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Leptospirosis in Humans

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Abstract

Leptospirosis is a widespread and potentially fatal zoonosis that is endemic in many tropical regions and causes large epidemics after heavy rainfall and flooding. Infection results from direct or indirect exposure to infected reservoir host animals that carry the pathogen in their renal tubules and shed pathogenic leptospires in their urine. Although many wild and domestic animals can serve as reservoir hosts, the brown rat (*Rattus norvegicus*) is the most important source of human infections. Individuals living in urban slum environments characterized by inadequate sanitation and poor housing are at high risk of rat exposure and leptospirosis. The global burden of leptospirosis is expected to rise with demographic shifts that favor increases in the number of urban poor in tropical regions subject to worsening storms and urban flooding due to climate change. Data emerging from prospective surveillance studies suggest that most human leptospiral infections in endemic areas are mild or asymptomatic. Development of more severe outcomes likely depends on three factors: epidemiological conditions, host susceptibility, and pathogen virulence (Fig. 1). Mortality increases with age, particularly in patients older than 60 years of age. High levels of bacteremia are associated with poor clinical outcomes and, based on animal model and in vitro studies, are related in part to poor recognition of leptospiral LPS by human TLR4. Patients with severe leptospirosis experience a cytokine storm characterized by high levels of IL-6, TNF-alpha, and IL-10. Patients with the HLA DQ6 allele are at higher risk of disease, suggesting a role for lymphocyte stimulation by a leptospiral superantigen. Leptospirosis typically presents as a nonspecific, acute febrile illness characterized by fever, myalgia, and headache and may be confused with other entities such as influenza and dengue fever. Newer diagnostic methods facilitate early diagnosis and antibiotic treatment. Patients progressing to multisystem organ failure have widespread hematogenous dissemination of pathogens. Nonoliguric (high output) renal dysfunction should be supported with fluids and electrolytes. When oliguric renal failure occurs, prompt initiation of dialysis can be life saving. Elevated bilirubin levels are due to hepatocellular damage and disruption of intercellular junctions between hepatocytes, resulting in leaking of bilirubin out of bile canaliculi. Hemorrhagic complications are common and are associated with coagulation abnormalities. Severe pulmonary hemorrhage syndrome due to

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extensive alveolar hemorrhage has a fatality rate of >50 %. Readers are referred to earlier, excellent summaries related to this subject (Adler and de la Peña-Moctezuma 2010; Bharti et al. 2003; Hartskeerl et al. 2011; Ko et al. 2009; Levett 2001; McBride et al. 2005).

1 Epidemiology and Surveillance

1.1 Sources of Infection

Pathogenic leptospires are widespread in nature, reflecting maintenance in the kidneys of many wild and domestic reservoir hosts. The leptospiral life cycle involves shedding in the urine, persistence in the ambient environment, acquisition of a new host, and hematogenous dissemination to the kidneys through the glomerulus or peritubular capillaries. Once leptospires gain access to the renal tubular lumen of the kidney, they colonize the brush border of the proximal renal tubular epithelium, from which urinary shedding can persist for long periods of time without significant ill effects on the reservoir host. For this reason, leptospiral infection of the reservoir host can be considered a commensal relationship (Fig. 1).

Small mammals are the most important reservoirs, with large herbivores as additional significant sources of infection. Pathogenic *Leptospira* species have been isolated from hundreds of mammalian species, including bats and pinnipeds (see the chapter by W.A. Ellis, this volume). In addition, leptospires have been recovered from poikilothermic animals such as frogs and toads, and it is possible that these animals play a role in the circulation of leptospirosis in the environment, although they may not be significant reservoirs of human infection. Only a few studies have reported isolation of leptospires from amphibians (Babudieri et al. 1973; Everard et al. 1988; Gravekamp et al. 1991). However, the results justify further attempts to understand the role of amphibians in maintaining leptospires in nature (Adler and de la Peña-Moctezuma 2010; Bharti et al. 2003; Hartskeerl et al. 2011; Ko et al. 2009; Levett 2001; McBride et al. 2005; Felzemburgh et al. 2014).

Leptospirosis is primarily a zoonosis, with humans serving as accidental hosts. However, it is worth noting that transient leptospiral shedding does occur during human infection and human-to-human infection, although extremely rare, has occurred through sexual intercourse (Doeleman 1932; Harrison and Fitzgerald 1988) and during lactation (Bolin and Koellner 1988). Transplacental transmission may occur if infection occurs during pregnancy, resulting in abortion (Chung et al. 1963) or still birth (Coghlan and Bain 1969; Faine et al. 1984).

1.2 Transmission

Portals of entry include cuts and abrasions or mucous membranes such as the conjunctival, oral, or genital surfaces. Exposure may occur through either direct contact with an infected animal or through indirect contact via soil or water contaminated with urine from an infected animal. Individuals with occupations at risk for direct contact with potentially infected animals include veterinarians, abattoir workers, farm workers (particularly in dairy milking situations), hunters and trappers, animal shelter workers, scientists, and technologists handling animals in laboratories or during fieldwork. The magnitude of the risk depends on

the local prevalence of leptospiral carriage and the degree and frequency of exposure. Most of these infections are preventable by the use of appropriate personal protective equipment such as rubber boots, gloves, and protective eyewear. Since many of these infections are covered by occupational health and safety regulations, local risk assessments and training are essential (Steneroden et al. 2011).

Indirect contact with water or soil contaminated with leptospires is much more common, and can be associated with occupational, recreational, or avocational activities. In addition to the risks associated with outdoor work listed above, sewer work, military exercises, and farming in high rainfall tropical regions are recognized; the latter is by far the most important numerically. Agricultural workers at risk for leptospirosis include rice field workers, taro farmers, banana farmers, and sugar cane and pineapple field harvesters (Levett 2001). These occupations involve activities likely to result in exposure of cuts and abrasions to soil and water contaminated with the urine of rodents and other animals attracted to food sources. For example, banana workers accounted for two-thirds of the reported leptospirosis cases in a tropical region of Queensland, Australia (Smythe et al. 1997).

Recreational exposures include all freshwater water sports including caving (Self et al. 1987), canoeing (Waitkins 1986), kayaking (Jevon et al. 1986; Shaw 1992), rafting (Wilkins et al. 1988), and triathlons (Morgan et al. 2002; Sejvar et al. 2003). The importance of this type of exposure has increased over the past 20 years as the popularity of adventure sports and races has increased, and also because the relative cost of travel to exotic destinations has decreased (Lau et al. 2010a). Competitive events create the potential for large outbreaks; 80 and 98 leptospirosis cases occurred as part of the 2000 Eco-challenge competition (Fig. 2a) (Sejvar et al. 2003) and 1998 Springfield triathlon (Morgan et al. 2002), respectively. Participants in international events may become ill after having returned home, often to multiple destination countries, which complicates recognition and investigation of outbreaks. In many series, the incidence of leptospirosis is much higher in males than females (Everard et al. 1992; Guerra-Silveira and Abad-Franch 2013; Katz et al. 2011). However, it seems likely that gender differences in leptospirosis incidence are due entirely to exposure-related bias, as reports of leptospirosis outbreaks related to athletic events where males and females have similar levels of exposure have found no significant effects of gender on development of illness (Morgan et al. 2002; Sejvar et al. 2003).

