Review Article

Leptospirosis - A Bird's Eye View

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ABSTRACT

Leptospirosis, the most widespread zoonotic disease is currently emerging as a major public health problem. The causative organism of this disease is a spiral bacterium known as Leptospira with many serovars. Leptospira are antigenically diverse due to unstable antigenic composition of lipopolysaccharide (LPS) that brings out more than 200 recognized antigenic types (serovars) of pathogenic leptospira . The outbreak of Leptospirosis is usually associated with occupational exposure and also can occur sporadically or in epidemics. The clinical manifestations of human Leptospirosis are diverse, ranging from mild fever to severe disease known as Weil's disease, characterized by hepato-renal damage with pulmonary distress and hemorrhage that can lead to death. Laboratory diagnosis is usually performed by serological tests such as Enzyme-linked immunosorbent assay and the Microscopic agglutination tests. Limitations of serological tests are that antibodies cannot be found in acute phase of the disease and only specialized laboratories can perform serologic tests. This eventually leads to delay in therapeutic interventions of the acutely ill patients.

Keywords: Leptospirosis, Zoonotic, MAT, Macroscopic Agglutination Test.

INTRODUCTION:

Leptospirosis is a bacterial zoonotic disease of global importance. It is caused by pathogenic *Leptospira* species which are hook shaped motile spirochetes that belong to the family Leptospiracaece. Leptospirosis encompasses a wide spectrum of clinical and sub clinical disease in both humans and animals. Rats and other rodents are the most important sources.[1, 2] Livestock farming plays an important role as a major occupational risk factor for human Leptospirosis. The genus Leptospira is divided into two species, L. interrogans consisting pathogenic strains and L. biflexa consisting saprophyte strains isolated from the environment. These two species are divided into serovars based on agglutination of homologous antigen (approximately 60 serovars for L. biflexa, more than 225 for L. interrogans). More than 300 serovars have been identified based on serological tests of which around 200 serovars are considered pathogenic.[3,4] The disease follows a trend in small outbreaks or sporadic.[4,5,6] Spread over the whole year, it shows a marked increase in summer-autumn.[7] Clinical presentations of Leptopspirosis among humans range from asymptomatic infection to fatal zoonosis. The majority of human infections are mild, systemic illnesses that bring headache, chills, fever, conjunctival injection and muscle pain. All animal pathogenic serovars can also be pathogenic to humans. Transmission to humans occurs through penetration of the organism into the blood stream

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via cuts, skin abrasions or mucus membranes. This review article elucidates in a nutshell, the pathogen, diagnosis and the disease management of Leptospirosis that would assist in better understanding of this global crisis.

Morphology:

Leptospires are spiral shaped bacteria, which differ from other spirochaetes by the presence of end hooks. They belong to the order of Spirochaetales, family Leptospiraceae, genus Leptospira, about 0.1 μm in diameter by 6-20 μm in length. They are aerobic motile bacteria that can be visualised under dark field microscopy or phase contrast microscopy.

Leptospira species are also divided serologically into more than 200 serovars by agglutination tests.[1] They are regularly and tightly coiled with characteristic hooked ends resembling a question mark. There is a single endoflagellum at the pole that is responsible for the active motility. There is typical double membrane structure in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated. Outer membrane is present consisting of Lipopolysaccharide (LPS) that constitutes the main antigen of Leptospira. It is structurally and immunologically similar to LPS of other Gram negative bacteria.

Classification:

There are two families in the order Spirochaetales, Spirochaetaceae and Leptospiraceae. The causative agent of Leptospirosis, the genus Leptospira, belongs to the family of Leptospiraceae. Leptospira is divided into several species, serogroups and serovars, based on the lipopolysaccharide (LPS) antigens. Leptospira interrogans is an important species causing human disease which in turn consists of several sero groups and serovars.

Pathogenesis:

Leptospirosis is a zoonotic disease and direct human to human transmission does not occur. Numerous animals, primarily rodents are sources of human infection. Rodents are the most important reservoirs of Leptospires in which leptospires are located in the kidneys, with or without detectable clinical manifestations and excreted in urine that contaminates water.

