**Leptospira Species (Leptospirosis)**

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Leptospirosis is a zoonosis of global distribution, caused by infection with pathogenic spirochetes of the genus *Leptospira*. The disease is greatly underreported, particularly in tropical regions, but attempts at surveillance suggest that it may be the most common zoonosis. The disease is maintained in nature by chronic renal infection of carrier animals, which excrete the organism in their urine, contaminating the environment. Human infection occurs by direct contact with infected urine or tissues or, more commonly by indirect exposure to the organisms in damp soil or water. Most human infections are probably asymptomatic; the spectrum of illness is extremely wide, ranging from undifferentiated febrile illness to severe multisystem disease with high mortality rates. The extreme variation in clinical presentation is partly responsible for the significant degree of underdiagnosis.

**Etiology**

“Leptospira” derives from the Greek *leptos* (thin) and Latin *spira* (coiled). Aply named, the leptospires are a mere 0.1 μm in diameter by 6 to 24 μm in length. The cells have pointed ends, one or both of which is usually bent into a characteristic hook (Fig. 240-1). Motility is conferred by the rotation of two axial flagella underlying the membrane sheath, which are inserted at opposite ends of the cell and extend toward the central region. Because of their small diameter, leptospires are best visualized by darkfield microscopy, appearing as actively motile spirochetes (Fig. 240-2). Leptospires are readily cultured in polysorbate-albumin medium if specimens are obtained prior to initiation of antibiotic therapy.

Historically, the genus *Leptospira* was classified into two species, *L. interrogans* and *L. biflexa*, comprised of pathogenic and nonpathogenic strains, respectively. Within each species, large numbers of serovars were differentiated using agglutinating antibodies. Serovar specificity is conferred by lipopolysaccharide (LPS) O antigens. More than 250 serovars of pathogenic leptospires have been described; because of the large number of serovars, antigenically related serovars were grouped into serogroups for convenience in serologic testing. Leptospires are now classified into a number of species defined by their degree of genetic relatedness, determined by DNA reassociation. There are currently 14 named species, including pathogens (e.g., *L. interrogans*), nonpathogenic saprophytes (e.g., *L. biflexa*), and species of indeterminate pathogenicity (e.g., *L. inadai*) (Table 240-1). Some species contain both pathogenic and nonpathogenic strains. This classification is supported by 16S RNA gene sequencing (Fig. 240-3), but is distinct from the former serologic classification.

The system of serogroup nomenclature has no taxonomic standing, but is retained because presumptive serogroup determination by serologic testing has some epidemiologic value. However, it is doubtful whether serologic responses can be extrapolated to identification of the infecting serovar in an individual patient. The genome sequences of several *Leptospira* species and strains have been determined and sequencing of other strains is underway. The availability of these genome sequences has already led to better understanding of leptospiral pathogenesis.

**Epidemiology and Transmission**

Leptospirosis is endemic throughout the world. Human infections are endemic in most regions; the peak incidence occurs in the rainy season in tropical regions and the late summer to early fall in temperate regions. Outbreaks may follow periods of excess rainfall. The incidence of leptospirosis is probably grossly underestimated, because of limited diagnostic capacity in the regions where the burden of disease is greatest. In the United States, the highest incidence is found in Hawaii; active surveillance in 1992 detected an annual incidence of approximately 128 cases/100,000. Leptospirosis is no longer a nationally notifiable disease in the United States, although it remains notifiable in more than 20 states. Leptospirosis is maintained in nature by chronic renal infection of carrier animals. The most important reservoirs are rodents and other small mammals, but livestock and companion animals are also significant sources of human infection. Infection of carrier animals usually occurs during infancy and, once infected, animals may excrete leptospires in their urine intermittently or continuously throughout life.

Infection occurs through direct or indirect contact with urine or tissues of infected animals. Direct contact is important in transmission to veterinarians, workers in milking sheds on dairy farms, abattoir workers, butchers, hunters, and animal handlers (transmission has been reported to occur to children handling puppies and to dog handlers). Indirect contact is more common, and is responsible for disease following exposure to wet soil or water. The great majority of cases are acquired by this route in the tropics, either through occupational exposure to water, as in rice or taro farming, flooding after heavy rains, or exposure to damp soil and water during avocational activities.

