Leptospirosis: an emerging global public health problem

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Leptospirosis has been recognized as an emerging global public health problem because of its increasing incidence in both developing and developed countries. A number of leptospirosis outbreaks have occurred in the past few years in various places such as Nicaragua, Brazil and India. Some of these resulted due to natural calamities such as cyclone and floods. It is a direct zoonotic disease caused by spirochetes belonging to different pathogenic species of the genus *Leptospira*. Large number of animals acts as carriers or vectors. Human infection results from accidental contact with carrier animals or environment contaminated with leptospires. The primary source of leptospires is the excretor animal, from whose renal tubules leptospires are excreted into the environment with the animal urine. Majority of leptospiral infections are either sub clinical or result in very mild illness and recover without any complications. However, a small proportion develops various complications due to involvement of multiple organ systems. In such patients, the clinical presentation depends upon the predominant organs involved and the case fatality ratio could be about 40% or more. Febrile illness with icterus, splenomegaly and nephritis (known as Weil's disease), acute febrile illness with severe muscle pain, febrile illness with pulmonary haemorrhages in the form of haemoptysis, jaundice with pulmonary haemorrhages, jaundice with heamaturea, meningitis with haemorrhages including sub conjunctival haemorrhage or febrile illness with cardiac arrhythmias with or without haemorrhages are some of the syndromes. Because of the protean manifestations of leptospirosis it is often misdiagnosed and under-reported. Although the basic principles of prevention such as source reduction, environmental sanitation, more hygienic work-related and personal practices etc., are same everywhere, there is no universal control method applicable to all epidemiological settings. Comprehensive understanding of the eco- epidemiological and cultural characteristics of a community that faces the problem of leptospirosis is an essential prerequisite for evolving an effective and acceptable control measure.

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1. Introduction

Leptospirosis has been recognized as an important emerging global public health problem because of its epidemic proportions and increasing incidence in both developing and developed countries (Meites *et al* 2004). It is an acute bacterial infection caused by spirochetes, with different pathogenic species of the genus *Leptospira* (Waitkins 1987). Leptospirosis has wide geographical distribution and occurs in tropical, subtropical and temperate zones. In the developed world, the incidence of the disease has come down substantially and most cases that occur now are associated with recreational exposure to the contaminated water. Contrastingly, the incidence appears to be increasing in developing countries (Tangkanakul *et al* 2000). Most countries in the South East Asia region are endemic to leptospirosis. On an average 10,000 severe cases requiring hospitalization occur world over annually (Faine 1994).

Several outbreaks of leptospirosis have been reported in the past few years in various places viz., Nicaragua (Zaki and Sheih 1996), Salvador (Ko *et al* 1999) and Rio de Janeiro (Barcellos and Sabroza 2001) in Brazil and Orissa (Faine

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Abbreviations used: CFR, case fatality ratio; MPT, microagglutination test; OMP, outer-membrane proteins; PCR, polymerase chain reactions

1994; Sehgal *et al* 2001), Mumbai (Karande *et al* 2002) and the Andaman archipelago, India (Sehgal *et al* 1995; Singh *et al* 1999). Some of these outbreaks were associated with natural calamities such as cyclone and flood.

Leptospirosis is a direct zoonosis. Leptospires are maintained in nature by a large variety of animal hosts. These include both wild and domestic animals. Leptospires shed in the urine of these carrier animals can survive in the environment for prolonged period. The source of human leptospiral infection is infected animal urine. Hence, the commonly considered risk factors and behaviours are those that expose people to animal reservoirs or contaminated environment. Contact with various species of animals, animal tissue, animal urine and wet environment and occupational and recreational exposure to contaminated water bodies have been implicated as risk factors.

Leptospirosis is known to be endemic in India since the early 20th century (Chowdry 1903; Woolley 1911, 1913; De Castro 1922; Barker 1926). The first comprehensive account on the clinical spectrum, etiological agents and epidemiological characteristics of confirmed leptospirosis in India originated from the Andaman archipelago (Taylor and Goyle 1931).

2. Historical aspects

Diseases clinically similar to leptospirosis were recognized as occupational hazards of the rice farmers in ancient China (Faine 1994). In 1886, Adolf Weil reported his description of a clinical syndrome characterized by splenomegaly, jaundice and nephritis (Weil 1886 as quoted in Levett 2001), commonly referred to as Weil's disease which became synonymous with leptospirosis.

Leptospires were first identified as the cause of Weil's disease in Japan, where it was common among coal miners (Faine 1994). Inada and Ido (1915) successfully demonstrated the transmission of infection to guinea pigs from the blood of the infected animals which produced the responsible organism. Unaware of this development, Huebener and Reiter reported the successful transmission of Weil's disease to guinea pigs couple of years later. Several years before these events, Stimson reported the presence of spiral organisms in kidney specimens stained with Levadeti technique from a patient, who was thought to have died of yellow fever (Stimson 1907 as reproduced in Faine 1994).

