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LEPTOSPIROSIS: AN OVERVIEW

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Leptospirosis is an emerging infectious disease caused by the spirochaete, *Leptospira interrogans (L. interrogans)*, with increasing incidence in both developing and developed countries.[1] The infection is ubiquitous and is considered the most common zoonosis. [2, 3] Epidemics of leptospirosis occur in addition to sporadic cases. Though it is sub- clinical or mild in most cases, severe illness can sometimes end fatally. Prompt treatment instituted early during the illness can save the patient. Though eradication is thought to be difficult, control measures have been practiced.[2, 4, 5, 6]

Leptospirosis was recognized as a distinct clinical entity by Adolf Weil, a German physician in 1886, who differentiated leptospiral jaundice from other causes of infective jaundice. Weil described the severe syndrome characterized by jaundice, myalgia, fever, acute renal failure and tissue haemorrhage. The causal organism was discovered by Japanese workers, Inada and Ido. They demonstrated that rats were the natural vectors, inoculated guinea pigs with tissues from infected rats and demonstrated the presence of the organisms at each stage of the experiment.[2] In India, leptospirosis was first identified in the Andaman Islands.[7]

MORPHOLOGY

Leptospires are so called because they are thin and spiral organisms (G. *Leptos*: fine + *speira*: a coil). They are 0.1μm in diameter and 6-20μm in length. The coils are tightly wound. The organisms are filterable. Leptospires appear as protoplasmic cylinders covered by a cell membrane, cell wall and are enveloped by a sheath of 3-5 layers. They possess two flagellae which arise from basal bodies situated at each pole. The flagellae wrap round the organism and the free end of the flagella is in the middle of the bacteria.

The pathogenic leptospira have either one or both ends bent in the form of a hook resembling a question mark. Leptospiral cell wall shares the properties of both Gram-negative and Gram-positive bacteria. Leptospires have a double membrane cell wall like Gram- negative bacteria, but they have the peptidoglycan attached to the inner membrane like Gram -positive bacteria. This property renders them susceptible to antibiotics which act on both Gram-positive and Gram-negative bacteria.[1, 4, 8]

METABOLISM AND BIOCHEMICAL PROPERTIES

Leptospirae are obligate aerobes with optimal growth temperature of 30ºC. They

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produce both oxidase and catalase. They require ammonium salts, and long chain fatty acids which are used as carbon and energy sources; they also require vitamins B_2 and B_{12} as growth factors.[8]

CULTURAL CHARACTERISTICS

Leptospires are chemo-organotrophs, they grow in media containing serum or albumin with polysorbate. Several liquid media containing rabbit serum have been described by Stuart, Fletcher, Korthof, and Noguchi. The most widely used medium is the oleic acid-albumin based EMJH (Ellinghausen, McCullough, Johnson, Harris) medium. It is supplemented with 1% agar to enhance primary isolation. It contains Tween- 80 and bovine serum albumin. The medium can be rendered selective by addition of 5- fluorouracil or neomycin.[8,9] Protein free synthetic media have also been developed for vaccine production.

Leptospires take 3-4 weeks to grow and cultures have to be retained for 13 weeks. In semisolid medium leptospires first grow as a discrete zone beneath the surface of the medium which becomes increasingly turbid as incubation proceeds. This is called Dingers ring or disc. The formation of Dingers ring is related to optimal oxygen tension. Leptospires stand lyophilization and storage at -70°C.[8]

CLASSIFICATION

Leptospires have been classified mainly into two species. Those which are pathogenic fall under the species *L.interrogans* (question mark like) and the non pathogenic saprophytes belong to the species *L.biflexa* (flexed at both ends). The saprophytes are found in small streams, lakes and stagnant water. They cannot grow in low temperatures. There are about 268 serological variants which belong to the species *L.interrogans* called serovars.[1] Serovar specificity rests with the lipopolysaccharide O antigen similar to other Gram-negative bacteria. These serovars are grouped into 25 sero groups. Each serovar is given a specific name: icterohaemorrhagiae, grippotyphosa, australis, autumnalis, etc. There appears to be an association of some serovars with certain animal species.[8]

