Leptospirosis now: epidemiology, progress, challenges and research gaps

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ABSTRACT

Leptospirosis continues to be a challenge to health worldwide, among farmers, municipal workers etc. Despite widespread implementation of effective therapy, leptospirosis has not been eliminated or keeping under the control. The outbreaks occurring every year with increasing mortality during the flood and other water oriented natural calamities. Identification of patients with leptospirosis is challenging among the clinicians, due to misdiagnosing or under diagnosing, including non consideration of PUO cases to leptospirosis. Children who develop leptospirosis continue to be especially disadvantaged, with rates of late diagnosis and mortality remaining high. Leptospirosis is not a specified disease in the Millennium development goals, but severity and multiple organ dysfunctions to multiple organ failure induce the researchers and clinicians to concentrate for education and level of improvement of the patient care including immediate therapy. We review data and make recommendations for research on diagnosis, treatment and prevention such us further use of molecular analysis of *Leptospira interrogans* genome, formulation of newer vaccines and administration of doxycycline chemoprophylaxis to contacts. We also suggest developing the tools for early diagnosis and detection of infection and organ damage and formulation of strategies to manage the chronic complications of leptospirosis such as hepatospleenomegaly, renal failure, respiratory distress and neuropathy.

Introduction

Leptospirosis is an acute febrile infectious disease caused by the spirochete Leptospira interrogans characterized as being a zoonosis which affects both wild and domestic animals. The epidemiology of the infection in humans is related to their direct or indirect contact with contaminated animals [1,2]. Environmental contamination with the urine of carrier animals is usually the immediate source of infection [3,4]. Because of the ability of the leptospires to survive in the environment and infect human beings, this infection is a potential health hazard of occupational groups exposed to the environment. This disease occurs throughout the world, but its incidence is highest in tropical regions, but cases have been reported in temperate climates and developed countries [5,6]. The spectrum of disease is extremely wide and varies from clinically inapparent to severe multisystemic disease characterized by jaundice and acute renal failure [7,8]. The broad range of clinical manifestations that leptospirosis is part of the differential diagnosis of many febrile illness syndromes. In most cases the leptospirosis is confused with malaria and dengue fever, but the differential diagnosis inevitably varies depending on the infectious diseases that are prevalent locally.

Epidemiology

Leptospirosis occurs throughout the world. However, the disease burden is difficult to assess due to a lack of epidemiological data. Known high risk areas of leptospirosis are Brazil, the Caribbean, China, India, Malaysia, the Pacific islands, Seychelles, SriLanka, Thailand, and Vietnam. The worldwide number of cases is estimated to be between 0.1 - 1 per 100,000 population per year in temperate climates to 10 or more per 100,000 population per year in the humid tropics.

During an outbreak this figure may rise to 100 or more per 100,000. Although Leptospirosis can occur worldwide, there are a number of risk factors associated with the disease. It is most common in urban slum areas, where there is inadequate sewage disposal and water treatment. It can also be an occupational hazard for those working outdoors or with animals and a recreational hazard for those participating in water-related activities. Epidemics are typically seen during flooding, and changing environmental trends, with extreme weather patterns, may perpetuate these epidemics.

Very little is currently known regarding the true incidence of Leptospirosis. It is estimated that 0.1 to 1 per 100 000 people living in temperate climates are affected each year, with the number increasing to 10 or more per 100 000 people living in tropical climates. If there is an epidemic, the incidence can soar to 100 or more per 100 000 people. The disease is underreported for many reasons, including difficulty in distinguishing clinical signs from those of other endemic diseases and a lack of appropriate diagnostic laboratory services.

It is endemic in many rural and urban slum communities and can also cause sporadic epidemics, little is actually known about the true disease burden and consequently, the disease has been neglected.

Possible Natural History model of leptospirosis

This is attributed mainly to longer survival of leptospires in warm and humid environments. Leptospirosis is not limited to developing countries; retrospective reviews of the disease epidemiology have been reported from Ireland, Denmark and India [9,10,11]. A pattern of disease seasonality has been described with a peak incidence occurring in rainy season in warm climate regions [7,11]. The humans get infection while

