Leptospirosis is a globally important zoonotic disease and an important public health problem in developing countries. Early diagnosis is essential because antibiotic treatment is most effective when initiated early in the course of the disease. Culture and the microscopic agglutination test are gold standard methods for leptospirosis diagnosis; however, they are not useful for early diagnosis. Current whole cell-based rapid serological tests have low sensitivity for early phase leptospirosis and may have low specificity in highly endemic areas. PCR is demonstrably useful for early diagnosis, but it is unavailable in most developing countries. Thus, diagnostic methods that not only have higher sensitivity and accuracy for early phase leptospirosis but are also widely applicable in developing countries remain to be developed. The availability of genome sequences and genetic tools of *Leptospira* spp. will accelerate our understanding of *Leptospira* pathogenesis and provide insights into the development of more efficient and accurate diagnostic tests for acute-phase leptospirosis.

Keywords: culture • diagnosis • *Leptospira* • leptospirosis • microscopic agglutination test • PCR • serological test

**Epidemiology**

Leptospirosis affects humans in rural and urban settings and in industrialized and developing countries [3–6]. Based on global data collection, the International Leptospirosis Society estimates that 300,000–500,000 cases of leptospirosis occur annually [9,10]. However, this number may be underestimated, and the true extent of leptospirosis remains unknown owing to a lack of surveillance worldwide. Global climate change affects the occurrence of leptospirosis, exemplified by outbreaks in Central and South America due to El Niño-related flooding [11–13]. Occupational exposure such as that occurring during rice farming and other agricultural activities is still significant in rural areas of developing countries [14,15]. Leptospirosis is also a health problem in urban areas in the tropics. Outbreaks occur in poor urban slum communities during annual seasonal periods of heavy rainfall [11,16,17,201]. Infrastructural deficiencies in urban slums produce ecological conditions for rodent-borne transmission and contribute to the transmission of leptospirosis during epidemics [11,16,17,201]. The morbidity and
mortality of leptospirosis have significantly decreased because of improved hygiene levels in industrialized countries. However, the risk of infection in urban dwellers is not limited to tropical environments as the importance of urban leptospirosis was recognized in American and Japanese inner-city populations [8,19]. Recreational activities have recently gained attention as significant risk factors for leptospirosis in industrialized countries. There is a significant risk associated with recreational exposure occurring in water sports, such as swimming, canoeing and rafting [20–22], in addition to travel and adventure tourism [23–25]. Large outbreaks of leptospirosis caused by recreational activities are associated with competitive outdoor events [26–28], including an international adventure race [23].

Clinical manifestations & diagnosis

Leptospirosis is an acute febrile illness that has an extremely broad clinical spectrum ranging from mild influenza-like illness symptoms to severe disease including jaundice, bleeding, renal failure and death. Most patients have subclinical or very mild illness [29,30]. The major symptoms of leptospirosis include fever, headache, myalgia and vomiting, which are nonspecific, and conjunctival suffusion, which appear after an incubation period of 5–14 days (Table 1). Diagnosis of leptospirosis is difficult because of the nonspecific symptoms [31,32]. Fever often occurs abruptly and is accompanied by chilly sensations, chills or shivering. Headache and myalgia are usually more apparent in leptospirosis than in other febrile illness. Headache in leptospirosis is similar to that in dengue fever with retro-orbital pain and photophobia [4]. Calf or lower back myalgia and conjunctival suffusion without purulent discharge are characteristic of leptospirosis. Gastrointestinal symptoms including anorexia, nausea, vomiting, diarrhea and abdominal pain occur less frequently in patients. Patients with leptospirosis less commonly present with lymphadenopathy, hepatomegaly, splenomegaly and rash. Patients occasionally show meningeal signs, and rarely show consciousness disturbance and mild pleocytosis on performing lumbar puncture.

Icteric leptospirosis, also known as Weil’s disease, represents the most severe form of leptospirosis, with symptoms such as jaundice, bleeding and renal failure, which develop after initial nonspecific symptoms. In typical cases of leptospirosis, jaundice appears after several days of illness, after which physicians can detect hepatosplenomegaly. In leptospirosis, jaundice does not appear to be associated with hepatocyte damage but appears to be related more to the cholestasis of sepsis [4]. Acute renal failure (ARF), which occurs in 10 to over 60% of cases [33], frequently accompanies hypokalemia, non-oliguria and even polyuria. Oliguria is a significant predictor of death in patients with ARF [34]. Thrombocytopenia occurs in up to 50% of leptospirosis patients but does not appear to result from disseminated intravascular coagulation [35,36]. Hemorrhage in leptospirosis involves subcutaneous hemorrhage, conjunctival bleeding, hemoptyisis and gastrointestinal bleeding.

