Chapter

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Leptospirosis: Recent Observations

R SAJITH KUMAR

SUMMARY

Leptospirosis, even though known to humanity for long, has been presenting with various modifications in clinical picture. As a disease affecting the working population of developing countries, it is producing damages in personal lives as well as economic prosperity. Ours being an agriculture based nation, the damage that Leptospirosis causes as an occupational hazard of farmers needs to be stressed. The classical clinical manifestations of Weil's disease are also accompanied by instances where predominant meningeal, cardiovascular or pulmonary manifestations are significant. They cause serious morbidity and mortality. The observation that early detection and management are the keys to reducing mortality has to be taken note of in organizing CMEs and health awareness programs for professionals and public alike. The available management systems have to be improvised with facilities for cardiac monitoring, ventilatory support etc.

Newer insights into why the disease behaves differently in different persons, communities and epidemics also need to be discussed. The availability and acceptance of preventive strategies in the form of vaccines and chemoprophylaxis are to be ensured. Environmental sanitation, vector and reservoir annihilation are important priorities. The diagnostic modalities and investigational therapies need regular upgradation as per the needs assessment. The close relationship with the climate helps predict certain clusters and help us in planning better.

The need for sensitizing the urban communities as well needs to be stressed, as Leptospirosis is being increasingly reported from metropolitan areas in the country and other developed nations. Finally, the differential diagnosis also must be chosen and excluded carefully in view of the many emerging diseases closely linked to poor sanitation.

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection with pathogenic *Leptospira*. It is a worldwide zoonotic infection with a much greater incidence in tropical regions and has become one of the major infectious diseases occurring almost every season in India. The spectrum of human disease caused by leptospires is extremely wide, ranging from subclinical infection to a severe syndrome of multiorgan infection with high mortality. This syndrome, icteric leptospirosis with renal failure, was first reported over 100 years ago by Adolf Weil of Heidelberg. The disease was first recognized as an occupational disease of sewer workers in 1883. In 1886, Weil described the clinical manifestations in 4 men who had severe jaundice, fever, and hemorrhage with renal involvement. Inada, et al identified the causal agent in Japan in 1916. It was first described by Larrey in 1812 of *fièvre jaune* among Napoleon's troops at the siege of Cairo. In early 19th century, the illness was known in Europe as "bilious typhoid." The disease has also been given names like swineherd's disease, sugarcane grower's disease, swamp fever, or mud fever.

The economic burden imposed by this disease is unknown, however it is true that the incidence of leptospirosis is remarkably underestimated in estimates from endemic regions. Leptospirosis is estimated to affect tens of millions of humans annually with case fatality rates ranging from 5 to 25%. Leptospirosis is underreported due to lack of clinical suspicion and barriers to diagnostic capacity. General physicians often lack familiarity with the broad clinical presentation of leptospirosis. Occupational exposure probably accounts

for 30-50% of human cases. Agricultural laborers (paddy, sugarcane, pineapple), veterinarians, pet owners, abattoir workers, plumbers, meat handlers and slaughterhouse workers, coal miners, workers in the fishing industry, military troops, milkers, and sewer workers are frequently infected. In Kerala people engaged in occupations related to underwater activity like river bed sandminers, under water oyster and fish catchers, mud handlers and coir workers are commonly affected. Crews in transport and automobile industry become affected when they use dirty water for washing the vehicles. Dairy workers and milkers are affected when the act of milking leads to splash on the conjunctiva. Water associated recreational activities also increase the risk. Cleaning and sanitation activities in unused ponds, lakes etc. lead to occurrence in the youth. In tropical areas the water being blocked from sunlight by surface floaters like salvinia has also been implicated. In many parts of the world and in Indian states, epidemics are closely linked to heavy rainfall and floods (as in Mumbai, Tamil Nadu, Andhra Pradesh and Kerala). Many cyclonic floods are followed by a rising occurrence of cases. The occurrence of many types of rats in and around the paddy fields and farms and the habit of many of them to live in burrows close to the water level also facilitate contamination at times of flood. The contaminated water which tends to pool following the receding of water after floods also paves the way for humans to come into contact with the organisms. Most cases occurred by cutaneous exposure of the legs and feet while walking in stagnant water or moist soil. It is suggested that leptospira multiply in the walking paths where water remained undrained for a period of two to three days after the rains and this was responsible for most cases. With urbanization and population growth a new environment for urban transmission has been created, mainly in slums and areas lacking proper sanitation. Infection in a significant number of persons cannot be linked to any source as such.

