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## Leptospirosis in Dogs - Current Status (19-Jul-2001)

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### Introduction

Leptospirosis is a bacterial disease caused by pathogenic members of the Genus *Leptospira*. The disease occurs worldwide in numerous animal hosts, including the dog. The canine disease presents as an acute infection of the kidney and liver and, sometimes, as a septicemia. Chronic kidney disease is a common sequel of infection and abortions may occur in pregnant dams. Because many aspects of the infection are poorly understood, there is the possibility that the disease in dogs may go undiagnosed. Recent events in the Northeastern USA have placed leptospirosis at the top of the list of differential diagnoses for dogs that present with signs of acute liver and/or kidney disease. While diagnostic methods have improved over the years, most are relatively insensitive. Information about leptospirosis is further complicated by major changes in the taxonomic classification of the Genus *Leptospira*. If the changing patterns of disease in dogs are to be understood, it is necessary to recognize that this re-emerging infection is influenced by the cycles of infection in wildlife, where the infection may spill over into domestic animal populations. Other factors affecting the pattern of disease in dogs are the vaccination history and antibiotic usage. This monograph on canine leptospirosis highlights recent findings on the disease in dogs, and it challenges veterinarians to learn more about this serious disease which affects both animals and man.

### *Leptospira* - Environmental Survival, Microbiology, and Taxonomy

*Leptospira* do not multiply outside of the host and their survival depends on environmental conditions in which leptospirae are found, e.g., soil and water conditions. *Leptospira* are highly susceptible to drying and to pH changes - pH<6 and pH>8 are inhibitory; temperatures < 7 - 10°C (44.6 - 50°F) and temperatures > 34 - 36°C ( 93 - 96°F) are detrimental. *Leptospira* organisms survive up to 180 days in wet soil, for many months in surface water and they survive better in stagnant than in free-flowing water. The source of infection to animals is either by direct contact with infected urine, fetal and placental material or fluids, uterine discharges, or indirect contact from a contaminated environment. A higher incidence of disease is more likely in soils with an alkaline pH, during the wet season (high rainfall areas), in low lying areas susceptible to run off conditions during rains, warm and humid climates, areas with an abundance of surface water resulting in marshy fields and muddy areas. Also, dogs in fenced yards may be exposed to urine from wildlife, including rodents; dogs which are exercised by walks in parks and those who roam in the countryside or swim in ponds and slow running streams are at greatest risk to exposure to leptospirae. Dogs such as hunting breeds, show animals, and all dogs with access to ponds or slow-moving streams are at higher risk than house pets.

### Etiology

*Leptospira* are aerobic or microaerophilic gram-negative bacteria and members of the Order *Spirochaetales*. *Leptospira* do not stain well with the conventional Gram's stain, but they are readily seen with fluorescent antibody (FA) stains of tissue touch preparations or urine sediments, the Warthin-Starry silver stain or immunohistochemistry staining of fixed tissues. Their microscopic morphology is spiral often with hooks visible at each end of the bacterial cell (Fig. 1).

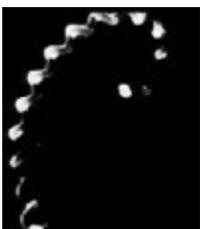


Figure 1. Typical morphology of leptospirae. Electron photomicrograph. *L. pomona* x20,000. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

The optimum growth temperature is 30°C and their generation time is 7 to 10 days for newly isolated field strains. Thus, they are difficult to recover by in vitro cultivation.

**Classification** - The traditional method of classification divided leptospirens into over 200 serovars based on antigenic (serologic) differences and all pathogenic *Leptospira* were classified as one species, *L. interrogans*; the free-living, non-pathogenic serovars were all included within the *L. biflexa* species. However, the new classification of the Genus *Leptospira* relies on genetic relatedness of the organisms e.g., restriction endonuclease analysis of chromosomal DNA. There are currently 7 genospecies, 28 serogroups and numerous serovars and genotypes. Three species of saprophytic *Leptospira* have been described.