Leptospirosis-associated pulmonary hemorrhage syndrome (LPHS) has recently gained attention [8]. However, it may be underdiagnosed even in high endemic regions [37]. Symptoms may be mild and nonspecific, including chest pain, cough and dyspnea, although the presence of severe pulmonary hemorrhage, with or without acute respiratory distress syndrome, is associated with higher mortality. The severity of respiratory disease is unrelated to the presence of jaundice. Respiratory symptoms usually appear between the fourth and sixth days of disease and may lead to death in less than 72 h [38]. Severe leptospirosis may cause high rates of mortality if treatment is not initiated early. The mortality rate can be as high as 5–15% in Weil’s disease [3] and 30–70% in LPHS [38,39].

Non-specific symptoms in acute-phase leptospirosis may cause misdiagnosis. Sasaki and colleagues reported that physicians suspected only 30% of the confirmed cases as leptospirosis at the initial clinical diagnosis in Hawaii where leptospirosis is relatively common [40]. Physicians should recognize that misdiagnosis may cause poor prognosis; 5–10% of patients with leptospirosis can potentially develop a severe form of the disease [3,4]. In addition to relatively characteristic clinical features such as myalgia, tenderness of calves or lumbar areas, and/or conjunctival suffusion, epidemiological histories such as contact with animals or contaminated environment through occupational and recreational activities and travel before onset can provide clues to diagnose leptospirosis in patients with nonspecific febrile illnesses. Physicians should consider leptospirosis as a differential diagnosis for patients who have the epidemiological histories mentioned earlier, even when the illness presents with unusual features [41].

Leptospirosis has diverse clinical manifestations that are easily confused with many other diseases in the tropics, such as dengue fever or dengue hemorrhagic fever, malaria and scrub typhus [42–47]. Physicians should consider leptospirosis in patients with traveler’s fever and in endemic areas, depending on the epidemic situation and risk behavior of leptospirosis. LaRocque and colleagues compared the clinical presentations of 938 patients with dengue fever with those of 63 patients with leptospirosis [44]. The study indicated that the presence of rash, pruritus and a positive tourniquet test were significantly associated with dengue fever, whereas fever with high temperature and long duration and subconjunctival hemorrhage were significantly associated with leptospirosis. Another study noted that clinical features such as myalgia, conjunctival suffusion and history of contact with flood water in children were significantly associated with leptospirosis, while manifestations such as giddiness, abdominal pain, rash and bleeding were associated with dengue fever [47].

To assist clinicians in diagnosing leptospirosis, some studies to formulate clinical- or laboratory-based criteria for diagnosis have been reported [48–52]. Bal reported that Faine’s Criteria [48] had moderately good sensitivity (81.8%) and specificity (72.9%) [51]. The positive and negative predictive values of the criteria were 40.9 and 94.5%, respectively, when compared with serological tests. Shivakumar and colleagues proposed modifications in the scoring system of Faine’s Criteria to consider epidemiological information and serological tests in greater detail [52]. The modifications produced statistically higher specificity and positive predictive values than those of standard Faine’s criteria. These criteria have high negative predictive values, which may help exclude leptospirosis from the differential diagnosis of febrile patients.
Because early initiation of antibiotic therapy is important to prevent disease progression, clinicians should familiarize themselves with the clinical presentations that may lead to poor prognosis. Independent prognostic markers for a poor outcome of leptospirosis have been reported in some studies as follows: old age, dyspnea, oliguria, presence of pulmonary rales, hyperkalemia, abnormal serum creatinine, leukocytosis, thrombocytopenia, elevated bilirubin, hypotension, arrhythmia, acute respiratory distress syndrome, pulmonary hemorrhage and altered mental status [11,53–58]. More recently, multivariate logistic regression revealed that five factors were independently associated with LPHS development: serum potassium (mmol/l; odds ratio [OR]: 2.6); serum creatinine (mmol/l; OR: 1.2); respiratory rate (breaths/min; OR: 1.1); presenting shock (OR: 69.9) and Glasgow Coma Scale Score less than 15 (OR: 7.7) [59]. It should be noted that previous studies included similar factors in prognostic models for leptospirosis. Identification of these factors upon hospital admission can help triage patients in need of intensive care, thus reducing mortality.

