J Vet Intern Med 2014;28:284–293

Serologic and Urinary PCR Survey of Leptospirosis in Healthy Cats and in Cats with Kidney Disease

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Background: Although there is serologic evidence of exposure of cats to *Leptospira* spp., clinical disease is rarely reported in cats.

Objective: To compare the seropositivity and urinary polymerase chain reaction (PCR) status for *Leptospira* spp. between healthy (H) cats and cats with kidney disease (KD), to investigate the serovars potentially involved, and to evaluate potential risk factors.

Animals: Two hundred and forty client-owned cats.

Methods: Cats were prospectively recruited and classified based on physical examination, complete blood count, serum biochemistry profile, and urinalysis (125 H and 115 KD cats). *Leptospira* spp. serology (titers ≥1 : 100 considered positive) and urinary PCR were performed in all cats. Data assessing risk factors, obtained from a questionnaire, were evaluated using logistic regression models.

Results: Seropositivity for *Leptospira* spp. was statistically different between groups: 7.2% (9/125) and 14.9% (17/114) in the H and KD, respectively ($P = .05$). The proportion of PCR-positive cats was not. The most common serovars detected serologically were Pomona (n = 16) and Bratislava (n = 8). Risk factors for seropositivity included outdoor and hunting lifestyles ($P = .03$ and $P < .001$, respectively), the presence of another cat in the household ($P < .01$), and the sampling period, with the greatest number of cases identified between June and August (P =.02).

Conclusions: Seropositivity was significantly greater in KD cats, suggesting that the role of *Leptospira* spp. in KD in cats should be further investigated. The detection of urinary shedding of leptospires in several cats identifies a potential role in the transmission of the organism.

Key words: Acute kidney injury; Chronic kidney disease; *Leptospira* spp.; Microscopic agglutination test; Renal disease.

Leptospirosis, which is caused by infection with pathogenic *Leptospira* species, is the most widespread zoonosis worldwide, and it has been found in almost all species of mammals examined.[1,2] In cats, the seroprevalence varies from 4.8 to 35% depending on the geographic location and the diagnostic methods used.[3–13] Although serologic evidence of exposure exists, clinical disease in cats is rarely reported and little information is known about leptospirosis in cats and its clinical significance. Cats can shed leptospires in their urine intermittently for several weeks after experimental infection and although different inoculation routes and leptospiral doses were used, these studies support cats as a potential source of infection for humans.[14–16]

Kidney disease (KD) has a major impact on the health of cats.[17,18] Although many cats with an outdoor lifestyle are in close contact with potential reservoir hosts for leptospirosis (mice and rats),[19] the role of *Leptospira* spp. as an etiologic agent for KD in cats has not been determined.[6,19,20] Most experimental studies suggest that cats are resistant to acute leptospirosis,[14–16] yet the description of some clinical cases proves that leptospires can be pathogenic to this species, causing mainly kidney[21–23] and liver damage.[22,24] In addition, a serologic study conducted in France found a statistical relationship between cats presenting with polyuria and polydipsia (PU/PD) and seropositivity for *Leptospira* spp.[6] In that study, 14/16 PU/PD cats were seropositive versus 32/80 without PU/PD. However, the long-term impact of infection on the renal function of cats is unknown as the longest experimental study lasted only 84 days.[15]

The aim of this study was to compare the seropositivity and the urinary PCR status for *Leptospira* spp. between healthy cats (H) and cats with KD (both acute kidney injury [AKI] and chronic KD),[a] and to determine the serovars probably involved in Quebec, Canada. In addition, factors potentially influencing the seropositivity and PCR status for *Leptospira* spp. were

Abbreviations:

A/G	albumin/globulin
AKI	acute kidney injury
CBC	complete blood count
CI	confidence interval
CKD	chronic kidney disease
H	healthy
KD	kidney disease
MAPAQ	Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
MAT	microscopic agglutination test
PCR	polymerase chain reaction
PU/PD	polyuria and polydipsia
SUN	serum urea nitrogen
USG	urine specific gravity
WBC	white blood cell

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Submitted May 21, 2013; Revised October 21, 2013; Accepted November 21, 2013.

10.1111/jvim.12287

evaluated, including age, sex, outdoor access, multipet households, a rural versus urban environment, contact with wildlife, and seasonality of sampling. Finally, among KD cats, several variables were evaluated for their potential influence on the seropositivity, PCR status, or both, including pertinent hematology, serum biochemistry, and urinalysis variables, and the type of KD (ie, AKI versus CKD of various stages).

