Zoonosis Update

Leptospirosis

Marta A. Guerra, DVM, MPH, PhD, DACVPM

Leptospirosis, a bacterial zoonotic disease with a worldwide distribution, is caused by spirochetes of the genus *Leptospira*. Leptospirosis encompasses a wide spectrum of clinical disease in humans, including multiorgan failure with a high mortality rate. Because of the lack of specific symptoms associated with this disease, it is difficult to make an accurate diagnosis in a timely manner. In 1886, Adolph Weil[1] was one of the first to describe the severe or icteric form of leptospirosis in Heidelberg, Germany. Descriptions of the disease among agricultural workers and miners in Japan and China were reported earlier.[2] Occupation was recognized as an important risk factor before animal host species were identified. Rodents were first identified as a potential source of human infection, followed by dogs. The role of livestock as reservoirs was not determined until several decades later.[3] Leptospirosis has been recently classified as an emerging disease because of large clusters of cases resulting from exposure during recreational activities and natural disasters.

Pathogen Characteristics

Leptospires are gram-negative, highly motile, obligate-aerobic spirochetes; these organisms are tightly coiled with characteristic hooked ends and are 0.2 μm in diameter and 6 to 20 μm in length. Histologically, silver staining is the method of choice for identification in tissue specimens. Unstained organisms can be viewed only via darkfield or phase-contrast microscopy.

The taxonomy of leptospires has been continually evolving. Historically, there were 2 species within this genus: *Leptospira interrogans,* which is generally pathogenic to humans and a variety of mammals, and *Leptospira biflexa,* which is saprophytic and nonpathogenic. *Leptospira interrogans* has been subclassified into serovars according to stable antigenic differences and into serogroups on the basis of common antigens.[4] Molecular techniques have allowed the identification of 17 genomospecies.[5–7] Currently, more than 200 serovars and 23 pathogenic serogroups have been identified.[8]

In human tissues infected with leptospires, the most common pathologic changes observed include swelling and necrosis of capillary endothelial cells, inflammation and cellular infiltration of renal tubules, and mild degenerative changes of hepatocytes.[9] Hemorrhage is a result of disruption of endothelial cellular membranes

ABBREVIATION	
MAT	Microscopic agglutination test

and not attributable to clotting abnormalities.[10] Jaundice develops because of hepatic cellular dysfunction; however, permanent liver damage rarely occurs. The exact mechanism of vascular injury is not well understood. It has been postulated that organ damage may be caused by a toxin produced by leptospires, which may mediate some pathophysiologic effects.[11] Injury to tissues may also occur during the development of antibodies and the deposition of immune complexes.

Ecology and Transmission

Leptospirosis has a global distribution, and leptospires have been detected in more than 180 species of animals. Mammals are the only class of animals capable of transmitting *Leptospira* organisms, even though leptospires have been identified in reptiles and birds.[12] The pathogenicity of a leptospiral serovar in a host animal varies depending on the host species and the geographic area in which the host is located. Some serovars have adapted to specific mammalian species, which are considered maintenance hosts, and cause mild to no disease. These maintenance hosts are capable of shedding large quantities of leptospires into the environment. A mammalian species may serve as a maintenance host for > 1 serovar. Animals will generally have more severe clinical signs when infected with serovars to which they are not adapted. These animals are considered incidental hosts and may shed leptospires in limited quantities.

Infection is usually acquired early in an animal's life, and the prevalence of chronic urinary excretion of leptospires increases with age.[12] Animals that survive an acute infection and have no clinical signs can go on to shed the organism in urine for months to years. Pathogenic leptospires can be found in the renal tubules of a wide variety of wild, peridomestic, and domestic animals.[13] The most important maintenance hosts are small mammals, especially rodents, which may transmit infection to domestic farm animals, dogs, and humans. Once excreted in the urine, leptospires can survive in fairly moist environments for months to years. For survival of the organisms in water, temperature in the range of 28° to 38°C and pH in the range of 6.2 to 8.0 are optimal conditions.[14,15] Survival in water is inhibited by contamination with sewage, high acidity, and high salinity.

Humans become infected with leptospires when the organisms are introduced into the body through abraded skin or through the mucosal surfaces of the eye, mouth, nasopharynx, or esophagus.[16] The most common sources of infection for humans are direct contact with the infected urine of animals or indirect contact with water or moist soil contaminated with infected urine. Another route of exposure is direct contact with blood or tissues of infected animals. Human-to-human transmission via ingestion of breast milk or via sexual contact has been documented, but is reported rarely.[17,18] Infection during pregnancy can result in infection of the fetus with various outcomes, ranging from midgestational fetal deaths to births of healthy infants following administration of appropriate antimicrobial agents.[19] Laboratory-acquired infections have also been reported.[20]