Avocational exposures are by far the most important exposures, affecting millions of people living in tropical regions. As illustrated in Fig. 2b, lack of adequate sanitation and poor housing combine to exacerbate the risk of exposure to leptospires in both rural and urban slum communities (Bharti et al. 2003; Felzemburgh et al. 2014; Hotez et al. 2008; Reis et al. 2008). The role of poor housing is also suggested by the study of Maciel et al. (2008), which showed a greatly increased risk (odds ratio 5.29) of leptospirosis exposure among individuals who live in the same household as a leptospirosis patient. These factors are most likely surrogates for rat exposure, as proximity to uncollected trash and sighting of rats increased the risk of leptospirosis among residents of urban slums (Reis et al. 2008). The recognition of large outbreaks following excess rainfall events (Ahern et al. 2005; Dechet et al. 2012; Ko et al. 1999; Zaki and Shieh 1996) led to the labeling of leptospirosis as an emerging infectious disease two decades ago (Levett 1999). More recently, the interaction of

urbanization and climate change has been identified as a significant risk for both increased incidence and increasing frequency of outbreaks of leptospirosis (Lau et al. 2010b). The need for interdisciplinary research to understand the effects of anthropogenic change and its effect on the epidemiology of leptospirosis has been proposed (Vinetz et al. 2005).

1.3 Global Burden of Disease

Early studies of leptospirosis incidence concentrated on occupational disease, primarily in developed countries related to leptospirosis in livestock animals (Alston and Broom 1958; Faine et al. 1999). As the importance of the disease in tropical countries became better recognized, guidelines were developed for the diagnosis and control of leptospirosis (Faine 1982). As diagnostic methods became more widely available, numerous epidemiologic studies were reported from many countries. An initial attempt to gather global data on the incidence of leptospirosis was published over 15 years ago (WHO 1999). Based on global data collected by International Leptospirosis Society surveys, the incidence was estimated to be 350,000–500,000 severe leptospirosis cases annually (Ahmed et al. 2012). Despite these efforts, the global burden of leptospirosis was felt to be largely underestimated for a number of reasons, including the fact that the vast majority of countries either lack a notification system or notification is not mandatory (Ahmed et al. 2012). To address these shortcomings, the WHO established the Leptospirosis Burden Epidemiology Reference Group (LERG) (Abela-Ridder et al. 2010). The LERG met for the first time in 2009 and for a second time in 2010. The specific objectives of the second LERG meeting were: (1) To review and appraise the revised systematic review of mortality, morbidity, and disability from human leptospirosis; (2) To review a draft disease transmission model for leptospirosis and provide technical input for the further development and refinement of the model; (3) To assemble preliminary estimates of the disease burden; (4) To identify gaps in knowledge and research; and (5) To advise WHO on the next steps for estimation of the burden of human leptospirosis and the implications for policy. The resulting LERG report included a systematic literature review that estimated the overall global annual incidence of endemic and epidemic human leptospirosis at 5 and 14 cases per 100,000 population, respectively (WHO 2011). Endemic human leptospirosis rates varied by region from 0.5/100,000 population in Europe to 95/100,000 population in Africa.

2 Pathology

The first step in the pathogenesis of leptospirosis is penetration of tissue barriers to gain entrance to the body. Potential portals of entry include the skin via a cut or abrasion and the mucous membranes of the conjunctivae or oral cavity. The importance of the oral mucosa as a portal of entry is indicated by a number of studies that found that swallowing while swimming in contaminated water is a risk factor for infection (Corwin et al. 1990; Lingappa et al. 2004; Stern et al. 2010).

The second step in pathogenesis is hematogenous dissemination. Unlike other pathogenic spirochetes such as *B. burgdorferi* and *T. pallidum*, which cause skin lesions indicating establishment of infection in the skin, pathogenic leptospires make their way into the bloodstream and persist there during the leptospiremic phase of the illness. Results from inoculation of blood into leptospiral medium and detection of leptospiremia by quantitative

PCR are more likely to be positive during the first 8 days of fever (Agampodi et al. 2012) prior to antibody formation and clearance of organisms from the bloodstream. Quantitative PCR has documented leptospiremia levels as high as 10^6/ml of blood (Agampodi et al. 2012), which is similar to the burden of spirochetes seen in the blood of patients with relapsing fever (Stoenner et al. 1982). Levels of $>10^4$ leptospires/ml in the bloodstream have been associated with severe outcomes (Segura et al. 2005; Truccolo et al. 2001), although a more recent larger study suggests that leptospires with lower virulence may be able to achieve even higher leptospiral bloodstream burdens without causing severe complications (Agampodi et al. 2012).

The levels of bacteremia that occur during leptospirosis are similar to those found in infections caused by the relapsing fever *Borreliae* (Stoenner et al. 1982), and very different from those found in bacteremia caused by *E. coli* and other *Enterobacteriaceae*, in which concentrations are typically <1 cfu/ml (Yagupsky and Nolte 1990). Part of the explanation for these differences is the human innate immune response. Human TLR4 is able to detect *E. coli* lipopolysaccharide (LPS) at extremely low concentrations, but is unable to recognize leptospiral LPS (Werts et al. 2001). A likely explanation for this difference in reactivity of human TLR4 is structural differences between the lipid A component of *E. coli* and leptospiral LPS; leptospiral LPS has a unique methylated phosphate residue not found in any other form of lipid A (Que-Gewirth et al. 2004). In contrast to human TLR4, mouse TLR4 is able to recognize leptospiral LPS (Nahori et al. 2005), suggesting that the murine innate immune response is adapted to leptospiral infection. This notion is consistent with differences in the pathogenesis of leptospirosis between humans and mice; humans are accidental hosts that experience potentially fatal outcomes and rarely transmit infection, while mice are resistant to fatal infection and serve as natural reservoirs.

The importance of TLR4 in determining the outcome of infection was demonstrated in studies showing that young (but not adult) C3H/HeJ mice lacking TLR4 are susceptible to lethal infection with *L. interrogans* (Viriyakosol et al. 2006). However, TLR4 is only one component of the innate immune response to leptospirosis. Both human and mouse TLR2 are able to recognize the polysaccharide or 2-keto-3-deoxyoctonoic acid (KDO) component of leptospiral LPS (Nahori et al. 2005; Werts 2010). Only when both TLR4 and TLR2 are mutated do adult C57BL/6 J mice experience lethal leptospirosis infections (Nahori et al. 2005). Presumably, TLR2 and other innate immune response mechanisms are responsible for the host response to leptospiral infection that leads to symptoms of disease. TLR2, TLR4, and TLR5 have been shown to be required for virulent leptospires to induce expression of the cytokines IL-6 and TNF-alpha in whole blood (Goris et al. 2011a).

When high levels of leptospiremia occur during infection, innate immune mechanisms eventually trigger tissue-based and systemic responses to infection that lead to severe outcomes such as a sepsis-like syndrome or organ failure. Patients with severe leptospirosis have evidence of a “cytokine storm” with higher levels of IL-6, TNF-alpha, and a number of other cytokines than patients with mild disease (Reis et al. 2013). In particular, IL-6 and IL-10 levels were independent predictors of death, suggesting that overproduction of IL-10 may inhibit a protective Th1 immune response. Superantigen stimulation of nonspecific T cell activation may also play a role in human leptospirosis. A study of athletes participating

in the Springfield triathalon found that the human leukocyte antigen (HLA) DQ6 was an independent risk factor for development of leptospirosis after exposure to lake water contaminated with virulent leptospires (Lingappa et al. 2004). The structural location of HLA-DQ6 polymorphisms associated with disease suggested a superantigen mechanism for this HLA-dependent susceptibility (Lingappa et al. 2004), although the identity of any such antigen(s) remains unknown.