Materials and Methods

Case Selection Criteria

The study was approved by the Université de Montréal Animal Care and Use Committee based on the Institutional Ethics Committee Guidelines provided by the Canadian Council on Animal Care. Healthy cats and cats with KD of unknown etiology were recruited from January 2010 to March 2012 from the client population of the Centre Hospitalier Universitaire Vétérinaire of the Université de Montréal, and from 3 local private veterinary clinics,[b] until the calculated sample size was attained for each group. Final classification between the H and KD groups was based on physical examination by a veterinarian, complete blood count (CBC), serum biochemistry profile, and urinalysis results. More precisely, cats included in the H group had normal kidneys on abdominal palpation, a urine specific gravity >1.035 (a morning urine sample was requested the following day if the urine specific gravity was below 1.035), no proteinuria, and a normal serum creatinine (<1.6 mg/dL). Only azotemic cats with a urine specific gravity <1.035, with or without clinical signs, were assigned to the KD group. KD cats were categorized by the attending clinician as being in AKI or in CKD based on the chronicity of their condition and the workup performed for each case. Chronic kidney disease cats were subcategorized for further analysis (CKD stage IIb, III, or IV). Therefore, some cats initially thought to be healthy were reclassified in the KD group. Cats suffering from KD of a known etiology (eg, exposure to a nephrotoxin, pyelonephritis, urinary tract obstructions, ureterolithiases, neoplasia, and congenital KD) were excluded from the study. Similarly, breeds with a high risk of congenital KD (eg, polycystic kidney disease [PKD] in Persian, Exotic, Himalayan and Oriental cats, and renal amyloidosis in the Abyssinian, Somali, Siamese, and certain Oriental breeds) were excluded unless a renal ultrasound (PKD) or a DNA test had been performed to exclude these diseases. Finally, cats having received antibiotics in the 3 months before the study were excluded.

After signed informed owner consent was obtained, a physical examination was performed, and blood (5 mL) and urine (5 mL via cystocentesis) were collected for analysis. Other pertinent data, such as home address, age, sex, outdoor access with or without likely contact with wildlife, rural versus urban environment and the presence of other pets in the household, were obtained by means of a questionnaire (Data S1). The date of sample collection was recorded. For analysis purposes, the cats were divided into 3 age-groups: ≤5, 6–10, and >10 years old.

Sample Size Determination

Based on results obtained in a preliminary survey[13] and previously published data,[6,19] the sample size by group (H, KD) was estimated on the basis of an assumed prevalence of positive microscopic agglutination test (MAT) of 24 and 48% per group, respectively. Sample sizes were calculated for seroprevalence estimation in each group (95% confidence interval [CI], 10% precision) and for comparison of seroprevalence between groups (95% CI, 80% power). For both groups, the largest sample size estimated was used, resulting in a minimum of 96 cats per group. Additional cats were recruited to offset the group change of several cats (H versus KD) once the CBC, serum biochemistry profile, and urinalysis results were available.

Procedures

CBC, Serum Biochemistry Profile, and Urinalysis. A CBC, serum biochemistry profile, and urinalysis were performed on every cat enrolled in the study.

Microscopic Agglutination Test. The MAT were performed by the Quebec government veterinary diagnostic laboratory (LEPAQ).[c] The serology samples were frozen and sent once a month for analysis. The samples were tested for *Leptospira interrogans* serovars Pomona, Canicola, Hardjo, Icterohaemorrhagiae, and Bratislava, and for *L. kirshneri* serovar Grippotyphosa. Titers ≥1 : 100 were considered positive. In seropositive cats, the serovar with the highest titer was identified.

***Polymerase Chain Reaction Assay for Leptospira* spp.** One to two milliliters of urine collected by cystocentesis were used to perform conventional PCR in all cats. The urine samples were refrigerated and processed within 72 hours of collection by the Laboratoire de Diagnostic Moléculaire of the Université de Montréal, using G1 and G2 and B64-I/B64-II primers, which can amplify a 285-bp DNA fragment from *L. interrogans* serovars Pomona, Canicola, Hardjo, Icterohaemorrhagiae, Automnalis, and Bratislava, and a 352-bp fragment from the DNA of *L. interrogans* serovar Sejroe and *L. kirshneri* serovar Gripptyphosa, respectively, as previously described.[25,26] The analytic sensitivity and specificity of this PCR technique were validated on urine of cats before the study, with a detection limit of 8.3×10^2 leptospira/mL of urine, which was the maximal dilution tested.[d] A positive result was considered to reflect urine shedding.[27]

Statistical Analysis

Prevalence Estimation. For both laboratory assays (MAT, PCR), the prevalence of positive cats with 95% CI was estimated separately for H and KD groups. The CI were adjusted for the clustered sampling design, ie, accounting for cats living in the same household. The Surveyfreq procedure of SAS version 9.3 was used for estimation.

