

Leptospirosis

(Rat Fever)



**Indian Council of Agricultural Research - National Institute of
Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI)**

(ISO 9001 - 2015 Certified)

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What is Leptospirosis?

Leptospirosis is a transmissible disease of animals and humans caused by infection with the spirochete *Leptospira*. It is one of the fastest re-emerging widespread and one of the leading neglected zoonosis in the world and is emerging as an important public health problem, which results in high morbidity and considerable mortality in areas of high prevalence. It affects both human and animals including cattle, buffalo, goat, sheep, horse and swine resulting in heavy economic losses to the farming community. This is mainly due to the great diversity of the *Leptospira*s, and their ability to infect and survive in a wide range of animal hosts. Livestock farming remains as a major occupational risk factor for human leptospirosis especially animal handlers.

Bovine leptospirosis has a global distribution, infected by a wide variety of serovars and with varied clinical outcome. Cattle are infected by pathogenic serovars of *Leptospira* and can act as potential carrier and excrete the bacteria in their urine in large quantity for a long time, which increases the risk of transmission to other animals and humans. The clinical signs can vary with infecting serovar and host. In dairy cattle it is responsible for great economic losses as a consequence of reduced milk yield, (agalactia), abortion, stillbirth, mortality in calves, decreased daily weight gain, birth of weak calves and reduced fertility. Similar to bovine majority of *Leptospira* infections in swine are subclinical, clinical signs is most often characterized by reproductive disorders including late term abortions, infertility, stillbirths, mummified or macerated fetuses, and increased neonatal mortality in the infected herds.

What is the etiology of leptospirosis?

The causative agent for Leptospirosis is the spirochete *Leptospira*. *Leptospira*s are spirochetes that may be saprophytes (free-living) found in freshwater or pathogens which may cause acute or chronic infection of humans and animals. *Leptospira*s belong to the order *Spirochaetales*, family *Leptospiraceae*, genus *Leptospira*. In the genus *Leptospira*, there are three clades of pathogens, an intermediate and nonpathogens group.

Pathogens: *Leptospira interrogans*, *Leptospira noguchii*, *Leptospira alexanderi*, *Leptospira borgpetersenii*, *Leptospira kirschneri*, *Leptospira kmetyi*, *Leptospira weilii*, *Leptospira alstonii*, *Leptospira santarosai*;

Intermediate: *Leptospira licerasiae*, *Leptospira inadai*, *Leptospira wolffii*, *Leptospira fainei*, *Leptospira broomii*;

Nonpathogen: *Leptospira biflexa*, *Leptospira vanthielii*, *Leptospira idonei*, *Leptospira wolbachii*, *Leptospira terpstrae*, *Leptospira yanagawae*, *Leptospira meyeri*.

*Leptospira*s are further divided into serovars divergent Leptospiral lipopolysaccharide (LPS) structures; antigenically there are more than 300 serovars which are further clustered into 30 serogroups for convenience. Species are traditionally classified into serogroups and over different serovars. Genetic methods like DNA-DNA hybridization studies have identified 21 *Leptospira* spp. to date. However, antigenically related serovars are classified in two or more different species and a serogroup is often found in several species of *Leptospira*.



A certain serovar may develop a commensal or comparatively mild pathogenic relationship with certain animal host species. For instance, cattle are often associated with serovar Hardjo, dogs with Canicola and rats with Icterohaemorrhagiae and Copenhageni.

How can pathogenic *Leptospires* be distinguished from saprophytic *Leptospires*?

Several tests based on culture conditions and on antigenic and genetic properties can be used to differentiate between pathogenic and saprophytic *Leptospires*. Growth at 13°C in the presence of 8-azaguanine and conversion to spherical forms in 1M NaCl suggests that *Leptospires* are saprophytes.

Do pathogenic *Leptospires* always cause disease?

Certain vertebrate animal species have a commensal relationship with *Leptospires*, in which they are the natural hosts for pathogenic *Leptospires* that live in their kidneys. Such *Leptospires* do little or no detectable harm to these hosts but they maintain the infection and are therefore known as natural maintenance hosts. If other animals that are not natural maintenance hosts (including humans) are infected by the same pathogenic *Leptospires*, they often become ill. In addition, if a maintenance host for a particular *Leptospira* is infected with another serovar, it may develop symptoms and signs of leptospirosis.

How do *Leptospires* look like?

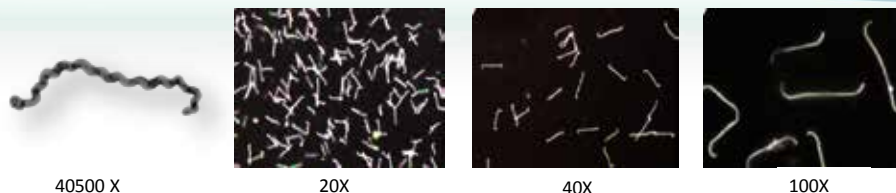
Leptospires are corkscrew-shaped bacteria which differ from other spirochaetes by the presence of end hooks. They are too thin, hence are to be observed under the dark-field microscope. All *Leptospires* look alike with only minor differences. So differentiation between pathogenic and saprophytic *Leptospires* or between the various pathogenic *Leptospires* is difficult.