The liver is a major target organ in leptospirosis. Pathology reports from autopsy specimens from fatal cases of leptospirosis have reported congested sinusoids and distention of the space of Disse, located between the sinusoids and hepatocytes (Arean 1962). Immunohistochemistry studies have documented large numbers of leptospires between hepatocytes in animal models. A recent, elegant study has documented leptospiral infiltration of Disse's space and preferential leptospiral attachment to and invasion of the perijunctional region between hepatocytes (Miyahara et al. 2014). Additionally, hepatocyte apoptosis has been documented in leptospirosis (Merien et al. 1998). Together, hepatocellular damage and disruption of hepatocyte intercellular junctions (Fig. 3a) leads to leakage of bile from bile canaliculi into sinusoidal blood vessels, which accounts for the elevated levels of direct bilirubin seen in icteric forms of leptospirosis. Occasionally, elevation of indirect bilirubin levels may also occur in the setting of leptospirosis-induced hemolysis (Avdeeva et al. 2002).

Pathological changes in the lung are extremely common in leptospirosis. In the 1962 study of fatal leptospirosis cases by Arean, all 33 cases were found to have pulmonary petechiae on the pleural surfaces and 60 % of patients had gross hemorrhage on the cut surfaces of the lungs (Arean 1962). Histologically, hemorrhage was found to occur in both the alveolar septa and intra-alveolar spaces (Arean 1962). A recent Brazilian study of patients with severe pulmonary hemorrhage syndrome (SPHS) performed immunohistochemistry on pulmonary tissue and found finely granular material representing leptospiral antigen within macrophages in septa and alveoli (Silva et al. 2002b). The guinea pig model of leptospirosis replicates the pulmonary hemorrhage seen in humans and studies of this animal model also revealed extensive deposition of immunoglobulin and complement along the alveolar basement membrane (Nally et al. 2004). Petechiae and hemorrhage are noted in a number of different organs beside the lungs and may be related, at least in part, to the coagulation abnormalities associated that frequently occur in severe leptospirosis (Wagenaar et al. 2010). The concept that SPHS is a manifestation of severe systemic disease rather than a strictly pulmonary problem is consistent with the finding that risk factors for SPHS include not only the respiratory rate but also hypokalemia, elevated serum creatinine, shock, and the Glasgow Coma Scale Score (Marotto et al. 2010).

Renal involvement varies in severity from mild nonoliguric renal dysfunction to complete renal failure, a hallmark of Weil's syndrome. The polyuria observed in mild leptospirosis appears to be due to reduced expression of the sodium–hydrogen exchanger 3, resulting in decreased reabsorption of sodium and fluid by the proximal tubule (Araujo et al. 2010). The histologic changes vary in intensity and typically involve tubular changes and interstitial nephritis (Fig. 3b). Tubular damage includes thinning and/or necrosis of the tubular epithelium and distention of the tubular lumen with hyaline casts and cellular debris (Arean

1962). In the reservoir host, the tubular lumen is a key site of colonization in the leptospiral life cycle and immunohistochemistry can show large numbers of organisms attached to the brush border of proximal tubular epithelium. In humans, an inflammatory response is triggered by recognition of leptospiral lipoproteins such as LipL32 by TLR2 on renal tubular epithelial cells, resulting in induction of nitric oxide synthase (iNOS) and monocyte chemoattractant protein-1 (Yang et al. 2006). Tubular inflammation results in interstitial nephritis characterized by edema and infiltration with lymphocytes, monocytes, and plasma cells, and occasionally neutrophils (Arean 1962; Sitprija and Evans 1970). This interstitial nephritis increases in extent and intensity during the first 2 weeks of illness. Most patients with acute renal failure due to leptospirosis who survive regain normal renal function. However, some patients have persistent renal dysfunction associated with tubular atrophy and interstitial fibrosis on kidney biopsy (Herath et al. 2014).

3 Clinical Features

Leptospirosis ranges in severity from a mild, self-limited febrile illness to a fulminant life-threatening illness. When illness occurs, a broad array of organ systems may be involved, reflecting the systemic nature of the infection. As a result, the signs and symptoms of leptospirosis are protean and frequently mistaken for other causes of acute febrile syndrome.

3.1 Incubation Phase

The incubation phase from exposure to onset of symptoms averages from 7 to 12 days, though it can be as short as 3 days or as long as a month. The remarkable variability in the duration of the incubation phase is evident in the 6–29 day lag between exposure and onset of symptoms among 52 athletes who developed laboratory-confirmed leptospirosis after participating (all on the same day) in the Springfield Triathalon (Morgan et al. 2002).

3.2 Presentation

Patients typically present with sudden onset of fever, chills, and headache. These signs and symptoms are nonspecific and also occur with other causes of acute febrile syndrome that, depending on the setting, could also be caused by influenza, dengue fever, or malaria. The headache is often severe and has been described as a bitemporal, frontal throbbing headache accompanied by retro-orbital pain and photophobia.

Muscle pain and tenderness is common and characteristically involves the calves and lower back. A tip-off to identification of leptospirosis is conjunctival suffusion (dilatation of conjunctival vessels without purulent exudate), which occurs frequently in leptospirosis, but is uncommon in other infectious diseases. Additional ocular findings typically include subconjunctival hemorrhages and icterus (Fig. 4a). Rash is uncommon in leptospirosis and when it occurs in the setting of an acute febrile illness, may suggest an alternative diagnosis such as dengue or chikungunya fever (Burt et al. 2012; Zaki and Shanbag 2010). An erythematous rash limited to the pretibial areas of both legs appearing on about the fourth day of illness was a feature of an outbreak of “Fort Bragg Fever” which also included headache, malaise, and splenomegaly among soldiers in North Carolina, the etiology of

which was later determined to be *L. interrogans* serovar Autumnalis (Gochenour et al. 1952).

A nonproductive cough has been noted in 20–57 % of leptospirosis patients and can potentially lead clinicians to incorrectly diagnose the patient with influenza or another respiratory illness. Gastrointestinal symptoms are frequently observed, and may include nausea, vomiting, diarrhea, and abdominal pain. Nausea and other gastrointestinal symptoms may contribute to dehydration in patients with high-output nonoliguric renal failure caused by leptospirosis. The abdominal pain may be due to acalculous cholecystitis and/or pancreatitis. In patients admitted to the hospital for leptospirosis, abdominal pain associated with abnormal serum amylase and/or lipase levels is relatively common (O'Brien et al. 1998). It should be kept in mind that impaired renal function alone can elevate pancreatic enzyme levels when the creatinine clearance is less than 50 ml/min (Collen et al. 1990). While most cases of pancreatitis due to leptospirosis are self-limited, some cases are more severe and associated with fatal outcomes (Spichler et al. 2007).

Severe leptospirosis is characterized by dysfunction of multiple organs including the liver, kidneys, lungs, and brain. The combination of jaundice and renal failure, known as Weil's disease, was first described in 1886 (Weil 1886) and remains one of the most clinically recognizable forms of leptospirosis (see the chapter by B. Adler, this volume). Evidence of organ dysfunction indicates a more advanced stage of infection, yet may develop suddenly and be present in a large percentage of patients at the time of presentation.

Leptospirosis patients are typically found to have mild to moderate elevations in levels of liver transaminases and direct (conjugated) bilirubin. The frequency of jaundice varies widely among case series, perhaps due in part to the virulence of the causative organism. Katz et al. (2001) found a strong association between infection with the Icterohaemorrhagiae serogroup and jaundice and elevated bilirubin levels. Acute hemolytic anemia can contribute to jaundice which, not surprisingly, is more common in leptospirosis patients with glucose-6-phosphate dehydrogenase deficiency (Avdeeva et al. 2002). Such patients have a higher percentage of unconjugated (i.e., indirect) bilirubin. Many patients have leukocytosis and thrombocytopenia, though usually not to the extent that would cause spontaneous hemorrhage. Leukopenia in the setting of thrombocytopenia and anemia can suggest bone marrow suppression.

