Leptospirosis - an overview

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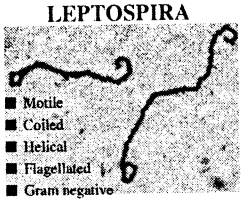
Leptospirosis is considered to be the most widespread, re-emerging zoonotic disease prevalent in the world. The disease is caused by a spirochete called Leptospira. The public health importance of this disease is extremely high in a Country like ours where there is close association between man and animals. Moreover the humid and wet tropical climate of Kerala is ideal for the growth of these deadly organisms. The high-density population, unhygienic surroundings and increased rate of construction of buildings in the urban and suburban areas of Kerala lead to an exponential growth in rodent population. Control of rodents by maintaining good sanitary conditions goes a long way in the control of spread of the disease. In India, leptospirosis is still labelled as "the lesser known greater malady" due to inadequate description of clinical manifestations.

Etiology

Leptospires represent uniquely featured organisms, which are often claimed to be difficult to isolate and to maintain over longer periods. Leptospires are elongated, thin and motile spirochetes. They may be free-living (saprophytic) or associated with animal hosts



(pathogenic) and they survive well in fresh water, soil, and mud in tropical areas. The Leptospira organisms belong to the Family *Leptospiraceae* and Genus *Leptospira*. The saprophytic organisms come under the species L. *Biflexa* and the pathogenic organisms belong to the *L. interrogans* species, which in turn contains more than 260 serotypes.

Transmission

Transmission occurs through direct or indirect route from a mammalian host. Rodents act as the reservoir host of these organisms. Wild animals and domestic animals like cattle, sheep, goat, cats, swine dogs, rodents, rabbits, guinea pigs, squirrels, hamsters, reptiles and marsupials, are the carriers of Leptospires. Once infected by Leptospirosis, the excreta of the reservoir hosts as well as the carrier animals will contain these organisms throughout their life. Anyone who comes into contact with this urine can get leptospirosis. Soil or water contaminated with the infected urine is also a potent source of the disease. The Leptospires enter the body through abraded skin, abraded mucous membrane of mouth, nose or through the conjunctiva of eye. The infections can occur through inhalation as well as through transplacental route. Human to human transmission has not been reported so far.

Leptospirosis exist as an occupational hazard to the workers in rice fields, sugar cane plantations, mines, sewer systems, and slaughterhouses; animal caretakers, veterinarians, travelers to tropical parts of the world, people involved in recreational activities in fresh water. Hence Leptospirosis has got synonyms like Weil's disease, Haemorrhagic jaundice, Canicola fever Swine handler's disease, Cane cutter's disease, rice field worker disease, Dairy worker fever etc. Farm labourers, cane workers, vegetable growers and cattle farmers Veterinarians and abattoir workers come under the high-risk group. You can catch leptospirosis even by swimming in ponds or rivers contaminated by the urine of carrier animals, during gardening in contaminated soil, while walking

in open sandals during rain in roads with open drains or even from walking barefoot in the early morning when the grass is wet.

Clinical Features

In humans the symptoms include fever, headache, chills, muscle pains especially of the neck, shoulders, back and legs, and eye irritation. There may also be nausea, vomition, loss of appetite and jaundice etc. Urgent medical attention is needed if you have persistent vomiting, have dark urine or pass much less urine than usual, or if your eyes turn yellow.

In animals leptospira infection causes severe economic loss in the animal production sector through abortion, lowered milk yields, infertility and death of newborns. Domestic animals such as cattle, pigs, sheep, goats and horses are severely infected.

Pathogenesis

Once the Leptospires enter the body it multiplies in blood and causes the Leptospiremic phase. The incubation period of the organism varies from 2 – 30 days. The initial septicaemic phase lasts for 3 – 5 days and is followed by complete recovery, or by a sudden worsening of symptoms – known as the immune phase. The immune phase varies from 4 – 30 days. If not treated, the patient could develop kidney damage, meningitis, liver failure, and respiratory distress leading to death of the patient. Hence early diagnosis of the disease is imperative for proper treatment of the condition.

Leptospirosis in Domestic Animals

Leptospirosis of domestic animals is a very complex disease.

Signs in cattle. Leptospirosis in cattle may vary in severity from a mild, in apparent infection to an acute infection that may cause death. The usual symptoms are high fever of 104° to 107°F, depression, loss of appetite, decreased milk production, and weakness. Haemoglobinuria, anemia, icterus, and bloody milk are also seen. In lactating cows a condition called flaccid mastitis occurs where the udder becomes flaccid and milk may contain thick yellow or reddish clots. Abortion, frequently the only clinical sign reported, generally occurs in the last trimester of pregnancy two to five weeks after initial infection. In some cases infection occurs mildly and the animal develops serologic titers without apparent clinical signs.