### **Epizootiology**

Leptospirosis occurs worldwide; however, it is not uncommon to find endemic disease in a particular geographic region caused by infections with only one, or several, serovars. Leptospirens become adapted to "primary reservoir hosts"; which are commonly wildlife species. These same *Leptospira* species also occur in almost any other mammalian hosts as "incidental or accidental hosts". The dog is the "primary reservoir host" to *L. canicola* (*L. canicola* is found in the incidental hosts rats, raccoons, hedgehogs, voles and skunks) and to *L. bataviae* (*L. bataviae* occurs in the incidental hosts hedgehogs and voles). Dogs also may become infected with several other serovars and serve as "accidental or incidental hosts". Historically, the serovars associated with clinical disease in the dog included *L. canicola* and *L. icterohaemorrhagiae* (primary reservoir host is the rat; incidental hosts are mice, raccoon, opossum, hedgehog, fox, woodchuck, skunks, and muskrats). However, the disease picture changed in the Northeastern USA when several hundred cases of leptospirosis were reported on Long Island, New York (USA) in 1996. Since that time, both *L. grippityphosa* (primary reservoir host is the vole; incidental hosts are mice, rat, raccoon, opossum, fox, squirrel, skunk, hedgehog, muskrat, mole) and *L. pomona* (primary reservoir hosts are the cow and pig; incidental hosts are deer, mice, raccoon, opossum, hedgehog, fox, woodchuck, vole) have become more prevalent in that region. *L. bratislava* (primary reservoir in pig and horse) emerged in 2000 as an additional problem. The reported prevalence/incidence of leptospirosis in dogs may be underestimated, for it is likely that much canine disease is undiagnosed since many infections are asymptomatic. Also, many veterinarians have not included leptospirosis in the differential diagnosis of acute renal disease, or owners have not sought veterinary help. It should be recognized that seroconversion does not always correlate with overt clinical disease in the dog. Leptospirens do not multiply outside of the host animal species, but they survive well in the environment under optimal conditions, as noted above. In order for direct infection to occur, dogs must be exposed to leptospirens from infectious urine, via transplacental and venereal routes, bite wounds, or ingestion of contaminated meat. The most common source of leptospirosis in dogs is contaminated water. Indirect transmission also occurs from vegetation, soil, or food contaminated by infectious urine.

### **Pathogenesis**

An understanding of the pathogenesis of leptospirosis is incomplete. Clinical forms of the disease are influenced by several factors, including the host, which may either be a primary reservoir host or an incidental host. The disease in primary reservoir hosts tends to be more chronic, or asymptomatic with weak antibody responses. In contrast, the disease in an incidental host tends to be acute and severe with marked antibody responses. The spectrum of disease in the dog ranges from subclinical, to subacute, acute (severe), or chronic; there also may be abortions with or without placentitis. Initially, leptospirens penetrate the mucus membranes or intact or abraded skin. Then, over the next 4 to 11 days, organisms rapidly invade the bloodstream, creating a leptospiremia. Early leptospiremia is associated with the clinical signs of fever, transitory anemia due to hemolysis, leukocytosis, hemoglobinuria and albuminuria. In susceptible dogs, leptospirens usually establish a septicemia and spread systemically to the internal organs, including the liver and kidneys, or to the placenta and fetus. The extent of development of specific lesions depends on the particular serovar and its virulence, as well as the dog's immune status. If a dog had been vaccinated, it still may have antibodies in its serum, or it may mount an anamnestic response in the absence of antibodies.

