

CANINE LEPTOSPIROSIS: PRIME SUSPECT

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Is Leptospirosis a Re-emerging Disease?

Over recent years, there has been published evidence that leptospirosis is an increasing threat across the USA. Serological surveys have documented that the most commonly identified antibodies are against serovars Autumnalis, Grippityphosa, Pomona and Bratislava (Gautam, et al., 2010), although this does not necessarily reflect the true prevalence of these serovars because of cross-reactivity between serovars. One study reported that 24.9% of healthy dogs from Michigan had significant *Leptospira* titers indicating exposure, with serovar Grippityphosa being the most common. Interestingly, there was no breed predilection for seropositivity to *Leptospira* in that study, and breeds ranging from German Shepherds (12.5%) to Yorkshire Terriers (11.0%) had positive MAT results (Gautam, et al., 2010).

There was also a significant increase in the proportion of positive *Leptospira* microscopic agglutination tests (MATs) in a prevalence study of 23,005 US dogs over 2002-2004 (Moore et al., 2006). Additionally, a survey of US and Canadian veterinary teaching hospitals between 1983 and 1998 reported a significant increase in canine leptospirosis prevalence (Ward et al., 2002).

There have been various hypotheses proposed to explain the recent increase in prevalence. More frequent testing could explain some of the increase, but the main reason is likely to be urban sprawl resulting in increased exposure to wildlife reservoir hosts (Ward et al., 2004). One Connecticut study of peridomestic wildlife showed exposure to leptospirosis in 36% of raccoons (*Icterohaemorrhagiae*) and 13% of skunks (*Grippityphosa*; Richardson, 2003), while another Illinois study reported that 222/459 (48%) of raccoons were seropositive for leptospirosis (Mitchell et al., 1999). Wildlife studies have revealed that nearly 50% of rats, coypus (*nutria*) and muskrats are seropositive and almost 30% of rats and muskrats are renal carriers for serovars *Icterohaemorrhagiae* and *Grippityphosa*, respectively. Additionally, the initial 2-serovar bacterin vaccine was not broadly protective, so vaccinated dogs were still at risk of infection.

***Leptospira* Serovars**

Serovar classification is based on the expression of lipopolysaccharide (LPS) surface epitope antigens, which is the main antigen for *Leptospira*. Serovars are differentiated by biochemical tests. Antigenically related serovars are grouped into serogroups. Information on serovars is useful for epidemiological understanding, but relationships between serovars and clinical presentation or severity of disease are not well characterized in the veterinary literature. However, one recent study reported significant associations between serovar *Grippityphosa* and renal disease and serovar *Icterohaemorrhagiae* and hepatic involvement (Tangeman and Littmann, 2013). Additionally, another study reported that dogs infected with serovar *Pomona* had more severe renal disease and were statistically less likely to be discharged from hospital alive (Goldstein et al., 2006). Serovar *Canicola* is highly adapted to dogs, but it is thought from experimental studies that, given time, kidney disease and chronic shedding eventually develop.

Epidemiology of *Leptospira*

Leptospirae are thin, flexible, spiral bacteria that are directly transmitted by contact with infected urine and intact mucous membranes, abraded skin, or skin that has been immersed in water for a prolonged time. Indirect transmission can also occur via contaminated water sources, soil, food, or contaminated bedding. Stagnant or slow-moving warm water provides ideal environmental conditions for *Leptospira*, and increased incidence has been reported after high rainfall or flooding. There is

Increased seasonal incidence during summer/late fall. During periods of drought, infection is more common around water sources. Leptospire remain viable for several months in moist soil and temperatures of 0-25 °C favor survival; freezing markedly decreases survival.

Pathogenesis of Leptospirosis

Organism numbers are highest in blood for the first 10 days after infection. Subsequently, the highest concentration of organisms is found in the urine. The incubation period is typically 7 days, but can range from 2 to 30 days. The severity of clinical disease is dependent on the infecting strain, number of organisms, and host age and susceptibility. The initial IgM antibody response develops within 1 week, increasing for up to 14 days before decreasing in favor of a rising IgG antibody-mediated response.

Clinical Signs of Leptospirosis

While many practitioners consider leptospirosis in dogs with acute renal failure and/or signs of hepatopathy, leptospirosis has also been responsible for pulmonary and hemorrhagic disease. Fever, uveitis, meningitis and immune-mediated disease have also been associated with leptospirosis. Laboratory abnormalities can include proteinuria, thrombocytopenia, coagulopathy, hypoalbuminemia and glucosuria (Tangeman and Littman, 2013).