MICROBIOLOGICAL ASPECTS

Leptospires are obligate aerobes with an optimum growth temperature of 28 to 30°C. These are finely coiled spirocheates, thin and motile. Their flagella allow them to burrow into tissues. The genus *Leptospira* belongs to the *Leptospiraceae* family of the order *Spirochaetales*. The nomenclature system has undergone changes. The genus is divided into 2 species: the pathogenic *Leptospira interrogans* and the nonpathogenic *Leptospira biflexa*. These species were divided further into serogroups, serovars, and strains, based on shared antigens. *L. interrogans* included more than 250 serovars.

The current classification system based on DNA homology recognizes the heterogeneity and divides *L interrogans* and *L biflexa* into 12 new species:

1. *L interrogans*
2. *L weilii*
3. *L santarosai*
4. *L noguchi*
5. *L borgpetersenii*
6. *L kirschner*
7. *L alexanderi*
8. *L inadai* (pathogenicity unclear)
9. *L fainei* (pathogenicity unclear)
10. *L meyeri* (pathogenicity unclear)
11. *L biflexa* (saprophytes)
12. *L wolbachi* (saprophytes)

Morphologically all leptospires are indistinguishable. Within these species, leptospires are further grouped by serogroups, serovars, and strains on the basis of microscopic agglutination testing (MAT), defined by agglutination after cross-absorption with homologous antigen. Using microagglutination techniques, the major serogroup in Kerala were grippotyphosa, australis, autumnalis, icterohaemorrhagiae and leuisiana. A new serovar Bharathy type Kolencherry has been identified in Kerala. Although certain species (e.g. *L. interrogans*) have a classic association with Weil disease, knowledge of the species type does not necessarily help predict disease severity.

These organisms infect at least 160 mammalian species. The major ones are rats, cattle, pigs, dogs, cats, squirrels, raccoons, mongooses, and bandicoots. Leptospiral species' and serogroups' host animals vary from region to region. Many serovars are associated with particular animals. To suggest a few; *L. pomona* and *L interrogans* are seen in cattle and pigs; *L. grippotyphosa* in cattle, sheep, goats, and voles; *L. ballum* and *L. ictero-haemorrhagiae* associated with rats and mice; and *L canicola* with dogs. Other important serotypes are autumnalis, hebdomadis, and australis. Leptospirosis in animals is usually subclinical, even when excreting leptospires in urine. Humans are dead-end hosts for the leptospirae. Excretion of leptospires in human urine months after recovery has been reported. The low pH of human urine limits survival of leptospires after excretion. Transmission by sexual intercourse has been reported. Both nosocomial and congenital leptospirosis have been described, but are very rare.

Leptospires are transmitted via infected urine. Leptospires infect humans by invasion across mucosal

surfaces or nonintact skin. Infection may occur via direct contact with urine or through contact with contaminated water and soil. In favorable conditions, leptospires can survive in fresh water for as many as 16 days and in soil for as many as 24 days. The organisms are killed by even minor changes in the temperature, acidity, salinity, chlorine content etc.

PATHOLOGY

Leptospiral entry through the skin or mucous membrane is followed by extensive proliferation in many tissues. The resulting leptospiremia causes widespread dissemination and further damage in multiple organs. After the leptospiremic stage the organisms disappear from blood. The next is the leptospiruric phase. Although microbiological invasion causes tissue damage, most of the manifestations are due to immunological injury. Complex interactions involving endotoxin, hemolysin, and lipase are thought to occur.

