common in dogs [8], [9], [10]. Some studies have investigated the prevalence of urinary shedding of leptospires based on PCR analyses in apparently healthy dogs or in a general referral hospital population. The percentage of positive samples has been reported to vary between 1.5 and 20% [11], [12], [13], [14], [15]. In those studies, the renal carrier state was evaluated at a single point in time and the vaccination status was not specifically addressed in two of the studies [12], [15]. The aim of the current study was to screen for *Leptospira* DNA in urine and blood samples from 30 apparently healthy, privately owned dogs with a well-known vaccination history and behavioural habits that favoured contamination and to recheck those with a positive result (if any) on a second occasion, without administering a course of antibiotics in between tests.

2 MATERIALS AND METHODS

2.1 Selection of the population

Thirty apparently healthy student-owned dogs from a veterinary university were included. The ethical committee of the University approved this study. Recruitment of the population was made on a voluntary basis in June 2011. The study population was selected based on answers to a questionnaire. Male dogs with a lifestyle that favoured exposure to [Leptospirosis](#) were eligible for inclusion. The risk of exposure was assessed based on the frequency of walks near water sources and/or bathing in these water sources during the past two months.

2.2 Vaccination status

Vaccination histories (date and type of vaccine), which were obtained from valid vaccination certificates, were recorded for each dog.

2.3 Epidemiological data

The data recorded for each dog included living place; lifestyle (number of dogs in the household and the main source of activity: work dog, hunting dog, breeder or pet);

walking, bathing and drinking habits (ditchwater, running water, lake, swamp, pond or tap water exclusively); and proximity to wild rodents, which was estimated by the owner.

2.4 Clinical status evaluation

A thorough medical history was recorded, and each dog underwent a complete physical examination, including a urinary dipstick (Combur 10 Test, Roche) test and specific gravity measurements using refractometer (URC-Ne, Atago). Only apparently healthy dogs were included in the study. [Glucosuria](#) was not an exclusion criterion.

2.5 Sample collection and DNA isolation

The owners collected urine via the free-catch method in a sterile plastic urine cup during the dog's first morning walk. Subsequently, one millilitre of urine was placed in a dry tube for PCR analysis. Two blood samples were collected at the University Hospital on the same day as the urine sampling: one in a clot tube for MAT serology and the other in a tube containing EDTA for PCR analysis. The urine samples were centrifuged at 3500 g for 10 min prior to DNA extraction to obtain 200 µL of pellet. DNA was isolated (QIAamp DNA Mini Kit, Qiagen, Courtaboeuf, France) from 200 µL of blood and the urine pellet within hours of sampling, following the manufacturer's instructions. The DNA template was stored at -20 °C.

2.6 PCR assay on blood and urine

A public laboratory¹ completed the real-time PCR analysis. Blood and urine were handled separately, and the assays were completed using a kit (Taqvet Pathogenic Leptospira Real-Time PCR kit (LEPT/50), LSI, Lissieu, France). The Taqvet kit included a specific internal control to check for PCR inhibitors. Each run included a no template control, a positive control using a reference laboratory strain (*L. interrogans* Icterohaemorrhagiae strain 9120) as a template and a negative control (PCR mix without the targeted DNA). The no template control was defined as a negative control that undergoes all the process including extraction of nucleic acids. A final volume of 25 µL, which consisted of 20 µL lepto mix (LSI) and 5 µL of the targeted DNA, was analysed via PCR using a Rotor-Gene Q system (Qiagen) using the following program: 10 min of enzyme activation at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. The detection threshold was 10³ leptospirae/mL of blood or urine. Each PCR was performed in duplicate to assess the reproducibility of the results. All samples have been tested in the same run.

2.7 Genotyping

The 16S *rRNA* gene was amplified with the primers LeptA (5'-GGCGGCGCGTCTTAAACATG-3') and LeptB (5'-TTCCCCCATTGAGCAAGATT-3') [16]. The amplified fragments were sequenced (see appendix), and the sequences were compared to 16S *rRNA* sequences available in Genbank². Further characterization was performed using the multispacer sequence typing (MST) method with MST1, MST3 and MST9 primers as described by Zilber et al. [17]. For one dog (dog N° 19), a variable-number tandem-repeat (VNTR) analysis was subsequently performed as an alternative molecular approach [18].

2.8 Serologic examination

Microscopic agglutination tests were performed by a public laboratory for 19 serovars (Australis, Bratislava, Munchen, Autumnalis, Bim, Canicola, Grippotyphosa, Vanderhoedoni, Icterohaemorrhagiae, Copenhageni, Panama, Mangus, Pomona, Mozdok, Pyrogenes, Sejroe, Saxkoebing, Hardjo, and Wolfii) representing nine serogroups (Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Panama, Pomona, Pyrogenes and Sejroe). The initial dilution used was 1/40.