There are four main forms of clinical leptospirosis –

1. **Peracute** – Affected animals can experience massive leptospiremia and death with few preliminary signs
2. **Acute** – This is characterized by fever, muscle pain, vomiting, dehydration, vascular collapse, tachypnea, hematemesis, hematochezia, melena, epistaxis, petechiae, icterus, and/or pain on renal palpation.
3. **Subacute** – In subacute infection, there can be fever, decreased appetite, vomiting, dehydration, polydipsia/polyuria, paraspinal hyperesthesia, petechial or ecchymotic hemorrhages, uveitis, icterus, coughing, and/or dyspnea.
4. **Chronic** – Chronic infection can be associated with uveitis, fever, progressive deterioration in renal or hepatic function, PD/PU, weight loss, and/or anorexia.

DIAGNOSIS OF LEPTOSPIROSIS

Serology - Antibody Testing

Point-of-Care Tests

There are two commercially available point-of-care tests for the detection of antibodies against *Leptospira* in dogs. WITNESS® Lepto is an IgM-based test designed to detect acute infection 4-6 days after the onset of clinical signs. Since IgM is only transitionally produced after vaccination, it will not produce false-positive results after vaccination for as long as other antibody-based detection methods (Winzelberg et al., 2015; Lizer et al., 2017; Table 1); vaccinated dogs test negative with WITNESS Lepto 3-6 months post vaccination. SNAP® Lepto detects antibodies against LipL32, a major outer membrane protein of *Leptospira*. The antibody Ig class detected is not specified (Curtis et al., 2015; Winzelberg et al., 2015).

Table 1: Agreement with Clinical Diagnosis and Post-Vaccination Positive Results (1. Lizer et al., 2017; 2. Winzelberg et al., 2015)

	WITNESS® Lepto ¹	SNAP® Lepto ²	MAT ²
First specimen from dogs clinically diagnosed with acute leptospirosis	89.7% positive test results (87/97)	80% positive test results overall	78% positive test results overall
Convalescent specimen from dogs clinically diagnosed with leptospirosis (12-19 days after first specimen)	100% positive test results (9/9)		
Specimen from healthy dogs vaccinated 4 weeks earlier	64% (16/25) False positives due to vaccination	79% (22/28) False positives due to vaccination	100% (28/28) False positives due to vaccination

Microscopic Agglutination Test (MAT) Titers

The MAT is the most commonly used test to diagnose canine leptospirosis. The test detects antibodies against specific serovars, by mixing serial dilutions of sera with cultured *Leptospira* organisms of different serovars representing different serogroups. The resulting titer is the highest dilution of the sera that causes 50% agglutination. Agglutination is visualized using dark field microscopy. Antibody titers become positive in about 1 week, peak in 3-4 weeks and remain elevated for months. If the titer is <1:800, retesting in 2-3 weeks is recommended; a four-fold increase in MAT titer is generally demonstrated in infected dogs. Low titers can be the result of an acute infection (in the first 7-9 days after initial infection); a less pathogenic strain; or antibiotic treatment. Generally, a single titer >1:800 in an unvaccinated dog with compatible clinical signs is adequate for the diagnosis of leptospirosis, although some workers feel that titers >1:1600 or ≥3200 represent a more reliable threshold for a positive test. One recent study demonstrated that: (1) dogs vaccinated with *Leptospira* vaccines have variable MAT titers over time; (2) antibodies should not be used to predict resistance to *Leptospira* infection; and (3) MAT titers ≥1 : 800 can develop after *Leptospira* spp. vaccination (Martin et al., 2014).

There are a number of challenges with the interpretation of MATs. A positive *Leptospira* antibody titer does not necessarily mean that the dog has clinical leptospirosis, since many canine *Leptospira* infections are subclinical; therefore, seroprevalence studies that only include dogs with clinical disease might underestimate exposure rates in the canine population as a whole. Additionally, vaccination can also produce detectable antibody titers. Cross-reaction between serovars in infected dogs can make identification of the infecting serovar difficult. For example, in one study of naïve puppies vaccinated against four serovars, the MAT titers were often highest to the nonvaccinal serovar Autumnalis. Therefore, high titers to a specific serovar may not definitively identify the causative serovar, because of cross-reactive antibodies. In a human study where urine cultures and MAT results were compared, the MAT accurately predicted serovar in only 46% of cases. Information on the infecting serovar is not crucial, as the disease appears to be similar and the treatment is identical, regardless of serovar. Serovar information is important mainly from an epidemiologic perspective. Additionally, intra- and inter-laboratory variability can be problematic, since there is no standardization for MAT methodology.

Polymerase Chain Reaction (PCR) Testing

Kansas State University Veterinary Diagnostic Laboratory offers PCR testing on urine and IDEXX Laboratories offer PCR testing on urine and blood. PCR allows identification of early infections prior to seroconversion and helps evaluate dogs for urinary shedding. While PCR testing is significantly more sensitive than culture and MAT for identifying dogs shedding leptospires and for diagnosing infection, it does not identify *Leptospira* to a serovar level and it cannot be used to rule out *Leptospira* infection. PCR testing has no cross reactivity with commercially available vaccines.