Epidemiology

Geographically, leptospirosis is ubiquitous; endemic foci correspond with areas where domestic and wild animals can serve as reservoirs. Seasonality of cases may be related to agricultural cycles and increased levels of outdoor recreation in the warmer months. In the United States, more than 50% of cases are reported from July through October.[21] In tropical regions, cases are reported year-round but predominantly during the rainy season.[22-24] The increased risk during the rainy season becomes even greater after flooding that accompanies natural disasters, when the human population may be exposed to water contaminated with urine from infected animals. Outbreaks associated with flooding and natural disasters have occurred in Nicaragua in 1995,[22] in Brazil in 1996,[25] and in India in 2002.[26]

Leptospirosis can be a frequent cause of acute febrile illness among humans in countries where this disease is endemic. Confirmed cases are detected sporadically or in clusters, giving rise to point-source outbreaks. Unrecognized asymptomatic or mild infections may also develop among humans, resulting in underestimation of incidence. The majority of affected humans (90%) develop a mild form of the disease; the more severe form of the disease may have a case fatality rate of 10% to 15%.[27] The prevalence of leptospirosis in some countries within tropical zones has been assessed and reported as ranging from 12.8% among children in Vietnam[28] to 23.3% among inhabitants of suburban neighborhoods in Colombia.[29]

Persons at risk for leptospirosis include those with occupations that involve direct contact with domestic animals (eg, livestock farmers, abattoir workers, and veterinarians) or those working outdoors where exposure to contaminated environments may occur (eg, agricultural laborers, miners, and fish workers). A case-control study[30] in Thailand revealed an increased risk of leptospiral infection among persons that performed various agricultural activities in wet fields for > 6 h/d.

Persons living in urban environments where rodents are present may be at increased risk for acquiring leptospirosis. Contact with infected urine from rodents or, potentially, pets may serve as a source of infection. Results of serosurveys among inner city populations in the United States indicated that the prevalence of anti-bodies against *Leptospira* organisms was approximately 30% among inner-city school children in Detroit[31] and 16% among adults in Baltimore.[32] In Brazil, an investigation of risk factors for leptospirosis among residents in a poor, crowded urban area revealed a positive association between acquiring infection and residency in close proximity to open sewers; similarly, there was a positive association between acquiring infection and the presence of rats near the home.[24] In studies[22,33] in Nicaragua, walking in creeks and having close contact with rodents and dogs were positively associated with acquiring infection. Having an indoor water source was negatively associated with development of leptospirosis.[33] The encroachment of human habitation into areas previously inhabited by wildlife or into areas with poor drainage and inadequate sewage systems has increased the risk of exposure to infective leptospires.

Avocational and recreational exposures such as pet ownership and gardening are also considered risk factors for infection.[34] Recent outbreaks of leptospirosis have involved sporting activities and events conducted in or near bodies of water. Individuals have developed leptospirosis as a result of exposure to contaminated waters while engaging in water sports that include swimming in rivers in Hawaii,[35] white-water rafting in Costa Rica,[36] and participating in multisport events such as a triathlon in Illinois[37] and the Eco-Challenge-Sabah 2000 in Malaysia.[38]

In the United States, leptospirosis ceased being a nationally notifiable disease in December 1994; thus, it is difficult to estimate the current disease incidence in the United States. Nevertheless, several states and territories with tropical climates (including Hawaii and Puerto Rico) have maintained mandatory reporting. In Hawaii, which consistently has one of the highest yearly incidence rates, leptospirosis was initially considered an occupational disease of agricultural workers[39]; however, in more recent years, cases have been associated with outdoor and recreational activities.[34] In Puerto Rico, sporadic cases and occasional clusters of leptospirosis-affected persons continue to be reported each year. A seroprevalence survey conducted more than 50 years ago yielded a prevalence of 14%.[23] During a recent outbreak in Puerto Rico of dengue, a disease with clinical signs similar to those associated with leptospirosis, prevalence of leptospirosis among ill patients that were serologically negative for dengue was 5%.[40] In that country, a much higher prevalence of 24% was determined from analysis of a series of dengue-negative samples obtained from ill patients after Hurricane Hortense in 1996.[23]

Clinical Signs of Leptospirosis in Humans

Leptospirosis in humans is characterized by a wide variety of symptoms and a biphasic course of illness.[41] The first phase corresponds to the multiplication and spread of the organism throughout the body. The second phase is characterized by the development of circulating antibodies and the detection of leptospires in the urine. The incubation period is typically 1 to 2 weeks (range, 2 to 30 days). Most infections appear to be subclinical or so mild that they are never reported. Among clinical cases, initial influenza-like signs during

the first phase include fever, headache, chills, myalgias, and, occasionally, a maculopapular skin rash and conjunctival suffusion (ie, redness of the conjunctiva without inflammatory exudates). This phase is followed by a 1- to 3-day period of defervescence and symptomatic improvement.

