Canine Diseases

Canine Distemper

Canine distemper is a highly contagious, systemic, viral disease of dogs seen worldwide. Clinically, it is characterized by a diphasic fever, leukopenia, GI and respiratory catarrh, and frequently pneumonic and neurologic complications. Its epidemiology is complicated by the large number of species susceptible to infection.

Etiology and Pathogenesis

Canine distemper is caused by a paramyxovirus closely related to the viruses of measles and rinderpest. The fragile, enveloped, single-strand RNA virus is sensitive to lipid solvents, such as ether, and most disinfectants, including phenols and quaternary ammonium compounds. It is relatively unstable outside the host. The main route of infection is via aerosol droplet secretions from infected animals. Some infected dogs may shed virus for several months.

Virus initially replicates in the lymphatic tissue of the respiratory tract. A cell-associated viremia results in infection of all lymphatic tissues, which is followed by infection of respiratory, GI, and urogenital epithelium, as well as the CNS and optic nerves. Disease follows virus replication in these tissues. The degree of viremia and extent of viral spread to various tissues is moderated by the level of specific humoral immunity in the host during the viremic period.

Clinical and Pathological Findings

A transient fever usually occurs 3–6 days after infection, and there may be a leukopenia (especially lymphopenia) at this time; these signs may go unnoticed or be accompanied by anorexia. The fever subsides for several days before a second fever occurs, which may be accompanied by serous nasal discharge, mucopurulent ocular discharge, lethargy, and anorexia. GI and respiratory signs, typically complicated by secondary bacterial infections, may follow; rarely, pustular dermatitis may be seen. Encephalomyelitis may occur in association with these signs, follow the systemic disease, or occur in the absence of systemic manifestations. Dogs surviving the acute phase may have hyperkeratosis of the footpads and epithelium of the nasal planum, as well as enamel hypoplasia in incompletely erupted teeth.

Overall, a longer course of illness is associated with the presence of neurologic signs; however, there is no way to anticipate whether an infected dog will develop neurologic manifestations. CNS signs include circling, head tilt, nystagmus, paresis to paralysis, and focal to generalized seizures. Localized involuntary twitching of a muscle or group of muscles (myoclonus, chorea, flexor spasm, hyperkinesia) and convulsions characterized by salivation and, often, chewing movements of the jaw ("chewing-gum fits") are considered classic neurologic signs.

A dog may exhibit any or all of these multisystemic signs during the course of the disease. Infection may be mild and inapparent or lead to severe disease with most of the described signs. The course of the systemic disease may be as short as 10 days, but the onset of neurologic signs may be delayed for several weeks or months as a result of chronic progressive demyelination within the CNS.

Clinicopathologic findings are nonspecific and include lymphopenia, with the possible finding of viral inclusion bodies in circulating leukocytes very early in the course of the disease. Thoracic radiographs may reveal an interstitial pattern typical of viral pneumonia.

Chronic distemper encephalitis (old dog encephalitis, [ODE]), a condition often marked by ataxia, compulsive movements such as head pressing or continual pacing, and incoordinated hypermetria, may be seen in fully vaccinated adult dogs without a history suggestive of systemic canine distemper infection. Although canine distemper antigen has been detected in the brains of some dogs with ODE by fluorescent antibody staining or genetic methods, dogs with ODE are not infectious, and replication-competent virus has not been isolated. The disease is caused by an inflammatory reaction associated with persistent canine distemper virus infection in the CNS, but mechanisms that trigger this syndrome are unknown.

Thymic atrophy is a consistent postmortem finding in infected young puppies. Hyperkeratosis of the nose and footpads is often found in dogs with neurologic manifestations. Depending on the degree of secondary bacterial infection, bronchopneumonia, enteritis, and skin pustules also may be present. In cases of acute to peracute death, exclusively respiratory abnormalities may be found. Histologically, canine distemper virus produces necrosis of lymphatic tissues, interstitial pneumonia, and cytoplasmic and intranuclear inclusion bodies in respiratory, urinary, and GI epithelium. Lesions found in the brains of dogs with neurologic complications include neuronal degeneration, gliosis, noninflammatory demyelination, perivascular cuffing, nonsuppurative leptomeningitis, and intranuclear inclusion bodies predominately within glial cells.