Visualization of *Leptospira* organisms

Bacterial Culture

If bacterial culture is to be attempted, suitable specimens need to be submitted early after infection, since leptospiremia occurs early, often before clinical signs appear, and leptospire have moved out of the blood and into the urine by the end of the first week of acute illness. Special techniques are required for culture and blood is inoculated a semi-solid medium containing 5-fluorouracil. Obtaining multiple cultures and using media dilutions may increase recovery.

Microscopic Demonstration

Dark-field microscopic examination is required to visualize leptospire. Suitable specimens are infected blood, urine, CSF or peritoneal dialysate fluid, but sensitivity and specificity are low.

Histopathology

Leptospire can be visualized in infected tissue sections using silver staining or immunohistochemical stains.

Ancillary Testing - Diagnostic Imaging

Pulmonary hemorrhage in infected dogs can result in an interstitial pattern on thoracic radiographs, especially in the caudal dorsal lung fields, which can progress to a nodular alveolar pattern. On abdominal radiology, there can be renomegaly, hepatomegaly, and/or abdominal effusion. Abdominal ultrasound can reveal renomegaly, perinephric effusion, increased cortical echogenicity, and/or a 'medullary rim sign'.

TREATMENT OF LEPTOSPIROSIS

The treatment of choice for leptospirosis is oral doxycycline (5 mg/kg PO q12h or 10 mg/kg q24h for 2 weeks), as only doxycycline will clear leptospire from the kidneys. However, some dogs cannot tolerate oral doxycycline, especially in the early stages of infection, so IV ampicillin (22 mg/kg q8h) or oral amoxicillin (22 mg/kg q12h) can be used initially. Therapy should switch to oral doxycycline as soon as the dog can tolerate it.

Supportive care is vital and general management of acute renal failure or liver failure should be instituted promptly. Management for acute renal failure includes IV fluid diuresis (hemodialysis an option for some oliguric or anuric dogs); close monitoring of volume of fluids administered and urine output; electrolyte supplementation to correct any electrolyte or acid-base abnormalities; mannitol, furosemide, dopamine can be considered for oliguric or anuric dogs; blood pressure monitoring; use of H2 blockers and anti-emetics if there is vomiting; nutritional support if it does not result in vomiting, possibly with concurrent use of phosphate binders.

Prognosis

Published data is limited and survival rates are variable, depending on the manifestations of infection, severity of disease and general health status of the dog at the time of infection. Dogs with renal and/or liver failure can have high mortality rates and chronic organ disease is possible in dogs surviving the infection. One study reported that the prognosis for dogs with mild to moderate azotemia was good with conservative therapy and that treatment with hemodialysis appeared to improve prognosis for dogs with severe azotemia (Adin et al., 2000).

ZOONOTIC POTENTIAL

Leptospirosis is a zoonotic disease and it can be an occupational hazard for people who work outdoors or with animals, campers and people who participate in outdoor sports. Since there is not a

vaccine available to prevent disease in people, avoiding contact with potentially infected material is crucial. Particular care should be exercised when handling urine from dogs with renal, liver or pulmonary disease, or those with coagulopathies. Any suspect case should be handled as if it had leptospirosis until proven otherwise and communication of any potential risks to all personnel involved is paramount. One study of 500 urine samples from dogs of any health status submitted to Kansas State University Veterinary Diagnostic Laboratory reported that 8.2% of specimens contained leptospire (Harkin et.al., 2003). Interestingly, a CDC survey of 3,000 veterinarians indicated a large proportion utilize 'very poor practices when handling urine'.

Proper protection when handling potentially infected material includes wearing disposable gloves and a gown. Eye protection and a mask or full-face shield should be worn if there could be potential aerosolization of leptospire e.g. a struggling, urine-soaked dog. Hands should be washed or hand sanitizer used after contact with a potentially infected dog, the dog's environment, and after glove removal. Special care is required for immunocompromised people or pregnant women as leptospirosis can cause miscarriage. If there has been potential exposure to infectious material, the person at risk should be monitored for early signs of infection (e.g. fever, flu-like signs) and should consult their physician. The incubation period for leptospirosis is 2-20 days.

PREVENTION – VACCINATION

Immunization is effective in reducing the prevalence and severity of canine leptospirosis by preventing renal colonization and shedding (Wilson et al., 2013). With increasing prevalence of leptospirosis, there is significant risk of morbidity and mortality among unvaccinated dogs. Additionally, since leptospirosis is a zoonotic disease, there is potential risk to the owners of unvaccinated dogs, and to veterinary hospital and laboratory staff. Human exposure to leptospire from vaccinated dogs has never been documented. Annual vaccination is recommended against the four most common *Leptospira* serovars currently diagnosed in dogs – Icterohaemorrhagiae, Canicola, Grippotyphosa and Pomona.

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