

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.¹ 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.

History

A syndrome of severe multisystem disease, presenting with profound jaundice and renal function impairment, was described by Weil in Heidelberg in 1886. Other descriptions of disease that probably represent leptospirosis were made earlier, but the cause could not be definitively ascribed to leptospiral infection.² Leptospirae were first visualized in autopsy specimens from a patient thought to have had yellow fever,³ but were not isolated until several years later, almost simultaneously, in Germany and Japan.⁴ Diagnostic confusion between severe icteric leptospirosis and yellow fever continued, with prominent researchers such as Stokes and Noguchi dying in their attempts to discover the causative agent.⁴ Several authoritative reviews have been published.^{2,4-7}

Etiology

“Leptospira” derives from the Greek *leptos* (thin) and Latin *spira* (coiled). Aptly named, the leptospirae are a mere 0.1 μ m in diameter by 6 to 20 μ m 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.⁸ Because of their small diameter, leptospirae are best visualized by darkfield microscopy, appearing as actively motile spirochetes (Fig. 240-2). Leptospirae are readily cultured in polysorbate-albumin medium if specimens are obtained prior to initiation of antibiotic therapy.⁹

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.¹⁰ More than 250 serovars of pathogenic leptospirae have been described; because of the large number of serovars, antigenically related serovars were grouped into serogroups for convenience in serologic testing.

Leptospirae are now classified into a number of species defined by their degree of genetic relatedness, determined by DNA reassociation.^{11,12} 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),¹³ but is distinct from the former serologic classification.³

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.¹⁴

The genome sequences of several *Leptospira* species and strains have been determined¹⁵⁻¹⁸ 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.¹⁹ The incidence of leptospirosis is probably grossly underestimated, because of limited diagnostic capacity in the regions where the burden of disease is greatest.¹ In the United States, the highest incidence is found in Hawaii; active surveillance in 1992 detected an annual incidence of approximately 128 cases/100,000.²⁰ 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 leptospirae 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.^{21,22} There has been an increase in leptospirosis cases in dogs in the eastern regions of North America and in the Midwest,²³ associated with a shift in the predominant serovars causing disease.^{24,25} Veterinary vaccine manufacturers have responded to this problem by licensing new canine vaccines containing these emerging serovars.

Pathogenesis

Leptospirae 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.²¹ 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,

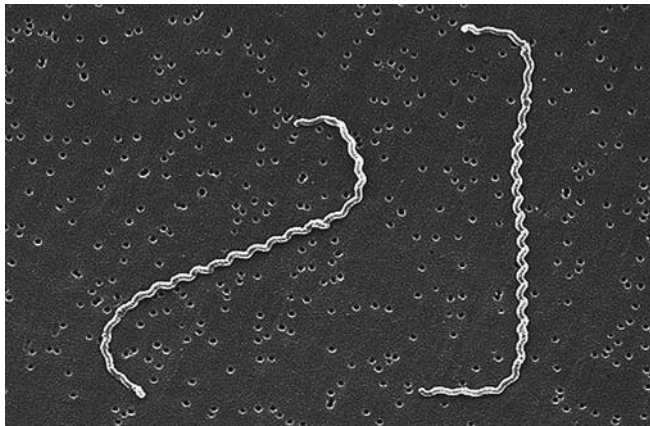


Figure 240-1 Scanning electron micrograph of cells *Leptospira interrogans* showing helical structure and curved (hooked) ends (original magnification $\times 60,000$). (Courtesy of Rob Weyant, Centers for Disease Control and Prevention.)

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,^{28,29} 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 as an independent risk factor for leptospirosis.³³ The structural location of HLA-DQ6 polymorphisms associated with disease suggested that leptospires produce a superantigen that can cause nonspecific T-cell activation in susceptible individuals. Other

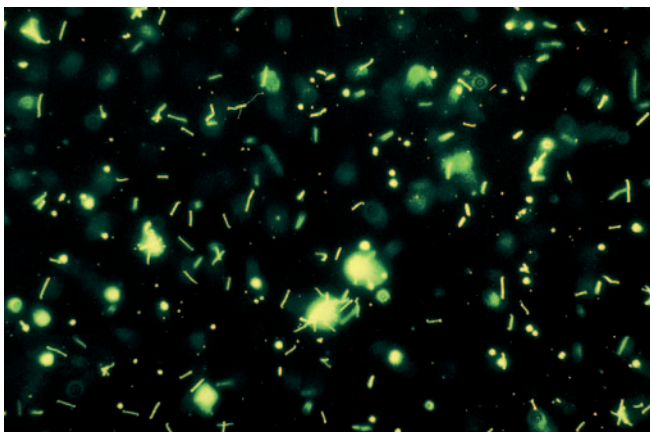


Figure 240-2 Leptospires viewed by darkfield microscopy (** original magnification $\times 100$). (Courtesy of Mildred Galton, Public Health Image Library, Centers for Disease Control and Prevention.)

