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## Leptospira: An emerging zoonotic pathogen of climate change, global warming and unplanned urbanization: A review

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### Abstract

Leptospirosis is a worldwide zoonotic infection with a much greater incidence in tropical regions and has now been identified as one of the emerging infectious diseases. The epidemiology of leptospirosis has been modified by changes in animal husbandry, climate and human behavior. Resurgent interest in leptospirosis has resulted from large outbreaks that have received significant publicity. The transmission cycle of leptospirosis involves maintenance hosts, carrier hosts, environment and human beings. The carrier animals in India include rats, pigs, cattle, bandicoots and dogs. Intervention strategies include vaccination, rodent control and improved environmental hygiene to eliminate possibility of water, soil and food contamination. Mass awareness or public education is of utmost importance. The development of simpler, rapid assays for diagnosis has been based largely on the recognition that early initiation of antibiotic therapy is important in acute disease but also on the need for assays which can be used more widely. The gold standard is microscopic agglutination test though serological studies are widely conducted for the diagnostic purpose. The antibiotics use for the treatment of leptospirosis includes oral doxycycline, azithromycin and penicillins. Among the parenteral therapy Intravenous penicillin G and third generation cephalosporins are used. We reviewed the clinical presentation, diagnosis, treatment and prevention of leptospirosis in this study.

**Keywords:** Leptospirosis, serovar, climate change, emerging zoonosis, environmental hygiene

### Introduction

Leptospirosis is a zoonotic, waterborne, neglected, reemerging bacterial disease of public health importance. Due to rapid, unplanned urbanization and poor sanitation, leptospirosis emerges as a leading cause of acute febrile illness in many of the developing countries. With rapid global urbanization about 68 percent of the world's human population projected to be living in urban areas by the year 2050<sup>[53]</sup> (55 percent as of 2018). Wild rats seem to benefit from urbanization and thrive in urban and domestic environments, leading to frequent human exposure to these species<sup>[4]</sup>. Leptospirosis is rat-associated zoonotic disease. *R. norvegicus* and *R. rattus* have become ubiquitous in urban environments and are significant sources of many zoonotic pathogens that can result in mortality and morbidity in humans and animals. Because of knowledge gaps in the ecology of rats, rat control is largely ineffective. India with an 8,129 km long coastline and with endowment of plenty of natural resources has one of the major important coastal, agro-ecosystem that supports livelihood of several million people thus outbreaks of leptospirosis had been reported from tropical and coastal districts of Gujarat, Maharashtra, Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, Andamans, Goa and Orissa. It is estimated that annual rates of infection vary from 0.02 per 100,000 in temperate climates to 10 to 100 per 100,000 in tropical climates, but it may occur anywhere and generally outbreaks may occur in slums of the developing world. An estimated 1.03 million cases of human leptospirosis occur worldwide, resulting in 2.9 million disability adjusted life years lost<sup>[8]</sup>. Leptospirosis is also known as Dairy worker fever, Autumnal fever, Cane cutter's disease, Canicola fever, Harvesting fever, Infectious jaundice, Lepro, Mud fever, Rice field worker's disease, Field fever, Eye cather's yellows disease, Seven day fever, Swamp fever, Walter fever, Nanukayami fever, Icterohemorrhagic fever, Swineherd's disease, Hemorrhagic jaundice, Stuttgart disease, Weil's disease, Flabby bag (sudden loss of all milk with flaccid

udder), Milk drop syndrome, Fort Bragg fever in U.S., Andaman haemorrhagic fever

### Historical aspect

Leptospirosis first described by physician Adolf Weil in 1886 in Germany when he described an “acute infectious disease with enlargement of spleen, jaundice and nephritis” [49]. The etiology of leptospirosis was demonstrated independently in 1915 in Japan. Inada and Ido detected both spirochetes and specific antibodies in the blood of Japanese miners with infectious jaundice thus Inada and Ito first identified *Leptospira* as the causative organism in 1908 [18, 19] later on Uhlenhuth and Fromme [52] detected spirochetes in the blood of guinea pigs inoculated with the blood of infected soldiers. During World War I, outbreaks confirmed in French, German and British troops as favorable conditions conferred by trench warfare including standing water and rat infestation. Leptospirosis outbreaks during World War II were linked to troops bathing in rivers and streams. *Leptospira icterohaemorrhagiae* was identified as the causative agent in pre- World War II outbreak in Japan [7].

