Topical Review

Seroprevalence of Leptospirosis in Working Dogs

S.F. Lau, DVM, PhD\textsuperscript{a,*}, J.Y. Wong, DVM\textsuperscript{a}, K.H. Khor, DVM, PhD\textsuperscript{a}, M.A. Roslan, DIBM\textsuperscript{b}, M.S. Abdul Rahman, DVM\textsuperscript{b}, S.K. Bejo, DVM, PhD\textsuperscript{b}, R. Radzi, DVM, PhD\textsuperscript{a}, A.R. Bahaman, DVM, PhD\textsuperscript{b}

Keywords: working dogs leptospirosis vaccination microscopic agglutination test handlers

\textsuperscript{a}Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia

\textsuperscript{b}Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia

*Address reprint requests to Seng Fong Lau, DVM, PhD, Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia. E-mail: lausengfong@hotmail.com (S.F. Lau)

Introduction

Working dogs are a group of canine animals trained to perform various tasks to assist human beings, such as guard dogs trained to protect property, rescue dogs that are used in rescue operations, and search dogs to detect for drugs, explosives, and others. Not only are they trained in protecting human beings but they can also aid people with disabilities such as blindness. Man’s oldest animal friend has come to help humans in remarkable ways. The handlers or caretakers of working dogs are very careful about the health and welfare of their dogs, and ensure that their dog’s physical health is optimal. The human-animal bond is pivotal to the modern working dog team, and dogs provide much more than just work to their human partners.

Leptospirosis is one of the most widespread zoonotic diseases worldwide, commonly occurring in tropical and subtropical regions.\textsuperscript{2} It produces a wide array of clinical signs in both human and animals, ranging from undifferentiated mild fever to multi-organ failure.\textsuperscript{3} The clinical features of canine leptospirosis can either be subclinical or clinical.\textsuperscript{1} Common clinical signs include lethargy, anorexia, and vomiting, and the clinical signs are believed to be related to different serovars, geographic area, pathogenicity, and immunity of host. For example, dogs infected with serovar Pomona were more likely to suffer from vomiting due to renal failure.\textsuperscript{4} Unfortunately, none of these symptoms is specific to the disease and diagnosis based solely on clinical signs is not sufficient. Therefore, accurate diagnosis is essential.

Methods used for the diagnosis of leptospirosis includes the microscopic agglutination test (MAT), polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA) test kits. In animals, this disease is always under-diagnosed, especially in developing countries due to economic burden, lack of facilities, and cultural barriers.\textsuperscript{7,8} The first canine leptospirosis in Malaysia was reported in 1928, a Hebdomadis serovar.\textsuperscript{9} Since the 1980s, 37 Leptospira serovars had been isolated from both animals and humans in Malaysia.\textsuperscript{10} The most recent studies on the seroprevalence of canine leptospirosis in selected dog populations from Klang Valley, Malaysia, revealed that the most common serovars were Canicola (15.8%, 3 out of 19 dogs with renal disease), Bataviae (3.8%, 3 out of 80 sheltered dogs), and Icterohaemorrhagiae (5.3%, 1 out of 19 healthy pet dogs).\textsuperscript{11,12} The evidence of seropositivity of leptospirosis among dog populations is of public health concern due to the increasingly close contact between reservoir dogs and human pet-owners, veterinarians, animal handlers, and people employed in animal husbandry related jobs. Although the working dog population belongs to the high-risk group, the seroprevalence of leptospirosis, to the best of our knowledge, has never been investigated in this population before. This group of dogs is generally assumed to be well protected against leptospirosis, as they are vaccinated yearly. However, this population of dogs actually work in high-risk environments, and the likelihood of them acquiring leptospirosis is in fact higher.

Therefore, the current preliminary study may help in elucidating the current status of canine leptospirosis in the working dog population in Malaysia. These data would also provide veterinarians with a general estimation of the spatial distribution, and trend of this infectious disease among the working dog
population, and to determine whether it is of public health concern especially to handlers. The objectives of this study are to investigate the prevalence of canine leptospirosis among the working dog population in Malaysia and to determine the most common Leptospira serovar in infected dogs. From the results obtained, appropriate preventive measures can be recommended for the future.

