Journal of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2018;32:201–207

Evaluation of 3 Serological Tests for Early Detection Of *Leptospira*-specific Antibodies in Experimentally Infected Dogs

J. Lizer, S. Velineni, A. Weber, M. Krecic, and P. Meeus

Background: Leptospirosis in dogs is a disease of global importance. Early detection and appropriate therapeutic intervention are necessary to resolve infection and prevent zoonotic transmission. However, its diagnosis is hindered by nonspecific clinical signs and lack of rapid diagnostic tests of early infection. Recently, 2 rapid point-of-care tests (WITNESS Lepto [WITNESS Lepto, Zoetis LLC, Kalamazoo, MI, USA] and SNAP Lepto [SNAP Lepto, IDEXX Laboratories, Westbrook, ME, USA]) for detection of *Leptospira*-specific antibodies in canine sera were developed.

Hypothesis: Immunoglobulin M-based WITNESS Lepto containing multiple detection antigens can detect *Leptospira*-specific antibodies to common leptospiral serovars earlier in the course of infection as compared to microscopic agglutination test (MAT) and SNAP Lepto.

Animals: Four groups of 8 6- to 8-month-old male Beagle dogs were used.

Methods: Thirty-two healthy seronegative dogs were inoculated experimentally with serovars Canicola, Grippotyphosa, Icterohaemorrhagiae, and Pomona (8 dogs/serovar). Acute-phase sera were collected at regular intervals and monitored for *Leptospira*-specific antibodies by WITNESS Lepto, MAT, and SNAP Lepto.

Results: Seroconversion was detected in all dogs by day 10 by WITNESS Lepto and in 30 of 32 dogs by day 14 by MAT. The SNAP Lepto test detected seroconversion in 3 dogs during the 2 weeks postchallenge.

Conclusions: Immunoglobulin M-based WITNESS Lepto detected immune responses specific to multiple leptospiral serovars early in the course of infection and identified seroconversion in all animals earlier than did the gold standard MAT. The SNAP Lepto test displayed considerably lower and inconsistent performance during the study period. At the point-of-care, WITNESS Lepto should be the test of choice for rapid and reliable screening of acutely ill dogs suspected to have leptospirosis.

Key words: Canine leptospirosis; IgM; Microscopic agglutination test; Seroconversion; SNAP Lepto; WITNESS Lepto.

Leptospirosis is a globally important zoonosis caused by pathogenic serovars of spirochetal bacteria belonging to the genus *Leptospira*.[1,2] It affects virtually all mammalian species including dogs, pigs, cattle, horses, and human beings. Historically, *Leptospira interrogans* serovars Canicola and Icterohaemorrhagiae have been the most common cause of leptospirosis in dogs in North America[3] and Europe.[4] The dog is considered a maintenance host for serogroup Canicola.[3,5] However, since the introduction of bivalent leptospiral vaccines, the serovars Autumnalis, Bratislava, Grippotyphosa, and Pomona have been suspected to be increasing in prevalence.[5–10] Clinical leptospirosis in dogs is common, and affected dogs present with a wide range of clinical signs including anorexia, vomiting, fever, diarrhea, myalgia, jaundice, and also reproductive failure and stillbirths in some long-term carriers. Additionally, pathologic complications may manifest as acute kidney injury and acute pulmonary hemorrhage, all of which contribute to a case fatality rate of 10–20%.[6,11] In chronically infected maintenance hosts, leptospires colonize the proximal convoluted renal tubules from which they are disseminated through the urine into the environment. Because of the large numbers of organisms shed in the urine of infected animals, environmental contamination can result in rapid propagation of infection from the index animal(s) to susceptible hosts.

Leptospirosis in dogs may be misdiagnosed because of its nonspecific clinical manifestations during the initial stages of illness and lack of early and definitive diagnostic tests.[12,13] Until recently, veterinarians have had to rely on the outcome of laboratory tests for diagnosis of dogs clinically suspected to have leptospirosis, which further delays initiation of antibiotic treatment.[14,15] Culture and direct demonstration of leptospires confirm active infection, but culture from clinical specimens is time-consuming, less sensitive[3,4,16] and does not satisfy the need of veterinarians for rapid

Abbreviations:

CI	confidence interval
ELISA	enzyme-linked immunosorbent assay
EMJH medium	Ellinghausen-McCullough-Johnson-Harris medium
IgG	immunoglobulin G
IgM	immunoglobulin M
LPS	lipopolysaccharide
MAT	microscopic agglutination test
PCR	polymerase chain reaction

From Veterinary Medicine Research and Development, Zoetis, Kalamazoo, MI (Lizer, Velineni, Weber, Meeus); and Diagnostics, Zoetis, Parsippany, NJ (Krecic).