Diagnosis

Distemper should be considered in the diagnosis of any febrile condition in dogs with multisystemic manifestations. Characteristic signs sometimes do not appear until late in the disease, and the clinical picture may be modified by concurrent parasitism and numerous viral or bacterial infections. A febrile catarrhal illness with neurologic sequelae justifies a clinical diagnosis of distemper.

In dogs with multisystemic signs, the following can be examined by immunofluorescent assay or reverse transcriptase (RT) PCR: smears of conjunctival, tracheal, vaginal, or other epithelium; the buffy coat of the blood; urine sediment; or bone marrow aspirates. Commercially available quantitative RT-PCR can usually distinguish natural infection from vaccinal virus. A combined two-step RT-PCR to distinguish vaccinal strains from emerging wild-type strains has also been described; this assay would be of particular value in epidemiologic investigations or in outbreaks in non-canine species. Antibody titers or ELISA can be performed on CSF and compared with peripheral blood; a relatively higher level in the CSF is typical of natural infection versus vaccination. Viral antigen immunofluorescent assay (IFA) or fluorescent in situ hybridization for viral DNA can be performed on biopsies from the footpads or from the haired skin of the dorsal neck.

At necropsy, diagnosis is usually confirmed by histologic lesions, IFA, or both. These samples are often negative when the dog is showing only neurologic manifestations or when circulating antibody

is present (or both), requiring that the diagnosis be made by CSF evaluation or RT-PCR as described above.

Infectious Canine Hepatitis

Infectious canine hepatitis (ICH) is a worldwide, contagious disease of dogs with signs that vary from a slight fever and congestion of the mucous membranes to severe depression, marked leukopenia, and coagulation disorders. It also is seen in foxes, wolves, coyotes, bears, lynx, and some pinnipeds; other carnivores may become infected without developing clinical illness. In recent years, the disease has become uncommon in areas where routine immunization is done, but periodic outbreaks, which may reflect maintenance of the disease in wild and feral hosts, reinforce the need for continued vaccination.

Etiology and Pathogenesis

ICH is caused by a nonenveloped DNA virus, canine adenovirus 1 (CAV-1), which is antigenically related only to CAV-2 (one of the causes of infectious canine tracheobronchitis). CAV-1 is resistant to lipid solvents (such as ether), as well as to acid and formalin. It survives outside the host for weeks or months, but a 1%–3% solution of sodium hypochlorite (household bleach) is an effective disinfectant.

Ingestion of urine, feces, or saliva of infected dogs is the main route of infection. Recovered dogs shed virus in their urine for ≥6 mo. Initial infection occurs in the tonsillar crypts and Peyer's patches, followed by viremia and disseminated infection. Vascular endothelial cells are the primary target, with hepatic and renal parenchyma, spleen, and lungs becoming infected as well. Chronic kidney lesions and corneal clouding ("blue eye") result from immune-complex reactions after recovery from acute or subclinical disease.

Clinical and Pathological Findings

Signs vary from a slight fever to death. The mortality rate ranges from 10%–30% and is typically highest in very young dogs. Concurrent parvoviral or distemper infection worsens the prognosis. The incubation period is 4–9 days. The first sign is a fever of >104°F (40°C), which lasts 1–6 days and is usually biphasic. If the fever is of short duration, leukopenia may be the only other sign, but if it persists for >1 day, acute illness develops.

Signs are apathy, anorexia, thirst, conjunctivitis, serous discharge from the eyes and nose, and occasionally abdominal pain and vomiting. Intense hyperemia or petechiae of the oral mucosa, as well as enlarged tonsils, may be seen. Tachycardia out of proportion to the fever may occur. There may be subcutaneous edema of the head, neck, and trunk. Despite hepatic involvement, there is a notable absence of icterus in most acute clinical cases.

Clotting time is directly correlated with the severity of illness and is the result of disseminated intravascular coagulation induced by vascular endothelial compromise, coupled with failure of the liver to rapidly replace consumed clotting factors. It may be difficult to control hemorrhage, which is manifest by bleeding around deciduous teeth and by spontaneous hematomas. CNS involvement is unusual and is typically the result of vascular injury. Severely infected dogs may develop convulsions

from forebrain damage. Paresis may result from brain stem hemorrhages, and ataxia and central blindness have also been described.