### General laboratory tests

The erythrocyte sedimentation rate is elevated in mild leptospirosis and white cell counts range from below normal to moderately elevated. Liver function tests show mild elevation of aminotransferases, bilirubin and alkaline phosphatase. Urinalysis shows proteinuria, pyuria and microscopic hematuria. In severe leptospirosis,
peripheral leukocytosis with a left shift occurs, and platelet counts usually decrease [4]. Renal function is impaired and hemodialysis therapy is occasionally needed. Serum bilirubin concentrations can reach up to 20 mg/dl, while the hepatic transaminase elevation is comparatively mild or moderate in comparison with significant jaundice [32]. Xanthochromia may develop in patients with jaundice [4]. Elevated serum creatine phosphokinase levels may be encountered (3), which may be helpful for diagnosis. Serum amylase levels may also increase, particularly in ARF patients.

Lumbar puncture usually shows normal or slightly elevated cerebrospinal fluid (CSF) pressure [3]. The CSF cell count is generally less than 500/mm³ with mononuclear cell predominance. In most cases, protein and glucose levels in CSF are normal or slightly increased and normal, respectively [3,4].

The nonspecific nature of these laboratory findings only suggests a diagnosis of leptospirosis. To confirm the diagnosis of leptospirosis, specific microbiological tests are necessary.

**Laboratory diagnosis**

Owing to the nonspecific and diverse presentation in the early phase or mild leptospirosis and the nonspecific nature of general laboratory test findings, diagnosis of leptospirosis depends on the specific laboratory tests described in the following sections.

**Microscopic demonstration**

Leptospires may be demonstrated by direct microscopic observation of clinical specimens. Dark-field microscopic examination of bodily fluids such as blood, urine, CSF, and dialysate fluid has been used and has advantages for early diagnosis [3]. However, the sensitivity of this method is low; approximately 1 x 10⁷ leptospires/ml are necessary to observe one cell per field. The result is also affected by the timing of sample collection and the skill of the laboratory personnel. Leptospires in blood can be detected only during the first few days after onset. Direct examination of blood by dark-field microscopy can lead to misclassification of threads of fibrin or other proteins as leptospires. Thus, results of direct microscopic examination may be supported by other laboratory methods, irrespective of positive results.

Immunofluorescence or light microscopy after appropriate staining have been applied for direct examination. Leptospires in tissues can be visualized by histopathological staining and immunohistochemical methods [3].

**Antigen detection**

Detection of leptosporal antigens in clinical specimens has not been applied widely for the diagnosis of leptospirosis. Several methods developed before the year 2000 were reviewed in a previous article [3]. More recently, antigen detection in urine has been performed by dot blot ELISA (dot-ELISA) using monoclonal antibody directed against the uncharacterized 35-kDa component of pathogenic leptospires [60]. The assay detected the antigen in the urine of patients whose sera tested negative in IgM serological tests. Sharma and colleagues succeeded in detecting leptosporal antigens in plasma from experimentally infected animals using sandwich dot-ELISA with polyclonal and monoclonal antibodies [61].

**Culture**

Leptospires can be isolated from clinical materials such as blood, CSF, urine, and tissues. Blood culture should be performed as soon as possible after onset of the disease, during the leptospiruria phase and before administration of antibiotics. For the blood culture, a few drops of blood are inoculated into 5–10 ml of a suitable culture medium such as EMJH medium or modified Korthof medium [8] and, if possible, separated into several tubes. Recently, Wuthiekanun and colleagues suggested the use of the culture using deposits from spun plasma in addition to whole blood [62]. This study also demonstrated that leptospires can be isolated from hepatised blood stored at room temperature for up to 109 days. Some *Leptospira* strains can survive in mycobacterial blood culture media, although these samples require subculturing into leptospiral media for growth [63]. Leptospires may be isolated by inoculating 0.5 ml of CSF into 5 ml of semi-solid culture medium during the first week of illness [8]. Urine is the most suitable specimen for the isolation of leptospires during the leptospiruria phase (~1 week after onset). As the viability of leptospires is limited in acidic urine, one drop of urine is inoculated into 5 ml of culture medium within 2 h after voiding. Alternatively, the urine sample is centrifuged and the pellet is resuspended in the culture medium described above, following which tenfold serial dilutions are prepared in one or two tubes [8]. 5-fluorouracil and/or antibiotics such as neomycin, nalidixic acid, actidione, sulfadiazol, rifampicin and amphotericin B may be used to reduce the risk of contamination [1,8]. Leptospires may be cultured from postmortem samples of various tissues.