General morphological features

“*Leptospires* are highly motile, obligate aerobic spirochetes that share features of both Gram-positive and Gram-negative bacteria”. *Leptospira* has a spiral shape with an average diameter of “approximately 0.1 µm, length range of 6–20 µm, helical amplitude of 0.1–0.15 µm, and wavelength of 0.5 µm. As with other spirochetes, *Leptospira* can alter their morphology according to the environmental variations.

- ✦ Shorter, more tightly coiled and highly motile: fresh isolated from a mammalian host and environment.
- ✦ Slightly elongated, motile: laboratory strains that have undergone repeated serial passage and saprophytic strains.
- ✦ Extremely elongated cells with decreased motility and poor cell health: Under stress, nutrition-limiting conditions, in detergents.
- ✦ Spherical bodies and granule formation: Further deterioration of cell health and under high NaCl concentration.

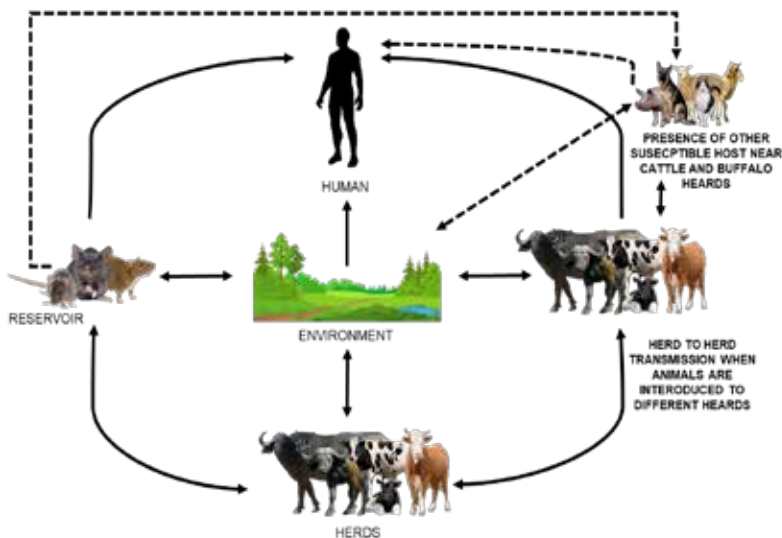




How and when leptospirosis spreads?

Leptospirosis has a worldwide distribution and is more spread in tropical regions than in temperate countries. This is attributed mainly to longer survival of *Leptospira* in warm and humid environments. A pattern of disease seasonality has been described with a peak incidence occurring in summer or fall in temperate regions and during rainy seasons (due to water logging conditions and contaminations of environment with urine of carrier animals) in warm-climate regions. Direct transmission can occur by contact with an animal or human host either by ingestion or skin contact, especially with mucosal surfaces. Indirect transmission is the most common mode of transmission and occurs with contaminated water or soil.

Transmission Cycle of Leptospirosis



What are the epidemiological factors involved in Leptospirosis?

Leptospirosis is caused by different serovars of *Leptospira*. Leptospirosis is usually a seasonal disease that starts at the onset of the rainy season and declines as



the rainfall recedes. Sporadic cases may occur throughout the year with outbreaks associated with extreme changing weather events such as heavy rainfall and flooding. Leptospirosis also spread in contaminated water supplies, food, pastures and soil. The animals that commonly develop or spread through leptospirosis are Rodents, Raccoons, Opossums, Cattle, Swine, Dogs, Horses, Buffaloes, Sheep and Goats.

The bacteria can live for a long time in surface fresh water, damp soil, vegetation and mud, but are very quickly killed on dry soil or by sunlight. *Leptospira* have been found in all farm animals, rodents and wild animals. They colonise the kidneys of infected animals and, in females, they also colonise the reproductive tract. The bacteria may infect animals and humans through damaged skin or through the membranes lining the nose, eyes or mouth. *Leptospira* has the ability to colonise and persist in the genital tract of infected cows and bulls suggesting that venereal spread may be a factor in transmission.

Animals are infected by direct contact with this contaminated water or soil or by drinking contaminated water. Infected animals can carry the bacteria for long periods, shedding them in urine and at birth or abortion, thus contaminating the animals' environment. Many infected animals do not display any illness. An animal may be infected by serovars maintained by its own species (maintenance host infection or host-adapted infection) or serovars maintained by other species (incidental infection or non host-adapted infection). Maintenance hosts carry the bacteria and expose other susceptible animals. Maintenance hosts can be cattle, pigs, dogs, rodents or horses. These apparently healthy carriers are the main source of infection for other cattle as well as for humans.

The environment plays a very important role in the transmission of the organism. Tropical region where there is plenty of rainfall, it is often difficult to avoid exposure of the people to animals or contaminated environment especially since the bacteria is well adapted to the environment. Flooding after heavy rainfall can spread the bacteria to previously uninfected farms. Outbreaks of leptospirosis infection are therefore more common in wet years. Even closed herds are not completely safe, as water from other properties could carry *Leptospira* organisms onto the farm.