Risk Factor Analyses. All potential risk factors collected by means of a questionnaire, as well as the geographic analyses, were categorized; the data are presented in Table 1. For the geographic analyses, the home address of each cat owner was converted into geographic coordinates using the GeoPinpoint software version 2011.3 (DMTI Spatial) and then manually validated. The population density within an 80-m radius of the cat's home, which represents the standard roaming territory of cats,[28] was estimated using data from Statistics Canada 2011 census.[e] The Euclidean distance between the cat's home and the nearest farm was estimated for swine, bovine (dairy cattle, beef cattle, or both) and small ruminants (sheep, goat, or both). The centroid of the farm lot where animals were housed was used for the calculation, based on 2007 data obtained from the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ). This database was updated voluntarily by farmers until 2010 (eg, newly registered enterprises, farming cessation, change in production type).[f] All spatial analyses were performed in ArcInfo version 9.3 (ESRI Inc.).

Generalized estimating equations (GEE) logistic regression was used for modeling. An exchangeable correlation structure was used in the model to account for a possible correlation in the predicted outcomes among cats residing in the same house.

Table 1. Percentage of seropositive and PCR-positive cats for *Leptospira* spp. according to potential risk factors.

	MAT			PCR		
Variable	Number of Cats	% Positive	*P* Value[a]	Number of Cats	% Positive	*P* Value[a]
Group						
Healthy	125	7.2	.05	125	1.6	.55
Kidney disease	114	14.9		113	5.3	
Time of year[b]						
September–November	58	15.5	.07	58	5.2	.51
June–August	73	15.1		73	1.4	
December–May	108	5.5		107	3.8	
Age (years)						
≤5	23	4.4	.23	23	4.3	.33
6–10	114	7.9		114	0.9	
>10	102	15.7		101	4.9	
Sex						
Female	130	10	.77	130	3.8	.64
Male	109	11.9		108	2.8	
Type of diet						
Canned	6	0	.55	5	0.0	N/A[c]
Mixed	68	14.7		68	4.4	
Dry	165	9.7		165	3	
Other pets in the household						
Yes	173	13.3	.03	172	4.1	.17
No	66	4.6		66	1.5	
Other cat in the household						
Yes	148	15.5	<.01	148	4.1	.20
No	91	3.3		90	2.2	
Dog in the household						
Yes	70	11.4	.36	69	4.3	.94
No	169	10.7		169	3	
Rodents in the household						
Yes	6	0	N/A[c]	6	0.0	N/A[c]
No	233	11.1		232	3.5	
Environment						
Rural	42	9.5	.93	43	0.0	N/A[c]
Urban	197	11.2		195	4.1	
Distance: household to nearest pig farms						
≤1 km	7	0	N/A[c]	7	0.0	N/A[c]
>1 km	232	11.2		231	3.5	
Distance: household to nearest dairy farms						
≤1 km	43	7	.52	43	2.3	.50
>1 km	196	11.8		195	3.6	
Distance: household to nearest goat farms, sheep farms, or both						
≤1 km	30	3.3	.29	30	0.0	N/A[c]
>1 km	209	12		208	3.8	
Outdoor access						
Yes	142	14.8	.03	142	5	.09
No	97	5.1		96	1	
Known hunter						
Yes	70	21.4	<.001	70	7.1	.50
No	169	6.5		168	1.8	
Contact with raccoons						
Yes	69	14.5	.27	69	8.7	.01
No	170	9.4		169	1.2	
Contact with skunks						
Yes	87	18.4	<.01	87	6.9	.03
No	152	6.6		151	1.3	
Contact with wild rodents						
Yes	110	16.3	<.01	110	5.5	.06
No	129	6.2		1.6	1.6	
Routine vaccination up-to-date						
Yes	132	13.6	.11	131	4.6	.14
No	107	7.5		107	1.9	

PCR, polymerase chain reaction; MAT, microscopic agglutination test; H, healthy; KD, kidney disease.

[a]*P* Value (Wald test) for the overall significance of the variable on the seropositivity. *P* Value <.05 was considered statistically significant. Generalized estimating equation logistic regression model including the group (H versus KD) adjusted for the cluster effect.

[b]The time of year September–November and June–August were compared with December–May.

[c]N/A, not available. This variable was excluded from statistical analysis because of difficulty in model convergence.

