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DIAGNOSIS AND EPIDEMIOLOGY OF LEPTOSPIROSIS LIMITATIONS OF PRESENT DIAGNOSTIC TOOLS- GENERAL REVIEW

Medical Microbiology

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ABSTRACT

Leptospirosis is commonly known as rat-urine disease, is a global but endemic zoonotic disease in the tropics. 1 Leptospira species are aerobic, spiral, Gram-negative bacteria that are distributed ubiquitously in the soil and environmental water. These organisms cause leptospirosis and are thereby associated with ill health and death of humans and animals. Leptospira are carried in the kidney tubules of a variety of animals and contaminate the environment (water, soil, marsh and other elements) by being excreted in large numbers in urine. Humans and other animals become infected, mainly through their skins and mucous membranes, when they encounter a leptospire-contaminated environment. Due to this correlation between organisms in the environment and disease transmission, isolation of leptospires from the environment is important for epidemiological studies as well as for prevention and control of the disease. However, isolation from environmental samples is challenging due to the slow growth of leptospires and overgrowth of co-existing microorganisms.[11]

KEYWORDS

INTRODUCTION

Leptospirosis is a zoonosis of global distribution, caused by infection with pathogenic spirochetes of the genus Leptospira. The disease is greatly underreported, particularly in tropical regions, but attempts at surveillance suggest that it may be the most common zoonosis. The disease is maintained in nature by chronic renal infection of carrier animals, which excrete the organism in their urine, contaminating the environment. Human infection occurs by direct contact with infected urine or tissues or, more commonly by indirect exposure to the organisms in damp soil or water. Most human infections are probably asymptomatic; the spectrum of illness is extremely wide, ranging from undifferentiated febrile illness to severe multisystem disease with high mortality rates. The extreme variation in clinical presentation is partly responsible for the significant degree of underdiagnosis.[3]

"Leptospira" derives from the Greek leptos (thin) and Latin spira (coiled). Aptly named, the leptospires are a mere 0.1 m in diameter by 6 to 20 mm in length.[3] It includes both saprophytic and pathogenic species comprising the genus Leptospira, which belongs to the family Leptospiraceae and order Spirochaetales.[2]

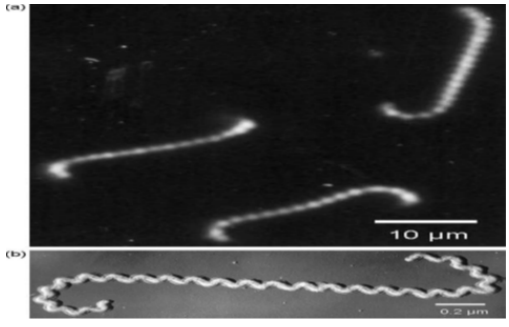
The cells have pointed ends, one or both of which is usually bent into a characteristic hook. Motility is conferred by the rotation of two axial flagella underlying the membrane sheath, which are inserted at opposite ends of the cell and extend towards the central region. Because of their small diameter, leptospires are best visualized by darkfield microscopy, appearing as actively motile spirochetes. Leptospires are readily cultured in polysorbate-albumin medium if specimens are obtained prior to initiation of antibiotic therapy.[3]

Historically, the genus Leptospira was classified into two species, L. interrogans and L. biflexa, comprised of pathogenic and non-pathogenic strains, respectively. Within each species, large numbers of serovars were differentiated using agglutinating antibodies. Serovar specificity is conferred by lipopolysaccharide (LPS) O antigens. More than 250 serovars of pathogenic leptospires have been described; because of the large number of serovars, antigenically related serovars were grouped into serogroups for convenience in serologic testing.[3]

Review Of Literature

Leptospirosis is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus *Leptospira*. In humans, it can cause a wide range of symptoms, some of which may be mistaken for other diseases. Some infected persons, however, may have no symptoms at all. Without treatment, Leptospirosis can lead to kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, respiratory distress, and even death.[8]

The *Leptospira* genus is classified in more than 300 serovars based on the structural heterogeneity of the O antigen lipopolysaccharide (LPS) detected by the Cross-agglutinin absorption test (CAAT) and 25 serogroups determined by the microagglutination test (MAT) Different molecular methods such as DNA-DNA hybridization, 16SrRNAanalysis, *Multilocus Sequence Typing* (MLST), and comparative genomics, have been used to identify 22 species of the genus *Leptospira.* Species are classified into three phylogenetic groups. 10 pathogenic, 5 intermediate, and 7 saprophytic, correlated also with the virulence of the bacteria.[7]



Pathogenic Leptospira[10]

Leptospira alstonii
Leptospira interrogans
Leptospira kirschneri
Leptospira noguchii
Leptospira alexanderi
Leptospira weilii
Leptospira borgpetersenii
Leptospira santarosai
Leptospira kmetyi
Leptospira mayottensis

Intermediates or opportunistic Leptospira[10]

Leptospira inadai
Leptospira fainei
Leptospira broomii
Leptospira licerasiae
Leptospira wolffii