Now *Leptospira* are classified into number of species defined by their genetic relatedness as seen with DNA reassociation. This classification is supported by 16s RNA sequencing and is different from serological classification into serovars. There are currently 13 named genomic species and 4 unnamed ones: *L.interrogans, L.kirschneri, L.borgpetersenii, L.santarosai, L.noguchii, L.weilii, L.inadai, L.biflexa, L.meyeri, L.fainei, L.wolbachii, L.alexanderi, L.parva*, Genomo species 1, Genomo species 3, Genomo species 4 and Genomo 5.[10] Some of the genomo species include both pathogenic and nonpathogenic strains. However the classification into serovars is retained for epidemiological purposes.[8,9]

GENOMIC STRUCTURE

The size of the leptospiral genome is approximately 5000 kb. The genome has two chromosomes one which is 4400 kb (C I) and another smaller one which is 350kb (C II).The genome has several repetitive elements and many of these are insertion sequences coding for transposases. There are two insertion sequences found in many serovars: Insertion sequence 1533 (with a single open reading frame) and insertion sequence 1500 (with 4 open reading frames). The copy number of these varies between different serovars and isolates of same serovar. Within serovar icterohaemorrhagiae the genome appears to be conserved.

Leptospires contain two sets of 16s RNA and 23s rRNA genes, but only one 5s rRNA gene. The ribosomal RNA genes are widely

spaced. A number of leptospiral genes have been cloned and analyzed. [1,8]

EPIDEMIOLOGY

Leptospirosis is a direct anthrapozoonosis, transmitted directly between animals and man or indirectly, mostly through the vehicle of contaminated water or soil. Rodents play a very important role, as do small animals, which maintain leptospirosis. Four rodent species seem to be involved in India: *Rattus norwegicus, Rattus rattus* (house rat), lesser bandicoot (*Bandicota bengalensis*) and larger bandicoot (*Bandicota indica*). [5] The rats are known to be infected for life time. [5] In addition to rodents, larger animals: cattle, pigs, goats and dogs are also known as important reservoirs. Animals that acquire infection do not usually develop illness; they have been termed as maintenance hosts. Leptospira lurk in the kidneys of these animals and are shed in urine. The association of some of the serovars with certain animals may be significant; rats with Icterohaemorrhagiae, cattle with Hardjo, Hebdomadis, Grippotyphosa, dogs with Canicola, pigs with Pomona, Tarassovi and Bratislava. [8]

The leptospires, shed in the animal urine, can survive in a neutral pH or slightly alkaline water and they can also survive in the soil even after the rats have disappeared. [7] Human beings acquire infection directly by contact with animal excreta, tissues, animal products or by indirect contact with contaminated water or soil. Thus pet fanciers, veterinarians, abattoirs, laboratory animal handlers and rat trappers may be exposed to leptospires directly. Sewage workers, farmers and coal miners may be exposed indirectly by contact with contaminated water and soil. [2] Leptospirosis was associated with rats in the trenches of 1st world war. [7] Unlike plague, humans get infection without any rats in sight. [7]

Leptospiral infection does not spread from man to man usually. However occasionally intra-uterine, sexual, transmission through mother's milk and by close contact with patients or patient's urine have been reported. But, for epidemiological purposes man is the dead end host. [1,7]

Leptospirosis is found worldwide, however, humid tropical and sub-tropical regions provide optimal environmental conditions. [3] Historically, Leptospirosis has been called by different names based on the clinical picture and the place where the disease was seen. It has been called the yellow fever of temperate zones, the mud fever, marsh fever or field fever in central Europe and is associated with *L.grippotyphosa*. It has been called 7 day fever in Japan and is associated with the serovar *L.hebdomadis*. It has been called swine herd disease in Australia and is associated with serovar *L.pomona*. The association of the fever with rats during the harvest has given the name "harvest fever" to the disease (associated with agriculture). It has also been called: mouse fever, fish handler's disease, rice field fever or water fever. [2]