TABLE 240-1 Species of *Leptospira* and Some Pathogenic Serovars

Species	Selected Pathogenic Serovars
<i>L. interrogans</i>	Icterohaemorrhagiae, Copenhageni, Canicola, Pomona, Australis, Autumnalis, Pyrogenes, Bratislava, Lai
<i>L. noguchii</i>	Panama, Pomona
<i>L. borgpetersenii</i>	Ballum, Hardjo, Javanica
<i>L. santarosai</i>	Bataviae
<i>L. kirschneri</i>	Bim, Bulgarica, Grippotyphosa, Cynopteri
<i>L. weilii</i>	Celledoni, Sarmin
<i>L. alexanderi</i>	Manhao 3
<i>Leptospira genomospecies 1</i>	Sichuan
<i>L. fainei</i>	Hurtsbridge
<i>L. meyeri</i>	Sofia
<i>L. inadai</i>	Indeterminate
<i>L. wolbachii</i>	Nonpathogens
<i>L. biflexa</i>	Nonpathogens
<i>Leptospira genomospecies 3</i>	Nonpathogens
<i>Leptospira genomospecies 4</i>	Nonpathogens
<i>Leptospira genomospecies 5</i>	Nonpathogens
<i>L. broomii</i>	Indeterminate
<i>L. licerasiae</i>	Indeterminate

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 response³⁸ and appears to be involved in the pathogenesis of tubulointerstitial nephritis.³⁹ 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.⁴⁰ 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.⁴¹ The endostatin-like LenA protein binds the complement regulatory protein, factor H, suggesting an important role in serum resistance.⁴²

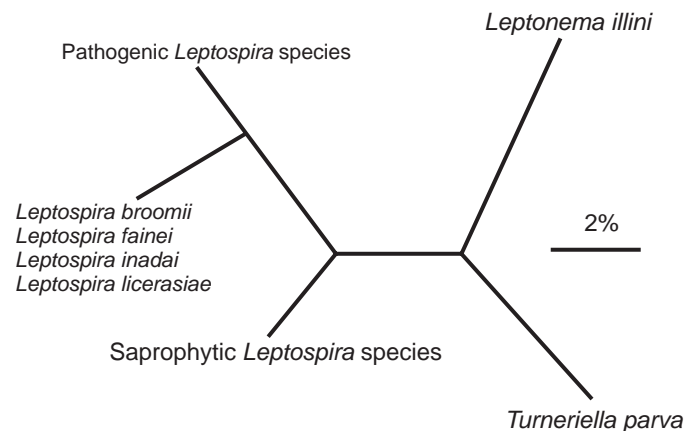


Figure 240-3 Unrooted phylogenetic tree based on 16s RNA gene sequences of the *Leptospiraceae* obtained from GenBank. Species comprised of pathogenic serovars (see Table 237-1) cluster separately from nonpathogenic species. *Leptospira inadai*, *L. fainei*, *L. broomii*, and *L. licerasiae* are intermediate 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.^{2,6,7} In some patients, the disease has two distinct phases, an initial septicemic stage followed by a temporary decline in fever followed by 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 myalgias; 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. Leptospire can be recovered from blood and cerebrospinal fluid (CSF) during the acute phase of illness, but meningeal signs are not prominent in this phase. Leptospire 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 leptospire from the blood and CSF coincides with the appearance of IgM antibodies.^{7,52} 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.⁵³ 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.⁵

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.⁷ Mortality rates in patients developing severe disease have ranged from 5% to 40%.^{2,5,6,47} 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.^{49,54} Altered mental status has been found to be the strongest predictor of death.^{49,55} 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.⁴⁹ 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, which rarely exceed 200 U/L.⁵⁶ 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 erythrophagocytosis and mononuclear cell infiltrates are observed.⁵⁷ 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

TABLE 240-2 Signs and Symptoms on Admission in Patients with Leptospirosis in Large Case Series

Sign or Symptom (%)	Puerto Rico, 1963 ⁴³ (N = 208)	China 1965 ⁴⁴ (N = 168)	Vietnam, 1973 ⁴⁵ (N = 93)	Korea, 1987 ⁴⁶ (N = 150)	Barbados, 1990 ⁴⁷ (N = 88)	Seychelles, 1998 ⁴⁸ (N = 75)	Brazil, 1999 ⁴⁹ (N = 93)	Hawaii, 2001 ⁵⁰ (N = 353)	India, 2002 ⁵¹ (N = 74)
Jaundice	49	0	1.5	16	95	27	93	39	34
Anorexia	—	46	—	80	85	—	—	82	—
Headache	91	90	98	70	76	80	75	89	92
Conjunctival suffusion	99	57	42	58	54	—	28.5	28	35
Vomiting	69	18	33	32	50	40	—	73	—
Myalgia	97	64	79	40	49	63	94	91	68
Arthralgia	—	36	—	—	—	31	—	59	12
Abdominal pain	—	26	28	40	43	41	—	51	—
Nausea	75	29	41	46	37	—	—	77	—
Dehydration	—	—	—	—	37	—	—	—	—
Cough	24	57	20	45	32	39	—	—	—
Hemoptysis	9	51	—	40	—	13	20	—	35
Hepatomegaly	69	28	15	17	27	—	—	16	—
Lymphadenopathy	24	49	21	—	21	—	—	—	15
Diarrhea	27	20	29	36	14	11	—	53	—
Rash	6	—	7	—	2	—	—	8	12

