Original Article

Journal of Feline Medicine and Surgery
2016, Vol. 18(10) 768–772

DOI: 10.1177/1098612X15593902
jfms.com
SAGE

Evaluation of the *Leptospira* species microscopic agglutination test in experimentally vaccinated cats and *Leptospira* species seropositivity in aged azotemic client-owned cats

Sarah B Shropshire, Julia K Veir, Arianne K Morris and Michael R Lappin

Abstract

Objectives The objectives of this study were to validate the microscopic agglutination test (MAT) using feline sera, determine cross-reactivity of *Borrelia burgdorferi* antibodies in the MAT, and evaluate if there is an association between *Leptospira* species seropositivity in aged (⩾10 years) client-owned cats with and without azotemia (creatinine >2 g/dl).

Methods A four-serovar canine leptospiral vaccine was administered to two specific pathogen-free (SPF) cats on days 0 and 14. The MAT was performed intermittently until day 42 for the serovars *Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona* and *Bratislava*, with a cut-off value of ⩾1:100. Five purpose-bred cats were infested with wild-caught *Ixodes scapularis* adults with an average *B burgdorferi* infection rate of 50%, and tested for antibodies against *B burgdorferi* C6 peptide and DNA in skin biopsies, as well as by MAT. Sera from 66 azotemic and 75 non-azotemic cats ⩾10 years of age were tested for *Leptospira* species antibodies using the MAT and results were compared by the χ^2 test.

Results Both SPF cats seroconverted by week 3 and formed antibodies against at least one serovar. There was no cross-reactivity in the MAT using samples from cats with antibodies to *B burgdorferi*. MAT results were positive for 4/66 azotemic cats and 8/75 non-azotemic cats; these results were not statistically different.

Conclusions and relevance The MAT can be interpreted using feline serum and does not appear to cross-react in cats with *B burgdorferi* antibodies. There was no association between *Leptospira* species MAT results and azotemia in this group of aged client-owned cats but further studies are needed to determine if leptospirosis contributes to feline chronic kidney disease.

Accepted: 2 June 2015

Introduction

Leptospira species are commonly associated with acute renal failure in dogs, and residual damage has also been theorized to result in canine chronic kidney disease (CKD).[1] CKD is a common cause of morbidity and mortality in cats worldwide, and has been suggested to be the number one cause of death in aged cats.[2,3] Although cats have historically been considered to be naturally resistant to developing leptospirosis, results of several studies have called this assumption into question. For example, experimental inoculation of cats with *Leptospira* species can result in nephritis, and *Leptospira* species seropositivity has been associated with clinical signs such as polydipsia and polyuria,[4–9] thus making it plausible that these organisms could be associated with CKD in this species. Additionally, the potential known exposure of cats (particularly outdoor cats) to reservoir hosts and the documentation that cats can shed leptospires in their urine

Center for Companion Animal Studies, Colorado State University College of Veterinary Medicine, Fort Collins, CO, USA

Corresponding author:
Sarah Shropshire DVM, Center for Companion Animal Studies, Colorado State University College of Veterinary Medicine, 300 West Drake Road, Fort Collins, CO 80526, USA
Email: sarah.shropshire@colostate.edu

raises additional concern for transmission and disease manifestations in humans.[4,10–12] These factors make investigation of leptospirosis of particular interest to practitioners invested in improving feline medicine and preventing potential zoonotic disease.

Currently, there are several ways to detect a leptospirosis infection in cats, including culture to help document an active infection, but this is rarely performed and not readily available.[13] Recently, however, the use of PCR assays to amplify *Leptospira* species DNA from feline urine has been used to suggest current infection in several studies.[10–12] Additionally, antibodies against *Leptospira* species in cat serum are detected by the modified agglutination test (MAT) and suggest either current or previous infection.[1,8–13,14–27] *Leptospira* species antibody prevalence rates in feline sera have varied between 4.8% and 48.0%, depending on the geographic location and sampling method.[8,10,11,14–27] A recent study utilizing both the MAT and PCR demonstrated a higher prevalence of antibodies to *Leptospira* species in cats with kidney disease than in healthy cats,[10] suggesting that *Leptospira* species infections could be associated with CKD in naturally exposed cats. However, while the MAT is thought to be accurate for use with serum from any mammal, information concerning validation of this assay in cats is minimal. For example, the magnitude and duration of *Leptospira* species antibodies as detected by the MAT in cat sera is largely unknown. While the MAT is believed to be specific for *Leptospira* species antibodies, it is unknown whether antibodies against other spirochetes in feline sera can lead to falsely positive results. When considering false-positive test results, it is known that naturally exposed and experimentally infected cats develop antibodies against *Borrelia burgdorferi* but there is no information available that evaluates whether *B burgdorferi* antibodies in feline sera are detected in the *Leptospira* species MAT. Furthermore, *B burgdorferi* is known to cause nephritis in dogs,[28,29] making the possibility of cross-reactivity a true concern when interpreting test results in cats.