EPIDEMIOLOGICAL TRANSMISSION PATTERNS[11]:

Sporadic infections and epidemics caused by leptospira have been recognized with four epidemiological patterns of transmission: a) rural, b) urban, c) recreational and d) natural disaster associated.

a) The rural pattern seen in agrarian communities of developing countries is associated with large number of farm animals, monsoon season, sowing and harvesting activities. b) The urban transmission pattern is seen in overcrowded cities of developing countries; it is associated with poor drainage system, stagnant water, and sewage canals swarming with rats and bandicoots. Upsurge of infections occur during the rainy season; infections in Chennai, Mumbai, Madurai[12] in

India, El Salvador in Brazil, and Hawaii in USA seem to represent this pattern. c) Recreational leptospirosis is associated with water sports such as swimming, boating, water skiing, rafting, and recreational activities like fishing, school children bathing in a water channel etc. d) Disasters such as floods and cyclones provide conditions for prolonged exposure; people wade through contaminated stagnant water and get infected in large numbers. Outbreaks that occurred in Orissa in1999 following super cyclone and floods and in Mumbai following heavy rainfall in 2000 and 2005 in addition to similar outbreaks reported from Philippines and Thailand provide examples.[11]

Leptospirosis is considered a single disease caused by many serovars with protean manifestations ranging from sub clinical or mild febrile illness to severe disease with multi-organ failure. Some serovars seem to affect predominantly certain organ systems such as liver causing jaundice, kidney causing renal failure, brain causing meningitis, or lungs causing pulmonary hemorrhage. However, the wide variation in clinical manifestations prevents one from tagging a particular serovar with disease involving a target organ. Thus Weil syndrome is preferred to Weil's disease.[7]

Leptospirosis is considered to occur more commonly in coastal regions of Kerala, Tamilnadu, Gujarat, Maharashtra, Karnataka and Andaman islands.[13] Sero-prevalence rates of 29.4% and 54.2% have been recorded among villagers of Kerala and Andaman respectively.[14,15] In northern India Leptospirosis is not considered as a major public health problem because of low transmission in arid weather.[16]

SEROGROUPS IN INDIA

Many serovars have been reported to be prevalent and causing disease in India. Leptospira belonging to 4 serogroups: Autumnalis, Icterohemmorhagiae, Grippotyphosa, and Australis were thought to cause febrile infections commonly in India in a multi centric study based on the results of the Microscopic Agglutination Test (MAT).[13] Leptospira belonging to these serogroups and other serogroups such as Pomona, Canicola, Terrassovi, Bataviae, Hebdomadis, Sejroe, Javanica, Ballam, and Pyrogenes have also been reported in patients from different parts of India.[6,16,17]

LABORATORY DIAGNOSIS

Laboratory diagnosis of leptospiral infections can be made by direct evidences or by indirect evidences. The direct evidences include isolation of the organism, demonstration of antigen in blood,[18] urine or amplification of a specific fragment of leptospiral DNA by PCR.[1,6,7] The indirect evidences include detection of leptospiral antibodies by serological tests.