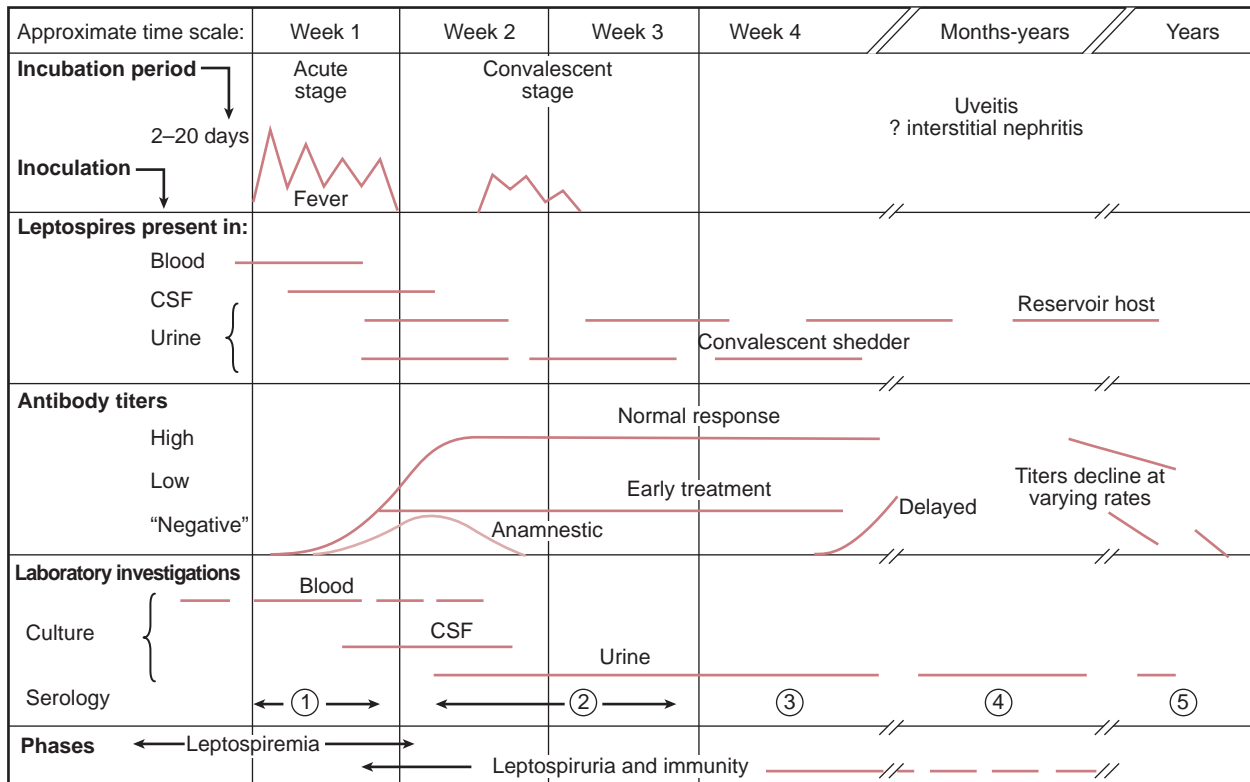


Figure 240-4 Biphasic nature of leptospirosis and relevant investigations at different stages of disease. Specimens 1 and 2 for serology are acute-phase specimens, 3 is a convalescent-phase sample which may facilitate detection of a delayed immune response, and 4 and 5 are follow-up samples that can provide epidemiological information, such as the presumptive infecting serogroup. CSF, cerebrospinal fluid. (Adapted from Levett PN. *Leptospirosis*. *Clin Microbiol Rev*. 2001;14:296-326, with permission of ASM Press.)

epithelium. The blood urea nitrogen level is usually below 100 mg/dL, and the serum creatinine level is usually below 2 to 8 mg/dL during the acute phase of illness.⁵⁸ Thrombocytopenia occurs in the absence of disseminated intravascular coagulation and may accompany progressive renal dysfunction.⁵⁹ Renal biopsy reveals acute interstitial nephritis; immune complex glomerulonephritis may also be present.⁶⁰ 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.²⁶ 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.⁵⁷

Severe pulmonary hemorrhage syndrome (SPHS) can be a prominent manifestation of infection and may occur in the absence of hepatic and renal failure.⁶¹ Frank hemoptysis can arise simultaneously with the onset of cough during the acute phase of illness.⁶² 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.⁶³ 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.⁶⁴ At autopsy, the lungs appear grossly congested and demonstrate focal areas of hemorrhage.⁵⁷ Histologically, damage to the capillary endothelium leads to congestion with foci of interstitial and intra-alveolar hemorrhage, diffuse alveolar damage, and severe air space disorganization.⁶⁵ Inflammatory infiltrates are usually absent.

Congestive heart failure occurs rarely. However, nonspecific electrocardiographic changes are common.⁶⁶ 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.⁶⁶ Atrial fibrillation is associated with more severe disease.⁶⁷ 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 seen⁶⁸; acute coronary arteritis and aortitis are also common at postmortem examination.⁶⁹

Laboratory Diagnosis

DIRECT DETECTION METHODS

Direct visualization of leptospire in blood or urine by darkfield microscopic examination has been used for diagnosis. However, artefacts are commonly mistaken for leptospire, and the method has both low sensitivity (40.2%) and specificity (61.5%).⁷⁰ 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 leptospire, but few have been evaluated in clinical studies,⁷¹⁻⁷³ 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 (leptospiemic) 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.⁷¹ Leptospiral

DNA has been amplified from serum, urine, aqueous humor, and a number of tissues obtained at autopsy.⁷⁴ 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,³ but immunohistochemical staining offers greater sensitivity and specificity.^{75,76}

ISOLATION AND IDENTIFICATION

Leptospire 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 medium at the bedside. Survival of leptospire in commercial blood culture media for several days has been reported.⁷⁷ 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 leptospire do not survive well in acidic environments.⁹

Cultures are performed in albumin-polysorbate media such as EMJH (Ellinghausen-McCullough-Johnson-Harris) medium, which is available commercially. Older media contained serum.⁵ 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.