### Etiology

*Leptospira* (Greek ‘leptos’=fine and Latin ‘spira’=coil) are ubiquitous spirochetes. Leptospirosis is caused by *Leptospira interrogans* (Group 1-Spirochaets). On the basis of pathogenicity genus *Leptospira* is divided into following two species: (1) *L. interrogans* consisting of pathogenic strains; (2) *L. biflexa* consisting of non-pathogenic saprophyte strains. But, the currently used genetically based classification indicates that there are at least 19 species (13 pathogenic and six saprophytic, identified through DNA hybridization analysis [3, 5]. Among them seven of these species (*L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. weilli*, *L. kirschneri* and *L. alexanderi*) are the main agents of leptospirosis [1]. These species again divided into different serovars (based on difference in agglutination reaction because of the surface lipopolysaccharide) of leptospira. There are approximately more than 225 serovars for *L. interrogans* and more than 60 serovars for *L. biflexa* [31]. The leptospira serovars predominantly present in India are *L. andamana*, *L. pomana*, *L. grippotyphosa*, *L. hebdomadis*, *L. semoranga*, *L. javanica*, *L. autumnalis*, *L. canicola*. Leptospira are thin, flexible, gram negative, motile (with cork-screw like rotating axial filament), helicoidal (spiral) rods (0.1x6-20µ) with one or both hooked end(s) give it distinctive question-mark shape. Leptospira is gram negative but it have surface structures that share features of both gram positive and gram negative bacteria. The double-membrane and the presence of LPS are characteristic of Gram-negative bacteria but have lower endotoxic activity [45, 46], while the close association of the cytoplasmic membrane with murein cell wall is reminiscent of Gram-positive envelope architecture [10, 17, 54].

### Epidemiology of disease

#### Host range

**1. Reservoir host:** Leptospirosis has a very wide range of natural rodent and non-rodent reservoir hosts which include foxes, rabbits, rat, mice, moles etc. Among them the brown rat (*Rattus norvegicus*) and house mouse (*Mus Musculus*) is the most important source of human infections as they usually remain carrier throughout their

life (permanent carrier). Rodents are therefore considered as the major reservoir of infection. Leptospire have been recovered from amphibians and poikilothermic animals such as frogs and toads [2, 12, 16].

- 2. Carrier hosts:** Domestic animals carry the microorganisms and therefore act as carrier host for several months (temporary carrier) which includes cattle, sheep, goat, buffalo, horse and dogs.
- 3. Accidental/ Incidental host:** Human act as accidental host of disease.

### Mode of transmission

#### Leptospirosis is transmitted by the following way

- Direct contact with an infected animal. Individuals with occupational risk for direct contact with potentially infected animals include: veterinarians, abattoir workers, farm workers (particularly in dairy milking situations), hunters, trappers, animal shelter workers, scientists and technologists handling animals in laboratories or during fieldwork, where there is presence of water lodging conditions [20]. No human to human transmission is reported [55].
- Indirect contact via soil, water and food contaminated with urine from an infected animal. It is much more common and can be associated with occupational, recreational or avocational activities. The disease has also been associated with swimming, wading and rafting in contaminated lakes and rivers.
- Transplacental transmission may occur if infection occurs during pregnancy.
- By infected semen during sexual intercourse.
- Droplet infection – breathing air polluted with droplets of urine, eg. During milking of cattle and buffalo.

### Factors favours emergence of Leptospirosis

The conditions that are favorable for maintenance and the transmission of the leptospirosis are as follows –

- Reservoir and carrier host - Together the rodents and the cattle excrete large number of organisms in their urine and thus are responsible for the contamination of soil as well large and small water bodies.
- Drainage congestion and water logging - Heavy concentrated rainfall leaves a lot of surplus water. Developmental activities like canal network, roads and railway lines obstruct natural drainage of rain water causing its accumulation for longer periods. The water logged areas force the rodent population to abandon their burrows and contaminate the stagnant water by their urine. The farmers and agricultural labourers working in the water logged contaminated fields catch the infection.
- Soil salinization - In fact, salinity and water logging are inter-linked problems. The salinity of the soil provides favourable environment for survival of leptospire for months together.
- Soil temperature - The soil of endemic areas in general has lower base saturation and the mean annual soil temperature at the depth of 50 cm is 22°C or more and the difference between mean summer (June-August) and mean winter (December-February) temperature is less than 50 °C. This favours the survival of leptospire for long durations.
- Urbanization - Incidence of leptospirosis infection among urban children appears to be increasing [28].