Recreational exposures have become relatively more important, often in association with adventure tourism to tropical endemic areas. Several large point-source waterborne outbreaks have occurred after athletic events. There has been an increase in leptospirosis cases in dogs in the eastern regions of North America and in the Midwest, associated with a shift in the predominant serovars causing disease. Veterinary vaccine manufacturers have responded to this problem by licensing new canine vaccines containing these emerging serovars.

**Pathogenesis**

Leptospires enter the body through cuts and abrasions, mucous membranes or conjunctivae, or aerosol inhalation of microscopic droplets. Swallowing contaminated lake water was the only behavioral risk factor identified in a case-control study of a large leptospirosis outbreak at the 1998 Springfield Illinois Triathlon. However, the oral mucosa are probably a more important route of entry after ingestion than the intestinal tract. On entering the body, there is widespread hematogenous dissemination and penetration of tissue barriers,
including invasion of the central nervous system and aqueous humor of the eye. Transendothelial migration of spirochetes is facilitated by a systemic vasculitis, accounting for a broad spectrum of clinical illness. Severe vascular injury can ensue, leading to pulmonary hemorrhage, ischemia of the renal cortex and tubular-epithelial cell necrosis, and destruction of the hepatic architecture, resulting in jaundice and liver cell injury, with or without necrosis.26

The mechanisms whereby leptospires cause disease are not clearly understood. Potential virulence factors include immune mechanisms, toxin production, adhesins, and other surface proteins. Human susceptibility to leptospirosis may be related to poor recognition of leptospiral LPS by the innate immune system.27,28 Human toll-like receptor (TLR) 4, which responds to extremely low concentrations of gram-negative LPS (endotoxin), appears to be unable to bind leptospiral LPS,29 perhaps because of the unique methylated phosphate residue of its lipid A.30 Direct tissue damage may also be caused by production of hemolytic toxins, which may act as sphingomyelinases, phospholipases, or pore-forming proteins.31

Immune-mediated mechanisms have been postulated as one factor influencing the severity of symptoms.32 Investigation of the triathlon outbreak mentioned earlier identified the human leukocyte antigen (HLA) DQ6 polymorphisms associated with leptospirosis.33 The outbreak mentioned earlier identified the human leukocyte antigen (HLA) DQ6 as an independent risk factor for leptospirosis.33 The structural location of HLA-DQ6 polymorphisms associated with disease suggested that HLA-DQ6 may function as a superantigen that can cause nonspecific T-cell activation in susceptible individuals. Other immune mechanisms, including circulating immune complexes, anti-cardiolipin antibodies, and antiplatelet antibodies, have been proposed but their significance is unproven. In horses, recurrent uveitis (moon blindness) may result from direct infection34 or from the production of antibodies against a host epitope that is shared by common equine pathogenic serovars.35

A number of studies have focused on the roles of surface lipoproteins in leptospiral pathogenesis.36 The major surface lipoprotein, LipL32, is highly conserved among pathogenic serovars.37 LipL32 is a major target of the human immune response and appears to be involved in the pathogenesis of tubulointerstitial nephritis.38 Virulent leptospires respond to the increased osmolarity of host tissues by inducing expression of the multifunctional Lig surface proteins that mediate interactions with fibronectin, fibrinogen, and other extracellular matrix factors.39 The Lig proteins are early antigens; IgM antibodies to their immunoglobulin-like repeats develop early in infection, offering an approach to improved detection of acute infection.40 The endostatin-like LenA protein binds the complement regulatory protein, factor H, suggesting an important role in serum resistance.41

![Figure 240-1](https://example.com/image1.png) Scanning electron micrograph of cells Leptospira interrogans showing helical structure and curved (hooked) ends (original magnification × 60,000). (Courtesy of Rob Weyant, Centers for Disease Control and Prevention.)

![Figure 240-2](https://example.com/image2.png) Leptospires viewed by darkfield microscopy (original magnification × 1000). (Courtesy of Mildred Galton, Public Health Image Library, Centers for Disease Control and Prevention.)