The described virulence factors of *Leptospira* include adherence factors associated with outer surface proteins (OSP) which allow attachment to host fibronectin and collagen, as well as unknown factors which allow invasion across mucus membranes or moist, softened skin. Additional factors include the endotoxic activity of *Leptospira* lipooligosaccharide (LOS) and its action on monocytes; release of lymphokines, eliciting of disseminated intravascular coagulation (DIC) reactions, including hemorrhage and bleeding abnormalities; thrombocytopenia and platelet aggregation; the amount of LOS present; the lipid A activity of LOS and its toxic effects; LOS and its protective effects against the bactericidal effects of normal serum; various hemolysins and their action in causing hemoglobinuria, hemolytic anemia, and other tissue damage;

sphingomyelinase C; phospholipase A and other cytotoxins. *L. icterohemorrhagiae* usually causes fever, hemorrhage, anemia, and jaundice; whereas severe acute kidney failure and/or chronic active hepatitis is common with *L. grippityphosa*, resulting in a more severe disease than that caused by *L. pomona*. Infections with *L. pomona* are often subclinical, but a chronic carrier state is common. Infection of dogs with the host adapted *L. canicola* commonly results in chronic interstitial nephritis (Fig. 2a and Fig. 2b).



Figure 2a. Interstitial nephritis and fibrosis resulting from chronic infection with *L. canicola*. On the left: kidney surface; light areas represent fibrosis; on the right: cut surface of the same kidney. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 2b. Interstitial nephritis and fibrosis resulting from chronic infection with *L. canicola*. Normal kidney (left), diseased kidney (right). - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

Young dogs who are unvaccinated, or whose dams were not vaccinated, are at greater risk of severe disease and death that may occur due to an acute septicemia or hemolytic anemia. Previously vaccinated older dogs who, later, become naturally infected with a field strain homologous to a vaccine serovar generally have minimal clinical signs. During the period of tissue invasion there may be liver necrosis as well as capillary and endothelial cell damage. As a consequence, petechial hemorrhages may occur in the renal parenchyma together with vascular damage, focal interstitial nephritis (Fig. 3), anemic anoxia, and hemoglobinuric nephrosis. At this stage death may occur due to renal failure caused by interstitial nephritis.

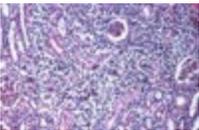


Figure 3. Acute-subacute interstitial nephritis; microscopic appearance. *L. canicola* infection (H&E stain, x25). - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

Towards the end of the bacteremic stage, 7 - 10 days post infection, the fever usually subsides and leptospirae are cleared from the bloodstream as antibodies emerge. Recovery occurs as antibodies increase in the blood and the bacteremia ends; the rapidity of recovery depends on the degree of organ damage. Leptospirae which have localized in the kidney tubules (Fig. 4), the eye, or the reproductive tract are sheltered from the bactericidal effects of serum antibody; a persistent leptospiuria may therefore develop, with periodic episodes of fever.



Figure 4. Leptospirae (dark, elongated spiral organisms) localized in renal tubules. Acute case. *L. canicola*. Silver stain. X40. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

Urine shedding may last for prolonged periods, but antibody levels eventually decline since the leptospirae, protected in the renal tubules, do not stimulate antibody production. Eventually, recovered but shedding dogs may be seronegative when tested; however, the organisms continue to multiply and persist.

### Clinical Signs

The severity of clinical signs is influenced by a dog's age, vaccination status, the inherent virulence of a particular leptospiral serovar, as well as the route and degree of exposure. In peracute to subacute disease, dogs may die without clinical signs. Such dogs commonly present with loss of appetite, fevers of 103 - 104°F (38.5 - 40°C), severe myalgia and a reluctance to move, stiffness, shivering, progressive weakness and depression. Dogs may vomit (Fig. 5) and/or have diarrhea resulting in rapid dehydration and excessive thirst. Injected mucus membranes are typical, often with widespread petechial and ecchymotic hemorrhages; icterus (Fig. 6) is uncommon, and it occurs more frequently in dogs infected with *L. icterohemorrhagiae*.



Figure 5. Vomiting is common in acute cases of leptospirosis. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 6. Icterus may be seen in dogs with leptospirosis, especially in acute cases infected with *L. icterohaemorrhagiae*. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

Dogs usually have conjunctivitis and congested oral mucous membranes. There also can be a dry spontaneous cough accompanied with difficulty in breathing. In addition, dogs may have frequent urination, often with hematuria and, later, anuria may occur. There also can be hematemesis, hematochezia, melena and epistaxis; eventually, infected dogs may have cold extremities and, finally, death in untreated cases. Acutely ill dogs also may have a gray color to stools, yellow skin and eyes, and develop chronic weight loss. In chronic cases, there may be no apparent illness, or only fever of unknown origin and mild to severe conjunctivitis ("red eyes").