2.9 Follow up

Owners were individually informed of the MAT and PCR results. For dogs that had positive PCR results on the initial test, informed consent of the pet owner was required to recheck the blood and urine ten months later using the same PCR method. The vaccination schedule was continued unchanged. All dogs with at least one positive PCR result either the first or second test were treated with doxycycline (5 mg/kg BID, 14 days) following the second sampling of blood and urine.

3 RESULTS

The study included 30 male dogs, 14 of which were neutered (47%). In total, two dogs (6%) were small breeds (< 10 kg), 20 (67%) were medium breeds (between 10 and 30 kg), and eight (27%) were large breeds (> 30 kg), including eight mixed-breed dogs, four Border Collies, four Australian Shepherds, two Samoyeds, two Labradors, two Golden Retrievers, one flat-coated Retriever, one Entlebuch Mountain Cattle dog, one Collie, one Brittany Spaniel, one Long-Haired Pyrenean Sheepdog, one Beagle, one Bernese Mountain dog and one Manchester Terrier. The age of the dogs varied from eight months to 14.5 years, and the median age was 26.5 months (8–177 months). All dogs primarily lived in suburban areas and had access to one or more water sources during walks. They also had access to the Veterinary Campus and its surrounding areas, which contain many sources of stagnant water (especially ponds, puddles and ruts) and is adjacent to areas where dairy cows and wild rodents lived. Certain dogs

also had access to lakes and/or running water (rivers, streams and springs) outside the Veterinary Campus area. During the 15 days preceding the first sampling, 20 (67%) dogs went for a swim, and all dogs drank from a natural water source.

Less than one year before the first sampling, all dogs had received a booster vaccine or completed a primo-vaccination against [Leptospirosis](#) with a two-serovar inactivated vaccine, including the Canicola and Icterohaemorrhagiae serovars (25 EURICAN LR (Merial), two CANIGEN L (Virbac), two ENDURACELL LR (Zoetis), one NOBIVAC L (MSD)) ([Table 1](#)).

Table 1. Summary table of vaccinal, serological and PCR data.

N° of dog	MAT serology																			Last booste	Type of booster	PCR (first sampling)		PCR (recheck 10 months later)	
	19	COP	MUN	AUS	BRAT	AKI	BIM	CAN	GRIP	VAN	PAN	MAN	POM	MOZ	PYR	SJ	SAX	HJ	WOLF			Blood	Urine	Blood	Urine
1	80	320	40	40	80			2560				40			160					< 3 months	EURICAN LR	Negative	Negative		
2	640	1280	2560	1280	1280	320	320	2560	40	40	1280	2560								< 3 months	EURICAN LR	Negative	Positive	Negative	Negative
3	320	640	640	40	160			640	1280	320		1280	80	40						< 3 months	EURICAN LR	Negative	Negative		
4	640	1280	40		1280			640							320					< 3 months	EURICAN LR	Negative	Negative		
5	80	640	40		40			640												< 3 months	EURICAN LR	Negative	Negative		
6	40	40						80												3–6 months	EURICAN LR	Negative	Negative		
7								1280												3–6 months	EURICAN LR	Negative	Negative		
8	80	80			40	40	80	80												3–6 months	ENDURACELL LR	Positive	Negative	Negative	Negative
9	40	40			40			80							80					3–6 months	EURICAN LR	Negative	Negative		
10	80	80						160												3–6 months	EURICAN LR	Negative	Negative		
11	40	40																		3–6 months	EURICAN LR	Negative	Negative		
12	40	40	40		40															3–6 months	EURICAN LR	Negative	Negative		
13	40	40				40	40	160												3–6 months	ENDURACELL LR	Negative	Positive	Negative	Negative
14	40	80	40	40	40			640												3–6 months	EURICAN LR	Negative	Negative		
15	80	160				40	80	80												6–9 months	EURICAN LR	Negative	Negative		
16	160	320	40		40			160												6–9 months	EURICAN LR	Negative	Negative		
17	40	40			40			80												6–9 months	EURICAN LR	Negative	Negative		
18	80	80						160												6–9 months	EURICAN LR	Positive	Negative	Negative	Negative
19	40	80	40		40			80												6–9 months	EURICAN LR	Negative	Positive	Positive	Negative
20	40	40	40		40															6–9 months	EURICAN LR	Negative	Negative		
21	80	160	40		40	40	40	160							80					6–9 months	EURICAN LR	Negative	Negative		
22															80					6–9 months	NOBIVAC L	Negative	Negative		
23	80	80			80				160	160			40	40						6–9 months	EURICAN LR	Negative	Positive	Positive	Negative
24	80	80			40	40	80	2560					40	40	160					6–9 months	EURICAN LR	Negative	Negative		
25	160	320						640	640	160				80	40					6–9 months	EURICAN LR	Negative	Negative		
26	80	160	40		80			80												9–12 months	EURICAN LR	Negative	Negative		
27	80	40	40		40			40												9–12 months	EURICAN LR	Negative	Negative		
28	160	80	80		40			160	40											9–12 months	EURICAN LR	Negative	Negative		
29	640	640	1280	1280	640	640	1280					80								9–12 months	CANIGEN L	Negative	Negative		
30	40	40	40	40	40										40					9–12 months	CANIGEN L	Negative	Negative		