Clinicopathologic findings reflect the coagulopathy (prolonged prothrombin time, thrombocytopenia, and increased fibrin degradation products). Severely affected dogs show acute hepatocellular injury (increased ALT and AST). Proteinuria is common. Leukopenia typically persists throughout the febrile period. The degree of leukopenia varies and seems to be correlated with the severity of illness.

On recovery, dogs eat well but regain weight slowly. Hepatic transaminase activities peak around day 14 of infection and then decline slowly. In ~25% of recovered dogs, bilateral corneal opacity develops 7–10 days after acute signs disappear and usually resolves spontaneously. In mild cases, transient corneal opacity may be the only sign of disease.

Endothelial damage results in "paint-brush" hemorrhages on the gastric serosa, lymph nodes, thymus, pancreas, and subcutaneous tissues. Hepatic cell necrosis produces a variegated color change in the liver, which may be normal in size or swollen. Histologically, there is centrilobular necrosis, with neutrophilic and monocytic infiltration, and hepatocellular intranuclear inclusions. The gallbladder wall is typically edematous and thickened; edema of the thymus may be found. Grayish white foci may be seen in the kidney cortex.

Diagnosis

Usually, the abrupt onset of illness and bleeding suggest ICH, although clinical evidence is not always sufficient to differentiate ICH from distemper (see Canine Distemper). Definitive antemortem diagnosis is not required before institution of supportive care but can be pursued with commercially available ELISA, serologic, and PCR testing. PCR or restriction fragment length polymorphism is required to definitively distinguish CAV-1 from CAV-2, if clinically necessary. Postmortem gross changes in the liver and gallbladder are more conclusive, and diagnosis is confirmed by virus isolation, immunofluorescence, characteristic intranuclear inclusion bodies in the liver, or PCR or fluorescence in situ hybridization studies of infected tissue.

Canine Parvovirosis

Canine parvovirosis is a highly contagious and relatively common cause of acute, infectious GI illness in young dogs caused by a relatively recently evolved virus, canine parvovirus 2 (CPV-2) which first emerged in the late 1970s. Although its exact origin is unknown, it is believed to have arisen from feline panleukopenia virus or a related parvovirus of nondomestic animals. The virus showed relatively rapid genetic evolution and within only a few years the first antigenic variants, CPV-2a and CPV-2b, appeared. These variants completely replaced the original CPV-2 in the field and spread worldwide. In 2000, a new variant, referred to as CPV-2c, was first identified in Europe and this variant has now also been identified in many other countries.

Etiology and Pathophysiology

CPV-2 is a nonenveloped, single-stranded DNA virus, resistant to many common detergents and disinfectants. Infectious CPV-2 can persist indoors at room temperature for a few weeks; outdoors, if protected from sunlight and desiccation, it persists for many months.

Young (6 wk to 6 mo), unvaccinated or incompletely vaccinated dogs are most susceptible. Rottweilers, Doberman Pinschers, American Pit Bull Terriers, English Springer Spaniels and German Shepherd dogs have been described to be at increased risk of disease. Assuming sufficient colostrum ingestion, puppies born to a dam with CPV-2 antibodies are protected from infection for the first few weeks of life; however, susceptibility to infection increases as maternally acquired antibody wanes. Stress (eg, from weaning, overcrowding, malnutrition, etc), concurrent intestinal parasitism, or enteric pathogen infection (eg, *Clostridium spp*, *Campylobacter spp*, *Salmonella spp*, *Giardia spp*, coronavirus) have been associated with more severe clinical illness.

Virus is shed in the feces of infected dogs within 4–5 days of exposure (often before clinical signs develop), throughout the period of illness, and for ~10 days after clinical recovery. Infection is acquired directly through contact with virus-containing feces or indirectly through contact with virus-contaminated fomites (eg, environment, personnel, equipment). Viral replication occurs initially in the lymphoid tissue of the oropharynx, with systemic illness resulting for subsequent hematogenous dissemination. CPV-2 preferentially infects and destroys rapidly dividing cells of the small intestinal crypt epithelium, lymphopoietic tissue, and bone marrow. Destruction of the intestinal crypt epithelium results in epithelial necrosis, villous atrophy, impaired absorptive capacity, and disrupted gut barrier function with the potential for bacterial translocation and bacteremia.

