Comparison of polymerase chain reaction assay, bacteriologic culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs

Kenneth R. Harkin, DVM, DACVM; Yvette M. Roshto, DVM; Jennifer T. Sullivan, DVM; Tanya J. Purvis; M. M. Chengappa, DVM, PhD, DACVM

Objective—To compare results of polymerase chain reaction (PCR) testing of urine samples, serologic testing, and bacteriologic culture of urine to determine prevalence of urinary shedding of leptospires in dogs.

Design—Serial case study.

Animals—500 dogs evaluated serially without regard to health status.

Procedure—Urine samples were examined via PCR assay and bacteriologic culture for leptospires. Blood samples were analyzed for antibodies against serovars canicola, bratislava, pomona,icterohemorrhagiae, grippotyphosa, and hardjo.

Results—Titters ≥ 1:100 against at least 1 serovar were detected in 104 (20.8%) dogs, and titers ≥ 1:400 were detected in 41 (8.2%) dogs. High titers were detected most commonly to serovar grippotyphosa, followed by icterohemorrhagiae, canicola, pomona, bratislava, and hardjo. High titters to > 1 serovar were detected in 14 dogs. A positive PCR assay result was obtained in 41 (8.2%) dogs, only 9 of which had a titer ≥ 1:100. Leptospires were not cultured from the urine of any dog. Only 4 dogs had clinical leptospirosis. Overall disease prevalence was 0.8% for the 6-month evaluation period. Compared with PCR assay, serologic testing for predicting shedding had a sensitivity of 22%, specificity of 79%, positive predictive value of 9%, and negative predictive value of 92%.

Conclusions and Clinical Relevance—Irrespective of health status, 8.2% of dogs were shedding pathogenic leptospires. Serologic testing was a poor predictor of urinary shedding. Clinically normal dogs that shed leptospires may pose a zoonotic risk to their owners. (J Am Vet Med Assoc 2003;222:1230–1233)

Although the prevalence of canine leptospirosis in the United States is unknown, it may be 1 of the most underdiagnosed diseases of dogs. One study reported a median annual prevalence of 26 cases/100,000 (0.026%) dogs examined at veterinary teaching hospitals. Results of serologic surveys suggest that exposure rates are substantially higher than the rate of clinical disease. A serologic survey of 1,400 dogs in Michigan detected high titers in 224 (16%) dogs, with a suspected natural exposure rate of 82 of 1,400 (5.8%) dogs. Similar findings were reported from Australia (9.8% seroprevalence) and Scotland (17.6% seroprevalence). In contrast, the seroprevalence in Barbados was 62% in unowned, healthy dogs and 75% in acutely ill dogs.

Although serologic surveys may provide an estimate of the exposure rate for dogs, it does not provide information regarding how many dogs are actively shedding leptospires and poses a potential zoonotic risk. Results of several studies suggest that dogs can be seronegative and clinically normal yet still actively shed leptospires. Those results suggest that serologic surveys may be inaccurate representations of the zoonotic potential of clinically normal dogs. Clinical application of the polymerase chain reaction (PCR) assay has been described for diagnosing leptospirosis in dogs, cows, horses, and humans.

The purpose of the study reported here was to evaluate the prevalence of urinary shedding of leptospires regardless of health status through the use of PCR assay and bacteriologic culture of urine and compare the results with serologic findings in dogs.

Materials and Methods

Case selection—Five hundred dogs that were either owned by staff and students of the Kansas State University College of Veterinary Medicine (KSU-CVM) or evaluated by the internal medicine, oncology, community practice, orthopedic surgery, or soft tissue surgery services at the KSU Medical Teaching Hospital (KSU-VMTH) were evaluated during a 6-month period from February through August of 2001. There were no selective criteria for inclusion in this study. The first 500 dogs that were evaluated and from which urine and blood could be collected were included. Staff and students of the KSU-CVM were encouraged to bring in their dogs for evaluation to maximize the number of healthy dogs that were included in the study. Vaccination history for leptospirosis was recorded at the time of sample collection if available, or retrospectively obtained if a titer ≥ 1:100 was found. All procedures were approved either by the Institutional Biosafety Committee or the Institutional Animal Care and Use Committee at KSU.

Sample collection and DNA isolation—A single urine sample of 6 to 20 mL was collected by cystocentesis or catheterization from each dog at the initial evaluation and...
analyzed as described. From the final suspended pellet, 200 µL was inoculated in Ellinghausen-McCullough-Johnson-Harris (EMJH) culture media, 60 µL was used for DNA isolation, and the remainder was stored at −80°C. Isolation of DNA was performed with a kit following the manufacturer's instructions. Primer selection, PCR assay, and determination of specificity were performed as described.

Leptospiral culture—For each sample, 200 µL of the resuspended pellet was inoculated into a culture tube containing EMJH media. This tube was incubated at 30°C in an ambient atmosphere incubator. The culture tubes were checked weekly for evidence of growth. The procedure for culture was evaluated by inoculating 200 µL of broth from a pure culture of leptospires into 20 mL of sterile urine collected from a Greyhound housed at the KSU-VMTH and processing the urine as described. Inoculation of the EMJH medium with 200 µL of the resuspended pellet in the evaluation procedure successfully resulted in growth and recovery of leptospires.

