Update on the Management of Leptospirosis

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Abstract. In this lecture, the attendees will be given an update on the most important new information concerning leptospirosis in dogs and cats. Emphasis will be placed on how to recognize, diagnose, and treat the infections. We will discuss why *Leptospira* spp. vaccination is almost "core", including for small breed dogs. The objectives are

1. To review the manifestations of leptospirosis in dogs and cats.
2. To understand how to use serology and PCR assay results to diagnose leptospirosis.
3. To learn the optimal treatment plan for leptospirosis.
4. To understand the importance of *Leptospira* spp. vaccination.

Keywords. Leptospira, serovar, ELISA, polymerase chain reaction, vaccine

Etiology and epidemiology. Leptospires are motile, filamentous spirochetes that infect animals and human beings. Leptospirosis can be caused by many different serovars of *Leptospira interrogans* and *Leptospira kirschneri* (Sykes et al, 2011). Seropositive dogs have been detected in many countries, and the most prevalent serovars vary by country and regions within countries (White et al, 2017). In the United States, antibodies against *L. autumnalis, L. bratislava, L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae,* and *L. pomona* have been detected most commonly. One study based on PCR analyses of *Leptospira* spp. DNA in urine from dogs in the United States showed *L. grippotyphosa* in 78 if the 98 positive samples (Harkin and Hays, 2016). Recently, the *L. santarosai* serogroup Sejroe was amplified from healthy dogs in Sao Paulo (Miotto et al, 2018). The *Leptospira* spp. that infect cats are less clear and cats appear to be more resistant to clinical disease than dogs.

Prevalence and risk factors for cases of canine leptospirosis have been evaluated in several studies. In the United States the number of seropositive dogs increased between 2002 and 2004 (Moore et al, 2006). *Leptospira* spp. exposure can be common in the United States; 8.1% of 33,119 canine serum samples had titers greater than 1:1600 in one study (Gautam, 2010). Infection by *Leptospira* spp. occurs in both rural and suburban environments in semitropical areas of the world with alkaline soil conditions. In one study in Kansas, an association between leptospirosis in dogs and urban environments was made, so leptospirosis should be considered in all appropriate clinical situations (Raghavan, 2011). Exposure to water outdoors, wetlands, and public open spaces were identified as risk factors in one case-control study (Ghneim et al, 2006). Clinical cases are most commonly diagnosed in the summer and early fall, and numbers of cases often increase in years with heavy rainfall.

Infection by host-adapted species results in subclinical infection; the host acts as a reservoir, shedding the organism intermittently. Infection by non-host-adapted species results in clinical illness. *Leptospira* spp. are passed in urine and enter the body through abraded skin or intact

mucous membranes. Transmission also occurs through bite wounds; by venereal contact; transplacentally; and by ingestion of contaminated tissues, soil, water, bedding, food, and other fomites. In an experimental study *L. pomona* but not *L. bratislava* was successfully transmitted by conjunctival inoculation and resulted in fever and lethargy starting within 7 days (Greenlee et al, 2005). Hosts with preexisting antibody titers usually eliminate the organism quickly and remain subclinically infected. *Leptospira* spp. replicate in multiple tissues of nonimmune hosts or hosts infected by a non-host-adapted species; in the dog, the liver and kidneys develop the highest levels of infection. Inflammation induced by organism replication and production of toxins leads to renal, hepatic, or pulmonary disease. Dogs that are treated or develop appropriate immune responses usually survive. Some animals clear the infection 2 to 3 weeks after exposure without treatment but develop chronic active hepatitis or chronic kidney disease. Cats are generally subclinically affected but may shed the organism into the environment for variable periods after exposure and occasionally develop polyuria, polydipsia, and renal insufficiency (Arbour, 2012; Shropshire et al, 2016)).