contact directly or indirectly with urine, blood or tissue from an infected animal containing virulent leptospires. Infection may also arise from bathing or accidental immersion in the fresh water of lakes, rivers or canals contaminated with the urine of the infected livestock that has been previously using the water [12]. After an incubation period that ranges from 1 to 3 weeks, a phase commences which marked fever to arthralgias [13]. The majority of infections remains undetected or misdiagnosed since leptospirosis is a zoonoses of protean and non specific manifestations. Still in some cases of clinical infections, can cause multiple organ involvement and lead to death [14].
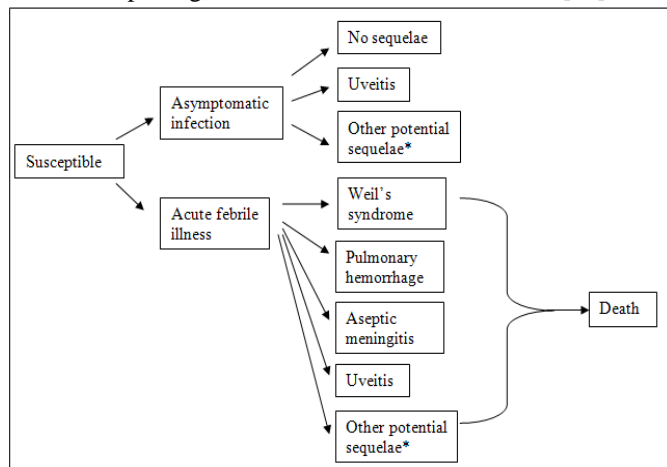
Animals

Feral and domestic animals including cattle, pigs, dogs, cats as well as reptiles and amphibians serve as a source of permanent maintenance hosts or reservoirs for the over 250 known serovars of leptospires [15,16]. Rats and other rodents are the most important sources of human infection [15,17,18]. This can be confirmed from the fact that several incidences of human and animal leptospirosis in over forty countries were attributed to the rodents. Several rodent species were associated with the disease including rats (*Rattus rattus*), mice (*Mus musculus*), bandicoots (*Bandicota indica*) and others [3,12,19]. In general, infected animals such as dogs, cats although vaccinated against *Leptospira*, can shed the organism in their urine for its entire lifetime and this may result in the domestic transmission in humans [20,21,22]. The infected animals transfer the infectious agent to their offspring wither in-utero or during neonatal period. Some leptospiral serovars are commonly associated with particular animal reservoirs; thus the prevalence of different population depends on the reservoirs present and the serovars they carry [23]. However no association has been found between infecting serovar and severity or manifestations of clinical symptoms [7,18].

Human subjects

Leptospirosis was thought to be primarily an occupational disease. Miners were the first occupational risk group to be recognized [24]. Farmers, rice mill workers, veterinarians, abattoir workers, rodent control workers, miners, soldiers, sewer workers, fish farmers, rice filed workers, banana farmers and others [3,25,26,27]. However, occupational risk has been significantly decreased since preventive measures have been implemented. In this regard, a high seroprevalence rate 32%, 28%, 17%, 12% among sewer workers, farmers, buchers and miners from India has been reported [4,11,18]. Further the use of protective clothing and other universal safety precautions may reduce leptospiral infection rate. A number of outbreaks have been related to heavy rainfalls in various rural locations including India, Philippines, Peru etc [18,28,29].

In India, particularly in Orissa, leptospirosis was reported following the cyclone in 1999 [30]. Moreover, there is a significant risk associated with recreational exposures occurring in water sports including swimming, rafting, canoeing, kayaking [31]. Furthermore, an outbreak of leptospirosis among athletes, travelers, barefoot gardening walking and number of disease outbreaks associated with drinking of urine contaminated water also recorded [32,33].

Progress

The characteristic biphasic illness may not be found in all patients, with only a fulminant monophasic illness being a predominating clinical course in few. These patients present with an acute undifferentiated illness which rapidly progresses to refractory shock, jaundice, renal failure and massive pulmonary haemorrhage [34]. Diagnosis is made on the basis of epidemiological, clinical and laboratory features.

Clinical Manifestations

Leptospirosis occurs as two clinically recognizable syndromes and most common is anicteric leptospirosis, a self limited illness that occurs in 85 – 90% of the cases [35]. There are two clearly defined stages in anicteric leptospirosis; the septicemic phase and immune phase. Icteric leptospirosis or Weil's syndrome is a more serious, potentially fatal, syndrome occurs in 5 to 10% of the cases. The demarcation between the septicemia phase and the immune phase is not as distinct in this syndrome [36]. Although subclinical infection is uncommon, the results of serological testing show that it occurs in some workers who have been occupationally exposed to leptospires. The incubation period for leptospirosis is usually 7 to 12 days, but it can range from 2 to 20 days.