Materials and Methods

Blood Sample Collection

Five representative canine units from different government agencies in Malaysia were recruited in this study. Before commencing the study, approval from both the Institutional Animal Care and Use Committee (UPM/IACUC/FYP.2015/FPV.005) and government agencies were obtained. The dogs were manually restrained by the handlers or caretakers from the canine units for blood collection. Approximately 3–5 ml of blood was collected via cephalic or saphenous venipuncture. Blood samples obtained were immediately transferred into plain and ethylenediaminetetraacetic acid (EDTA) tubes. Information of the working dogs, such as name, sex, breed, age, vaccination status, and medical history, were recorded. All blood samples were stored in polystyrene ice boxes with ice packs, and immediately transported to the Bacteriology Laboratory in Faculty of Veterinary Medicine, University Putra Malaysia. The blood samples in plain tubes were immediately centrifuged at 4000 rpm for 5 minutes. After centrifugation, both serum and plasma samples were isolated and transferred into 1.5 ml Eppendorf tubes and then stored at –20°C for further analysis.

Microscopic Agglutination Test (MAT)

Eleven serovars of live leptospires were subcultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium, and incubated at 30°C for 5–7 days. The serovars tested included Icterohaemorrhagiae, Canicola, Pomona, Grippotyphosa, Australis, Bataviae, Javanica, Tarassovi, Hebdomadis, Lai, and Pyrogenes. These live leptospires were examined under a dark microscopic before application for the MAT.

Serial dilutions of the sera samples, negative and positive controls were prepared in the sterile microtitre plate wells. A total of 14 samples were loaded into each microtitre plate, including a positive and negative control, and a serial dilution up to 1:640. All wells were filled with 50 μl of phosphate buffer saline (PBS). Hyperimmune serum was used as a positive control. Serial dilutions were carried out by pipetting 50 μl starting from the first column until 12th column. The last dilution was 1:640, and the 50 μl dilution was discarded. These steps were repeated for all the serum samples. In total, 50 μl of live leptospiral solutions were loaded into each well, and subsequently incubated at 37.1°C for 2 hours. After incubation, all the serum sample solutions were placed onto glass slides and examined under a dark-field microscope. Any evidence of microscopic agglutination was determined until the highest antibody titer for each serum sample. The cutoff point was determined at 1:80. If >50% agglutination was detected at this cutoff point or more, it was recorded as positive.

Polymerase Chain Reaction (PCR)

DNA extraction from blood plasma was carried out using the QIAamp DNA blood Mini Kits (QIAGEN, Germany) according to the methods described in a previous study. Through PCR detection of the Leptospira gene, all the 96 blood samples of working dogs were negative for both pathogenic and nonpathogenic Leptospira strains.
antibodies against *Leptospira* serovar, either from a recent natural infection, past exposure, or recent vaccination.