All work was performed at Zoetis, Inc facilities in Kalamazoo, MI or Lincoln, NE or Veterinary Diagnostic Laboratory-University of Illinois at Urbana-Champaign (MAT testing).

An abstract from this study was presented at the 2017 ACVIM Forum, National Harbor, MD.

Corresponding author: J. Lizer, Veterinary Medicine Research and Development, Zoetis, 333 Portage St., Kalamazoo, MI 49007; e-mail: joshua.lizer@zoetis.com.

Submitted March 15, 2017; Revised August 29, 2017; Accepted September 27, 2017.

DOI: 10.1111/jvim.14865

diagnosis of suspected cases. Therefore, the diagnosis of leptospirosis in dogs mainly is based on MAT for serological detection of *Leptospira*-specific antibodies and PCR for detection of their nucleic acids.[3,4,6] Leptospiral infections usually are confirmed by a 4-fold increase in MAT titers between acute- and convalescent-phase sera, which is considered the current gold standard methodology. Nevertheless, MAT is laborious and complex to perform and interpret, requiring specialized expertise, a panel of live serovars, and ideally paired sera for confirmation.[1,3,4] Polymerase chain reaction has the potential to overcome some of the disadvantages of culture and MAT, but it also requires specialized expertise and its use is hindered if antibiotics have already been administered. Therefore, there is a need for a rapid and reliable point-of-care diagnostic test that shortens diagnostic turnaround time and is more sensitive during the early stages of clinical illness.

Leptospira-specific Immunoglobulin M (IgM) antibodies are ideally suited for the diagnosis of acute leptospirosis because their presence indicates current or recent infection, and IgM appears as early as 4–6 days after infection and remains detectable for only a few months. Furthermore, IgM also is only transiently produced after vaccination.[17,18] The SNAP Lepto test, a LipL32-based in-clinic enzyme-linked immunosorbent assay (ELISA), is used by clinicians in the United States for the rapid detection of *Leptospira*-specific antibodies in dogs, but it is not IgM specific.[14,15] Recently, a rapid point-of-care immunodiagnostic test referred to as WITNESS Lepto[19,20] was developed to detect *Leptospira*-specific IgM antibodies in dogs.

The objective of our study was to comparatively evaluate the diagnostic potential of WITNESS Lepto, MAT, and SNAP Lepto for the early and accurate detection of *Leptospira*-specific humoral immune responses in acute-phase sera of dogs experimentally inoculated with 4 common leptospiral serovars.

Materials and Methods

Leptospira Strains

Leptospira interrogans serovar Canicola strain Moulton, *Leptospira kirschneri* serovar Grippotyphosa strain 109285, *L. interrogans* serovar Icterohaemorrhagiae strain IC-02, and *L. interrogans* serovar Pomona type kennewicki strain RM211 were used as challenge strains. Virulence of serovars Canicola and Grippotyphosa was maintained by serial passage in hamsters, and a virulent Pomona strain was derived by passage through cattle. Animal passages were performed according to previous reports with some modifications.[21,22]

Production of Challenge Inoculum

To produce the challenge inoculum, stocks stored in liquid nitrogen were rapidly thawed and cultured by aseptically inoculating 0.5 mL into 9.5 mL of fresh Ellinghausen-McCullough-Johnson-Harris (EMJH) media. The cultures then were incubated at 28–30°C under aerobic conditions and examined at regular intervals to determine leptospiral growth. Cultures were considered ready when motility was high, and they attained a desired density of $\geq 10^9$ leptospires/mL as determined by direct counting with a Thoma-ruled cell counter.[a] On the day of challenge, the challenge inoculum was prepared from logarithmic growth phase cultures by diluting in EMJH media to the desired concentration of 10^9 leptospires/mL.

Animal Infection and Sample Collection

Animal experiments were performed using 6- to 8-month-old male Beagle dogs[b] with no history of vaccination against *Leptospira* spp. and confirmed seronegativity on MAT with titers <1 : 100. Groups of 8 dogs for each of the 4 selected leptospiral serovars were housed in 4 pens (2 dogs per pen) in a single room, 1 room per serovar. Dogs were sedated for challenge with either dexmedetomidine HCl[c] or tiletamine HCl – zolazepam HCl administered according to the manufacturer's recommendation. Challenge inoculum containing approximately 10^9 leptospires/mL was administered PO (1.0 mL), conjunctivally in each eye (0.1 mL per eye) and intranasally in each nostril (0.2 mL per nostril) on days 0, 1, and 2. All of the dogs were monitored once daily by animal care staff for clinical signs and general health during the study period. Dogs were monitored for clinical signs including vomiting, diarrhea, lethargy, anorexia, dehydration, jaundice, hematuria, or any other abnormal clinical signs. Blood samples were collected on days 0, 4, 7, 10, and 14 postchallenge to determine the *Leptospira*-specific immune responses. Some dogs (Table 2) that were highly seropositive were removed from the study before its end to allow collection of larger quantities of serum, which was stored as bulk critical reagent for future use. The Institutional Animal Care and Use Committee of Zoetis reviewed and approved this study (IACUC numbers: 12-NARDO-02 and KZ-3060e-2015-08-smw).