Lymphopenia and neutropenia develop secondary to destruction of hematopoietic progenitor cells in the bone marrow and lymphopoietic tissues (eg, thymus, lymph nodes, etc) and are further exacerbated by an increased systemic demand for leukocytes. Infection in utero or in pups <8wk old or born to unvaccinated dams without naturally occurring antibodies can result in myocardial infection, necrosis, and myocarditis. Myocarditis, presenting as acute cardiopulmonary failure or delayed, progressive cardiac failure, can occur with or without signs of enteritis. However, CPV-2 myocarditis is infrequent because most bitches have CPV-2 antibodies from immunization or natural exposure.

Clinical and Pathological Findings

Clinical signs of parvoviral enteritis generally develop within 3–7 days of infection. Initial clinical signs may be nonspecific (eg, lethargy, anorexia, fever) with progression to vomiting and hemorrhagic small-bowel diarrhea within 24–48 hr. Physical examination findings can include depression, fever, dehydration, and intestinal loops that are dilated and fluid filled. Abdominal pain warrants further investigation to rule out the potential complication of intussusception. Severely affected animals may present collapsed with prolonged capillary refill time, poor pulse quality, tachycardia, and hypothermia—signs potentially consistent with septic shock. Although CPV-2-associated leukoencephalomalacia has been reported, CNS signs are more commonly attributable to hypoglycemia, sepsis, or acid-base and electrolyte abnormalities. Inapparent or subclinical infection is common.

Gross necropsy lesions can include a thickened and discolored intestinal wall; watery, mucoid, or hemorrhagic intestinal contents; edema and congestion of abdominal and thoracic lymph nodes; thymic atrophy; and, in the case of CPV-2 myocarditis, pale streaks in the myocardium. Histologically, intestinal lesions are characterized by multifocal necrosis of the crypt epithelium, loss of crypt architecture, and villous blunting and sloughing. Depletion of lymphoid tissue and cortical

lymphocytes (Peyer's patches, peripheral lymph nodes, mesenteric lymph nodes, thymus, spleen) and bone marrow hypoplasia are also observed. Pulmonary edema, alveolitis, and bacterial colonization of the lungs and liver may be seen in dogs that died of complicating acute respiratory distress syndrome, systemic inflammatory response syndrome, endotoxemia, or septicemia.

Diagnosis

CPV-2 enteritis should be suspected in any young, unvaccinated, or incompletely vaccinated dog with relevant clinical signs. Over the course of the illness, most dogs develop a moderate to severe leukopenia characterized by lymphopenia and neutropenia. Leukopenia, lymphopenia, and the absence of a band neutrophil response within 24 hr of initiating treatment has been associated with a poor prognosis. Prerenal azotemia, hypoalbuminemia (GI protein loss), hyponatremia, hypokalemia, hypochloremia, and hypoglycemia (inadequate glycogen stores in young puppies, sepsis), and increased liver enzyme activities may be noted on serum biochemical profile. Commercial ELISAs for detection of antigen in feces are widely available. Most clinically ill dogs shed large quantities of virus in the feces. However, false-negative results can occur early in the course of the disease (before peak viral shedding) and after the rapid decline in viral shedding that tends to occur within 10–12 days of infection. False-positive results can occur with 4–10 days of vaccination with modified live CPV-2 vaccine. Alternative methods of detecting CPV-2 antigen in feces include PCR testing, electron microscopy, and virus isolation. Serodiagnosis of CPV-2 infection requires demonstration of a 4-fold increase in serum IgG titer over a 14-day period or detection of IgM antibodies in the absence of recent (within 4 wk) vaccination.