The onset of anicteric leptospirosis is abrupt where the septicemic phase lasts 3 to 7 days where fever is high and remitting, headache is intense, unremitting and possibly throbbing. Anorexia, nausea, vomiting and abdominal pain occur in most patients [4]. The most common physical finding is conjunctival suffusion in the absence of purulent discharge. Other signs include masculopapular skin rash, pharyngeal injection, lymphadenopathy, splenomegaly, hepatomegaly and muscle tenderness. The symptoms are prominent for 4 to 7 days during septicemic phase, at which time defervescence due to lysis occurs [18,36]. Leptospirosis can be isolated from the blood and CSF during this phase. The immune phase of anicteric leptospirosis is preceded by a one to three day asymptomatic period. The onset of the immune phase coincides with the appearance of IgM antibodies. Fever, headache and vomiting are less severe at the onset of the immune phase than during the septicemic phase. The duration of the immune stage ranges from 4 to 30 days and the leptospires are cleared from the blood and the CSF after the first days of the phase, where leptospiruria develops and persists for 1 to 3 weeks.

Aseptic meningitis is the hallmark of the immune phase where mild pleocytosis present with or without meningeal signs and symptoms [11,17]. The CSF cell count is $<500/mm^3$ in most cases. Polymorphonuclear cells may predominate early in the illness, but mononuclear cells predominate later. The protein levels in the CSF ranges from <40mg/dl (normal) to 300 mg/dl and the CSF glucose concentration is generally normal. Uvetitis, iritis, iridocyclitis and chorioretinitis may also appear during the immune phase [36].

Icteric leptospirosis or Weil's syndrome is a form of disease characterized by symptoms of hepatic, renal and vascular dysfunction. The clinical manifestations vary in terms of severity and symptomatology. Some of the patients with jaundice may have no renal manifestation. Any serotype of *L.*

interrogans may cause icteric leptospirosis. During the leptospiraemic phase of icteric leptospirosis, the symptoms do not suggest leptospirosis until the third and seventh day of illness, when jaundice and azotaemia develops. The biphasic course of the disease is obscured by severe and persistent fever, jaundice and azotaemia. Jaundice appears, but there is no evidence of hepatocellular destruction. Hepatic dysfunction occurs, bit it resolves and it is rarely the cause of death.

Microbiology

Leptospirosis has diverse clinical manifestations that resemble many other tropical infectious diseases such as dengue fever, malaria, and scrub typhus which are prevalent in the region. Though a large number of fever of unknown origin are reported to the health facilities, investigation for leptospirosis is not carried out partly due to poor knowledge of clinical manifestations of the disease or lack of proper laboratory diagnostic facilities. Thus a large number of leptospirosis cases are reported without laboratory confirmation which directly affects the estimated disease burden in the region. Laboratory diagnosis of leptospirosis involves two groups of tests. One group is designed to detect anti-leptospiral antibodies, while the other group is to detect leptospires, leptospiral antigens, or leptospiral nucleic acid in body fluids or tissues [7]. Culture using EMJH semisolid medium and microscopic agglutination test (MAT) are the gold standard methods for its laboratory diagnosis. However, these methods are laborious for the routine use.

The MAT is the most widely used diagnostic serological test. Although MAT detects serogroup-specific antibodies, it appeared to be of little value for predicting infecting serogroup (serovar) of patients [8,37,38]. MAT requires paired sera for definitive diagnosis of leptospirosis. Seroconvension or at least fourfold increase in the titer must be observed between acute and convalescent serum samples. Anti-leptospiral antibodies detected by MAT are present for months to years after infection. Thus, it is difficult to confirm acute infection from a single serum sample. In endemic areas, a high titer of 400 or more in a symptomatic patient is generally accepted as a criterion for disease confirmation [7]. Furthermore, MAT requires maintenance of a panel of *Leptospira* cultures prevalent in a particular geographical area, and appropriate quality control must be employed [18].

Several whole *Leptospira* cell-based rapid screening tests for antibody detection in acute infection have been developed, including enzyme linked immunosorbent assay (ELISA), latex agglutination test, lateral flow assay, and IgM dipstick [7,23,39,40]. These assays have been used as alternatives to MAT but have low sensitivity especially during the acute phase [41,42,43,44]. Furthermore, the diagnostic accuracies of these techniques are poor in some areas where leptospirosis is endemic [45,46].