Signs in swine.

The disease in swine is largely subclinical except for abortions, which usually occur during the last two to three weeks of pregnancy. The sow aborts pigs rapidly with no apparent signs of illness. Some aborted fetuses may have been dead a short time. Piglets may be born weak and die shortly after birth.

Signs in horses.

The acute phase of the disease in horses following exposure is frequently subclinical. The infected animal may have a slight rise in temperature and mild loss of appetite. Within 12 to 14 months after the initial infection, the eyes of many horses show evidence of uveitis -a disease commonly known as periodic ophthalmia or "moon blindness." The amount of eye involvement is variable, and the disease may be arrested after one or two acute attacks, or may proceed through several acute episodes to total blindness.

Sheep and goat.

Leptospirosis occurs in sheep and goats with less frequency than in cattle and swine. The signs reported are similar to those described for cattle.

Dogs.

The incidence of canine leptospirosis is widespread. The acute disease in dogs is recog- nized as a bacterial disease causing elevated body temp erature, vomi- ting, muscular stiffness, weakness, and nephritis. In severe cases, jaundice and death may occur. Central nervous system signs may occur with or without other clinical signs, and organisms may be present in the brain tissue for extended periods. Chronic leptospirosis is primarily associated with chronic kidney degeneration. Shedding of leptospires in the urine may continue for over a year.

Acute renal failure in Dog

Normal dog kidney Haemorrhage in medulla

Clinical Materials

Since the organisms will be circulating in the blood of the patient, blood is the ideal clinical material in the initial stage of disease. Isolation from blood is not often successful because bacteraemia is transient and not always accompanied by clinical signs. Blood can be collected on the first day of fever it self. Any anticoagulant can be used for the collection of blood but heparin is the

preferred one. CSF is also a good clinical material in the acute phase of the disease. Serum should be collected after 2-3 weeks only as the antibodies take this much time to develop. The role of urine as a clinical material starts only after 2-3 weeks of infection when the leptospiruria stage starts. Demonstration of leptospires in the genital tract, kidneys, or urine merely indicates that the animal is a carrier. The organisms will survive in alkaline urine. But acidity is detrimental to leptospires. Hence care should be taken to alkalinize the urine of carnivores with PBS immediately after collection. Collection of urine after giving a diuretic will increase the chance of detecting the organism. Failure to demonstrate leptospires in the urine merely indicates that the animal was not excreting detectable numbers of leptospires at the time of testing. From dead animals, collect the pooled the tissue samples in glycerin.

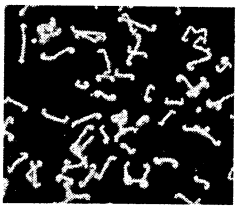
Diagnosis

Laboratory diagnosis of Leptospirosis is a tricky business as these organisms are extremely slow to grow. They require special nutrients like long chain fatty acids, vitamin B1, B6 etc for growth. More over, since they are extremely thin, the Leptospires cannot be viewed under ordinary light microscope and they do not take ordinary aniline dyes. So the diagnosis of Leptospirosis consists of tests for the demonstration of organism itself or their genetic material in animal tissues or body fluids. Antibody detection is another method for diagnosis. The selection of tests to be carried out depends on the purpose for which a diagnosis is to be made and the resources available.

The methods of diagnosis of Leptospira organisms are detailed below.

1. Dark field microscopy.

This is the fastest and easiest method to detect Leptospira organisms in clinical samples like urine and blood. The organisms appear as thin hair like structures with characteristic corkscrew movement and hooked ends under a dark field microscope. But unfortunately, DFM has got only 50% reliability. Most of the clinical samples will often contain artifacts that closely resemble the leptospires. Even an experienced worker may find it difficult to differentiate the organisms and the artifacts. The number of organisms present in the sample taken need not be that high to make a correct diagnosis. So, with the method of dark field microscopy, there is a



chance of getting false positive and false negative results if we are not careful.

2. Staining (Fontana Method)

Leptospires do not stain satisfactorily with aniline dyes, as they are too thin. Hence a special staining method known as the modified silver impregnation staining method called Fontana staining method was developed for staining the Leptospires. In this staining method the smear is fixed on to the slide with the help of glacial acetic acid and the thickness of the organism is increased by layering the silver salts on the soma of the organism and then they are stained. The Leptospires take the brown colour. This method also requires considerable experience to get a good quality slides.