Soon after the breakthrough that Weil's disease was caused by leptospires several other disease entities were being recognized to have leptospiral aetiology. These include '*nanukayami'* or the Japanese seven-day fever, '*akiyami'* the harvest fever and more recently Andaman fever or Andaman haemorrhagic fever (AHF). Much of the basic current knowledge about leptospires and leptospirosis was understood within a decade following the discovery of leptospires and several types of them were recognized during this period (Kmety and Dicken 1988, 1993). The same Japanese group that identified leptospires illustrated the zoonotic nature of the disease and rats as vectors. Subsequently several wild and domestic animals were identified as carriers (Faine 1994).

3. Public health significance -- sporadic cases, epidemics and sero-prevalence

Leptospirosis is currently identified as a worldwide public health problem. Increase in the incidence of the disease has been recorded in countries where leptospirosis surveillance exists (WHO 2003). In endemic areas, leptospirosis is a major cause of various clinical syndromes such as jaundice, renal failure, myocarditis and atypical pneumonia. (Muthusethupathy *et al* 1995). The annual incidence of leptospirosis has increased from 0.3/100,000 persons (between 1982 and 1995) to 3.3/100,000 persons (between 1997 and 1998) in Thailand (Faine 1994). Multi-centric investigations in India indicate that leptospirosis account for about 12.7% of cases of acute febrile illness reporting to the hospitals (Sehgal *et al* 2003). Besides, leptospirosis is the cause of a significant proportion of cases of non-hepatitis A and E jaundice, non-malarial febrile illnesses and non-dengue haemorrhagic fever in South East Asia (Laras *et al* 2002). During the past several years, large outbreaks of leptospirosis have occurred in many countries, particularly in Southeast Asian countries, Central and South America. Several outbreaks were reported from the Andaman archipelago, India since 1988 (Sehgal *et al* 1995). Leptospiral aetiology was attributed to the acute febrile illness associated with pulmonary haemorrhage in a large outbreak in Nicaragua in 1995 following floods (Zaki and Sheih 1996). El Salvador, Brazil witnessed large outbreaks (Ko *et al* 1999). During an eight-month period in 1996, the surveillance system detected 326 cases of leptospirosis among the two million population of El Salvador. The case fatality rate in this case series was 15%. About 42% of the cases detected by the surveillance system were initially misdiagnosed as dengue fever at the outpatient clinic. Another outbreak was reported in the same year in Rio de Janeiro following heavy rainfall (Barcellos and Sabroza, 2001). Following the super-cyclone that hit the coastal villages in Orissa, nearly 14% of the studied subjects had febrile illness and serological evidence of leptospiral infection (Faine 1994). A random sample of 1067 persons in Seychelles showed a sero-prevalence rate of 37% (Bovet *et al* 1999), whereas 54% sero-prevalence rate was observed among healthy population from the North Andaman, Andaman and Nicobar archipelago (Murhekar *et al* 1998). Consequent to an outbreak of bovine leptospirosis in Chennai, serological evidence of leptospirosis was

evident among human subjects (Ratnam *et al* 1983). High sero-prevalence has also been noticed in some sub-tropical and temperate regions. Sero-epidemiological studies from North-eastern Alpine regions of Italy detected 10%–12% sero-prevalence of leptospirosis among farmers and forestry workers (Faine 1994), while studies from Yucatan State, Mexico situated in the inter-tropical belt, reported 14.25% (57/400) sero-positvity from randomly selected subjects (Vado-solis *et al* 2002).

4. Leptospira – a spirochete with surface architecture resembling gram negative and gram positive bacteria

Leptospira, a spiral shaped bacterium exhibits a surface architecture that resembles Gram- negative and Gram-positive bacteria. Double membrane constitution supports Gram-negative bacteria whereas attachment of peptidoglycan to the inner membrane resembles gram positive nature. Thus this bacterium is susceptible to the antibiotics which are used for both Gram-negative as well as gram positive bacteria. The bacteria are sensitive to wide range of antibiotics except chloromphenical as some of the serovars were found to be resistant.