Investigated outcomes were the MAT status (positive versus negative) and PCR status (positive versus negative). Potential risk factors were first tested individually in a model including the intercept and the group (H versus KD). Variables with a P value ≤.25 (Wald test for the overall significance of the variable) were considered for inclusion in a full model. In the presence of colinear variables based on biological knowledge and data exploration, only the one with the smallest P value was selected. A backward procedure was then used for final model selection, with $P > .05$ as criterion for rejection. Because of sample size limitations, no interactions were tested. Alternative models were explored by considering other variables previously rejected for colinearity issues. Odds ratios (OR) with 95% CI and predicted probabilities were used to present the results. Analyses were performed using the Genmod procedure of SAS version 9.3.

Associations with Indicators of Disease Severity. Based on data from experimental and clinical leptospirosis in cats,[14,21] and from clinical studies in dogs,[20] selected hematology, serum biochemistry, and urinalysis variables were further investigated in the KD group (ie, PCV, platelet count, hepatic enzyme activities (alkaline phosphatase, alanine transaminase, gamma-glutamyltransferase) and total bilirubin concentration, as well as urine specific gravity, presence of proteinuria and glucosuria and their respective concentrations). These variables were compared between the seropositive and seronegative KD cats using the Wilcoxon test. The association between KD types (AKI versus CKD) or CKD IRIS stages, and MAT or PCR status was tested using the exact chi-square test. Analyses were performed in SAS version 9.3.

Results

A total of 251 cats were recruited, 11 of which were excluded for one of the following reasons: receiving or having received antibiotics in the previous 3 months (n = 9), presence of a urinary tract infection in 1 H cat and diagnosis of obstructive nephrolithiasis in 1 KD cat. Therefore, 240 cats belonging to 194 households (mean value of 1.24 cats per family; range of 1–10 cats) were enrolled in the study: 125 H cats and 115 cats with either AKI (n = 19; 16.5%) or CKD (n = 96; 83.5%). Three KD cats were excluded from part of the statistical analysis because of missing results for MAT (n = 1) or PCR (n = 2). The mean ages of the H and KD groups were 8.8 and 11.6 years old, respectively. The cats' distribution by age-group was as follows: ≤5 years old (H = 10 cats; KD = 13 cats), 6–10 years old (H = 83 cats; KD = 32 cats), and >10 years old (H = 32 cats; KD = 70 cats). The CBC, serum biochemistry profile, and urinalysis results are summarized in Tables 2 and 3 for each group.

Table 2. Hematology, serum biochemistry, and urinalysis laboratory values in H and KD cats.

		Group			
		H (n = 125[a])		KD (n = 115[b])	
Laboratory Values	Reference Range	Median	(Min–Max)	Median	(Min–Max)
Hematology					
PCV (%)	28–47	40	(22–53)	33	(18–47.8)
Hb (g/L)	81–42	126	(12–157)	106	(54–154)
RBC (*10E12/L)	6–10.1	8.74	(5.8–11.0)	7.025	(3.9–10.2)
WBC (*10E9/L)	6.3–119.6	6.85	(3.7–24.9)	7.63	(3.0–42.4)
Platelet (*10E9/L)	156–626	252	(17–855)	281	(64–591)
Biochemistry					
ALT (U/L)	16–63	52	(22–278)	51	(18–305)
ALP (U/L)	0–50	24	(6–94)	20	(0–746)
GGT (U/L)	0–10	2	(0–5)	2	(0–6)
Total bilirubin (μmol/L)	0–10	6.1	(0–15)	5	(0–23.9)
Total protein (g/L)	59.6–80.8	71	(57.8–89.0)	71.3	(41.6–91.0)
Albumin (g/L)	26–39	30.3	(23.8–37)	30	(21.8–41.6)
Globulin (g/L)	29–47	40.9	(29–60.5)	41.9	(11.1–66)
A/G ratio	0.58–1.16	0.74	(0.43–1.16)	0.73	(0.37–3.64)
SUN (mmol/L)	4.1–10.8	8.78	(4.26–16.1)	15.29	(0.39–512)
Creatinine (μmol/L)	51–180	131	(68–198)	243	(144–1895)
Phosphorus (mmol/L)	0.96–1.96	1.41	(0.71–2.17)	1.59	(0.92–8.92)
Sodium (mmol/L)	145–158	151.9	(142–162)	152	(131–173)
Potassium (mmol/L)	3.6–5.3	4.40	(3.50–5.67)	4.45	(1.55–9.95)
Urinalysis[c]					
Protein (g/L)	Neg	0.3	(0–3)	0.3	(0–5)
Blood (RBC/hpf)	Neg	0	(0–250)	5	(0–250)
USG[d]	1.035–1.060	1.052	(1.035–1.060)	1.015	(1.007–1.034)

H, healthy; KD, kidney disease; min, minimum; max, maximum; Hb, hemoglobin; PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; A/G, albumin/globulin; SUN, serum urea nitrogen; USG, urine specific gravity.