Current diagnostic tools available for leptospirosis include direct dark-field microscopy examination of blood, bacteriological culturing, polymerase chain reaction (PCR), isothermal methods, IgM or IgG enzyme-linked immunosorbent assay (ELISA), immuno-fluorescence assay, macroscopic or rapid slide agglutination test (MSAT or RSAT), indirect hemagglutination assay (IHA), complement fixation test (CF), and lateral flow assay (LFA).20,22 Selecting a diagnostic test depends on several factors, such as diagnostic accuracy (sensitivity, specificity, and predictive value), financial feasibility, technical or practical feasibility, and whether early or rapid results are required.21 Both the leptospiiral culture and MAT are still used as the gold standard for leptospirosis diagnosis. Leptospiiral culture is less practical in clinical diagnosis, when rapid results are required, as Leptospires are slow-growing bacteria and require longer time for culturing. MAT, however, is used to assess the antibody titers of serum samples against *Leptospira* from different serovar groups.23 It has been recommended that, a paired serum sample at 14 days interval be used, as a four-fold or higher antibody titer will help in diagnosing leptospirosis.23 The cutoff antibody titers used in different countries varies, ranging from 1:20 to 1:3200, depending on the condition of infection, or whether Leptospirosis is endemic in the country.24,25 The MAT, however, is not useful for early diagnosis as anti-Leptospira antibodies can only be detected by the second week after exposure to infection. Other limitations of MAT is that it is time consuming, can be potentially hazardous to laboratory workers, requires well-trained personnel, and repeated subculturing of many strains of *Leptospira*.21 Both MAT and ELISA cannot be used to differentiate between vaccine antibodies and natural infection. In addition to that, vaccinated dogs developed variable seroconversion after vaccination, and there were no correlation between postvaccination titers and protection. Previous study reported that dogs vaccinated with a bivalent vaccine developed MAT titers < 1:80, which lasted only 1–4 months, but in another study, dogs vaccinated with quadrivalent vaccine developed MAT titers > 1:1600. Hence, there is an urgent need to improve current diagnostic methods for investigating leptospirosis and differentiating vaccine antibodies and natural infection.26,27

A low titer (< 1:80) against different *Leptospira* serovars was detected in 8 dogs. However, healthy dogs with detectable levels of antibodies must be interpreted carefully, and the interpretation of the results highly depends upon the endemicity of the disease in an area as well as the vaccination history. The possibility of previous exposure in this group of dogs cannot be ruled out either. The lower titers can be interpreted as antibody cross-reactivity between serovars.25 Cross-reaction between leptospires of the same serogroup might occur, as reported in a previous study of seroprevalence of cattle leptospirosis. The authors reported that infection by *L. balaenica* led to a false-positive *L. hardjo* reaction.25 According to Gautum,25 cross-reaction between *Leptospira* serovars could occur due to the production of both IgM and IgG antibodies that can agglutinate to multiple leptospira serovars. Cross-protection with the use of commercial vaccines for Canicola and Icterohaemorrhagiae serovars has been reported, but not for other serovars.25 Therefore, a low cutoff antibody titer used in MAT may overestimate seroprevalence of canine leptospirosis and the prevalence of clinical leptospirosis. According to Klaasen33 and Schreiber,34 the duration of immunity of vaccinated dogs for *Leptospira* can last up to 13 months, but is not necessarily associated with the presence of circulating anti-*Leptospira* antibodies, 15 weeks postvaccination. The vaccinated dogs were protected from leptospiiral infection despite of its low or even undetectable levels of agglutinating serum antibodies.33

**Discussion**

Dogs are a good indicator of the distribution of different leptospiral serovars in nature. Therefore, occasional serological screening of dogs is warranted, to detect possible serovar changes and its distribution, despite the fact that the prevalence of canine leptospirosis has decreased worldwide with the use of bivalent vaccines.12 Various countries have reported the re-emergence of canine leptospirosis, mostly due to changes in the infecting serovars in the last decade, indicative that, most of the infections were caused by *Leptospira* serovars which are not covered by the vaccine.13 In Malaysia, there is a paucity of information regarding the current circulating serovars, among the dog population. Most of the dogs are vaccinated with Pomona, Icterohaemorrhagiae, Canicola, and Grippotyphosa serovars, based on a protocol adopted from other countries. A vaccination protocol should be based on the major serovars causing infection in a local setting. Different serovars are known to adapt to specific reservoir or maintenance hosts and vary according to the geographical location.17 Dogs in Europe are potentially exposed to the serogroups Icterohaemorrhagiae (maintenance host = rat), Australis (maintenance host = wildlife), Canicola (maintenance host = dog), Grippotyphosa and Sejroe (maintenance host for both serogroups = rodents). The incidence of the latter 2 groups varies with the presence of the maintenance host. More recent studies in Germany and Greece have identified antibodies against the serogroup, Pomona, in ill dogs.