5. Basic taxon of leptospires, intrasubspecific ranks – serovars and serogroups

Leptospires belonging to division-gracillicutes, class-scotobacteria order – Spirochaetales and family –Leptospiraceae which has three genera viz., Leptospira, Leptonema and Turneria. The strain Ictero No. 1 of serovar Icterohaemorrhagiae (Spirochaeta *Icterohaemorrhagiae japonica*) was the first isolate of leptospira, which was recovered by Inada and Ido in 1915 from a patient suffering from Weil's disease. Since then about 268 pathogenic serovars have been described and each one has reference strains (table 1). Serovar Sichvan (serogroup Sichvan), Serovar Hurstbridge (serogroup Hurstbridge) serovar

Table 1. Serogroups, serovars and genomospecies

Serogroup	Representative serovar	Reference strain	Species
Australis	australis	Ballico	*L. interrogans*
Autumnalis	Rachmati	Rachmat	*L. interrogans*
Ballum	Ballum	Mus 127	*L. borgpetersenii*
Bataviae	Bataviae	Swart	*L. santarosai*
Canicola	Canicola	H.Uterecht IV	*L. interrogans*
Celledoni	Cellodoni	Cellodoni	*L. celledoni*
Cynopteri	Cynopteri	3522 C	*L. kirschneri*
Djasiman	Djasiman	Djasinman	*L. interrogans*
Grippotyphosa	grippotyphosa	Moskva V	*L. interrogans*
Hebdomadis	hebdomadis	Hebdomadis	*L. interrogans*
Icterohaem.	icterohaem.	RGA	*L. interrogans*
Javanica	poi	Poi	*L. borgpteresenii*
Louisiana	louisiana	LSU 1945	*L. naguchii*
Manhao	manhao	L 60	*Leptospira*
Mini	mini	Sari	*L. borgpteresenii*
Panama	panama	CZ 214 K	*L.noguchii*
Pomona	pomona	Pomona	*L. interrogans*
Pyrogenes	pyrogenes	Salinem	*L. interrogans*
Sarmin	rio	Rr 5	*L.weilii*
Sejroe	hardjo	Hardjopraj.	*L. borgpetersenii*
Shermani	shermani	1342 K	*L. santarosai*
Tarassovi	bakeri	LT 79	*L. krishneri*
Ranarum	ranarum	ICF	*L. interrogans*
Sichuan	sichuan	Sichuan	*Leptospira*
Sehgali	portblairi	DS 2	*L. interrogans*

Portblairi (serogroup Sehgali) are the recent entities (Brenner *et al* 1999; Vijayachari *et al* 2004).

The classification and nomenclature of Leptospira is complex. Presently two different classification systems – one based on phenotypic characters and other on the genetic homology are being used. In the phenotypic classification there are two species namely, the *L. interrogans* (pathogenic) and the *L. biflexa* (non-pathogenic). Both the species have several serovars and serovar is the basic taxon, which is defined on the basis of surface antigenic makeup. *"Two strains are said to belong to different serovars if after cross absorption with adequate amount of heterologous antigen more than 10% of the homologous titre regularly remains in at least one of the two antisera in repeated test"*. Closely related serovars are arranged into serogroups. However serogroup designation has no official taxonomic status and is intended for laboratory use. The binominal classification system is strictly followed. However serovar and serogroup name may be added (figure 1).

6. Leptospiral genome and classification based on genetic homology

Genomic species is a group of Leptospiraceae serovars who's DNAs show 70% or more homology at the optimal reassociation temperature of 55ºC or 60% or more homology at a stringent reassociation temperature of 70ºC and in which the related DNAs contain 5% or less unpaired bases.

A complete sequencing data is now available for the two leptopsires (serovar Lai and serovar Copenhegeni). Leptopsiral genome has two chromosomes – large chromosome (CI) and small chromosome (CII). The size of the large chromosome ranges from 4,332,241 bp to 4,277,185 bp whereas the size of the small chromosome is in the range of 358,943 bp to 350, 181 bp. Based on genetic homology in DNA hybridization experiments, 15 genomic species (*L. interrogans, L. kirschneri, L. borgpetersenii, L. santarosai, L. noguchii, L.weilii, L.inadai, L.biflexa, L.meyeri, L.wolbachii, Genomo species 1, Genomo species*

