# Reliable prevention. **Less stress.**

**Nobivac® Intra-Trac® Oral Bb**

## Nobivac® Intra-Trac® Oral Bb delivers convenience that works.

For social dogs and puppies, Nobivac® Intra-Trac® Oral Bb was shown to be effective against canine infectious tracheobronchitis caused by *Bordetella bronchiseptica* (Bb).[1]

- Easy to administer in the buccal cavity
- Syringes with a green plunger provided to differentiate from injectable vaccines
- No booster required for primary immunization
- For use in dogs seven weeks of age or older


## SIGNIFICANT PROTECTION

21-day efficacy challenge data study results[1]:

- Significantly reduced duration of coughing and shedding of Bb in vaccinates compared with control dogs

## PROVEN TO BE SAFE

- In a field safety trial that included over 300 dogs, there were no adverse events deemed related to Nobivac® Intra-Trac® Oral Bb[1]

**WITH COMFORT IN MIND, CHOOSE NOBIVAC® INTRA-TRAC® ORAL Bb.**

To learn more, contact your Merck Animal Health sales representative or your distributor representative.

**Customer Service:** 1-800-521-5767
(Monday–Friday, 9:00AM–6:00PM EST)

**Technical Services:** 1-800-224-5318
(Monday–Friday, 8:30AM–7:00PM EST)

Nobivac®
Protection unites us.

**Reference: 1.** Summary of studies supporting USDA product licensure for Nobivac Intra-Trac Oral Bb. USDA website. Available at: www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-biologics/product-summaries/vet-label-data/83c6706d-ffd8-4e32-ba7a-706e1bcb9333. Accessed October 28, 2020.



MERCK Animal Health

**CALL OF THE WILD** Dogs that may come in contact with wildlife or outdoor water sources are among the group that should receive a leptospirosis vaccine.

VACCINATION STATION

# Vaccination Overview: Leptospirosis

***Jane Sykes***, *BVSc(Hons), PhD, MBA, DACVIM (SAIM)*
*University of California, Davis, School of Veterinary Medicine*

## WHAT IS LEPTOSPIROSIS?

Leptospirosis is a zoonotic, multisystemic disease of worldwide significance caused by pathogenic species of the spirochete *Leptospira*. The main species that cause disease in dogs and humans are *Leptospira interrogans* and *Leptospira kirschneri*. *Leptospira* species are thin, motile, spiral-shaped bacteria with hook-shaped ends that penetrate abraded skin or intact mucous membranes. They are transmitted by direct contact with infected urine, bite wounds, or predation of infected wildlife, or indirectly through contact with contaminated water, soil, food, or bedding. Some strains of *Leptospira* survive several weeks in the environment when conditions are optimal.[1,2]

Some strains of *Leptospira* survive several weeks in the environment when conditions are optimal.[1,2]

Leptospirosis is considered a seasonal disease, with outbreaks often linked to heavy rainfall or flooding. The peak seasonal distribution in parts of North America where freezing winters occur is late fall, but in more temperate regions, it is after months of high rainfall (such as in late winter in northern California).[3,4] Where it rains heavily throughout the year, there may be no seasonality to infections. However, outbreaks of leptospirosis in dogs can also occur in relatively arid regions, possibly due to predation of infected wildlife, presence of irrigation, or group housing. For example, in an outbreak of leptospirosis in dogs in Arizona, affected dogs were 7.7× and 2.9× more likely than unaffected control dogs to have visited doggy daycare and boarding facilities, respectively, in the 30 days prior to the onset of clinical signs or diagnosis, suggesting a possible source of infection in these facilities.[5-7]

## WHY SHOULD VETERINARIANS PROMOTE PREVENTION OF LEPTOSPIROSIS?

Pathogenic leptospires multiply rapidly in the body, causing acute kidney injury (AKI), hepatic injury, and vasculitis. Other organs may also be affected (**FIGURE 1 AND BOX 1**). Dogs that develop severe AKI with oliguria or anuria may require renal replacement therapy for survival, which may not be accessible or

affordable for their owners. Similarly, those that develop severe pulmonary hemorrhage may require mechanical ventilation; sometimes both mechanical ventilation and renal replacement therapy are required. Prevention of disease through vaccination is preferred to such costly treatments with an uncertain outcome.