In the second phase, the signs of leptospirosis are more organ specific,[42] and the disease can be categorized into anicteric and icteric forms. The milder anicteric form of the disease is diagnosed in approximately 90% of patients, whereas the severe, icteric form is diagnosed in 5% to 10% of patients. In persons with anicteric leptospirosis, aseptic meningitis is the most common clinical syndrome and is characterized by severe headache and neck stiffness; this syndrome is more common in younger patients.[43] Uveitis may develop during this phase or may develop weeks to years after the onset of disease.

The more severe form, icteric leptospirosis (also called Weil's syndrome), has a less pronounced biphasic course. After initial nonspecific signs, the second phase is characterized by jaundice, renal dysfunction, pulmonary dysfunction, or hemorrhagic manifestations. Untreated patients with the icteric form have a higher mortality rate than those with the anicteric form of leptospirosis. The most severe complication of icteric disease is the development of oliguria; subsequently, anuria and renal failure develop, the latter being the most common cause of death.[44] The most commonly detected liver-related serum biochemical abnormalities are high bilirubin concentration and mildly high activities of transaminases. In patients that recover, there is no chronic liver dysfunction. Pulmonic involvement is often manifested by cough and hemoptysis. Severe pulmonary distress that culminates in pulmonary hemorrhage had been previously detected only among affected persons in Asia,[45] but has now been reported among patients in outbreaks in Nicaragua.[22,46]

Clinical Signs of Leptospirosis in Nonhuman Animals

In animals other than humans, leptospirosis can be subclinical; subclinical infection usually develops when an animal is infected by a serovar to which it is adapted. In adult cattle, often the only observable clinical sign of leptospirosis is abortion or stillbirth.[47] Infected calves usually develop a more severe, acute form of the disease (with clinical signs such as fever, jaundice, and hematuria) that is frequently fatal. Among infected horses, abortions and stillbirths can occur, and nonspecific signs (eg, fever and jaundice) may develop; however, the most frequently reported clinical sign is uveitis.[48,49] Abortions and stillbirths also occur among swine that develop leptospiral infections, and those affected animals are capable of shedding large amounts of leptospires.[50]

In dogs, clinical signs of leptospirosis may vary depending on the age and immunologic status of the animal and on the serovar of the leptospire.[51–54] In acute infections, early signs include fever, stiffness, and vomiting; dehydration, pulmonary hemorrhage, and shock may develop later. In subacute infections, anorexia and signs of depression and respiratory tract effects, such as conjunctivitis and rhinitis, may be evident. In chronically infected dogs, renal function may decline accompanied by weight loss, vomiting, polydipsia, and polyuria. Hepatic dysfunction may result in icterus, and severe hepatic failure may include the development of ascites and encephalopathy. Meningitis and uveitis develop infrequently in dogs with leptospirosis.

Diagnosis in Humans

The diagnosis of leptospirosis in humans can be challenging because of the protean nature of the disease. In tropical climates, many diseases (eg, malaria, typhoid fever, scrub typhus, hantavirus infection, and dengue) may have clinical characteristics similar to those associated with leptospirosis. Humans with leptospirosis usually have a history of contact with animals or contaminated water. The first step toward establishing an accurate diagnosis of leptospirosis is to collect accurate information regarding a patient's travel, recreational, and avocational activities. Definitive diagnosis is typically based on results of serologic testing. Anti-*Leptospira* antibodies can be detected in blood samples collected from an infected person at 5 to 7 days after onset of symptoms. The MAT is currently the diagnostic method of choice.[8] However, because as many as 24 serovar antigens are used to determine the most likely serogroup that is causing illness, the complexity of this test limits its use to reference laboratories. Case confirmation requires detection of a 4-fold or greater increase in titers of serum anti-*Leptospira* antibodies between acute and convalescent samples obtained at least 2 weeks apart. A serum antibody titer > 200 in a single sample defines a probable human case according to the current case definition accepted by the CDC and the Council of State and Territorial Epidemiologists.[55] Determination of the infecting serovar by use of an MAT may not always be predictive. The antibodies in serum may cross-react with several different serovars in the MAT (paradoxical reaction), particularly in specimens collected from patients with acute infections.[56] As the immune response matures, the antibody cross-reactivity lessens, and the test becomes relatively serogroup specific.

