Leptospirosis: Epidemiology, Diagnosis, and Control

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ABSTRACT

Leptospirosis is a worldwide emerging zoonosis caused by the spirochete of the genus *Leptospira* affecting humans and animals; humans are incidental hosts. The determinants of incidence and prevalence are the suitability of the environment for the survival of the organism and the behavioral and occupational habits of human beings. The diagnosis of leptospirosis is as elusive as it was when it was first recognized. An isolation of organism from clinical specimens is still difficult, and serological techniques remain the cornerstone of diagnosis. There is a need to further unravel the pathogenesis of leptospirosis and to develop effective cross-protective recombinant vaccines. (J Infect Dis Antimicrob Agents 2008;25:93-103.)

History

The history of *Leptospira* dates back to nineteenth century when there were many reports of outbreak of icteric fever. These were described under a variety of names such as Griesinger bilious typhoid, bilious or hepatic fever, hepatic typhoid, icteric typhoid, catarrhal icteris, and febrile icterus.1 In 1886, Adolf Weil, Professor of Medicine at Heidelberg whose name has been given to the disease in humans, first described this disease but the distinction between leptospirosis, yellow fever, and other diseases with icterus was not clear.2 In 1914, Ryo Kichei Inada and his colleagues in Kyushu, Japan, observed the spiral organisms in the liver of a guinea pig inoculated with blood taken from four Japanese minors presumed to have Weil’s disease. They named the organisms *Spirochaita icterohaemorrhagiae*.3

In 1917, Hideyo Noguchi proposed the genus be renamed *Leptospira* (slender coil).4 In the next 15 years or so from the discovery until the 1930s, many of the important serovarieties prevalent throughout the world and their host sources were discovered.5 By 1950, milder forms of leptospirosis had been elucidated in Japan and Germany. The detailed structure of *Leptospira* was studied under electron microscope during the 1960s and 1970s.4

In 1972, leptospirosis researchers developed a preoccupation with serological classification, based only...
on absorption and cross-agglutination of antisera. By 1980, enzyme-linked immunosorbant assay (ELISA) methods were developed to analyze non-agglutinating as well as agglutinating antigens and applied with monoclonal antibodies to identify epitopes involved in the immunity and useful for classification. For many years, leptospires were classified in either non-pathogenic group as *L. biflexa* or the pathogenic group as *L. interrogans*. The current system of genetic classification was adopted in 1994.²

In the last 15 years, developments have included lipopolysaccharide derivation of the antigens involved in the immunity, and molecular techniques like polymerase chain reaction (PCR) has been developed for identification and genetic speciation.⁶ Recently, in 2006 multilocus sequence typing method has been used for genotyping of *Leptospira*.⁷

**Classification**

- **Order**: Spirochaetales
- **Family**: Leptospiraceae
- **Class**: Spirochaetes
- **Genera**: *Leptospira*
  - *Leptonema*
  - *Turneria*

The genus was formerly divided into two species *L. interrogans*, comprising all pathogenic strains, and *L. biflexa* containing the saprophytic strains isolated from the environment. These species were further divided into serogroups, serovarieties, and strains, based on shared antigens. Over 60 serovarieties of *L. biflexa* and more than 250 serovarieties of *L. interrogans* have been recognized.⁸,⁹

**Genotypic classification**

The phenotypic classification of leptospires has been replaced by a genotypic classification in which 12 named species and 5 unnamed species comprise all serovarieties of *Leptospira* spp..¹⁰,¹¹ The genetically defined species of *Leptospira* do not correspond to the previous two species (*L. interrogans* and *L. biflexa*), and both pathogenic and non-pathogenic serovarieties occur with in same species. The reclassification of leptospires on genotypic grounds is taxonomically correct and provides a strong foundation for future classification. However, the molecular classification is problematic for clinical microbiologists. Thus, they will have to retain the serological classification of pathogenic leptospires until simpler DNA-based identification methods are developed and validated.¹²

