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Vaccine-Associated *Leptospira* Antibodies in Client-Owned Dogs*Leptospira* Vaccine Responses in DogsMartin et al

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Background: Long-term microscopic agglutination test (MAT) results after vaccination with 4-serovar *Leptospira* vaccines are not available for all vaccines used in client-owned dogs.

Hypothesis/Objectives: To determine antibody responses of client-owned dogs given 1 of 4 commercially available *Leptospira* vaccines.

Animals: Healthy client-owned dogs (n = 32) with no history of *Leptospira* vaccination for at least the previous year.

Methods: Dogs were given 1 of 4 *Leptospira* vaccines on week 0 and then approximately on week 3 and week 52. Sera were collected before vaccine administration on week 0 and then within 3 days of week 3, within 2 days of week 4, and approximately on weeks 7, 15, 29, 52, and 56. Antibody titers against *Leptospira* serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona* and were determined by MAT.

Results: When compared among vaccines, MAT results varied in maximal titers, the serovars inducing maximal titers, and the time required to reach maximal titers. Each vaccine induced at least some MAT titers ≥1 : 800. Most dogs were negative for antibodies against all serovars 1 year after vaccination, and anamnestic responses were variable.

Conclusions and Clinical Importance: Dogs vaccinated with *Leptospira* vaccines have variable MAT titers over time, and antibodies should not be used to predict resistance to *Leptospira* infection. MAT titers ≥1 : 800 can develop after *Leptospira* spp. vaccination, which can complicate the clinical diagnosis of leptospirosis.

Key words: Serology; Titers.

Leptospirosis is a worldwide zoonotic bacterial disease, affecting several species, including dogs.[1,2] Clinical illness in dogs in the United States most commonly is associated with serovars within *Leptospira interrogans* and *L. kirschneri*.[2] *Leptospira* infection occurs after contact with infected urine or contaminated water.[1] Clinical signs of leptospirosis are highly variable in dogs, ranging from no clinical signs (subclinical) to renal failure and death.[2] There are currently 4 commercially available vaccines in the United States that contain serovars *canicola*, *grippotyphosa*, *icterohemorrhagiae*, and *pomona*. These vaccines are recommended for all dogs with risk of exposure to *Leptospira* spp.[2,3]

The microscopic agglutination test (MAT) is considered the diagnostic test of choice in dogs with suspected leptospirosis.[2] MAT results, however, can be negative in acute infections and do not differentiate vaccinated from infected dogs, making interpretation of results challenging.[2]

Abbreviations:

MAT microscopic agglutination test

Previous studies of *Leptospira* vaccine responses in dogs utilized specific-pathogen-free animals housed in laboratory settings.[4–7] In these dogs, vaccination-associated antibody titers generally are low (<1 : 800), and when higher titers are detected in the field, results may be interpreted as indicating *Leptospira* spp. infection. Research dogs, however, may have decreased immune responses to vaccination compared to client-owned animals as a result of decreased antigenic exposure and overall immune system stimulation. Little data exist concerning the MAT titer magnitudes that develop in client-owned dogs after vaccination. In addition, data on temporal MAT titers from vaccinated client-owned dogs after primary and booster immunization are not widely available. The purpose of this study was to determine the *Leptospira* spp. antibody responses of client-owned dogs using MAT after administration of 4 commercially available *Leptospira* vaccines.

Materials and Methods

The study protocol was approved by the Colorado State University Institutional Animal Care and Use Committee. The study, which was to use healthy client-owned dogs, was announced to veterinary students at Colorado State University by email. Inclusion criteria that stipulated dogs be between 1 and 8 years of age, >15 kg, healthy, and have a known vaccination history with no *Leptospira* spp. vaccination in the previous year. Ultimately, 32 dogs were enrolled in the study, 23 of which were purebred representing 14 breeds, and 9 of which were mixed breed dogs. The median age of dogs in the study was 6 years (range, 2–8 years) and the median weight was 25.8 kg (range, 15–48.7 kg). Some dogs had never been given a *Leptospira* spp. vaccine (n = 21) and the remainder of the dogs (n = 11) had not

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been given a *Leptospira* spp. vaccine in the previous year. The dogs were randomly assigned to be given 1 of 4 commercially available vaccines that all contain the *canicola*, *grippotyphosa*, *icterohemorrhagiae*, and *pomona* serovars.[a,b,c,d] After the initial vaccine administration (week 0), all dogs were given a second vaccine of the same type within 3 days of week 3. All vaccinations were delivered SC over the left shoulder. Approximately 52 weeks later, 25 of the dogs still were available and were given the same vaccine as used previously, within 28 days of week 52.

Blood was collected by jugular or cephalic venipuncture and placed into serum tubes before vaccination on week 0 and then depending on the dog, serum was collected approximately on weeks 3, 4, 7, 15, 29, 52, and 56. After clot formation, sera were separated and stored at −80°C until tested. Antibody titers against serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona* were determined using MAT performed by standard operating procedures at a reference laboratory.[e] For the purposes of this study, MAT titers ≥1 : 100 were considered positive.

