Canine Diseases

Canine Leptospirosis

Leptospirosis is a zoonotic disease with a worldwide distribution caused by infection with any of several pathogenic serovars of Leptospira. The infection and disease is more prevalent in warm, moist climates and is endemic in much of the tropics. In temperate climates, the disease is more seasonal with the highest incidence associated with periods of rainfall. Essentially all mammals are susceptible to infection with pathogenic Leptospira, although some species are more resistant to disease. Chronic infection without apparent clinical disease is common in many wildlife species, especially rodents. These species act as reservoirs for infection and are primarily responsible for spreading the infection to non-reservoir, incidental hosts who can suffer clinical disease. Leptospira infection in an incidental host may be subclinical but can cause profound multisystemic disease involving the hepatic, renal, and coagulation systems.

Etiology and Pathogenesis

Leptospira are aerobic, gram-negative spirochetes that are fastidious, slow growing, and have characteristic corkscrew-like motility. The taxonomy of Leptospira is complex and can be confusing. Traditionally, Leptospira were divided into 2 groups; the pathogenic Leptospira were all classified as members of *L. interrogans* and the saprophytic Leptospira were classified as *L. biflexa*. Within each of these species, leptospiral serovars were recognized, with over 250 different serovars of pathogenic Leptospira identified (based on surface antigens) throughout the world. Antigenically related serovars are further grouped into serogroups. With the increased use of genomic information for the classification of bacteria, the genus Leptospira was reorganized with the pathogenic leptospires now identified in seven species of Leptospira. Some of the common leptospiral pathogens of domestic animals now have different species names. For example, *L. interrogans* serovar grippotyphosa is now *L. kirschneri* serovar grippotyphosa. The revised nomenclature is now increasingly reflected in the scientific literature, however the serovar / serogroup classification remains useful when discussing the epidemiology, clinical features, treatment, and prevention of leptospirosis. Different serovars are adapted to different wild or domestic animal reservoir hosts and immunity to leptospires is serogroup specific. Thus knowledge of serogroups that commonly cause disease within a particular geographic region is important for vaccine development.

Dogs are the reservoir host for serovar canicola, and prior to widespread vaccination programs, serovars canicola and icterohaemorrhagiae were the most common serovars in dogs. The prevalence of canine serovars has shifted significantly in the last 15 years and clinical disease caused by serovars

grippotyphosa, pomona, and bratislava is being increasingly diagnosed, with the relative proportion of these serovars differing geographically. However serovar canicola still circulates in the canine population, particularly in unvaccinated stray dogs and serovar icterohaemorrhagiae is still commonly identified in unvaccinated dogs with exposure to rats.

Asymptomatic reservoir hosts shed infection chronically via their urine and infection of incidental hosts is usually indirect, by contact with areas contaminated with infected urine from a reservoir host. Environmental conditions are critical in determining the frequency of indirect transmission. Survival of leptospires is favored by moisture and moderately warm temperatures; survival is brief in dry soil or at temperatures <10°C or >34°C.

In a susceptible incidental host leptospires invade the body after penetrating exposed mucous membranes or damaged skin. After a variable incubation period (4–20 days), leptospires circulate in the blood and replicate in many tissues including the liver, kidneys, lungs, genital tract, and CNS for 7–10 days. During the period of bacteremia and tissue colonization, the clinical signs of acute leptospirosis occur. Agglutinating antibodies can be detected in serum soon after leptospiremia occurs and coincide with clearance of the leptospires from blood and most organs. As the organisms are cleared, the clinical signs of acute leptospirosis begin to resolve, although damaged organs may take some time to return to normal function. However leptospires can persist in the renal tubules of incidental hosts for a longer period and from here can be shed in the urine for a few days to several weeks.

Clinical and Pathological Findings

There are relatively minor clinically relevant differences in disease produced by the common serovars. There is significant variation in pathogenicity among isolates within a serovar. Therefore, dogs with leptospirosis can be expected to exhibit a spectrum of clinical signs confounding clinical diagnosis. Early clinical signs are nonspecific and may include depression, lethargy, anorexia, vomiting, diarrhea, conjunctivitis, fever, and arthralgia or myalgia. Hours to days later, specific signs of renal and/or hepatic disease are observed, with mild to moderate elevations in BUN, creatinine, and bilirubin to profound jaundice, oliguric renal failure, hyperphosphatemia, thrombocytopenia, and death. Less commonly, uveitis, pancreatitis, pulmonary hemorrhage, and chronic hepatitis are recognized.

