Commentary

Leptospirosis - a reemerging disease?

The turn of the century will be remembered for its many emerging and reemerging infections. The resurgence of interest in leptospirosis has been due to its potential to occur in epidemics in both tropical and temperate climates and in both developing and developed countries. Epidemics of the disease have been reported from India and many parts of the world at the turn of the century. Various factors that have contributed to the reemergence of this infection include disturbances in natural ecological niches, increase in international travel and an improvement in diagnostic facilities resulting in better detection of these infections. Though the syndrome of icteric leptospirosis presenting with renal failure was first described over a century ago, it still poses a challenge to the diagnostic laboratory.

Leptospirosis is ubiquitous in distribution and has the dubious distinction of being both an occupational disease and a zoonosis. All occupations related to animal handling are at a great risk. Individuals at risk include farmers, abattoir workers, sewer workers and miners. The organism enters through minor cuts and abrasions, which are contaminated with discharges of infected animals. Indirect contact with infected animals via water or soil contaminated with infected urine is a more common source of human infection than direct animal contact. Worldwide, rats are the most common source of human infection than direct animal contact. Worldwide, rats are the most common source of human infection though dogs, livestock and cats also contribute. Even immunized dogs can excrete leptospires in their urine over long periods.

The causative organism of the disease is the finely coiled motile spirochete with bent or hooked ends. All pathogenic leptospires belong to the species Leptospira interrogans. More than 250 serovars of pathogenic leptospires are recognized. The free living saprophytic Leptospira belong to the species L. biflexa and though widely prevalent in the environment, are not usually associated with disease. Though a reclassification of the genus on molecular basis has been done, it is incompatible with the serovar-based identifications being done by clinical microbiologists and epidemiologists. In the absence of simple DNA based identification systems, the new nomenclature is likely to add to the difficulties prevailing in the laboratory diagnosis of leptospirosis. Thus the approach recommended by Natarajaseenivasan et al in this issue using outer membrane proteins, which are conserved in all the serovars, for the diagnosis of leptospirosis may be a useful beginning towards developing simple diagnostic tests for the clinical diagnosis of leptospirosis. This would result in a more realistic estimate of the disease burden.

Leptospirosis at present is grossly underreported and a diagnostic dilemma because of its protean clinical manifestations. Thus, the term ‘great imposter’ has been applied to this spirochetal infection. The spectrum of disease ranges from a mild inconsequential febrile illness to a severe fatal form of the disease presenting with multi organ failure conventionally called ‘Weil’s disease’.

The more common syndrome, anicteric leptospirosis, is a self-limited illness that occurs in 90 per cent of the cases. The illness usually starts after an average incubation period of 10 days with headache, fever and severe muscular pain with anorexia, nausea, vomiting and abdominal pain in most patients. The most common physical finding is conjunctival suffusion. The symptoms are prominent for 4-7 days i.e., during the septicaemic phase of the disease. At the end of this phase defervescence occurs and the patient is usually afebrile for a day or two before the immune phase begins. This phase is characterized by the presence of circulating antibodies and its duration ranges from 4 to 30 days. Involvement of other organs also occurs in this phase. Aseptic meningitis is the hallmark of the immune phase.
Icteric leptospirosis or Weil’s disease is seen in about 10 per cent of the patients. The biphasic course of the disease is obscured by severe and persistent fever, jaundice and azotaemia. The jaundice is not associated with hepatocellular necrosis. There is marked elevation of serum bilirubin and creatinine phosphokinase with only modest elevation of transaminases in these patients. Severely jaundiced patients are the ones more likely to exhibit renal failure, haemorrhages and cardiovascular collapse. Thrombocytopenia occurs in most patients in renal failure.

The myriad clinical manifestations of leptospirosis make it imperative that the microbiology plays a major role in diagnosis.

Isolation of leptospires from clinical samples gives a definitive diagnosis and also aids in identifying the infective serovar. Leptospires grow well on Fletcher’s or the commercially available Ellinghausen, McCullough, Johnson and Harris (EMJH) medium. However, a prolonged incubation, from 3 to 13 wk may be required. Leptospiraemia occurs only in the first 10 days of the disease therefore blood cultures need to be done soon after the onset of clinical manifestations to be useful. Leptospiruria is present from the second week onwards. However survival of the leptospires in voided urine is limited unless its acidic pH is neutralized. Thus, culture techniques have not played a significant role in clinical diagnosis.