### Immunity to infection

Dogs in different parts of the world may be infected by many different serovars, but the local prevalences vary. Vaccines currently used in dogs in most countries contain the serovars *Leptospira canicola* and *L. icterohaemorrhagiae*. In the newer vaccines, *L. grippityphosa* and *L. pomona* have been added. The development of protective immunity to leptospirosis is believed to be associated with opsonizing and bactericidal antibodies directed to the LOS and associated protein antigens. Older vaccines may produce immunity which is adequate to suppress systemic invasion by homologous serovars, but not to prevent colonization of a dog's kidneys, resulting in renal carrier states. The localization of leptospire in the proximal tubules of the kidney, and survival in cerebrospinal fluid (CSF) and vitreous humor of the eye in some infected animals, reflects the inability of antibodies to penetrate into those sites without causing inflammation. It should be recognized that protection by vaccines is serovar specific and, to a lesser extent, serogroup specific. Protection against leptospirosis is related to the level of agglutinating and/or opsonizing antibodies. Despite the availability of vaccines for several decades, the duration of vaccine induced immunity is not known since data from long-term challenge studies are not available. The most commonly used serological test for leptospirosis is the *Leptospira* Microscopic Agglutination Test (L-MAT). It detects IgM responses well, but it is not as efficient in detecting IgG responses. The decline of L-MAT titers often commences about 16 weeks post-vaccination, but low titers may not indicate lack of immunity since anamnestic responses may be sufficient to engender protection against clinical illness. The protection afforded by whole cell bacterins is short (anecdotally, about 9 months) suggesting that dogs at high-risk of infection require boosters at least twice a year. As indicated above, the question whether or not to vaccinate an animal must take into account the leptospiral serovars in a particular region and ascertain that the appropriate serovars are contained in a vaccine. As with other bacterins, adverse vaccine reactions may occur which are likely due to the effects of the leptospiral LOS which is different in structure from other gram-negative bacterial LPS. Current vaccine research is concerned with subunit products and is aimed at determining which fraction(s) of the leptospiral cell wall are immunogenic and protective without being toxic to the animal. An ideal vaccine would reduce the rate of adverse reactions, yet provide protection against both homologous and heterologous serovars.

### Diagnosis

The diagnosis of leptospirosis in dogs depends on detecting the leptospire in clinical specimens and/or demonstrating an increase in antibody titers to one or more leptospiral serovars. Subclinical infections are unlikely to be diagnosed. The differential diagnosis of peracute or acute disease in the dog includes heartworm disease, autoimmune hemolytic anemia, bacteremia (from bite wounds, prostatitis, dental disease), infectious canine hepatitis virus, hepatic neoplasia, trauma, lupus, Rocky Mountain spotted fever, Ehrlichiosis, toxoplasmosis, renal neoplasia, and renal calculi. The differential diagnosis of chronic disease, e.g., abortions, weak puppy syndrome, includes canine brucellosis, canine herpesvirus infection and distemper. Laboratory tests include hematology and serum chemistry profiles, urinalysis, serology and bacterial and viral

studies of appropriate specimens.

Serology - The current "gold standard" diagnostic test for leptospirosis is the *Leptospira* Microscopic Agglutination Test (L-MAT) performed during the acute stage of disease; a second (convalescent) serum should be obtained within 3 to 4 weeks.

*Leptospira* serology is imprecise, but generalizations may be made regarding the interpretation of L-MAT results.