Abbreviations of serovars: 19: Icterohaemorrhagiae; COP: Copenhageni (serogroup Icterohaemorrhagiae); MUN: Munchen; AUS: Australis, BRAT: Bratislava (serogroup Australis); AKI: Autumnalis; BIM: Bim (serogroup Autumnalis); CAN: Canicola (serogroup Canicola); GRIP: Grippotyphosa; VAN: Vanderhoedoni (serogroup Grippotyphosa); PAN: Panama; MAN: Mangus (serogroup Panama); POM: Pomona; MOZ: Mozdok (serogroup Pomona); PYR: Pyrogenes (serogroup Pyrogenes); SJ: Sejroe; SAX: Saxkoebing; HJ: Hardjo; WOLF: Wolfii (serogroup Sejro).

No dog had history of [renal](#) and/or hepatic diseases. Clinical examinations were normal for all dogs. [Urine specific gravity](#) varied between 1.012 and 1.050. In total, one dog (dog N° 23) had [glucosuria](#) without clinical symptoms. Glycaemia was normal (4.5 mmol/L, 0.8 g/L) for this dog (Accu-Chek Active, Roche). In total, 19 dogs (63%) had [proteinuria](#) (one + for 17 dogs and two + for two dogs), with urine specific gravities > 1.035 in 15/19 of these dogs. All dogs except one (dog N° 2) had hypersthenuric urine. For dog N° 2, uraemia and creatinaemia were subsequently checked and were within the reference ranges (urea = 6.4 [2–7 mmol/L]; [creatinine](#) = 102 [60–135 µmol/L]).

Serological and PCR data are presented in [Table 1](#).

Six out of 30 (20%) dogs had a unique positive PCR result at the first sampling (two from blood, four from urine). At the recheck ten months later, four of those six dogs had negative PCR results for both their blood and urine samples, and two had a unique positive PCR result for their blood sample. These last two dogs had both positive PCR results for their urine at the first sampling. The detailed results are described below. At the first sampling, twenty-four dogs (80%) had negative PCR results for both their blood and urine samples. Among them, 15 (62.5%) had MAT titres that were all ≤ 160 , and five (20.8%) had MAT titres ≥ 640 for the vaccinal serogroups only (1/640 for Canicola for one dog, 1/1280 for Canicola for one dog, 1/2560 for Canicola for two dogs and 1/640 for Canicola and Icterohaemorrhagiae for one dog). Two of these five dogs (dog N° 1 with a titre of 1/2560 for Canicola and dog N° 5 with a titre of 1/640 for Canicola and Icterohaemorrhagiae) had received their last booster less than three months prior to the study. Four dogs (16.6%) had multiple titres ≥ 640 for at least one non-vaccinal serogroup (1/1280 for Panama and Grippotyphosa and 1/640 for Australis for one dog, 1/1280 for Australis and Autumnalis for one dog, 1/1280 for Australis for one dog and 1/640 for Grippotyphosa for one dog).

