IDEXX LABORATORIES

DIAGNOSTIC UPDATE

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Diagnosing and Managing Canine Leptospirosis

IDEXX Reference Laboratories Introduces a RealPCR™ Test for Canine Leptospirosis

Background

Leptospirosis, a zoonotic disease of worldwide significance, is caused by spirochetes of the genus *Leptospira*. Leptospirosis has been thought to most commonly affect young-adult, large-breed, outdoor dogs; however, small dogs in urban areas can also contract the disease. Pathogenic serovars infecting dogs include icterohaemorrhagiae, canicola, pomona, bratislava, grippotyphosa and autumnalis. Although serovar identification is of interest from an epidemiologic stand point, clinical disease is similar for all serovars and treatment is the same. Therefore, determining whether a dog has leptospirosis is much more important than identification of which specific serovar is involved.

Prevalence

The prevalence of canine leptospirosis has increased in recent years. Prevalence varies by region. Results of one study in Michigan indicated that >20% of healthy, client-owned dogs had been exposed to Leptospira serovars.[1] In another study, 8.2% of dogs were shedding pathogenic leptospires irrespective of the health status.[2] It is unknown what proportion of dogs with acute kidney disease have leptospirosis, however, given the high rate of exposure, *leptospirosis should be considered in every dog presenting with acute renal abnormalities regardless of the dog's signalment, environment or geography.*

Transmission

Infected animals shed spirochetes in their urine that subsequently contaminates the environment. Susceptible animals and humans are most often infected through contact with contaminated water. Bacteria enter through damaged skin or mucous membranes.

Clinical signs

Acute kidney disease is the most commonly recognized disease in dogs, accounting for more than 90% of reported cases of leptospirosis. Hepatic disease occurs concurrently in 10%–20% of dogs with acute renal failure but can also occur independently. Anorexia, lethargy, vomiting, polyuria and polydipsia are common signs. Icterus, fever, abdominal pain, muscle pain and stiffness, uveitis, dyspnea and coagulopathies occur as well but with less frequency.[3]

Clinicopathologic findings

Anemia, leukocytosis characterized by neutrophilia and thrombocytopenia are the most common findings on the complete blood count (CBC). Azotemia, increased liver enzymes, hyperbilirubinemia and electrolyte disturbances are the most common biochemical changes. Coagulation abnormalities, including prolongation of PT and PTT, are not uncommon. Decreased specific gravity and markers of tubular injury, including glucosuria, granular casts and low-grade proteinuria, are often present on urinalysis.[4]

Serology

Detection of antibodies using the microscopic agglutination test (MAT) has been the most common diagnostic method used for the diagnosis of canine leptospirosis.[5] There are several difficulties that confound interpretation of MAT titers. Many dogs with leptospirosis will present with clinical signs of disease prior to the development of antibodies measurable by MAT. On the other hand, there is a high prevalence of subclinical infections that result in the persistence of antibodies. Lastly, vaccination of dogs with commercially available leptospirosis vaccines will produce detectable MAT titers.

In a dog with suspect leptospirosis a single MAT titer of 1:800 or greater has been considered supportive of the diagnosis. In a recent study, however, the sensitivity of an initial titer of ≥1:800 (for any serogroup) ranged from 22%–67% and the specificity determined at this same titer was 69%–100% in ill dogs without leptospirosis.[6] Another study revealed that serology was a poor predictor of urinary shedding.[2] However, a four-fold increase or greater in MAT titer between paired acute and convalescent sera can be used to confirm the diagnosis of leptospirosis. In most cases, therapeutic decisions are needed prior to acquiring a convalescent sample.

PCR

Real-time polymerase chain reaction (PCR) is considered as sensitive and specific as culture but significantly faster (hours) and more reliable.[7] The diagnostic advantage of PCR over serology occurs primarily during the very early stages of the infection prior to the development of antibodies in most dogs and for the detection of urinary shedding in sick and healthy animals. PCR will be positive on blood

very early in infection usually prior to seroconversion. Urine will become positive 7–14 days after infection at which time leptospires may or may not be detected in the blood.

According to R. E. Goldstein, DVM, DACVIM, DECVIM (Oral communication, June 2009), a recent study performed at Cornell University College of Veterinary Medicine in collaboration with IDEXX Reference Laboratories in which the currently available bacterin-based leptospirosis vaccines were used, dogs remained PCR-negative when tested on days 3 and 7 and weekly for 8 weeks, post-vaccination.

IDEXX *Leptospira* spp. RealPCR Test

SPECIMEN REQUIREMENTS

To increase the diagnostic utility in clinically sick dogs, the IDEXX *Leptospira* spp. RealPCR Test requires both whole blood and urine samples collected prior to antibiotic administration. Serovar- specific results are not available, but a quick, reliable positive or negative PCR result for leptospirosis allows for early and accurate diagnosis and appropriate management of individual patients.

INTERPRETING RESULTS

Positive blood and negative urine RealPCR results

Dog is infected. A positive RealPCR result on whole blood in the presence of a negative urine result can occur during the first 7–14 days of infection. Leptospiraemia is detectable within days after infection. Negative urine samples can be explained by a preleptospiruria sample or intermittent shedding. Treatment is recommended. A follow-up RealPCR test in 2 weeks is recommended.