PCR is demonstrably useful for early diagnosis of leptospirosis before its antibody production has commenced. PCR protocols for detection of leptospiral DNA in clinical materials have been developed [47]. Conventional or real time PCR assays targeting a range of genes, such as 16SrRNA, 23SrRNA, LipL32, LipL21, RpoB, GyrB, OmpL1, LigA and B, and flagellin, have been described [17,48,49,50,51]. However, PCR may not be widely applied in resource-poor countries due to its high operational cost [52]. Thus, diagnostic methods that not only have higher sensitivity and accuracy for early-phase leptospirosis but also are applicable widely in resource-poor countries remain to be developed.

Biochemistry

The serum bilirubin level is usually <20 mg/dl but can be as high as 60 mg/dl to 80 mg/dl. Hypoprothrombinemia occurs in a minority of patients and responds to the administration of vitamin K. Serum transaminase levels are mildly elevated rarely exceeding 100 U/L to 200 U/L. serum bilirubin levels peak within seven days and the increase persists for a few days to several weeks. Renal involvement is common in both icteric and anicteric leptospirosis, but symptoms are present only in the patients with icteric diseases. Azotemia, oliguria and anuria commonly occur during the second week of illness, but may appear as early as 3 to 4 days after onset. Blood urea nitrogen levels are below 100mg/dL in most cases, but may occasionally exceed to 300mg/dL. Serum creatinine levels are usually 2mg/dL to 8mg/dL although they may reach 18mg/dL. Results of urinalysis are abnormal in 70 to 80% of cases; proteinuria, hyaline or granular casts, hematuria and pyuria are typical findings. The onset of anuria is a poor prognostic sign and dieresis usually signals resolution [53,54].

Hypotension due to vascular collapse occurs only in patients with icteric leptospirosis, hemorrhage occurs in severe cases, congestive heart failure occurs rarely but nonspecific ECG changes are observed in most patients. Changes in sensorium may occur. Other laboratory abnormalities include anemia, thrombocytopenia, leucocytosis with neutrophilia and an increase in the level of creatinine phosphokinase [4,55].

Radiology

Respiratory manifestations have been reported in 20 – 70% of patients but such features for the disease are often overshadowed by other more serious expressions [56]. Chest radiographic abnormalities have been observed in 11 – 64 % of these patients [56,57]. Reports of chest radiographic findings in leptospirosis patients in Asian countries are few [6]. Pulmonary hemorrhage is usually mild in the course of disease, with spontaneous resolution and no permanent damage to the lungs. Hemoptysis due to hemorrhagic pneumonitis is occasionally an early and prominent clinical feature and may present the clinician with an unusual diagnostic problem [58]. Pulmonary and cardiovascular involvements are common where air space nodules detected by chest radiograph indicate severe leptospirosis [59]. Patients with leptospirosis may present with predominant pulmonary symptoms, ranging from cough, chest pain, breathlessness and mild to severe hemoptysis to acute respiratory distress syndrome (ARDS).

The pulmonary symptoms usually appear between fourth and sixth day of illness. The evolution of the disease may be very rapid and may result in death in less than 72 hours [60]. There are various patterns of abnormalities evident on chest X-rays. These are a combination of pulmonary and cardiac abnormalities. Cardiomegaly and congestive heart failure highlight a cardiac cause of radiographic abnormalities [59]. However, pulmonary hemorrhage and ARDS are two of the most fatal conditions in leptospirosis

Challenges

Hepatobiliary challenges

Liver involvement is seen as centrilobular necrosis with proliferation of Kupffer cells. Jaundice may occur as a result of hepatocellular dysfunction. Patients progress to the severe form of the disease which is also known as Weil's disease [61,62]. This comprises jaundice due to hepatocellular dysfunction rather than hepatic necrosis and multi-organ involvement. Diagnosing hepatobiliary disease in small animals can be challenging. Due to the dual blood supply of the organ (systemic and portal) it is susceptible to insult from systemic disorders as well as primary

organ disease making clinical signs of liver disease often very non-specific (inappetance, lethargy, weakness, vomiting). More specific hepatobiliary signs often occur with severe or end-stage disease such as icterus, hypoglycemia, bleeding tendencies, hepatic encephalopathy and ascites [63].