[a]Maximum sample size, ranging from 121 to 125 depending on the assay.

[b]Maximum sample size, ranging from 108 to 115 depending on the assay.

[c]Urinalysis categorical variables.

[d]USG ≥1.056 were set at 1.060.

Table 3. Percentage of H and KD cats with glucosuria, bilirubinuria, and cristalluria.

	Group	
	H (%) (n = 125)[a]	KD (%) (n = 115)
Glucosuria	7.2[b,c]	8.7
Bilirubinuria	0.8	1.7
Cristalluria[b,c]	6.5	4.4

H, healthy; KD, kidney disease.

[a]Maximum sample size, ranging from 124 to 125 depending on the assay.

[b]Only struvites were identified in all cases.

[c]Nine healthy cats presented with some mild glucosuria, which was attributed to stress hyperglycemia (increased blood glucose documented in 6/9, which normalized on follow-up blood glucose evaluation). No other laboratory or clinical abnormalities were detected in any of those cats, and all 9 cats were microscopic agglutination test negative.

Overall, 26 cats were seropositive and 8 were PCR-positive. The seropositivity for *Leptospira* spp. was estimated as 7.2% (n = 9/125; 95% CI: 2.2–12.2) in the H group and 14.9% (n = 17/114; 95% CI; 8.3–21.6) in the KD group. The proportion of PCR-positive cats was estimated as 1.6% (n = 2/125) and 5.3% (n = 6/113) in the H and KD cats, respectively.

The serovars with the highest titers were Pomona (n = 16), Bratislava (n = 8), and Grippotyphosa (n = 1), with median titers significantly higher for Pomona ($P = .04$). One cat had equivalent titers for Bratislava and Grippotyphosa (titers = 100; Table 4).

According to the GEE models, the seroprevalence was higher in the KD group compared with the H group ($P = .05$), but no difference was observed between groups for the PCR status ($P = .55$) (Table 1). The seroprevalence and PCR status did not vary significantly according to the age ($P = .23$ and $P = .33$, respectively) or sex ($P = .77$ and $P = .64$, respectively) of the cats. Among the variables selected for the multivariate analysis, the variables "other cat in the household" and "other pets in the household" were strongly correlated. Only the former was included in the multivariate model.

Predicted seropositivity for *Leptospira* spp. remained statistically different between groups: 5 and 13.7% in the H and KD, respectively (OR = 2.8, $P = .02$) (Table 5). Known hunters had a predicted seroprevalence of 15.2% compared to 4.5% in cats not considered hunters by their owner ($P < .01$). The presence of another cat in the household significantly increased the risk of seropositivity for leptospirosis ($P < .01$), although the presence of a dog did not. Cats were more likely to be seropositive between the months of June–August and December–May ($P = .02$). No difference was found between the months of September–November and December–May ($P = .06$). Because of the potential that age could be a confounding factor for the group (H versus KD) effect, the age variable was tested in the final model. As it was not statistically significant ($P = .09$) and age did not appear to be a confounding factor for the effect of group (<18% changes in the OR estimate for the group variable), age was not kept in the final model. The variable "hunter" selected in this model was correlated with the variables "outdoor access," "contact with wild rodents," and "contact with skunks." Thus, 3 alternative multivariate models were developed while

Table 4. Individual MAT titer results of seropositive cats.

Number of Cats with Similar Titers Pattern	Serovars			
	Pomona	Bratislava	Grippotyphosa	Icterohaemorragiae
1	≥1 : 12,800	–	–	–
1	≥1 : 12,800	1 : 3,200	–	–
1	≥1 : 12,800	1 : 400	–	–
1[a]	≥1 : 12,800	1 : 100	1 : 100	1 : 100
2	≥1 : 6,400	1 : 200	–	–
1[a]	≥1 : 6,400	1 : 100	–	–
1[a]	1 : 1,600	1 : 100	–	–
1	1 : 800	–	–	–
1	1 : 400	1 : 100	–	–
1	1 : 400	–	–	–
2	1 : 200	–	–	–
3	1 : 100	–	–	–
1	1 : 100	1 : 3,200	–	–
1	–	1 : 1,600	1 : 100	–
1[a]	–	1 : 100	1 : 100	–
6	–	1 : 100	–	–
1	–	–	1 : 200	–

PCR, polymerase chain reaction; MAT, microscopic agglutination test.