**Epidemiology**

Leptospires are ubiquitous spirochetes. Based on global data collected by International Leptospirosis Society (ILS) surveys, there are currently 300,000 to 500,000 severe cases of leptospirosis annually. Leptospirosis is now recognized as a globally re-emerging disease with a marked increase in the number of cases and frequent outbreaks in Southeast Asia (Thailand, India, Malaysia, and Indonesia) and the United States of America, particularly in Hawaii.¹³,¹⁴ It remains a grossly neglected disease and is under-recognized clinically because of the difficulty of diagnosis and the wide variety of clinical manifestations.¹⁵

Leptospirosis is a worldwide zoonosis. The climatic conditions in tropical and subtropical regions help provide an optimal environment to support the survivability of leptospires.¹⁶ Saprophytic species usually contaminate surface waters, whereas the primary habitat of pathogenic species is the distal tubules of the kidneys of rodents, the reservoir host, and various other animal species. The pathogenic leptospires which are
shed in the urine of the carrier animals contaminate the environment and cause human and animal infection throughout the world. Animals that acquire infection may not develop discernible disease, but become long-term carriers maintenance host. For example rats are maintenance hosts for *L. icterohaemorrhagiae*, cattle for *L. hardjo*, dogs for *L. canicola*, and pigs for *L. pomona* or *L. bratislava*. The reason for this tolerance is unclear since infection with other serovars may cause illness of varying severity followed by transient shedding of leptospires in the urine for a few weeks. Moreover, an animal may become a long-term maintenance host for one serovariety and yet develop disease and transient carriage after infection with another.

Viable leptospires are present in the semen of infected animals. In rodents, a significant increase in the carriage of leptospires is seen after sexual maturity is reached. Also a spread across the placenta occurs in several animal species, leading to infection and fetal death in utero.

Outside the animal host, leptospiral survival is favoured by the warm, moist condition at neutral or slightly alkaline pH. This no doubt contributes to the seasonal pattern of human infections, which peak in the summer months in both hemisphere. Even small reductions below pH 7.0 markedly reduce their survival. For example the anaerobic conditions and low pH of raw sewage cause a rather short survival time, compared with aerated sewage. Salt water is also relatively toxic to leptospires. They do not survive well in undiluted cow’s milk, and therefore drinking unpasteurized milk poses minimal risk. However, they will survive in water at pH 7.0 or damp soil for up to one month. In the soil saturated with urine they may survive for up to 6 months, indicating the potential for long-term exposure to an infection risk even if the reservoir host has been removed for some time.

Changes in industrial, agricultural, and social practices may result in the rapid change of both the density and type of animal population in an area. The source of infection in an area is determined by factors such as rodent density, farm size, presence of other domestic animals, and sanitation of animal habitats. Other factors are the availability of veterinary services for prompt detection and treatment of animal leptospirosis and control programs for animal leptospirosis. In human, the spread of the disease is influenced by behavioural, socio-cultural, and occupation factors; i.e. personal hygiene, practices such as bathing in unprotected water bodies, recreational activities associated with water, use of protective gear, agricultural, and other occupational practices.

Leptospirosis is a disease of the environment; transmission depends on the interactions between humans and mammalian reservoir hosts. The mode of transmission of leptospirosis is often categorized as direct or indirect depending upon the immediate source of infection. When the immediate source of infection is animal tissue, body fluids or urine, the transmission is termed as direct. Cattle and pig farmers, veterinarian, butchers, laboratory personnel who handle laboratory animals are at high risk of contracting leptospirosis. When the immediate source of infection is an environment contaminated with urine of carrier animals, the transmission is termed as indirect. Agricultural workers, sewage workers, people walking barefoot in water logged areas, sports person who participate in water-related sports are at high risk of acquiring the disease by indirect transmission.

**Morphology**

Leptospires are about 6-20 μm long but only
about 0.1 μm in diameter, which allows them to pass through the filters that retain most other bacteria. They are Gram-negative, however they poorly take up conventional stains. They can be seen by Giemsa or silver deposition methods or by use of fluorescent antibody. They are best viewed by the dark-field or electron microscopy, with one or both ends appearing hooked. And they rotate rapidly around their long axis. They have many closely set primary coils that are often difficult to see in living bacteria.