## WHICH DOGS ARE AT RISK FOR LEPTOSPIROSIS?

Consistent with the transmission cycle of leptospires, a 2007 study found that dogs with leptospirosis in the United States were more likely to live near outdoor water, swim in or drink from outdoor water sources, and have indirect exposure to wildlife (**FIGURE 2**).[8] However, over the past decade, residence in urban areas has emerged as a risk factor for *Leptospira* infection in dogs in some parts of North America, possibly due to exposure to rodents.[9,10] In other words, all dogs may be at risk.

Risk factors identified for age, sex, and breed of dogs with acute leptospirosis have yielded conflicting results and might be subject to changes over time.[11] Males, herding dogs, hounds, working dogs, and mixed breeds have previously been reported to be at increased risk in the United States.[12] In a U.S. study using the Veterinary Medical Database, dogs weighing less than 6.8 kg (15 lb)—in particular, Yorkshire terriers—had the highest hospital prevalence for leptospirosis between 2000 and 2009.[11] This may be due to the fact that small breeds are suspected to have a higher risk for adverse effects of vaccination and therefore were less likely to be vaccinated.

Factors such as the density of reservoir hosts, the concentration of organisms in their urine, and specific leptospiral strains may be important in determining whether disease occurs in dogs and humans.[13,14] In one study, strains from humans clustered with those detected in dogs but not mice, and the profile of one human isolate was identical to that from a dog.[15] Disease in incidental hosts tends to be more severe, and the duration of shedding is generally shorter.

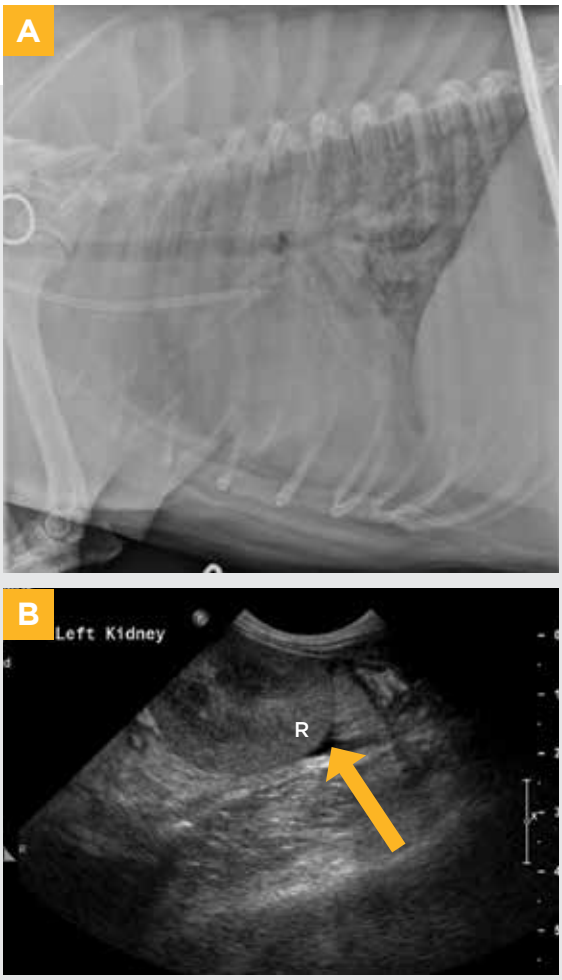


**FIGURE 1.** Diagnostic images from dogs with leptospirosis. **(A)** Lateral thoracic radiograph of a 6-year-old female spayed pitbull terrier with leptospiral pulmonary hemorrhage syndrome showing a diffuse alveolar infiltrate due to massive pulmonary hemorrhage. **(B)** Ultrasound image of the kidney in a 10-month-old Shiba Inu with leptospirosis that had hyperechoic renal cortices **(R)** and perirenal free abdominal fluid **(arrow)**.

**FIGURE 2.** Although exposure to standing water is a classic risk factor for leptospirosis and large-breed, outdoor dogs are often considered at risk, disease is increasingly being recognized in small-breed dogs from urban environments.

From left: courtesy of University of California, Davis Veterinary Diagnostic Imaging Service (2); courtesy of Dr. Zach Mills

## WHY IS IT IMPORTANT TO KNOW ABOUT SEROVARS, SEROGROUPS, AND SEQUENCE TYPES?

More than 250 serovars of *L interrogans* have been described and further classified into serogroups based on relatedness of the outer lipopolysaccharide antigen. (By convention, serovar names are capitalized and not italicized.) These have been historically useful for epidemiologic tracking and understanding of leptospirosis, because each serovar is adapted to one or more animal reservoir host species. Maintenance hosts include dogs (serovar Canicola); rats (Icterohaemorrhagiae); small wildlife mammalian species such as voles, skunks, and raccoons (Grippotyphosa); cattle (Hardjo); and mice (Ballum). Worldwide, small rodents are considered the most important reservoir hosts.