In areas in which the disease is highly endemic, a low anti-*Leptospira* antibody titer may reflect past infections; therefore, a higher titer may need to be considered as a cutoff point for a probable case.[57] Immunoglobulin M-specific ELISA rapid dipstick diagnostic tests are now available for use in leptospirosis-endemic areas and can provide a reliable diagnosis quickly during potential outbreaks. In an evaluation of 4 commercially available tests, a microplate ELISA (for IgM) and a dot-ELISA (for IgM) dipstick test each had high sensitivity and specificity for detection of leptospirosis.[58]

Other diagnostic methods for leptospirosis include PCR assays for detection of leptospiral DNA in blood, sera, CSF, aqueous humor, or urine samples[59,60]; however, no PCR assay has been validated for use with clinical specimens. Bacterial culture and isolation of the organism are not practical approaches for rapid diagnosis because of the relatively slow growth of the organism. Isolation of the organisms from blood is possible only during the first 10 days of illness. However, organisms

may be isolated from urine after the second week of illness and, potentially, for several months.

Diagnosis in Nonhuman Animals

The diagnostic methods used in other animals are comparable to those used in humans—serologic detection of anti-*Leptospira* antibodies or identification of the leptospires by use of PCR assays or bacterial culture.[61] Results of serologic testing are frequently used to diagnose leptospirosis, and the MAT is performed most often. However, establishing a diagnosis based on MAT results may be complicated because of the possibility of cross-reactivity among serovars, the presence of antibodies elicited by vaccination, and the difficulty inherent in the interpretation of titers determined in single samples. A 4-fold difference in antibody titers in paired acute and convalescent serum samples collected at least 2 weeks apart is essential for the confirmation of acute leptospiral infections. The ELISA kits have been developed as simple screening tests for leptospirosis; however, they cannot determine the infecting serovar. Bacterial culture is not a rapid diagnostic method for leptospires, but isolation of the organism allows identification of the serovars. Use of a combination of these tests can improve the ability to establish a diagnosis.

Treatment of Humans

Antimicrobial treatment is indicated for all patients with leptospirosis. There is an extensive range of antimicrobial agents that are active against this disease. In patients with mild disease, the drug regimen of choice is oral administration of doxycycline for 2 weeks.[62] However, doxycycline is not recommended for treatment of pregnant women. Initiation of antimicrobial treatment within 7 days following the onset of clinical signs has been associated with a shorter duration of illness.[63] For patients with the more severe form of the disease, IV administration of penicillin G is indicated until improvement is observed and oral administration of the drug is tolerated. Studies in Thailand have revealed that ceftriaxone[64] and cefotaxime[65] are as effective as penicillin in the treatment of severe infections. Treatment guidelines for children > 8 years old are similar to those for adults. Results of recent studies appear to indicate that short courses of doxycycline administration in children < 8 years old may not cause clinically important staining of permanent teeth.[66,67] Controversy has existed as to whether antimicrobials should be used to treat individuals with leptospirosis, especially with regard to persons who seek treatment late in the course of disease. The consensus among leptospirosis experts has been that physicians should not withhold antimicrobial treatment, even for suspect cases.[62,68]

Appropriate and timely administration of antimicrobials and supportive care are essential for a favorable outcome in the treatment of patients with moderate to severe leptospirosis. Aggressive fluid therapy and maintenance of electrolyte balance are also highly important. Among patients with leptospirosis, the mortality rate is higher in persons who present late in the course of the disease and do not receive antimicrobials and in persons with impairment of renal function, especially anuria.[69] Blood transfusion may be necessary if hemorrhagic conditions develop or occult blood loss is evident. Patients with pulmonary hemorrhage frequently require intubation and mechanical ventilation.

Preexposure prophylaxis is recommended for persons who are traveling to areas in which leptospirosis is endemic and who have a high risk of exposure. Doxycycline may be administered orally at a dose of 200 mg/wk during the time of exposure.[70,71] Results of a study[72] in which doxycycline was administered as prophylaxis to inhabitants of a leptospirosis-endemic area indicated that infection with leptospires is not prevented; however, the development of clinical disease is decreased. Currently, guidelines for preexposure prophylaxis in children have not been established.

Treatment of Nonhuman Animals

Successful antimicrobial treatment of cattle with leptospirosis has been reported.[73–75] After administration of a single IM dose of dihydrostreptomycin in 1 study,[73] urinary shedding of leptospires by cattle ceased within a week. Other antimicrobials that are approved for food-producing animals, such as ceftiofur, oxytetracycline, tilmicosin, and tulathromycin, have also been successful in eliminating leptospire shedding.[74,75] The rapid response to a single treatment makes it feasible (considering cost and safety) to treat animals in small herds if they become infected with leptospirosis.

In dogs with leptospirosis, antimicrobial treatment that is started early in the course of infection is effective in shortening the duration of clinical disease and preventing permanent liver and kidney damage and can minimize the risk of transmission to humans.[76] Penicillin, or one of its derivatives, is the antimicrobial agent of choice for initial treatment. Upon improvement, doxycycline is often administered to prevent potential development of a long-term carrier state. Supportive fluid therapy is essential to correct dehydration while the concurrent liver or kidney problems are treated.[77] When available, use of peritoneal dialysis or hemodialysis can be lifesaving.