Leptospires have an envelope around them composed of 3-5 layers of protein, polysaccharide, and lipid. Within the outer sheath is the protoplasmic cylinder, bounded by a cell membrane and cell wall. Leptospires have two flagellae with their free ends towards the middle of the bacteria. The flagellae lie in the peri-plasmic space between the cell wall and the outer envelope and are wrapped around the cell wall. Each flagellum is attached to a basal body located at either end of the cell. They are responsible for the motility of the leptospires, but the exact mechanism of motility is still incompletely understood.

Antigenic structure
Leptospires have a complex antigenic structure. There are somatic antigens, surface antigen, outer membrane lipopolysaccharides, flagellar antigens, and some serovarieties have Vi antigen. The somatic antigen is genus specific whereas the surface antigen is a polysaccharide and serovar specific. The flagellar antigen is composed of genus and serotype specific antigens.

Leptospires show both humoral and cell mediated immune responses. On entry of the bacteria into the host, both the B and T-cell dependent areas are stimulated. Leptospires are first eliminated by phagocytosis, and cell-mediated immunity is thought to play a role in preventing renal localization.

IgM antibodies are first to appear, followed by IgG antibodies which persist longer than IgM. IgA antibodies appear on the fifth day and persist up to 9 months. The IgG antibody response is often erratic and occasionally is not detected.

Cultural Characteristics
Leptospires require aerobic or microaerophilic conditions for growth. Adequate sources of nitrogen, phosphate, calcium, magnesium, and iron are essential. They can use fatty acids as the major source of energy, but they are unable to synthesize long-chain fatty acids with 15 or more carbon atoms. Vitamin B1 (thiamine) and B12 (cyanocobalamin) are also required, and the addition of biotin is needed for the growth of some strains.

The media used for isolation and cultivation of leptospires can be liquid or solid enriched with rabbit serum or bovine serum albumin and protein free media. The most commonly used media for cultures are Fletcher’s semisolid medium, Korthof’s liquid medium, and Ellinghausen-McCullough-Johnson-Harris medium (EMJH). Antibiotics are usually added when the specimen such as urine, is likely to be contaminated with other bacteria. A combination of fostomycin and 5-fluorouracil has been found effective when used with Korthof’s medium.

The optimal growth of pathogenic species in cultures takes place at 28-30°C at pH 7.2-7.6 in media supplemented with 0.1 percent agar to enhance primary isolation. The growth is often slow, with the periods of 3-4 weeks required after inoculation.

Manca and colleagues reported recovery of L. interrogans from simulated specimens and from human blood, using a commercial radiometric blood culture system. They used Stuart’s and Middlebrook
TB media that have been supplemented with bovine serum albumin, catalase, and casein hydrolysate and labeled with C-fatty acids. The time of recovery in human blood was 2-5 days.

**Pathogenesis**

The pathogenesis of leptospirosis is incompletely understood, but a vasculitis resulting in damage to the endothelial cells of small blood vessels is probably the main underlying pathology.

The infection is acquired by direct or indirect contact with infected urine, tissues, or secretions. An ingestion or inhalation of leptospires does not pose a risk to man, and human-to-human spread is very rare. Leptospires may gain entry through small areas of damage in the skin or via mucus membranes. They may also pass through waterlogged skin.

The term ‘leptospirosis’ should be used to describe all infections in both humans and animals. According to the occupational groups involved and the nature of the disease presentation, the different names have been used, including epidemic pulmonary hemorrhagic fever, cane cutter disease, Fort Bragg fever, Weil’s disease, and autumnal fever. This does not represent the full range of the disease, and leptospiral infection may not be suspected unless the patients have classically severe disease.

The primary lesion in leptospirosis is described of failure of integrity of the cell membrane of endothelial cells lining the small blood vessels in all parts of the body. Both capillary leakages and hemorrhages occur. These effects are due to the action of the glycoprotein toxin of *Leptospira* (GLP). Wide-spread petechial haemorrhages are present in all organs and tissues. Renal involvement is common in leptospirosis. Ischaemia from damage to blood vessels in renal cortex leads to renal tubular necrosis particularly of proximal convoluted tubules. The resulting anatomical damage causes renal failure that can be fatal. Liver cell necrosis caused by ischemia and the destruction of hepatic architecture leads to the characteristic jaundice of the severe type of leptospirosis. Blood clotting mechanisms are affected by liver failure aggravating the hemorrhagic tendencies.