The serogroup/serovar classification system is confusing because the same serovar can be found in more than one *Leptospira* species (e.g., serovar Grippotyphosa can be found in *L interrogans* and *L kirschneri*) by virtue of similar lipopolysaccharide antigens.[16] In addition, each serogroup contains a serovar of the same name (e.g., serogroup Icterohaemorrhagiae contains serovar Icterohaemorrhagiae; serogroup Canicola contains serovar Canicola).[17]

Accurate identification of infecting serovars entails culture and serotyping, which requires special expertise and can take many weeks. Because of these limitations, molecular typing methods that classify leptospires into sequence types (STs) are now being used for epidemiologic purposes. Application of these methods has revealed that there may be even tighter associations between certain STs and specific reservoir host species. One widely used method is multilocus sequence typing (MLST). In classic MLST, a series of DNA segments (typically 7) from different parts of the genome (loci) are amplified using polymerase chain reaction (PCR) and sequenced. Unfortunately, several different MLST schemes have been used, making comparisons difficult. In 2019, a core genome MLST scheme was described that involves analysis of 545 core gene sequences following whole genome sequencing.[18] This method has high discrimination power and allows identification of species, STs, and most serogroups, allowing tracking of *Leptospira* strains in different hosts and over time.[19]

Understanding circulating serogroups and serovars is still important because immunity after vaccination with inactivated vaccines is serogroup specific and possibly serovar specific, with only partial immunity to heterologous serogroups.[20] Improved understanding of serogroups infecting dogs therefore has the potential to lead to more protective vaccines. More than 10 different serovars have been associated with disease in dogs worldwide, although the exact serovars responsible in different geographic locations remains poorly understood due to the difficulties associated with culture of leptospires. With improvement of molecular identification techniques that allow correlation between STs and serogroups, it is likely that even more efficacious vaccines could be designed.

**BOX 1 Clinical Abnormalities Associated With Canine Leptospirosis**

- Acute kidney injury/renal failure
- Hepatic injury
- Pancreatitis
- Pulmonary hemorrhage (leptospiral pulmonary hemorrhage syndrome)
- Uveitis
- Myositis (and increased serum creatine kinase concentrations)
- Thrombocytopenia
- Normal serum potassium concentration despite oliguria/anuria
- Glucosuria in the absence of diabetes mellitus

## WHICH SEROVARS INFECT DOGS?

Several decades ago, before the introduction of canine *Leptospira* vaccines, the most common serovars thought to infect dogs belonged to the serogroups Canicola and Icterohaemorrhagiae. Since that time, widespread seroconversion to other serovars, especially Pomona and Grippotyphosa in North America, has been noted in sick dogs. This apparent shift in the immune response could be due to the inclusion of additional serovars into the serologic testing panels (based on the microscopic agglutination test [MAT]), as well as increased contact between dogs, wildlife, and farm animal reservoir hosts.

Most of the knowledge regarding serovars that infect dogs has been inferred from MAT results, but unfortunately these do not accurately reflect the infecting serovar because there is extensive cross-reactivity among serovars in the assay, frequently leading to paradoxical results (higher titers to serovars

Dogs in urban backyards may also be at risk if there is significant exposure to wildlife (including rodents) in the immediate home environment.

other than the infecting serovar).[17] Also, veterinary assays typically include only 6 or 7 serovars (compared with >20 in panels for human diagnostics), so there may be higher titers present to serovars not included in the panel. The advent of molecular typing methods has allowed more accurate identification of serovars and STs of *Leptospira* species that cause disease in domestic dogs from different geographic locations, and in some circumstances, typing may be possible following amplification of DNA directly from clinical specimens, without previous culture.

Currently, only a few published studies have reported serovars and STs infecting dogs, and studies from North America are lacking. A 2010 study suggested an association between infection with serovars Ballum and Icterohaemorrhagiae and human disease in the United States.[21] In this study, 4 of 6 isolates from the continental United States and 21 of 41 isolates from Hawaii were of unknown identity. A 2016 study suggested that serovar Grippotyphosa may be the most prevalent serovar in dogs from the midwestern United States, based on molecular analysis (78 of 98 dogs),[22] although the molecular typing method used had limited discriminatory power compared with more recently developed schemes. As typing methods have been refined and the number of STs in electronic databases has grown, the ability to correlate data has improved. For example, a 2020 Italian study of dogs with leptospirosis that used classic MLST without the need for culture was able to identify 6 different STs belonging to the serogroups Icterohaemorrhagiae, Australis, Sejroe, and Pomona.[23] The possible transmission chains involved rats, mice, hedgehogs, and pigs.