Microscopic demonstration of leptospires by dark ground microscopy (DGM), has been claimed to be a standard screening test for early and rapid diagnosis of leptospirosis.[19,20] Leptospires appear as a series of small dots under DGM.[2] Subjecting the fluid samples to double centrifugation at low speed to sediment the cellular elements first and then followed by high speed centrifugation may concentrate leptospires and increase sensitivity.[9,19] DGM has not been accepted universally for diagnostic purposes; as it is considered insensitive and the results non specific.[2,8] However, DGM is helpful in monitoring growth of leptospira in liquid media and to detect leptospiral agglutination in MAT. Staining methods have the same limitations as of DGM.[21] Culture is known to give low isolation rates and is time consuming. Inoculation of specimen into the peritoneal cavity of guinea pigs and hamsters, though sensitive is not recommended to be used routinely.[2]

Among the antibody detection tests, MAT is considered the gold standard when paired samples are tested and sero-conversion or a four-fold rise in titre is demonstrated. It has been used to report serovars against which the antibodies are directed. The MAT may not accurately identify the serovar causing infection in an individual patient. However presumptive serogroup reactivity data obtained by MAT is useful to gain a broad idea of serogroups infecting the population of a geographical region.[22]

There are many rapid diagnostic tests for diagnosis of leptospirosis: IgM ELISA, Micro-capsule agglutination test, Lepto dip-stick (IgM assay), Lepto lateral flow (IgM assay), macroscopic slide agglutination test (MSAT)[23], indirect haemagglutination, Lepto Tek Dri-Dot latex agglutination test.[24,25] These rapid tests have 82-96% sensitivity and 89-98% specificity when compared to the gold standard. These serological tests have low sensitivity in the 1st week of illness and acceptable sensitivity during 2nd week of illness .The disadvantages of these tests is that they are known to cross-react with a host of non- specific antibodies such as those in autoimmune disease, Hanta virus infection, HIV infection, Dengue etc.[25] A patient with a compatible history may be considered to have current leptospirosis if he has IgM antibodies to leptospira and a MAT titre of ≥ 80.[26]

Molecular techniques are helpful for early diagnosis of leptospirosis and also have a role in molecular epidemiology. The DNA analysis can sometimes distinguish between antigenically similar types.[7] PCR based DNA finger printing methods are being routinely used for characterizing leptospiral isolates.[24] These include random amplified polymorphic DNA (RAPD) finger printing, arbitrarily primed PCR (AP-PCR), single nucleotide polymorphism of specific PCR product, repetitive extragenic PCR(REPPCR), Fluorescent amplified fragment length polymorphism (FAFLP).[24] FAFLP analyses of isolates from patients during epidemics and from sporadic cases in Andaman Islands have shown that the outbreak associated isolates formed a single tight cluster.[27]

PATHOGENESIS AND CLINICAL FEATURES

Leptospires gain entry into the body through cuts in skin or mucous membranes.[4,8] They are thought to pass through water logged skin; bathing in contaminated ponds, using stream water for domestic purposes,[6,15] barefoot walking and immersing feet with abrasions during agricultural operations carry the risk of leptospirosis. Ingestion and inhalation do not pose a risk.[4] The incubation period is 2-30 days.[8]

Infection by leptospires results in sub-clinical or a mild febrile clinical illness, in about 90% of cases.[28] Leptospirosis was diagnosed in 14.7% of acute febrile illness from different parts of India.[13] The clinical presentation of leptospirosis is biphasic: an acute leptospiraemic phase lasting for a week, followed by an immune phase characterized by antibodies in blood and excretion of leptospires in urine.[8] The complications occur due to localization of leptospires in the immune phase during the second week of illness.

The illness during the first week is called anicteric illness. It is characterized by fever (100°-105° F), chills, headache, myalgia, abdominal pain, conjunctival suffusion and an evanescent skin rash lasting for less than 24 hours.[1] The clinical picture during this phase is non-specific and resembles viral infections like Dengue or Influenza. Intense myalgia affects the lower back, thighs and calf muscles with raised CPK levels. Skeletal muscles of the leg may show focal necrosis of isolated muscle fibres with neutrophil, plasma cell and macrophage infiltration. Leptospires can be found in the CSF in the latter part of the anicteric phase causing

aseptic meningitis. Aseptic meningitis is seen more often in children. Death is unusual during the first week; however 2.4% of Chinese patients during an outbreak died of pulmonary haemorrhage in the first week.[8]

The icteric illness is seen usually in 5-10% of infected patients. The leptospires localize commonly in liver, kidney, lung and heart which results in multi-organ involvement. The presentation depends upon the predominant organ involved. The main pathological event is vasculitis with endothelial damage and infiltration with monocytes, plasma cells, polymorphs and macrophages.

