Protect yourself from Leptospirosis

Early reporting to health facility can prevent illness and deaths

Leptospirosis is caused by bacteria which is found in urine of rodents, cattle, pigs etc

Do not bathe, wash face or hands in dirty water

Do not enter or work in dirty water without rubber boots/gloves

If you have walked through dirty/flood water bare foot, thoroughly wash the feet with soap and water

Contact the health facility immediately if you have fever with headache, eye suffusion, calf muscle pain, chest pain

Do not litter food grains and left over food

Keep your surroundings clean and clutter free to avoid rodent infestation

Take medicines as advised by your doctor

Programme for Prevention and Control of Leptospirosis

National Guidelines

Diagnosis, Case Management
Prevention and Control of Leptospirosis
Leptospirosis had long been considered a rare zoonotic disease in India with only sporadic cases being reported. However, since 1980's, the disease is increasingly being reported from some coastal states during monsoon months in mini-epidemic proportions.

Considering the rising burden of the diseases, a Pilot Project on prevention and control of Leptospirosis was approved as a New Initiative under XI Five year plan for period 2008-12. The project was implemented in five Leptospirosis-endemic states with an objective to reduce the morbidity and mortality due to Leptospirosis in humans. The control strategy adopted in the pilot project was found feasible and reproducible and with the lessons learnt in the pilot project, the Government of India launched the programme for prevention and control of Leptospirosis in the endemic states under the 12th Five Year Plan.

As the clinical presentation of Leptospirosis vary from mild illness to severe life threatening illness and laboratory tests to diagnose the Leptospirosis are complex therefore definite guidelines for diagnosis of Human Leptospirosis needed to be developed to ensure the uniformity in the case management of the Leptospirosis.

I congratulate Director NCDC, Dr Veena Mittal, Head (Zoonoses) and her team for bringing out these guidelines. I am sure these guidelines will be extremely useful for the health officials for preparedness, planning and delivery of prevention and control measures of Leptospirosis.

(Dr. Jagdish Prasad)
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 and contributes substantially to the national economy. Due to the rapid ecological changes in the region during the past decade many new zoonotic diseases have emerged and resulted in epidemics leading to significant morbidity and mortality in humans. Leptospirosis is one among them. The change in the distribution and incidence rate of leptospirosis has occurred proportionately to the alterations in the eco-system. Reclamation of wastelands, afforestations, irrigation, changes in crops and agricultural technology have been important factors. The areas which would have remained free of this infection have converted into potentially endemic zones either by the changes brought out by man or the nature. The outbreaks of leptospirosis have been reported from coastal districts of Gujarat, Maharashtra, Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, Andamans & Nicobar, Dadar & Nagar Havelli, Daman & Diu & Puducherry from time to time. In addition, the cases have been reported from Goa and Odisha.

The high burden of disease has been reported from Andaman & Nicobar, Gujarat (4 districts affected) Kerala (14 districts affected), Maharashtra (4 districts and Mumbai city affected), Karnataka (9 districts affected) and Tamil Nadu (2 districts and Chennai city affected).
2. Epidemiology

2.1 Causative Agent
Leptospirosis is primarily a disease of animals, occasionally infecting humans. It is caused by pathogenic spirochete of the genus leptospira that traditionally consist of two species, Leptospira interrogans and Leptospira biflexa. The former includes all pathogenic serovars and the latter includes the saprophytic strains. Leptospira strains have been divided into 26 serogroups, of which 2 belong to saprophytic leptospires. Each serogroup consists of several strains designated as seorovars. Nearly 300 host adopted leptospiral serovars are naturally carried by more than a dozen species of rodents, wild and domestic animals. The moderate to highly conducive abundantly available variety of hosts, results in successful perpetuation of this organism. The leptospira serovars predominantly present in India are L. andamana, L. pomona, L. grippotyphosa, L. hebdomadis, L. semoranga, L. javanica, L. autumnalis, L. canicola.

2.2 Risk Factors & Determinants
The conditions that are favorable for maintenance and the transmission of the leptospirosis are as follows:

2.2.1 Reservoir and carrier hosts
Leptospirosis has a very wide range of natural rodent and non-rodent reservoir hosts which include rabbits etc. The domestic animals such as cattle, buffalo, goat, sheep and pigs carry the microorganisms and therefore act as carriers of the leptospires. 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 as large and small water bodies.

2.2.2 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 acquire the infection.

2.2.3 Soil salinization
Soil salinity and water logging are inter-linked problems. The salinity of the soil and alkaline pH provides favorable environment for survival of leptospires for months.

2.2.4 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 5˚C. This favors the survival of leptospires for long durations.

2.3 Mode of transmission
Infection is acquired through contact of abraded skin and/or mucus membrane with the environment contaminated with urine of rodents, carrier or diseased animals. Direct transmission of leptospirosis is rare.