Cultures are incubated at 30°C and checked regularly by dark-field microscopy. On primary isolation, growth of leptospires is often slow and their generation times are approximately 20 h, and the cultures are recommended to be maintained for up to 13 weeks [3]. Although isolation constitutes the definitive diagnosis of leptospirosis, it is not useful for early diagnosis owing to the above reason. In addition to the slow growth rate, requirement for special media and the relatively low sensitivity hamper routine application of isolation in many laboratories. However, isolation and characterization of leptospires are essential for epidemiological studies. *Leptospira* isolates are characterized by serotyping and molecular typing. The conventional method of serotyping involves the determination of serogroups by the microscopic agglutination test (MAT; see later) using a panel of rabbit antisera and the cross agglutinin absorption test (CAAT) for serovar definition. However, CAAT is complicated and time-consuming, preventing most laboratories from performing the test [1]. Although identification of serovars is important for epidemiological investigations, molecular typing has been widely used recently. A number of methods have been developed, which have been extensively reviewed in a recent article [2].

**Antibody detection**

Most cases of leptospirosis are diagnosed by serology. Antibodies are detected in the blood approximately 5–7 days after the onset of symptoms [3]. Although a number of serological methods have been applied for diagnosing leptospirosis [3], definitive serological diagnosis is still performed using MAT, which was developed soon after the first isolation of *Leptospira*. 
In MAT, diluted patient serum samples are mixed with leptospiral cultures at 1:1 ratios, and the mixtures are incubated for 2–4 h at 30°C or room temperature. The serum–antigen mixtures are then examined for agglutination by dark-field microscopy. The end point is the highest dilution of serum that shows 50% agglutination when compared with the control mixture. Alternatively, it is easier to determine the end point by the proportion of free, unagglutinated leptospires in the mixture. The reaction is positive when the proportion of free leptospires is less than 50% compared with the control suspension. MAT detects both serogroup-specific antibodies IgM and IgG. Thus, the panel of antigens should include serovars representative of all serogroups and locally common serovars. The WHO recommends 19 serovars of 16 serogroups [8]. The serovar (serogroup) that shows the highest titer in MAT is considered the infecting serovar (serogroup). However, based on comparison of the presumptive serogroup (serovar) determined by MAT with serovars of the isolates from single patients, recent studies revealed that MAT appeared to be of little value for predicting infecting serovars of patients [64–66].

Paired sera are required for definitive diagnosis by MAT. Seroconversion of at least fourfold increases in titer must be observed between acute and convalescent serum samples. It is difficult to confirm acute infection from a single serum sample. A low titer equal to 100 in a febrile patient may indicate current infection in areas where leptospirosis is uncommon. Conversely, in endemic areas of leptospirosis, a high titer of 400 or more in a symptomatic patient is generally accepted, but titers as high as 1600 or more have been recommended [3]. In addition to the issues of determining the cutoff point to indicate current infection, anti-leptospiiral antibodies are present for months to years after infection.