Control and Prevention in Cattle, Swine, and Dogs

In the United States, vaccines have been available for use in cattle, swine, and dogs for several decades. In cattle herds in which vaccination is performed as part of a herd management program, the disease can be controlled. However, vaccination does not completely prevent infection or shedding of leptospires in urine.

Infections in cattle herds are frequently attributed to commingling different species of livestock together on pasture, introduction of untested infected animals to a herd, and possible transmission from local infected wildlife. Vaccination and implementation of measures to decrease contact with wildlife and contaminated environments can be useful for controlling the disease in livestock.

Leptospirosis can be considered a reemerging infectious disease in dogs. In the United States and Canada, the number of dogs with leptospirosis evaluated at veterinary teaching hospitals has increased signifi-

cantly from 1983 through 1998.[78] This increase may be attributed to climatic factors, serovar shifts, or greater contact with wildlife.[79]

Historically, leptospirosis in canids has been associated with the serovars canicola and icterohemorrhagiae, which led to the inclusion of these serovars in vaccines for use in dogs. The frequency with which other serovars, such as grippotyphosa,[80] pomona, hardjo, and bratislava,[81] are being detected in healthy and clinically ill canids is increasing. New vaccines have been formulated to reflect these changes. Results of challenge studies[82,83] have indicated that commercial vaccines can prevent shedding of leptospires in urine. However, dog owners should be aware that their pets may not be fully protected because serovars that cause disease may vary temporally and geographically. Veterinarians should continue to consider including leptospirosis in a differential diagnosis of febrile illness in dogs with clinical signs compatible with this disease.

Control and Prevention in Humans

Among humans, exposures to leptospirosis may occur when veterinarians and their staffs and pet and livestock owners are handling infected animals. If a potential exposure occurs, local or state health department staff can be contacted for guidance. Recommendations for postexposure prophylaxis should be made on an individual basis (with consideration of the environmental conditions and the health status of the person) by the exposed person's physician in consultation with public health officials. Exposed persons should be monitored for the abrupt development of influenza-like symptoms that are accompanied by rash, headache, or photophobia.

To decrease risk of transmission, veterinary personnel and others working with animals should avoid contact with urine and other body fluids from infected animals through the use of gloves. Additional equipment, such as masks and protective eyewear, should be worn when performing activities that may cause splashes (eg, cleaning cages). All blood, urine, and tissues from infected animals should be considered biologically hazardous waste. Hands should be washed thoroughly with soap and water after handling or cleaning up after infected animals. Iodine- or chlorine-based disinfectants may be used to clean contaminated bedding, cages, and surfaces. The viability of leptospires decreases via desiccation or exposure to direct sunlight and a low pH.

Precautions should be taken by veterinary staff and owners to minimize contact of infected dogs that may be shedding *Leptospira* organisms with other animals; this applies both during periods of hospitalization and after the infected dogs are returned home. For purposes of urination, infected and recovering dogs should be taken to areas where no other dogs, other animals, or children have access and that are away from pools and natural bodies of water. Other dogs residing in the same household as a dog with leptospirosis should be tested for leptospirosis, and prophylactic antimicrobial treatment may be considered. Administration of appropriate antimicrobials can decrease the duration of clinical signs and urine shedding in dogs; however, in some instances, shedding of leptospires in the urine may persist

for as long as 3 months after infection as a result of inadequate or lack of treatment.[84]

For persons who may be occupationally or recreationally exposed to leptospires in the environment, protective clothing and boots are recommended. Exclusion of rodents and other small mammals from domiciles will decrease the risk of contact with infected urine. During episodes of flooding or other natural events that can cause disruption of the public health infrastructure, measures should be taken to ensure a safe water supply and physical protection from a contaminated environment.

Public Health Implications

Leptospirosis can be characterized by 3 epidemiologic patterns.[2] In temperate climates, a limited set of serovars causes human infection, mostly through direct contact with infected animals. In tropical, humid areas, numerous serovars and host animal species are present, and humans are usually infected from contaminated environments. In urban and rural slum environments, rodents are the primary host responsible for transmitting leptospirosis to humans. The epidemiologic importance of any specific animal source at a given time is often a function of the local ecology, the human activities in that environment, and dynamic shifts in the prevalence and virulence of *Leptospira* serovars. Knowledge of the most common serovars and their maintenance hosts is necessary for understanding the epidemiology of the disease in an endemic region.

Prevention efforts against leptospirosis have focused primarily on the vaccination of domestic animals, both livestock and pets. The control of rodents in urban and rural areas can decrease environmental contamination and the risk of transmission to susceptible hosts. However, leptospirosis may continue to circulate in wildlife populations because of the persistence of the organism in the environment. Public health officials, particularly those in known endemic areas, must take into consideration all these factors when developing control and prevention programs. Prompt investigation of reported human cases by epidemiologists can identify other persons at risk and allow the initiation of antimicrobial prophylaxis for exposed individuals. Education of those who are occupationally exposed and those who could be potentially exposed during their recreational activities or via their pets is essential to reduce the risk of transmission. In addition to their involvement in the prevention, diagnosis, and treatment of diseases in animals, veterinarians serve an important role in public health by providing guidance and information on zoonotic diseases, including communication of risk factors and prevention and control measures to their clients and the general public.