![Figure 240-3](https://example.com/image3.png) Unrooted phylogenetic tree based on 16s RNA gene sequences of the Leptospiraceae obtained from GenBank. Species comprised of pathogenic serovars (see Table 237-1) cluster separately from nonpathogenic species. Leptospira inadai, L. fainei, L. broomii, and L. licerasiae are intermediate between pathogens and nonpathogens.
Clinical Manifestations

Leptospiral infection is associated with a very broad spectrum of severity, ranging from subclinical illness followed by seroconversion to two clinically recognizable syndromes—a self-limited systemic illness seen in approximately 90% of infections, and a severe, potentially fatal illness accompanied by any combination of renal failure, liver failure, and pneumonitis with hemorrhagic diathesis. In some patients, the disease has two distinct phases, an initial septicemic stage followed by a temporary decline in fever followed in an immune phase in which the severe symptoms occur. However, in many severe cases, the distinction between these two phases is not apparent; in addition, many patients present only with the onset of the second phase of the illness.

The mean incubation period is 10 days (range, 5 to 14 days); determination of precise exposures may be difficult, leading to significant imprecision in estimated incubation times. The acute septicemic phase of illness begins abruptly with a high remittent fever (38° to 40°C) and headache, chills, rigors, and myalgia; conjunctival suffusion without purulent discharge; abdominal pain; anorexia, nausea, and vomiting; diarrhea; and cough and pharyngitis; a pretibial maculopapular cutaneous eruption occurs rarely (Table 240-2). Conjunctival suffusion (redness without exudate) and muscle tenderness, most notable in the calf and lumbar areas, are the most characteristic physical findings, but may occur in a minority of cases (see Table 240-2). Other less common signs include lymphadenopathy, splenomegaly, and hepatomegaly.

The acute phase lasts from 5 to 7 days. Routine laboratory tests are nonspecific but indicative of a bacterial infection. Leptospires can be recovered from blood and cerebrospinal fluid (CSF) during the acute phase of illness, but meningeval signs are not prominent in this phase. Leptospires may also be recovered from urine, beginning about 5 to 7 days after the onset of symptoms (Fig. 240-4). Urinalysis reveals mild proteinuria and pyuria, with or without hematuria, and hyaline or granular casts. Death is rare in the acute phase of illness.

The immune phase of illness generally lasts from 4 to 30 days (see Fig. 240-4). The disappearance of leptospires from the blood and CSF coincides with the appearance of IgM antibodies. The organisms can be detected in almost all tissues and organs, and in urine for several weeks, depending on the severity of the disease. In addition to the acute-phase symptoms described, the immune phase may be characterized by any or all of the after-signs and symptoms: jaundice, renal failure, cardiac arrhythmias, pulmonary symptoms, aseptic meningitis, conjunctival suffusion with or without hemorrhage; photophobia; eye pain; muscle tenderness; adenopathy; and hepatosplenomegaly (see Table 240-2). Abdominal pain is not uncommon and may be an indication of pancreatitis.

Aseptic meningitis, with or without symptoms, is characteristic of the immune phase of illness, occurring in up to 80% of cases. In endemic areas, a significant proportion of all aseptic meningitis cases may be caused by leptospiral infection. Symptomatic patients present with an intense, bitemporal, and frontal throbbing headache, with or without delirium. A lymphocytic pleocytosis occurs, with total cell counts generally below 500/mm³. CSF protein levels are modestly elevated, between 50 and 100 mg/mL; the CSF glucose concentration is normal. Severe neurologic complications such as coma, meningoencephalitis, hemiplegia, transverse myelitis, or Guillain-Barré syndrome occur only rarely.