Microscopic Agglutination Test (MAT)

Aliquots of sera were submitted to the Veterinary Diagnostic Laboratory of the University of Illinois at Urbana-Champaign to determine the antibody responses to the 4 leptospiral challenge strains by MAT. This laboratory participates in the National Veterinary Services Laboratory's (NVSL) proficiency testing scheme to maintain quality assurance for the MAT. The MAT was performed using a panel of live reference serovars belonging to 7 serogroups: Autumnalis, Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona. Briefly, in a 96-well round-bottom polystyrene microwell plate, 50 μL of sera diluted 2-fold starting at 1 : 100 was incubated with equal volumes of each serovar separately. The serovar control included 50 μL of live antigen without addition of antibody. The endpoint titer was the highest dilution of the serum in which 50% of the leptospiral cells were agglutinated as compared to the control. Dogs were considered to have seroconverted to any serovar when a minimum of 4-fold increase in antibody titer with paired sera or a titer ≥1 : 400 in a single serum sample was observed.

WITNESS Lepto Test

The WITNESS Lepto test was performed according to the manufacturer's instructions. It detects IgM antibody specific to multiple *Leptospira* antigens derived from filtered whole cell extracts of serovars Grippotyphosa and Bratislava. To perform the test, 5 μL of serum was added to the sample well of the lateral flow device, followed by 3 drops of chase buffer. Canine IgM in sera initially is bound to the colloidal gold-labeled anti-dog IgM to form complexes. The immune complexes then migrate on the nitrocellulose test strip where they cross the test line containing the *Leptospira* antigen extract. Colloidal gold complexed with *Leptospira*-specific IgM present in the sample accumulates at the test line resulting in

the formation of a red line, which indicates a positive result. The absence of a test line indicates a negative result. The formation of a control line for all tests indicates proper function of the test. The presence or absence of *Leptospira*-specific IgM in the test sample was determined by visual interpretation after 10 minutes at ambient temperature.

SNAP Lepto Test

The SNAP Lepto test was performed according to the manufacturer's instructions. Briefly, 3 drops of serum were dispensed into a sample tube, followed by 4 drops of the recombinant LipL32-HRP conjugate and were mixed thoroughly by inverting 3–5 times. The entire contents of the sample tube were carefully added to the sample well of the SNAP device. *Leptospira*-specific antibodies in sera first bind to the LipL32-HRP conjugate to form immune complexes. The immune complexes flow across the result window and accumulate at the recombinant LipL32 on the sample spot of the test membrane. When the fluid flow reaches an activation window, the test then is subjected to wash solution and substrate reagents are released by snapping down the top of the device. The presence or absence of antibody was determined by visual interpretation after 10 minutes at ambient temperature. Color of the sample spot more intense than color of the background indicated that the sample was positive for *Leptospira*-specific antibodies, whereas a negative result was interpreted by the absence of color in the sample spot. The positive control spot on all tests indicated proper function of the test.

Statistical analyses

Diagnostic sensitivity and Jeffrey's 95% confidence interval (CI) were calculated for all 3 tests at each sample collection point during the course of the study by SAS Version 9.4 software.[d]

Results

After experimental inoculation with serovars Canicola and Pomona, some dogs manifested mild clinical signs that included diarrhea, vomiting, and hematuria. At least 1 dog in each of 2 pens inoculated with serovar Canicola exhibited an episode of diarrhea on day 1 or 2, whereas in the case of dogs exposed to serovar Pomona, vomitus was observed in a pen on day 3 and hematuria was observed in a different pen on day 12 postchallenge. None of the dogs exposed to serovars Grippotyphosa and Icterohaemorrhagiae exhibited any clinical signs.

Aliquots of serum samples from all dogs were tested by MAT, and agglutinating serovars with the highest MAT titers per seropositive dog are shown in Table 1. Before experimental challenge, all dogs had negative MAT titers (≤1 : 100). Seroconversion by MAT was observed in a majority of dogs by day 7 after exposure and peaked between days 10 and 14 for all dogs, with the exception of 2 dogs exposed to serovars Grippotyphosa or Canicola that had not seroconverted by this time. The serovar with the highest MAT titer often was different from that of the infecting serovar, and strong cross-agglutination was observed among all serovars except Hardjo (Table 1).