Ward reported a higher incidence of leptospirosis in middle-aged male dogs (between 4- and 10-year-olds). He suggested that, male middle-aged dogs and large-breed dogs were usually more active outdoors, and this could increase their risk to exposure to *Leptospira* serovars. Working dogs, as compared to the pet dog population, have a higher risk of infection to leptospirosis. All the seropositive dogs belonged to the search and rescue group that work outdoors. Their jobs include, searching for lost victims in the forest, walking along riverbanks, damp soil, sniffing cadavers, rescuing victims trapped in collapsed soil structures and landslides, as well as others. All of these activities can potentially expose working dogs to water and soil contaminated with *Leptospira*. Leptospires is known to survive for several months in the environment under favorable conditions.19 One of the working dog units in this study reported an outbreak of leptospirosis in 2013. One survivor tested positive using MAT (1:80), for serovar Australis, and might act as carrier. As a limitation in this study, the sera samples were only analyzed in 1 laboratory, and no follow-up had been done in all these 3 dogs that were considered positive based on the cutoff point at 1:80. Hence, in the current study, a positive MAT result was indicative of the presence of circulating antibodies against *Leptospira* serovar, either from a recent natural infection, past exposure, or recent vaccination.
The PCR technique was used to amplify leptospiral DNA, for the detection in blood and urine samples. It is important to detect pathogenic *Leptospira* genes, such as Lpl 32, Lig A, and Lig B, and is effective for early diagnosis with clinical symptoms. According to Harkin, the sensitivity and specificity to detect *Leptospira* genes from urine samples through PCR can be as high as 100% and 88.3%, respectively. In contrast, the PCR assay has a 90% sensitivity in the first 5 days of illness, and greatly decreases to 50% if blood samples are used. Since the dogs in this study all appeared healthy with no clinical signs, the negative PCR results were not unexpected. Urine samples should be used in the future study as it has higher sensitivity in comparison to the blood samples through PCR.

Owing to the widespread nature of this zoonotic disease, Leptospirosis is of public health concern. Therefore, it is important to determine the serovars that infect the dog population, as office handlers or caretakers are constantly in contact with these animals. According to Masuzawa, the seroprevalence of *L. australis* and *L. javanica* among human beings in Philippines was 8.5% and 1.4% (n = 71), respectively. In addition, the seroprevalence of *L. australis* and *L. javanica* has also been detected among dairy farm workers in India, which was approximately 12.8% and 17.9% (n = 41), respectively. *L. batavia* was also reported causing tuberculosis in 2 children. The seropositive results found in the current study, among the working dog population, suggests that humans are potentially at risk of exposure, and the possibility of direct or indirect transmission of leptospirosis from dogs to human cannot be ruled out.

In addition to the routine annual vaccination, chemoprophylaxis using doxycycline might be useful in preventing and controlling canine leptospirosis among working dogs. The advantages of this preventive measure have been reported by the U.S. military. Strict kennel sanitation, isolation of infected dogs, and rodent control are appropriate strategies to reduce the spread of leptospirosis. Routine herd screening of leptospirosis using MAT and PCR assay for working dogs should be conducted to monitor the eradication of other serovars, and to provide early diagnosis for immediate and appropriate antimicrobial treatment.

### Conclusion

The seroprevalence of canine leptospirosis among the working dog population in Malaysia was 3.1% (n = 3/96). The pathogenic infecting serovars detected include, *L. australis, L. bataviae, and L. javanica*. To the best of our knowledge, this study is the first to report the seroprevalence of leptospirosis in working dogs from Malaysia. Effective preventative and control protocols for leptospirosis should be implemented in order to maintain a healthy condition in both dogs and handlers.

### Acknowledgments

The authors would like to thank the staff from the Bacteriology Laboratory of the Faculty of Veterinary Medicine, Universiti Putra Malaysia, for their assistance in processing the samples and technical help.

### References

15. Ellis WA. Control of leptospirosis in canine Europe: time for a change? *Vet Rec* 167:602–605, 2010