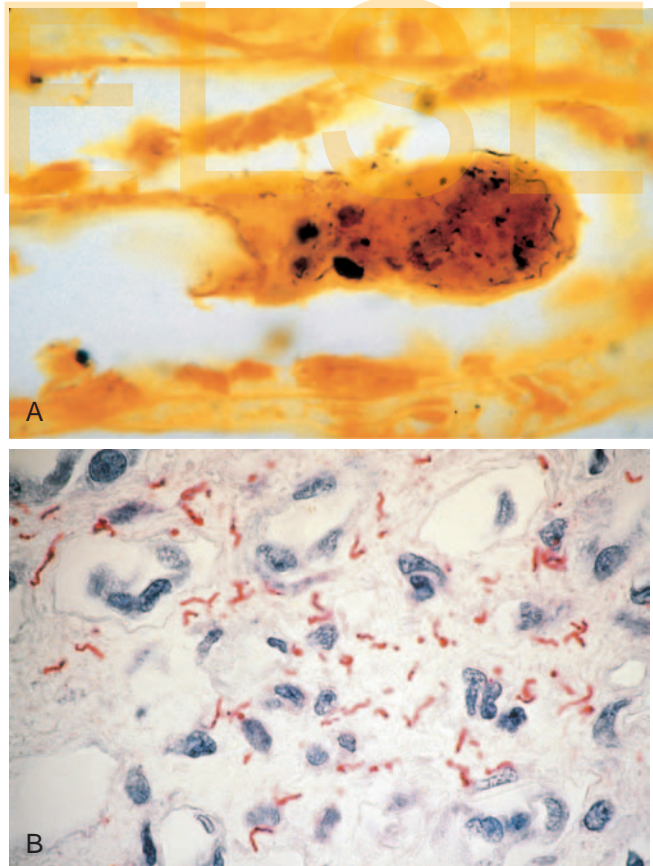


Figure 240-5 Kidney sections stained by silver staining (A) and immunohistochemical staining (B) showing presence of multiple leptospire in tubules. (A, Courtesy of Dr. Martin Hicklin, Public Health Image Library, Centers for Disease Control and Prevention.; B, courtesy of Juaune Layne, University of the West Indies, Barbados.)

Isolated leptospire are identified to serovar level by traditional serologic methods or by molecular methods, such as pulse field gel electrophoresis.⁷⁸ These techniques are limited in availability to a few reference laboratories. Powerful molecular techniques such as multi-locus sequence typing (MLST) and multiple-locus variable number tandem repeat analysis (MLVA) have been applied to the epidemiologic analysis of leptospire, but have yet to be widely used.⁷⁹⁻⁸²

Indirect Detection Methods

Most leptospire cases are diagnosed by serology. The reference standard assay is the microscopic agglutination test (MAT), in which live antigens representing different serogroups of leptospire are reacted with serum samples and then examined by darkfield microscopy for agglutination.⁹ 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 leptospire is defined by a four-fold 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.⁸³ Suggestive evidence for recent or current infection includes a single titer of at least 1:200 obtained after the onset of symptoms.⁸⁴ 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.⁸⁵

The interpretation of the MAT is complicated by cross-reaction between different serogroups, especially in acute-phase samples.⁵ Cross-reactivity in acute samples is attributable to IgM antibodies, which may persist for several years.⁸⁶ The MAT is a serogroup-specific assay, and should not be used to infer the identity of the infecting serovar.¹⁴ 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.⁸⁷ 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.⁸⁸ IgM antibodies are detectable after about the fifth day of illness, and IgM detection assays are available in several formats.⁸⁸⁻⁹¹ 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 leptospire 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,⁹²⁻⁹⁵ 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.⁹⁶ Jarisch-Herxheimer reactions have been reported in patients treated with penicillin.⁹⁷ 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 leptospire is typically completely reversible.⁹⁸ Patients requiring intubation for SPHS have

6 TABLE 240-3 Antimicrobial Agents Recommended for Treatment and Chemoprophylaxis of Leptospirosis

Indication	Compound	Dosage
Chemoprophylaxis	Doxycycline	200 mg PO once weekly
Treatment of mild leptospirosis	Doxycycline	100 mg bid PO
	Ampicillin	500-750 mg q6h PO
	Amoxicillin	500 mg q6h PO
Treatment of moderate to severe leptospirosis	Penicillin G	1.5 MU IV q6h
	Ceftriaxone	1 g IV q24h
	Ampicillin	0.5-1 g IV q6h

decreased pulmonary compliance and should be managed as cases of ARDS. Protective ventilation strategies involving low tidal volumes (lower than 6 mL/kg) to avoid alveolar injury caused by high ventilation pressures have been shown to improve survival rates in ARDS dramatically.⁹⁹

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.² Removal of leptospires 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.¹⁰⁰