Leptospira can also be visualized in clinical material by dark field microscopy, immuno fluorescent staining or bright field microscopy after appropriate silver impregnation staining. Microscopy of blood, urine, CSF has been attempted but found to be insensitive and lacking in specificity as it requires expertise to differentiate the organisms from fibrils and other extraneous elements. DNA amplification using PCR assays can detect as few as 1-10 leptospires per ml of sample. However these techniques are still being standardized and are out of reach of most diagnostic laboratories.

So, serology continues to be the mainstay of diagnosis. Antibodies are detected in the blood by the 6th to 12th day of illness and reach to the maximum titre in 3rd or 4th wk. Though the microscopic agglutination test (MAT) continues to remain the gold standard for diagnosis, it has its limitations. The immense serovar diversity among pathogenic leptospires necessitates maintaining of a large battery of serovars and thus restricting the use of the test. The test is also complex to perform and interpret. Paired sera are required to confirm a diagnosis with certainty. A four-fold or greater rise in titre between paired sera confirms the diagnosis. Titres following acute infection may be extremely high or seroconversion may be delayed up to several weeks after recovery. The lack of a good and user friendly diagnostic test has led to a plethora of tests being developed which include latex agglutination (LA), haemagglutination assays (HA) and microagglutination assays (MCAT). Recently ELISA, dot ELISA and gold immuno blot techniques have been developed. The complexity of the antigens present in the genus Leptospira has contributed to limiting the usefulness of all these techniques. In the present issue Natarajaseenivasan et al have tried to increase the sensitivity and specificity of currently available techniques by identifying outer membrane proteins (OMPs), which elicit a humoral response during the acute and convalescent phases of the disease. They extracted and identified 15 different OMPs. Nine of these antigens stimulated an IgM response while 14 of them produced an IgG response. The p14 antigen stimulated only an IgM response and could thus be used for diagnosis in the early stages of the disease. However, if a combination of P14, p25, p32, p41/42 were used as antigens in an immunoblot format, the positive predictive value (PPV) of the test increased to 94.3 per cent, which was much higher than the PPV of all existing serological kits. A different combination of antigens, which would detect IgG antibodies, have been advocated for seroepidemiological studies.

The availability of simple and efficient tests for diagnosis would assist in an early diagnosis of a patient of leptospirosis, a more accurate estimate of the disease burden and better demarcation of endemic areas of the disease. This is important as early institution of therapy with penicillin or ampicillin results in a good prognosis and low mortality. The same antibiotics if instituted late in the disease may
not be able to reduce mortality, as the destructive effect of the organism and immune complexes on the various organs and tissues is difficult to reverse. Doxycycline when used in conjunction decreases the renal excretion of leptospires.

The authors have put forward a concept of identifying specific protein subunits which are immunogenic and using them as diagnostic markers. Enlarging on the same concept probably these subunits could also be evaluated as potential vaccine candidates.

At present the prevention of human leptospirosis is very difficult at it is impossible to eliminate animal reservoirs. Effective rat control, and vaccination of livestock and pet animals can assist in decreasing the widespread prevalence of this disease. An effective human vaccine has still not been developed. Preparations of leptospiral lipopolysaccharide (LPS) can elicit protective immunity, but this is generally serovar specific and a vaccine would require using a combination of commonly prevalent serovars in an area. Given the inherent difficulty in preparing multivalent LPS vaccines the identification of conserved protein antigens for use in vaccination is of critical importance.

With the identification of conserved leptospiral surface proteins, development of a broadly protective vaccine may soon be possible. OmpL1 and Lip L41 of L. kirshneri serovar grippotyphosa have been shown to be protective in animal models. The leptospiral OMPs which stimulate an IgM response and other recently described surface expressed proteins the Lig proteins could also be evaluated as candidates for preparation of subunit vaccines which would have broad-based actions in preventing leptospirosis in humans and animals.

References


Renu Bharadwaj
Department of Microbiology
B.J. Medical College
Pune 411001
e-mail : bharadwaj@vsnl.com