At the first sampling, four dogs (13.3%) (dogs N° 2, 13, 19 and 23) had positive PCR results for their urine samples (cycle threshold (Ct) = 35.62, 37.07, 38.11 and 40.0, respectively) and negative PCR results for their blood samples. One dog had glucosuria without hyperglycaemia (dog N° 23). One out of four dogs (dog N° 2) had multiple MAT titres ≥ 640 (1/2560 for Panama, Australis and Canicola; 1/1280 for Icterohaemorrhagiae). The other three dogs, including the dog with glycosuria (dog N° 23), had MAT titres that were all ≤ 160 . The 16S rRNA gene amplification results indicate that all the sequences were similar and that the genomospecies found in these four dogs was *L. interrogans*. Further molecular characterization via MST failed because an insufficient amount of DNA was available. Ten months later, PCR results for both blood and urine samples were negative for two dogs (dogs N° 2 and 13). For the other two (dogs N° 19 and 23), PCR results were negative from urine and positive

from blood samples (Ct = 30.20 for dog N° 19, Ct = 32.64 for dog N° 23). The 16S *rRNA* gene amplification results indicate that the genomospecies was *L. interrogans*. Further molecular characterization via MST for these two dogs failed. For dog N° 19, who had the most substantial Leptospiral DNA burden, another molecular typing method (variable-number tandem-repeat analysis) was completed, and the *L. interrogans*Grippotyphosa Valbuzzi genotype (results for VNTR locus 4, 7, 10: 2, 1, 1, respectively) was identified [18].

At the first sampling, two dogs (6.7%) (dogs N° 8 and 18) had positive PCR results for their blood samples (Ct = 34.55 for dog N° 8 and Ct = 37.78 for dog N° 18) and negative PCR results for their urine samples. For those two dogs, all MAT titres were ≤ 160. The 16S *rRNA* gene amplification results indicate that the genomospecies was *L. borgpetersenii* for dog N° 8 and *L. interrogans* for dog N 18. Further molecular characterization via MST failed because an insufficient amount of DNA was available. Ten months later, the PCR results for both blood and urine samples were negative for these two dogs.

4 DISCUSSION

In this study, 4/30 (13.3%) vaccinated, apparently healthy, privately owned, male dogs had positive PCR assay results from urine samples. This indicates that asymptomatic urinary shedding of leptospires in male dogs with behavioural habits that favour contamination is not an anecdotal phenomenon, even when the dogs are vaccinated. Overall, 4/30 dogs (13.3%, 95% Confidence Interval 5.3–29.6%) had positive PCR results from urine samples. This frequency is among the highest of those previously reported in asymptomatic dogs: 1.5% [13], 4.4% [11], 7% [15], 8% [12] and 20% [14]. However, the small number of dogs in the current study and its design, which favoured the recruitment of urinary shedders, precludes further comparisons of the frequency found in this study with those of previous studies. Indeed, only male dogs were included because they have an increased risk of contamination [19], [20], [21]. The selected dogs were mainly living in suburban areas and had access to one or more water sources during their walks. Living in an urban area, the proximity of outdoor water sources, swimming or drinking from outdoor water sources and indirect exposure to wild rodents have all been suggested to promote Leptospirosis contamination [20], [22].

In our study, all urine samples showing positive PCR results had a quantity of DNA that was notably lower than that reported in the pioneer study describing the MST method [17]. A positive PCR result does not determine whether the pathogen is viable, and the low Leptospiral burden observed here in the urine of apparently healthy dogs is of questionable biological significance. Ideally, cultures would have been

recommended to investigate this issue. However, this option was rejected because of the difficulties in obtaining positive culture results, even in acute cases with high *Leptospiral* burdens. The sequencing results showed that there were no false positive results. The diagnostic kits used appeared to be very sensitive, which is a technical advantage. According to the manufacturer's notice for the Taqvet PCR kit, the sample is positive if Ct values are < 45. However, a positive test with such a low *Leptospiral* burden possibly calls into question the biological significance of the results. Limits of detection (LODs) as low as three genome equivalents per 5 µL (6×10^2 *Leptospires*/mL) have been reported for specific real-time PCR assays for detecting *Leptospira* that have been validated for diagnostic application [23]. Because of this, the positive PCR results observed in this study reflect those obtained under clinical conditions and should be considered in the same regard.

All four dogs with initial positive PCR results for their urine samples had negative PCR results from urine ten months later, without antibiotic treatment. Possible explanations for this include intermittent shedding, natural cleansing or methodological limits due to the detection threshold of the test and the low infectious burden. Surprisingly, two of these dogs had positive results for their blood samples at the recheck.

At the time of the current study, only bivalent inactivated vaccines were available in France. Three of the four dogs with positive PCR results from urine samples were vaccinated with the same vaccine (EURICAN LR, Merial) and one with a different one (ENDURACELL LR, Zoetis). The relative efficiency of vaccines in preventing urinary shedding could not be compared based on the results presented here because of the small size of the study population and because the majority of the study group (25/30) was vaccinated with the same vaccine (EURICAN LR, Merial). The genomospecies identified in all four cases was *L. interrogans*. *L. interrogans* contains both pathogenic vaccinal and non-vaccinal serovars associated with canine *Leptospirosis*. Further genotyping at the serovar level would have been of particular interest here and is absolutely necessary to extend the assessment of vaccination status and [renal](#) carrier state. However, the amount of DNA was insufficient for molecular characterization at the serovar level.