6. Climate change - 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 [29]. Environment and climatic factors also influence the survival of organism in environment and thus its subsequent transmission. These factors include rainfall, humidity, ambient temperature, water retaining capacity of the soil, pH and salinity of the soil and surface waters and forest cover [42, 43].
7. Gender - Incidence of leptospirosis is much higher in males than females [12, 15, 23]. However, 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 [32, 44].
8. Age - *Leptospira* infections occur more frequently in persons 20-30 years of age group. Leptospirosis rarely occurs in young children and infants, possibly, because of minimal exposure.
9. Seasonal variation - Leptospirosis is usually a seasonal disease that starts at the onset of the rainy season and declines as the rains recede. Sporadic cases may occur throughout the year. More than 97% of the 2,934 reported leptospirosis cases occurred during the harvest season between August and October, and most commonly affected farmers [9] (83%). Tropical climate and high temperatures from March to May, with rainy and humid season between mid-June and November and the annual rainfall is around 3188 mm favours the transmission of *Leptospira* [34].
10. High risk group - Agricultural workers such as rice field planters, sugar cane and pineapple field harvestors, livestock handlers, labourers engaged in canal cleaning operations are subjected to exposure with leptospires which have reservoir in rodents, cattle, swine, sheep, goats etc. Some occupational groups are – Fishermen, sewer workers and all those persons who are liable to work in rodent infested environment. Lorry drivers and masons - As lorry drivers may use contaminated water to wash their vehicles and masons may come in contact with the organisms while preparing the cement and sand mixture for construction work with contaminated water.
11. Rodent density - The overall prevalence of *Leptospira* spp. in our rodent sample was 26.7%, a finding in accordance with former studies [6, 50]. No correlation was shown between sex and prevalence but age had a major impact on prevalence, adult animals being much more frequently infected (33.6%) than juveniles (17%), as already described in other locations [45, 46]. Mice were more frequently infected than rats.
12. Population size of the farm and other domestic animals
13. Sanitation of animal habitats
14. Availability of veterinary services for prompt detection and treatment of animal leptospirosis and control programs
15. Personal hygiene - Personal practices such as bathing in unprotected water bodies; water associated recreational activities, use of protective gear, agricultural and other occupational practices, animal rearing practices, level of hygiene in milking and slaughtering places, etc.

#### Disinfectant property of leptospira

Leptospirosis cannot survive dry environment, heat, acids and

basic disinfectants but can sustain alkali pH up to pH 7.8 [21]. Leptospirosis are very intolerant of acid – a solution of acetic acid (vinegar) or citric acid (lemon juice) will work as a disinfectant, for example to wash hands on a camping trip. Alcohol is also effective but requires a greater concentration. Swimming pools and some water features will use chlorination tablets. For disinfecting, a dilute bleach solution (1:1 solution of water with 10% bleach) effectively kills leptospires and can be used for these areas. Quaternary ammonium solutions, alcohol, 1 percent sodium hypochlorite, 70 percent ethanol, glutaraldehyde, formaldehyde, detergents and acid are also among the disinfectants that can also be used for equipment, runs/cages, floors etc [35]. *Leptospira* species is sensitive to moist heat (121°C for a minimum of 15 min) and is also killed by pasteurization.

**Global distribution of leptospirosis:** Leptospirosis are reported from all the continent except Antarctica. Recent information on worldwide human leptospirosis differs but shows that approximately 1 million severe cases occur per year, with a mean case-fatality rate of nearly 10 per cent. The majority of cases occur in men. Estimated 100-200 cases are recognized yearly in the United States, with about 50% of cases in Hawaii, Indonesia as a nation with high leptospirosis cases, third rank of death rate in the world. Indonesia Ministry of Health gave details that there were 641 human cases in 2013 with casefatality was 9.36%. Outbreak of leptospirosis is not well understood. Epidemic may effect from exposure to flood waters contaminated by urine from infected animal, as has been made clear from several countries. Recreational disclosure and domestic animal contact are well-known sources of leptospirosis. Occupational exposure may be accounts for leptospirosis [41].