Three experiments were designed to address these clinical questions. The objectives of the first two experiments were to gather and report new information validating the use of MAT for detection of *Leptospira* species antibodies in cats. We hypothesized that cats would have variable *Leptospira* species antibody responses when administered a four-serovar vaccine marketed for dogs and that serum antibodies from cats experimentally infected with *B burgdorferi* would not be detected in the *Leptospira* species MAT. The objective of the third experiment was to evaluate if there is an association between antibodies to *Leptospira* species and azotemia in aged client-owned cats. We hypothesized that cats ⩾10 years of age with azotemia would be more likely to be positive for *Leptospira* species antibodies than cats ⩾10 years of age without azotemia.

Materials and methods

Detection of Leptospira *species antibodies in vaccinated cats*

Adult (n = 2) purpose-bred laboratory-housed cats with no potential exposure to *Leptospira* species reservoir hosts were transferred from another study to the current study with approval from the Institutional Animal Care and Use Committee. Both cats were administered a subcutaneous dose of a commercially available canine vaccine (Vanguard L4; Zoetis Animal Health), which contains the *Leptospira* serovars *Canicola, Grippotyphosa, Icterohaemorrhagiae* and *Pomona*, on days 0 and 14. Sera were collected from the cats prior to inoculation and intermittently until day 42. Each serum sample was evaluated for *Leptospira* species antibodies (serovars *Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona* and *Bratislava*) by MAT performed by standard operating procedures at an accredited laboratory (Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO, USA). Samples with agglutination at ⩾1:100 were considered positive for the respective serovar.

Evaluation for B burgdorferi *antibody cross-reactivity with* Leptospira *species using MAT*

Laboratory-reared young adult specific pathogen-free cats (n = 5) were infested with wild-caught *Ixodes scapularis* adults (from Rhode Island) with an average *B burgdorferi* infection rate of 50% in an unrelated experiment.[29] All of the cats developed antibodies against *B burgdorferi* C6 peptide and were positive for *B burgdorferi* DNA in skin biopsies taken at the sites of *I scapularis* attachment. The sera had been stored at –80°C until assayed for *Leptospira* species antibodies by MAT, as previously described.

Leptospira *species antibodies in serum of aged client-owned cats with and without azotemia*

In a separate study on vaccine-associated renal antibodies, clinicians at Colorado State University and a select group of feline practitioners in the USA were contacted and asked to submit serum from cats ⩾10 years of age with serum creatinine concentrations >2 mg/dl that were believed to have stable CKD. The veterinarians were also asked to send serum from cats ⩾10 years of age evaluated in the clinic during the same time as the azotemic cats but with serum creatinine concentrations ⩽2 mg/dl. On arrival, the serum creatinine concentration was determined and then the samples were stored at –80°C until assayed in the *Leptospira* species MAT. Positive MAT prevalence rates were compared between groups by the χ^2 test with a P value <0.05 considered significant.

Table 1 *Leptospira* species modified agglutination test antibody titers from two adult specific pathogen-free cats administered two doses of a commercially available canine vaccine

	Day 0		Day 12		Day 19		Day 36		Day 42	
Serovar	Cat 1	Cat 2	Cat 1	Cat 2	Cat 1	Cat 2	Cat 1	Cat 2	Cat 1	Cat 2
Canicola	0	0	0	0	0	0	0	0	0	0
Grippotyphosa	0	0	0	0	0	0	800	0	800	0
Hardjo	0	0	200	0	100	100	400	100	400	0
Icterohaemorrhagiae	0	0	0	0	0	0	0	0	0	0
Pomona	0	0	0	0	0	0	0	0	0	0
Bratislava	0	0	0	0	0	0	100	0	200	0

Results

Detection of Leptospira *species antibodies in vaccinated cats*

Antibodies against at least one *Leptospira* species serovar were detected from both vaccinated cats, as reported in Table 1. One cat was positive for antibodies against serovar *Hardjo* by day 12 and ultimately developed serum antibodies against serovars *Hardjo, Grippotyphosa* and *Bratislava*. The other cat was positive for antibodies against serovar *Hardjo* on postinoculation days 19 and 36 (1:100) but was negative by day 42.