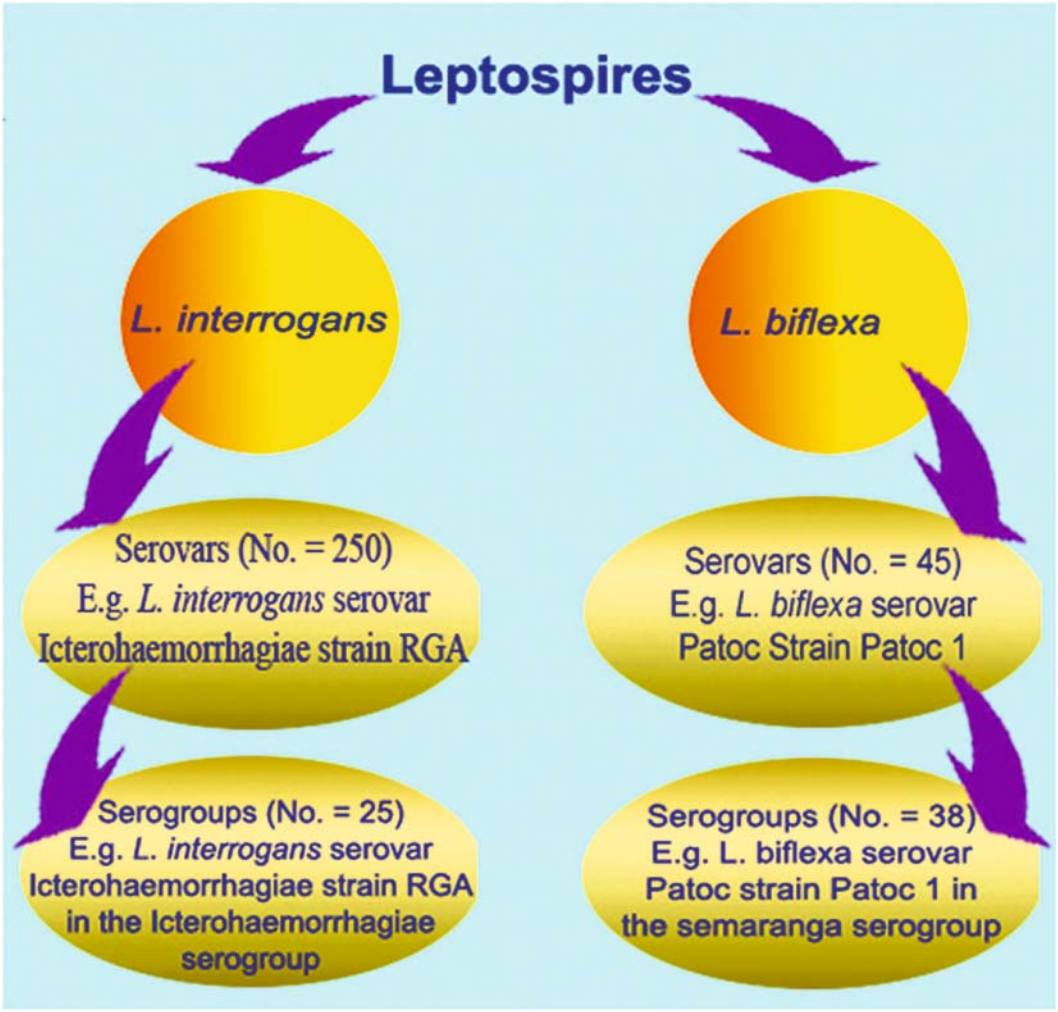

Figure 1. Phenotypic classification and nomenclature.

3, Genomo species 4 and *Genomo species* 5) have been described in the genus Leptopsira (*see* figure 4) whereas *Leptonema* and *Turneria* have one species each (*L. illini* and *T. parva*).

7. Animal reservoirs or vectors

The leptospires dwell in the renal tubules of their animal host. Although they are susceptible to environmental factors, in particular drying, they can survive for long periods in water and wet soil. The transmission cycle of leptospirosis involves the maintenance hosts, the carrier hosts, the environment and human beings (Waitkins 1987). Almost every known species of rodent, marsupial and mammal can be carrier and excretor of leptospires (Faine 1994).

7.1 *Rodents*

Rodents were first to be recognized carriers of leptospires. They are often incriminated as the source of infection to human beings. Although serovar Icterohaemorrhagiae has been often associated with rodents, other serovars have also been isolated. (Matthias and Levett 2002). Very high sero-prevalence rate and carrier rates (90.9% and 82.9% respectively) were observed among house mice in Terceira Island in Azores (Collares-Pereira *et al* 2000). A multiple logistic regression analysis with environmental and biological factors as independent variables and leptospiral infection as dependant variable identified male *Mus domesticus*, sexually active and living in humid biotopes 500 m above the sea level as the most likely reservoir. Rats and bandicoots have shown evidence of anti-leptospiral antibodies following isolation of leptospires from suspected human patient from the suburbs of Chennai (Saravanan *et al* 2000). Elsewhere, in Tamil Nadu anti-leptospiral antibodies (52.1%) were evident from the field rodents (Natarajaseenivasan *et al* 2002). While relatively low sero-prevalence was observed among the rat population, yielding two isolates from the Andaman and Nicobar archipelago (Sharma *et al* 2003).