Clinical signs of bleeding are common and occur in the majority of patients with severe leptospirosis. Most bleeding manifestations are mild, including petechiae, ecchymoses, and epistaxis. However, some patients have severe gastrointestinal (melena or hematemesis) or pulmonary hemorrhage. Thrombocytopenia frequently occurs, although usually not to the extent that would cause spontaneous hemorrhage. In a study of severe leptospirosis performed in the Netherlands, all patients had coagulation disorders, including prolongation of the prothrombin time (PT) and the length of the PT was associated with severe bleeding manifestations (Wagenaar et al. 2010).

The kidney is a major target organ in leptospirosis, perhaps due to the intrinsic renal-tropic homing ability of leptospires in their reservoir hosts. The kidneys are commonly involved,

as manifested by elevations in serum blood urea nitrogen and creatinine levels and findings on urinalysis of pyuria, hematuria, and elevated urine protein levels (Katz et al. 2001). Leptospirosis causes a unique nonoliguric potassium wasting nephropathy characterized by impaired sodium reabsorption and potassium wasting (Seguro et al. 1990). When poor oral intake due to nausea and high-output renal failure combine to cause dehydration, patients are at risk of oliguria and renal failure, a frequent cause of death in areas where peritoneal or hemodialysis is not available.

Progression to severe leptospirosis and circulatory collapse may be accompanied by acute respiratory distress syndrome (ARDS). As in other causes of ARDS, leptospirosis causes diffuse lung injury characterized by impaired gas exchange and the need for mechanical ventilation. Massive hemoptysis, representing extensive alveolar hemorrhage, is an ominous complication of leptospirosis associated with fatality rates >50 % (Gouveia et al. 2008). Pulmonary hemorrhage associated with leptospirosis was first reported in Switzerland in 1943 (Moeschlin 1943), and since then has been reported with increasing frequency from a variety of locations (Park et al. 1989). Leptospirosis-associated severe pulmonary hemorrhage syndrome (SHPS) can occur sporadically or in outbreaks that can be confused clinically with viral pneumonitis (Sehgal et al. 1995; Trevejo et al. 1998). For example, a 1995 outbreak of SPHS that occurred after heavy rainfall and flooding in Nicaragua was initially thought to be due to hantavirus pulmonary syndrome until silver staining and immunohistochemistry of postmortem lung tissue revealed leptospires (Trevejo et al. 1998). SPHS can present as hemoptysis associated with cough or may be discovered after patients undergo pulmonary intubation (Yersin et al. 2000). Chest radiographs show diffuse alveolar infiltrates (Fig. 4b). Epidemiologic evidence suggests that SPHS may be a relatively new problem, suggesting emergence of a new clone of *L. interrogans* with enhanced virulence. However, it is also possible that SPHS is an old problem that is finally being recognized and documented.

As noted above, headache is frequently severe and when accompanied by meningismus may prompt performance of lumbar puncture. Typical findings on CSF examination include a lymphocytic predominance with total cell counts of up to 500/mm^3, protein levels between 50 and 100 mg/mL, and normal glucose levels, consistent with aseptic meningitis (Berman et al. 1973). Depending on the epidemiologic setting, leptospirosis may be a predominant cause of aseptic meningitis in some areas (Silva et al. 2002a). Patients with aseptic meningitis due to leptospirosis may be anicteric, making the diagnosis more challenging (Berman et al. 1973; Karande et al. 2005). In severe leptospirosis, altered mental status may be an indicator of meningoencephalitis. A variety of other neurologic complications may also occur including hemiplegia, transverse myelitis, and Guillain–Barré syndrome (Levett 2001).

3.3 Risk Factors for Morbidity and Mortality

In an active surveillance study of 326 cases of leptospirosis in Salvador, Brazil, the strongest independent predictor of a fatal outcome was altered mental status (odds ratio 9.12), which typically began with confusion and obtundation without focal neurologic signs (Ko et al. 1999). Other independent risk factors for death identified in the Salvador study included

oliguria (odds ratio 5.28), age over 36 years (odds ratio 4.38), and respiratory insufficiency (2. 56) (Ko et al. 1999). The risk of a fatal outcome increases with increased age; compared to individuals aged 19–29, the increased risk of death rose from 3.7-fold for 40–49 year olds to 7.3-fold among those 60 or older (Lopes et al. 2004). Lung involvement, as indicated by dyspnea (odds ratio 11.7) or alveolar infiltrates on chest X-ray (odds ratio 7.3), was found to be associated with mortality in a retrospective study of 68 leptospirosis cases at Pointe-à-Pître Hospital in the French West Indies, along with oliguria (odds ratio 9), repolarization abnormalities on electrocardiogram (odds ratio 5.95), and a white blood count >12,900/mm^3 (odds ratio 2.54) (Dupont et al. 1997). A retrospective review of leptospirosis cases associated with an outbreak of leptospirosis in India identified pulmonary involvement and altered mental status as independent predictors of death (Pappachan et al. 2004). Additional poor prognostic signs identified in other studies include acute renal failure, hypotension, and arrhythmias (Daher et al. 1999; Panaphut et al. 2002).

3.4 Recovery Phase

With proper supportive care (see Management below), most leptospirosis patients recover completely (Spichler et al. 2011). Patients with acute renal failure who require dialysis typically regain most of their renal function, although there may be evidence of persistent mild renal impairment (Covic et al. 2003). In addition, there is growing recognition that many patients suffer from chronic postleptospirosis symptoms. In a recent study of laboratory-confirmed leptospirosis patients in the Netherlands, 30 % of patients experienced persistent complaints after acute leptospirosis (PCAC) characterized by fatigue, myalgia, malaise, headache, and weakness (Goris et al. 2013a). Of patients with PCAC, 21 % reported that their complaints lasted for more than 24 months.

Ocular involvement in the form of uveitis is well-known to occur during the convalescent phase of leptospirosis. Eye involvement ranges in severity from insidious onset of mild anterior uveitis to acute, severe panuveitis involving the anterior, middle, and posterior segments of the eye (Rathinam 2005). Leptospiral uveitis may occur either as a single, self-limited event or as a series of recurrent episodes, which appears to occur more frequently in patients with severe uveitis. In one study, 80 % of patients had leptospiral DNA in the aqueous humor, detected by PCR (Chu et al. 1998). However, the relative contributions of infection and autoimmunity are uncertain. There are parallels between recurrent uveitis in humans and equine recurrent uveitis (ERU) and autoimmunity to lens proteins has been suggested to play a role in ERU (Verma et al. 2010).

4 Diagnosis

Diagnosis of leptospirosis may be accomplished by direct detection of the organism or its components in body fluid or tissues, by isolation of leptospires in cultures, or by detection of specific antibodies (Hartskeerl et al. 2011; Schreier et al. 2013). The collection of appropriate specimens and selection of tests for diagnosis depend upon the timing of collection and the duration of symptoms (Fig. 5). For detailed descriptions of historical methods see the following publications (Faine et al. 1999; Galton 1962; Levett 2001; Sulzer and Jones 1978; Turner 1968, 1970; Wolff 1954).

4.1 Molecular Diagnosis

Leptospiral DNA has been amplified from serum, urine, aqueous humor, CSF, and a number of organs post mortem (Levett 2004). Conventional PCR and other assays such as LAMP and NASBA were reviewed recently (Ahmed et al. 2012) and will not be discussed further. Many quantitative PCR assays have been described, which target a number of different genes (Ahmed et al. 2009; Merien et al. 2005; Palaniappan et al. 2005; Smythe et al. 2002; Stoddard et al. 2009). Assays developed for diagnostic use can be considered in two broad categories, targeting either housekeeping genes, such as *rrs*, *gyrB*, or *secY*, or pathogen-specific genes such as *lipL32*, *lig*, or *lfb1* (Ahmed et al. 2012). Examples of these two types of quantitative assay were evaluated in a large case-control study in a high-prevalence population in Thailand (Thaipadunpanit et al. 2011), that confirmed earlier reports that PCR detection in blood samples collected at admission to hospital was more sensitive than culture, but serology using the microscopic agglutination test (MAT) ultimately detected more cases (Brown et al. 1995). Real-time PCR assays have been used to quantify the bacterial load in leptospirosis (Agampodi et al. 2012; Segura et al. 2005; Tubiana et al. 2013).