Human Leptospirosis: The incubation period is usually 2-26 days, but usually (7-12 days) days. Characterized by broad spectrum of clinical manifestation varying from self-limiting anicteric illness to severe form (Weil's syndrome). Humans with leptospirosis usually excrete the organism in the urine for 4-6 weeks and occasionally for as long as 18 weeks. Person-to-person transmission is considered extremely rare.

Animal Leptospirosis: Found in a wide variety of wild and domestic animals including marine mammals. The disease can affect cattle, sheep, goats, pigs, horses, and dogs but is rare in cats. Rats, rodents, cattle, pigs and dogs serving as the most important animal reservoirs for the organism. Urine of infected animals is the main source of infection. Leptospirosis in animals is often sub-clinical, and the infected animal may appear normal and healthy even though it excretes *Leptospire*s in its urine. Carrier host-animals- Wild and domestic animals rodents, livestock (cattle, horses, sheep, goats, swine), canines, and wild mammals are the reservoir for leptospirosis. Many animals have prolonged leptospiuria without suffering from the disease themselves.

In bovine, some of the predominant serovars include Hardjo and Pomona. A wide variety of other serovars belonging to the Icterohaemorrhagiae, Canicola, Hebdomadis, Sejroe, Pyrogenes, Autumnalis, Australis, Javanica, Tarassovi, and Grippotyphosa serogroups have been reported as causing incidental infections in cattle in some parts of the world. Cattle are the maintenance hosts for Hardjo, but as this is specialised to survive within cattle, the infection is less severe. Animals infected with other strains (such as Pomona) suffer more severe illness.

Generally, the pigs are considered as the maintenance host for serovars of serogroups Pomona, Tarassovi and Australis, while other serovars such as Canicola, Icterohaemorrhagiae and Grippotyphosa in pigs are considered more likely to be accidental infections. Strains of serovar Pomona have well adapted to the swine and it is the most common serovars isolated from the swine worldwide.

How leptospirosis is significant in public health?

The significance of the disease in public health aspects acquires more importance, especially in countries like India because of large livestock, rodent and wildlife populations, poor sanitary conditions and animal health practices, and close association between man and animals, providing a congenial environment for the spread of the disease. Various factors influencing animal activity, suitability of the environment for the survival of the organism and behavioral and occupational habits of human beings can be the determinants of incidence and prevalence of the disease. The disease was considered inconsequential till recently, but it is emerging as an important public health problem during the last decade or so due to sudden upsurge in the number of reported cases and outbreaks. Leptospirosis is an important public health problem in large urban



centres of India. *Leptospira* serovars responsible for seropositivity among most of the animals and man in India have been identified as Icterohaemorrhagiae, Hardjo, Australis, Canicola, Grippotyphosa, Pyrogenes, Pomona, Tarassovi and Ballum.

How does a person get leptospirosis?

Leptospirosis is spread mainly by contact with water or soil contaminated by the urine of infected animals. Persons can get the disease by swimming or wading in fresh unchlorinated water contaminated with animal urine or by coming into contact with wet soil with animal urine. The disease also can be transmitted through direct contact with urine, blood or tissue from an infected animal. The bacteria can enter through broken skin or through the soft tissues on the inside of the mouth, nose or eyes. It is generally not transmitted from person to person.

What are the Risk factors associated with Leptospirosis?

The important risk factors associated with leptospirosis are socio-cultural, freshwater fishing, behavioural and environmental factors, population, personal hygiene, domestic animal, sanitation of animal habitats, human behavioural practices, outdoor environment, forestry, animal rearing, rodent density, occupational, etc.,

Human: The associated risk factors in human are generally grouped in to three categories viz., host, environmental and occupational related activities.

Occupational groups: Farmers and ranchers, abattoir workers, trappers, veterinarians, loggers, sewer workers, rice field workers and military personnel. Dairy farmers and animal handlers are particularly at risk of infection from urine splashing onto the face whilst milking the cows.

Recreational Activities: Freshwater swimming, Canoeing and kayaking, trail biking and hunting

Household environments: Pet dogs, domestic livestock. Rainwater catchment and rodent infestation.





Bovine: The important risk factors include sharing bulls, mixed grazing with sheep, shared grazing with common watercourses. Animal factors- Fatness, Pregnancy, Weight, Temperament, Breed / hoof colour; Purchase and/or introduction of new cattle; History of abortions in the farm; Rodents in the farm; Adult cows in contact with calves/heifers; Calves rearing system either natural or artificial; Cleanliness in the calving area, Level of hygiene in milking and status of *Leptospira* vaccination; Breeding herd size or presence of bulls used for natural service; Presence of other animals in the farms like dogs, sheep and goats, horse, pigs, etc.