[a]Seropositive cats also positive on urinary PCR (n = 4); the additional PCR-positive cats are not identified in the table, as they were seronegative (n = 4). Titers ≥1 : 6,400 and 1 : 12,800 represent the maximal dilution evaluated by LEPAQ at the time the MAT was performed. All cats included in the study were seronegative for the serovars Hardjo and Canicola.

Table 5. Results from multivariate logistic regression analysis predicting a positive MAT status in cats (n = 239).[a]

	OR			Predicted Outcome (%)[b]	
Variable	Estimate	95% CI	*P* Value	Estimate	95% CI
Group					
KD	2.8	(1.2–6.6)	.02	13.7	(6.6–28.7)
H	Ref.			5.0	
Known hunter					
Yes	3.4	(1.4–8.3)	<.01	15.2	(6.7–33.8)
No	Ref.			4.5	
Other cat in the household					
Yes	6.0	(1.6–22.2)	<.01	20.3	(12.5–32.7)
No	Ref.			3.4	(0.1–11.7)
Time of year					
September–November	3.0	(0.9–11.7)	.06	11.1	(12.5–32.7)
June–August	3.6	(1.2–11.0)	.02	13.5	(5.8–31.5)
December–May	Ref			3.7	(1.3–10.8)

MAT, microscopic agglutination test; OR, odd ratio; CI, confidence interval; H, healthy; KD, kidney disease.
[a]One KD cat was excluded from this part of the statistical analysis because of missing MAT results.
[b]The predicted outcome (%) was adjusted for the other variables (mean value).

considering only one of the above variables at a time (results not shown). In all models, the variable was kept in the model if $P < .05$.

Because of the low number of PCR-positive cats, no multivariate logistic regression model was developed. When evaluating the PCR status of cats in the GEE model for individually tested variables, only the variables "contact with raccoons" and "contact with skunks" were statistically significant ($P \leq .03$).

For the KD group, no significant difference was observed in the selected hematology, serum biochemistry, and urinalysis variables, including liver enzyme activities, between the seropositive and seronegative cats (Wilcoxon test, $P \geq .47$). Only the total bilirubin was statistically significantly different, with higher bilirubin concentrations, which remained within reference range, found in the seronegative KD cats (Wilcoxon test, $P < .01$). The seroprevalence in cats with AKI was 21.1% (4/19) and with CKD was 13.7% (13/95), which were not statistically different (Exact chi-square test, $P = .48$). Of the 96 CKD cats, 61 cats were classified in stage IIb (63.5%), 33 cats in stage III (34.4%) and 2 cats in stage IV (2.0%). The seroprevalence in cats with CKD did not vary statistically according to their IRIS stage (Exact chi-square test, $P = .41$) and was as follows: 13.3% (8/60) for stage IIb, 12.1% (4/33) for stage III, and 50% (1/2) for stage IV. The analysis was repeated with the type and stage of KD reclassified into 3 categories (AKI; CKD stage IIb; and CKD stage III/IV), which remained unassociated with the seroprevalence (Exact chi-square test, $P = .72$) or with the urine PCR status (Exact chi-square test, $P = .14$).

Discussion

In this study, the seropositivity for *Leptospira* spp. was significantly greater in KD cats (14.9% in 17/114) than in H cats (7.2% in 9/125). Positive PCR test was detected in 5.3% (6/113) KD cats and 1.6% (2/125) healthy cats ($P = 0.55$). These findings foster the idea that leptospirosis should be further regarded as a potential underdiagnosed cause of KD in cats. The potential role of *Leptospira* spp. in the pathophysiology of KD in cats remains to be elucidated. For instance, cats with KD could simply be at increased risk of contracting leptospirosis compared with healthy cats.

The seroprevalences obtained in this study are similar to those reported in most other studies, ie, from 4.8 to 16.9%.[3–5,7,9–13] Only 2 studies reported seroprevalences >30%,[6,8] but lower cut-off values were utilized, ie, antibody titers of 1 : 50 and 1 : 80, respectively.