2.4 Age and sex distribution
Gender difference in susceptibility is not apparent under conditions where both men and women are at equal risk. Males suffer more frequently from leptospirosis than females because of greater occupational exposure to infected animals and contaminated environment. Leptospiral infections occur more frequently in persons 20-45 years of age group. Leptospirosis rarely occurs in young children and infants, possibly, because of minimal exposure.

2.5 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. In India the disease has been found more commonly associated during post-monsoon period. In natural disasters such as floods it may assume epidemic potential.

2.6 High risk groups
Agricultural workers such as rice field planters, sugar cane and pineapple field harvesters, labourers engaged in canal cleaning operations and livestock handlers are subjected to exposure with leptospires.

Other occupational high risk groups are – Fishermen, sewer workers and all those persons who are liable to work in rodent infested environment. Lorry drivers as they may use contaminated water to wash their vehicles and masons, who may come in contact with the organisms while preparing the cement and sand mixture for construction work with contaminated water.
3. Clinical presentation

3.1 Clinical types:
The clinical spectrum of leptospirosis is very wide, with mild anicteric presentation at one end to severe leptospirosis with severe jaundice and multiple organ involvement on the other. Many infections may go unnoticed because of lack of significant clinical illness. Various clinical presentations of leptospirosis are as follows -

### 3.1.1 Anicteric leptospirosis
- It is the milder form of the disease. Patients present with -
  - Fever - Patients have remittent fever with chills. It may be moderate to severe.
  - Myalgia - It is characteristic finding in leptospirosis. Calf, abdominal & lumbosacral muscles are very painful & severely tender. This symptom is very useful in differentiating leptospirosis from other diseases causing fever. This is associated with increase in serum Creatinine Phosphokinase (C.P.K.) which helps in differentiating leptospirosis from other illnesses.
  - Conjunctival suffusion - There is reddish coloration of conjunctiva which is a very predominant sign in leptospirosis. It is usually bilateral and is most marked on palpebral conjunctiva. It may be associated with unilateral or bilateral conjunctival hemorrhage.
  - Headache - Usually intense, sometimes throbbing, commonly in frontal region. It is often not relieved by analgesics
  - Renal manifestations - Some form of renal involvement is invariable in leptospirosis. It usually occurs as asymptomatic urinary abnormality in the form of mild proteinuria with few casts and cells in the urine. Severe renal involvement in the form of acute renal failure, (which occurs in icteric leptospirosis) is rare
  - Pulmonary manifestations –Cough and chest pain are primary manifestations and in few cases haemoptysis may occur. Severe involvement leading to respiratory failure does not occur in anicteric leptospirosis.
  - Hemorrhage - Hemorrhagic tendencies are also present in some cases

Note: All the clinical features either decrease or disappear within two to three days and then they reappear and may progress to severe disease

In endemic area all cases of fever with myalgia and conjunctival suffusion should be considered as suspected cases of leptospirosis.

### 3.1.2 Icteric leptospirosis
- This is the more severe form of leptospirosis. As the name suggests, patients present with jaundice. Others features are-
  - Fever – Same as in anicteric leptospirosis but may be more severe and prolonged.
  - Myalgia – Calf muscle tenderness becomes more evident. Severe myalgia may force the patient to stop walking and may even be mistaken as paraplegia. Muscle pain may occur due to myositis, myonecrosis or bleeding into the muscles.
Headache - 50% of the patients present with diffuse headache which is seldom severe.

Conjunctival suffusion – Many patients will have reddish yellow discoloration, caused by icterus and congested block vessels or sub conjunctival hemorrhage.

Acute renal failure manifests as oliguria/anuria and/or proteinuria

Nausea, vomiting, diarrhea, abdominal pain

Hypotension and circulatory collapse.

3.1.3 Severe leptospirosis

The more severe form of disease with severe liver and kidney involvement is known as Weil's disease. Salient features of the organ involvements are described below.

**Hepatic:** Jaundice is the most important clinical feature. It may be mild to severe. It starts after 4 to 7 days of illness. Hepatic encephalopathy or death due to hepatic failure is rare. Hepatomegaly & tenderness in right hypochondrium are usually detected.

**Renal:** Renal involvement is almost invariably present in Leptospirosis. It presents as acute tubular necrosis (ATN) and interstitial nephritis. Hematuria with complaints of Cola colored urine and RBC casts in urine microscopy is common. In severe cases patients have acute renal failure and present with:

- Decreased urine output (oliguria or even anuria)
- Edema on face and feet.
- Features of uremia like breathlessness, convulsion, delirium and altered level of consciousness in very severe cases.

The renal dysfunction worsens during the first week to the end of 2nd week, after which it starts improving and complete recovery occurs by the end of the 4th week if the patient is maintained on renal support. Severe acute failure cases will need dialysis to tide over the acute phase. There is usually no residual renal dysfunction.