## WHAT VACCINES ARE AVAILABLE TO PREVENT LEPTOSPIROSIS?

Current canine leptospirosis vaccines are inactivated bacterins that have been purified to reduce the prevalence of adverse reactions. Quadrivalent vaccines that contain serovars Canicola, Icterohaemorrhagiae, Grippotyphosa, and Pomona have been available in North America since 2001.

## HOW EFFECTIVE ARE CURRENT CANINE LEPTOSPIROSIS VACCINES AND WHAT IS THEIR DURATION OF IMMUNITY?

In experimental challenge studies, leptospiral vaccines effectively prevent disease and reduce shedding after challenge with the serovar included in the vaccine. As the quantity and nature of experimental challenge studies for vaccine approval do not mimic natural exposure, prevention claims should be interpreted with caution. Documentation of urine shedding in vaccinated dogs following natural exposure is very limited. The duration of immunity following vaccination with bacterins is at least 12 months; some studies show protection 15 months after vaccination.[24-27]

Anecdotally, quadrivalent vaccines appear to protect dogs from leptospirosis because the disease is now almost exclusively diagnosed in unvaccinated dogs.[28] In a retrospective case-control study in a Swiss cohort of 469 dogs with AKI, vaccination with a quadrivalent vaccine including serovars from serogroups Icterohaemorrhagiae, Canicola, Grippotyphosa, and Australis was associated with significantly lower odds of leptospirosis diagnosis.[29] However, the 2020 epidemiologic study from Italy that used MLST suggested several instances of vaccine failure.[23]

Anecdotally and in the scientific literature,[30] leptospirosis has been reported widely in dogs vaccinated with bivalent Icterohaemorrhagiae and Canicola vaccines; thus, vaccines that contain only 2 serovars do not sufficiently cross-protect against serogroups responsible for most infections in dogs.

## HOW SAFE ARE LEPTOSPIROSIS VACCINES?

In the past, vaccination with *Leptospira* vaccines was associated with type I hypersensitivity reactions such as anaphylaxis, especially in small-breed dogs.[31] Anecdotal evidence from industry and veterinary practitioners in North America suggests that the prevalence of these reactions has considerably decreased in recent years (to less than 1%, with most reactions being local reactions

at the injection site rather than anaphylaxis) following efforts from the industry to remove residual cell culture constituents (e.g., bovine proteins) that have been associated with vaccine reactivity.[32,33]

## WHICH DOGS SHOULD BE VACCINATED FOR LEPTOSPIROSIS?

Immunization is recommended for all dogs at risk of exposure. In some regions, this may be dogs that may come in contact with wildlife or farm animals or that may be exposed from environmental water sources. Dogs in urban backyards may also be at risk if there is significant exposure to wildlife (including rodents) in the immediate home environment.

A study of vaccination compliance in dogs in Germany revealed that only 50.1% of 3874 dogs had been vaccinated for leptospirosis, and the primary risk factor for lack of vaccination was recommendation from a veterinarian not to vaccinate for leptospirosis.[34]

Evidence to show the protective effect of currently available leptospirosis vaccines beyond 12 or 15 months is lacking. Until more data become available, it has been recommended to restart a basic vaccination schedule with 2 doses administered 3 or 4 weeks apart in dogs that have not been revaccinated against leptospirosis for more than 18 months.[35] However, it is possible that a single booster at this time would restore immunity, as is generally recommended even for delays of many years in vaccination with bacterins for humans.

For dogs that experience natural infection, immunization could commence after recovery, assuming that natural immunity is also serogroup-specific and the possibility of exposure to unrelated serogroups exists. However, the duration of immunity and the degree and duration of cross-protection to heterologous serogroups that follow natural infection require further investigation.