Immunization of animals with killed vaccines is widely practiced, but the immunity is short-lived and animals require periodic (usually annual) boosters.¹⁰¹ Moreover, although these vaccines prevent against disease, they do not prevent infection and renal colonization; 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, Canciola, 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^{102,103} and appear to protect against renal colonization and urinary shedding.¹⁰⁴

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.¹⁰⁵ 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.¹⁰⁶ The use of doxycycline prophylaxis after excess rainfall in local populations in endemic areas has been studied.^{107,108} Symptomatic disease was significantly reduced in one study, but serologic evidence of infection was found equally in subjects and controls.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 azithromycin¹⁰⁹ 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

- World Health Organization. Leptospirosis worldwide, 1999. *Wkly Epidemiol Rec.* 1999;74:237-242.
- Faine S, Adler B, Bolin C, et al. *Leptospira and leptospirosis*. 2nd ed. Melbourne: MedSci; 1999.
- Stimson AM. Note on an organism found in yellow-fever tissue. *Publ Health Rep.* 1907;22:541.
- Everard JD. Leptospirosis. In: Cox FEG, ed. *The Wellcome Trust Illustrated History of Tropical Diseases*. London: The Wellcome Trust; 1996:111-119, 416-418.
- Levett PN. Leptospirosis. *Clin Microbiol Rev.* 2001;14:296-326.
- Edwards GA, Domm BM. Human leptospirosis. *Medicine.* 1960;39:117-156.
- Feigin RD, Anderson DC. Human leptospirosis. *CRC Crit Rev Clin Lab Sci.* 1975; 5:413-467.
- Goldstein SF, Charon NW. Motility of the spirochete *Leptospira*. *Cell Motil Cytoskeleton.* 1988;9:101-110.
- Levett PN. Leptospira. In: Murray PR, Baron EJ, Jorgensen JH, et al, eds. *Manual of Clinical Microbiology*. 9th ed. Washington, DC: American Society for Microbiology Press; 2007:963-970.
- Bulach DM, Kalambaheti T, de La Peña-Moctezuma A, et al. Lipopolysaccharide biosynthesis in *Leptospira*. *J Mol Microbiol Biotechnol.* 2000;2:375-380.
- Yasuda PH, Steigerwalt AG, Sulzer KR, et al. Deoxyribonucleic acid relatedness between serogroups and serovars in the family Leptospiraceae with proposals for seven new *Leptospira* species. *Int J Syst Bacteriol.* 1987;37:407-415.
- Brenner DJ, Kaufmann AF, Sulzer KR, et al. Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. *Int J Syst Bacteriol.* 1999;49:839-858.
- Morey RE, Galloway RL, Bragg SL, et al. Species-specific identification of *Leptospiraceae* by 16S rRNA gene sequencing. *Journal of Clinical Microbiology.* 2006;44:3510-3516.
- Levett PN. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clin Infect Dis.* 2003;36:447-452.
- Ren SX, Fu G, Jiang XG, et al. Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. *Nature.* 2003; 422:888-893.
- Nascimento AL, Ko AI, Martins EA, et al. Comparative genomics of two *Leptospira interrogans* serovars reveals novel insights into physiology and pathogenesis. *Journal of Bacteriology.* 2004;186:2164-2172.
- Bulach DM, Zuerner RL, Wilson P, et al. Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. *Proceedings of the National Academy of Sciences USA.* 2006;103:14560-14565.
- Picardeau M, Bulach DM, Bouchier C, et al. Genome sequence of the saprophyte *Leptospira biflexa* provides insights into the evolution of *Leptospira* and the pathogenesis of leptospirosis. *PLoS ONE.* 2008;3:e1607.
- Trejejo RT, Rigau-Perez JG, Ashford DA, et al. Epidemic leptospirosis associated with pulmonary hemorrhage-Nicaragua, 1995. *J Infect Dis.* 1998;178:1457-1463.
- Sasaki DM, Pang L, Minette HP, et al. Active surveillance and risk factors for leptospirosis in Hawaii. *Am J Trop Med Hyg.* 1993;48:35-43.