**Pulmonary:** In mild illness patients present with only cough, chest pain and blood tinged sputum. In severe cases patients have cough, hemoptysis, rapidly increasing breathlessness which may lead to respiratory failure and death. On examination, these patients have increased respiratory rate with crepitation in the basal region, which rapidly spread upwards to middle and upper lobes. X-ray shows basal and mid zone opacity in severe cases. It may be normal in mild cases. Hemorrhagic pneumonitis with interstitial and intra alveolar hemorrhage surrounded by focal capillary injury are common pathologic changes. Death can occur within hours to two days due to pulmonary hemorrhage and severe respiratory distress. There are wide variations in pulmonary presentation. It is the commonest cause of death due to Leptospirosis. Case fatality rate in leptospirosis is 0-15% and more than ninety percent (90%) of deaths due to leptospirosis occur due to pulmonary alveolar hemorrhage and renal complications.

**Cardiovascular system involvement:** Patients present with one or more of the following features:

Shock: Patient develop severe hypotension, cold clammy extremities, and tachycardia. Echocardiography reveals normal systolic function of left ventricle hence hypotension is due to either dehydration or peripheral vasodilatation.

Arrhythmias: Patient presents with palpitations, syncope and irregular pulse. Common arrhythmias seen are supraventricular tachyarrhythmia and various degrees of A.V. blocks. Ventricular tachy-arrhythmias are infrequent. Segment depression and T wave inversion may be present in some patients.

**Central nervous system:** CNS involvement in leptospirosis commonly present as meningitis. Headache may be the only manifestation or irritability, restlessness, seizures and coma can occur. Encephalitis, focal deficits, spasticity, paralysis, nystagmus, peripheral neuropathies, nerve palsy, radiculitis, myelitis all have been reported.

**Skin:** Macular, maculopapular erythematous skin eruptions are seen in the face, trunk and/or extremities in many patients with occasional cases of purpura. It may be noted that bleeding manifestations in leptospirosis are not directly related to the level of thrombocytopenia. They resolve in two to three days without any specific intervention.

**Leptospirosis in Pregnancy:** Leptospirosis during pregnancy has a bad prognosis and fetal loss had been reported to be high in the first trimester and near term mothers.

*All patients with severe, multiple organ involvement should be referred to tertiary care centre*

3.2 Differential diagnosis

Falciparum malaria, Dengue fever, Dengue hemorrhagic fever, Scrub typhus, Typhoid and Viral hepatitis closely resemble leptospirosis and are prevalent in areas reporting leptospirosis. Other conditions to be differentiated include viral pneumonia, viral hepatitis, alcoholic hepatitis, acute encephalitis syndrome and pyelonephritis. Possibility of coinfections should be kept in mind. SGOT (AST) and SGPT (ALT) are either normal or mildly elevated usually in hundreds only (in IU/L) in leptospirosis. This helps to differentiate leptospirosis from viral hepatitis where SGPT is markedly elevated and also from alcoholic hepatitis where SGOT is markedly elevated. High level of Creatinine Phosphokinase (CPK) is suggestive of leptospirosis. It is normal in viral hepatitis and alcoholic hepatitis and hence helps to
3.3 Recommended case definition

The recommended case definition for the management of cases of leptospirosis as follows:

_Suspected_: Acute febrile illness with headache, myalgia and prostration associated with a history of exposure to infected animals or an environment contaminated with animal urine with one or more of the following

- Calf muscle tenderness
- Conjunctival suffusion
- Anuria or oliguria and/or proteinuria
- Jaundice
- Hemorrhagic manifestations (intestines, lung)
- Meningeal irritation
- Nausea, Vomiting, Abdominal pain, Diarrhoea.

_Probable:_ Suspected case with positive presumptive laboratory diagnosis.

_Confirmed:_ Suspect/Probable case with confirmatory laboratory test.
(Note: The classification of suspected, probable and confirmed does not in any way explain the severity and that has to be assessed based on the severity and rapidity of organ involvement.)

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4. Laboratory Diagnosis

4.1 Criteria for diagnosis

**Presumptive diagnosis**

- A positive result in IgM based immune- assays, slide agglutination test or latex agglutination test or immunochromatographic test.
- A Microscopic Agglutination Test (MAT) titre of 100/200/400 or above in single sample based on endemicity.
- Demonstration of leptospires directly or by staining methods.

**Confirmatory diagnosis**

- Isolation of leptospires from clinical specimen
- Four fold or greater rise in the MAT titer between acute and convalescent phase serum specimens run in parallel.
- Positive by any two different type of rapid test.
- Sero-conversion.
- PCR test.

4.2 Collection and Transportation of samples

4.2.1 Blood sample

While collecting blood and separating serum proper procedures should be followed to avoid lysis or contamination. The important steps are-

- Use sterile syringe and needle
- Syringe, needle and vial must be dry
- Collect 5 ml blood
- Transfer from syringe to sterile vial after removing needle
- Allow the blood to clot at room temperature. Do not shake
- Separate serum by dislodging retracted clot with a sterile Pasteur pipette.
- If facilities for serum separation are not available then refrigerate at 4 to 8°C. Samples should not be frozen
- Transfer the liquid portion to sterile centrifuge tube. Centrifuge at 3000 rpm for 5 minutes.
- Transfer supernatant (serum) to sterile plastic disposable leak proof screw
4.2.2 CSF sample
- CSF should be collected in a sterile container by lumbar puncture under aseptic conditions before the institution of antibiotics.
- Preferably CSF should be collected in three different vials, one for cell count, one for biochemical examination and one for culture.
- CSF should be transported immediately to laboratory without delay.