The most common hematologic abnormality is a mild to moderate neutrophilic leukocytosis without a left shift, although a normal WBC count may be seen. A mild anemia is seen in 25–35% of cases, often as a result of subclinical hemolysis. Thrombocytopenia occurs in only 10–20% of dogs but is rarely severe enough to be a source of bleeding. Vasculitis is typically the cause of hemorrhage associated with leptospirosis. Azotemia is the most common finding on a serum biochemistry profile. When liver values are abnormal, elevations in serum alkaline phosphatase are typically more pronounced than elevations in ALT and AST. Serum bilirubin is elevated in ~20% of cases. Isosthenuria or hyposthenuria is typically present on the urinalysis, and hematuria, proteinuria, and granular casts are identified in ~30% of cases.

Gross findings can include petechial or ecchymotic hemorrhages on any organ, pleural, or peritoneal surface; hepatomegaly; and renomegaly. The liver is often friable with an accentuated lobular pattern and may have a yellowish brown discoloration. The kidneys may have white foci on the subcapsular surface. Microscopic findings in the liver may include hepatocytic necrosis, nonsuppurative hepatitis, and intrahepatic bile stasis, while swollen tubular epithelial cells, tubular necrosis, and a mixed inflammatory reaction may be seen in the kidneys. Chronic hepatitis and chronic interstitial nephritis are described in less severe cases.

Diagnosis

Serology is the most frequently used diagnostic test for dogs. Acute and convalescent titers may be necessary to confirm a diagnosis. Other diagnostic tests such as immunofluorescence, PCR, and culture are useful, but collection of samples prior to the administration of antibiotics should be considered for maximal sensitivity.

Diagnosis of leptospirosis depends on a good clinical and vaccination history and laboratory testing. Diagnostic tests for leptospirosis include those designed to detect antibodies against the organism and those designed to detect the organism in tissues or body fluids. Serologic testing is recommended in each case, combined with one or more techniques to identify the organism in tissue or body fluids.

Serologic assays measuring anti-leptospiral antibodies are the most commonly used techniques for diagnosing leptospirosis in animals. The microscopic agglutination test (MAT) is most frequently used but ELISA tests are also available in many countries. Interpretation of serologic results is complicated by a number of factors including cross-reactivity of antibodies, antibody titers induced by vaccination, and lack of consensus about what antibody titers indicate infection. MAT antibodies produced in an animal in response to infection with a given serovar of Leptospira often cross-react with other serovars. In some cases, these patterns of cross-reactivity are predictable based on the antigenic relatedness of the various serovars of Leptospira, but the patterns of cross-reactive antibodies vary between host species. However, in general, the infecting serovar is assumed to be the serovar to which that animal develops the highest titer. However, paradoxical reactions may also occur with the MAT early in the course of an acute infection, with a marked agglutinating antibody response to a serovar other than the infecting serovar.

Widespread vaccination of dogs with leptospiral vaccines also complicates interpretation of leptospiral serology. In general, vaccinated animals develop relatively low agglutinating antibody titers (1:100 to 1:400) in response to vaccination, and these titers persist for 1–3 mo after vaccination. However, some animals develop high titers after vaccination which persist for ≥6 mo.

Consensus is lacking as to what titer is diagnostic for leptospiral infection. A low antibody titer does not necessarily rule out a diagnosis of leptospirosis because titers are often low in acute disease and in maintenance host infections. In cases of acute leptospirosis, a 4-fold rise in antibody titer is often observed in paired serum samples collected 7–10 days apart. Diagnosis of leptospirosis based on a single serum sample should be made with caution and with full consideration of the clinical picture and vaccination history of the animal. In general, with a compatible clinical history and vaccination >3 mo ago, a titer of 1:800 to 1:1,600 is good presumptive evidence of leptospiral infection. Consultation with the diagnostic laboratory is often useful for titer interpretation. Antibody titers can persist for months following infection and recovery, although there is usually a gradual decline with time.

Immunofluorescence can be used to identify leptospires in tissues, blood, or urine sediment. The test is rapid and has good sensitivity but interpretation requires a skilled laboratory technician. Immunohistochemistry is useful to identify leptospires in formalin-fixed tissue but, because there may be small numbers of organisms present in some tissues, the sensitivity of this technique is variable. A number of PCR procedures are available, and each laboratory may select a slightly different procedure. These techniques allow detection of leptospires but do not determine the infecting serogroup or serovar. Culture of blood, urine, or tissue specimens is the only method to definitively identify the infecting serovar. Blood may be cultured early in the clinical course; urine is more likely to be positive 7–10 days after clinical signs appear. Culture is rarely positive after antibiotic therapy has begun. Culture of

leptospires requires specialized culture medium, and diagnostic laboratories rarely culture specimens for the presence of leptospires.