Vasculitis of capillaries, endothelial edema, necrosis, and lymphocytic infiltration occur. Capillary vasculitis leads to loss of RBCs and fluid through enlarged junctions and fenestrae. This causes secondary tissue injury. Low platelet count is a common observation. Vascular congestion and disorganization of the cell plates occur in liver. The hyper bilirubinemia seen in leptospirosis is contributed also by muscle damage (releasing myoglobin), intra- and extravascular hemolysis, hemorrhages.

In the kidneys, the interstitium, renal tubules, and tubular lumen are affected. This causes interstitial nephritis and tubular necrosis. Various factors like tubular damage, hypovolemia from dehydration and from altered capillary permeability lead to renal failure.

Alveolar capillary injury in the lungs is common, focal or diffuse. Interstitial and intra-alveolar edema and bleeding can occur. Adult Respiratory Distress Syndrome (ARDS) can claim many lives, if not properly handled. Epicardial and endocardial petechiae, myocardial interstitial edema, lymphocyte/plasma cell infiltrate (consistent with myocarditis) and coronary arteritis have been described.

In skeletal muscles, particularly of the leg, focal necrosis of isolated muscle fibers is a common occurence with infiltration of histiocytes, neutrophils, and plasma cells. This correlates with the intense calf muscle pain and local tenderness reported by some patients.

CLINICAL FEATURES

The incubation period is usually 7-12 days, with a range of 2-20 days. Approximately 90% of patients manifest a mild anicteric form of the disease, and approximately 5-10% have the severe form with jaundice, otherwise known as Weil's disease. The natural course of leptospirosis falls into 2 distinct phases, leptospiremic and immune. During a brief period of 1-3 days between the 2 phases, the patient shows some improvement.

The leptospiremic phase resembles a viral fever and is associated with high fever, generalized myalgia, sore throat, cough, chest pain, hemoptysis, rash and frontal headache. Recovery is usual and the patient becomes afebrile. The next phase also called as immune or leptospiruric phase is associated with the appearance of antibodies and leptospira can be isolated only from urine. Symptoms may persist for 6 days to more than 4 weeks, with a mean duration of 14 days.

There are two well defined patterns, icteric and non-icteric. The non icteric illness is more common. The presenting feature is usually headache, attributable to meningitis (usually aseptic) with confusion, irritability and occasionally delirium. Calf muscle tenderness and subconjuctival hemorrhages appear early in classical Weil's disease. The involvement of kidneys leads to oliguric renal failure. Patient may progress faster and may require dialysis for survival. Hyperkalemia and metabolic acidosis are common. Cutaneous manifestations include hemorrhages and maculopapular eruptions on the trunk. The icteric illness is usually associated with acalculous cholecystitis, pancreatitis, enteritis and GI bleeding. Bilirubin levels in patients with Weil's disease can exceed 30 mg/dL. Mild elevations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) are seen. Cardiac complications are well described in patients with severe-to-fatal disease. Myocarditis and coronary arteritis may occur. First-degree atrioventricular (AV) block is most common (30%), presents early in the disease course, and resolves in patients who survive. Atrial fibrillation has been correlated with a bad prognosis. T-wave inversions occur in 18% of patients, ST-segment elevation in 15%, and dysrhythmias in 11%, but the latter are not associated with progression to shock or congestive heart failure. Cervical, axillary and mediastinal lymph nodes may be enlarged. Different types of bleeding can occur ranging from epistaxis and gingival oozing to hematuria, hemoptysis, and pulmonary hemorrhage. Visible subconjunctival hemorrhage is a common occurrence, though not related to platelet count or coagulation abnormalities.

Almost all clinical manifestations can appear irrespective of the species or subtype. However, the following associations are worth remembering.

Hepatorenal lesions in icterohemorrhagiae, pretibial skin lesions in autumnalis (Fort Bragg fever), and meningeal symptoms in canicola.