References

1. Weil A. Ueber eine eigenilimiliche, mitMilztumor, Icterus, and Nephritis einhergehends acute Infektionskrankheit. *Dtsch Arch Klin Med* 1886;39:209–232.
2. Faine S. Chapter 1. In: *Leptospira and leptospirosis.* Boca Raton, Fla: CRC Press Inc, 1994;1–2.
3. Alston JM, Broom JC. *Leptospirosis in man and animals.* Edinburgh: E & S Livingstone, 1958.

4. Dikken K, Kmety E. Serological typing methods of leptospires. In: Bergan T, Norris J, eds. *Methods in microbiology*. Vol 11. London: Academic Press Inc, 1978;259–307.

5. Ramadass P, Jarvis BDW, Corner RJ, et al. Genetic characterization of pathogenic *Leptospira* species by DNA hybridization. *Int J Syst Bacteriol* 1992;42:215–219.

6. 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.

7. 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.

8. Levett PN. Leptospirosis. *Clin Microbiol Rev* 2001;14:296–326.

9. Zaki SR, Spiegel RA. Chapter 4: leptospirosis. In: Nelson AM, Horsburgh CR, eds. *Pathology of emerging infections 2*. Washington, DC: American Society for Microbiology, 1998;73–92.

10. Arean VM. The pathological anatomy and pathogenesis of fatal human leptospirosis (Weil's disease). *Am J Pathol* 1962;40:393–423.

11. Arean VM, Sarasin G, Green JH. The pathogenesis of leptospirosis: toxin production by *Leptospira icterohaemorrhagiae*. *Am J Vet Res* 1964;25:836–843.

12. Babudieri B. Animal reservoirs of leptospirosis. *Ann N Y Acad Sci* 1958;70:393–413.

13. Hartskeerl RA, Terpstra WJ. Leptospirosis in wild animals. *Vet Q* 1996;18(suppl 3):S149–S150.

14. Gordon Smith CE, Turner LH. The effect of pH on the survival of leptospires in water. *Bull WHO* 1961;24:35–43.

15. Kirschner L, Maguire T. Survival of *Leptospira* outside their hosts. *N Z Med J* 1957;56:385–391.

16. Thiermann AB. Leptospirosis: current development and trends. *J Am Vet Med Assoc* 1984;184:722–725.

17. Bolin CA, Koellner P. Human-to-human transmission of *Leptospira interrogans* by milk. *J Infect Dis* 1988;158:246–247.

18. Harrison NA, Fitzgerald WR. Leptospirosis—can it be a sexually transmitted disease? *Postgrad Med J* 1988;64:163–164.

19. Chedraui PA, San Miguel G. A case of leptospirosis and pregnancy. *Arch Gynecol Obstet* 2003;269:53–54.

20. Sugunan AP, Natarajaseenivasan K, Vijayachari P, et al. Percutaneous exposure resulting in laboratory-acquired leptospirosis—a case report. *J Med Microbiol* 2004;53:1259–1262.

21. Kaufman AF. Epidemiologic trends of leptospirosis in the United States, 1954–1974. In: Johnson RC, ed. *The biology of parasitic spirochetes*. New York: Academic Press Inc, 1976;177–190.

22. Trevejo RT, Rigau-Perez JG, Ashford DA, et al. Epidemic leptospirosis associated with pulmonary hemorrhage—Nicaragua, 1995. *J Infect Dis* 1998;178:1457–1463.

23. Sanders EJ, Rigau-Perez JG, Smits HL, et al. Increase of leptospirosis in dengue-negative patients after a hurricane in Puerto Rico in 1996. *Am J Trop Med Hyg* 1999;61:399–404.

24. Sarkar U, Nascimento SF, Barbosa R, et al. Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. *Am J Trop Med Hyg* 2002;66:605–610.

25. Barcellos C, Sabroza PC. Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: a geographical approach. *Int J Environ Health Res* 2000;10:301–313.

26. Karande S, Gandhi D, Kulkarni M, et al. Concurrent outbreak of leptospirosis and dengue in Mumbai, India, 2002. *J Trop Pediatr* 2005;51:174–181.

27. Heath CW, Alexander AD, Galton MM. Leptospirosis in the United States: 1949–1961. *N Engl J Med* 1965;273:857–922.

28. Thai KT, Binh TQ, Giao PT, et al. Seroepidemiology of leptospirosis in southern Vietnamese children. *Trop Med Int Health* 2006;11:738–745.