Swine: The important risk factors include introduction of infected gilts and boars. Herd to herd infection from neighbouring pens, pooling of urine due to poor surface, sharing of workers from in one pen to another. Outer grazing exposed to wet ground. Large number of breeding sows; Not performing quarantine in case of abortion among the pig herds. Cohabitation of cattle and other in the farm and by other animals; rats, mice and dogs. The presence of Porcine reproductive and respiratory syndrome (PRRS) in the herd and artificial insemination are the risk factor in swine leptospirosis.

When leptospirosis can be suspected?

Human Leptospirosis: Abrupt onset of high fever, sore throat, headache, myalgia, conjunctivitis, abdominal pain, albuminuria, jaundice, skin rashes, superficial lymph node enlargement etc. Leptospirosis affects humans causing influenza-like symptoms with severe headaches but can be treated effectively. Clinical signs depend on the immunity virulence and serovars to which they are exposed.

Animal Leptospirosis: In general, disease in cattle, sheep, goats and swine may include a fever, abortions, reproductive problems/disorders etc.

Cattle: Pyrexia, anorexia, malaise, haemorrhages, anaemia, jaundice, haemoglobinuria, abortion, mastitis etc.

Sheep and Goats: Pyrexia, conjunctivitis, jaundice, anaemia, anuria, hemoglobinuria, diarrhoea, mastitis, haemoagalactia, abortion etc.

Pigs: Pyrexia, weakness, depression, haemoglobinuria, periodic ophthalmia, jaundice etc.

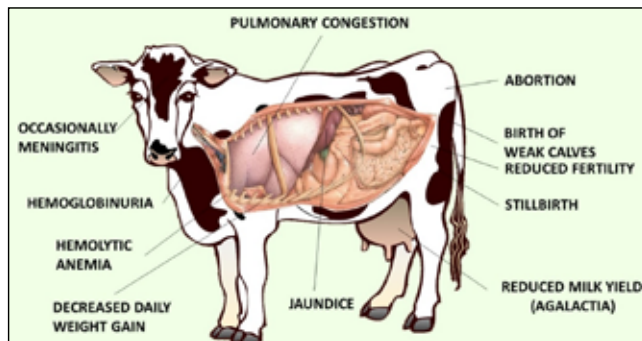


Dogs: Pyrexia, weakness, depression, conjunctivitis, vomiting, convulsion, anaemia, haemorrhages, jaundice etc.

Horses: eye is most commonly affected. The eyelids may be reddened, the horse will be sensitive to light and may blink frequently or clouding of the eye may be seen.

Bovine Leptospirosis: In bovine leptospirosis generally associated with clinical signs of agalactia (drop in milk), production of stillbirth or weak calves, abortion at 4-7 months, reduced conception rates, repeat breeding, infertility, failure to thrive, etc., Further, the clinical signs of leptospirosis is depending on the herd's degree of resistance or immunity, the infecting serovar, and the age of the animal infected.

Leptospirosis is a common cause of an abortion in dairy and beef herds. It can cause milk drop affecting a large proportion of the herd. Infection may cause an increased number of repeat breeder cows, infertility, abortion and poor milk yield. A sudden drop in milk yield may occur two to seven days after infection of susceptible cows. There is circumstantial evidence of infertility following isolation of *Leptospira Hardjo* from the reproductive tract of a high percentage of repeat breeder cows. *Leptospira Hardjo* may also cause embryonic death. Venereal transmission is also possible but may not significantly affect the pregnancy rate because *Leptospira* susceptible to uterine defences during oestrus.



Leptospira Hardjo is the only host-adapted *Leptospira* in cattle and can infect animals at any age, including young calves. Thus often produce a carrier state with chronic kidney infection associated with long-term urinary shedding. Infections with Hardjo can also persist in the reproductive tract and cause infertility. In case of persisting Hardjo infection the detection and diagnosis is often difficult due to low antibody titers. Cattle are incidental hosts for *Leptospira* serovars such as Pomona, Icterohaemorrhagiae, Grippityphosa and Canicola and the clinical signs are typically very different than infection with Hardjo serovar.

In case of non host-adapted *Leptospira* serovars in calves it results in high fever, anemia, red urine, jaundice and sometimes death in three to five days. In older cattle,

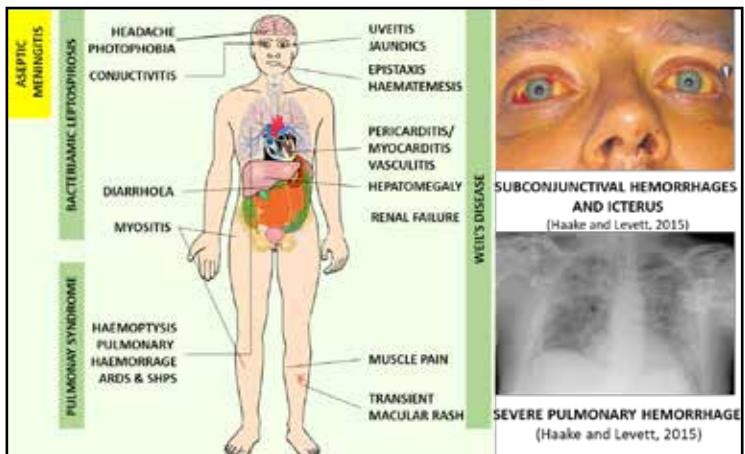


the initial symptoms such as fever and lethargy are often milder and usually go unnoticed. In addition, older animals' death due to leptospirosis is unlikely. Lactating cows produce less milk which is thick and yellow for some days. These serovars also affects pregnant cows causing embryonic death, abortions, retained placenta, stillbirths and the birth of weak calves. Abortions usually occur three to ten weeks after infection.