Serologic examination—A blood sample was collected from all dogs at the initial evaluation for serologic testing. The microscopic agglutination test was performed for serovars canicola, bratislava, pomona, icterohemorrhagiae, hardjo, and grippotyphosa. Results were recorded as seronegative if the titer was < 1:100. For reporting, the serovar with the highest titer was recorded as the important serovar and was the only serovar reported for that dog, unless multiple serovars had equivalent high titers.

Statistical analyses—Sensitivity, specificity, negative predictive value, and positive predictive value of the ability of serologic testing to detect urinary shedding of leptospires were evaluated by use of results of PCR assay as true positives and true negatives. Distribution of strength of titers (≥ 1:100, ≥ 1:400) and serovars identified were independently compared for vaccinated and unvaccinated dogs by use of the χ² test. Significance was defined as P < 0.05.

Results
Serologic evaluation of 500 dogs revealed titers ≥ 1:100 in 104 (20.8%) dogs and titers ≥ 1:400 in 41 (8.2%) dogs. High titers to serovar grippotyphosa were most common; 39 dogs had a titer ≥ 1:100, and 21 dogs had a titer ≥ 1:400. For the remaining serovars, titers were detected (number of samples with titer ≥ 1:100/number of samples with titer ≥ 1:400) to icterohemorrhagiae (36/13), canicola (26/8), pomona (9/5), bratislava (8/4), and hardjo (1/0). Multiple high titers were detected in 14 dogs, 7 of which had equivalent titers to serovars canicola and icterohemorrhagiae, and 3 dogs had equivalent titers to serovars grippotyphosa and bratislava.

Vaccination status against leptospirosis was obtained for 75 of 104 dogs with a titer ≥ 1:100. Thirty-one dogs had been vaccinated, and 44 dogs had not been vaccinated. The distribution of titers by numerical value was (number of vaccinated dogs/number of unvaccinated dogs) 1:100 (9/10), 1:200 (10/15), 1:400 (6/8), 1:800 (2/7), 1:1,600 (1/1), 1:3,200 (3/1), and 1:6,400 (0/2). Differences among these groups were not significant (P = 0.549). The distribution of titers by serovar with the highest titer was (number of vaccinated dogs/number of unvaccinated dogs) canicola (5/4), icterohemorrhagiae (9/10), bratislava (0/3), grippotyphosa (9/20), hardjo (0/1), pomona (1/4), canicola and icterohemorrhagiae equally high (4/2), and multiple serovars (3/0). Differences among these groups were not significant (P = 0.096). Vaccination status was not obtained from enough seronegative dogs to be reported.

A positive PCR assay result was obtained from urine samples from 41 (8.2%) dogs. Titters were detected in 9 (22%) dogs with positive PCR assay results. Of these 9 dogs, 6 had titers to serovar grippotyphosa with titers of 1:200 (n = 1), 1:400 (3), 1:1,600 (1), and 1:31,200 (1). The remaining 3 dogs had titers to serovar icterohemorrhagiae (1:3,200 and 1:200) and serovar canicola (1:100). No positive results of bacteriologic culture of urine were obtained with any of the samples, irrespective of PCR results.

During the 6-month period of collection, leptospirosis was diagnosed in 4 dogs. Two dogs had acute renal failure, positive results of PCR assay and serologic tests, and negative results of cultures. The first dog had an acute titer of 1:31,200 for serovar grippotyphosa, and the second had an acute titer of 1:800 and an 8-week convalescent titer of 1:6,400 for serovar grippotyphosa. The remaining 2 dogs were evaluated for polyuria and polydipsia without being azotemic. One dog had a positive PCR assay result, negative results of culture, and was seronegative; clinical signs resolved rapidly with administration of doxycycline (5 mg/kg [2.3 mg/lb], PO, q 12 h, for 2 weeks). The other dog became seropositive for serovar pomona, had negative results of PCR assay and culture, and also responded rapidly to the same dosage of doxycycline.

Overall prevalence for leptospirosis was 0.8% for the 6-month evaluation period. Prevalence of titers for leptospirosis for this period was 20.8% for titers ≥ 1:100 and 8.2% for titers ≥ 1:400. As estimated on the basis of PCR assay results, 8.2% of the dogs were actively shedding organisms. Using PCR assay results as the gold standard, serologic results had sensitivity of 22%, specificity of 79%, positive predictive value of 9%, and negative predictive value of 92% in determining the risk of shedding leptospires in the urine.

Discussion
Results of this study suggest that serologic results are poor for prediction of the risk of an individual dog actively shedding leptospires in the urine, with poor sensitivity and poor positive predictive value. Van den Broek et al4 recovered leptospires by bacteriologic culture of urine from 7 of 84 healthy dogs; 3 of those dogs were seronegative, and only 1 dog had high titers to the isolated serovar, which was similar to our findings.