4.2.3 Urine sample
- Urine should be collected in sterile widemouth container
- Carefully clean the peri-urethral area with soap and plenty of water.
- Discard first voided sample and subsequent midstream urine is collected in sterile widemouth container.
- Transport the sample immediately to avoid multiplication of contaminants

Each sample should be properly labeled mentioning name, date of collection, and accompanied with a duly filled proforma with relevant clinical details should be included. If delay is expected, specimen should be kept cool preferably at 4 to 8°C (serology and molecular tests) and ambient temperature (culture) and sent to laboratory as early as possible.

4.3 Protocol for laboratory investigations

4.4 Referral Laboratories
- Regional Medical Research Center (ICMR), Port Blair (A&N), Tel: 03192-251158/251159
- National Centre for Disease Control, 22-Sham Nath Marg, Delhi, Tel: 011-23971272/23971060/23912901
- National Institute of Epidemiology, Chennai, Tel: 044-26820517, 044-26821600
- Government Medical College, Surat, Tel: 0261-2244175, 0261-2208373
- BJ Medical College, Ahmedabad, Tel: 079-22680074
- Madurai Medical College, Madurai, Tel: 0452-2533235

(Details of Laboratory investigations are at Annexure I)
5. Leptospirosis – Case management

5.1 Treatment at PHC (in leptospirosis endemic areas)

STEP-1: How to clinically suspect Leptospirosis?
- Refer to case definition
- Guidelines for fever case management at field level are annexed at Annexure-2

STEP-2: How to treat clinically suspected Leptospirosis?
Adults: Doxycycline 100 mg twice a day for seven days. Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.
Children < 8 years: Amoxycillin/Ampicillin 30-50 mg/kg/day in divided doses for 7 days.

STEP-3: Laboratory screening of all suspected leptospirosis cases by rapid immunodiagnostic test:
- Certain rapid tests are available for diagnosis of leptospirosis. They do not require expertise or any expensive instruments. However, they require confirmation by ELISA.

STEP-4: Treatment at PHC for mild disease and rapid immunodiagnostic test positive cases
Adults: Doxycycline 100 mg twice a day for seven days. Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.
Children < 8 years: Amoxycillin/Ampicillin 30-50 mg/kg/day in divided doses for 7 days.

Guidelines for fever case management at PHC level are annexed at Annexure 3

STEP-5: How to treat patients with negative ELISA and negative rapid immunodiagnostic test and clinically stable?
Adults: Doxycycline 100 mg twice a day for seven days. Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.
Children < 8 years: Amoxycillin/Ampicillin 30-50 mg/kg/day in divided doses for 7 days.
STEP-6: When to shift patients to higher centre?

All suspected leptospirosis cases whether positive or negative with rapid immunodiagnostic test having feature of organ dysfunction as follows should be IMMEDIATELY shifted to higher centre.

- Hypotension
- Decreased urine output (<400 ml per day)
- Jaundice (serum bilirubin >3.0 mg%)
- Haemoptysis or breathlessness
- Bleeding tendency
- Irregular pulse
- Altered level of consciousness

While shifting patients to higher centre, individual patient’s record should be furnished in the following order:

- Age, Sex
- Occupation
- Clinical symptoms
- Date of onset of clinical symptoms
- Serological result
- Hospitalization details—treatment given and outcome

Guidelines for fever case management at higher centers are annexed at Annexure-4

5.2 Treatment at CHC/District Hospital

STEP-1: How to clinically suspect Leptospirosis?

Refer to case definition

STEP-2: How to treat clinically suspected Leptospirosis?

Adults: Doxycycline 100 mg twice a day for seven days. Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.

Children< 8 years: Amoxycillin/Ampicillin 30-50 mg/kg/day in divided doses for 7 days.

STEP-3: Laboratory screening of all suspected leptospirosis cases by rapid immunodiagnostic test:

Certain rapid tests are available for diagnosis of leptospirosis. They do not require expertise or any expensive instruments. However, they require confirmation by ELISA.

STEP-4: Treatment at CHC for mild disease and rapid immunodiagnostic positive cases

Adults: Doxycycline 100 mg twice a day for seven days. Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.

Children<8 years: Amoxycillin/Ampicillin 30-50 mg/kg/day in divided doses for 7 days.

STEP-5: How to treat patients with negative ELISA and negative rapid immunodiagnostic test and clinically stable cases?

Adults: Doxycycline 100 mg twice a day for seven days. Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.

Children< 8 years: Amoxycillin/Ampicillin 30-50 mg/kg/day in divided doses for 7 days.