In this study, the seropositive cats often had extremely increased titers, with 7 out of 16 titers for Pomona reaching the maximal dilutions normally conducted by LEPAQ (1 : 6,400 and 1 : 12,800; Table 4). This finding differs from what is reported in the literature, including both experimental and epidemiologic studies, as cats are thought to respond to infection with low antibody titers ranging from 1 : 30 to 1 : 400.[3,4,7,8,10,12] To the best of our knowledge, less than 5 cats in the literature have been reported to have titers exceeding 1 : 3,200.[5,11,21,30] In addition to low serological response, cats are reported to rarely develop clinical leptospirosis.[21,24,31] The very high titers identified in our study may reflect either a recent or active infection, or a re-infection, especially in outdoor cats. Monitoring all seropositive cats using paired titers would have helped better explain our results. It is possible that the high antibody titers corresponded to an efficient humoral response, which might explain why only 15% of seropositive cats were also PCR-positive (Table 4). Nevertheless, 10 of the 26 seropositive cats

had titers of 1 : 100, which is consistent with both experimental and previous epidemiologic studies.[11–14,16] The reason for such low titers in experimentally and in some naturally infected cats remains unknown; it might represent a cross-reactivity or paradoxical reactions with serovars not tested for, or simply that low antibody titers are sufficient to control the infection in cats. As no information is available on how long-lived are leptospiral antibodies in cats, paired serum titers should always be used for clinical interpretation of the results.

Based on antibody testing, the most common serovars detected were Pomona (n = 16) and Bratislava (n = 8; Table 4). This finding might reflect the importance of bovine and porcine livestock in the province of Quebec, which are known to be primary reservoirs of both serovars.[32–36] However, the geographic analyses did not reveal any relationship between seropositive cases and the proximity of farms. Thus, this finding could also reflect cross-reactivity or paradoxical reactions with other serovars.

Similar to previous studies,[16,29] outdoor cats, notably hunters, were more likely to be seropositive, probably because of their increased contact with potential reservoirs of the disease. In addition, wild animals, including raccoons and skunks, were identified as risk factors in PCR-positive cats ($P \leq .03$). The former are also known maintenance hosts of the serovars Pomona and Bratislava and might indirectly infect cats.[37–41] In fact, the population of raccoons in Quebec is massive, particularly in the Montérégie region[42] where most of the cats were recruited, and raccoons are known carriers of Pomona and Bratislava.[38–40,43] Moreover, a recent serosurvey conducted by the MAPAQ in the area revealed a 56.1% seroprevalence of *Leptospira* spp. in 107 raccoons and 25% in 112 striped skunks, with the *L. interrogans* serovars Pomona, Bratislava, and Grippotyphosa detected most frequently.[44]

The presence of another cat in the household significantly increased the risk of seropositivity for leptospirosis, although the presence of a dog did not. Sharing a litter box was not evaluated as a risk factor on its own, but might be of concern in the perpetuation of the infection.

Based on meteorologic risk factors previously described,[45,46] the following 3 periods of the year were used for analysis: June–August, September–November, and December–May. The seroprevalence was statistically greater between June and August compared with December–May. The period between June and August corresponds to the warmest and most humid months of the year in Quebec.[g,h] This enables the persistence of *Leptospira* spp. in the environment.[46–49] Many questions remain to be elucidated concerning specific clinical signs and clinicopathologic findings associated with leptospirosis in cats, how serologic results should be interpreted and what treatment plan should be implemented in seropositive or PCR-positive cats. Nevertheless, the risk factors identified in this study, such as an outdoor and hunting lifestyles, the likely contact with raccoons, skunks, or both, the presence of another cat in the household, and appropriate seasonality, should prompt veterinarians to test for *Leptospira* spp. in cats with either acute or chronic KD. Results of testing should, however, be interpreted with caution. We recommend testing suspected cases with acute and convalescent titers along with a urine PCR, blood PCR, or both. As little information is available on leptospirosis in cats, we think that it is reasonable to use guidelines recommended in dogs for interpretation of results and treatment recommendations.[20]

The excretory status was confirmed by positive urine PCR in 8 cats, mainly in the KD group, but also in 2 healthy cats. Similar to the chronic carrier state suggested in PCR-positive, MAT-negative healthy dogs,[50] the PCR-positive healthy cats identified in our study and in others,[ij] suggest that cats could be asymptomatic hosts, thereby excreting leptospires in their urine and becoming a potential source of infection for both the owners and the environment. While this finding raises obvious public health concerns, 2 studies have demonstrated the protective role of cat ownership in reducing the risk of infection in their owners, likely because of the scavenging role of cats for rodents, which are considered the main reservoir for humans.[41,51] Other explanations for the identification of PCR-positive healthy cats include false-positive results or the detection of leptospiral DNA from dead organisms in the urine.[9,14]

Limitations

Limiting our study to 6 serovars might have resulted in some false-negative serologic results,[20] although the seroprevalence in this study is similar, if not slightly greater than that in other studies reporting seroprevalence rates of 4.8–12.8%.[3,5,11] Moreover, our study included the 4 most commonly identified *Leptospira* serovars in domestic animals in Quebec, which are Pomona, Hardjo, Bratislava, and Grippotyphosa.[44]