A limitation of PCR-based diagnosis of leptospirosis is the current inability of PCR assays to identify the infecting serovar. While this is not significant for individual patient management, the identity of the serovar has both epidemiological and public health value. Serovar identification requires isolation of the infecting strain from patients or carrier animals. However, whole genome sequencing has recently been applied to the diagnosis of neurological leptospirosis (Wilson et al. 2014) and it is probable that direct serovar identification will be possible in the near future, limited only by the quality of sequences obtained from specimens.

4.2 Isolation and Identification of Leptospires

Culture of leptospires requires specialized media (see the chapter by C.E. Cameron, this volume). Leptospires can be recovered from humans during the acute phase of the illness and during the so-called immune phase. Leptospiremia occurs during the first stage of the disease, beginning before the onset of symptoms and has usually declined by the end of the first week of the acute illness. Timing of culture of different specimens depends upon an accurate date of onset of symptoms, so a careful history is essential. Blood cultures should be taken as soon as possible after the patient’s presentation. One or two drops of blood are inoculated into 5–10 ml semisolid or liquid medium at the bedside (Turner 1970). Multiple cultures yield higher recovery rates, but this is rarely possible. Inoculation of media with dilutions of blood samples may increase recovery (Sulzer and Jones 1978). Leptospires have been shown to survive in commercially available conventional blood culture media for periods of time ranging from 48 h to 4 weeks (Palmer and Zochowski 2000). Blood cultures with no growth can be used to inoculate leptospiral culture medium (Turner 1970).

Other samples that may be cultured during the first week of illness include CSF and peritoneal dialysate. Urine should be cultured from the beginning of the second week of symptomatic illness. The duration of urinary excretion varies, but may be several weeks (Bal et al. 1994). Survival of leptospires in voided human urine is limited, so urine should be

collected into sterile phosphate buffered saline (Turner 1970). Contamination of urine cultures is a major problem and the use of selective media containing 5-fluorouracil or other antimicrobial agents (see the chapter by C.E. Cameron, this volume) is strongly recommended. Cultures are incubated at 28–30 °C and examined weekly by dark field microscopy, for up to 13 weeks.

Isolated leptospires are identified either by serological methods, or more recently, by molecular techniques. Traditional methods relied on cross-agglutinin absorption (Dikken and Kmety 1978). The number of laboratories which can perform these identification methods is small. Monoclonal antibodies are available for identification of many, but not all, serovars (Korver et al. 1988). The limitations of these approaches are discussed by Hartskeerl and Smythe (see the chapter by R.A. Hartskeerl and L.D. Smythe, this volume).

Molecular methods for identification and subtyping have been studied extensively. Increasingly, sequence-based identification of *Leptospira* is becoming the standard (Ahmed et al. 2012) and this can be performed on the products of diagnostic PCR (Ganoza et al. 2010; Perez and Goarant 2010). Pulsed-field gel electrophoresis (PFGE) has been shown to identify most serovars (Galloway and Levett 2010; Herrmann et al. 1992), but complements, rather than replaces, serological identification (Ahmed et al. 2012). Identification of serovars by whole genome sequencing will likely become standardized in the near future (Ahmed et al. 2012).

Strain subtyping for epidemiological purposes can be accomplished by simple methods using restriction enzymes or variations of PCR conditions that can generate banding patterns that allow strains to be differentiated (Ahmed et al. 2012). However, reproducibility is poor, particularly between laboratories. More recently, sequence-based methods such as MLVA (Pavan et al. 2008; Salaun et al. 2006; Slack et al. 2005) and MLST (Ahmed et al. 2006, 2011; Boonsilp et al. 2013; Leon et al. 2009; Thaipadungpanit et al. 2007) have been applied. These methods are reproducible and can yield significant information at a subserovar level (Boonsilp et al. 2013). MLST data can be analyzed online (http://leptospira.mlst.net).

4.3 Serological Diagnosis

Most cases of leptospirosis are diagnosed by serology, because capacity for culture and PCR is limited. IgM antibodies are detectable in the blood 5–7 days after the onset of symptoms. Serological methods can be divided into those which are genus-specific and those which are serogroup-specific. The use of agglutination tests was described soon after the first isolation of the organism and the microscopic agglutination test remains the definitive serological investigation in both human and animals.

4.3.1 Microscopic Agglutination Test—In the microscopic agglutination test (MAT), patients' sera are reacted with live antigen suspensions of leptospiral serovars. After incubation, the serum/antigen mixtures are examined microscopically for agglutination and the titers are determined. The MAT can be a complex test to control, perform, and interpret (Turner 1968). Live cultures must be maintained of all the serovars required for use as antigens. The range of antigens used should include serovars representative of all serogroups

(Faine 1982; Turner 1968) and locally common serovars (Torten 1979). A wide range of antigens is used in order to detect infections with uncommon, or previously undetected, serovars (Katz et al. 1991). The MAT is a serogroup-specific assay and cannot be relied upon to detect the infecting serovar (Levett 2003; Murray et al. 2011; Smythe et al. 2009).

The MAT is read by dark field microscopy. The endpoint is the highest dilution of serum in which 50 % agglutination occurs. Because of the difficulty in detecting when 50 % of the leptospires are agglutinated, the endpoint is determined by the presence of approximately 50 % free, unagglutinated leptospires, by comparison with the control suspension (Faine 1982). Considerable effort is required to reduce the subjective effect of observer variation, even within laboratories.

Interpretation of the MAT is complicated by the high degree of cross-reaction that occurs between different serogroups, especially in acute-phase samples. Patients often have similar titers to all serovars of an individual serogroup, but “paradoxical” reactions, in which the highest titers are detected to a serogroup unrelated to the infecting one, may also occur (Alston and Broom 1958; Levett 2001). The broad cross-reactivity in the acute phase, followed by relative serogroup specificity in convalescent samples, results from the detection in the MAT of both IgM and IgG antibodies (Adler and Faine 1978).

Paired sera are required to confirm a diagnosis with certainty. A fourfold or greater rise in titre between paired sera confirms the diagnosis, regardless of the interval between samples. The interval between first and second samples depends very much on the delay between onset of symptoms and presentation of the patient. If symptoms typical of leptospirosis are present, then an interval of 3–5 days may be adequate to detect rising titers. However, if the patient presents earlier in the course of the disease, or if the date of onset is not known precisely, then an interval of 10–14 days between samples is more appropriate. Less often, seroconversion does not occur with such rapidity, and a longer interval between samples (or repeated sampling) is necessary. MAT serology is insensitive in early acute-phase specimens (Appassakij et al. 1995; Brandão et al. 1998; Cumberland et al. 1999). Moreover, patients with fulminant leptospirosis may die before seroconversion occurs (Brown et al. 1995; Cumberland et al. 1999; Ribeiro et al. 1994).

Acute infection is suggested by a single elevated titer detected in association with an acute febrile illness. The magnitude of such a titer is dependent upon the background level of exposure in the population, and hence the seroprevalence. The application of single titers for presumptive diagnosis has been reviewed (Levett 2001) and will not be discussed further. Titers following acute infection may be extremely high (≥25,600) and may take months, or even years, to fall to low levels (Alston and Broom 1958; Blackmore et al. 1984; Cumberland et al. 2001; Lupidi et al. 1991; Romero et al. 1998). Rarely, seroconversion may be delayed for many weeks after recovery, and longer serological follow-up will be necessary to confirm the diagnosis.