In our study, only 3 of 41 (7%) dogs that were actively shedding leptospires were clinically ill. It appears that dogs shed leptospires in their urine for a period of time after infection, even if they do not develop clinical illness. In 1 study9 in which dogs were inoculated with serovar bataviae (5 × 10⁷ organisms/mL, 5 mL, IP), only 5 of 24 (21%) dogs developed severe clinical disease, whereas the remainder had mild to moderate clinical signs and recovered without treatment. It is possible that some of the dogs in our study that were actively shedding leptospires had transient clinical signs that were not noticed by the owner. Follow-up PCR assays on urine were performed on 4
dogs that initially had positive results. None of these dogs had been treated with doxycycline, yet all 4 had negative results of PCR assays 2 weeks later. This may suggest that the leptospiruric phase in healthy dogs is brief, although it is not known when these dogs were initially infected. It is also possible that these dogs were persistently infected and intermittently shedding; however, that seems unlikely, because all 4 dogs had negative results at the 2-week reevaluation. It is unclear why 1 dog that was polyuric and polydipsic and serum-positive at the 2-week reevaluation had titers to serovar pomona and a negative PCR assay result, although this dog may have been shedding leptospires in numbers less than the limit of detection for the test.

One additional healthy dog that had positive results of PCR assay had a titer of 1:3,200 to serovar icterohemorrhagiae. This dog had a diagnosis of acute renal failure as a result of serovar pomona infection, based on serologic testing 1 year prior to the study, and was vaccinated against serovars pomona, grippotyphosa, icterohemorrhagiae, and canicola 1 month prior to inclusion in the study. In addition to the high titer to serovar icterohemorrhagiae, titers to serovars grippotyphosa, pomona, and bratislava were 1:800, and the dog was seronegative to serovars canicola and hardjo. The dog was not treated and tested negative via PCR assay on urine 5 months later. At that time, the dog only had titers to serovars pomona and icterohemorrhagiae (1:200 for both). Positive results of PCR assays of urine were not detected in any other dog that had been previously vaccinated, and vaccination would not be anticipated to cause a positive result. It has been suggested that humans treated for leptospirosis may shed the organism for up to 1 year after treatment; whether the same is true for dogs is not known.

For our study, the protocol for leptospirosis culture as described by Bal et al.³ was used. The standard protocol used by most laboratories involves a 5-tube dilution protocol; however, that technique was cost prohibitive for our study. Despite the fact that the technique worked on leptospirosis-inoculated urine, no dog in our study that had positive results of PCR assay had positive results of culture. Disappointingly, even the dog with acute renal failure, a titer to serovar grippotyphosa of 1:51,200, and a strong band detected via PCR assay had negative culture results. It is likely that the culture method used for our study was unsatisfactory or ineffective for leptospirosis culture. The higher rate in our study cannot be explained by case selection alone, because in our study it would have been necessary to include an additional 14,885 healthy dogs during the 6-month period to obtain an overall disease prevalence of 0.026%.

The distribution of serovars in our study contrasted with the distribution from the Michigan study,³ which identified serovar canicola as the most common, followed by serovars grippotyphosa and bratislava. In our study, serovar grippotyphosa was the most common, followed by serovars canicola and icterohemorrhagiae. Serovar grippotyphosa has been identified in a number of studies of naturally occurring leptospirosis as the most common serovar, although other studies³⁴-²³ have found serovar pomona to be more common. The serologic findings in our study were similar to previous findings that indicated the importance of serovar grippotyphosa. Additionally, in our study, 6 of 9 dogs that were seropositive and had positive results of PCR assay had high titers to serovar grippotyphosa.

Because of the poor positive predictive value for serologic testing to determine whether a dog is actively shedding leptospires in the urine, it may be appropriate to consider using the PCR assay. Additionally, the PCR assay appears to have more applicability in determining potential zoonotic risk for immunocompromised or immunosuppressed pet owners.

significant differences among groups of dogs with various titers suggests that establishing a cutoff value for determining natural exposure versus vaccinal response is imprecise. Vaccination histories were often unreliable, however, raising doubt as to the validity of these findings. Vaccines were given up to 17 months prior to testing in certain dogs, although those dogs were still classified as vaccinated, and certain dogs that were classified as unvaccinated had been evaluated by multiple veterinarians in the past, and their vaccine records were often incomplete. For purposes of this study, they were classified as unvaccinated for leptospirosis if they had supposedly not received the vaccine at least 3 years prior to the study, although this time frame was also arbitrary. Likewise, even vaccinated dogs may have had natural exposure and developed an antibody response, although they were still considered to have had an antibody response from vaccine.

Results of the study by van den Broek et al.¹ suggested that 8% of clinically normal dogs could shed leptospires, which compares favorably to the 8.2% prevalence of positive PCR assay results that were obtained in our study. The overall disease prevalence of 0.8% in our study was substantially higher than the median prevalence (0.026%) identified by Ward et al.³ The higher rate in our study cannot be explained by case selection alone, because in our study it would have been necessary to include an additional 14,885 healthy dogs during the 6-month period to obtain an overall disease prevalence of 0.026%.

References


³¹National Animal Disease Center, Ames, Iowa.
³²QIAamp viral RNA kit, Qiagen Inc, Valencia, Calif.
³³Prism 3.0, Graph Pad Software Inc, San Diego, Calif.