OTHER RECENT OBSERVATIONS

Ocular Changes

Anterior uveitis, either unilateral or bilateral, occurs after recovery from the acute illness in a minority of cases. Uveitis may present weeks, months, or occasionally years after the acute stage. Chronic visual disturbance, persisting 20 years or more after the acute illness, has been reported. In most cases uveitis is presumed to be an immune phenomenon, but leptospires have been isolated from human and equine eyes, and more recently, leptospiral DNA has been demonstrated in aqueous humor by PCR. Late-onset uveitis may result from an autoimmune reaction to subsequent exposure.

Pregnancy

Acute infection in pregnancy has been reported to cause abortion and fetal death but not invariably so. The organisms have been recovered from amniotic fluid, placenta, cord blood and even breast milk.

Others

Cerebrovascular accidents, rhabdomyolysis, thrombotic thrombocytopenic purpura, erythema nodosum, aortic stenosis, Kawasaki syndrome, reactive arthritis, epididymitis, male hypogonadism, antiphospholipid syndrome, transverse myelitis and Guillain-Barré syndrome, Cerebral arteritis, resembling Moya Moya disease etc. have also been seen. Although asymptomatic aseptic meningitis is a common feature of leptospirosis, severe symptomatic involvement is rare. Rare clinical presentations include myeloradiculopathy, myelopathy and cerebellar dysfunction.

Hemorrhagic Pneumonitis

Pulmonary manifestations include cough, hemoptysis, and pneumonia. Chest X-ray may show multifocal infiltrates, as well as pleural effusions. Respiratory symptoms may progress to acute respiratory distress syndrome (ARDS), requiring intubation and mechanical ventilation. There has been a significant increase recently of cases presenting with hemorrhagic pneumonitis. This may be the initial manifestation or may occur while on treatment. Interstitial pneumonia has also been reported. Radiological features in chest include small "snowflake-like" nodular densities corresponding to areas of alveolar hemorrhage, large confluent consolidations, and diffuse, ill-defined ground-glass pattern that may represent resolving hemorrhage. Serial radiographs may show progression from a nodular pattern to confluent consolidation. ARDS like presentation is more common now, as better management of renal failure prolongs the survival. In many instances hemorrhagic pneumonitis is associated with a very high mortality. Many deaths occur within one to two days due to pulmonary hemorrhage and severe respiratory distress

DIFFERENTIAL DIAGNOSIS

Leptospirosis mimicks almost any febrile illness at different stages. These include dengue fever, chikungunya fever, encephalitis, hantavirus cardiopulmonary syndrome, scrub typhus, hepatitis, cholecystitis, yellow fever, puumaala hemorrhagic fever, rickettsial diseases, falciparum malaria, meningitis, infectious mononucleosis and even enteric fever. Leptospirosis has been reported to coexist with many of these illnesses also.

LABORATORY DIAGNOSIS

In anicteric disease, the erythrocyte sedimentation rate is elevated, and white cell counts range from below normal to moderately elevated. Very high values producing a leukemoid reaction are also seen. Liver function tests show a slight elevation in aminotransferases, bilirubin, and alkaline phosphatase in the absence of jaundice. Urinalysis shows proteinuria, pyuria, and often microscopic hematuria. Hyaline and granular casts may also be present during the first week of illness.

In late stages, polymorphonuclear neutrophilia (occasionally with toxic granules and band forms) with raised ESR is seen in about 90% cases. Thrombocytopenia may occur in up to 50% cases, usually mild. Urine examination reveals proteinuria with leukocytes, erythrocytes, hyaline casts, and granular casts in the sediment. As noted earlier the gross elevation in serum bilirubin is associated with a mild or moderate elevation in transaminase values. Creatine phospho-kinase (CPK) is markedly elevated, particularly in patients with muscle necrosis.

Lumbar puncture reveals a normal or slightly elevated CSF pressure and may serve to reduce the intensity of headache. Predominance of polymorphs or lymphocytes in early and a lymphocytic predominance later is usual. CSF protein may be normal or elevated, while CSF glucose is usually normal. In patients with severe jaundice, xanthochromia occurs. CSF abnor-

malities are common in the second week of illness, and CSF pleocytosis can last for weeks.