STEP-6: When to shift patients to higher centre?

All suspected leptospirosis cases whether positive or negative with rapid immunodiagnostic test having feature of organ dysfunction as follows should be IMMEDIATELY shifted to higher centre.

Renal:
- Decreased urine output (<400 ml per day)
- High blood urea (> 60 mg. %)
- High S. Creatinine (> 2.5 mg%)
- Clinical features of uremia, breathlessness, convulsion, delirium, and / or altered level of consciousness

Hepatic:
- Jaundice
- High S. Bilirubin (>3.0mg. %)

Pulmonary:
- Breathlessness
- Haemoptysis
- Increased respiratory rate
- X-ray chest showing opacities

Blood:
- Bleeding tendency
- Low platelet count
Neurological:

- Altered level of consciousness

While shifting patients to the higher centres, relevant clinical profile along with the treatment given should be furnished.

5.3 Treatment at medical college/tertiary level health care facility

Treatment should be started as early as possible. Any case of fever in leptospirosis endemic areas during monsoon and post-monsoon season should be administered antibiotics as follows:

**Adults:** Doxycycline 100 mg twice a day for seven days

Inj. Crystalline penicillin 20 lacs IU IV every 6 hourly after sensitivity test.

(For the individuals who are sensitive to penicillin group of drugs following alternative regimes may be used)

Ceftriaxone 1 gm IV x 6 hourly for 7 days

OR

Cefotaxime 1 gm IV x 6 hourly for 7 days

OR

Erythromycin 500 mg IV x 6 hourly for 7 days

Pregnant & lactating mothers should be given capsule ampicillin 500 mg every 6 hourly.

**Children** < 8 years: Amoxycillin/ Ampicillin 30-50 mg/kg/day should be given in divided doses for 7 days

Inj. Crystalline penicillin should be given 2–4 lacs IU/kg/ day for 7 days.

(For individuals who are sensitive to penicillin group of drugs following alternative regimes may be used)

Ceftriaxone 50-75 IV mg/kg/day for 7 days

OR

Cefotaxime 50-100 IV mg/kg/day for 7 days

OR

Erythromycin 30-50mg/kg/day in divided dose for 7 days

In disease progression multiple organs such as kidney, liver, lungs, CVS and CNS may be involved. The management of organ involvement does not differ than that of non-leptospirosis causes.

6.1 Personal protection

Hygienic methods such as avoidance of direct and indirect human contact with animal urine are recommended as preventive measures. Workers in flooded fields should be cautioned against direct contact with contaminated water or mud and should be advised to use rubber shoes and gloves. In case of any cuts or abrasion on the lower extremities of the body, the worker should apply an antiseptic ointment e.g. betadine, before entering the field and after exit.

6.2 Health education

The main preventive measure for leptospirosis is to create awareness about the disease and its prevention. This has to be conceptualized through intensive educational campaign, IEC templates/software for audio visual, print, press, outdoor outreach modes, new and emerging electronic media.

6.3 Chemoprophylaxis

During the peak transmission season Doxycycline 200 mg, once a week, may be given to agricultural workers (e.g. paddy field workers, canal cleaning workers in endemic areas) from where clustering of cases has been reported. The chemoprophylaxis should be for six weeks and never to be extended for more than eight weeks.

6.4 Rodent control

It is established beyond doubt that rodents are the major reservoirs of bacterium *Leptospira interrogans*. Four species of rodents *Rattus rattus* (House rat), *Rattus norvegicus* (Norway rat), *Bandicota bengalensis* (Lesser bandicoot) and *Bandicota indica* (Larger bandicoot) are so far found to be reservoirs for this bacterium in India. Hence controlling these reservoir species with proper strategy planning and management planning will reduce the incidence of the disease in the affected areas. The strategic planning should cover the following:

1. Identifying the reservoir species of affected area
2. Delineating areas for anti rodent activities
3. Completion of activities in pre monsoon months.
4. Adopting appropriate technology for anti rodent operations. This includes correct inputs and appropriate application technology.
### 6.5 Mapping of water bodies for establishing a proper drainage system

The mapping of water bodies and human activities in water logged areas should be carried out. This will help to identify the high risk population. Farmers may be educated to drain out the urine from the cattle shed into a pit, instead of letting it flow and mix with water bodies (rivers, ponds etc.).

### 6.6 Health impact assessment of developmental projects

Health impact assessment should be made mandatory for all developmental projects along with environmental assessment.

### 6.7 Vaccination of animals

Leptospiral vaccines confer a limited duration of immunity. Boosters are needed every one to two years. Vaccination should however be very selective and used only in endemic situations having high incidence of leptospirosis. The vaccine must contain the dominant local serovars. While this prevents illness, it does not necessarily protect from infection and renal shedding.