Non-pathogenic Leptospira (Saprophytic)[10]

Leptospira biflexa
Leptospira idonii
Leptospira meyeri
Leptospira wolbachii
Leptospira vanthielii
Leptospira terpstrae
Leptospira yanagawae

Sources of Infection

Pathogenic leptospires are widespread in nature, reflecting maintenance in the kidneys of many wild and domestic reservoir hosts. The leptospiral life cycle involves shedding in the urine, persistence in the ambient environment, acquisition of a new host, and hematogenous dissemination to the kidneys through the glomerulus or peritubular capillaries. Once leptospires gain access to the renal tubular lumen of the kidney, they colonize the brush border of the proximal renal tubular epithelium, from

which urinary shedding can persist for long periods of time without significant ill effects on the reservoir host. Small mammals are the most important reservoirs, with large herbivores as additional significant sources of infection. Pathogenic Leptospira species have been isolated from hundreds of mammalian species, including bats and pinnipeds.[5]

In addition, leptospires have been recovered from poikilothermic animals such as frogs and toads, and it is possible that these animals play a role in the circulation of leptospirosis in the environment, although they may not be significant reservoirs of human infection.[5]

Transmission

Portal of entry include cuts and abrasions or mucous membranes such as the conjunctival, oral, or genital surfaces. Exposure may occur through either direct contact with an infected animal or through indirect contact via soil or water contaminated with urine from an infected animal. Individuals with occupations at risk for direct contact with potentially infected animals include veterinarians, abattoir workers, farm workers (particularly in dairy milking situations), hunters and trappers, animal shelter workers, scientists, and technologists handling animals in laboratories or during fieldwork.[5]

Current Status of Leptospirosis

Currently, leptospirosis is a disease of significant concern. The World Health Organization (WHO) estimates that globally the endemic human leptospirosis rate is 5 cases per 100,000 people annually and the epidemic human leptospirosis rate is 14 cases per 100,000 people annually. However, more recent estimates suggest that leptospirosis is "among the leading zoonotic causes of morbidity and mortality" and causes around 1,000,000 cases annually.[6]

Leptospirosis in the Environment: Soil

Leptospira, both pathogenic and not, have been found in soils globally. Contact with Leptospira in the soil is one of the leading routes of infection with leptospirosis, resulting in outbreaks across the globe. As mentioned previously, outbreaks of leptospirosis following floods, storms, and other mass precipitation events have been documented globally.

However, just as with the current understanding of Leptospira in water, very little is currently understood about the growth, survival, and long-term persistence in soils.[6]

Leptospirosis in the Environment: Water

While leptospirosis is reliant on the continuous cycle of transmission of Leptospira from infected to uninfected animals, there is growing evidence that there is long-term survival and viability of the pathogen in the environmental stage of the transmission cycle. This is important because the opportunity for transmission of the pathogen increases the longer that Leptospira survives and remains a persistent feature in the environment.[6]

Leptospirosis Enzootic Persistence in the Environment: Organisms of Concern

Based on our current understanding of the lifecycle of Leptospira, the enzootic persistence of the pathogen in water and soil is reliant on the continual excretion of the pathogen by infected animals. Leptospirosis affects many animal species, both domestic and wild . Despite most mammals serving as competent hosts and vectors of Leptospira, very little is known about the pathogen load excreted by different species or the role that specific species play in establishing and maintaining environmental sources of the disease.[6]

Leptospirosis in the Environment: Across the Urban–Rural Gradient

The prevalence, rate of transmission, and route of transmission of Leptospira is not uniform across the landscape. This is unsurprising as there are significant environmental differences across the urban–rural gradient, including to (but not limited to) differences in hydrology, soils, and animal life.[6]

Pathogenesis

In humans, leptospirosis has a very wide spectrum of clinical manifestations ranging from mild flu-like symptoms to serious complications such as Weil's disease and Hemorrhagic Pulmonary Syndrome (HPS), with a 40% fatality rate. This disease presents complex and dynamic epidemiology, due to the characteristics of the life cycle of the bacteria, which is involved among humans (susceptible hosts), animals (asymptomatic reservoirs), and ecosystems (environment).[7]

Leptospirosis consists of two phases: the leptospiremic (acute) phase and the immune (delayed) phase. During the leptospiremic phase (also called the septicemic phase), patient may experience a sudden onset of flu-like symptoms. This usually starts within two to 14 days after Leptospira infection.