It is very difficult to demonstrate or grow leptospira from a patient's blood or urine samples. Dark field examination and/or use of immunoflourescent techniques are helpful in early stages. Approximately 10^4 leptospires/ml are necessary for one cell per field to be visible by dark-field microscopy. A quantitative buffy coat method was recently shown to have a sensitivity of approximately 10^3 leptospires/ml. A method which involved repeated microscopic examination of double-centrifuged anticoagulated blood demonstrated leptospires in 32% of patients. Microscopy of blood is of value only during the first few days of the acute illness, while leptospiremia occurs. Radioimmunoassay (RIA) could detect 10^4 to 10^5 leptospires/ml and an enzyme-linked immunosorbent assay (ELISA) method would detect 10^5 leptospires/ml. More recently, immunomagnetic antigen capture was combined with fluoro-immunoassay to detect as few as 10^2 leptospires/ml. Inhibitory substances may have to be neutralized prior to testing.

The serologic tests become positive by the end of first week. These include macroscopic and microscopic agglutination tests using antigens from live strains and ELISA. False positive and false negative results are common. Rising titres are more helpful in reaching a conclusion. Interpretation of the MAT is complicated by the high degree of cross-reaction that occurs between different serogroups, especially in acute-phase samples. "Paradoxical" reaction, can occur with highest titers detected to a serogroup unrelated to the infecting one. Acute infection is suggested by a single elevated titer detected in association with an acute febrile illness. The magnitude of such a titer is dependent on the background level of exposure in the population and hence the seroprevalence. Thus, in the current CDC case definition, a titer of 1 in 200 is used to define a probable case with a clinically compatible illness. Although this may be appropriate for use in a population in which exposure to leptospirosis is uncommon, a higher cut-off titer is necessary for defining probable cases of leptospirosis in most tropical countries. In areas where leptospirosis is endemic, a single titer of 1/800 in symptomatic patients is generally indicative of leptospirosis, but titers as high as 1/1600 have been recommended. Some patients have serological evidence of previous infection with a different leptospiral serogroup. In these cases, serological diagnosis is complicated further by the "anamnestic response," in which the first rise in antibody titer is usually directed against the infecting serovar from the previous exposure.

Titers following acute infection may be extremely high and may take months or even years to fall to low levels. Complement fixation (CF) was widely used, but methods were not standardized. CF tests have generally been replaced by ELISA methods. IgM antibodies become detectable during the first week of illness facilitating the diagnosis to be confirmed and treatment initiated while it is likely to be most effective. IgM detection has repeatedly been shown to be more sensitive than MAT when the first specimen is taken early in the acute phase of the illness. Other techniques applied to the detection of leptospiral antibodies include immunofluorescence, RIA, counterimmunoelectrophoresis, and thin-layer immunoassay. These methods have not been widely used. More recently, rapid commercial tests have been made available, such as the Dipsticks which detects leptospira antibodies.

Management

Treatment should be started as soon as possible and may be effective even after the first 4 days of illness onset. Monitoring the fluid intake and output can give valuable inputs to management, including indications for dialysis. All steps must be taken to prevent hypovolemic shock, fluid overload and hyperkalemia.

Antimicrobial therapy is indicated for the severe form of leptospirosis, but it is controversial for the mild form of leptospirosis. Evidence from randomized clinical trials is insufficient to provide clear guidelines for the use of antibiotics in leptospirosis. Mild leptospirosis is treated with doxycycline, ampicillin, or amoxicillin. For severe leptospirosis, the primary therapy is penicillin G, which is used widely in clinical practice. Alternative regimens are ampicillin, amoxicillin, or erythromycin. A Jarisch-Herxheimer reaction may develop rarely, which should be treated with supportive measures.

Patients with renal failure may require dialysis; renal function is restored in most. Large numbers of deaths have been averted with timely dialysis support. Supportive therapy and careful management of renal, hepatic, hematologic, and CNS complications are important. In cases of ARDS or hemorrhagic pneumonitis, bolus methylprednisolone one gram intravenously for three days followed by oral prednisolone 1 mg/kg for seven days has been shown to improve survival.