Results

Before initial vaccination, 1 dog had antibodies (1 : 200) to serovar *icterohemorrhagiae* but appeared healthy on physical examination, and had no clinically relevant abnormalities on CBC, serum biochemical profile, or urinalysis. All of the other dogs were negative for antibodies against the 6 serovars. After vaccination, proportions of dogs developing positive titers of different magnitudes varied by serovar and by vaccine over time (Table 1). The maximal MAT titer detected for each serovar was as follows: *bratislava* (1 : 6,400), *canicola* (1 : 6,400), *grippotyphosa* (1 : 6,400), *hardjo* (1 : 400), *icterohemorrhagiae* (1 : 6,400), and *pomona* (1 : 3,200). After the initial vaccination, all dogs had the highest titers for serovars *canicola*, *grippotyphosa,* and *icterohemorrhagiae* during week 4 with 100%, 72% and 94% of dogs having titers ≥1 : 100 and 78%, 59% and 63% of dogs having titers ≥1 : 800, respectively. During weeks 3 and 4, titers of ≥1 : 100 against serovars *bratislava*, *hardjo*, and *pomona* were observed in 44%, 3% and 81% of dogs, respectively. At week 7 (4 weeks after booster), titers for serovars *canicola*, *grippotyphosa*, and *icterohemorrhagiae* decreased for most dogs, with 72%, 63%, and 72% of dogs having titers ≥1 : 100, and 53%, 22%, and 25% of dogs having titers ≥1 : 800, respectively. Overall, 66% of dogs maintained a MAT titer ≥1 : 800 for at least 1 serovar at week 7. By week 15, 3% of dogs maintained titers ≥ 1 : 800 for serovars *bratislava*, *canicola*, *grippotyphosa*, and *icterohemorrhagiae*. At week 29, dogs were seropositive only for serovars *canicola* (19%), *grippotyphosa* (6%), and *icterohemorrhagiae* (6%). One dog was not returned at week 29, so no sera were collected.

At 52 weeks postvaccination, 84% of dogs were seronegative for all serovars. Positive titers were recorded only for serovars *canicola* (8%), *grippotyphosa* (4%), and *icterohemorrhagiae* (12%), and none of these were ≥1 : 800. At 56 weeks (4 weeks after revaccination), titers for serovars *canicola*, *grippotyphosa*, and *icterohemorrhagiae* were highest, with 70%, 35%, and 48% of dogs having titers ≥1 : 100 and 30%, 17%, and 17% having titers ≥1 : 800, respectively. At week 56, 22%, 0%, and 13% of dogs were seropositive for serovars *bratislava*, *hardjo*, and *pomona*, respectively. Overall, 48% of dogs had MAT titers ≥1 : 800 for at least 1 serovar at week 56. Two dogs were not returned during week 56, and no sera were collected.

Table 1. Percentage of client-owned dogs with MAT titers ≥1 : 100, ≥1 : 400, ≥1 : 800, ≥1 : 1,600 grouped by week before and after the administration of 1 of 4 commercially available 4-serovar leptospire vaccines. After the initial vaccine administration (Week 0), all dogs were given a second vaccine of the same type within 3 days of week 3. Approximately 52 weeks later, the 25 available dogs were given the same vaccine as used previously.

	Week							
Serovar	0	3	4	7	15	29	52	56
MAT ≥1 : 100								
bratislava	0	44	44	13	9	0	0	22
canicola	0	66	100	72	34	19	8	70
grippotyphosa	0	63	72	63	13	6	4	35
hardjo	0	3	3	6	0	0	0	0
icterohemorrhagiae	3	75	94	72	31	6	12	48
pomona	0	81	81	50	0	0	0	13
MAT ≥1 : 400								
bratislava	0	9	22	6	3	0	0	4
canicola	0	53	84	53	6	0	0	39
grippotyphosa	0	41	63	41	3	0	0	17
hardjo	0	3	0	3	0	0	0	0
icterohemorrhagiae	0	31	88	50	6	0	0	22
pomona	0	41	59	16	0	0	0	4
MAT ≥1 : 800								
bratislava	0	6	13	3	3	0	0	4
canicola	0	44	78	53	3	0	0	30
grippotyphosa	0	22	59	22	3	0	0	17
hardjo	0	0	0	0	0	0	0	0
icterohemorrhagiae	0	6	63	25	3	0	0	17
pomona	0	34	38	9	0	0	0	4
MAT ≥1 : 1,600								
bratislava	0	3	9	0	3	0	0	4
canicola	0	28	66	31	0	0	0	13
grippotyphosa	0	16	38	9	3	0	0	0
hardjo	0	0	0	0	0	0	0	0
icterohemorrhagiae	0	3	44	9	0	0	0	9
pomona	0	22	16	3	0	0	0	0

Discussion

Although the 4 vaccines used in this study were designed to protect against the same 4 serovars, the timing and degree of seroconversion did not appear to be equivalent. Vaccine response was highly variable not only among and within vaccine groups, but also among individuals. Because it is possible that other factors such as natural exposure or immune status could have affected the responses of individual dogs, we did not attempt to statistically compare the results among vaccines.