The organisms may invade the meninges and conjunctivae.

**Clinical features**

Classical leptospirosis is a biphasic disease which consists of an initial septicemic phase and a secondary immune phase. The severity ranges from subclinical infection to fatal systemic disease known as Weil’s disease. In severe cases, the two phases merge together and an asymptomatic interval may not be recognized.

The initial septicemic phase of the disease is abrupt in onset with high fever, severe headache, malaise, and muscle aches. Eye pain, photophobia, and conjunctival suffusion may be present. Leptospiral uveitis is a common entity in tropical countries. Ocular manifestations are noted in the second phase of illness, but these remain underdiagnosed mainly because of prolonged symptom-free period that separates the systemic manifestations from the detection of ocular manifestations. Pulmonary involvement in leptospirosis varies in its incidence and presentation; the incidence varies from as low as 20 percent to as high as 70 percent. It may be mild in the form of a cough or manifests as severe forms including alveolar hemorrhage and acute respiratory distress syndrome. The severe forms have always been associated with the high mortality, inspite of invasive mechanical ventilation in a critical care setting. Other signs include maculopapular,
urticarial or hemorrhagic skin rashes, pharyngeal injection, lymphadenopathy, splenomegaly, and hepatomegaly.

After an asymptomatic interval of 1-3 days, the immune phase of infection develops. The duration of the immune phase ranges from 4 to 30 days, and the leptospires are cleared from the blood and cerebro-spinal fluid (CSF). CSF pleocytosis will develop in 90 percent of patients during the second week of illness but only half of these patients have symptoms of aseptic meningitis.

The severe forms, referred to as Weil’s disease, commonly involve the kidneys, liver, lungs, brain, and heart. The jaundice and other signs of liver dysfunction appear as early as the third week and late as the ninth week after infection. The typical biphasic course of the disease is observed by severe and persistent fever, jaundice, and azotemia.

After infection, the immunity develops against the infecting strain, but may not fully protect against infection with unrelated strains.

**Laboratory diagnosis**

Laboratory tests are necessary to confirm the diagnosis of clinically suspected leptospirosis due to its varied symptomatology. Moreover, leptospirosis must always be considered upon the differential diagnosis of other tropical febrile illnesses. Laboratory analysis depends on the samples available and temporal stage of the illness. Various laboratory tests are described for the detection of this spirochaete.

Leptospirosis may be diagnosed by:

1. Demonstration of the organisms in the clinical specimens.
2. Cultures of the spirochaetes.
3. Detection of leptospiral antigens or nucleic acids.
4. Serology.
5. Animal inoculation.

**1. Demonstration of the organisms in the clinical specimens**

1.1 Direct detection

Direct demonstration of *Leptospira* may be attempted by the dark-field examination of the blood, CSF, or urine. Centrifugation at low speed of oxalated or heparinized blood to remove cellular elements and then at high speed to concentrate leptospiro has been recommended. The dark-field microscopy of the blood is technically demanding since Brownian movement of collagen fibrils, red blood cell, and other cells can resemble viable leptospires. Although the examination of the CSF and urine are somewhat less treacherous, similar caveats apply. Direct identification of *Leptospira* with labelled antisera has been described but reagents are not available commercially.

1.2 Staining methods

Leptospires in smears of tissues or fluids on slides can be stained using silver impregnation methods including modified Warthin-Starry’s method. Well stained preparations show black spirochaetes in pale yellow or brown tissue elements.

Immunofluorescence staining of leptospires is often preferable to silver staining because it is easier to see leptospires even in small numbers and the serovar or serogroup can be determined when a combination of antisera labeled with different fluorochromes is used.