The principle of MAT is simple, but the procedure is laborious and requires the maintenance of a panel of Leptospira cultures, and quality control must be employed [67]. Furthermore, the sensitivity of MAT in the acute phase is low, and paired sera are needed to confirm diagnosis. Therefore, a number of rapid screening tests for antibody detection in acute infection have been developed [3,68]. Commercially available tests are whole Leptospira cell-based assays such as ELISA, dipstick, lateral flow, indirect hemagglutination assay and latex agglutination test [68–72]. Most of the assays employ antigens from nonpathogenic Leptospira biflexa serovar Patoc (strain Patoc I). It has been demonstrated that sera from patients with leptospirosis cross-reacted with antigens from this nonpathogenic strain [73,74], in which the antigenic components of lipopolysaccharide had a repeating disaccharide of (→3)-β-D-Manp-(1→4)-β-D-Manp-(1→75). Thus, these assays are believed to be genus-specific and detect IgM (and/or IgG) antibodies from patients, regardless of infective serovars or serogroups, with easy and rapid formats. As indicated previously [68,76–85], these assays are used as alternatives to MAT but have low sensitivity during the acute phase. Therefore, they are suitable for antibody detection in the late acute phase (after the tenth day of illness). Furthermore, recent studies indicate that the diagnostic accuracies of these techniques are poor in some areas where leptospirosis is endemic [86,87]. This may be attributed to persistence of anti-leptospiral IgM after infection, frequent reinfection with leptospires in endemic areas, or cross-reaction with other infectious agents.

In addition to antibodies against lipopolysaccharide, sera from patients with leptospirosis contain antibodies against several protein antigens [88,89]. Protein antigens that are recognized in human patient sera or experimentally infected or immunized-animals sera have been identified [90]. Recombinant protein-based serological tests can attain high sensitivity and specificity because of the high concentration of antigens that can be used in these tests. Furthermore, these tests are free of nonspecific moieties, which are present in whole-cell preparations. Protein antigens that are conserved among pathogenic leptospires can be potential diagnostic markers. Recombinant protein-based assays for diagnosing leptospirosis have been developed, but the evaluation of these assays is controversial. Two research groups demonstrated that ELISA, using recombinant (r) LipL32, detected IgG antibodies in more than 90% of patients [91,92]. On the other hand, one group failed to detect IgM by ELISA, whereas the other group detected it in more than 90% of samples. The sensitivities and specificities of rLipL41 ELISA and rOmpL1 ELISA vary among studies [91–94]. Three independent experiments using rLipL41 revealed its sensitivities (IgM: 82 and 89% [92,93]; IgG: 44 and 84% [91,92]) and specificities (IgM: 98 and 100%; IgG: 78 and 100%). Three independent studies using rOmpL1 also indicated its sensitivities (IgM: 0, 87 and 91% [91,92,94]; IgG: 72 and 95% [91,94]) and specificities (IgM: 97 and 100%; IgG: 96 and 100%). The study that was performed using serum samples in which the days of collection after onset were known indicated that the sensitivity of these assays was low for acute-phase samples [91]. Leptospiral immunoglobulin-like (Lig) proteins, which are surface-exposed outer membrane proteins with antigenic and immunogenic properties that function in binding to extracellular matrix proteins [95–99], have proven to be diagnostic markers for early diagnosis [100]. The Lig-based immunoblot assay had improved sensitivity and specificity over both the other recombinant protein-based and whole Leptospira cell-based assays. Other recombinant-based assays such as ELISA using Lp29, MPL17, MPL21 and LipL32 [92,101,102], latex agglutination test, flow-through immunoassay using LipL41 [103] and dipstick assay using LipL32 [104] have been described.

**DNA detection**

Isolation of leptospires from clinical specimens requires a couple to several weeks for growth, and current serological tests exhibit low sensitivity in the acute phase and require paired sera for definitive serodiagnosis. Therefore, detection of leptospiral DNA by PCR has been applied for early diagnosis of leptospirosis in the last two decades. Various genes including secY, the flagellin gene, rrs, flaB, rrl and genomic locus LA3521 in Leptospira interrogans serovar Lai have been used as the targets of PCR-based diagnosis [105–110]. Leptospiral DNA has been amplified from blood, urine, CSF, aqueous humor and tissues
A PCR protocol using two primer sets, G1/G2 and B64I/B64II for the \textit{secY} and flagellin gene, is described in the WHO manual [8]. Recently, real-time PCR has been introduced not only as a rapid and sensitive tool for leptospiral DNA detection but also as a technique to reduce the risk of carryover contamination. Real-time PCRs targeting \textit{rrs}, \textit{ligA} and \textit{B}, \textit{gyrB}, genomic locus LA0322 in \textit{L. interrogans} serovar Lai, \textit{lipL32} and \textit{secY} have been developed using TaqMan® probes or SYBR green fluorescence [112–120]. It has been demonstrated that both conventional and real-time PCR are useful for early diagnosis during which antibody production has not begun; the sensitivity of PCRs for DNA detection in blood declines over the course of the disease as a corollary [105,108,109,117,119]. More recently, a loop-mediated isothermal amplification (LAMP) method has been developed for detecting pathogenic leptospires [121]. Unlike PCR, the LAMP method amplifies a target DNA sequence under isothermal conditions for approximately 1 h with high specificity and efficiency, and the results can be assessed with the naked eye [122], promising lower expenses for equipment. The LAMP method was applied for detecting leptospiral DNA from mouse kidneys but remains unevaluated for its sensitivity and specificity for human clinical specimens.