Prevention

Prevention is difficult because the organism has not been eradicated from wild animals, which constantly infect domestic animals. To prevent future outbreaks of

leptospirosis, rodent control measures and improvement in sewerage and drainage facilities are necessary. Important control measures include control of livestock infection with good sanitation, immunization, and proper veterinary care. Preventing infected animals from urinating in waters where humans have contact, disinfecting contaminated work areas, providing worker education, practising good personal hygiene, and using personal protective equipment (PPE) when handling infected animals or tissues are important actions for prevention of the disease. Ensuring that the feet are kept dry soon after coming out of water and quick washing with soap has been suggested to reduce chances for skin penetration. Quantifying the presence and determining types of pathogenic Leptospira in environmental surface waters would be an important tool for guiding leptospirosis control efforts in endemic regions. Doxycycline, in the dose of 200 mg every week, has demonstrated efficacy of 95% against leptospirosis and may be given to help prevent the disease in those exposed. This regimen is recommended for those with short-term exposure and is not for repeated exposure over protracted periods of time.

Leptospiral Vaccines

Killed whole cell leptospiral vaccines for humans are available in some countries, including Japan. The Japanese leptospiral vaccine consists of formalin-killed leptospires. The immunogenic proteins, especially the outer membrane surface proteins, of pathogenic leptospira, may be effective as vaccinogens. The identification of proteins, which are conserved among pathogenic leptospires and can generate cross-protection against various serovars, has become a major focus of leptospirosis research. Exploration of the full-length leptospiral genome to detect genes encoding candidate vaccine proteins and attempts at vaccines using cell mediated immunity are other new steps in Leptospiral vaccine research.

Prognosis

Most patients with leptospirosis recover. The highest mortality rates are in elderly patients and in those with ARDS or Weil's syndrome. Pregnant women also face a high rate of fetal mortality, as infected women have a higher chance of landing in spontaneous abortion if the infection is acquired in the early months of pregnancy. Patients with hepatic dysfunction and renal failure have a good chance of recovering renal and hepatic dysfunction as long term sequelae. The report from an autopsy series of 62 cases from Mumbai provides good insight. Massive intra-alveolar hemorrhage (48 cases), acute interstitial nephritis and/or acute tubular necrosis (45 cases) and myocarditis (24 cases) were the main autopsy findings. Hemorrhages in various organs like the heart, gastrointestinal tract, brain, pancreas and adrenals were also seen. Thirty of 54 kidney sections were positive for leptospiral antigens by IHC. There were extensive hemorrhages in the lungs in 48 (77%) cases and that was the cause of death in most of these cases.

Why only some people develop any clinical manifestations of leptospirosis such as undifferentiated fever, and of these, few (less than 5-10%) develop severe disease despite being infected with highly pathogenic leptospires such as *L. interrogans* serovar icterohemorrhagiae? The discrepancy between infection and symptomatic and severe disease remains a central mystery. It has long been thought that certain leptospiral serovars are particularly pathogenic. Human host genetics are likely to determine clinical outcomes of leptospiral infection. The quantum of inoculum, protective immunity from previous exposures, status of nutrition (alcoholism, malnutrition etc.) may be related factors. One report suggests the association of a major histocompatibility complex allele, HLA-DQ6, with risk of infection due to exposure to Leptospira-contaminated water in an outbreak. It is likely that some people living in leptospirosis-endemic regions develop naturally acquired protective immunity against severe disease. Serovar-specific antibodies targeted against lipopolysaccharide, antibodies against cross-species conserved proteins such as LipL32 acquired cellular immunity with either T cells with ab T cell receptors or T cells with gd T cell receptors have all been suggested as the mechanisms of such protective immunity in humans playing important roles.