Human leptospirosis

Leptospirosis presentation ranges from often mild self-limited febrile illness to severe life-threatening illness with involvement of multi organ system. The leptospirosis symptoms in human are protean and overlapping with other acute fibrile syndrome such as dengue, chikungunya, malaria, influenza etc.

The incubation period varies from 7 to 12 days. Leptospirosis typically presents with sudden onset of fever, chills, and headache, muscular pain and tenderness calves and lower back. Additional features include ocular conjunctival suffusion, subconjunctival hemorrhages and icterus. Whereas a nonproductive cough has been noted in 20–57 % of leptospirosis patients. Gastrointestinal symptoms are common and may include nausea, vomiting, diarrhea, and abdominal pain. Which may be due to acalculous cholecystitis and/or pancreatitis. While most of the cases of pancreatitis due to leptospirosis are self-limited, some cases are more severe and associated with fatal outcomes. Rash is uncommon.



In contrast, severe leptospirosis is associated with multiple organs dysfunction involving the liver, kidneys, lungs, and brain. The most common forms are known as Weil's disease with combination of jaundice and renal failure, was first described in 1886. Leptospirosis typically have mild to moderate elevations in levels of liver transaminases and direct (conjugated) bilirubin causing varied frequency of jaundice.



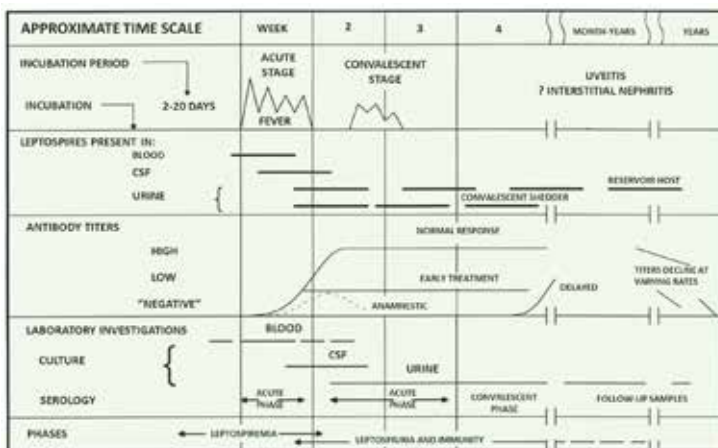
Clinical signs of bleeding are common and occur in the majority of patients with severe leptospirosis. Most are mild including petechiae, ecchymoses, and epistaxis. Severe bleeding includes gastrointestinal (melena or hematemesis) or pulmonary haemorrhage (haemoptysis) and some levels for thrombocytopenia can also occur. Renal complications are most common which, involves elevations in serum blood urea nitrogen, creatinine levels, pyuria, hematuria, oliguria and elevated urine protein levels and in fatal cases cause renal failure. Circulatory complication may progress to acute respiratory distress syndrome (ARDS) leading to breathlessness, hemoptysis, representing extensive alveolar hemorrhage, associated with >50 % fatality rates. Leptospirosis-associated severe pulmonary hemorrhage syndrome (SHPS) can occur sporadically or in outbreaks. SHPS can present as hemoptysis associated with cough showing diffuse alveolar infiltrates. Leptospirosis may be a predominant cause of aseptic meningitis in some areas. In severe leptospirosis, altered mental status may be an indicator of meningoencephalitis and neuro leptospirosis.

What are the samples to be collected from suspected individuals or animals?

Blood: During first phase of illness (upto 10 days), blood in anticoagulant preferably heparin, for culture isolation.

Urine, Cerebrospinal fluid (CSF), Milk: To be collected aseptically in sterile containers. Midstream urine in equal quantity of sterile phosphate buffer saline (PBS).

Post Mortem samples: A piece of kidney, liver and spleen in sterile PBS.



Guidelines on specimen collection for the diagnosis of leptospirosis

Samples are to be sent to the laboratory as soon as possible (immediately for DFM or culture) in duly sealed ice pack.

Blood culture more than 10 days after disease onset is not worthwhile as *Leptospirae* have mostly disappeared from the blood and antibodies will have become detectable in the serum allowing sero-diagnosis. One or two drops of blood are inoculated into 10 ml of semisolid medium containing 5-fluorouracil at bedside/penside. For the greatest recovery rate, multiple cultures should be performed, but this is rarely possible. Inoculation of media with dilutions of blood samples may increase recovery. Samples for culture should be stored and transported at ambient temperatures, since low temperatures are detrimental to pathogenic *Leptospirae*.

Clotted blood or serum for serology to be collected twice at an interval of several days. The testing of paired sera is necessary to detect a rise in titres between the two samples or seroconversion to confirm the diagnosis of leptospirosis. A negative serological result in the early phase of the disease does not exclude leptospirosis.