This is an occupational disease for veterinarians, farmers, butchers, rodent control workers, and other occupations requiring contact with animals. Indirect contact with contaminated wet soil or water is responsible for the great majority of cases. Floods, rainfall, lower socio economic status, occupational exposure to rodent infested environment are the risk factors for Leptospirosis.

The usual mode of transmission is through mucous membrane, abrasions or cuts in the skin when they come in contact with urine or tissues of infected animals. After an incubation period of 8 to 10 days, it is presented with febrile illness due to the presence of Leptospires in the bloodstream known as septicaemic phase. Further hematogenous dissemination of the organism into various organs like liver, kidney, spleen and meninges causes damage to the endothelium of small blood vessels. It leads to localised ischemia in those organs, resulting in renal tubular necrosis, hepatocellular and pulmonary damage. Leptospires persist in the internal organs most abundantly in the convoluted tubules of kidneys and therefore excreted in the

urine in later stages of the disease. The patients present with fever, albuminuria, jaundice, conjuctivitis and rarely aseptic meningitis.[8,9] It is a fatal disease known as Weil's Disease with hepatorenal damage.[10]

LABORATORY DIAGNOSIS:

Specimen:

Blood and urine are the samples from which Leptospires can be demonstrated by microscopy, isolated in culture or animal inoculation and serological tests.

Microscopy:

Leptospires can be demonstrated from blood by dark field or phase contrast microscopy in the first week as they disappear after that period and it is helpful only in the early stage of the disease. Leptospires can be demostrated from urine from second week and they appear intermittently for 4 to 6 weeks. Centrifuged deposit of urine should be examined under dark ground or phase contrast microscopy soon after voiding as the organism will get lysed in urine.

Isolation:

Samples for culture should be collected prior to the administration of antibiotics. Blood, cerebrospinal fluid and dialysate should be cultured in the first 10 days of the illness, and urine from the second week of the illness. Culture is usually done from blood as urine culture is not successful due to contamination. Few drops of blood are inoculated into special media like Ellinghausen-McCullough-Johnson-Harris (EMJH) at 37°C for two days and left in dark at room temperature for two weeks. Primary isolation takes a long time, sometimes even months. Inoculated sample should be examined under dark field microscope every third day for the presence of Leptospires .

Animal Inoculation:

Intraperitoneal inoculation of patient's blood into guinea pig leads to development of fever with jaundice and hemorrhage into the lungs and animal dies within 8-12 days. The peritoneal fluid is examined under darkground microscope to demonstrate Leptospires.

Serology:

Antibodies start appearing at the end of first week and keep increasing till fourth week. There are two types of serological tests used to diagnose Leptospirosis.

Genus Specific Tests detect the infection without identifying the serovar. Enzyme-linked immunosorbent assay (ELISA) has been done for this purpose which detects IgM antibodies[11] in recent infection and IgG antibodies in past infection that react with a broadly reactive genus-specific antigen.

Type Specific tests identify the infecting serovar by demonstrating specific antibodies by Macroscopic Agglutination Test and Microscopic Agglutination Test (MAT). MAT is the gold standard test for serological diagnosis of Leptospirosis, in which live antigens representing different serogroups are reacted with serum samples and the agglutination is examined by dark field microscopy. MAT may be positive from day 10–12 after the onset of illness, sometimes later if specific antibiotics have been prescribed. MAT was reported to have a sensitivity of 41% during the 1st week, 82% during the 2nd to 4th week, and 96% beyond the 4th week of illness.[12] Several rapid tests have been used for diagnosis of

Leptospirosis, but because of their low sensitivity there are considered to be of limited value.[13]

The PCR assays are useful in rapid diagnosis of disease at early stages prior to the detection of antibodies. PCR detects DNA in blood in the first 5–10 days after the onset of the disease and upto the 15th day.[14]

Treatment :

Leptospires are sensitive to Penicillin and Tetracycline. Doxcycycline 100mg twice daily is the drug of choice currently.

Prevention :

General measures of prevention include rodent control, disinfection of water and wearing protective clothing. For chemoprophylaxis, Doxycycline 200mg can be used once a week.

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