- Morgan J, Bornstein SL, Karpati AM, et al. Outbreak of Leptospirosis among Triathlon participants and community residents in Springfield, Illinois, 1998. *Clin Infect Dis.* 2002; 34:1593-1599.
- Sejvar J, Bancroft E, Winthrop K, et al. Leptospirosis in "Eco-Challenge" athletes, Malaysian Borneo, 2000. *Emerg Infect Dis.* 2003;9:702-707.
- Ward MP, Glickman LT, Guptill LF. Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970-1998). *J Am Vet Med Assoc.* 2002;220:53-58.
- Prescott JF, McEwen B, Taylor J, et al. Resurgence of leptospirosis in dogs in Ontario: Recent findings. *Can Vet J.* 2002; 43:955-961.
- Brown CA, Roberts AW, Miller MA, et al. *Leptospira interrogans* serovar *grippotyphosa* infection in dogs. *J Am Vet Med Assoc.* 1996;209:1265-1267.
- Areán VM. The pathologic anatomy and pathogenesis of fatal human leptospirosis (Weil's disease). *Am J Pathol.* 1962; 40:393-423.
- de Souza L, Koury MC. Isolation and biological activities of endotoxin from *Leptospira interrogans*. *Can J Microbiol.* 1992;38:284-289.
- Werts C, Tapping RI, Mathison JC, et al. Leptospiral endotoxin activates cells via a TLR2-dependent mechanism. *Nature Immunol.* 2001;2:346-352.
- Nahori MA, Fournie-Amazouz E, Que-Gewirth NS, et al. Differential TLR recognition of leptospiral lipid A and lipopolysaccharide in murine and human cells. *J Immunol.* 2005;175: 6022-6031.
- Que-Gewirth NL, Ribeiro AA, Kalb SR, et al. A methylated phosphate group and four amide-linked acyl chains in *Leptospira interrogans* lipid A. *J Biol Chem.* 2004;279:25420-25429.
- Lee SH, Kim S, Park SC, Kim MJ. Cytotoxic activities of *Leptospira interrogans* hemolysin SphH as a pore-forming protein on mammalian cells. *Infect Immun.* 2002;70:315-322.
- Abdulkader RC, Daher EF, Camargo ED, et al. Leptospirosis severity may be associated with the intensity of humoral immune response. *Revis Inst Med Trop São Paulo.* 2002;44: 79-83.
- Lingappa J, Kuffner T, Tappero J, et al. HLA-DQ6 and ingestion of contaminated water: possible gene-environment interaction in an outbreak of leptospirosis. *Genes Immun.* 2004;5:197-202.
- Faber NA, Crawford M, LeFebvre RB, et al. Detection of *Leptospira* spp. in the aqueous humor of horses with naturally acquired recurrent uveitis. *J Clin Microbiol.* 2000;38: 2731-2733.
- Lucchesi PM, Parma AE, Arroyo GH. Serovar distribution of a DNA sequence involved in the antigenic relationship between *Leptospira* and equine cornea. *BMC Microbiol.* 2002;2:3.
- Haake DA. Spirochaetal lipoproteins and pathogenesis. *Microbiology.* 2000;146:1491-1504.
- Haake DA, Chao G, Zuerner RL, et al. The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infect Immun.* 2000;68: 2276-2285.
- Guerreiro H, Croda J, Flannery B, et al. Leptospiral proteins recognized during the humoral immune response to leptospirosis in humans. *Infect Immun.* 2001;69:4958-4968.
- Yang CW, Wu MS, Pan MJ, et al. The *Leptospira* outer membrane protein LipL32 induces tubulointerstitial nephritis-mediated gene expression in mouse proximal tubule cells. *J Am Soc Nephrol.* 2002;13:2037-2045.
- Choy HA, Kelley MM, Chen TL, et al. Physiological osmotic induction of *Leptospira interrogans* adhesion: LigA and LigB bind extracellular matrix proteins and fibrinogen. *Infect Immun.* 2007;75:2441-2450.
- Croda J, Ramos JG, Matsunaga J, et al. *Leptospira* immunoglobulin-like proteins as a serodiagnostic marker for acute leptospirosis. *J Clin Microbiol.* 2007;45:1528-1534.
- Stevenson B, Choy HA, Pinne M, et al. *Leptospira interrogans* endostatin-like outer membrane proteins bind host fibronectin, laminin and regulators of complement. *PLoS ONE.* 2007;2:e1188.