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**Annexure 1**

**Protocols for Laboratory investigations**

1. **Isolation of Leptospires**

   Isolation of leptospires from clinical specimens is the strongest evidence for confirmatory diagnosis. Isolation and identification is the method of choice to identify circulating serovars in a particular geographical region.

   a) **Blood culture**

   **Ideal time:** Within 10 days of the onset of the disease. Sample should be collected before antibiotics are started.

   **Media:** EMJH, Fletcher's and Stuart's (commercially available or obtain from the designated regional/district laboratory)

   **Procedure:**
   - Swab the area with the spirit
   - Draw the blood using sterile syringe and needle by vein puncture
   - Take 2 tubes containing 5 ml EMJH medium and inoculate two drops of blood in the first tube and four drops in the second tube.
   - Incubate at 30°C for 4-6 weeks.
   - Examine the culture using dark field illumination initially on 1\(^{st}\), 3\(^{rd}\) and 5\(^{th}\) days followed by at 7-10 days interval upto 6 weeks.
   - Selective culture media containing 5FU 50-1000 µg/ml can be used to avoid contamination.
   - Sub culture should be made within 48 hrs to minimize the inhibitory effect of the selective agents on Leptospires. Growth of the fastidious isolates is encouraged by adding to the medium 0.1% -0.15% agarose & 0.4%-1% of rabbit serum or fetal calf serum.

   b) **Urine culture:**

   **Time:** 10-30 days after the onset of the disease

   **Media:** EMJH, Fletcher's and Stuart's

   **Procedure:**
   - Collect fresh midstream sample. The sample should be tested within 2 hours of collection.
   - Dilute the urine as follows: using sterile test tubes and sterile phosphate buffer (pH 7.2).
     - Add 0.4 ml of urine to 3.6 ml of PBS (1 in 10)
     - Add 3 ml of urine to 3 ml of PBS (1 in 20)
     - Add 2 ml of urine to 2 ml of PBS (1 in 40)
     - Add 1 ml of urine to 1 ml of PBS (1 in 80)
Take 5 ml of medium in 4 separate tubes and add 0.5 ml each of a, b, c, d solutions of PBS in 4 different medium tubes.

Label the tubes mentioning the dilution

Incubate at 30°C

Examine the culture using dark field illumination at intervals of 7-10 days upto 6 weeks.

The above procedure should be repeated 2 or 3 times with urine samples collected at different times/days to increase the probability of isolation.

Urine can be filtered (through 0.22 µm filter) and/or inoculated into selective culture media to avoid contamination.

Urine may have acidic pH in many cases. Therefore urine should be collected in tubes containing equal amount of PBS with pH 7.2.

c) CSF Culture

Time: Within 5 - 10 days of the onset of the disease

Medium: EMJH, Fletcher's and Stuart's

Procedure:

- Inoculate 0.5 ml of CSF into 5 ml of culture media
- Follow the same procedure as blood culture

Advantages of isolating leptospires from clinical specimens:

- Definite proof of infection
- Circulating serovars can be identified
- Local isolates can be used as antigen in MAT
- Local isolates can be used in vaccine development.

Limitations:

- Fastidious organism requires special medium for isolation
- Leptospires grow slowly. Isolation of leptospires from clinical specimens takes several days to several weeks.
- The technique is laborious, time consuming and is not possible in small laboratories
- Contamination of culture media by other micro-organisms or by saprophytic leptospires is common in routine practice
- The successful isolation rate is less due to prior use of antibiotic, imperfectly cleaned glass ware or wrong incubation temperature and pH.

2. Demonstration of Leptospires

A. Dark Field Microscopy

Demonstration of leptospires by using Dark Field Microscopy appears to be a simple and rapid procedure. Though the organism is present in the blood during acute stage of the disease, the concentration is too low to allow detection by direct microscopy. The leptospiral shedding in urine is intermittent. Moreover serum proteins or cell fragments may mimic leptospires. Even experienced personnel may be confused with these artifacts as in majority of the clinical samples leptospires may not exhibit typical motility due to reactive antibodies or due to mechanical injury during the process of specimen for examination. Critical evaluation of this technique as a diagnostic tool has shown that the test has low sensitivity (40.2%) and specificity (61.5%). Therefore DFM is not recommended as a sole diagnostic tool for the diagnosis of leptospirosis.

Specimens: The specimens should be taken aseptically and sent to laboratory without delay, they must not be frozen. Oxalate, citrate or heparin may be used as anticoagulant for blood or pleural fluid.

Procedure (Blood)

- Centrifuge 5 ml of blood (treated with an anticoagulant) at 1000 g for 15 min.
- Add approximately 10 µl of plasma on a thin microscopic slide and apply cover slip.
- Examine under dark field microscope with low power and high power (x 200 and x 400).
- If no leptospires are seen, centrifuge plasma at 3000-4000 g for 20 min.
- Carefully remove the supernatant and examine a drop of sediment microscopically as above.

Procedure (Urine)

- Centrifuge a portion of freshly voided urine at 3000 g for 10 min.
- Examine a drop of deposit by DFM (x 200 and x 400).