**Distribution of leptospira in India:** According to a NICD report (1997), prevalence rate of leptospirosis is high in Andaman, Bengal, Gujarat, Karnataka, Andhra Pradesh, Tamil Nadu and Kerala. The incidence rate of leptospirosis in the Andaman Islands was estimated to be between 50-65 cases/100,000 per year, which is believed to be the highest in India [43]. Prevalence studies being carried out by Indian Veterinary Research Institute during the last 35 years have shown an overall prevalence of 10.1% during 1975-90, with highest in sheep (20.6%). The common serovars identified in animals during the period were *Leptospira Pomona*, *Patoc*, *Australis*, *Grippotyposa*, *Hardjo* and *Autumnalis*. During 1991-2000 the overall sero-positivity marginally increased to 13.4% which was due to an increase in cases of leptospirosis in cattle (15.8%) and dogs (19.1%) and the common serovars were *RGA*, *Patoc*, *Australis*, *Autumnalis*, *Grippotyposa*, *Pyrogens*, *Ballum*, *Hardjo*, *Pomona* and *Canicola*. During the next 5 years (2000-2005) the disease status has remained at an approximated 12 percent. Based on the prevalence studies and distribution of leptospira in animals in different regions, the states have been classified as high prevalence, moderate prevalence and rarely reporting states [56]. Leptospirosis can occur in both urban and rural areas. In urban areas of developing countries, a contaminated environment due to various factors such as overcrowding, inadequate drainage and sanitation facilities for man and animals, presence of stray animals, domestic rats, bandicoots, unhygienic slaughter houses and people walking bare foot contribute to the spread of the illness [47]. Persons of all ages and races are susceptible. In India, urban leptospirosis has been reported from Chennai

and Mumbai while rural leptospirosis has been reported from Gujarat, Kerala and Andamans. Non-reporting of leptospirosis from other states of India does not mean that it is absent in

those parts [47]. Indian scenario leptospirosis is reported to be of endemic importance from many States of India.

**Table 1** - Distribution of Leptospira serovar in animal in different regions

State	Animal species	Serovar
<b>High prevalence</b>		
Tamil Nadu, Kerala, Andaman	cattle, buffalo, sheep, goats, pig	pyrogenes, pomona, australis, utumnalis, hebdomadis, hardjo, icterohaemorrhagiae
<b>Moderate Prevalence</b>		
Maharashtra, U.P., M.P., Gujarat, Karnataka	cattle, buffalo, goats, sheep, pigs, dogs, horse	pomona, hardjo, canicola, javanica, icterohaemorrhagiae, pyrogenes
<b>Rarely Reported</b>		
Punjab, J&K, Rajasthan, North Eastern Hills, Himachal Pradesh	cattle, sheep	icterohaemorrhagiae, pyrogenes, canicola

**Andaman and Nicobar Islands:** Andaman and Nicobar Islands are endemic for leptospirosis. Outbreaks of Andaman Hemorrhagic fever were reported since 1988, 524 cases of AHF (leptospirosis) were reported from 1988-97 [42, 43, 47, 48]. This was proved to be leptospirosis in 1994.

**Gujarat:** The disease is endemic in south Gujarat since 1994. The endemic districts are Valsad, Navsari and Surat [38, 47].

**Maharashtra:** Leptospirosis has been reported repeatedly since 1998. 2355 cases and 167 deaths were reported in 2005, mainly due to large outbreak during the post monsoon floods. The number of districts in Maharashtra reporting leptospirosis has increased from two in 1998 to ten districts in 2005 [22, 47].

**Kerala:** Leptospirosis is endemic in various parts of Kerala. Kolenchery is in the midlands of Kerala. In this area leptospirosis was rarely diagnosed before 1987 [27, 47].

**Tamilnadu:** Leptospirosis has been reported from Chennai since 1980's. The leptospirosis laboratory at Institute of Microbiology, Madras Medical College was established in 1994 [39, 40, 47].

**Puducherry:** In a study of 33 icteric patients from Puducherry, 22 had altered sensorium and 20 had multi-organ failure and thrombocytopenia and 13 patients died (39.3%) [10, 47].