Clinical findings. Dogs of any age, breed, or gender can develop leptospirosis if not previously immune. Male, middle-aged, herding dogs; hounds; working dogs; and mixed-breed dogs were at greater risk than companion dogs younger than 1 year in one study (Ward et al, 2002). But more recently, small breed dogs were over-represented (Lee et al, 2014). Most dogs have subclinical infection. Dogs with peracute clinical disease are usually presented for evaluation of anorexia, depression, generalized muscle hyperesthesia, tachypnea, and vomiting. Fever, pale mucous membranes, and tachycardia are usually present. Petechiae, ecchymoses, melena, and epistaxis occur frequently from thrombocytopenia and disseminated intravascular coagulation. Peracute infections may rapidly progress to death before marked renal or hepatic disease is recognized.

Fever, depression, and clinical signs or physical examination findings consistent with hemorrhagic syndromes, hepatic disease, renal disease, or a combination of hepatic and renal disease are common in subacutely infected dogs. Conjunctivitis, panuveitis, rhinitis, tonsillitis, cough, and dyspnea occur occasionally. Oliguric or anuric renal failure can develop during the subacute phase. Clinical findings can vary on the basis of the infecting serovar (Goldstein et al, 2006). The pulmonary hemorrhagic syndrome described in people is likely to occur in dogs as well, so leptospirosis should be on the differential list for dogs with dyspnea (Klopfleisch, 2010).

Some dogs that survive peracute or subacute infection develop chronic interstitial nephritis or chronic active hepatitis. Polyuria, polydipsia, weight loss, ascites, and signs of hepatic encephalopathy secondary to hepatic insufficiency are the most common manifestations of chronic leptospirosis.

Diagnosis. Multiple nonspecific clinicopathologic and imaging abnormalities occur in dogs with leptospirosis and vary depending on the host, the serovar, and whether the disease was peracute, subacute, or chronic. Leukopenia (peracute leptospiremic phase), leukocytosis with or without a left shift, thrombocytopenia, regenerative anemia (from blood loss), or nonregenerative anemia (from chronic renal or hepatic disease) are common hematologic abnormalities. Hyponatremia; hypokalemia; hyperphosphatemia; hypoalbuminemia; hypocalcemia; azotemia; hyperbilirubinemia; decreased total carbon dioxide concentrations; and increased activities of

alanine transaminase, alkaline phosphatase, and aspartate transaminase are common serum biochemical abnormalities that develop from renal disease, hepatic disease, gastrointestinal losses, or acidosis. Hyperglobulinemia is detected in some dogs with chronic leptospirosis. Dogs with myositis may have increased creatine kinase activity. Urinalysis abnormalities include bilirubinuria, suboptimal urine specific gravity in the face of azotemia, granular casts, and increased numbers of granulocytes and erythrocytes. The organism is not seen in the urine sediment by light microscopy. Renomegaly, hepatomegaly, and interstitial or alveolar pulmonary infiltrates are common radiographic abnormalities. Mineralization of the renal pelves and cortices can occur with chronic leptospirosis. On histopathologic evaluation of renal tissues, mesangial proliferative glomerulonephritis with or without interstitial nephritis were the most common lesions in one study (Ortega-Pacheco, 2011).
Detection of anti-*Leptospira* antibodies is commonly performed by a microscopic agglutination test (MAT). In some countries, there are also now 2 commercially available point of care assays (Zoetis Animal Health; IDEXX Laboratories). One of the commercial kits is optimized to detect IgM antibodies and in one study was the first to become positive in most dogs during acute infection (Lizer et al, 2018). If MAT is used, because of the wide range of leptospires infecting dogs, as many serovars as possible should be used for screening. *Leptospira bratislava, L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae,* and *L. pomona* are commonly used. Positive titers can result from active infection, previous infection, or vaccination. Antibody titers can be negative in animals with peracute disease; seronegative dogs with classic clinical disease should be retested in 2 to 4 weeks. The serovar with the highest titer is usually considered the infecting serovar, but this should be interpreted cautiously. When the same sera were sent to different laboratories, the results were not always in agreement for the serovar giving the highest titer (Miller et al., 2011) and some vaccinated, client-owned dogs have high titer responses (Martin et al, 2014).