Limitations:

- Low sensitivity and specificity.
- Serum proteins and fibrin strands in blood resembles leptospires.
- The concentration of organism is frequently too low in the specimens.
- Requires technical expertise.

B. Silver Impregnation techniques

Various silver impregnation techniques are used for the staining of leptospires in body fluids and tissues.

Fontana Staining

Requirements

- Fixative- Acetic acid 1ml+ Formalin (40% HCHO) 2 ml+ Distilled water 100 ml
- Mordant- Phenol 1 gm+ tannic acid 5 gm+ Distilled water 100ml
- Ammoniated silver nitrate (to make fresh): Add 10 % ammonia to 0.5% solution of silver nitrate in distilled water until the precipitate formed just
dissolves. Now add more silver nitrate solution drop by drop until the precipitate returns and does not re-dissolve.

**Procedure**
- Treat the film three times, 30 seconds each time, with fixative.
- Wash off the fixative with absolute alcohol and allow the alcohol to act for 3 min.
- Drain off the excess alcohol and carefully burn off the remainder until the film is dry.
- Pour on the mordant, heated till steam rises, and allow it to act for 30 sec.
- Wash well in distilled water and again dry the slide.
- Treat with ammoniated silver nitrate, heated till steam rises, for 30 sec, till the film becomes brown in colour.
- Wash well in distilled water, dry and mount in Canada Balsam.

It is essential that the specimen be mounted in balsam under a cover slip before examination, as some immersion oils cause the film to fade at once. The spirochetes are stained brownish black on a brownish-yellow background.

### 3. Detection of specific antibodies of leptospires

#### a) Detection of serovar specific antibodies

Microscopic Agglutination Test (MAT): MAT is the gold standard test for detection of serovar/serogroup specific antibodies. One of the critical issues of MAT is the cut off or a significant titer for diagnosis, when the test is done on a single sample. A battery of antigens covering the range of serovars that are expected or likely to be circulating in a particular geographical area, where the patient becomes infected, should be used.

**Preparation of Antigens:**
- The stock for collection of leptospires is best maintained in screw capped test tube containing 5-6 ml of liquid media.
- Fresh subculture are made by inoculating 0.5 ml from each strain/serovar into tubes.
- A loop full of culture should be examined by dark field microscopy to confirm the presence of viable leptospires and the absence of contaminant.
- The inoculated cultures are incubated at 30°C and checked for the presence of growth after 5-7 days.

**Procedure:**
- Fill all 96 wells of microtitre plate with 50µl PBS
- Add another 140 µl PBS to the wells of column 2
- Add 10 µl of serum to the wells of column 2 (now dilution becomes 1:20), mix and discard 100 µl.
- Dilute by pipetting 50 µl from one well to the next, discard the final 50 µl.

**Add 50 µl leptospira cultures to all wells.**
- Mix thoroughly on a micro shaker.
- Incubate for 2 - 4 hour at 30°C

**Reading of the test results:** The serum antigen mixtures are examined under a dark field microscope for agglutination. For observation, one drop mixture is transferred with a platinum loop or pipette from a well to a microscopic slide and examined under dark field microscope with 20x objective without cover slip. Compare with a control suspension of leptospires diluted 1 in two in PBS without serum. Agglutination is measured indirectly by establishing the reduction of Leptospiral density with 50% in comparison with the density of free leptospires in control.

**Advantages:**
- It is serovar/serogroup specific test. Some clue about the infecting serovar can be obtained
- Once infected the person stays MAT positive for several years so the test is useful for epidemiological purpose.

**Limitations:**
- 14-21 strains have to be maintained in cultures which are often very difficult.
- Procedure is complex and time consuming.
- It is not possible to distinguish between IgM indicative of current infection and IgG indicative of past infection.
- Finding agglutination antibodies in single serum sample does not necessarily prove current leptospirosis. An antibody titer may be due to residual antibodies of a past infection. Therefore the interpretation of a single titer is not easy so a second serum sample is required for demonstrating a rising titer which has a diagnostic significance.

#### b) Detection of genus specific antibodies

1. **ELISA**

ELISA is one of the techniques commonly used for the diagnosis. The test can detect specific antibodies earlier than MAT. The advantage of the test is that it can differentiate between recent and past infection by detecting the type of antibodies (IgM or IgG) present in the clinical specimen. In this test, broadly, reactive antigen is used. The antigen antibody reaction is visualized or measured by spectrophotometer/ELISA reader using a conjugate (enzyme conjugated to anti-IgM or IgG) and a colour reagent.

(Kits are commercially available. Procedure of the test should be as per the manufacturers' instructions.)

**Advantages**
- Single antigenic preparation can be used.
- Allows rapid processing of large number of samples.

**Limitations:**
Infecting serovar cannot be assessed.

2. Rapid Diagnostic Test
   - Latex based Agglutination Test
   - Immunochromatography
   The tests should be performed as per manufacturer's instructions.