Comparative analyses of the performance of 3 serological tests along with their sensitivities and 95% CI are summarized in Table 2. Seroconversion was detected in all dogs by day 10 by WITNESS Lepto, and in 30 of 32 (93.8%) dogs by day 14 by MAT (Table 2; Fig. 1). WITNESS Lepto detected *Leptospira*-specific

Table 1. Highest MAT titers in sera of dogs experimentally inoculated with 4 common leptospiral serovars.

		Day After Exposure				
Challenge Serovar	MAT Test	0	4	7	10[a]	14[a]
Canicola	Highest MAT titer	<100	<100	800 (I)[b]	3,200 (I)	3,200 (I)
	Agglutinating serovars[c]	–	–	I (2/8)[d]	A (1/8) C (2/8) I (4/8)	C (2/4) I (2/4)
Grippotyphosa	Highest MAT titer	<100	<100	3,200 (G)	3,200 (G)	3,200 (G)
	Agglutinating serovars	–	–	G (6/8)	G (7/8)	G (5/6)
Icterohaemorrhagiae	Highest MAT titer	<100	<100	1,600 (B)	3,200 (A, B, I)	3,200 (A, B, I)
	Agglutinating serovars	–	–	A (2/8) B (5/8) I (3/5)	A (4/8) B (8/8) I (6/8)	A (3/8) B (8/8) I (8/8)
Pomona	Highest MAT titer	100	<100	3,200 (A, B, I, P)	3,200 (A, B, I, P)	3,200 (A, B, P)
	Agglutinating serovars	–	–	A (8/8) B (1/8) I (1/8) P (3/8)	A (7/7) B (2/7) I (1/7) P (6/7)	A (6/6) B (1/6) P (4/6)

[a]Some seropositive dogs were removed to allow for the collection of larger quantities of serum for future use.

[b]The serovar with the highest MAT titer observed is denoted in parentheses (A, Autumnalis; B, Bratislava; C, Canicola; G, Grippotyphosa; I, Icterohaemorrhagiae; P, Pomona).

[c]Agglutinating serovars with highest MAT titers per seropositive dog.

[d]Number of seropositive dogs are denoted in parentheses.

Table 2. Evaluation of WITNESS Lepto, MAT, and SNAP Lepto for early detection of *Leptospira*-specific antibodies in acute-phase sera from dogs experimentally infected with 4 common leptospiral serovars

	WITNESS Lepto		MAT		SNAP Lepto	
Serum	Positive	Sensitivity (95% CI)	Positive	Sensitivity (95% CI)	Positive	Sensitivity (95% CI)
Canicola						
Day 0	0/8	–	0/8	–	0/8	–
Day 4	0/8	–	0/8	–	0/8	–
Day 7	8/8	100.0% (73.8–100.0)	2/8	25.0% (5.6–59.2)	0/8	–
Day 10	8/8	100.0% (73.8–100.0)	7/8	87.5% (54.6–98.6)	0/8	–
Day 14[a]	4/4	100.0% (55.5–100.0)	4/4	100.0% (55.5–100.0)	0/4	–
Grippotyphosa						
Day 0	0/8	–	0/8	–	0/8	–
Day 4	0/8	–	0/8	–	0/8	–
Day 7	8/8	100.0% (73.8–100.0)	6/8	75.0% (40.8–94.4)	1/8	12.5% (1.4–45.4)
Day 10	8/8	100.0% (73.8–100.0)	7/8	87.5% (54.6–98.6)	1/8	12.5% (1.4–45.4)
Day 14[a]	6/6	100.0% (67.0–100.0)	5/6	83.3% (44.2–98.1)	0/6	–
Icterohaemorrhagiae						
Day 0	0/8	–	0/8	–	0/8	–
Day 4	0/8	–	0/8	–	0/8	–
Day 7	4/8	50.0% (19.9–80.1)	5/8	62.5% (29.5–88.1)	0/8	–
Day 10	8/8	100.0% (73.8–100.0)	8/8	100.0% (73.8–100.0)	1/8	12.5% (1.4–45.4)
Day 14	8/8	100.0% (73.8–100.0)	8/8	100.0% (73.8–100.0)	0/8	–
Pomona						
Day 0	0/8	–	0/8	–	0/8	–
Day 4	0/8	–	0/8	–	0/8	–
Day 7	8/8	100.0% (73.8–100.0)	8/8	100.0% (73.8–100.0)	0/8	–
Day 10[a]	7/7	100.0% (70.8–100.0)	7/7	100.0% (70.8–100.0)	1/7	14.3% (1.6–50.1)
Day 14[a]	6/6	100.0% (67.0–100.0)	6/6	100.0% (67.0–100.0)	0/6	–