It is important to note on physical exam size of liver, jaundice, abdominal pain, fluid wave, fundic abnormalities (chorioretinitis) and pyrexia. Clinicopathologic evaluation can help to identify hepatobiliary disease as well as other organ systems that are affected. Complete blood count can reveal microcytosis without anemia. Anemia can develop due to a coagulopathy, anemia of chronic disease or bleeding gastric ulcers. Thrombocytopenia can develop due to decreased hepatic thrombopoietin production [64]. Hepatic biopsy is considered if there are persistent serial increases in liver enzymes, abnormal hepatic function tests, hepatomegaly of unknown cause, ultrasonographic abnormalities of the hepatic parenchyma and to evaluate for breed specific hepatopathies.

The conditions like cholangitis to cholangiohepatitis because the primary inflammation in felines surrounds the bile ducts. The four categories are most commonly neutrophilic cholangitis – acute and chronic form, lymphocytic cholangitis, cholangitis associated with liver flukes and lymphocytic portal hepatitis. The acute neutrophilic form is seen mostly in young to middle age male cats, causes vomiting, diarrhea, anorexia and lethargy with fever, dehydration, icterus and abdominal pain with or without hepatomegaly.

Pulmonary challenges

Sudden remission of fever and development of hepato-renal-pulmonary involvement due to capillary vasculitis was first described as Leptospirosis by Weil in 1886. Leptospirosis, an emerging zoonosis, is usually transmitted to humans by contact with soil or water contaminated with urine of rat. Usually it presents as flu like illness with mild hepatic and renal impairment. Acute/septicemic phase for one week followed by immune phase for another one week characterizes the biphasic pattern of the illness. The immune phase is marked by production of antibodies and excretion of leptospires in urine. The characteristic biphasic illness may not be found in all patients, with only a fulminant monophasic illness being a predominating clinical course in few. These patients present with an acute undifferentiated illness which rapidly progresses to refractory shock, jaundice, renal failure and massive pulmonary haemorrhage.

Diagnosis is made on the basis of epidemiological, clinical and laboratory features. Since, leptospirosis has protean manifestations; it is frequently misdiagnosed even in areas of high prevalence. In patients presenting with less common forms of leptospirosis, the diagnosis is frequently either not considered or only discovered at autopsy. A delay in diagnosis leads to progression of disease and development of its complications. Since then a number of studies have identified the association of leptospirosis with lung. Pulmonary involvement usually occurs in immune phase and the overt pulmonary manifestations occur in 20-70% of patients, most of which resolve without any sequelae [65,66,67,68,69,70,71].

Ocular challenges

Ocular manifestations are noted in the second phase of illness, but these remain under-diagnosed mainly because of the prolonged symptom-free period that separates the systemic manifestations from detection of ocular manifestations. Varying ophthalmic presentations and the intrinsic nature of different types of uveitis to mimic one another also challenge the accuracy of the diagnosis. Of the individual ocular signs, the combination of acute, non-granulomatous, panuveitis, hypopyon, vasculitis, optic disc edema, membranous vitreous opacities and absence of choroiditis or retinitis have high predictive value for the clinical diagnosis of leptospiral uveitis. Geographic location of the patient, occupation, socio-economic status, risk factors related to exposure, past history of fever or jaundice also aid in diagnosis. Steroids are the mainstay of treatment for leptospiral uveitis. Depending upon the severity and anatomical location of inflammatory lesion, topical, peri-ocular and/or systemic steroids are given. The prognosis is generally good, even when the inflammation is severe [72].

Cataract is a well-recognized complication of uveitis and the steroid treatment of uveitis [73,74,75,76]. Once the inflammation is controlled, a majority of uveitic cataracts remain stable or progress only slightly. In a previous study 14% of patients with sero-positive leptospiral uveitis developed cataract, of them 76% of the patients had visually significant cataract on their first visit even before the steroid treatment [77]. The spontaneous absorption of opacified lens material is rare except in patients with traumatic cataract, congenital rubella and in age related leaking Morganian cataract [78,79,80]. Holloway and Gowen reported a case of spontaneous absorption of cataract. The patient also had vitreous veils and vitreous opacities. However etiological diagnosis of uveitis was not known [81].

Progression of cataract in leptospirosis was rapid in these young patients and the lens material was found absorbed in 2.5% [77] of patients with leptospirosis. There is a single case report from the United States of a missing lens nucleus in a 31-year-old patient who suffered from leptospiral uveitis [82]. Horses are well recognized to develop severe uveitis associated with rapid cataract formation in the setting of equine recurrent leptospiral uveitis. Laboratory studies have demonstrated the presence of anti- *Leptospira* serum antibodies in these horses that react with lens antigens, suggesting the presence of an antigenic relationship between the leptospires and lens antigens [83], the mechanism of cataract formation in human is not known. The leptospiral uveitis usually responds promptly to treatment and cataract removal and intra-ocular lens implantation result in complete recovery of vision [77].