4. Molecular Methods:
   **Polymerase Chain Reaction (PCR):**
   PCR method involves in vitro amplification of genus-specific target, DNA sequence, if present, in clinical samples. A pair of short DNA fragments, known as primers is used for specific amplification of DNA fragments from the pathogen in blood, urine or CSF. Positive diagnosis results from the amplification of the target sequence whereas negative samples fail to produce amplified DNA in PCR. PCR can be used to detect leptospiral infection in both animals and human beings, especially during the first few days of the disease when antibodies are not fully detectable in serological tests. The primers used for the PCR are G1 5’ – CTG AAT CGC TGT ATA AAA GT-3’ & G2 5’-GGA AAA CAA ATG GTC GGA AG-3’ and B 64I5’-CTG AAT TCT CAT CTC AAC TC-3’ & B64II5’-GCA GAA ATC AGA TGG ACG AT-3’.

   **Advantages :**
   Gives relatively quick results in the early stage of the disease when antibodies have not yet developed in detectable levels

   **Limitations :**
   Sophisticated equipment and trained manpower is required.

   **Note :**
   1. The sero-diagnostic tests being used for Leptospirosis has shown cross-reactivity with hepatitis E and A. Thus, caution is necessary in the interpretation of serological data.
   2. The health facilities undertaking sero-diagnosis should send 5% of their sera samples to the designated laboratory for cross-verification to ensure correct diagnosis.

### Annexure 2

**Community Surveillance by Paramedics/ Volunteers/Field workers**

Acute febrile illness with headache, myalgia and prostration associated with a history of exposure to infected animals or an environment contaminated with animal urine with one or more of the following:
Calf muscle tenderness/Conjuctival suffusion/Anuria or oliguria and/or proteinuria/Jaundice/Hemorrhagic manifestations (intestines, lung)/Meningeal irritation/Nausea, Vomiting, Abdominal pain, Diarrhoea.

```
Suspected leptospirosis

No

Look for other causes of fever

Yes

Ask/ Look for
- Severe myalgia
- Oliguria/Anuria
- Jaundice
- Blood in cough
- Breathlessness
- Confusion
- Cold extremities

Absent

Present

Refer to PHC

Refer to CHC/ District hospital
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**Note:** Field Worker shall daily report (A) Number of persons surveyed (B) Number of fever cases (C) Fever cases referred and (D) Any death following fever
Annexure 3

**Guidelines for fever case management at PHC level for Medical officers**

Acute febrile illness with headache, myalgia and prostration associated with a history of exposure to infected animals or an environment contaminated with animal urine with one or more of the following:
- Calf muscle tenderness/Conjuctival suffusion/Anuria or oliguria and/or proteinuria/Jaundice/Hemorrhagic manifestations (intestines, lung)/Meningeal irritation/Nausea, Vomiting, Abdominal pain, Diarrhoea.

- **Yes**
  - Suspected case
    - Rapid diagnostic test
      - Positive
        - Prepare slide for malarial parasite
          - Doxycycline 100 mg BD for 7 days
      - Negative
        - Probable case
          - Ensure complete course of doxycycline 100 mg BD for 7 days
    - Ask/Look for
      - Severe myalgia
      - Oliguria/Anuria
      - Jaundice
      - Blood in cough
      - Breathlessness
      - Confusion
      - Cold extremities
      - Organ involvement
        - Present
          - Report to Chief district medical officer for line listing (LL) number
      - Absent
        - Ensure complete course of doxycycline 100 mg BD for 7 days

**Note:**
1. Doxycycline should be avoided in pregnancy, lactation and children less than 8 years
2. Pregnant & lactating female should be given capsule ampicillin 500 mg every 6 hourly.
3. Amoxycillin/ Ampicillin 30-50 mg/kg/day should be given in divided doses 6 hourly for 7 days.

Annexure 4

**Guidelines for Suspected/ Probable case management at Higher Centre (CHC/ District Hospital/ Medical College)**

- **Suspected/Probable case**
  - Doxycycline 100 mg BD for 7 days
  - Perform rapid diagnostic test
  - Perform biochemical test
  - Ask/ Look for
    - Severe myalgia
    - Oliguria/Anuria
    - Jaundice
    - Blood in cough
    - Breathlessness
    - Confusion
    - Cold extremities
  - Rapid diagnostic test Positive/ Negative
    - Organ involvement present
      - Complete seven day course Inj. Crystalline penicillin after negative test dose
      - Treat the patient according to organ involvement
      - Report to Chief district medical officer for line listing (LL) number
    - No organ involvement present
      - Ensure complete course of doxycycline 100 mg BD for 7 days
  - General condition worsening
    - Bleeding tendency
    - Confusion
    - Breathlessness
  - Any one of above
    - Refer to higher centre

**Note:**
1. Doxycycline should be avoided in pregnancy, lactation and children less than 8 years
2. Pregnant & lactating female should be given capsule ampicillin 500 mg every 6 hourly.
3. Amoxycillin/ Ampicillin of 30-50 mg/kg/day should be given in divided doses 6 hourly for 7 days.
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