Evaluation for B burgdorferi *antibody cross-reactivity with* Leptospira *species using MAT*

None of the five cats with antibodies against *B burgdorferi* C6 peptide were positive for antibodies against any *Leptospira* species by MAT.

Leptospira *species antibodies in serum of aged client-owned cats with and without azotemia*

Of the 66 cats with serum creatinine concentrations >2 mg/dl, 4/66 (6%) were positive for antibodies against one *Leptospira* serovar. None of the cats had antibodies to more than one serovar. Antibodies against serovar *Icterohaemorrhagiae* were detected in the serum of two cats from Ohio (1:100 titers), and antibodies against serovar *Bratislava* were detected in one cat from Ohio (1:200 titer) and one cat from West Virginia (1:100 titer). Of the 75 cats with serum creatinine concentrations ≤2 mg/dl, 8/75 (11%) were positive for antibodies against one *Leptospira* serovar. None of the cats had antibodies to more than one serovar. Antibodies against serovar *Canicola* were detected in the serum of one cat from Ohio (1:100), one cat from New York (1:100) and one cat from Oklahoma (1:200). Antibodies against serovar *Icterohaemorrhagiae* were detected in the serum of one cat from Ohio (1:100 titer). Antibodies against serovar *Bratislava* were detected in the serum of one cat from Colorado (1:100), one cat from South Dakota (1:100 titer) and two cats from Ohio (1:100 titers). The difference in *Leptospira* species antibody prevalence rates between the two groups of cats was not statistically different ($P = 0.3281$).

Discussion

In this study, it was documented that cats can produce *Leptospira* species agglutinating antibodies when exposed to the inactivated organisms within a commercially available four-serovar *Leptospira*-containing canine vaccine. However, only one of the cats maintained a positive titer to one serovar over the 42 day study period, and the highest titer documented was 1:800. The magnitude of the titers observed in this study was drastically lower than in a recent study performed in presumably naturally exposed healthy cats and cats with kidney disease that documented a maximal titer of ≥1:12,800 depending on the serovar.[10] A previous study in *Leptospira* serovar-vaccinated dogs also documented higher titer magnitudes where maximal titers of 1:6400 were detected transiently, depending on the serovar.[30] In addition, antibodies against three of the serovars contained within the vaccine were not detected, whereas antibodies against two serovars (*Bratislava* and *Hardjo*), which are not contained within the vaccine, were detected. The same problems also occur when interpreting canine *Leptospira* species MAT results as variable titer magnitudes are observed and cross-reactivity to serogroups not within the vaccine can occur. Results using cat sera should also be interpreted carefully as the *Leptospira* species MAT detection assay is optimized for use in dogs not cats.

Vaccinated cat sera were used in this study exclusively to help assess the validity of the MAT by attempting to induce *Leptospira* species antibodies in research cats. Thus, this information should not be overinterpreted and should not be used as a recommendation to administer this canine vaccine to cats. As this vaccine was not titrated for use in cats, these serological test results should not be interpreted as a lack of response of cats to optimized vaccines but could help to explain the observed differences in titer magnitude. If a feline *Leptospira* species vaccine is

to be developed, further research will be needed to determine initial and booster dosing recommendations, effect of adjuvants, duration of immunity characteristics, and degree of cell-mediated or humoral immunity response. Along this line, the immune system status could also have influenced the titer magnitude depending on whether a weak or strong cell-mediated immunity response occurred following vaccination. All of these factors may help to explain the differences in titer magnitudes seen in cats and underscore the need for further studies to determine what factors affect *Leptospira* serovar titer magnitude and duration in cats.

B burgdorferi has been documented to cause nephritis in some dogs, and it is now known that it can induce antibody production against the C6 peptide in cats, but whether this organism causes kidney disease in cats is unknown.[28,29,31] *B burgdorferi*-endemic areas are also endemic for leptospirosis, making distinguishing these infections from each other of clinical importance in both dogs and cats.[32] We hypothesized that it would be unlikely for *B burgdorferi* antibodies to cross-react with *Leptospira* species antibodies, but it has not been extensively investigated in dogs and has never been evaluated in cats. The results of our study show that cats experimentally infested with wild-caught *I scapularis* with an average *B burgdorferi* infection rate of 50% in the absence of *Leptospira* species reservoir exposure produce antibodies against *B burgdorferi* but not *Leptospira* species. Thus, positive *Leptospira* species MAT results from cats in the field likely reflect antibodies against leptospires not *B burgdorferi*.