Urine for culture to be inoculated into an appropriate culture medium not more than 2 hours after voiding- *Leptospirae* die quickly in urine. Survival of *Leptospirae* in acid urine may be increased by making it neutral. Urine should be processed immediately not more than 2 hours after voiding by centrifugation, followed by resuspending the sediment in phosphate buffered saline (to neutralize the pH) and inoculating into semisolid medium containing 5-fluorouracil.

Cultures are incubated at 28 to 30°C and examined weekly by DFM for up to 13 weeks before being discarded. Contaminated cultures may be passed through a 0.2-µm or 0.45-µm filter before subculture into fresh medium.

CSF and dialysate for culture in the first week of illness *Leptospirae* may be observed by DFM and culture isolation by inoculating 0.5 ml cerebrospinal fluid into 5 ml semi-solid culture medium during the first weeks of illness.

Post-mortem samples to be collected aseptically as soon as possible after death. The specimens collected will depend on the resources available and cultural restrictions. They should also be inoculated into culture medium. The samples should be stored and transported at +4 °C.

Sample collection for *Leptospira* culture: Sample should be collected from suspected leptospirosis cases from endemic areas before antibiotics treatments. One to two drops of blood should be aseptically transferred into EMJH (Ellinghausen and McCullough modified Johnson and Harris) transport media during the acute septicemia phase (ie, within 10 days of fever). During immune phase (ie, after 10 days) 2-3 drops of Urine

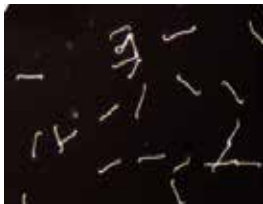


can be transferred into EMJH transport media containing tubes. Specimen should be labeled with sample type, Date & time of collection and completely filled laboratory request form should be submitted along with the specimen. Sample inoculated in the transport media can be transported at normal room temperature by courier or speed post to ICAR-NIVEDI within 2-3 days of collection for isolation of leptospirosis. EMJH transport media can be stored at room temperature

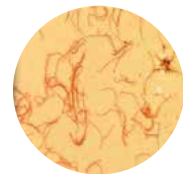
What are the commonly available techniques for diagnosis?

Microscopy examination

- ✦ Low sensitivity and specificity, serum proteins and fibrin strands in blood may resemble *Leptospires* and requires technical expertise to differentiate.
- ✦ *Leptospires* can be demonstrated in urine samples using Dark field microscope (DFM).
- ✦ In case of *Leptospira* three types of motility observed viz., Flexion and extension- Corkscrew motility; alternative rotation; and translation-different direction movement.
- ✦ The morphological feature of the *Leptospira* is extremely thin, elongated and thickly spiral. Hence basic aniline dyes fail to stain the bacteria. To overcome such disadvantage Fontana developed silver impregnation technique. Hence a modified silver staining was developed using mordant as an additional reagent.



Dark filed microscope (DFM) 40 X



STAINED SLIDE UNDER MICROSCOPE

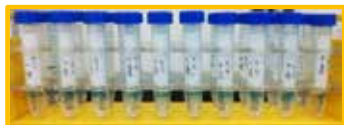
Microscopic agglutination test (MAT)

- ✦ Relatively serovar/ serogroup specific and test of choice for seroepidemiologic studies.
- ✦ Antibodies to *Leptospira* in blood samples with serum MAT titres of >1 in 100 considered to be significant.
- ✦ Second serum sample is essential and false negativity may occur in the early course of the disease.





STOCK CULTURE (SEMISOLID EMJH MEDIUM)



MAT PANNEL (LIQUID EMJH MEDIUM)



MAT DILLUTION



NO REACTION



MAT READING



WEEK REACTION



STRONG REACTION

Isolation

- ✦ Confirmatory proof of infection.
- ✦ Slow growth rate may hinder quicker diagnosis.
- ✦ Isolation of *Leptospires* from clinical material and identification of isolates is time-consuming and is a task for specialised reference labs.
- ✦ Isolation followed by typing from renal carriers is important and very useful in epidemiological studies to determine which serovars are present within a particular group of animals, an animal species, or a geographical region.



Enzyme-linked immunosorbent assay (ELISA)

- ✦ Allows rapid processing of large number of samples.
- ✦ Comparatively less specific.



Pen side test:

- ✦ Lateral flow immunoassays (LFA), point-of-care tests are simple to use, provide rapid results with minimum amount of sample preparation
- ✦ Simple to use with out need of any equipment in the field conditions.
- ✦ Latex Agglutination Test (LAT) and LFA are pen side or point of care test for rapid detection of *Leptospira* antibodies and results can be obtained within 5 minutes.