Humans are not considered asymptomatic renal carriers of leptospires after infection. However, asymptomatic urinary shedding of leptospires has recently been identified in Peruvian Amazon natives without evidence of recent infection, which may confound the diagnosis of acute infection by PCR using urine samples in highly endemic areas [123].

**Expert commentary & five-year view**

As discussed in this article, leptospirosis has become an important public health problem in developing countries in the tropics [4–7], and its nonspecific and varied presentation in the early phase hampers clinical diagnosis and can lead to misdiagnosis with many other infectious diseases such as dengue fever, malaria and scrub typhus. Therefore, a high index of suspicion among physicians and availability of a rapid and accurate point-of-care diagnostic test is required to identify leptospirosis. Early diagnosis is essential because antibiotic treatment is most effective when it is initiated early in the course of the disease [1,8]. A total of 5–10% of patients with leptospirosis can potentially develop a severe form of the disease, with a fatality rate of more than 10% in Weil’s disease and up to 70% in LPHS [5,38,39].

However, many experts agree that adequate laboratory tests for early diagnosis are still lacking. Current serological tests have low sensitivities in the acute phase, which limits their contribution to early diagnosis [68,76–85]. Owing to the limited sensitivity of current whole-cell-based serological tests, it has been theoretically indicated that empirical antibiotic treatment (doxycycline) was the most-cost effective treatment among therapies started after a positive result of a serological test [124]. In addition to their low sensitivity during the acute phase, recent findings suggest low specificity of these tests in some highly endemic areas [86,87]. Therefore, to improve the sensitivity and specificity of whole cell-based tests, identification of protein antigens that are conserved in pathogenic leptospires has been intensively investigated. Furthermore, the availability of full-length genome sequences of pathogenic and nonpathogenic \textit{Leptospira} spp. has provided new avenues for the aforementioned purposes (see later).

A number of studies have demonstrated the usefulness of detecting leptospiral DNA by conventional or real-time PCR for early diagnosis [105,108,109,117,119]. However, PCR is expensive and requires technical expertise and equipment, with particular care for avoiding false-positive results caused by contamination. Real-time PCR can reduce the risk of carryover contamination, but the instruments used in real-time PCR are significantly more expensive compared with those used in conventional PCR. Therefore, they may be unavailable to most health facilities in developing countries. Considering the advantages of rapid amplification, simple operation and easy detection [122], LAMP has potential applications for diagnostic methods in developing countries without requiring sophisticated equipment. Quality control should be introduced to assess the accuracy of PCR testing because multicenter studies have indicated that the use of PCR without validation is not justifiable [125].

Detection of leptospiral antigen in clinical specimens has not been applied widely to diagnosing leptospirosis. Urinary antigen detection assays provide simplicity and rapidity as well as less invasiveness and have been applied for diagnosing pneumococcal pneumonia and legionellosis [126,127]. The work done by Saengjaruk \textit{et al.} indicated that monoclonal antibodies can capture leptospiral antigens in the urine of patients a few days after onset [60]. These findings suggest the possibility of the development of urinary antigen assays with a rapid and easy format for early diagnosis of leptospirosis. As mentioned earlier, the existence of asymptomatic renal carriers may confound the diagnosis of acute infection in highly endemic areas [123].

It is impossible to predict whether a patient will develop the severe form of leptospirosis, although several prognostic factors of mortality have been identified [53–59]. In addition to a rapid and robust diagnostic method, the development of clinical prediction capacities allows physicians to initiate rational treatment for all forms of leptospirosis.