The MAT is the most appropriate test to employ in epidemiological sero-surveys, since it can be applied to sera from any animal species, and because the range of antigens utilized can be expanded or decreased as required. It is usual to use a titer ≥100 as evidence of past

exposure (Faine 1982). However, conclusions about infecting serovars cannot be drawn without isolates; MAT data can give only a general impression about which serogroups are present within a population (Everard and Everard 1993).

4.3.2 Other Serological Tests—Because of the complexity of the MAT, rapid screening tests for leptospiral antibodies in acute infection have been developed. IgM antibodies become detectable during the first week of illness, allowing the diagnosis to be confirmed and treatment to be initiated while it is likely to be most effective. IgM detection has repeatedly been shown to be more sensitive than MAT when the first specimen is taken early in the acute phase of the illness (Cumberland et al. 1999; Goris et al. 2011b; Ribeiro et al. 1994; Winslow et al. 1997).

Detection of IgM using ELISA has been employed widely, most often using antigen prepared from cultures of *L. biflexa*, although pathogenic species have also been used. Several products are available commercially. Recombinant antigens have also been employed, but none has been evaluated widely (Signorini et al. 2013). Specificity of IgM detection by ELISA is affected by the antigen used in the assay, by the presence of antibodies due to previous exposure (in endemic regions), and by the presence of other diseases (Bajani et al. 2003).

More recently, IgM detection assays have been developed in several rapid test formats intended for use in laboratories without extensive instrumentation, or potentially in field settings. These have included two dipstick formats (Smits et al. 2000a; Levett and Branch 2002), latex agglutination (Smits et al. 2000b, 2001a), lateral flow (Smits et al. 2001b) and dual path platform (Nabity et al. 2012).

However, there are significant limitations to early diagnosis using any serological test (Goris et al. 2011b; Signorini et al. 2013) and testing of a second sample should be considered mandatory. Moreover, confirmation of rapid diagnostic test results by a reference test has been recommended (Goris et al. 2013b).

4.3.3 Evaluation of Serological Tests—Evaluation of serological tests for leptospirosis has been problematic because there are few laboratories equipped to perform the definitive serological test (MAT), and there are fewer laboratories with the capacity to isolate and identify leptospires from patients. A large body of the literature consists of reports on studies that have been ill-designed and which use less than perfect case definitions, leading to misleading estimates of sensitivity and specificity. Ideally, new serological assays should be evaluated in clinical trials of consecutive patients investigated using a case definition which includes both MAT and culture results, and which are conducted in multiple regions, where different leptospiral serovars are prevalent and where the differential diagnoses may vary widely (Smits et al. 2000a, b). Assays may perform differently in different populations (Desakorn et al. 2012; Levett and Branch 2002). Alternatively, well-designed studies conducted in individual centers may be compared, providing the limitations of this approach are recognized (Levett 2001). Evaluations performed using collections of sera in reference laboratories may be useful for determining sensitivity of assays, but specificity is dependent upon the selection of noncase sera representative both of other diseases and the normal

population. Parallel studies in clinical and reference settings may yield quite different results (Bajani et al. 2003; Hull-Jackson et al. 2006).

5 Management

Most leptospirosis cases are mild and resolve spontaneously. Early initiation of antimicrobial therapy may prevent some patients from progressing to more severe disease. Identification of leptospirosis in its early stages is largely a clinical diagnosis and relies on a high index of suspicion based on the patient's risk factors, exposure history, and presenting signs and symptoms. Rapid diagnostic tests for leptospirosis are improving, but a negative result should not be relied on to rule out early infection. For these reasons, empirical therapy should be initiated as soon as the diagnosis of leptospirosis is suspected.

Therapy for patients with leptospirosis severe enough to merit hospitalization usually involves intravenous penicillin (1.5 million units IV every 6 h), ampicillin (0.5–1 g IV every 6 h), ceftriaxone (1 g IV every 24 h), or cefotaxime (1 g IV every 6 h). Ceftriaxone has been shown to be noninferior to penicillin for serious leptospirosis (Panaphut et al. 2003) and in addition to once daily dosing has the added benefit of intramuscular administration as an alternative to intravenous therapy in settings where hospitalization is not possible. Adult outpatients with early disease should receive either doxycycline 100 mg orally twice per day or azithromycin 500 mg orally once per day. When the dosage is adjusted for weight, either azithromycin or amoxycillin can also be given to pregnant women and children. These recommendations are based on in vitro susceptibility data (Hospenthal and Murray 2003; Ressner et al. 2008), animal studies (Alexander and Rule 1986; Truccolo et al. 2002), and clinical experience including a randomized, placebo-controlled, double-blinded study which found that doxycycline therapy shortened the duration of illness due to leptospirosis by 2 days and improved fever, malaise, headache, and myalgias (McClain et al. 1984). Doxycycline treatment also prevented shedding of organisms in the urine.

There are strong grounds for administering antibiotics as soon as possible to patients with risk factors and clinical features of severe leptospirosis. A placebo-controlled trial of intravenous penicillin for leptospirosis conducted in the Philippines found that penicillin shortened the duration of fever, abnormal renal function, and hospitalization and prevented leptospiral shedding in the urine (Watt et al. 1988). A flaw in this study was that a number of patients in both groups had received antibiotics prior to entry into the study. A second placebo-controlled study of intravenous penicillin for leptospirosis patients was conducted in Barbados, most of whom were icteric. Although the Barbados study failed to show significant differences between the penicillin and placebo groups, patients receiving penicillin had a lower mortality rate than patients receiving placebo (2.6 vs. 7.3 %, respectively) (Edwards et al. 1988). It can be difficult to demonstrate a beneficial effect of antibiotics in patients who have already begun to experience some degree of organ dysfunction, which of course cannot be reversed with antibiotics. As imperfect as these studies are, they are likely to be the only placebo-controlled studies that will ever be conducted, given the ethical barriers to placebo-controlled studies involving life-threatening illnesses caused by antibiotic-susceptible bacteria.

Severe leptospirosis is a medical emergency requiring both antibiotics and proper supportive therapy to improve mortality rates. Patients with severe leptospirosis are frequently found to have a unique form of potassium wasting high-output renal dysfunction (Abdulkader et al. 1996; Seguro et al. 1990). For this reason, patients should receive intravenous hydration to correct dehydration and prevent oliguric renal failure. Potassium supplementation should be included for patients with hypokalemia. When oliguric renal failure occurs, early initiation of peritoneal or hemodialysis can be lifesaving and is usually needed only on a short-term basis (Andrade et al. 2008). In a comparative study, prompt initiation of daily dialysis in critically ill leptospirosis patients reduced mortality from 67 to 17 % (Andrade et al. 2007). Patients with respiratory failure who require intubation typically have poor pulmonary compliance (i.e., “stiff lungs”) and have been found to benefit from ventilation with low tidal volumes (<6 mL/kg) to reduce ventilation pressures, protect patients from alveolar injury, and improve survival rates (Amato et al. 1998).

5.1 Antimicrobial Susceptibilities

Leptospires are susceptible to β-lactams, macrolides, tetracyclines, fluoroquinolones, and streptomycin (Alexander and Rule 1986; Faine et al. 1999). Problems in the determination of susceptibility include the long incubation time required, the use of media containing serum, and the difficulty in quantifying growth accurately. These constraints have limited the development of rapid, standardized methods for susceptibility testing. Most studies have used a limited range of laboratory strains and/or a small number of antimicrobial agents. However, microdilution methods have been described recently (Murray et al. 2004; Ressner et al. 2008), which will facilitate the study of large numbers of isolates against a wide range of antimicrobial agents, with the potential of identifying new agents for prophylaxis or treatment of leptospirosis.