Documentation of seroconversion (negative result becoming positive over time), a single microscopic agglutination test titer greater than 1:3200, or a fourfold increase in antibody titers combined with appropriate clinicopathologic abnormalities and clinical findings, are suggestive of clinical leptospirosis. A definitive diagnosis is made by demonstrating the organism in urine, blood, or tissues. The organism can be seen in urine using darkfield or phase-contrast microscopy, but because of intermittent shedding of small numbers of organisms these procedures can be falsely negative. The organism can be cultured from urine collected by cystocentesis, blood, or renal or hepatic tissue but this is of low sensitivity. Materials for culture should be collected before administration of antibiotics, placed in transport media immediately after collection, and transported to the laboratory as quickly as possible. Leptospiremia can be of short duration, and urine shedding of the organism can be intermittent, giving false-negative results. PCR assay can be used to amplify DNA of the organism in urine, blood, or tissues (Harkin et al., 2003a, 2003b). In one study of 500 dogs, 41 (8.2%) were PCR positive for a *Leptospira* spp. in urine, and some of these dogs were clinically normal (Harkin et al., 2003a). None of the PCR-positive dogs was culture-positive, and titers were not always high. *Leptospira* spp. DNA can also be amplified from renal tissues that have no evidence of inflammatory disease (Dash et al, 2018). Recent vaccination should not result in positive PCR assay results (Midence 2012). Antibodies induced by Leptospira spp. vaccine did not cross react with *Borrelia burgdorferi* peptides in one study (Caress et al, 2017).

Treatment. Fluid therapy is required for most dogs; intense diuresis for renal involvement may be required. Hemodialysis may increase the probability of survival in dogs with oliguric or anuric renal failure. Dogs should be treated during the initial treatment period with ampicillin administered intravenously at 22 mg/kg q8h. Some quinolones have an effect against leptospires and can be used in combination with penicillins during the acute phase of infection, in particular if other gram negative organisms are on the differential list. Ampicillin and enrofloxacin were used concurrently in one study, and 83% of infected dogs survived (Adin et al., 2000). Penicillins such as amoxicillin or amoxicillin clavulanate should be administered for 2 weeks. Doxycycline administered orally at 5 mg/kg q12h for 2 weeks should be used to eliminate the renal carrier phase (Sykes et al, 2011).

Zoonotic aspects and prevention. All mammalian serovars should be considered potentially zoonotic to human beings. Some human beings have antibodies against canine serovars, suggesting the dog can be a reservoir for human infection (Brod et al., 2005). However, results from studies attempting to associate dog contact with leptospirosis in humans have varied. For example, 0/91 people exposed to dogs with proven leptospirosis were seropositive suggesting the risk was minimal (Barmettler 2011). As leptospirosis is an occupational risk for veterinarians, the organism should be on the list of differential diagnoses if appropriate clinical signs of disease develop (Whitney 2009). Infected urine, contaminated water, and reservoir hosts should be avoided. Infected dogs should be handled with the clinician wearing gloves. Contaminated surfaces should be cleaned with detergents and disinfected (see Chapter 93).

To lessen risk of exposure, owners should attempt to restrict dogs from drinking potentially contaminated water. Healthy dogs can be shedding *Leptospira* spp. in urine; 7% of 525 urine samples from dogs in Dublin were positive in one study (Rojas 2010). Thus, contact with dog urine should always be avoided. Vaccines available for some serovars reduce the severity of disease and lessen leptospire shedding in urine. Several products containing serovars *L. canicola, L. icterohaemorrhagiae, L. grippotyphosa,* and *L. pomona* are now available and should be used rather than two serovar vaccines to provide the greatest spectrum of protection (see Chapter 93). Numbers of long term duration of immunity studies are small, but recently, one commercially available product was should to induce significant protection against *L. grippotyphosa* for 15 months (Grosenbaugh and Pardo, 2018). Dogs in endemic areas should be administered at least 2 vaccines 2 to 4 weeks apart and annual boosters are recommended (www.aahanet.org). Canine side effects associated with the current 4 serovar containing vaccines are generally transient and mild (Spiri et al, 2017; Yao et al, 2015). In one study, hypersensitivity reactions to *Leptospira* spp. vaccines was estimated at 6.5/10,000 vaccinated dogs (Yao et al, 2015).

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