7.2 *Cattle*

Leptospiral infection among cattle was first recorded in Russia (Faine 1994). Cattle world over may be infected with serovars Hardjobovis, Pomona, and Grippotyphosa. Infection with Icterohaemorrhagiae, Bratislava, Hebdomadis, Autumnalis, Australis, Sejroe, Canicola and Bataviae also occurs (Faine 1982). Leptospirosis in cattle could be totally unapparent or may result in acute febrile illness or severe complications. Infection among buffalos and deer is also similar to that in cattle (Bahaman *et al* 1988; Flint *et al* 1986). Several studies on cattle leptospirosis were conducted in other countries such as Spain and Australia also. Alonso-Andicoberry *et al* (2001) studied 762 dairy cattle belonging to 81 herds in Spain using microagglutination test (MAT) with 11 leptospiral serovars as antigens. Leptospiral antibodies were detected in 8% of the cattle. A serosurvey among animal populations of Andaman and Nicobar Islands (Sharma *et al* 2003) showed that about 40% of the cows and 26% of the bulls were seropositive. Ratnam *et al* (1983) screened 40 cows in a village near Chennai following an outbreak of leptospirosis in cattle. Antibodies against leptospires were found in 68% of the cows.

7.3 *Other domestic animals*

Pigs are commonly infected with serovars Pomona, Tarassovi, Grippotyphosa, Bratislava, Sejroe, Icterohaemorrhagiae and Canicola (Faine 1994). Adult non-pregnant infected pigs are usually symptom-free and become chronic carriers. Leptospires were also isolated from the kidneys and genital tracts of both the cows and the boar of the herd. A study conducted in Mekong delta in Vietnam, 73 (29%) of the 424 cows screened were seropositive (Boqvist *et al* 2002). The commonest serovar was Bratislava. Contact with cows in neighbouring pens, being born in herds with infected animals, lack of rodent control measures and artificial insemination were identified as the risk factors of acquiring leptospiral infection. Cousins *et al* (1989) followed up 15 sheep over one year period by urine culture. Initial cultures yielded leptospires in 10 out of 15 sheep. At the end of one year follow up, three of these were still excreting leptospires. Isolated leptospires were of serovar Hardjo, commonly seen in cattle. The study shows that sheep can also act as long-term carriers of leptospires. Historically, leptospirosis was recognized as a disease of dogs before it was known in any other animals. Canicola and Icterohaemorrhagiae are the commonest serovars that infect dogs (Faine 1994). Acute leptospirosis in dogs is known as Stuttgart disease, which is characterized by vomiting, dehydration, bloodstained faeces, mucosal sloughing and death. Some dogs survive with chronic nephritis and continue to excrete leptospires.

Leptospirosis due to a variety of serovars is reported rarely in cats and it is not significantly different in course from the disease in dogs. In a serosurvey conducted among 245 kennelled dogs in Italy (Scanziani *et al* 2002), 72 were found to be seropositive. Bratislava and Grippotyphosa were the common infecting serovars. Venkataraman and Nedunchelliyan (1992) reported an outbreak of leptospirosis in human beings and dogs in Chennai City. Following the outbreak, a serosurvey was conducted among humans and dogs. Seroprevalence was 50.5% among humans and 21.3% in dogs. Leptospira belonging to serovar Icterohaemorrhagiae was isolated from a human patient and Canicola from a dog.

7.4 *Wild animals*

Leptospires have been isolated from almost all warm-blooded animals. Ruiz-Pina *et al* (2002) reported a 4.9% seroprevalence among 91 captured opossums (*Didelphis virginiana*) in Mexico. Colagross-Schouten *et al* (2002) screened 225 free-ranging California sea lions presented to coastal marine mammal rehabilitation centres using MAT. They found an overall seroprevalence rate of 38.2%. Bunnell *et al* (2000) conducted a study among wild mammals in Peruvian Amazon basin. Wild mammals were trapped and their kidney samples were tested for leptospiral DNA using polymerase chain reaction (PCR). A total of 148 wild mammals of various species were trapped. This included 72 rodents, 55 marsupials (opossums of various species), bats and carnivores. Seroprevalence was highest among the opossums (39%) followed by bats (35%) and rodents (20%).

8. Transmission dynamics – direct and indirect modes of transmission

Leptospires are ubiquitous. They are found wherever experts in leptospirosis, astute and aware medical and veterinary practitioners and epidemiologists, and adequate specialist laboratory facilities exist (Faine 1994). The primary source of leptospires is the excretor animal, from whose renal tubules leptospires are excreted into the environment with the animal urine.