3. Cultural isolation and identification

Isolation of leptospires by culture is the most sensitive method of demonstrating their presence. The Leptospires are very fussy in their nutritional requirement and they require special nutrients like long chain fatty acids along with Vitamin B1 and B12 for its growth. Bovine serum albumin containing 5-fluorouracil and Tween 80 or pooled rabbit serum provides the nutrients for the growth. Culture is usually carried out in EMJH media, which is a semisolid (0.1-0.2% agar) media containing BSA and Tween 80. Inoculated cultures should be incubated at 30°C for a period of minimum 6-8 weeks. Examine the culture at weekly intervals by dark-field microscopy. Dinger's ring can be observed at the sub surface level.

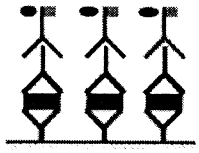
4. Microscopic agglutination tests – MAT.

MAT is the most commonly employed serological method for the diagnosis of leptospires. This is a simple agglutination test based on the principle of Ag-Ab reaction. In serial dilutions a drop of the live organisms from young culture (6-8 day old) is mixed with the patient's serum under controlled conditions. If the serum contains Ab, agglutination takes place, which can be observed after an incubation period under dark field. MAT is usually done on paired serum samples. A four-fold rise in Ab titre in the convalescent serum sample clearly indicates a current infection. MAT is serovar specific also. But because of its serovar specificity, a sample has to be tested against a large number of pathogenic serovars of leptospira in order to arrive at a result. The

biggest drawback of MAT is the difficulty in maintaining the young cultures of leptospires. The culture media is very costly and continuous sub culturing is time consuming. Chance of contamination of the culture is also very high.

5.ELISA Test.

Enzyme-linked immunosorbent assays for detection of leptospiral antigens as well as antibodies have been developed using a number of assay protocols and assay platforms like plates and dipsticks. AgELISA is based in the principle that when Ag and Ab react, an immune complex is formed. This reaction is done on a solid base. Another Ab, i.e. the monoclonal Ab detects this immune complex formed. Yet another Ab, which covalently linked to an enzyme, (conjugate) is allowed to react with the immobilized complex in the solid phase. Finally the sites of enzymatic activity are detected by the addition of a substrate when a coloured product is formed. This colour development is visible to naked eye and the intensity of colour can be measured with the help of an ELISA reader.



Detection of Leptospira antigen in the initial pyrexia stage itself by Antigen ELISA technique will save many lives of animals as well as humans. A single test will cost about 30 /- Dot ELISA kits are also available for Ag and Ab detection of Leptospirosis. But ELISA s lack the serovar specificity of MAT.

6. Molecular techniques

There are many advanced molecular techniques like Polymerase Chain Reaction, DNA fingerprinting and RAPD-PCR, which can be used for typing Leptospira serovars from clinical samples. All these methods, even though highly reliable, are expensive. Polymerase Chain Reaction (PCR) is an invitro method for the enzymatic synthesis of specific DNA sequence of the Leptospira organism, using oligonucleotide primers specific for pathogenic serovars. It can be done on the day of fever itself. PCR will give accurate results even if the sample contains a single molecule of the organism. So PCR is useful even in putrefied clinical sample. The rapid multiplication of target DNA sequence is achieved by a repeated cycling process under controlled temperatures. The amplified products can be viewed by conducting Gel electrophoresis and the result can be documented.

But the biggest drawback of PCR is that even contamination with saprophytic strains also give positive results. Moreover the test is expensive. A single test will cost around Rs.150/-

Treatment

Penicillin, Ampicillin, Doxycycline, Erythromycin, 3rd/4th generation Cephalosporins and Quinolones are found to be effective both in vivo and in vitro studies. Early antibiotic therapy has been shown to shorten the duration of illness. Simple analgesics and antipyretics like acetaminophen are adequate for the myalgia and fever. Dihydrostreptomycin, 10 mg per pound has been reported to be effective for termination of the carrier or shedder state in animals. In humans consider the possibility of leptospirosis in all fever cases with sudden onset and in animals think of leptospirosis in abortions, death of newborns, rose milk condition known as flaccid mastitis etc.

Control of leptospirosis

There is no widely accepted human vaccine.

- Strict sanitary measures should be adopted in the farms.
- People in high-risk group should wear appropriate protective clothing such as waterproof boots, aprons, and gloves and should cover cuts and scratches.
- Infected animals should be segregated and treated and recovered animals should be quarantined for a minimum period of 2 months.
- Avoid contact with urine of pet animals.
- Routine vaccination of pet animals
- Keep animals on well-drained land and supply drinking troughs with clean water.
- Buildings for food storage and processing should be rodent-proof.
- Routine screening for Leptospirosis

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