[a]Some dogs that seroconverted on MAT were removed from the study to allow for the collection of larger quantities of serum to be stored as bulk critical reagent for future use.

antibodies in 28 of 32 (87.5%) dogs as early as 7 days after exposure, whereas MAT scored positive in 21 of 32 (65.6%) dogs during that period (Table 2). In contrast, the SNAP Lepto test detected seroconversion in only 1 dog (1/32; 3.1%) at day 7 and 1 dog each from 2 groups that were inoculated with serovars Icterohaemorrhagiae and Pomona by day 10 (3/31; 9.7%). None of the dogs inoculated with serovar Canicola were positive by SNAP Lepto during the study period.

Discussion

Early diagnosis of acute leptospirosis in dogs is of importance to veterinarians because of the higher probability of treatment success early in the infection as well as the substantial zoonotic risk to humans.[3,23] WITNESS Lepto and SNAP Lepto are 2 commercially available rapid immunodiagnostic tests that detect *Leptospira*-specific IgM and leptospiral LipL32-specific immune responses in dogs, respectively. Earlier studies indicated that these 2 tests are highly sensitive and specific.[14,15,19,20] We compared the performance of the WITNESS Lepto, MAT, and SNAP Lepto tests in the clinically relevant, acute phase of infection with 4 common leptospiral serovars. Because vaccination, past exposure to leptospiral serovars or both will induce antibody concentrations that confound serodiagnostic interpretation, and owing to difficulty in obtaining well-characterized convalescent sera from dogs with known history of vaccination and past exposure, sera from seronegative dogs experimentally inoculated with the different serovars were used in our study.

Dogs in our study began seroconversion to *Leptospira* as early as day 7 for all serovars, with peak titers between days 10 and 14. However, the serovar with the highest MAT titer was often different from that of the infecting serovar and strong cross-agglutination was observed among all serovars except Hardjo (Table 1). These data also demonstrate the unreliability of the MAT for predicting the infecting serogroup.[3,4] Overall, WITNESS Lepto demonstrated superior performance as compared to MAT and SNAP Lepto and detected *Leptospira*-specific antibodies in 87.5% of dogs by day 7, followed by MAT in 65.6% of dogs. The SNAP Lepto test identified only 1 dog (1/32; 3.1%) as positive in the same postchallenge period (Table 2; Fig. 1). Furthermore, WITNESS Lepto detected seroconversion in all dogs by day 10 and MAT detected seroconversion in 30 of 32 dogs for all serovars by day 14 postchallenge, whereas only 3 dogs (3/32; 9.7%) were positive by SNAP Lepto during the same postchallenge period. The point-of-care WITNESS Lepto and SNAP Lepto tests were rapid, simple to perform, and required minimal operator technical ability. The test lines on the WITNESS Lepto test were obvious to interpret and initially appeared with weak-to-moderate visual intensity that increased through days 10–14. Some of the positive results on the SNAP Lepto test were difficult to