Canine Infectious Tracheobronchitis (Kennel Cough)

Canine infectious tracheobronchitis (CITB), also colloquially known as kennel cough, is a highly contagious multifactorial disease characterized by acute or chronic inflammation of the trachea and bronchial airways. It is usually a mild, self-limiting disease but may progress to fatal bronchopneumonia in puppies or to chronic bronchitis in debilitated adult or aged dogs. It is commonly seen where dogs are in close contact with each other, e.g. boarding kennels or rescue centres, although CITB can also occur in dogs with no history of having been in such a situation. The disease can spread rapidly among susceptible dogs housed in close confinement and signs can persist for some weeks.

Etiology and Pathogenesis

Canine parainfluenza virus, canine adenovirus 2 (CAV-2), or canine distemper virus can be the primary or sole pathogen involved. Canine reoviruses (types 1, 2, and 3), canine herpesvirus, and canine adenovirus 1 (CAV-1) are of questionable significance in this syndrome. *Bordetella bronchiseptica* may act as a primary pathogen, especially in dogs <6 mo old; however, it and other bacteria (usually gram-negative organisms such as *Pseudomonas* sp, *Escherichia coli*, and *Klebsiella pneumoniae*) may cause secondary infections after viral injury to the respiratory tract. Concurrent infections with several of these agents are common. The role of *Mycoplasma* sp has not been clearly established. Stress and extremes of ventilation, temperature, and humidity apparently increase susceptibility to, and severity of, the disease.

Clinical and Pathological Findings

The prominent clinical sign is paroxysms of harsh, dry coughing, which may be followed by retching and gagging. The cough is easily induced by gentle palpation of the larynx or trachea. Affected dogs demonstrate few if any additional clinical signs except for partial anorexia. On auscultation, respiratory sounds may be essentially normal. In advanced cases, inspiratory crackles and expiratory wheezes are heard. Body temperature may be only slightly increased and WBC counts usually remain normal. Development of more severe signs, including fever, purulent nasal discharge, depression, anorexia, and a productive cough, especially in puppies, indicates a complicating systemic infection such as distemper or bronchopneumonia. Stress, particularly due to adverse environmental conditions and improper nutrition, may contribute to a relapse during convalescence.

During the acute and subacute inflammatory stages, the air passages are filled with frothy, serous, or mucopurulent exudate. In chronic bronchitis, they contain excessive viscid mucus. The epithelial linings are roughened and opaque, a result of diffuse fibrosis, edema, and mononuclear cell infiltration. There is hypertrophy and hyperplasia of the tracheobronchial mucous glands and goblet cells. The act of coughing is an attempt to remove the accumulations of mucus and exudate from the respiratory passages.

Diagnosis

CITB should be suspected whenever the characteristic cough suddenly develops 5–10 days after exposure to other susceptible or affected dogs. Severity usually diminishes during the first 5 days, but the disease can persists for some weeks. The diagnosis is usually made from the history and clinical signs and by elimination of other causes of coughing. In chronic bronchitis, chest radiographs may show an increase in linear and peribronchial markings. Bronchoscopy reveals inflamed epithelium and often mucopurulent mucus in the bronchi. In addition, the procedure allows collection of biopsy and swab samples for in vitro assay. Bronchial washing is an additional diagnostic aid that may demonstrate causative agents or significant cellular responses (eg, eosinophils).

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.

Canine Lyme Disease

Lyme borreliosis is a bacterial, tick-transmitted disease of animals (dogs, horses, probably cats) and humans. Areas of greatest incidence in the USA are regions in the northeast (particularly the New England states), the upper Midwest, and the Pacific coast. Lyme borreliosis also occurs in moderate climatic regions of Europe and Asia. The importance of borreliosis as a zoonotic disease is increasing.

Etiology and Pathogenesis

Currently, on the basis of DNA-DNA reassociation analysis, 12 different species fall within the *Borrelia burgdorferi* sensu lato complex. Within this complex the most important spirochete species are *B. burgdorferi* sensu stricto (North America, Europe), *B. afzelii* (Europe, Asia), and *B. garinii* (Europe, Asia). Tick vectors of *B burgdorferi* sensu lato are hard-shelled Ixodes ticks. In the USA these are primarily *Ixodes pacificus* on the Pacific coast and *I. scapularis* in the Midwest and northeast. *I. ricinus* and *I. persulcatus* are primary vectors in Europe and Asia.