The full-length genome sequences of two strains of \textit{L. interrogans} serovars Lai and Copenhageni, and two strains of \textit{Leptospira borgpetersenii} serovar Hardjo, as well as two strains of nonpathogenic \textit{L. biflexa} serovar Patoc, have been published [128–133]. High-throughput screening with convalescent patient sera in immunoblotting identified novel antigens based on full-length genome analysis [132]. \textit{In silico} analyses [133], combined with microarray analysis [134], have identified leptospiral genes that encode potentially surface-exposed outer membrane proteins that are conserved in pathogenic leptospires and may be used as novel targets for the development of diagnostic markers. The availability of genome sequences of \textit{Leptospira} spp. also enables investigating transcriptomes using microarray technology, which identifies genes responding to various conditions mimicking host environment conditions such as temperature, osmolarity, exposure to serum and iron concentrations [135–139]. Proteomic analyses combined with available genome sequences have been used to identify several novel outer membrane proteins [140].
The mechanisms of Leptospira pathogenesis are still poorly understood owing to the lack of genetic tools, which became available in 2005 [141]. Bourhy and colleagues showed that the Himar1 mariner transposon permits random mutagenesis in pathogenic L. interrogans, and the system has been applied to various pathogenic strains to generate mutants with attenuated virulence [141-143]. Genome sequences of pathogenic and non-pathogenic Leptospira strains have revealed that 656 genes are pathogen-specific, among which the functions of approximately 60% of the genes are unknown [144]. This suggests the existence of virulent mechanisms unique to pathogenic Leptospira. This method will accelerate the identification of virulence factors as well as our understanding of the pathogenesis of leptospires. As new information evolves, insights will be gained into methods to develop more efficient and accurate diagnostic tests for acute-phase leptospirosis.

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Key issues

- Leptospirosis is a globally important zoonotic disease that affects humans in rural and urban settings and in industrialized and developing countries. Leptospirosis has become an important public health problem in developing countries in the tropics.
- Leptospirosis is an underdiagnosed disease, and its identification requires a high index of suspicion among physicians.
- Culture and microscopic agglutination test remain the gold standard of leptospirosis diagnosis. However, both methods are not useful in early diagnosis.
- Current whole cell-based rapid serological tests have low sensitivity for early phase leptospirosis and may have low specificity in highly endemic areas.
- PCR is demonstrably useful for early diagnosis before antibody production has commenced, but PCR may not be widely applied in developing countries.
- Diagnostic methods that not only have higher sensitivity and accuracy for early phase leptospirosis but are also widely applicable in developing countries remain to be developed.
- The availability of full-length genome sequences and genetic tools for pathogenic Leptospira will provide new methods to elucidate its pathogenesis as well as to develop more efficient and accurate diagnostic tests for acute-phase leptospirosis.

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Papers of special note have been highlighted as:
- of interest
- of considerable interest

- Comprehensive review on current typing methods for Leptospira spp.
- Comprehensive review on leptospirosis based on several important and historical papers.
- Clearly demonstrated high-risk activities by assessing the seroprevalence of leptospirosis in the Andaman Islands, India.
New trial to establish a more useful in urban areas of Tokyo, 11, 58, 1227–1230

Review

Human leptospirosis cases and the Koizumi N, Muto M, Tanikawa T


Revealed the under-recognition of leptospirosis and pulmonary complications by a prospective, population-based study in Peru.


Suggested the possibility of the introduction or emergence of a clone that has enhanced virulence within the same serovar.


Describes comparisons of clinical characteristics between dengue fever and leptospirosis in children through an observational study in India.


New trial to establish a more useful scoring system to improve the diagnosis of leptospirosis using epidemiological and clinical criteria.


**Identified predictive factors for the development of leptospirosis-associated pulmonary hemorrhage syndrome by using multivariate analysis for the first time.**
61 Indicated that monoclonal antibodies can capture leptospiral antigens in the urine of patients a few days after disease onset.
64 Demonstrated that leptospires can survive in heparinized blood stored at room temperature for a long period.


101 Clearly indicates the presence of anti-Lig IgM in acute-phase leptospirosis patients.


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Diagnosis of acute leptospirosis

Review


- Demonstrates asymptomatic urinary shedding of leptospires in Peruvian Amazon natives without evidence of recent infection.


- Comprehensive review on current progress in Leptospira genetics and pathogenesis.


Website