In the experiment studying aged client-owned cats with and without azotemia, the non-azotemic cats were more likely to have antibodies against *Leptospira* species than cats with azotemia. This is in contrast to a recent study that showed that cats with kidney disease were statistically more likely to be seropositive than healthy cats. This study included cats with CKD, as well as cats with acute kidney injury (AKI),[10] whereas the azotemic cats in our study were only considered to have stable CKD. The geographic location was also different between these two studies; Rodriguez et al sampled cats from various areas in Quebec, Canada,[10] and the present study sampled cats from various regions within the USA. The previous study also had a much larger sample set than the present one. The disease classification (CKD vs AKI), geographic location and number of cats all could explain the different results observed in these two studies.

Several papers have shown that cats with antibodies to *Leptospira* species have either signs associated with renal disease or histopathologic evidence of renal inflammation.[4–9,10] It is unknown, however, if leptospirosis causes an AKI that then leads to the development of CKD in cats. Therefore, determining if an active infection is present or not would help to better characterize the clinical disease course in cats. Similar to the recommendations for dogs and based on recent studies in cats, additional testing other than antibody testing is likely needed to determine if an active infection is present.[1,10] In dogs, PCR testing for leptospires in the urine can help to document an active infection but false-negatives do occur.[1] Combining the *Leptospira* species MAT and urine PCR assays could help increase the likelihood of detecting active leptospirosis in cats. This is also of relevance to human health owing to the known zoonotic risk of pathogenic leptospires in humans.[1] As a result, additional studies for accurate detection of leptospirosis in cats utilizing MAT and urine PCR are needed to help determine if feline leptospirosis plays a role in the development of feline CKD.

There were several limitations of this study. This study was partially retrospective rather than a double-blinded controlled prospective study. Additionally, the housing status and hunting tendencies of the cats in our study were not known, which could certainly have affected the results as Rodriguez et al showed that cats which were outdoors or which had hunting tendencies were considered to be at risk for *Leptospira* species seropositivity.[10] The sample set of cats was also not large, which would likely affect the ability to draw conclusions from the statistics performed. It is also unknown for the azotemic cats how their CKD was classified as stable disease. Additionally, for the aged client-owned non-azotemic cats, it was unknown why sera were collected and if they were clinically ill from something other than renal disease. This information could have aided in the interpretation of the prevalence rates observed in cats with and without azotemia.

Conclusions

This study showed that cats given a commercially available canine *Leptospira* species vaccine form antibodies that are detectable via a MAT and that variable titer magnitudes are observed against different serovars similar to what is observed in dogs.

To our knowledge, this is the first time that it has been documented that cats experimentally infected with *B burgdorferi* do not form antibodies that cross-react with the *Leptospira* species MAT.

There was no difference between *Leptospira* species seropositivity in aged client-owned cats with and without azotemia.

Acknowledgements We would like to thank the feline practitioners at the participating animal hospitals who supplied the sera from their patients for analysis.

Conflicts of Interest The authors do not have any potential conflicts of interest to declare.

Funding The funding for this study came from the Center for Companion Animal Studies at Colorado State University in Fort Collins, CO, USA.

References

1 Sykes JE, Hartmann K, Lunn KF, et al. **2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention**. *J Vet Intern Med* 2011; 25: 1–13.

2 Lulich JP, O'Brien TD, Osborne CA, et al. **Feline renal failure: questions, answers, questions**. *Comp Contin Educ Pract Vet* 1992; 14: 127–152.

3 White JD, Malik R and Norris JM. **Feline chronic kidney disease: can we move from treatment to prevention?** *Vet J* 2011; 190: 317–322.

4 Andre-Fontaine G. **Canine leptospirosis – do we have a problem?** *Vet Microbiol* 2006; 117: 19–24.

5 Fessler JF and Morter RL. **Experimental feline leptospirosis**. *Cornell Vet* 1964; 54: 176–190.

6 Hemsley LA. ***Leptospira canicola* and chronic nephritis in cats**. *Vet Rec* 1956; 68: 300–301.

7 Rees HG. **Leptospirosis in a cat**. *N Z Vet J* 1964; 12: 64.

8 Luciani O. **Receptivity and sensitivity of cats to *Leptospiras*** [in French]. Thesis, National Veterinary School of Nantes, 2004.

9 Arbour J, Blais MC, Carioto L, et al. **Clinical leptospirosis in three cats (2001–2009)**. *J Am Anim Hosp Assoc* 2012; 48: 256–260.

10 Rodriguez J, Blais M, Lapointe C, et al. **Serologic and urinary PCR survey of leptospirosis in healthy cats and in cats with kidney disease**. *J Vet Intern Med* 2014; 28: 284–293.