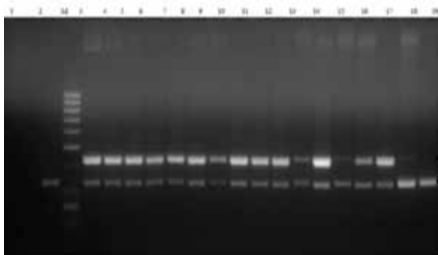
Lateral Flow Assay



In-house Bovine leptolAT

Polymerase Chain Reaction (PCR)

- ✦ Gives relatively quick results in the early stage of the infection.
- ✦ Expensive and sophisticated equipment is needed.
- ✦ Duplex PCR/real time PCR targeting LipL 32 / LipL 41 / 16S RNA gene for detection of pathogenic and nonpathogenic *Leptospira* organism is routinely being used.
- ✦ Multiplex PCR targeting the *genus* specific region as well as immune globulin coding region for the detection and differentiation of pathogenic *Leptospira* using two sets of primers and subsequent identification of the 5 serogroups levels (Canicola, Hardjo, Hebdomadis, Icterhaemorrhagiae and Grippotyphosa) using 10 sets of primers was also developed for routine use.



How is leptospirosis diagnosed?

A array of laboratory tests/assays are available for diagnosing leptospirosis in human and animals. However, selection of the right specimens and tests and correct interpretation of test results are important. Different approaches of laboratory diagnosis of Leptospirosis include bacteriological, microscopical, molecular and immunological assays. During *Leptospira* infection, leptospiraemic phase last for one week (< 7 Days), whereas *Leptospiruria* & Immune phase will commence after one week (> 7 days) of infection.

- ✦ Isolation from human samples can be attempted in initial stages of infection before antibiotic treatment. In case of reservoir/carrier host urine samples can be attempted.
- ✦ PCR can be used within one week of infection (fever) for early diagnosis (antigen detection-based test).
- ✦ IgM rapid assay can be used for detection of antibody (IgM) as serological test after 2-3 days of pyrexia. MAT can be considered with single serum along with a PCR or IgM ELISA or IgM rapid assay.
- ✦ MAT (gold standard test) with titre of >1 in 400 with single serum or seroconversion (if the first sample is negative) and raise in titre (if the first sample is having low titre) in case of paired sera samples from human and animals is conclusive for leptospirosis.
- ✦ Carrier animal can be identified by PCR using urine samples along with MAT titre.
- ✦ In acute infection with drop of milk production paired serum samples taken three to four weeks apart will normally demonstrate increased MAT or ELISA concentrations.
- ✦ Dam serology is of limited use because the MAT titre may fall rapidly after acute infection and be negative at the time of abortion; a positive result may only reflect previous exposure. During an abortion outbreak MAT titre >1/400 in some aborted cows are likely to be meaningful. ELISA titres are reported to remain positive for much longer following infection so may simply indicate previous exposure.
- ✦ Antibodies in foetal fluids may indicate exposure to *Leptospira* Hardjo in utero after four months' gestation however the foetus may die before mounting an immune response.
- ✦ Fluorescent antibody test (FAT) also useful to detect *Leptospira* Hardjo antigen in foetal tissues, e.g. kidney and lung is the best available test to confirm a diagnosis of abortion but delays in sample submission lead to rapid sample autolysis adversely affecting the test.
- ✦ A bulk milk ELISA test is available for herd screening and can be monitored regularly as part of surveillance programme in a naïve herd. Pooling milk samples



from first lactation heifers is a useful way of monitoring the infection status in a herd.

- ✦ Differential diagnosis also should be made with the common causes of abortion, which include *Neospora caninum*, BVDV infection, *Salmonella spp.*, *Bacillus licheniformis* and *Campylobacter*. Some of these conditions are BVD, IBR, *Neospora*, EBA (foothill abortion), *Campylobacter*, Trichomonosis, selenium deficiency, etc.

How leptospirosis can be treated?

Human: Severe cases of leptospirosis should be treated with high doses of intravenous penicillin. Jarisch-Herxheimer reactions may occur after penicillin treatment. Less severe cases can be treated with oral antibiotics such as amoxicillin, ampicillin, doxycycline or erythromycin. Third-generation cephalosporins, such as ceftriaxone and cefotaxime, and quinolone antibiotics also appear to be effective.

Animals: Tetracycline and oxytetracycline, erythromycin, enrofloxacin, tiamulin, and tylosin have been reported to be successful in acute cases. Oxytetracycline, amoxicillin, and enrofloxacin may be useful to treat chronic infections.

Antibiotic treatment of milk-drop cases is recommended to reduce excretion of *Leptospire*s and zoonotic risk. A single intramuscular injection of streptomycin/dihydrostreptomycin at 25mg/kg will eliminate infection from most cattle. However, vaccination is the better approach avoiding unnecessary use of antibiotics. Past infection with leptospirosis make animal immune. There are several serovars of the *Leptospira* infection with one usually provides immunity to that serogroup but not to other serogroup.

How can leptospirosis be prevented?