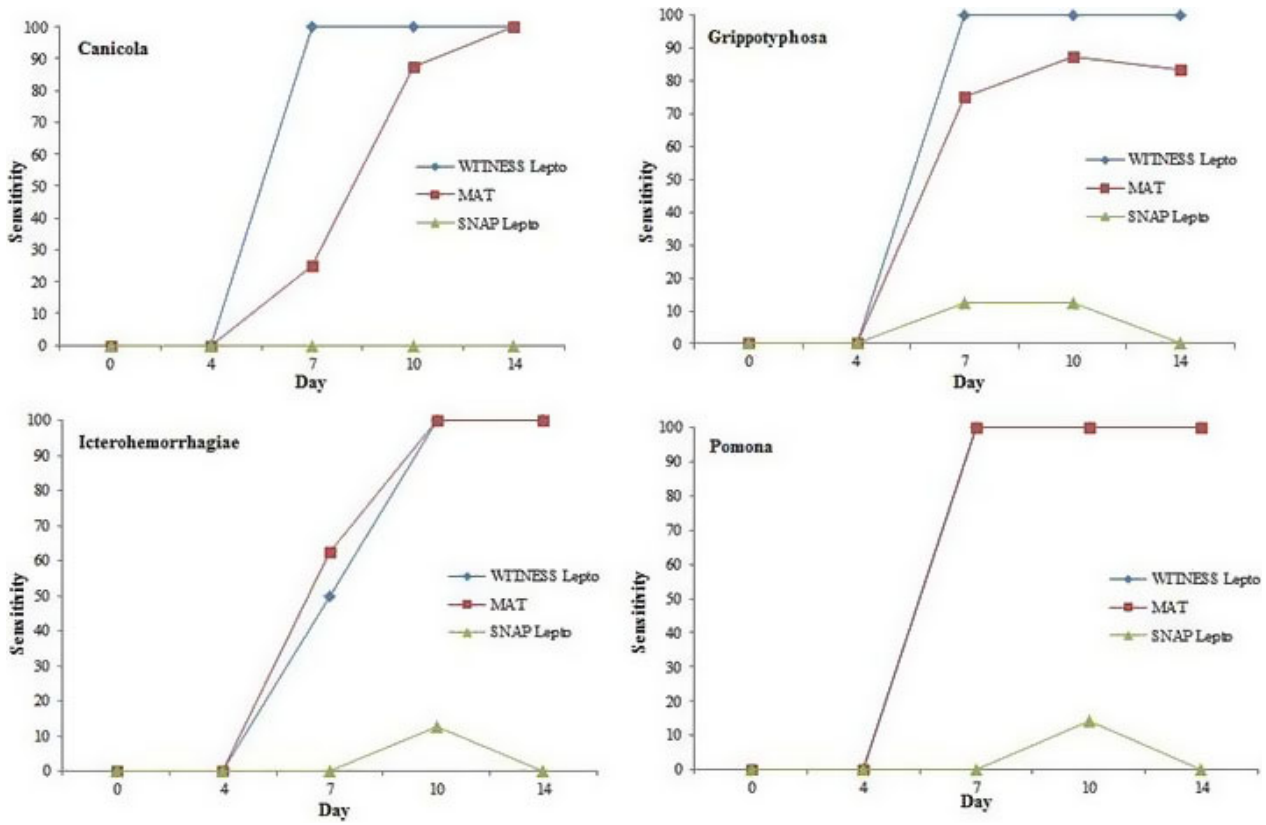

Fig 1. Diagnostic sensitivities of 3 serological tests in detecting *Leptospira*-specific humoral immune responses in experimentally infected dogs.

interpret and formed as faint spots that were not easily discernible from the membrane background.

The superior performance of WITNESS Lepto and MAT were expected because seroconversion of IgM class antibodies in dogs can occur as early as 4–6 days after exposure and may require 7–14 days for successful demonstration.[3,17] Immunoglobulin M class antibodies against genus-specific leptospiral antigens usually appear earlier than IgG class antibodies and generally remain detectable for several months. Immunoglobulin M antibodies specific to carbohydrate epitopes[16,24,25] are believed to be the predominant humoral immune responses during the acute phase of active infections. Although the detection antigen in WITNESS Lepto consisted of whole cell extracts of serovars Grippotyphosa and Bratislava, the secondary antibody of the test specifically detects canine IgM antibodies.[19,20] By contrast, agglutination in the MAT is triggered by both IgM and IgG classes.[26] The number of false-negative results for MAT was higher during the first week after challenge as compared to WITNESS Lepto. This observation could be explained by the fact that the magnitude of circulating IgM specific to leptospiral lipopolysaccharide (LPS) plays a major role in the agglutination of leptospires in MAT, and their optimum titer usually appears after day 8 of the illness.[27] Conversely, multiple IgM-specific epitopes exposed in the extracted antigen used in WITNESS Lepto might have contributed to higher performance. Although WITNESS Lepto contained antigens derived from whole cell extracts of serovars Grippotyphosa and Bratislava, the test also detected IgM antibodies specific to serovars Canicola, Icterohaemorrhagiae, and Pomona.

Although SNAP Lepto has the potential to detect both IgM and IgG, the performance of this test was poor at identifying antibodies induced during the acute phase of experimental infection in dogs. The SNAP Lepto test detects antibodies specific to LipL32, the most abundant outer membrane 32-kDa lipoprotein expressed by pathogenic, but not by nonpathogenic, leptospires.[28–30] Although LipL32 has the potential to bind both IgM and IgG classes of antibodies, its affinity is predominantly for *Leptospira*-specific IgG as compared to IgM.[31] The production of *Leptospira*-specific antibodies during the acute phase of disease is mainly of the IgM class, whereas IgG class antibodies are less prevalent.[17,32] Immunoglobulin M titers increase within 1 week and peak at 2 weeks after exposure, whereas IgG titers do not appear until 2–3 weeks and peak at 1-month postinfection.[33] The aforementioned observations together with the earlier appearance of *Leptospira*-specific IgM antibodies could explain the performance shortfall of SNAP Lepto.