6 Prevention

Strategies for prevention of leptospirosis are based on awareness of leptospirosis epidemiology and transmission mechanisms, as presented earlier in this chapter. Once the local epidemiology and transmission risks have been defined, it is possible to greatly mitigate risk by taking steps to reduce exposure and implement protective measures, immunization, and pre- or postexposure chemoprophylaxis.

From a global perspective, human leptospirosis is strongly linked to poverty wherever poor housing standards and local infrastructure result in exposure to rodent reservoirs. Rodent abatement efforts may have short-term benefit but rodenticides create risks for children and wildlife and are not good long-term solutions. Housing construction that prevents rodents from invading residential living spaces greatly reduces risk. Flood control projects that prevent inundation of residential areas would greatly reduce the potential for leptospirosis outbreaks. These measures are difficult to implement, but should be recognized as an important part of an overall prevention strategy.

Occupational activities that put workers at risk through exposure to contaminated water or infected animals should be identified. Personal protective equipment such as gloves, boots, goggles, and overalls for workers in high-risk occupations are important to prevent exposure

of mucous membranes and skin, but can be difficult to implement in hot and humid environments. Abrasions, cuts, and damaged skin are particularly important as portals of entry. Walking barefoot and water sports in endemic areas are notoriously high-risk activities. The 2001 Eco-challenge multi-sport competition in Borneo involving jungle trekking and leach bites followed by prolonged emersion in the rain-swollen Segama River resulted in an astounding 42 % attack rate and illustrates how endemic factors and susceptible hosts combine to create high-risk exposures (Sejvar et al. 2003).

Source reduction through immunization of agricultural and companion animals with killed whole-cell vaccines is an extremely important strategy for reducing the risk of human leptospirosis. Humans may also become infected through exposure to acutely or chronically infected animals that are shedding leptospires in their urine. Diagnosis and treatment of infected animals, and immunization of uninfected companion and agricultural animals is another cornerstone of leptospirosis prevention and is covered in chapter by W.A. Ellis, this volume.

6.1 Human Leptospirosis Vaccines

Immunization of humans with killed, whole-cell vaccines has generally been restricted to individuals in high-risk occupations and in response to floods and epidemics. One of the first reports of human leptospirosis immunization involved the vaccination of thousands of miners in Japan using a culture-derived *L. interrogans* serovar Icterohaemorrhagiae vaccine (Wani 1933). Although local and generalized reactions were common, a significant decrease in the incidence of leptospirosis among the miners was observed. Immunization of large populations at risk of leptospirosis due to extensive flooding has been performed in China (Chen 1985). A Cuban leptospirosis vaccine trial involving >100,000 persons reported that local pain and “general discomfort” were significantly greater than in a control group given a recombinant hepatitis B vaccine (Martinez et al. 2004). The vaccine showed an efficacy of >97 % against the prevalent local serovars. Concern over reactions to host proteins led to the development of a leptospiral vaccine derived from leptospires grown in a chemically defined medium (Shenberg and Torten 1973); however, growth in protein media is generally poorer and such media have not gained widespread use.

Some of the most detailed safety and efficacy studies involved a leptospirosis vaccination program for Parisian sewer workers. In response to a request by the City of Paris, the Pasteur Institute developed a killed, whole-cell vaccine derived from *L. interrogans* serovar Icterohaemorrhagiae strain Verdun. Mailloux et al. (1983) examined the safety of this vaccine and reported three systemic (nausea) reactions and seven local reactions among 1,157 immunizations of 454 vaccines. Importantly, after the vaccine was introduced in 1979, the incidence of leptospirosis dropped from 1.3 cases per year (29 cases from 1951 to 1979) to zero (no cases reported from 1981 to 1988) during a 7 year follow-up period. The recommended vaccination protocol involves two booster doses after the initial immunization followed by reimmunization every 2 years. More recent reports of safety and efficacy have been published since the vaccine was marketed as Spirolept™ (Benbrick et al. 2001; Laurichesse et al. 2007; Pouliquen and Catilina 2000).

As described in chapter by B. Adler, this volume, the active component of killed, whole-cell vaccines is leptospiral LPS, a serovar-specific antigen (Chapman et al. 1990). LPS-based immunity is generally considered to provide protection against homologous or closely related, but not heterologous, serovars. For example, Fukumura (1984) reported that individuals immunized with a serovar Pyrogenes vaccine were protected from infection by that serovar but not from serovars Autumnalis and Hebdomadis with antigenically unrelated LPS, leading to development of a trivalent vaccine consisting of all three serovars. Research on development of leptospirosis vaccines with a low side-effect profile that induce long-lasting, cross-protective immunity is focused on an improved understanding of the leptospiral outer membrane (see the chapters by D.A. Haake and W.R. Zückert and by B. Adler, this volume).

6.2 Chemoprophylaxis

Unavoidable short-term exposure can be mitigated by chemoprophylaxis. Pre-exposure prophylaxis with doxycycline (200 mg orally once per week) was effective for military personnel undergoing high-risk jungle training exercises (Takafuji et al. 1984). Doxycycline has also been studied for postexposure prophylaxis of local populations after heavy rainfall in endemic areas (Gonsalez et al. 1998; Sehgal et al. 2000). One of these two studies found that postexposure doxycycline prophylaxis reduced the incidence of symptomatic disease (Sehgal et al. 2000). Alternatives to doxycycline, such as azithromycin or amoxicillin, have not been studied, but may be considered in pregnant women and children and individuals at risk of photosensitivity.

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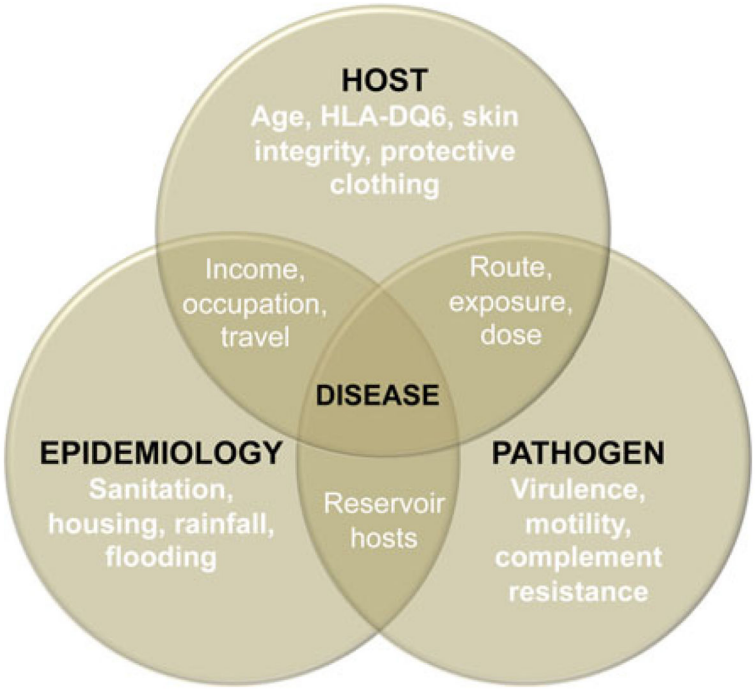

Fig. 1.

Factors contributing to leptospirosis. Development of leptospirosis depends on three types of factors (*epidemiology*, *host*, and *pathogen*) and their interactions. Epidemiologic factors include sanitation, housing, rainfall, and whether flooding occurs. Incidence is linked to income level, occupation, and travel, representing epidemiologic factors linked to specific hosts. Hosts vary in susceptibility depending on age, genetic factors (e.g., HLA-DQ6), skin integrity, and whether protective clothing (e.g., gloves and boots) are worn. The ways in which the host and leptospires interact determine the route, exposure, and dose of the pathogen. Leptospiral pathogens differ in their ability to cause disease, a reflection of their virulence, motility, and ability to survive in the host, a reflection (at least in part) of complement resistance. The types of reservoir hosts determine the types of pathogens present in a particular epidemiologic setting

Fig. 2.