Transmission can be direct or indirect (figure 2). Direct transmission occurs when leptospires from tissues, body fluids or urine of acutely infected or asymptomatic carrier animals enter the body of the new host and initiate infection. Direct transmission among animals can be transplacental, haematogenous, by sexual contact or by suckling milk from infected mother. Presence of leptospires in genital tracts as well as transplacental transmission has been demonstrated in animals (Ellis *et al* 1978, 1985, 1986). Direct transmission from animals to human beings is common amongst the occupational groups who handle animals and animal tissue such as butchers, veterinarians, cattle and pig farmers, rodent control workers etc. Accidental infection to veterinarians has been recorded (Bolin and Koellner 1988). Demers *et al* (1985) studied the risk of leptospiral exposure to rodent control workers in Detroit by a comparative cross-sectional study among the workers and two control groups. A statistically significant higher risk was found in rodent control workers (OR: 4.37; 95% CI: 3.0, 6.3 and OR: 11.08, 95% CI: 5.6, 22). Human-to-human transmission through breast-feeding has also been recorded (Bolin and Koellner 1988).

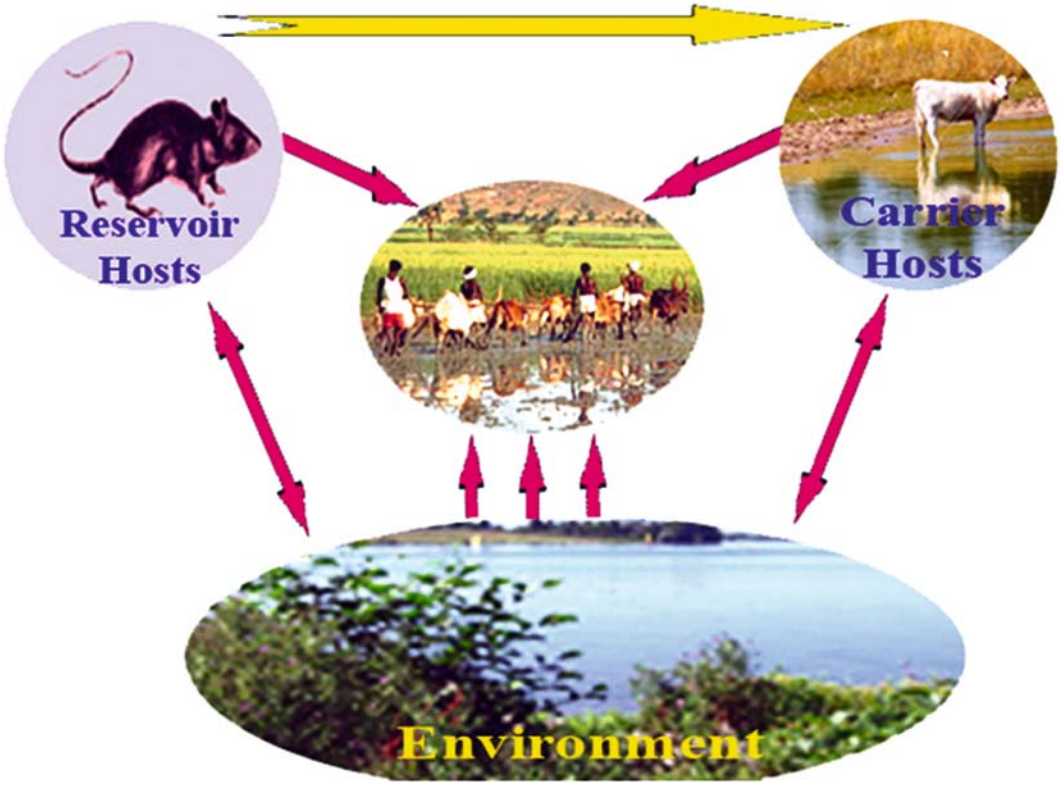

Figure 2. Transmission dynamics.

Indirect transmission occurs when an animal or human being acquires leptospirosis from environmental leptospires, originating in the urine of excretor animals (Faine 1994). Leptospires can survive for long periods of time in the environment (Smith and Self 1955) and probably multiply when the conditions are favourable (Baker and Baker 1970). It is considered that the most common portal of entry of leptospires into the host body is through intact skin (Faine 1994). High incidence has been recorded among people who are exposed to wet environments because of occupational or other activities. Leptospirosis is a known health hazard of rice farmers in countries such as Indonesia and Thailand. High incidence of leptospirosis has been recorded in provinces with large populations of farmers (Tangkanakul *et al* 2000a, b). Outbreaks have occurred in Korea on several occasions when the fields were flooded before harvest (Park *et al* 1989). Outbreaks have occurred among general population when people are exposed to floodwaters that have high chance of leptospiral contamination (WHO 2000; Sehgal 2001; Zaki and Sheih, 1996).