In a previous report [12], serologic testing was shown to be a poor predictor of urinary shedding. Three of the four dogs with positive PCR results from urine samples had MAT titres that were all ≤ 160 . One of them was glucosuric, suggesting tubular lesions, possibly caused by leptospires. The dog with a positive PCR result for the urine sample and multiple MAT titres ≥ 640 , including non-vaccinal titres, was the sole dog with isosthenuric urine, but he was not azotemic.

At the population scale, a low prevalence of renal carriage ($\leq 1\%$) and a low rate of excretion are expected in incidental hosts, whereas a high prevalence ($\geq 10\%$) and a

high sustained excretion rate are expected in maintenance hosts [2], [24]. Whether dogs can be considered as a maintenance host for serovars other than Canicola has not been determined [11], [25]. A recent publication suggests that, except for Canicola, dogs should be considered as vectors rather than as maintenance hosts [11]. Dogs represent an epidemiological link between the environment, wild fauna and humans, and therefore, they might play a significant role in human contamination in endemic areas [7].

The positive PCR results from blood in four dogs (two at the first sampling and two others at the recheck) were unexpected in France, which is a non-endemic region. The leptospiremic phase has been shown to be short in models susceptible to acute clinical infections and in a rat model of persistent renal colonization [26], [27], [28]. As a consequence, a positive PCR result from blood should correspond to a recent infection. Positive PCR results from blood have been previously reported in Brazil in apparently healthy dogs from a control centre for zoonoses (CCZ) and in dogs living in a neighbourhood with a high prevalence of human leptospirosis with respective frequencies of 7.7 and 14.8% [14]. The prevalence of Leptospiraemia was higher than that of Leptospiruria in the dogs living in the endemic area for human leptospirosis. Explanations proposed by the authors included a recent outbreak in this area. In our study, which was conducted in a non-endemic area, it seems unlikely that the four positive results obtained from blood were due to recent asymptomatic infections. Non-specific amplification was also excluded because all positive results obtained with the PCR diagnostic kit were confirmed via sequencing, and the negative controls were valid. Furthermore, for one dog, the *L. interrogans* Grippotyphosa Valbuzzi genotype was identified using the VNTR method. Vaccination also did not affect the PCR results because a recent vaccination does not interfere with the use of real-time PCR in the diagnosis of canine leptospirosis [29]. The leptospiral burden was low for the four dogs with a positive result for their blood samples, which renders the biological significance of these findings questionable. The amplification of residual DNA from a past infection seems very unlikely as DNA is degraded very quickly. The physiopathological process involved might also be more complex than is commonly thought: recurrent phases of Leptospiraemia in asymptomatic chronic carriers could be a possibility. The fact that two dogs with positive PCR results for their urine at the first sampling had positive PCR results from their blood at the recheck could suggest a complex phenomenon of recurrence. However, evidence for this hypothesis has never been documented, and it requires further investigation.

The limitations of the present study were the small population size, the absence of supporting culture results, the lack of PCR monitoring for the 24 dogs with negative PCR results from blood and urine at first sampling and the failure to genotype the

Leptospira species identified at the serovar level. It would also have been interesting to test the blood PCR-positive dogs for antibodies a few weeks after the first sample to explore the assumption of a recent infection.

Despite these limitations, this study supports the fact that asymptomatic urinary shedding of leptospires in apparently healthy vaccinated dogs does occur in non-endemic areas and that it may be transient. Whether dogs can be a reservoir for serovars other than Canicola remains controversial. A larger prospective study with close and repeated monitoring of dogs via PCR would be advisable, with a specific effort to determine the serovars involved and to screen for possible associated renal damage. Serological status was found to be a poor predictor of urinary shedding, as has been shown previously [\[12\]](#), [\[13\]](#). The significance of positive PCR results from blood samples for Leptospiral DNA in dogs from a non-endemic area warrants further investigation. The very low but detectable amounts of Leptospiral DNA in the blood and urine of some apparently healthy dogs raises questions regarding the diagnostic consequences of the extremely high sensitivity of PCR assays. It is also potentially important from a public health point of view. Given the potential veterinary and public health implications of these observations, repeated monitoring in a cohort study would be advisable.

5 ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

6 DISCLOSURE OF INTEREST

The authors declare that they have no competing interest.

7 ACKNOWLEDGEMENTS

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