29. Ferro BE, Rodriguez AL, Perez M, et al. Seroprevalence of *Leptospira* infection in inhabitants of peripheral neighborhoods in Cali, Colombia [in Spanish]. *Biomedica* 2006;26:250–257.

30. Tangkanakul W, Tharmaphornpil P, Plikaytis BD, et al. Risk factors associated with leptospirosis in northeastern Thailand, 1998. *Am J Trop Med Hyg* 2000;63:204–208.

31. Demers RY, Thiermann A, Demers P, et al. Exposure to *Leptospira icterohaemorrhagiae* in inner-city and suburban children: a serologic comparison. *J Fam Pract* 1983;17:1007–1011.

32. Childs JE, Schwartz BS, Ksiazek TG, et al. Risk factors associated with antibodies to leptospires in inner-city residents of Baltimore: a protective role for cats. *Am J Public Health* 1992;82:597–599.

33. Ashford DA, Kaiser RM, Spiegel RA, et al. Asymptomatic infection and risk factors for leptospirosis in Nicaragua. *Am J Trop Med Hyg* 2000;63:249–254.

34. Anderson BS, Minette HP. Leptospirosis in Hawaii: shifting trends in exposure, 1907–1984. *Int J Zoonoses* 1986;13:76–88.

35. Katz AR, Manea SJ, Sasaki DM. Leptospirosis on Kauai: investigation of a common source waterborne outbreak. *Am J Public Health* 1991;81:1310–1312.

36. CDC. Outbreak of leptospirosis among white-water rafters—Costa Rica, 1996. *MMWR Morb Mortal Wkly Rep* 1997;46:577–579.

37. 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.

38. Sejvar J, Bancroft E, Winthrop K, et al. Leptospirosis in "Eco-Challenge" athletes, Malaysian Borneo, 2000. *Emerg Infect Dis* 2003;9:702–707.

39. 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.

40. Bruce MG, Sanders EJ, Leake JAD, et al. Leptospirosis among patients presenting with dengue-like illness in Puerto Rico. *Acta Trop* 2005;96:36–46.

41. Turner LH. Leptospirosis. *Br Med J* 1969;1:231–235.

42. Edwards GA, Domm BM. Human leptospirosis. *Medicine* 1960;39:117–156.

43. de Souza AL. Neuroleptospirosis: unexplored and overlooked. *Indian J Med Res* 2006;124:125–128.

44. Abdulkader RC. Acute renal failure in leptospirosis. *Ren Fail* 1997;19:191–198.

45. Xim JG, Yeon KM, Han MC, et al. Leptospirosis of the lung: radiographic findings in 58 patients. *AJR Am J Roentgenol* 1989;152:955–959.

46. CDC. Outbreak of acute febrile illness and pulmonary hemorrhage—Nicaragua, 1995. *MMWR Morb Mortal Wkly Rep* 1995;44:841–843.

47. Hassig M, Lubsen J. Relationship between abortions and seroprevalences to selected infectious agents in dairy cows. *Zentralbl Veterinarmed [B]* 1998;45:435–441.

48. Bernard WV. Leptospirosis. *Vet Clin North Am Equine Pract* 1993;9:435–443.

49. 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.

50. Mitchell D, Robertson A, Corner AH, et al. Some observations on the diagnosis and epidemiology of leptospirosis in swine. *Can J Comp Med Vet Sci* 1966;30:211–217.

51. Rentko VT, Clark N, Ross LA, et al. Canine leptospirosis: a retrospective study of 17 cases. *J Vet Intern Med* 1992;6:235–244.

52. Geisen V, Stengel C, Brem S, et al. Canine leptospirosis infections—clinical signs and outcome with different suspected Leptospira serogroups. *J Small Anim Pract* 2007;48:324–328.

53. Adin CA, Cowgill LD. Treatment and outcome of dogs with leptospirosis: 36 cases (1990–1998). *J Am Vet Med Assoc* 2000;216:371–375.

54. Boutilier P, Carr A, Schulman RL. Leptospirosis in dogs: a serological survey and case series 1996–2001. *Vet Ther* 2003;4:178–187.

55. CDC. Recommendations and reports. Case definitions for infectious conditions under public health surveillance. *MMWR Morb Mortal Wkly Rep* 1997;46:1–55.

56. Faine S. Leptospirosis. In: Balows A, Hausler WJ, Ohashi M, et al, eds. *Laboratory diagnosis of infectious diseases: principles and practice*. Vol 1. New York: Springer-Verlag, 1988;344–352.

57. Faine S. Chapter 16. In: *Leptospira and leptospirosis*. Boca Raton, Fla: CRC Press Inc, 1994;267–277.

58. Bajani MD, Ashford DA, Bragg SL, et al. Evaluation of four com-

mercially available rapid serologic tests for diagnosis of leptospirosis. *J Clin Microbiol* 2003;41:803–809.