Diagnostic challenges

To reduce leptospirosis risk, awareness of the disease, understanding of limitations of rapid diagnostic tests and more regional laboratory capacity are needed. Diagnosis is usually performed by serology; enzyme-linked immunosorbent assay and the microscopic agglutination tests are the laboratory methods generally used, rapid tests are also available. Limitation of serology is that antibodies are lacking at the acute phase of the disease. In recent years, several real-time polymerase chain reaction assays have been described [84]. These can confirm the diagnosis in the early phase of the disease prior to antibody titers are at detectable levels, but molecular testing is not available in restricted resources areas. There is need to investigate the patients in the microbiology laboratory to confirm the clinical suspicion of leptospirosis in order to institute treatment early and effectively. Culture is technically demanding in leptospirosis, and the sensitivity reported is quite low as compared to PCR and serology [85].

Research Gaps

The urgent need of implementing the following procedures are very important for filling the gap of leptospiral research.

• Review and appraise the revised systematic epidemiological review for mortality, morbidity and disability of human leptospirosis,

- Review draft transmission disease model for leptospirosis and provide technical input for the further development and refinement of the model,
- Understand the preliminary burden estimates,
- Identify present knowledge and research gaps and
- Inform WHO on next steps for human leptospirosis burden estimation of the particular area and their translation into policy.

In recent years, considerable attention has been devoted to this infection but efforts to control and eliminate it, especially from natural foci, are hindered by gaps in our knowledge. The major research gaps are

❖The usage of proper epidemiological tools for appropriate surveillance
❖Suspicion of leptospirosis in all PUO cases by the clinicians
❖Standardizing the laboratory oriented diagnostic tool for the early diagnosis
❖Standardizing the molecular techniques for the appropriate diagnosis
❖Prompt antibiotic usage with correct dosage
❖Counseling the patients for the completion of the course
❖Develop vaccines for transmission control
❖Provide awareness about the reservoirs to the human population
❖Sensitizing the need of more regional reference centres

Prophylactic measures
❖There is no existing human vaccine helpful against leptospirosis.
❖For people who may be at high risk for short periods (eg occupational risk, high-risk water sports activities in known endemic areas or living or working in areas after natural disasters), taking doxycycline (200 mg weekly) may be effective.
❖Immunization of animals with *Leptospira* vaccines: an animal vaccine is available and immunizing and treating infected animals is worthwhile.
❖Reduce rodent populations, eg by clearing rubbish and preventing rodent access into buildings.
❖The risk of infection can be greatly reduced by not swimming or wading in water that might be contaminated with animal urine.
❖If there is contact with fresh, surface waters, eg canals, ponds or rivers, or with rats, then advise the person to:
➢Cover cuts, scratches or sores with a waterproof plaster and thoroughly clean any cuts or abrasions caused during the water activity.
➢Wear appropriate protective clothing, gloves or protective footwear.
➢Wash or shower promptly after water sports.
➢Avoid capsize drill or rolling in stagnant or slow-moving water.
➢Wear thick gloves when handling rats.
➢Wash hands after handling any animal, and before eating.

Conclusion and Recommendations
Leptospirosis is an emerging zoonotic disease of public health importance in countries of the WHO's South-East Asia Region. In some Member States, the disease has been endemic for many decades and causes sporadic outbreaks. The disease epidemiology is tightly linked to regional climatic factors and major occupational sectors such as agricultural and livestock workers. Interventions need to give special attention to at-risk geographic areas with a high case fatality rate and to individuals of particular socio-demographic characteristics (e.g., men and agricultural workers). Further research needs to be carried out

concerning more pathophysiological information of the disease to prevent leptospirosis deaths that could be prevented with proper and timely treatment after an early and accurate diagnosis of the disease. However, adequate laboratory tests for early diagnosis are still lacking [18,40].

Diagnostic methods that not only have higher sensitivity and accuracy for early phase leptospirosis but also are applicable widely in resource poor countries need urgently to be developed. The existence of large numbers of reservoir animals and route of disease transmission make activities in prevention and control of leptospirosis. The quality of data on which the control and prevention of leptospirosis in the region is based will hinge upon a periodic assessment of the efficacy with which the sentinel surveillance system captures, analyzes and disseminates information. Data quality along with accurate results from laboratory investigations will determine the true disease burden.

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