**Karnataka:** Leptospirosis outbreaks have been reported from 15 districts of Karnataka. The highest incidence of cases have occurred in Bangalore city, Udupi, dakshina kannada, Uttara kannada, Shimoga, Bidar and Gulbarga district. During the year 2004, 152 cases and 11 deaths were reported and during 2005, 224 cases and 19 deaths were reported.

**Odisha:** After the cyclone during the October-November

1999, 142 patients with febrile illness and hemorrhagic manifestations were evaluated. 28 (19.2%) had evidence of leptospirosis which was confirmed by Microscopic Agglutination Test [47].

**Seropositivity rate of leptospira in India (2000-2001) – Overall seroprevalence in India was 0 to 46.8%**

**Table 2** Seropositivity rate of leptospira in different regions of India (2000-2001)

South India	25.6%
North India	8.3%
West India	3.5%
East India	3.1%
Central India	3.3%

Other States Data from Andhra Pradesh, Uttar Pradesh, West Bengal and Delhi were analysed. Evaluation of acute febrile patients in Uttar Pradesh revealed that 7% had leptospirosis (25/ 346). 17 of the 25 patients had jaundice. In a study of 55 cases of leptospirosis in Hyderabad, 52% had renal failure and jaundice occurred in 42%. Out of 42 persons with jaundice who were evaluated in Calcutta, 10 (23.8%) were found positive for leptospirosis. 75 patients from Delhi with symptoms of leptospirosis were evaluated, 32 were found positive for leptospirosis and 5 died. 180 febrile patients from urban slums of Delhi were evaluated and 27 (15%) were positive for leptospirosis [47].

**Diagnosis:** World Health Organization (WHO) has introduced "Faine's criteria" for diagnosis of leptospirosis, based on clinical history (Part A) and epidemiological history (Part B) supported by laboratory parameters [14] (Part C). Faine's criteria has since been amended to increase the sensitivity of diagnosis [25]. Modified Faine's criteria (with amendment) was introduced in 2012.

**Table 3** - Faine's criteria for diagnosis of leptospirosis, based on clinical history (Part A) and epidemiological history (Part B) supported by laboratory parameters (Part C).

Part A - Clinical data	Part B - Epidemiological factor	Part C - Bacteriological and laboratory finding
Headache (2)	Rainfall	Isolation of leptospira in culture – diagnosis certain PCR
Fever (2)	Contact with contaminated environment	ELISA IgM positive or SAT positive or MAT- Single positive in high titer
Temperature <39°C (2)	Animal contact	MAT- rising titre
Conjunctival suffusion (4)		Other rapid tests
Meningism (4)		
Myalgia (4)		
Conjunctival suffusion + meningism + myalgia (10)		
Jaundice (1)		

Albuminuria (2)		
Hemoptysis/dyspnea (2)		
A score of 26 or more in parts A+B or a score 25 or more in parts A+B+C is considered diagnostic of leptospirosis, OR - Odds ratio, PCR - polymerase chain reaction, IgM - Immunoglobulin M, MAT - Microscopic Agglutination Test, SAT - Slide agglutination test		

## Laboratory diagnosis

### Specimen collection

Blood samples were collected in non-additive or gel separator tubes for chemistry and serology and EDTA tube for blood count. For blood culture, blood is inoculated directly into blood culture bottles including culture medium for leptospires.

### Microscopic demonstration

- Dark field microscopy - Approximately  $10^7$  leptospires/L is required to visualize leptospira. Through examination of blood and urine has both low sensitivity and specificity, it is issue to misunderstanding of fibrin or protein threads. Leptospires are not stained by conventional gram staining.
- Immunofluorescence staining
- Immunoperoxidase
- Silver staining
- Warthin-Starry staining
- Immunohistochemistry
- in situ hybridization

Samples for culture should be collected earlier to the administration of antibiotics. Blood, cerebrospinal fluid and

dialysate should be cultured in the first 7 days of the illness (as leptospires disappear from blood after 8 days) and mid-stream urine from the second week of the illness (leptospires present in urine in the second week of disease and intermittently thereafter upto 6 weeks) but survival of leptospires in human urine is restricted so urine should be processed instantly. To visualize the organism from urine samples after 20 minute neutralize the urine sample by 1/10 NaOH or buffered it by using 1 per cent bovine serum albumin and to visualize the morphology add 1.5 ml of 10% formalin (for killing of live microorganism) in 20 ml of urine.