## TO WHAT EXTENT DOES VACCINATION INTERFERE WITH DIAGNOSTIC TESTING FOR LEPTOSPIROSIS?

Available diagnostic tests include PCR testing, serology using the MAT, and in-clinic serologic assays that detect IgG/IgM (SNAP Lepto; IDEXX, idexx.com) or IgM (WITNESS Lepto; Zoetis, zoetis.com). In the MAT, titers are provided for each of several serovars to increase the chance of antibody detection. Postvaccinal titers against any serovar (even those other than in the vaccine, owing to cross-reactivity) occasionally rise as high as 1:6400 for a few months after vaccination, and these can interfere with interpretation.

In-clinic serologic assays are useful for screening dogs for the presence or absence of antibodies. Should these kits yield positive results, the clinician should consider whether the dog was previously vaccinated. Previous subclinical exposure should also be considered as a reason for positive results. Although the WITNESS test is less likely to be influenced by previous exposure or vaccination, some dogs can still be positive several weeks after vaccination.[36] If leptospirosis is suspected and in-clinic serologic tests show positive results, clinicians should consider reflex testing with the MAT to obtain a quantitative titer, followed by convalescent serology 1 to 2 weeks later to document a 4-fold or greater change in titer (e.g., 1:16 to 1:64, 1:128 to 1:512). Because vaccines are inactivated, they should not create false-positive results if PCR testing for leptospiral DNA is performed on blood or urine specimens. TVP

### References


1. Thibeaux R, Geroult S, Benezech C, et al. Seeking the environmental source of leptospirosis reveals durable bacterial viability in river soils. *PLoS Negl Trop Dis*. 2017;11(2):e0005414.
2. Andre-Fontaine G, Aviat F, Thorin C. Waterborne leptospirosis: survival and preservation of the virulence of pathogenic *Leptospira* spp. in fresh water. *Curr Microbiol*. 2015;71(1):136-142.
3. Hennebelle JH, Sykes JE, Carpenter TE, Foley J. Spatial and temporal patterns of *Leptospira* infection in dogs from northern California: 67 cases (2001-2010). *JAVMA*. 2013;242(7):941-947.
4. Lee HS, Levine M, Guptill-Yoran C, et al. Regional and temporal variations of *Leptospira* seropositivity in dogs in the United States, 2000-2010. *J Vet Intern Med*. 2014;28(3):779-788.
5. Guagliardo SAJ, Iverson SA, Reynolds L, et al. Despite high-risk exposures, no evidence of zoonotic transmission during a canine outbreak of leptospirosis. *Zoonosis Publ Health*. 2019;66(2):223-231.
6. LaFerla Jenni M, Woodward P, Yaglom H, et al. Knowledge, attitudes, and practices among veterinarians during an outbreak of canine leptospirosis-Maricopa County, Arizona, 2017. *Prev Vet Med*. 2019;172:104779.
7. Iverson SA, Levy C, Yaglom HD, et al. Clinical, diagnostic, and epidemiological features of a community-wide outbreak of canine leptospirosis in a low-prevalence region (Maricopa County, Arizona). *JAVMA*. 2021;258(6):616-629.
8. Ghneim GS, Viers JH, Chomel BB, et al. Use of a case-control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. *Vet Res*. 2007;38(1):37-50.
9. Azocar-Aedo L, Monti G. Meta-analyses of factors associated with leptospirosis in domestic dogs. *Zoonoses Publ Health*. 2016;63(4):328-336.
10. Raghavan R, Brenner K, Higgins J, et al. Evaluations of land cover risk factors for canine leptospirosis: 94 cases (2002-2009). *Prev Vet Med*. 2011;101(3-4):241-249.
11. Lee HS, Guptill L, Johnson AJ, Moore GE. Signalment changes in canine leptospirosis between 1970 and 2009. *J Vet Intern Med*. 2014;28(2):294-299.

### Jane Sykes

Dr. Sykes is a professor of small animal medicine at the University of California, Davis, School of Veterinary Medicine with a special interest in small animal infectious diseases. She obtained her veterinary degree and PhD at the University of Melbourne in Australia, her residency in small animal internal medicine at the University of Minnesota, and her MBA from the University of Georgia. She has coauthored more than 100 peer-reviewed publications and is the editor of *Canine and Feline Infectious Diseases* and the 5th edition of *Greene's Infectious Diseases of the Dog and Cat*. Her research interests include antimicrobial drug resistant bacterial infections, bloodborne infectious diseases, and deep mycoses.