59. Merien F, Baranton G, Perolat P. Comparison of polymerase chain reaction with microagglutination test and culture for diagnosis of leptospirosis. *J Infect Dis* 1995;172:281–285.

60. Merien F, Perolat P, Mancel E, et al. Detection of leptospiral DNA by polymerase chain reaction in aqueous humor of a patient with unilateral uveitis. *J Infect Dis* 1993;168:1335–1336.

61. World Organisation for Animal Health (OIE). Chapter 2.1.9. In: *OIE manual of diagnostic tests and vaccines for terrestrial animals.* 5th ed. Paris: World Organisation for Animal Health (OIE), 2004;251–255.

62. Pappas G, Cascio B. Optimal treatment of leptospirosis: queries and projections. *Int J Antimicrob Agents* 2006;28:491–496.

63. 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.

64. Panaphut T, Domrongkitchaiporn S, Suttinot C, et al. Ceftriaxone compared with sodium penicillin G for treatment of severe leptospirosis. *Clin Infect Dis* 2003;36:1507–1513.

65. Suputtamongkol Y, Niwattayakul K, Suttinont C, et al. An open, randomized, controlled trial of penicillin, doxycycline and cefotaxime for patients with severe leptospirosis. *Clin Infect Dis* 2004;39:1417–1424.

66. Volovitz B, Shkap R, Amir J, et al. Absence of tooth staining with doxycycline treatment in young children. *Clin Pediatr (Phila)* 2007;46:121–126.

67. Lochary ME, Lockhart PB, Williams WT. Doxycycline and staining of permanent teeth. *Pediatr Infect Dis J* 1998;17:429–431.

68. Vinetz JM. A mountain out of a molehill: do we treat acute leptospirosis, and, if so, with what? *Clin Infect Dis* 2003;36:1514–1515.

69. Costa E, Lopes AA, Sacramento E, et al. Penicillin at the late stage of leptospirosis: a randomized controlled trial. *Rev Inst Med Trop Sao Paulo* 2003;45:141–145.

70. Takafuji ET, Kirkpatrick JW, Miller RN, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *N Engl J Med* 1984;310:497–500.

71. Gonsalez CR, Casseb J, Monteiro FGV, et al. Use of doxycycline for leptospirosis after high-risk exposure in São Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 1998;40:59–61.

72. Sehgal SC, Sugunan AP, Murhekar MV, et al. Randomized control trial of doxycycline prophylaxis against leptospirosis in an endemic area. *Int J Antimicrob Agents* 2000;13:249–255.

73. Gerritsen MJ, Koopmans MJ, Dekker TCEM, et al. Effective treatment with dihydrostreptomycin of naturally infected cows shedding *Leptospira interrogans* serovar *hardjo* subtype *hardjobovis*. *Am J Vet Res* 1994;55:339–343.

74. Alt DP, Zuerner RL, Bolin CA. Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar hardjo. *J Am Vet Med Assoc* 2001;219:636–639.

75. Cortese VS, Behan S, Galvin JE, et al. Evaluation of two antimicrobial therapies in the treatment of *Leptospira borgpetersenii* serovar hardjo infection in experimentally infected cattle. *Vet Ther* 2007;8:201–208.

76. Rentko VT, Ross LA. Canine leptospirosis. In: Bonagura JD, ed. *Kirk's current veterinary therapy XI*. Philadelphia: WB Saunders Co, 1992;260–263.

77. Greene C, Miller M, Brown C. Leptospirosis. In: Greene C, ed. *Infectious diseases of the dog and cat*. Philadelphia: WB Saunders Co, 1998;273–281.

78. 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.

79. Ward MP. Clustering of reported cases of leptospirosis among dogs in the United States and Canada. *Prev Vet Med* 2002;56:215–236.

80. Ward MP, Guptill LF, Prahl A, et al. Serovar-specific prevalence and risk factors for leptospirosis among dogs: 90 cases (1997–2002). *J Am Vet Med Assoc* 2004;224:1958–1963.

81. Stokes JE, Kaneene JB, Schall WD, et al. Prevalence of serum antibodies against six *Leptospira* serovars in healthy dogs. *J Am Vet Med Assoc* 2007;230:1657–1664.

82. Schreiber P, Martin V, Najbar W, et al. Prevention of renal infection and urinary shedding in dogs by a *Leptospira* vaccine. *Vet Microbiol* 2005;108:113–118.

83. Andre-Fontaine G, Branger C, Gray AW, et al. Comparison of the efficacy of three commercial bacterins in preventing canine leptospirosis. *Vet Rec* 2003;153:165–169.

84. Wohl JS. Canine leptospirosis. *Compend Contin Educ Pract Vet* 1996;18:1215–1225.