11 Kun-Wei C, Yun-Hsiu H, Wei-Ling H, et al. **Serological and PCR detection of feline leptospira in Southern Taiwan**. *Vector Borne Zoonotic Dis* 2014; 14: 118–123.

12 Fenimore A, Carter K and Lunn K. **Detection of leptospiruria in shelter cats in Colorado** [abstract]. Proceedings of the 30th Annual Congress of the American College of Veterinary Internal Medicine (ACVIM); 2012 May 30–Jun 2; New Orleans, LA. p 783.

13 Felt SA, Wasfy MO, El-Tras WF, et al. **Cross-species surveillance of Leptospira in domestic and peri-domestic animals in Mahalla City, Gharbeya Governorate, Egypt**. *Am J Trop Med Hyg* 2011; 84: 420–425.

14 Agunloye CA and Nash AS. **Investigation of possible leptospiral infection in cats in Scotland**. *J Small Anim Pract* 1996; 37: 126–129.

15 Batza HJ and Weiss R. **Occurrence of *Leptospira* antibodies in cat serum samples**. *Kleintierpraxis* 1987; 32: 171–172.

16 Dickeson D and Love DN. **A serological survey of dogs, cats and horses in south-eastern Australia for leptospiral antibodies**. *Aust Vet J* 1993; 70: 389–390.

17 Everard CO, Cazabon EP, Dreesen DW, et al. **Leptospirosis in dogs and cats on the Island of Trinidad: West Indies**. *Int J Zoonoses* 1979; 6: 33–40.

18 Jamshidi S, Akhavizadegan MA, Bokaie S, et al. **Serologic study of feline leptospirosis in Tehran, Iran**. *J Microbiol* 2009; 1: 32–36.

19 Larsson CE, Santa Rosa CA, Hagiwara MK, et al. **Prevalence of feline leptospirosis: serologic survey and attempts of isolation and demonstration of the agent**. *Int J Zoonoses* 1984; 11: 161–169.

20 Lapointe C, Plamondon I and Dunn M. **Feline leptospirosis serosurvey from a Quebec referral hospital**. *Can Vet J* 2013; 54: 497–499.

21 Lucke VM and Crowther ST. **The incidence of leptospiral agglutination titres in the domestic cat**. *Vet Rec* 1965; 77: 647–648.

22 Markovich JE, Ross L and McCobb E. **The prevalence of leptospiral antibodies in free roaming cats in Worcester County, Massachusetts**. *J Vet Intern Med* 2012; 26: 688–689.

23 Mosallanejad B, Ghorbanpoor Najafabadi M, Avizeh R, et al. **A serological survey of leptospiral infection of cats in Ahavaz, southwestern of Iran**. *Int J Vet Res* 2011; 5: 49–52.

24 Millan J, Candela MG, Lopez-Bao JV, et al. **Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain**. *Vector Borne Zoonotic Dis* 2009; 9: 549–554.

25 Murphy LC, Cardeilhac PT and Carr JW. **The prevalence of leptospiral agglutinins in sera of the domestic cat**. *Cornell Vet* 1957; 48: 3–9.

26 Mylonakis ME, Bourtzi-Hatzopoulou E, Koutinas AF, et al. **Leptospiral seroepidemiology in a feline hospital population in Greece**. *Vet Rec* 2005; 156: 615–616.

27 Shophet R. **A serological survey of leptospirosis in cats**. *N Z Vet J* 1979; 27: 245–236.

28 Levy SA, O'Connor TP, Hanscom JL, et al. **Evaluation of a canine C6 ELISA Lyme disease test for the determination of the infection status of cats naturally exposed to *Borrelia burgdorferi***. *Vet Ther* 2003; 4: 172–177.

29 Lappin MR and Huesken R. ***Anaplasma phagocytophilum* and *Borrelia burgdorferi* infections in cats exposed to *Ixodes scapularis* twice**. *Parasites Vectors* 2015.

30 Martin LE, Wiggans KT, Wennogle SA, et al. **Vaccine-associated *Leptospira* antibodies in client-owned dogs**. *J Vet Intern Med* 2014; 28: 789–792.

31 Dambach DM, Smith CA, Lewis RM, et al. **Morphologic, immunohistochemical, and ultrastructural characterization of a distinctive renal lesion in dogs putatively associated with *Borrelia burgdorferi* infection: 49 cases (1987–1992)**. *Vet Pathol* 1997; 34: 85–96.

32 Gautam R, Guptill LF, Wu CC, et al. **Spatial and spatiotemporal clustering of overall and serovar-specific *Leptospira* microscopic agglutination test (MAT) seropositivity among dogs in the United States from 2000 through 2007**. *Prev Vet Med* 2010; 96: 122–131.