### Isolation of Leptospira

Leptospira are aerobic and microaerophilic. Optimum temperature for their growth is 28-32 °C and optimum pH 7.2-7.5. Culture should be observed weekly by dark field microscopy for up to 13 weeks prior to being discarded [33]. Vitamins B1 and B12, and long chain fatty acids, are the only organic compounds are required for their growth. The fatty acids are the sole source of carbon and are utilized by  $\beta$ -oxidation [11]. Addition of 5-fluorouracil and antibiotics such as nulidixic acid or rifampicin makes the medium inhibitory for common laboratory contaminants [11].

**Table4** Culture medium used for isolation of Leptospira spp.

Nature of the media	Serum enriched	Serum replaced by albumin and twin	Chemically defined medium
Liquid	Korthof's	Ellinghausen- McCullough-Johnson-Harris (EMJH) medium - contains oleic acid, bovine serum albumin and polysorbate EMJH basal medium, EMJH enrichment medium (10% enrichment), EMJH selective medium (5 fluorouracil is added 200-400 mg/ml), PLM-5, Leptospira-5x	Shenberg's, Vogel and Hunter
	Stuart's		
	Vervoort's		
Semisolid	Fletcher's		
	Noguchi's		
Solid	Cox's		
	Korthof's		

Growth is detected usually after 6-14 days of incubation. The generation time of leptospires in laboratory media is 12-16 hours and 4-8 hours in inoculated animals. Growth is readily initiated in these media and usually is easily visualized as one or more rings of dense growth several mm to cm below the surface of the medium, although a lack of rings of growth does not necessarily mean an absence of leptospires.

### Serological diagnosis

1. The MAT (Micro Agglutination test) – It is considered as the gold standard test in diagnosing leptospirosis according to CDC. MAT test was first explained in 1918 by Martin and Pettit. Live antigens representing diverse serogroups are reacted with serum samples and the agglutination is examined by dark field microscopy. This test is very much specific for particular serovar and sensitivity is also very high.
  - Suspected for leptospirosis – If leptospira agglutination titre >200 and <800
  - Confirmed for leptospirosis – If leptospira agglutination titre >800
2. Macroscopic agglutination test – Killed antigens representing diverse serogroups are reacted with serum

samples and the agglutination is examined by darkfield microscopy. Test is less specific and less sensitive but it is a herd screening test for leptospira.

3. Complement fixation test – Test is very much tedious and complex. Specialized laboratory is needed for this test.
4. ELISA - Antigens of only pathogenic leptospires can react with the monoclonal F9-4 antibodies, can also be used<sup>[51]</sup>. It can detect IgG (chronic infection) and IgM (acute infection) antileptospira antibodies.
5. Latex agglutination test – In this test latex beads are used for adherence of leptospira antigen.
6. Pathogenicity test – Morphologically it is very difficult to differentiate pathogenic and saprophytic leptospira thus conversion to spherical forms in 1M NaCl, growth in presence of 8-azaguanine (225 mg/l) and growth at 13°C are conducted. Saprophytes are able to grow at 13°C and in presence of 8-azaguanine and also are converted to spherical forms in presence of 1M NaCl which the pathogenic bacteria fails to do though *L. interrogans Icterohaemorrhagiae* can grow at 10°C.
7. Sensitized erythrocyte lysis
8. Patoc slide agglutination test
9. Indirect hemagglutination

10. Counterimmunoelectrophoresis
11. Microcapsule agglutination
12. Dot-ELISA
13. I<sub>g</sub>M dipstick

### Molecular diagnosis

1. Polymerase chain reaction (PCR) - Detects DNA in blood in the first 5-10 days after the start of the disease and up to the 15th day by targeting either housekeeping genes, such as *rrs*, *gyrB*, or *secY* or pathogen-specific genes such as *lipL32*, *lig* or *lfbI* [1].
2. Loop mediated isothermal amplification method (LAMP)
3. NASBA (Nucleic acid sequence based amplification test)

### Differential diagnosis

Leptospirosis should be differentiated from Brucellosis, Dengue, Enterovirus infections, Hantavirus pulmonary syndrome, Hepatitis A, Kawasaki disease, Malaria, Measles, Meningitis, Q fever.