The most distinctive form of severe illness that may develop after the acute phase of illness is Weil’s disease, characterized by impaired hepatic and renal function. More severe cases may progress directly from the acute phase without the characteristic brief improvement in symptoms to a fulminant illness, with fever higher than 40°C and the rapid onset of liver failure, acute renal failure, hemorrhagic pneumonitis, cardiac arrhythmia, or circulatory collapse. Mortality rates in patients developing severe disease have ranged from 5% to 40%. In a study of 840 hospitalized patients with severe leptospirosis (14% case-fatality rate), the risk of death was found to increase with age, especially in adults 40 years of age or older. Altered mental status has been found to be the strongest predictor of death. Other poor prognostic signs include acute renal failure (oliguria, hyperkalemia, serum creatinine > 3.0 mg/dL), respiratory insufficiency (dyspnea, pulmonary rales, chest x-ray infiltrates), hypotension, and arrhythmias.

In jaundiced patients, disturbance of liver function is out of proportion to the rather mild and nonspecific pathologic findings. Conjugated serum bilirubin levels may rise to 80 mg/dL, accompanied by more modest elevations of serum transaminases, alanine aminotransferase, and aspartate aminotransferase, and aspartate aminotransferase, which rarely exceed 200 U/L. This is in marked contrast to viral hepatitis. Jaundice is slow to resolve, but death caused by liver failure almost never occurs in the absence of renal failure. At autopsy, degenerative changes are seen in hepatocytes, Kupffer cells may be hypertrophied, cholestasis is evident, and erythropagocytosis and mononuclear cell infiltrates are observed. Hepatocellular necrosis is absent.

Kidney involvement is initially characterized by a unique nonoliguric hypokalemic form of renal insufficiency. Hallmarks are impaired sodium reabsorption, increased distal sodium delivery, and potassium wasting. The impairment in sodium reabsorption appears to be caused by selective loss of the ENaC sodium channel in the proximal tubular...

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<th>Table 240-2 Signs and Symptoms on Admission in Patients with Leptospirosis in Large Case Series</th>
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epidemiological information, such as the presumptive infecting serogroup. CSF, cerebrospinal fluid.

Severe pulmonary hemorrhage syndrome (SPHS) may be a prominent manifestation of infection and may occur in the absence of hepatic and renal failure. Frank hemoptysis can arise simultaneously with the onset of cough during the acute phase of illness. Renal biopsy reveals acute interstitial nephritis; immune complex glomerulonephritis may also be present. If electrolyte and volume losses are not replaced, patients progress to oliguric renal failure. In fatal cases, the kidneys are swollen and yellow, with prominent cortical blood vessels. Histologic findings include a diffuse, mixed tubulointerstitial inflammatory cell infiltrate of lymphocytes, plasma cells, macrophages, and polymorphonuclear leukocytes, accompanied by focal areas of tubular necrosis.

Severe pulmonary hemorrhage syndrome (SPHS) can be a prominent manifestation of infection and may occur in the absence of hepatic and renal failure. Frank hemoptysis can arise simultaneously with the onset of cough during the acute phase of illness. However, hemorrhage is often inapparent until patients are intubated; clinicians should suspect SPHS in patients with signs of respiratory distress, whether or not they have hemoptysis. With progressive pulmonary involvement, radiographic abnormalities seen most frequently in the lower lobes evolve from small nodular densities (snowflake-like) to patchy alveolar infiltrates; confluent consolidation is uncommon but may occur. The pathophysiology of SHPS is consistent with acute respiratory distress syndrome (ARDS) with diffuse lung injury, impaired gas exchange, and hemodynamic changes indicative of septic shock. At autopsy, the lungs appear grossly congested and demonstrate focal areas of hemorrhage. Histologically, damage to the capillary endothelium leads to congestion with foci of interstitial and intra-alveolar hemorrhage, diffuse alveolar damage, and severe air space disorders. Inflammatory infiltrates are usually absent. Congestive heart failure occurs rarely. However, nonspecific electrocardiographic changes are common. In more than 50% of patients receiving continuous cardiac monitoring, cardiac arrhythmias may occur, including atrial fibrillation, flutter and tachycardia, and cardiac irritability, including premature ventricular contractions and ventricular tachycardia.

Atrial fibrillation is associated with more severe disease. Cardiovascular collapse with shock can develop abruptly and, in the absence of aggressive supportive care, can be fatal. At autopsy, interstitial myocarditis with inflammatory involvement of the conduction system is seen; acute coronary arteritis and aortitis are also common at postmortem examination.