Antibodies are first detected within 7 to 10 days post-infection in the dog. In unvaccinated dogs titers may initially be low, 1:100 to 1:200, but may rise in the convalescent sample to 1:800 to 1:1600 or higher if a homologous *Leptospira* serovar is used as antigen. In vaccinated animals, low-level acute titers (>1:400) are often found, but they depend on when a dog was last vaccinated. Responses to infection in previously vaccinated dogs generally result in anamnestic responses only to the homologous serovars. Generally, a four-fold rise in antibody titer to a *Leptospira* serovar is considered significant. When titers to a particular serovar reach high levels, e.g., 1:3200 to 1:6400, it is not unusual to see elevated titers to other serovars, which is likely due to cross reactions. For accurate comparisons, all serum samples should be tested at the same time.

Antimicrobial treatment adversely affects the development of antibody titers. Therefore, the first serum samples should be obtained before antibiotic treatment has begun.

Darkfield microscopy versus fluorescent antibody (FA) testing of urine - Often the darkfield examination of urine is inconclusive. It is difficult to read, and requires fresh urine in order to observe intact leptospiral cells. In contrast, FA examination of centrifuged urine sediments is a more definitive test and leptospirae do not need to be viable. Urine should be submitted to the laboratory on ice by overnight courier to ensure that the specimen is of good quality. It is essential to correlate FA results with the clinical and vaccine history since leptospirae are commonly seen in the urine of seronegative carrier dogs and in dogs with clinical disease as early as 1 week post-infection.

Culture - Antemortem culture of body fluids (urine, blood, aqueous humor) and postmortem culture of tissues (kidney, liver, fetus, placenta) is usually not practical due to the fastidiousness of leptospirae. If culture is to be attempted, veterinarians should contact their diagnostic laboratory for the proper *Leptospira* transport medium.

FA examination - FA should be done on all tissues submitted for postmortem examination, especially important are kidney and liver specimens.

Polymerase chain reaction (PCR) - With the advent of PCR tests, rapid and genus and serovar specific detection of leptospirae from clinical specimens should be possible. This method is gaining in use in diagnostic laboratories and allows precise and rapid identification.

Histopathology - Special stains, e.g., the Warthin-Stary silver stain, and immunohistochemistry, using monoclonal antibodies, should be attempted on formalin-fixed sections of kidney, liver, and fetal/placental tissues.

### **Treatment and control**

The aims of treatment for acute cases of canine leptospirosis are to control the infection before irreparable damage is done to the liver and kidneys, and to suppress the leptospiruria. Severely ill, acute cases require a high degree of supportive care for survival; the prompt administration of fluids is essential. The prognosis is guarded for patients with acute renal failure and/or liver disease. Owners should be advised that leptospirosis is a zoonotic disease that is spread mainly by the urine of infected dogs. An infected dog's housing and outside areas need to be treated with a suitable disinfectant. Also, dogs should avoid muddy, stagnant water and rodents. Rodent control should be instituted. Vaccination (see above) is recommended in endemic areas.

Dogs usually recover after 2 weeks, if treated promptly with antibiotics and intravenous fluids. However, if kidney or liver damage is severe the infection may be fatal.

Successful treatment depends on an assessment of the severity of the dog's disease. Initial antimicrobial therapy, where there is evidence of renal dysfunction and/or leptospiremia, should include the use of procaine penicillin G (40,000 to 80,000 Units per kg IM, sid, or in divided doses, bid) until kidney function returns. Alternative drugs such as ampicillin or amoxicillin also may be used in place of penicillin. Elimination of leptospirae from the renal interstitial tissues to control the carrier state is best achieved with dihydrostreptomycin (10 to 15 mg per kg, IM, bid for 2 weeks) or streptomycin; however these drugs are not available in the United States for routine therapy. Doxycycline is not formally approved, but oral administration of 5.0 mg per kg SID has been proposed. Aminoglycosides cannot be used in patients until kidney function has been restored.

Control methods should, therefore, include vaccination; special attention to kennel sanitation to eliminate contact with potential sources of infected urine; knowledge that high risk dogs are hunter breeds, show dogs, and other dogs with access to water such as ponds; institute rodent control of households and kennels.

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