The classical Weil's disease includes fever, jaundice, renal involvement and splenomegaly. Jaundice is due to involvement of liver which is seen in as many as 80% of patients in some studies and has been a feature of patients seen in epidemics.[6,27,29] Serum bilirubin levels may be very high and take weeks to settle down.[8] The pathological picture shows intrahepatic cholestasis with hypertrophy and hyperplasia of Kupffer cells. However in paediatric patients, absence of jaundice in leptospirosis may be important, though hepatomegaly may be commonly seen.[30]

Renal involvement occurs in 16-40% of cases.[8] Renal manifestations may range from pyuria, albuminuria, hematuria and granular casts to severe renal failure. Kidney shows *interstitial nephritis with intensive* polymorphonuclear and monocyte infiltration. Leptospires can be seen in renal tubules.

Pulmonary involvement may be a major manifestation of Leptospirosis in some parts of the world like Andaman Islands.[8,27] Varying degrees of cough, breathlessness and haemoptysis are seen in this disease. The disease may be severe enough to cause death in 10-15% of patients.[27] Pulmonary congestion and haemorrhage are seen, if lungs are involved. There is infiltration by neutrophils and monocytes into the alveolar spaces.[8,10] Leptospires can be found in the capillaries of intra-alveolar septa.

Cardiac involvement in Leptospirosis is said to be common but underestimated. Fatal myocarditis has been reported with a mortality rate of 54%.[8] ECG may record abnormal T waves. Interstitial myocarditis, petechial haemorrhages, coronary arteritis and pericardial effusion may be seen if heart is involved.

Transient thrombocytopaenia i.e. a platelet count less than 10^5/ml occurs in more than 50% of the cases and is thought to predict renal failure.[8]

Unlike other spirochaetal infections, there is no objective evidence for chronic or latent infection in Leptospirosis unlike other spirochaetal infections. However uveitis seems to be an exception; it is hypothesized that recurrent leptospiral uveitis in man could be due to an autoimmune mechanism. A large cluster of uveitis cases have been reported from Madurai during an outbreak following heavy floods.[12] Some patients develop gastroenteritis characterized by abdominal pain and vomiting associated with diarrhoea or constipation.[1]

PATHOLOGY

Four pathological mechanisms have been incriminated in the causation of the above clinical features: Attachment, surface proteins, toxins and immunopathology.[8] Leptospires attach to vascular epithelial cells, renal epithelial cells and they also adhere to the neutrophils and platelets. Neutrophils phagocytose leptospires but the organisms are not killed. Their adhesion to platelets may be related to thrombocytopaenia seen in the illness.

The surface proteins which constitute the outer envelope appear to be involved in

pathogenesis of tubulo-interstitial nephritis. Haemolysins are chemically sphingomyelinases. They have been demonstrated in serovars Pomona and Ballam which are known to cause haemolytic disorder in cattle and hamsters respectively. Serovars Tarassovi and Hardjo are also known to elaborate haemolysins. Phospholipase-C, sodium and potassium ATPase inhibitors have been shown to occur in serovar Canicola.[8]

Immune complexes are thought to cause inflammation and have been postulated to be of importance in CNS Leptospirosis. In horses, the antibodies produced against epitopes of equine strains are known to cross react with ocular tissues and cause recurrent uveitis. Similar autoimmune mechanism has been postulated to be operating in human uveitis also.[2,12] Anti platelet antibodies, anti cardiolipin antibodies, and anti neutrophil cytoplasmic antibodies have been demonstrated in leptospirosis; their pathological role is not conclusively proved. Cytokines such as TNF-α liberated in response to leptospiral lipopolysaccharide are also hypothesized to play a role.