The highest MAT titers (≥1 : 800) were detected 4 weeks after vaccination (weeks 4 and 56). Although the majority of dogs developed positive MAT titers, a

minority of dogs remained seropositive by week 15, and at 1 year after vaccination, most dogs were seronegative for all serovars. These findings were similar between groups of dogs with and without *Leptospira* vaccination before entering the study and support the results of previous studies documenting that vaccine-associated *Leptospira* MAT titers can be short-lived.[2]

On week 56, *canicola* was the only serovar associated with MAT titers >1 : 100 in >50% of all dogs, regardless of vaccine. The magnitude of MAT titers at week 56 compared to those at week 52 generally was less than that seen at week 4 compared to week 0. The lower anamnestic response at week 56 compared to week 4 likely is because of the fact that dogs received a booster vaccine at week 3 of the study. However, even at week 7 (4 weeks postbooster), the number of dogs with titers ≥ 1 : 100 and ≥ 1 : 800 was higher than at week 56, and a higher proportion of dogs had at least 1 titer ≥ 1 : 800. The peak anamnestic response after revaccination at week 52 may not have been captured when sera were collected at week 56. Additionally, because this study used client-owned dogs with access to the outdoors, there was potential for natural exposure to leptospires in the environment. Contact with naturally occurring leptospires may have provided additional antigentic stimulation, ultimately blunting the last response to vaccination. Few dogs, however, had MAT titers to any serovar at week 52 and none of these titers was ≥1 : 800, thus natural exposure to *Leptospira* spp. during the interval between vaccines was considered to be unlikely.

Although none of the vaccines used in this study contained serovars *bratislava* or *hardjo*, several dogs developed titers against them. This was not unexpected, inasmuch as dogs in a study with a bivalent vaccine showed cross-reactivity to nonvaccine serovars, demonstrating that MAT titers are not serovar-specific.[6] In another study using quadrivalent *Leptospira* vaccines, dogs often developed the highest titers to nonvaccinal serovars.[8] There is also the potential for cross-contamination of *Leptospira* spp. in laboratory stocks in reference laboratories. In addition, the serovar giving the maximal titer can vary among laboratories and over time.[9] Based on these results, dogs inoculated with these 4 vaccines also may develop titers to other serovars not tested in this study. Positive titers to nonvaccinal serovars should be interpreted with caution if a vaccinated dog develops clinical signs consistent with leptospirosis.

The goal of this study was not to determine vaccine efficacy, and the results of this study should not be interpreted as variations in vaccine efficacy. Evaluating *Leptospira* vaccine efficacy by use of MAT titers is known to be inaccurate, and numerous studies have shown no correlation between postvaccination titers and protection.[4,5,7] For example, 1 challenge study demonstrated dogs to be protected against leptospirosis when challenged 13 months after vaccination with a bivalent vaccine, despite low or undetectable MAT titers at the time of challenge.[5] Vaccination with quadrivalent vaccines in a previous study resulted in 18 of 20 dogs becoming MAT titer positive, with roughly half developing a titer >1 : 1,600.[8] Single MAT titer values therefore are difficult to interpret in a recently vaccinated dog.[8] In another study, dogs vaccinated with a bivalent vaccine developed MAT titers ≤1 : 80, which lasted only 1–4 months, and were largely seronegative 1 year later.[7] Dogs in this study, however, were challenged 1 year after vaccination and found to be protected from leptospiremia and a renal carrier state.[7] Another study with challenges at 2 weeks and 14 months postvaccination with a bivalent vaccine showed similar antibody responses (low and short in duration), but dogs were protected from clinical disease and a renal carrier state.[4] Duration of immunity appears to be much longer than what would be anticipated solely from evaluation of MAT titers. However, a recent retrospective study documented 9 client-owned dogs that developed clinical signs consistent with leptospirosis despite vaccination at least 3 months prior but within 1 year.[10] Thus, duration of immunity against naturally occurring leptospirosis appears to be more difficult to predict, especially without a diagnostic method to differentiate between vaccine antibodies and natural infection.

The dogs in this study demonstrated variable seroconversion within and between vaccination groups as well as a poor anamnestic response to vaccination against *Leptospira* when measured by MAT titers. A majority of dogs lacked measurable antibodies to the investigated serovars at 52 weeks after initial vaccination, but inasmuch as previous studies have demonstrated that commercially available vaccines provide at least 1-year immunity, susceptibility to *Leptospira* cannot be reliably determined from serology. Given the strong, but variable, antibody response of some individual dogs in this study, a single MAT titer ≥ 1 : 800 cannot be reliably attributed to clinical leptospirosis, particularly if there is a history of recent vaccination. This study highlights the need for improved diagnostic methods for identifying leptospirosis and differentiating vaccinated from infected dogs.

Footnotes

[a] LeptoVax 4, Boehringer-Ingelheim, St. Joseph, MO
[b] Nobivac Lepto4, Merck Animal Health, Whitehouse Station, NJ
[c] RECOMBITEK 4 Lepto, Merial, Duluth, GA
[d] Vanguard L4, Pfizer Animal Health, New York, NY
[e] Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO

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