Ixodid ticks hatch from eggs as uninfected larvae. Both larvae and nymphs may acquire spirochetes from Borrelia-carrying hosts. Small mammals, especially rodents, often play a major role as reservoir hosts. Birds and lizards may also harbor certain Borrelia species and serve as reservoir hosts. Infection rates of the vectors vary according to region and season and can be as high as 50% in adult ticks. After tick attachment, >24 hrs elapse before the first *B. burgdorferi* sensu lato organisms are transmitted into the host's skin. Stable infection of the host occurs at >53 hrs into the blood meal. Therefore, early removal of attached ticks reduces the potential for spirochete transmission. *B. burgdorferi* sensu lato organisms are not transmitted by insects, body fluids (urine, saliva, semen), or bite wounds. Experimental studies have shown that dams infected prior to gestation may transmit spirochetes to their pups *in utero*.

Clinical and Pathological Findings

Numerous clinical syndromes have been attributed to Lyme borreliosis in domestic animals, including limb and joint disease and renal, neurologic, and cardiac abnormalities. In dogs, intermittent, recurrent lameness; fever; anorexia; lethargy; and lymphadenopathy with or without swollen, painful joints are the most commonly observed clinical signs. The second most common syndrome associated with Lyme borreliosis is renal failure and is generally fatal. It is characterized by uremia, hyperphosphatemia, and severe protein-losing nephropathy, often accompanied by peripheral edema. Bernese Mountain Dogs and Labrador Retrievers in particular often show high Borrelia-specific antibody levels; immune complexes in kidney tissues lead to severe inflammation. In human medicine, single case reports have described abnormalities with bradycardia with the cardiac form of Lyme borreliosis, while facial paralysis and seizure disorders are thought to be expressions of the neurologic form.

Diagnosis

Diagnosis is based on history, clinical signs, elimination of other diagnoses, laboratory data, epidemiologic considerations, and response to antibiotic therapy. Autoimmune panels, CBC, blood chemistry, radiographs, and other laboratory data are generally normal, except for results pertaining directly to the affected system (eg, soft-tissue swelling in limbs, neutrophil accumulation in synovial fluids of affected joints, uremia in renal disease).

Clinical signs for Lyme borreliosis are nonspecific. In addition to other orthopedic disorders (eg, trauma, osteochondritis dissecans, immune-mediated diseases), other infections should be considered. *Anaplasma phagocytophilum* can also induce intermittent, recurrent lameness. *A. phagocytophilum* is transmitted by the same ticks, and epidemiologic studies have revealed that up to 30% of all dogs in central Europe carry antibodies specific for this agent. Mixed infections should be considered when clinical signs are apparent.

Serologic testing for antibodies specific for *B. burgdorferi* sensu lato is an adjunct to clinical diagnosis. Antibodies can be detected with ELISA (including rapid test systems) and protein immunoelectrophoresis (Western blot). Due to their low specificity, indirect immunofluorescent antibody assays are no longer recommended. The standard procedure for antibody detection is a two-tiered approach in which samples are screened with a sensitive ELISA, and only positively reacting samples are rechecked with a specific Western blot assay. Western blot testing helps to differentiate the immune response elicited by infection from that induced by vaccination.

Alternatively, blood or serum samples can be tested with peptide-based assays (C6 peptide), which is specific for infection-induced antibodies. However, demonstration of specific antibodies indicates exposure to bacterial antigen only and does not equate to clinical disease. About 5–10% of dogs in central Europe carry Borrelia-specific antibodies with no clinical signs. Additionally, false-negative results can occur with the C6 peptide assays shortly after infection. Long incubation periods, persistence of antibodies for months to years, and the disassociation of the antibody response from the clinical stage of disease make diagnosis by blood testing alone impossible.

Isolation of *B. burgdorferi* sensu lato by culture or detection of specific DNA by PCR from joints, skin tissue samples, or other sources may also be helpful in diagnosis. However, direct detection of the organism is difficult, time consuming (up to 6 wk for culture), and in most cases produces negative results. Only a positive result is meaningful. Blood samples are generally negative, because the organism resides in tissue and not in the circulation.

Source: Merck Vet Manual