Leptospirosis has been recognized as a potential hazard of water sports and other recreational activities that exposes people to possible contaminated waters. Outbreaks associated with recreational exposure to water have been reported from several countries. Anderson *et al* (1978) investigated a cluster of seven cases of leptospirosis among the residents of Stewart County, Tennessee. Frequency of exposure to a creek was the only factor significantly associated with leptospirosis. Sejvar *et al* (2003) investigated an outbreak of febrile illness among the athletes who participated in the eco-challenge Sabah 2000 multi-sport expedition race in Borneo, Malaysia. Among the athletes studied, 54% were found to have serological evidence of leptospiral infection. Swimming in the river was significantly associated with leptospirosis.

9. Biologic spectrum of disease – subclinical infections, clinical syndromes and clinical courses

There is a wide spectrum of clinical presentations. The incubation period is usually 7-14 days but may range from 2-30 days. Vast majority of leptospiral infections are either subclinical or result in very mild illness and recover without any complications. However, a small proportion develops various complications due to involvement of multiple organ systems (Faine 1982, 1994). In such patients, the clinical presentation depends upon the predominant organs involved and the case fatality ratio could be about 40% or more. Febrile illness with icterus, splenomegaly and nephritis (known as Weil's disease), acute febrile illness with severe muscle pain, febrile illness with pulmonary haemorrhages in the form of haemoptysis, jaundice with pulmonary haemorrhages, jaundice with heamaturea, meningitis with haemorrhages including sub conjunctival haemorrhage or febrile illness with cardiac arrhythmias with or without haemorrhages are some of the syndromes. Protean manifestations of leptospirosis lead more often to misdiagnosis and eventually are under-reported.

The onset of disease is abrupt with fever, which is associated with severe headache and body ache. Temperature ranges from 100-105ºF. Persistent and severe headache, usually frontal, less often retro-orbital, may be the first symptom in a small proportion of patients. Body pains are most marked in the lower limbs especially in the calves and thighs. Prostration may also be marked, leading the patient to stop work and frequently take to bed. Anorexia, nausea and vomiting are frequent and may be associated with constipation or diarrhoea. Epitasis may occur during the early stage. Mental symptoms of restlessness, confusion, delirium and hallucinations and occasional psychotic behaviour may be some prominent features in few patients. Some patients develop acute gastroenteritis with abdominal pain, vomiting and diarrhoea. Conjuctival suffusion occurs at an early stage of the disease. It is bilateral; most marked on the palpebral portion and is usually associated with unilateral or bilateral subconjuctival haemorrhage. There is no inflammatory exudate and true conjunctivitis does not occur. Myalgia is severe and commonly observed in the lower limbs especially the calves. A transient macular, maculopapular, erythematous, purpuric or urticarial rash may occur, usually on the trunk but it may be localized on the upper limbs or the shins.

10. Clinical syndromes associated with high morbidity and case fatality ratio

10.1 *Hepato-renal syndrome or Weil's disease*

A small proportion of patients develop severe icteric illness with renal failure. Jaundice occurs between the fourth and sixth day but may occur as early as the second day or as late as the second to third week. The liver is often enlarged and tender. Jaundice is due to hepatocellular necrosis, intrahepatic cholestasis and increased bilirubin load from absorption of tissue haemorrhage. Marked elevations of bilirubin with mildly elevated transaminases are some characteristic features. Death rarely occurs due to hepatic failure. Renal involvement is the most serious complication and is the most common cause of death. Oliguria occurs in the second week, but may occur as early as fourth day of illness. A significant number of patients develop non oliguric renal failure and have a better prognosis than oliguric renal failure. Renal manifestations range sediment changes (pyuria, albuminurea, heamaturea and granular casts) to severe renal failure. Anorexia and vomiting worsen and

hiccups may occur. Confusion, restlessness, hallucinations, delusion and convulsions may occur. Cardiac and pulmonary complications are frequent. Death may occur at this stage due to renal failure. Occasionally sudden death may occur from arrhythmias, cardiac failure or adrenal haemorrhage. Massive bleeding from the alimentary and respiratory tract could also lead to death. In those who are not severely ill, recovery takes place in the second week. Diuresis occurs and the blood urea level falls gradually. Fever subsides and the general conditions improve, however, jaundice takes a longer time to clear.

10.2 *Haemorrhagic pneumonitis*

The onset is sudden with rapidly raising fever which is associated with headache, generalized body ache, and cough which is dry in the beginning but becomes streaked with blood after two to three days. The patient becomes breathless and toxic. Clinical examination reveals a toxic patient with temperature ranging between 100-105ºF, tachycardia, tachypnoea and hypertension in few. Fine crepitations, which in the initial stage are confined to the bases but soon become extensive involving bilateral lung fields. Massive haemoptysis may cause asphyxiation and death. Mortality in these cases is very high and it may be as high as 50-70% in cases who report late to the hospital. Chest X-ray in these patients show bilateral alveolar shadowing which appears to be denser in the mid and lower zones. However the radiological abnormalities may range form a single ill defined opacity, through multiple areas of infiltration to a large area of consolidation. These abnormalities clear up within weeks without any residual damage.