Positive blood and urine RealPCR results

Dog is infected. Positive whole blood and urine RealPCR results for *Leptospira* are rare but can occur within the first weeks of infection during an overlapping blood and tissue phase of infection. Positive urine samples are considered a source of infection for other animals and humans. Treatment is recommended. A follow-up RealPCR test in 2 weeks is recommended.

Negative blood and positive urine RealPCR results

Dog is infected. A negative whole blood result in the presence of a positive urine RealPCR result for *Leptospira* in a dog with clinical signs of infection suggests that the dog was likely infected at least 2 weeks prior to sample collection. Asymptomatic chronic carriers can also shed Leptospira in the urine intermittently for weeks to months. Positive urine samples are considered a source of infection for other animals and humans. Treatment is indicated. A follow-up RealPCR test in 2 weeks is recommended.

Negative blood and urine RealPCR results

Dog is likely not infected. A dog with negative whole blood and urine RealPCR results for *Leptospira* is likely not infected if the samples were collected prior to antibiotic therapy. However, whole blood RealPCR results are only positive early in infection and urinary shedding can be intermittent; therefore, if leptospirosis is still suspected, MAT testing (test code 712) is recommended.

WHEN TO USE

1. In all dogs with acute kidney disease unless known toxin exposure or pyelonephritis is definitively diagnosed.
2. In acutely ill dogs where MAT testing has been performed and results are negative or equivocal for infection, but leptospirosis is still suspected.
3. To identify dogs shedding leptospires in their urine. This would include testing:
   - Dogs after treatment that previously tested positive by a RealPCR test or MAT
   - Dogs in the same household or environment as a dog diagnosed with leptospirosis
   - High-risk dogs, e.g., hunting dogs, dogs in contact with stagnant water

Treatment

For dogs presenting with acute kidney disease, supportive therapy with intravenous fluids is indicated. The dog should be rehydrated and fluids given to support diuresis and replace ongoing losses. Electrolyte disturbances and acid-base abnormalities should be corrected. Most dogs with leptospirosis are polyuric, however, urinary output should be monitored closely. In severe cases, especially if oliguria or anuria develops, referral for hemodialysis should be considered if available.

Antibiotic therapy is key to specifically treating leptospirosis. Antibiotics should be initiated as soon as possible when leptospirosis is suspected, after diagnostic samples have been collected, even prior to confirmation of the diagnosis. Doxycycline (administered orally) or penicillin and its derivatives (i.e., ampicillin [intravenously] or amoxicillin [orally]) are the antibiotics of choice for initial treatment. These drugs terminate leptospiremia within 24 hours, which in turn prevents urinary shedding and transmission of the organism and significantly decreases the risk of zoonotic transfer. To clear renal infections and eliminate the carrier state and chronic shedding, doxycyline should be administered for 3 weeks once oral medication is possible, or if doxycycline is not tolerated, a fluoroquinolone can be administered in conjunction with a penicillin derivative.

Prognosis

Establishing a definitive diagnosis of leptospirosis is critical. Without specific therapy, permanent renal damage is more common and the disease is more likely to be fatal. With early recognition and appropriate treatment, survival rates for dogs with acute kidney disease are approximately 80%.[4,8]

Public health considerations

Urinary shedding of leptospires poses a zoonotic risk to dog owners. Urine from infected dogs can infect humans if it comes in contact with mucosal surfaces or a break in the epidermal barrier. One study evaluating 500 dogs used PCR on urine to detect urinary shedding of leptospires. The results revealed that, irrespective of health status, 8.2% of

dogs were shedding pathogenic leptospires.[2] Identifying dogs shedding leptospires allow veterinarians, their staff and the pet owner to take appropriate precautions (e.g., latex gloves, face mask, goggles) when handling the dog's urine and urine contaminated areas.

Ordering information

2628	***Leptospira* spp. RealPCR™ Test**

Sample Requirements: 2 mL EDTA whole blood, 2 mL urine in a sterile container; keep refrigerated

Turnaround time: 1–3 days

Limitations: A negative RealPCR result may be caused by the numbers of organisms being below the limit of detection, decreased numbers of organisms following treatment, a chronic carrier state or the occurrence of new strain variations.

Contacting IDEXX

Laboratory Customer Support

If you have any questions regarding test codes, turnaround times or pricing, please contact our Laboratory Customer Support Team at 1-888-433-9987, option 3, option 5.

Expert Feedback When You Need It

Our team of internal medicine specialists is always available for complimentary consultation. Please call 1-888-433-9987, option 4, option 2, if you have questions.

Turnaround time

The IDEXX nationwide network of reference laboratories provides daily courier service or IDEXX-Direct® service to pick up your samples and forward them to our IDEXX Molecular Diagnostics Laboratory in California. IDEXX RealPCR tests are run daily, Monday–Friday. Samples received on Saturday or Sunday are processed on Monday. You can expect results within 1–3 working days, depending on shipping time.

References

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The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions and cautions.

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