In the immune phase, bacteria have moved from your blood to your organs. The bacteria is most concentrated in your kidneys, which make pee (urine). Urine tests will show signs of the bacteria and have antibodies to Leptospira in blood.[7]

METHODOLOGY

Conventional laboratory diagnosis usually depends on culture and serological techniques like microscopic agglutination test (MAT), ELISA and dipstick assay. Briefly, biological confirmation of leptospirosis is laborious and does not provide a rapid diagnosis. Isolation of leptospires from human blood is possible in the acute phase of the disease that lasts for up to about 10 days. Culture in EMJH (Ellinghausen McCullough Johnson and Harris) medium may take up to 2 months and does not provide an emergency diagnosis. Serological techniques are mainly used, however antibodies are undetectable before 8–10 days after disease onset. Moreover, MAT requires the maintenance of a large number of live Leptospira strains as source of antigens and paired serum samples are needed for the correct interpretation of the results.[4]

Microscopy

Direct Microscopic observation is used to detect leptospires in body fluids, check culture growths etc. Dark Field Microscopy is the usual method, but immunostaining is useful in certain special circumstances.[9]

Darkfield and phase contrast: Leptospires are seen as thin, bright, actively motile rods, moving with characteristic rapid spinning and jerking motility. Approximately, 10 leptospires/mL are necessary for one cell per field to be visible by darkfield microscopy. [9]

Culture

Fluid media are used for primary culture. Greater yields and faster growths are obtained in Tween (oleate)-albumin media such as EMJH (Ellinghausen, McCullough, Johnson, Harris) than media with rabbit serum.[9]

Molecular methods

Direct Polymerase Chain Reaction (PCR) on specimens enables rapid and direct diagnosis, at least in the early and convalescent stages of infection. The reaction detects leptospiral DNA in the specimen, down to extremely small amounts equivalent to the DNA content of about 10 leptospires or less. [9]

Microscopic Agglutination Test (MAT)

In the microscopic agglutination test (MAT), patients' sera are reacted with live antigen suspensions of leptospiral serovars. After incubation, the serum/antigen mixtures are examined microscopically for agglutination and the titers are determined.[5] The MAT is a sensitive assay, but because of the antigenic heterogeneity of Leptospira spp. requires a large number of serovars as antigens. In addition, it would not be useful at the early stages of the disease when the antibody to Leptospira spp. is not present or, if present, is at a low level in the CSF. Positive results are defined as a 4-fold rise in titre between acute and convalescent specimens.[9]

Enzyme Linked Immunosorbent Assay (ELISA)

This test relies on the detection of IgM antibodies which appear in the blood a day or so earlier than those used in MAT. There is often poor correlation between MAT and ELISA results on sera of individuals. Though Microscopic agglutination test is considered to be the gold standard in the diagnosis of leptospirosis, its use as a routine diagnostic test in a clinical laboratory is limited. The test is both complex and tedious for routine use.[9]

Indirect Haemagglutination Assay (IHA)

IHA testing is a rapid and easily performed method of diagnosis that is based on genus-specific antibodies. However, contrasting results have been obtained through various studies done to find the sensitivity and

specificity of IHA in early infections. It has been shown to have a sensitivity of 92% and specificity of 95% compared with MAT. [9]

Leptodipstick Assay

This is an assay that detects Leptospira-specific IgM antibodies in human sera.[9]

Antimicrobial Susceptibility

Leptospires are susceptible to β-lactams, macrolides, tetracyclines, fluoroquinolones, and streptomycin. Problems in the determination of susceptibility include the long incubation time required, the use of media containing serum, and the difficulty in quantifying growth accurately.[5]

Advantages and disadvantages of diagnostic tests for the detection of Leptospirosis[9]

Test	Advantages	Disadvantages
Dark Field Microscopy (DFM)	Visualize Leptospirosis	Lack of sensitivity and specificity. 104 Leptospires/ml is necessary for one organism/field to be visible under DFM.
IgM ELISA	Most widely used	False positive, IgM cannot be detected in early stages of infection and can persist in blood for years.
Microscopic Agglutination Test (MAT)	Gold Standard	Less sensitive in early phase of disease. Labour intensive and complicated procedure as there is a need to maintain Leptospira strain for preparing live antigen.
Polymerase Chain Reaction (PCR)	Successful in detecting Leptospira DNA in serum and urine samples of patients	Expensive reagents, Requires large quantity of DNA. Cannot identify the infecting serovar.

CONCLUSION

Leptospirosis is an uncommon disease that usually causes mild symptoms but can cause serious illness in a small number of people. It's important to know if your job or hobbies put you at risk. Knowing your risks, taking precautions and recognizing symptoms can help keep healthy and safe wherever life takes you.

Leptospirosis remains a major public health issue in many developing countries. Leptospirosis is expected to become more important due to a rapid urbanization in developing countries (slums), global warming, and extreme climatic events (floods).[10]

Determining the circulating species is important to understand its epidemiology, thereby to strategize appropriate control measures through public health interventions, diagnostics, therapeutics and vaccine development.[1]

Limitations

Despite significant ongoing advances in the field, a "gold standard" test to diagnose leptospirosis has yet to be established. Based on lack of consensus, even the "standard test" is debated. Issues related to the diagnosis of leptospirosis have led to a wide variation of practices across the globe, partially based on the resource availability to diagnose leptospirosis definitively. While the diagnosis directly affects the clinical management of the individual cases, it also affects the disease burden estimates, essential for informing public health policy.[12]

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