PREVENTION

Four strategies that have been explored for the control of leptospirosis are mass immunization of domestic livestock, vaccination of humans, rodent control and personal protective measures.

For mass immunization of domestic livestock, the vaccine has to be serovar specific. This strategy is useful when fewer serovars are prevalent. An ideal vaccine for livestock should prevent clinical disease in the livestock and also prevent dissemination through their urine. Killed leptospiral vaccines have been used for this purpose. Vaccination of dogs has caused serovar Canicola to disappear from UK and vaccination of cattle has reduced serovar Hardjo infections.[4] Twice yearly vaccination with killed vaccine directed against the serovars Pomona and Tarassovii have been used in pigs.[2]

Human leptospiral killed vaccines have been used in Vietnam, China and Japan. The indications for vaccine include people living under wet tropical conditions in proximity of rodents, military personnel, sewage workers and farmers cultivating rice.[2]

Rodents are the most important animals in the zoonosis of leptospirosis.[5] Rodent control involves removing rubbish especially waste food and prevention of access of rats to buildings. Rodent control programs have to be synchronized with the rodent breeding season. Rodents breed with the start of South West monsoon leading to more leptospiral infestation related to flood waters in India. Therefore ideally rodent control should be undertaken during the pre-monsoon season. When live burrows are more than 50/hectare (severe infestation), the drug to be used is Zinc phosphide. If less than 50 burrows/hectare (moderate infestation) single dose of Bromadiolone in cereal baits may be used. In villages in Gujarat, Tamilnadu, and Andhra Pradesh in India, rodent control has been attempted by using Bromadiolone mixed with broken rice with a control success of above 80%.[5] Farm houses may be treated with multiple dose anticoagulant Coumatetralyl TP in cereal baits, where non target animals are many in number.[5]

Personal protective measures such as prevention of exposure of cuts to water, wearing footwear and showering promptly after immersion of any part in dirty water are recommended.[4] An efficiency of 95% has been recorded for pre-exposure chemoprophylaxis with Doxycycline in the soldiers visiting an endemic area.[28] Chemoprophylaxis brought down the incidence of clinical illness in Andaman Islands.[27]

Various attempts in development of leptospiral vaccines are in progress-recombinant leptospiral proteins, OMP lipoproteins and virulence factors have been tested for their usefulness as vaccine candidates. Leptospiral external membrane protein LipL 32 conserved in many serovars, which is the major target of human immune response has been cloned and expressed in mycobacterial vectors and is undergoing trials. [1] At least two DNA vaccines-one encoding for haemolysin protein and another which is a gene encoding for endoflagella are under study. [1]

TREATMENT

Antibiotic treatment is effective within 7-10 days of infection and it is to be given immediately on diagnosis or suspicion, because organ damage sets in by the 2nd half of 1st week and late antibiotic treatment does not have any influence on the outcome. [14] The antibiotic of choice is Benzyl Penicillin IV in a dose of 5-6 million units /day for 5 days. In patients who are allergic to Penicillin, Erythromycin 250mg, 4 times daily for 5 days is recommended. [28] Azithromycin 15mg/kg body weight twice daily for one week was tried and 72% of patients responded completely. [31] Alternatively, Doxycycline 100mg twice daily for 10 days may be given. Cefotaxime has been used for severe leptospirosis. [28] *Tetracycline* can be used as an alternative; however one has to keep in mind its contraindication in children, pregnant women and patients with renal insufficiency. [1]

Supportive measures which include early hydration to prevent hypotension, oliguric renal failure and electrolyte imbalance are required. For renal failure, peritoneal dialysis or haemodialysis may be needed. Thrombocytopaenia is usually self limiting, but some studies have shown use of corticosteroids with varying results. [28]

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