Effective prevention and control measures can be achieved through proper diagnostic and prophylactic aids to curtail further spread as in most of the zoonotic diseases. Improved sanitary conditions including proper treatment and disposal of human waste, higher standards for public water supplies, improved personal hygiene procedures and sanitary food preparation are vital to strengthen the control measures. The risk can be minimized to humans by avoiding contact of water with animal urine, control of rodents and ensuring the proper vaccination of pets. Annual vaccinations, confinement rearing, and chemoprophylaxis are to be employed for animals. Management methods to reduce transmission include rat control, fencing cattle from potentially contaminated streams and ponds, separating cattle from pigs and wildlife, selecting replacement stock from herds that are seronegative for leptospirosis, and chemoprophylaxis and vaccination of replacement stock. Further, control rats and mice in areas around the animal sheds and farms, drain areas that have still, standing water and wearing gloves when disposing of dead animals and when gutting (cleaning) livestock or animals are vital to the control measures.



Guidelines for designing preventive measure

- To increase awareness among the healthcare personnel on the importance of leptospirosis.
- To guide in diagnostic procedures in order to obtain early diagnosis so that prompt and appropriate management can be instituted and prevention and control measures can be carried out at the earliest possible stage to reduce morbidity and mortality.
- To quantify and monitor leptospirosis disease burden and its distribution throughout the country.
- To obtain good epidemiological and clinical data on leptospirosis which is important for improving strategies in prevention and control of the disease.

Highlights of ICAR-NIVEDI *Leptospira* Research Laboratory

- ✦ Established state of the art facility in BSL2 ++ Laboratory of ICAR-NIVEDI for conducting basic, applied and molecular research work on *Leptospira* viz., Dark field microscopic examination, Microscopic Agglutination Test, Isolation and Maintenance of reference *Leptospira* serovars, Molecular Diagnostic PCR techniques and Typing of *Leptospira* isolates to species level by molecular based approaches.
- ✦ The *Leptospira* laboratory in NIVEDI has made a mark for its technical expertise in the field of veterinary fraternities in the country and recognised as referral laboratory / Nodal centre for surveillance, diagnosis and training for animal leptospirosis in one health programme.
- ✦ The research activities in leptospirosis since inception has led to: Development of simple *Leptospira* staining kit for diagnosis of leptospirosis; Development of transport medium for sending the field materials to laboratory and Recording of the *Leptospira* abortions in bovines and other animal species.
- ✦ Established the MAT for seroepidemiological study in different livestock species and humans using 18 reference pathogenic serovars specific strain.
- ✦ The seroepidemiological study of leptospirosis by MAT provided an overall prevalence of leptospirosis in livestock and the zoonotic potential of leptospirosis in endemic states of India.
- ✦ Providing need-based diagnostic services to livestock farmers, Veterinarians, Medical doctors, different stakeholders, etc., for leptospirosis.
- ✦ Surveillance /prevalence of leptospirosis study in livestock in endemic states of India using serum repository facility of institute.
- ✦ Recombinant multi-antigenic proteins based latex agglutination test for diagnosis of leptospirosis.
- ✦ Molecular diagnostic methods antigen detection made available for the specific



identification of *Leptospira* livestock and human (*rpoB* / LipL41 gene based analysis with other PCR diagnostic techniques.

- ✦ Multiplex PCR for detection and differentiation of pathogenic and non-pathogenic *Leptospira* species.
- ✦ First Report: Prevalence of *Leptospira wolffi* species in animals and humans in India was identified besides *L. borgpetersenii*; *L. interrogans*; *L. kirschneri* species based on the *rpoB* gene-based sequence and phylogenetic analysis.
- ✦ Established the prevalence of leptospirosis in livestock species in endemic states of India- Baseline data generated on the status of prevalence of *Leptospira* serogroup specific antibodies as well as sero-prevalence in livestock in endemic coastal states of India.
- ✦ Baseline data generated /determined on the status of prevalence of *Leptospira* serogroup specific antibodies in organized cattle dairy farm in India in the nine (9) states of country.
- ✦ Imparting training program or “hands-on” training to the research scholars, or research/medical officers /personnel in the *Leptospira* research area.
- ✦ A stakeholders meeting and workshop on laboratory capacity building for leptospirosis was jointly organized by ICAR-NIVEDI and Centers for Disease Control and Prevention (CDC), Atlanta, USA during 11th to 15th September, 2017 at ICAR-NIVEDI, Bengaluru, India highlighted the importance of surveillance and capacity building and ICAR-NIVEDI was identified to collaborate in all the aspects as a lead centre for animal leptospirosis surveillance.
- ✦ In the minutes of meeting of the National Technical advisory group for “Programme for Prevention and Control of Leptospirosis” formulated by National Centre of Disease Control (NCDC) held on 13th June 2019 has recommended ICAR-NIVEDI as one of the laboratory to be included in the program to strengthen as a regional human leptospirosis laboratory.
- ✦ ICAR-NIVEDI has been identified by NCDC, as a regional coordinator for the programme “Intersectoral Coordination for Prevention and Control of Zoonotic Disease” for catering the needs of Karnataka, Kerala, Lakshadweep, Telangana for Training, IEC & strengthening of intersectoral coordination activities such as laboratory support for diagnosis of identified zoonotic disease, facilitation of the meeting of state zoonosis committees, joint training of medical and veterinary professional, preparation of relevant IEC material etc.





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