SUGGESTED READING

1. Bal AM. Unusual clinical manifestations of leptospirosis. J Postgrad Med 2005;51:179-83.
2. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. Leptospirosis:A zoonotic disease of global importance. Lancet Infect Dis 2003;3:757-71.
3. Branger CB, Chatrenet A, Gauvrit F, Aviat A, Aubert JM, Bach G, Andre-Fontaine. Protection against *Leptospira interrogans* sensu lato challenge by DNA immunization with the gene encoding hemolysin-associated protein 1. Infect Immun 2005; 73:4062-9.
4. Divate SA, Chaturvedi R, Jadhav NN, Vaideeswar P. Leptospirosis associated with diffuse alveolar haemorrhage. J Postgrad Med 2002;48:131-2.
5. John TJ. The prevention and control of human leptospirosis. J Postgrad Med 2005;51:205-9.

6. Katz AR, Effler PV. "Probable" versus "Confirmed" leptospirosis. An epidemiologic and clinical comparison utilizing a surveillance case classification. Ann Epidemiol 2003;13:196-203.
7. Koizumi N, Watanabe H. Leptospirosis vaccines: Past, present, and future. J Postgrad Med 2005;51:210-4.
8. Kulkarni, Renu Bharadwaj, Sae Pol, Jyotsna Thakare, Anuradha De. Concurrent Outbreak of Leptospirosis and Dengue in Mumbai, India. J Trop Pediatr 2002;51:174-81.
9. Kuriakose M, Eapen CK, Paul R. Leptospirosis in Kolenchery, Kerala, India: Epidemiology, prevalent local serogroups and serovars and a new serovar. Eur J Epidemiol 1997;13:691-7.
10. Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14:296-326.
11. Luks AM, Lakshminarayanan S, Hirschmann JV. Leptospirosis presenting as diffuse alveolar hemorrhage: Case report and literature review. Chest 2003;123:639-43
12. Nascimento AL, Ko AI, Martins EA and 44 other authors. Comparative genomics of two Leptospira interrogans serovars reveals novel insights into physiology and pathogenesis. J Bacteriol 2004;186:2164-72.
13. Pai ND, Adhikari PM. Hemorrhagic pneumonitis: A rare presentation of leptospirosis. J Postgrad Med 2001;47:35-6.
14. Pappachan MJ, Sheela M, Aravindan KP. Relation of rainfall pattern and epidemic leptospirosis in the Indian state of Kerala. J Epidemiol Community Health 2004;58:1054.
15. Ren SX, Fu G, Jiang XG, Zeng R, Miao YG, Xu H, et al. Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. Nature 2003;422:888-93.
16. Salkade HP, Divate S, Deshpande JR, Kawishwar V, Chaturvedi R, Kandalkar BM, et al. A study of autopsy findings in 62 cases of leptospirosis in a metropolitan city in India. J Postgrad Med 2005;51:169-73.
17. Segura E, Ganoza C, Campos K, Ricaldi JN, Torres S, Silva H, et al. Clinical spectrum of pulmonary involvement in leptospirosis in an endemic region, with quantification of leptospiral burden. Clin Infect Dis 2005;40:343-51.
18. Sehgal SC, Murhekar MV, Sugunan AP. Outbreak of leptospirosis with pulmonary involvement in north Andaman. Indian J Med Res 1995;102:9-12
19. Shenoy VV, Nagar VS, Chowdhury AA, Bhalgat PS, Juvale NI. Pulmonary leptospirosis: An excellent response to bolus methylprednisolone. Postgrad Med J 2006;82:602-6.
20. Vijayachari P, Ahmed Niyaz, Sugunan AP, Ghousunnissa Sheikh, Rao K Rajender, Hasnain Seyed E, Sehgal, Subhash C. Use of fluorescent amplified fragment length polymorphism for molecular epidemiology of leptospirosis in India. J Clin Microbiol 2004;42:3575-80.
21. Watt George, Jongsakul Krisada, Ruangvirayuth Ronnatrai, Kantipong Pacharee, Silpapojakul Khachornsakdi. Short report: Prospective evaluation of a multi-test strip for the diagnoses of scrub and murine typhus, leptospirosis, dengue fever, and *salmonella* typhi infection. Am J Trop Med Hyg 2005;72:10-12.