Epidemiologic settings for leptospirosis. **a** A high proportion of contestants in the 2000 Eco-Challenge multisport race held in Malaysian Borneo developed leptospirosis. Of 189 participants contacted by the Centers for Disease Control, 80 (42 %) met the case definition for leptospirosis. Risk factors included exposure for extended periods of time to the rain-swollen Segama river (*photograph credit* Reed Hoffmann). **b** This rural village in Laos is a typical epidemiologic setting for leptospirosis. Residents of tropical regions of the world with high levels of rainfall are at increased risk of leptospirosis, particularly when standing water is contaminated by urine from wild or domesticated animals, which may serve as reservoir hosts for pathogenic *Leptospira* species (*photograph credit* Ben Adler)

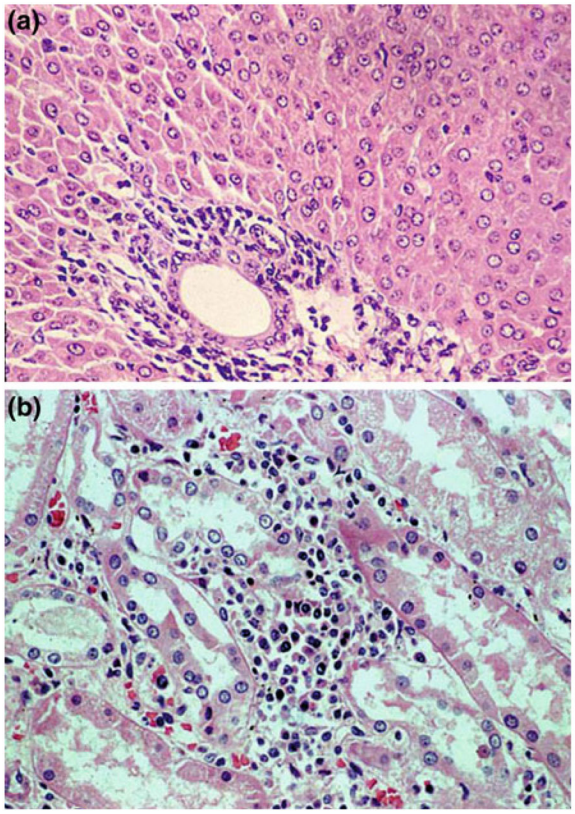

Fig. 3.

Histopathology of leptospirosis. **a** Histology of the liver typically shows lack of the normal adhesion between hepatocytes, a hallmark of the disease (*photograph credit* Thales De Brito). **b** Typical renal histopathology showing acute tubular necrosis and interstitial nephritis. The glomerulus is essentially unremarkable. Reproduced from Abdulkader and Silva, The kidney in leptospirosis. Pediatr Nephrol 2008; 23:2111–2120, with permission of the publisher, Springer

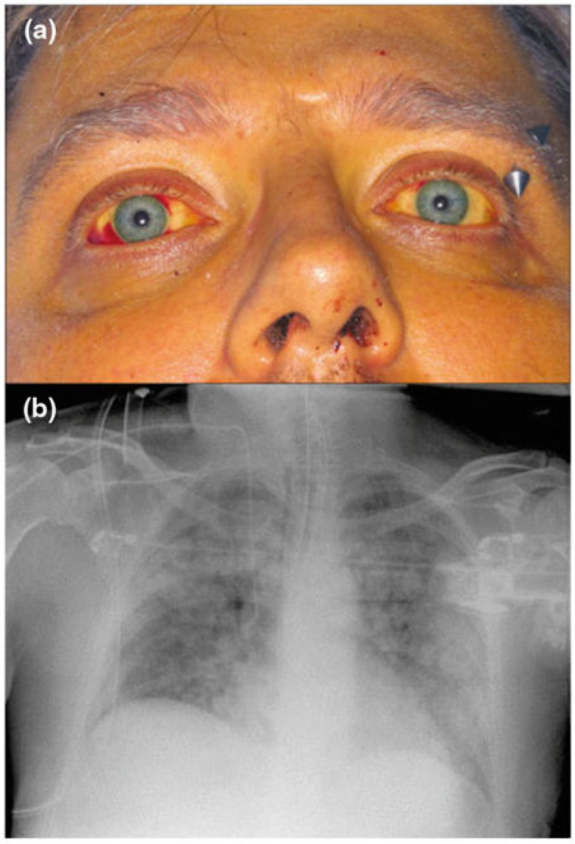

Fig. 4.

Clinical presentation of leptospirosis. **a** Subconjunctival hemorrhages and icterus in a 37-year-old man who kept pet rats presented with sudden onset of fever, myalgia, and severe headache. On admission he had abnormal liver and kidney function. Serological tests for leptospiral antibodies converted from negative to positive 1 week after presentation. He was treated with intravenous penicillin and recovered completely. Reproduced from Jansen and Schneider, Weil's disease in a rat owner. Lancet Infect Dis 2011; 11:152, with permission of the publisher, Elsevier Ltd. **b** Severe pulmonary hemorrhage in a 50-year-old man who had recently returned from vacation in Malaysia where he had waded through mangrove forests. Respiratory deterioration occurred on day 2 of hospitalization requiring mechanical ventilation complicated by severe hemoptysis requiring blood transfusion. He was treated initially with doxycycline followed by amoxicillin and made a slow but complete recovery. Blood culture in leptospiral growth medium became positive 4 months after inoculation. Reproduced from Wagenaar et al. Leptospirosis with pulmonary hemorrhage, caused by a new strain of serovar Lai: Langkawi. J Travel Med 2004; 11:379–382. With permission of the publisher, John Wiley and sons. No portion of this figure may be reproduced without permission of the publisher

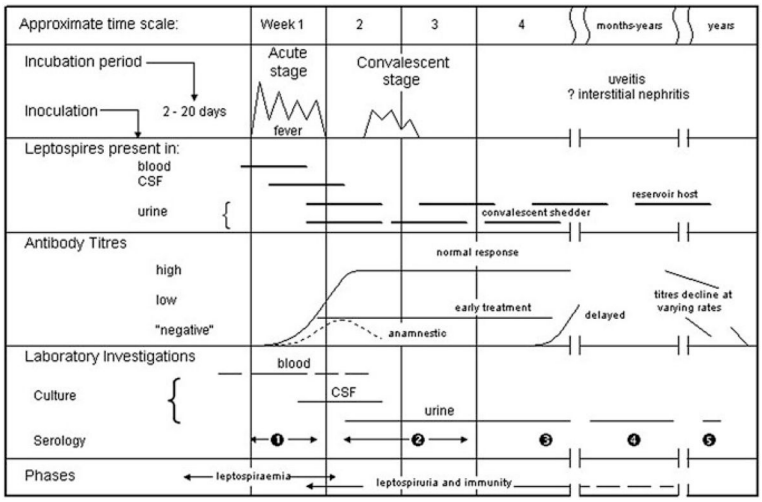

Fig. 5.

Biphasic nature of leptospirosis and relevant investigations at different stages of disease. Specimens *1* and *2* for serology are acute-phase specimens, *3* is a convalescent-phase sample which may facilitate detection of a delayed immune response, and *4* and *5* are follow-up samples which can provide epidemiological information, such as the presumptive infecting serogroup. Adapted from Turner LH (1969). Leptospirosis. Br Med J i:231–235, with permission of the publisher. Copyright © American Society for Microbiology, (Clin Microbiol Rev 2001, 14 (2):296–326. doi:10.1128/CMR.14.2.296-326.2001)