### Laboratory Diagnosis

#### DIRECT DETECTION METHODS

Direct visualization of leptospires in blood or urine by darkfield microscopic examination has been used for diagnosis. However, artefacts are commonly mistaken for leptospires, and the method has both low sensitivity (40.2%) and specificity (61.5%). A range of staining methods has been applied to direct detection, including immunofluorescence staining, immunoperoxidase staining, and silver staining. These methods are not widely used because of the lack of commercially available reagents and their relatively low sensitivity. Detection of leptospiral antigen in blood or urine has been attempted, but without significant success. Several polymerase chain reaction (PCR) assays have been developed for the detection of leptospires, but few have been evaluated in clinical studies, and there have been no multicenter studies of multiple molecular diagnostic methods. The chief advantage of PCR is the prospect of confirming the diagnosis during the early acute (leptospiremic) stage of the illness, before the appearance of immunoglobulin M (IgM) antibodies, when treatment is likely to have the greatest benefit. In fulminating cases, in which death occurs before seroconversion, PCR may be of great diagnostic value.
DNA has been amplified from serum, urine, aqueous humor, and a number of tissues obtained at autopsy. For early diagnosis, serum is the optimal specimen. Urine from severely ill patients is often highly concentrated and contains significant inhibitory activity. Histologic diagnosis (Fig. 240-5) traditionally relied on silver impregnation staining, but immunohistochemical staining offers greater sensitivity and specificity. 

**ISOLATION AND IDENTIFICATION**

Leptospires can be isolated from blood, CSF, and peritoneal dialysate fluids during the first 10 days of illness. Specimens should be collected while the patient is febrile and before antibiotic therapy is initiated. One or two drops of blood should be inoculated directly into culture media at the bedside. Survival of leptospires in commercial blood culture media for several days has been reported. Urine can be cultured after the first week of illness. Specimens should be collected aseptically into sterile containers without preservatives and must be processed within a short time of collection; best results are obtained when the delay is less than 1 hour, because leptospires do not survive well in acidic environments.

Cultures are performed in albumin-polysorbate media such as EMJH (Ellinghausen-McCullough-Johnson-Harris) medium, which is available commercially. Older media contained serum. Primary cultures are performed in semisolid medium, to which 5-fluorouracil is usually added as a selective agent. Cultures are incubated at 30°C for several weeks, because initial growth may be very slow.

**Indirect Detection Methods**

Most leptospirosis cases are diagnosed by serology. The reference standard assay is the microscopic agglutination test (MAT), in which live antigens representing different serogroups of leptospires are reacted with serum samples and then examined by darkfield microscopy for agglutination. This is a complex test to maintain, perform, and interpret, and its use is restricted to a few reference laboratories.

A serologically confirmed case of leptospirosis is defined by a fourfold rise in MAT titer to one or more serovars between acute-phase and convalescent serum specimens run in parallel. A titer of at least 1:800 in the presence of compatible symptoms is strong evidence of recent or current infection. Suggestive evidence for recent or current infection includes a single titer of at least 1:200 obtained after the onset of symptoms. Delayed seroconversions are common, with up to 10% of patients failing to seroconvert within 30 days of the clinical onset. Cross-reactive antibodies may be associated with syphilis, relapsing fever, Lyme disease, viral hepatitis, human immunodeficiency virus (HIV) infection, legionellosis, and autoimmune diseases.

The interpretation of the MAT is complicated by cross-reaction between different serogroups, especially in acute-phase samples. Cross-reactivity in acute samples is attributable to IgM antibodies, which may persist for several years. The MAT is a serogroup-specific assay, and should not be used to infer the identity of the infecting serovar. However, knowledge of the presumptive serogroup may be of epidemiologic value in determining potential exposures to animal reservoirs.