12. Ward MP, Glickman LT, Guptill LE. Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970-1998). *JAVMA*. 2002;220(1):53-58.
13. Barragan V, Olivas S, Keim P, Pearson T. Critical knowledge gaps in our understanding of environmental cycling and transmission of *Leptospira* spp. *Appl Environ Microbiol*. 2017;83(19):e01190-17.
14. Barragan V, Nieto N, Keim P, Pearson T. Meta-analysis to estimate the load of *Leptospira* excreted in urine: beyond rats as important sources of transmission in low-income rural communities. *BMC Res Notes*. 2017;10(1):71.
15. Koizumi N, Izumiya H, Ohnishi M. Genetic relatedness of *Leptospira interrogans* serogroup Autumnalis isolated from humans, dogs, and mice in Japan. *BMC Res Notes*. 2020;13(1):369.
16. Levett PN. Systematics of leptospiraceae. *Curr Top Microbiol Immunol.* 2015;387:11-20.
17. Levett PN. Leptospirosis. *Clin Microbiol Rev.* 2001;14(2):296-326.
18. Guglielmini J, Bourhy P, Schiettekatte O, et al. Genus-wide *Leptospira* core genome multilocus sequence typing for strain taxonomy and global surveillance. *PLoS Negl Trop Dis.* 2019;13(4):e0007374.
19. Grillova L, Picardeau M. Core genome multi-locus sequence typing analyses of *Leptospira* spp. using the bacterial isolate genome sequence database. *Methods Mol Biol.* 2020;2134:11-21.
20. Sonrier C, Branger C, Michel V, et al. Evidence of cross-protection within *Leptospira interrogans* in an experimental model. *Vaccine*. 2000;19(1):86-94.
21. Galloway RL, Levett PN. Application and validation of PFGE for serovar identification of *Leptospira* clinical isolates. *PLOS Negl Trop Dis.* 2010;4(9):e824.
22. Harkin KR, Hays MP. Variable-number tandem-repeat analysis of leptospiral DNA isolated from canine urine samples molecularly confirmed to contain pathogenic leptospires. *JAVMA*. 2016;249(4):399-405.
23. Bertasio C, Boniotti MB, Lucchese L, et al. Detection of new *Leptospira* genotypes infecting symptomatic dogs: is a new vaccine formulation needed? *Pathogens*. 2020;9(6):484.
24. Klaasen HLBM, van der Veen M, Sutton D, Molkenboer MJCH. A new tetravalent canine leptospirosis vaccine provides at least 12 months immunity against infection. *Vet Immunol Immunopathol*. 2014;158(1-2):26-29.
25. Grosenbaugh DA, Pardo MC. Fifteen-month duration of immunity for the serovar Grippotyphosa fraction of a tetravalent canine leptospirosis vaccine. *Vet Rec*. 2018;182(23):665.
26. Wilson S, Stirling C, Thomas A, et al. Duration of immunity of a multivalent (DHPPi/L4R) canine vaccine against four *Leptospira* serovars. *Vaccine*. 2013;31(31):3126-3130.
27. Minke JM, Bey R, Tronel JP, et al. Onset and duration of protective immunity against clinical disease and renal carriage in dogs provided by a bi-valent inactivated leptospirosis vaccine. *Vet Microbiol*. 2009;137(1-2):137-145.
28. Prescott J. Canine leptospirosis in Canada: a veterinarian's perspective. *CMAJ*. 2008;178(4):397-398.
29. Francey T, Schweighauser A, Reber A, Schuller S. Evaluation of changes in the epidemiology of leptospirosis in dogs after introduction of a quadrivalent antileptospiral vaccine in a highly endemic area. *J Vet Intern Med*. 2020;34(6):2405-2417.
30. Kohn B, Steinicke K, Arndt G, et al. Pulmonary abnormalities in dogs with leptospirosis. *J Vet Intern Med*. 2010;24(6):1277-1282.
31. Moore GE, Guptill LF, Ward MP, et al. Adverse events diagnosed within three days of vaccine administration in dogs. *JAVMA*. 2005;227(7):1102-1108.
32. 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):1-13.
33. Yao PJ, Stephenson N, Foley JE, et al. Incidence rates and risk factors for owner-reported adverse events following vaccination of dogs that did or did not receive a *Leptospira* vaccine. *JAVMA*. 2015;247(10):1139-1145.
34. Eschle S, Hartmann K, Rieger A, et al. Canine vaccination in Germany: A survey of owner attitudes and compliance. *PloS One*. 2020;15(8):e0238371.
35. Schuller S, Francey T, Hartmann K, et al. European consensus statement on leptospirosis in dogs and cats. *J Small Anim Pract*. 2015;56(3):159-179.
36. 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(21):517.