43. Alexander AD, Benenson AS, Byrne RJ, et al. Leptospirosis in Puerto Rico. *Zoon Res.* 1963;2:152-227.
44. Wang C, John L, Chang T, et al. Studies on anicteric leptospirosis. I. Clinical manifestations and antibiotic therapy. *Chin Med J.* 1965;84:283-291.
45. Berman SJ, Tsai CC, Holmes KK, et al. Sporadic anicteric leptospirosis in South Vietnam. *Ann Intern Med.* 1973;79:167-173.
46. Park S-K, Lee S-H, Rhee Y-K, et al. Leptospirosis in Chonbuk province of Korea in 1987: A study of 93 patients. *Am J Trop Med Hyg.* 1989;41:345-351.
47. Edwards CN, Nicholson GD, Hassell TA, et al. Leptospirosis in Barbados: A clinical study. *W Indian Med J.* 1990;39:27-34.
48. Yersin C, Bovet P, Mérien F, et al. Human leptospirosis in the Seychelles (Indian Ocean): A population-based study. *Am J Trop Med Hyg.* 1998;59:933-940.
49. Ko AI, Galvao Reis M, Ribeiro Dourado CM, et al; Salvador Leptospirosis Study Group. Urban epidemic of severe leptospirosis in Brazil. *Lancet.* 1999;354:820-825.
50. Katz AR, Ansdell VE, Effler PV, et al. Assessment of the clinical presentation and treatment of 353 cases of laboratory-confirmed leptospirosis in Hawaii, 1974-1998. *Clin Infect Dis.* 2001;33:1834-1841.
51. Bharadwaj R, Bal AM, Joshi SA, et al. An urban outbreak of leptospirosis in Mumbai, India. *Jpn J Infect Dis.* 2002;55:194-196.
52. Turner LH. Leptospirosis I. *Trans R Soc Trop Med Hyg.* 1967;61:842-855.
53. Silva HR, Tanajura GM, Tavares-Neto J, et al. Aseptic meningitis syndrome due to enterovirus and *Leptospira* sp in children of Salvador, Bahia. *Revis Soc Brasil Med Trop.* 2002;35:159-165.
54. Lopes AA, Costa E, Costa YA, et al. Comparative study of the in-hospital case-fatality rate of leptospirosis between pediatric and adult patients of different age groups. *Rev Inst Med Trop Sao Paulo.* 2004;46:19-24.
55. Esen S, Sunbul M, Leblebicioglu H, et al. Impact of clinical and laboratory findings on prognosis in leptospirosis. *Swiss Med Wkly.* 2004;134:347-352.
56. Edwards GA, Domm BM. Leptospirosis. II. *Med Times.* 1966;94:1086-1095.
57. Zaki SR, Spiegel RA. Leptospirosis. In: Nelson AM, Horsburgh CR, eds. *Pathology of Emerging Infections*, vol 2. Washington, DC: American Society for Microbiology Press; 1998:73-92.
58. Abdulkader RCRM, Seguro AC, Malheiro PS, et al. Peculiar electrolytic and hormonal abnormalities in acute renal failure due to leptospirosis. *Am J Trop Med Hyg.* 1996;54:1-6.
59. Edwards CN, Nicholson GD, Hassell TA, et al. Thrombocytopenia in leptospirosis: The absence of evidence for disseminated intravascular coagulation. *Am J Trop Med Hyg.* 1986;35:352-354.
60. Lai KN, Aarons I, Woodroffe AJ, et al. Renal lesions in leptospirosis. *Aust N Z J Med.* 1982;12:276-279.
61. Zaki SR, Shieh W-J; Epidemic Working Group. Leptospirosis associated with outbreak of acute febrile illness and pulmonary haemorrhage, Nicaragua, 1995. *Lancet.* 1996;347:535.
62. Yersin C, Bovet P, Mérien F, et al. Pulmonary haemorrhage as a predominant cause of death in leptospirosis in Seychelles. *Trans R Soc Trop Med Hyg.* 2000;94:71-76.
63. Im J-G, Yeon KM, Han MC, et al. Leptospirosis of the lung: Radiographic findings in 58 patients. *Am J Roentgenol.* 1989;152:955-959.
64. Marotto PC, Nascimento CM, Eluf-Neto J, et al. Acute lung injury in leptospirosis: clinical and laboratory features, outcome, and factors associated with mortality. *Clin Infect Dis.* 1999;29:1561-1563.
65. Nicodemo AC, Duarte MIS, Alves VAF, et al. Lung lesions in human leptospirosis: Microscopic, immunohistochemical, and ultrastructural features related to thrombocytopenia. *Am J Trop Med Hyg.* 1997;56:181-187.
66. Parsons M. Electrocardiographic changes in leptospirosis. *Br Med J.* 1965;2:201-203.
67. Sacramento E, Lopes AA, Costa E, et al. Electrocardiographic alterations in patients hospitalized with leptospirosis in the Brazilian city of Salvador. *Arquiv Brasil Cardiol.* 2002;78:267-270.
68. Areán VM. Leptospirosis myocarditis. *Lab Invest.* 1957;6:462-471.
69. de Brito T, Morais CF, Yasuda PH, et al. Cardiovascular involvement in human and experimental leptospirosis: Pathologic findings and immunohistochemical detection of leptospiral antigen. *Ann Trop Med Parasitol.* 1987;81:207-214.
70. Vijayachari P, Sugunan AP, Umapathi T, et al. Evaluation of darkground microscopy as a rapid diagnostic procedure in leptospirosis. *Indian J Med Res.* 2001;114:54-58.
71. Brown PD, Gravekamp C, Carrington DG, et al. Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. *J Med Microbiol.* 1995;43:110-114.
72. Mérien F, Baranton G, Pérolat P. Comparison of polymerase chain reaction with microagglutination test and culture for diagnosis of leptospirosis. *J Infect Dis.* 1995;172:281-285.
73. Slack A, Symonds M, Dohnt M, et al. Evaluation of a modified Taqman assay detecting pathogenic *Leptospira* spp. against culture and *Leptospira*-specific IgM enzyme-linked immunosorbent assay in a clinical environment. *Diag Microbiol Infect Dis.* 2007;57:361-366.
74. Brown PD, Carrington DG, Gravekamp C, et al. Direct detection of leptospiral material in human postmortem samples. *Res Microbiol.* 2003;154:581-586.
75. Alves VAF, Vianna MR, Yasuda PH, et al. Detection of leptospiral antigen in the human liver and kidney using an immunoperoxidase staining procedure. *J Pathol.* 1987;151:125-131.
76. Guarner J, Shieh W-J, Morgan J, et al. Leptospirosis mimicking acute cholecystitis among athletes participating in a triathlon. *Hum Pathol.* 2001;32:750-752.