Diagnostic application of the MAT is limited by the relatively low sensitivity when acute serum samples are tested. Other agglutination assays that detect total immunoglobulins, such as the indirect hemagglutination assay, suffer from similarly low sensitivities in acute specimens, but have high case sensitivities when acute and convalescent specimens are tested. IgM antibodies are detectable after about the fifth day of illness, and IgM detection assays are available in several formats. Use of these assays as screening tests offers the potential to enhance the diagnostic capacity of many laboratories, particularly in developing countries, where most cases of leptospirosis occur.

**Treatment**

Antibiotic therapy should be initiated as early in the course of the disease as suspicion allows. There have been few randomized or placebo-controlled trials, and these have produced conflicting results. Therapeutic benefits of antibiotics may be difficult to demonstrate in populations in which patients present for medical care with late and/or severe disease. Nevertheless, severe disease is usually treated with IV penicillin and mild disease with oral doxycycline (Table 240-3). Once-daily ceftriaxone has been shown to be as effective as penicillin. Jarisch-Herxheimer reactions have been reported in patients treated with penicillin. Patients receiving penicillin should be monitored because of the increased morbidity and mortality of such reactions.

Supportive therapy is essential for hospitalized patients. Patients with early renal disease with high-output renal dysfunction and hypokalemia should receive aggressive volume repletion and potassium supplementation to avoid severe dehydration and acute tubular necrosis. In patients who progress to oliguric renal failure, rapid initiation of hemodialysis reduces mortality and is typically required only on a short-term basis. Renal dysfunction caused by leptospirosis is typically completely reversible.

Patients requiring intubation for SPHS have
Infectious Diseases and Their Etiologic Agents

6

Prevention

Prevention of leptospirosis may be achieved by avoidance of high-risk exposures, adoption of protective measures, immunization, and use of chemoprophylaxis, in varying combinations depending on environmental circumstances and the degree of human activity. High-risk exposures include immersion in fresh water, as in swimming, and contact with animals and their body fluids. Removal of leptospirosis from the environment is impractical, but reducing direct contact with potentially infected animals and indirect contact with urine-contaminated soil and water remains the most effective preventive strategy available. Consistent application of rodent control measures is important in limiting the extent of contamination. Appropriate protective measures depend on the activity, but include wearing boots, goggles, overalls, and rubber gloves. In tropical environments, walking barefoot is a common risk factor.\(^\text{109}\)

Immunization of animals with killed vaccines is widely practiced, but the immunity is short-lived and animals require periodic (usually annual) boosters.\(^\text{110}\) Moreover, although these vaccines prevent disease, they do not prevent infection and renal disease. Thus, they have little effect on the maintenance and transmission of the disease in the animal population in which they are applied. Current bovine and porcine vaccines used in the United States contain serovars Icterohaemorrhagiae, Canicola, Grippotyphosa, Pomona, and Hardjo, whereas canine vaccines contain all except serovar Hardjo. New vaccines for bovine use stimulate a type 1 cell-mediated immune response against serovar Hardjo.\(^\text{102,105}\) and appear to protect against renal colonization and urinary shedding.\(^\text{114}\)

Human immunization is not widely practiced. A vaccine containing serovar Icterohaemorrhagiae is available in France for workers in high-risk occupations, and a vaccine has been developed for human use in Cuba.\(^\text{116}\) Immunization has been more widely used in Asia, to prevent large-scale epidemics in agricultural laborers.

For those who will be unavoidably exposed to leptospires in endemic environments, chemoprophylaxis is recommended (see Table 240-3). Weekly doxycycline (200 mg) has been shown to be effective in military personnel without previous exposure who underwent jungle training.\(^\text{117}\) The use of doxycycline prophylaxis after excess rainfall in local populations in endemic areas has been studied.\(^\text{107,118}\) Symptomatic disease was significantly reduced in one study, but serologic evidence of infection was found equally in subjects and controls.\(^\text{107}\) Limitations of doxycycline are its photosensitivity, high frequency of gastrointestinal side effects, dietary calcium restrictions, and contraindications for pregnant women and children. In vitro susceptibility of leptospires to azithromycin\(^\text{119}\) and its longer serum half-life suggest that this agent would be a reasonable alternative to doxycycline; however, clinical trials are needed to validate this approach.

### REFERENCES


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