In our study, mild clinical signs such as vomiting, diarrhea, and hematuria, as well as seroconversion on MAT provided evidence that the experimental challenge

model was successful. Complete blood counts (CBC), serum biochemical profiles, and urinalyses were not performed on the clinical samples derived from these dogs. Although previous studies have indicated that clinical disease in experimentally infected dogs can be variable and mild or inapparent,[34,35] occurrence of mild clinical signs is a limitation of our study because the timing of seroconversion could not be associated with the onset of clinical signs. Earlier, we reported that acute-phase sera from 20 of 37 client-owned dogs with confirmed clinical leptospirosis tested positive on WITNESS Lepto but not on MAT, whereas only 8 dogs tested positive on both tests.[20] Although the paucity of observed clinical signs may limit the clinical utility of our study, data from our previous study (showing earlier seroconversion with WITNESS Lepto) support the importance of the earlier seroconversion reported here.[20] Nonetheless, controlled experimental challenge with a known serovar is necessary to test serovar-specific antibody responses.

In practice, veterinarians should test at the first presentation of clinical signs suggestive of leptospirosis. Although WITNESS Lepto detected *Leptospira*-specific IgM as early as 7 days after exposure, the day of exposure is likely unknown in client-owned dogs and the time to onset of clinical signs may vary depending on the infectious dose, strain, and host.[3] Another study indicated that WITNESS Lepto detected *Leptospira*-specific IgM antibodies 2–4 days after onset of clinical signs in 31 of 41 (76%) client-owned dogs with clinical leptospirosis.[36] However, when suspicion of leptospirosis remains high despite a negative result on WITNESS Lepto, the dog should be retested in 3–7 days by the same test as well as an alternative confirmatory method.

In conclusion, rapid and reliable screening tests that are sensitive and specific early in the course of leptospirosis in dogs are of great benefit to veterinarians for timely initiation of treatment and possible mitigation of zoonotic transmission. Consistent with our previous observations,[20] the present study provided further evidence that WITNESS Lepto should be considered as the serological test of choice at the point-of-care for rapid and early detection of *Leptospira*-specific antibodies in acutely ill dogs suspected to have leptospirosis.

Footnotes

[a] Hawksley, Lancing, Sussex, England
[b] Ridglan Farms Inc., Mt. Horeb, Wisconsin, USA
[c] Dexdomitor, Zoetis LLC, Parsippany, NJ, USA
[d] SAS Institute Inc., Cary, NC, USA

Acknowledgments

We thank Dan Lin for her assistance in statistical analyses, and Laurel Bowersock and Therese Hildebrand for their help in study and animal facility planning, and logistics. We also thank the Animal Research Support staff at Zoetis for their help in providing high-quality care to the animals used in the study.

Conflict of Interest Declaration: This work was funded by Zoetis, Inc. All the authors are employed by Zoetis, and WITNESS Lepto is a product of the company with a business and/or financial interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobial.

References

1. Levett P. Leptospirosis. Clin Microbiol Rev 2001;14:296–326.

2. Bharti AR, Nally JE, Ricaldi JN, et al. Leptospirosis: A zoonotic disease of global importance. Lancet Infect Dis 2003; 3:757–771.

3. 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.

4. Schuller S, Francey T, Hartmann K, et al. European consensus statement on leptospirosis in dogs and cats. J Small Anim Pract 2015;56:159–179.

5. Rentko VT, Clark N, Ross LA, Schelling SH. Canine leptospirosis: A retrospective study of 17 cases. J Vet Intern Med 1992;6:235–244.

6. Bolin CA. Diagnosis of leptospirosis: A reemerging disease of companion animals. Semin Vet Med Surg (Small Anim) 1996;11:166–171.

7. Ward MP, Guptill LF, Prahl A, Wu CC. Serovar-specific prevalence and risk factors for leptospirosis among dogs: 90 cases (1997–2002). J Am Vet Med Assoc 2004;224:1958–1963.

8. Moore GE, Guptill LF, Glickman NW, et al. Canine Leptospirosis, United States, 2002–2004. Emerg Infect Dis 2006;12:501–503.

9. Stokes JE, Kaneene JB, Schall WD, et al. Prevalence of serum antibodies against six *Leptospira* serovars in healthy dogs. J Am Vet Med Assoc 2007;230:1657–1664.

10. Alton GD, Berke O, Reid-Smith R, et al. Increase in seroprevalence of canine leptospirosis and its risk factors, Ontario 1998–2006. Can J Vet Res 2009;73:167–175.

11. Klaasen HLBM, Adler B. Recent advances in canine leptospirosis: Focus on vaccine development. Vet Med Res Rep 2015;6:245–260.

12. Xu C, Loftis A, Ahluwalia SK, et al. Diagnosis of canine leptospirosis by a highly sensitive FRET-PCR targeting the *lig* Genes. PLoS One 2014;9:e89507.