**2. Cultures of the spirochaetes**

*Leptospira* can be isolated freely from the blood and CSF during the first week of illness. During the
immune phase the organisms disappear from the blood and CSF. The kidney, however, is a privileged site, the *Leptospira* are excreted in the urine for up to 1 month. The cultures are incubated in the dark at 28-30°C for up to 6 weeks and examined weekly by the dark-field microscopy for the presence of *Leptospira*.30

### 3. Detection of leptospiral antigens or nucleic acids

Monoclonal antibodies have been used to detect leptospiral antigens in the urine. One of the molecular techniques used for the early diagnosis of leptospirosis is the amplification of specific fragments of leptospiral genomic DNA in clinical samples using polymerase chain reaction. Other molecular techniques are nucleic acid probes and hybridization and pulse-field gel electrophoresis. Other molecular techniques used for characterization include DNA restriction enzyme analysis (REA), random amplified polymorphic DNA finger printing (RAPD), arbitrarily primed PCR (APPCR), repetitive extragenic PCR (REP-PCR), and fluorescent amplified fragment length polymorphism (FALFP).33

### 4. Serology

Antibodies appear in the serum towards the end of the first week of the disease, they continue to rise for several weeks and then begin to decline. Serological tests fall into 3 categories:

#### 4.1 Genus-specific, tests.

#### 4.2 Serogroup- or serovariety-specific tests.

#### 4.3 Newer techniques.

#### 4.1. Genus-specific tests

These tests detect leptospiral infection without indicating the exact infecting serovar. The antigens for these tests are prepared from non pathogenic *L. biflera* Patoc 1 strain. These tests include macroscopic agglutinations test (MSAT), indirect fluorescent antibody test (IFAT), indirect hemagglutination test (IHA), counter-immuno electrophoresis (CIEP), complement fixation test (CFT), and ELISA.

MSAT is a rapid macroscopic slide agglutination test which can be used to screen human and animal serum samples. These tests are carried out with a dense suspension of leptospires which agglutinate into clumps visible to the naked eye. It helps in making a provisional diagnosis of acute leptospirosis within a few minutes, but it is not suitable for retrospective or survey work.30 Positive reactions should be confirmed by CFT or MAT.

#### 4.2 Serogroup- or serovariety-specific tests

These tests identify the infecting serovariety by demonstrating specific antibodies. They include microscopic agglutination (MAT) and serotype-specific ELISA.

#### 4.2.1 MAT

MAT is a reference standard assay in which live antigens representing different serogroups of leptospires are reacted with serum samples and then examined by dark-field microscopy for agglutination. It is a complex test to maintain; perform and interpret; and its use is restricted to a few reference laboratories. A serologically confirmed case of leptospirosis is defined by a four fold rise in MAT titer to one or more serovars between acute phase and convalescent serum specimens run in parallel. The Centers for Disease Control and Prevention (CDC) has suggested a titer of >1:200 in a single serum, using MAT as presumptive evidence of leptospirosis in a patient with a compatible clinical illness, but other experts have suggested a titer of 1:800 or 1:1,600 as better cut offs. Titers may reach extremely high levels and take
months or years to decrease so the height of the titer cannot be used for determination of recent infection. Cross-reactive antibodies may be associated with syphilis, relapsing fever, Lyme’s disease, viral hepatitis, human immunodeficiency virus (HIV), legionellosis, and autoimmune diseases. The interpretation of MAT is complicated by cross reaction between different serogroups, especially in acute phase samples which is attributable to IgM antibodies.

4.2.2 Serotype-specific ELISA

Several attempts have been made to develop serotype-specific ELISA tests with a variety of extracted antigens. The tests based on boiled whole cell antigens tend to be genus specific but those based on ultrasound-disintegrated or phenol-extracted preparations show considerable serotype specificity. It is clear that ELISA is more sensitive than live antigen MAT. However, MAT is still the commonly used test for diagnosing leptospirosis.

4.3 Newer techniques

Newer diagnostic tests for leptospirosis include IgM-EIA, microcapsule agglutination test (MCAT), LEPTO dipstick, LEPTO lateral flow, and LEPTO Dri Dot. LEPTO dipstick and LEPTO lateral flow are IgM immunoassays, while LEPTO Dri Dot is a latex agglutination test. The principle of MCAT is similar to that of latex agglutination assay. All these tests detect antibodies and the sensitivity of these tests is usually low during the first week of illness but these tests have acceptable sensitivities during the second week of the disease.

China and Brazil, countries in which leptospirosis is a major health problem have completed the sequence of L. interrogans genome. Together with new genetic tools and proteomics, new insight have been made into the biology of Leptospira and the mechanism used to adopt to host and external environments.