77. Palmer MF, Zochowski WJ. Survival of leptospires in commercial blood culture systems revisited. *J Clin Pathol.* 2000;53:713-714.
78. Galloway RL, Levett PN. Evaluation of a modified pulsed-field gel electrophoresis approach for the identification of *Leptospira* serovars. *Am J Trop Med Hyg.* 2008;78:628-632.
79. Ahmed N, Devi SM, Valverde Mde L, et al. Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. *Ann Clin Microbiol Antimicrob.* 2006;5:28.
80. Salaun L, Merien F, Gurianova S, et al. Application of multilocus variable-number tandem-repeat analysis for molecular typing of the agent of leptospirosis. *J Clin Microbiol.* 2006;44:3954-3962.
81. Slack A, Symonds M, Dohnt M, et al. An improved multilocus variable number of tandem repeats analysis for *Leptospira interrogans* serovar Australis: a comparison with fluorescent amplified fragment length polymorphism analysis and its use to redefine the molecular epidemiology of this serovar in Queensland, Australia. *J Med Microbiol.* 2006;55:1549-1557.
82. Thaipadungpanit J, Wuthiekanun V, Chierakul W, et al. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. *PLoS Negl Trop Dis.* 2007;1:e56.
83. Faine S. *Guidelines for the control of leptospirosis (offset publication No. 67)*. Geneva: World Health Organization, 1982.
84. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. *Morbidity Mortal Wkly Rep MMWR.* 1997;46(RR-10):49-55.
85. Bajani MD, Ashford DA, Bragg SL, et al. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. *J Clin Microbiol.* 2003;41:803-809.
86. Cumberland PC, Everard COR, Wheeler JG, et al. Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979-1989. *Eur J Epidemiol.* 2001;17:601-608.
87. Cumberland PC, Everard COR, Levett PN. Assessment of the efficacy of the IgM enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. *Am J Trop Med Hyg.* 1999;61:731-734.
88. Levett PN, Branch SL, Whittington CU, et al. Two methods for rapid serological diagnosis of acute leptospirosis. *Clin Diagn Lab Immunol.* 2001;8:349-351.
89. Levett PN, Branch SL. Evaluation of two enzyme-linked immunosorbent assay methods for detection of immunoglobulin M antibodies in acute leptospirosis. *Am J Trop Med Hyg.* 2002;66:745-748.
90. Smits HL, Ananyina YV, Chereschsky A, et al. International multicenter evaluation of the clinical utility of a dipstick assay for detection of *Leptospira*-specific immunoglobulin M antibodies in human serum specimens. *J Clin Microbiol.* 1999;37:2904-2909.
91. Smits HL, van Der Hoorn MA, Goris MG, et al. Simple latex agglutination assay for rapid serodiagnosis of human leptospirosis. *J Clin Microbiol.* 2000;38:1272-1275.
92. McClain JBL, Ballou WR, Harrison SM, et al. Doxycycline therapy for leptospirosis. *Ann Intern Med.* 1984;100:696-698.
93. Edwards CN, Nicholson GD, Hassell TA, et al. Penicillin therapy in icteric leptospirosis. *Am J Trop Med Hyg.* 1988;39:388-390.
94. Watt G, Padre LP, Tuazon ML, et al. Placebo-controlled trial of intravenous penicillin for severe and late leptospirosis. *Lancet.* 1988;ii:433-435.
95. Costa E, Lopes AA, Sacramento E, et al. Penicillin at the late stage of leptospirosis: A randomized controlled trial. *Revis Inst Med Trop Sao Paulo.* 2003;45:141-145.
96. Panaphut T, Domrongkitchaiporn S, Vibhagool A, et al. Ceftriaxone compared with sodium penicillin G for treatment of severe leptospirosis. *Clin Infect Dis.* 2003;36:1507-1513.
97. Friedland JS, Warrell DA. The Jarisch-Herxheimer reaction in leptospirosis: Possible pathogenesis and review. *Rev Infect Dis.* 1991;13:207-210.
98. Andrade L, de Francesco Daher E, Seguro AC. Leptospirosis nephropathy. *Semin Nephrol.* 2008;28:383-394.
99. Amato MB, Barbas CS, Medeiros DM, et al. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med.* 1998;338:347-354.
100. Douglin CP, Jordan C, Rock R, et al. Risk factors for severe leptospirosis in the parish of St. Andrew, Barbados. *Emerg Infect Dis.* 1997;3:78-80.
101. Bey RF, Johnson RC. Current status of leptospiral vaccines. *Prog Vet Microbiol Immunol.* 1986;2:175-197.
102. Naiman BM, Alt D, Bolin CA, et al. Protective killed *Leptospira borgpetersenii* vaccine induces potent Th1 immunity comprising responses by CD4 and gamma delta T lymphocytes. *Infect Immun.* 2001;69:7550-7558.
103. Brown RA, Blumberman S, Gay C, et al. Comparison of three different leptospiral vaccines for induction of a type 1 immune response to *Leptospira borgpetersenii* serovar Hardjo. *Vaccine.* 2003;21:4448-4458.
104. Bolin CA, Alt DP. Use of a monovalent leptospiral vaccine to prevent renal colonization and urinary shedding in cattle exposed to *Leptospira borgpetersenii* serovar hardjo. *Am J Vet Res.* 2001;62:995-1000.
105. Martínez R, Pérez A, Quiñones MdC, et al. [Eficacia y seguridad de una vacuna contra la leptospirosis humana en Cuba.] *Rev Panam Salud Publica.* 2004;15:249-255.
106. Takafuji ET, Kirkpatrick JW, Miller RN, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *N Engl J Med.* 1984;310:497-500.
107. Sehgal SC, Sugunan AP, Murhekar MV, et al. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. *Int J Antimicrob Agents.* 2000;13:249-255.
108. Gonzalez CR, Casseb J, Monteiro FG, et al. Use of doxycycline for leptospirosis after high-risk exposure in Sao Paulo, Brazil. *Revis Inst Med Trop Sao Paulo.* 1998;41:59-61.
109. Hospenthal DR, Murray CK. In vitro susceptibilities of seven *Leptospira* species to traditional and newer antibiotics. *Antimicrob Agents Chemother.* 2003;47:2646-2648.

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