### Pathogenesis

The bacteria enter the body through small abrasions, mucosa, conjunctiva and genital tracts. It involves some chemotactic interactions and then transmembrane passage. The bacteria settle down in the convoluted tubules of the kidney and thus keep on shedding the pathogen in urine. The period of shedding varies from a few weeks to many months. After the bacteria reach a higher concentration in blood and tissue, there is tissue damage due to endotoxins secreted by the pathogen. Hemolysin is also secreted by the bacteria and leads to damage of blood cells. Endothelium gets damaged which leads to ischemia and other complications. The exact molecular basis of virulence is yet unknown however the humoral response has been observed to be active in first week of infection leading to phagocytosis by macrophages and neutrophils [31].

### Signs and Symptoms

Symptoms can take 2 to 26 days (average 10 days) to develop and may include:

**In humans:** The disease is characterized by variety of symptoms, including high fever, vomiting, jaundice (yellow skin and eyes), red eyes, headache, chills, muscle aches, abdominal pain, diarrhoea, rash etc. Some infected persons may have no symptoms at all. Illness usually begins abruptly with fever and other symptoms. The symptoms appear after an incubation period of 7–12 days. Leptospirosis may occur in two phases-

- The first phase (acute or septic phase) ends after 3–7 days of illness with the appearance of antibodies against leptospira and the disappearance of all the bacteria from the bloodstream.
- The patient is asymptomatic for 3–4 days until the second phase begins with another episode of fever. The hallmark of the second phase is meningitis (inflammation of the membranes covering the brain) the illness lasts from a few days to 3 weeks or longer. Severe leptospirosis is known as Weil's disease, which is characterized by liver damage, kidney failure and bleeding. The disease affects brain causing meningitis, encephalitis and affects lung as the most serious and life-threatening of all leptospirosis complications.

### Treatment

Antibiotics like amoxicillin, doxycycline, ceftriaxone etc. are used. Fluid therapy can be advised as per the requirement. Persons with symptoms suggestive of leptospirosis should contact a health care provider as soon as possible.

### Prevention and control

The global leptospirosis environmental action network (GLEAN) is a one health approach to reduce the impact that leptospirosis outbreaks have on communities through providing cost-effective, implementable and sustainable solution. Early warning system and improved prevention and control strategies increase the knowledge of the relationship between environmental, biological, ecological, economic, demographic factors. Intervention strategies can target many points in the transmission cycle of leptospirosis:

1. Leptospirosis in domestic animals can be controlled through vaccination with inactivated whole cells or an outer membrane preparation [37].
2. Rodent control preferably through the use of slow acting rodenticides and improved environmental hygiene to eliminate possibility of water, soil and food contamination are some of the measures for diminishing the risk of leptospirosis transmission.
3. Occupational hygiene that includes the use of water proof shoes and gloves is fundamental for preventing human leptospirosis [24].
4. The mass awareness or public education is of utmost importance.
5. Do not allow animals to urinate in or near ponds or pools.
6. Keep animals away from gardens, playgrounds, sandboxes, and other places children may play.
7. Chlorination is seldom useful, as virulent leptospire withstand up to 4 ppm in potable water. Filtered water is not safe thus using boiled or ultraviolet irradiated water is advisable.
8. Water tanks must be sealed if possible. Bathroom and kitchen waste pipes must be blocked at night.
9. Try avoiding walking in flood water. If you have to, wear gum boots.
10. Heavy drinking and smoking predispose damage to the liver and the kidneys which in turn become vulnerable to leptospiral infection.
11. Proper collection, transport, treatment and secured disposal of garbage.

### Conclusion

A climatic condition influences the transmission of disease by altering human exposure, animal host population dynamics and environmental conditions that allow *Leptospira* spp. to persist in the environment. Incidence of leptospirosis especially high amongst farmers may be associated with rice harvest and modulated by prior bacterial accumulation within the soil under moist conditions. Reservoirs control measures, environmental control programs and animal vaccination, in combination with a strong surveillance system may significantly reduce the disease. The extensive and good understanding of the eco-epidemiological and cultural characteristics of a community that faces the problem of leptospirosis is an essential prerequisite for evolving an effective and acceptable control measure.

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