5. Animal inoculation

Laboratory animals are useful for isolating the organisms from contaminated material and for maintaining recent isolates, and may be used to recover a single serotype from a mixed culture. Young animals preferably weanlings should be used which must be free from endemic leptospiral infection; guinea pigs, hamsters, gerbils, young rabbits, swiss white mice, albino American deer mice, and 1-3-day-old chicks may be used. The material should be inoculated intraperitoneally through one of the lower quadrants of the abdominal wall. The animals should be examined twice daily and a drop of peritoneal fluid can be examined with dark field microscopy for active leptospires from the third to the seventh day.

Treatment

Leptospirosis in all its forms is amenable to treatment with antibiotics. There have been few randomized or placebo controlled trials, and some of these studies have produced conflicting results. In severe illness, intravenous benzyl penicillin is the drug of first choice. For milder infections, a 7-10-day course of oral amoxicillin is appropriate. Patients allergic to penicillins can be treated with erythromycin. Cefotaxime, ceftriaxone, or azithromycin is also an effective alternative for penicillin in the treatment of leptospirosis. Mild cases can also be treated with oral doxycycline. In some parts of the world, differentiating between typhus and leptospirosis is difficult without a ready access to sophisticated laboratories. In this circumstance, doxycycline is may be the drug of first choice upon awaiting for the
laboratory results.\textsuperscript{35}

**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 the environmental circumstances and the degree of human activity. Removal of leptospires from the environment is obviously impractical. But the measures to reduce the direct contact with infected animals and indirect contact with urine contaminated soil and water remains the most effective preventive strategy available. Appropriate protective measures include wearing boots, goggles, overalls, and rubber gloves.\textsuperscript{20}

The purpose of immunization is to protect them from leptospirosis to maximize productivity and in order to protect humans who are in contact with these animals. There are various animal vaccines including inactivated whole culture, live attenuated, acellula, and DNA-based vaccines. Various commercially available vaccines are listed in the Table.\textsuperscript{41}

Human immunization is not widely practiced. A vaccine containing serovariety icterohaemorrhagiae is available in France for workers in high risk occupations, and a vaccine has been developed recently for human use in Cuba.\textsuperscript{20}

For individuals who will be unavoidably exposed to leptospires in endemic environment, chemoprophylaxis with doxycycline 200 mg weekly is recommended.\textsuperscript{20}

**Rodent control measures**

A consistent application of rodent control measures is important in limiting the extent of contamination. The various rodent control measures include:

1. **Understanding the breeding pattern of rodents**

   Rodents exhibit two types of breeding pattern including normal (K) and fast breeding (r). Whenever rodent control measures are planned, rodents exhibit

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**Table. List of commercial vaccines available for use in animals.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Serovars incorporated</th>
<th>Animal host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptavoid H</td>
<td>Scherring-plough animal health, United Kingdom</td>
<td>Hardjoprajitno and hardjobovis</td>
<td>Cattle</td>
</tr>
<tr>
<td>Leptoferm-5</td>
<td>Pfizer animal health</td>
<td>Canicola, grippotyphosa, hardjo, pomona, and icterohemorrhagiae</td>
<td>Pigs, cattle</td>
</tr>
<tr>
<td>Farrowsure</td>
<td>Pfizer animal health</td>
<td>Pomona, grippotyphosa, icterohaemorrhagiae, hardjo, canicola, porcine, parovirus, and erysipelas</td>
<td>Pigs</td>
</tr>
<tr>
<td>Suileptovac TPC1</td>
<td>Not known</td>
<td>Tarassovi, canicola, pomona, icterohemorrhagiae</td>
<td>Pigs</td>
</tr>
<tr>
<td>Nobivac</td>
<td>Intervet International</td>
<td>Canicola</td>
<td>Dogs</td>
</tr>
</tbody>
</table>
fast breeding mode and re-establish themselves faster in areas that have been treated with rodenticide.

**ii. Appropriate rodenticides**

These include zinc phosphide, bromadiolone (C), coimaetralyl TP, and bromadolone RB.

**iii. Proper application of rodenticide**

This should be applied in the fields as well as in residential situations.42

**References**