Recent studies suggest that the MAT does not accurately predict the infective serovar. This in turn limits our ability to infer the most likely source of infection amongst previously reported reservoirs based on the identification of a common serovar. In a human clinical study, the serovar with the highest titer was the infective serovar in only 46% of cases.[52] A study in vaccinated dogs and dogs infected with leptospirosis also found considerable interlaboratory variation in MAT results.[53] Molecular typing techniques, such as real-time PCR, would have been useful in identifying the causative serovars in our study.[27,28,54–57]

Although the prevalence of PCR-positive cats was higher in the KD group (5.3%; 6/113) compared with the H group (1.6%; 2/125), the difference between groups did not reach statistical significance. This is possibly because of the limited number of cats included in the study, which had been based on the preliminary serologic data, as no PCR study had previously been conducted in North American cats

before 2012.[i] Simultaneous PCR testing of blood and urine would have probably increased the diagnostic sensitivity.[20] In addition, a negative PCR result does not exclude the possibility of intermittent excretion,[28,58] short duration or low level of shedding, notably in seropositive cats. Along the same vein, repeating urine PCR in PCR-positive cats could have permitted confirmation if these cats were chronic, sporadic, or one-time shedders. Repeated MAT and PCR testing, particularly of seropositive cats, could have helped to shed light on these matters, but was not performed because of both limited finances and access to the cats.

Similarly, some risk factors could have been missed because of lack of statistical power resulting from the limited sample size and low proportion of positive cats. Also, controlling for age was considered important, as both the likelihood of exposure events to *Leptospira* and of developing KD would increase with age. For this reason, we initially intended to have age-matched groups, but were unable to do so because several presumably H cats had to be reclassified in the KD group once the urinalysis and blood work results were available. This was not surprising as CKD is a common disease in cats.[59] For instance, a prospective study conducted in initially healthy geriatric cats (≥9 years old; median of 13 years) revealed that a third of them (29/95) developed azotemia within the following year of enrollment.[17] However, no strong evidence of the age effect as a confounding factor for the group effect was observed during multivariate model building, although we cannot completely exclude this possibility.

Conclusion

Although the precise role of *Leptospira* spp. as an etiologic agent of KD in cats remains unclear, the significant difference found in the serologic status between H and KD cats suggests that leptospirosis might be an underdiagnosed cause of acute or chronic KD in cats. Based on the results of this study, leptospirosis should be investigated as a potential cause of KD in cats, notably if risk factors are present, such as an outdoor lifestyle, particularly if the cat is a known hunter, or is likely to have contact with wild animals, if another cat is present in the household, there is a corresponding seasonality or both. Two asymptomatic carriers and several healthy cats with very high titers were identified in this study; therefore, the role of cats in the transmission of leptospirosis should be reevaluated, as it might in fact be underestimated.

Acknowledgments

This research was supported by the Association des Médecins Vétérinaires du Québec en pratique des petits animaux and the generous donation from Mrs Valeria Rosenbloom and Mr Mike Rosenbloom (Fond de santé des animaux de compagnie).

Conflict of Interest Declaration: The authors disclose no conflict of interest.

Footnotes

[a] International Renal Interest Society (IRIS): http://iris-kidney.com

[b] Clinics and the hospital included in the study located in the Montérégie area of Québec, Canada: Hôpital vétérinaire Rive-Sud, Brossard. Clinique vétérinaire Johannaise, St-Jean-sur-Richelieu. Clinique vétérinaire Marieville, Marieville

[c] Laboratoire d'Expertise en Pathologie Animal du Québec (LEPAQ) of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ)

[d] Rodriguez, J. La leptospirose féline: sondage sérologique et de PCR urinaire chez des chats sains et des chats atteints de maladie rénale. Canada: Faculté de médecine vétérinaire, Université de Montréal; 2013. Master's thesis

[e] 2011 Canadian population census Statistics Canada (www.statcan.gc.ca)

[f] Data extracted from the MAPAQ May 2012 database on registered agricultural farms

[g] National climate data and information archive (http://www.climate.weatheroffice.gc.ca)

[h] Climat-Québec (http://www.climat-quebec.qc.ca/index.php)

[i] Fenimore A. et al. Detection of Leptospiruria in Shelter Cats in Colorado. Abstract presented at ACVIM, New Orleans, 2012;783

[j] Fenimore A. et al. Leptospira Interrogans Serovar Hardjo-Pra-Jitno DNA in the Urine of a Cat in Colorado. Abstract presented at ACVIM, Seattle, 2013;726

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Role of *Leptospira* spp. as an etiologic agent in feline renal insufficiency.