13. Azócar-Aedo L, Smits HL, Monti G. Leptospirosis in dogs and cats: Epidemiology, clinical disease, zoonotic implications and prevention. Arch Med Vet 2014;46:337–348.

14. Curtis KM, Foster PC, Smith PS, et al. Performance of a recombinant LipL32 based rapid in-clinic ELISA (SNAP Lepto) for the detection of antibodies against *Leptospira* in dogs. Int J Appl Res Vet Med 2015;13:182–189.

15. Winzelberg S, Tasse SM, Goldstein RE, et al. Evaluation of SNAP Lepto in the diagnosis of leptospirosis infections in dogs: Twenty two clinical cases. Int J Appl Res Vet Med 2015;13:193–198.

16. Faine S, Alder B, Bolin C, Perolar P. *Leptospira* and Leptospirosis. Melbourne, Austraila: MedSci; 1999.

17. Adler B, Faine S. The antibodies involved in the human immune response to leptospiral infection. J Med Microbiol 1978;11:387–400.

18. Hartman EG. Epidemiological aspects of canine leptospirosis in the Netherlands. Zentralbl Bakteriol Mikrobiol Hyg A 1984;258:350–359.

19. Kodjo A, Calleja C, Loenser M, et al. A rapid in-clinic test detects acute leptospirosis in dogs with high sensitivity and specificity. Biomed Res Int 2016;2016:3760191.

20. Lizer J, Grahlmann M, Hapke H, et al. Evaluation of a rapid IgM detection test for diagnosis of acute leptospirosis in dogs. Vet Rec 2017;180:517.

21. Haake DA. Hamster model of leptospirosis. Curr Protoc Microbiol 2006;Chapter 12:Unit 12E.12.

22. Zuerner RL, Alt DP, Palmer MV, et al. A *Leptospira borgpetersenii* serovar Hardjo vaccine induces a Th1 response, activates NK cells, and reduces renal colonization. Clin Vaccine Immunol 2011;18:684–691.

23. Goldstein RE. Canine leptospirosis. Vet Clin North Am Small Anim Pract 2010;40:1091–1101.

24. Cumberland PC, Everard COR, Levett PN. Assessment of the efficacy of the IgM enzyme-linked immunosorbent assay (ELISA) and the microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. Am J Trop Med Hyg 1999;61:731–734.

25. Guerreiro H, Croda J, Flannery B, et al. Leptospiral proteins recognized during the humoral immune response to leptospirosis in humans. Infect Immun 2001;69:4958–4968.

26. Faine S. Guidelines for the Control of Leptospirosis. WHO offset publication no. 67, 1982: 21–26. Geneva: World Health Organization.

27. Sehgal SC, Vijayachari P, Sugunan AP, Umapathi T. Field application of Lepto lateral flow for rapid diagnosis of leptospirosis. J Med Microbiol 2003;52:897–901.

28. Haake DA, Chao G, Zuerner RL, et al. The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. Infect Immun 2000;68:2276–2285.

29. Vivian JP, Beddoe T, McAlister AD, et al. Crystal structure of LipL32, the most abundant surface protein of pathogenic *Leptospira* spp. J Mol Biol 2009;387:1229–1238.

30. Pinne M, Haake DA. LipL32 is a subsurface lipoprotein of *Leptospira interrogans*: Presentation of new data and reevaluation of previous studies. PLoS One 2013;8:e51025.

31. Flannery B, Costa D, Carvalho FP, et al. Evaluation of recombinant *Leptospira* antigen-based enzyme-linked immunosorbent assays for the serodiagnosis of leptospirosis. J Clin Microbiol 2001;39:3303–3310.

32. Chernukha YG, Shishkina ZS, Baryshev PM, Kokovin IL. The dynamics of IgM and IgG antibodies in leptospiral infection in man. Zentralbl Bakteriol Orig A 1976;236:336–343.

33. Langston C, Heuter K. Leptospirosis, a re-emerging zoonotic disease. Vet Clin North Am Small Anim Pract 2003;33:791–807.

34. Low DG, Hiatt CW, Gleiser CA, Bergman EN. Experimental canine leptospirosis: I. *Leptospira* Icterohaemorrhagiae infections in immature dogs. J Infect Dis 1956;98:249–259.

35. Greenlee JJ, Alt DP, Bolin CA, et al. Experimental canine leptospirosis caused by *Leptospira interrogans* serovars pomona and bratislava. Am J Vet Res 2005;66:1816–1822.

36. Gloor CI, Schweighauser A, Francey T, et al. Diagnostic Value of two commercial chromatographic "patient-side" tests in the diagnosis of acute canine leptospirosis. J Small Anim Pract 2017;58:154–161.