With this in perspective, a prospective study (Singh *et al* 1999) on 58 patients confirmed to have current clinical infection reveals that there are two separate clinical syndromes; the hepato-renal form and the pulmonary form. However, overlapping of these two syndromes was observed in small proportion of patients. Central nervous system involvement (neck stiffness and altered sensorium) were observed in about 12.0% of patients but none of them developed complications of meningitis. Hypotension was observed in 40% of patients. Thirty patients of each (51.7%) had abnormal liver function test or abnormal renal function test and 45.6% of them have both abnormalities. 61.1% patients' Chest X-ray showed bilateral fluffy opacities (figure 3). Forty four patients (75.8%) recovered with antibiotic therapy (two million units of CP six hourly or 3-4 days followed by 1 million units of CP intra-muscular for 7-10 days) and conventional therapy. Remaining 14 patients (24.2%) died. Early antibiotic treatment reduces the severity of complications considerably. In 28 patients, chest X-ray

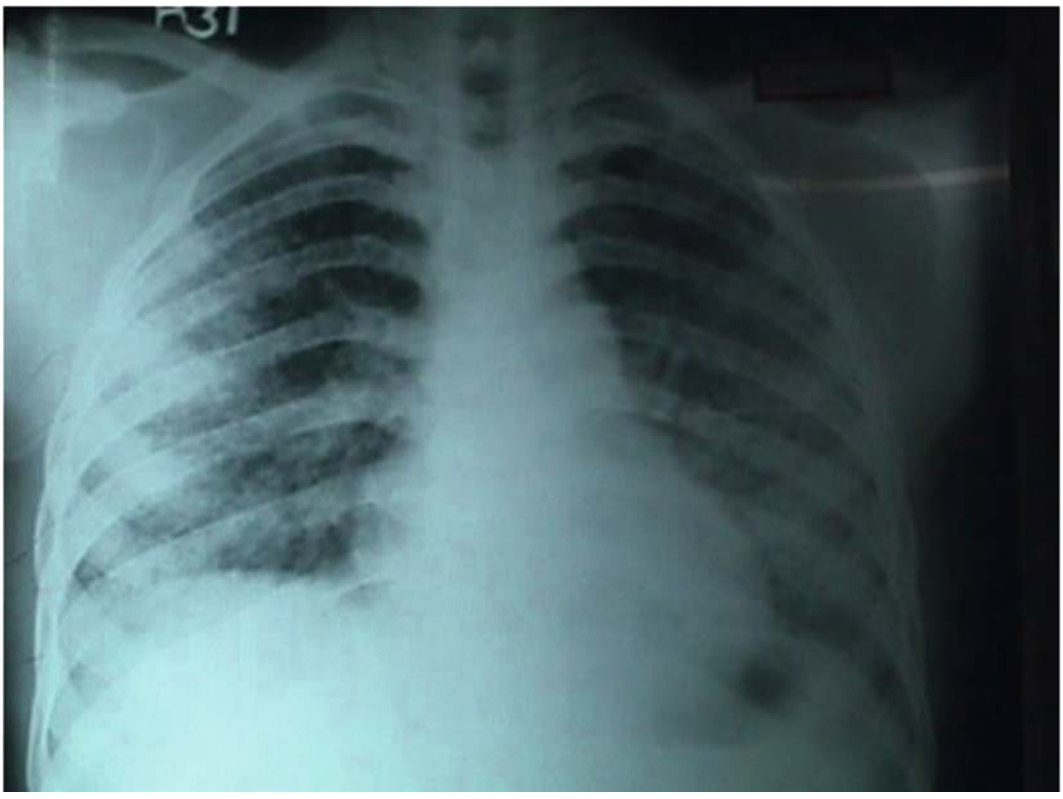

Figure 3. Chest X-ray showing bilateral fluffy opacities.

findings were suggestive of pneumonitis and all of them had pulmonary haemorrhages and 12 died giving case fatality ratio (CFR) of 42.9%, remaining 30 patients who presented with hepatic or renal involvement or both, two died (CFR 6.7%). Thus the mortality rate was considerably low among the patients with hepatic or renal or both complications when compared with patients having pulmonary complications.

11. Laboratory diagnosis

Laboratory diagnosis of leptospirosis is an area not often well understood by the scientific fraternity. Selection of the right specimens, tests and correct interpretation of test results are of paramount importance to provide better patient care. Laboratory diagnosis is broadly classified into direct evidences (isolation of organism or demonstration of leptospires by dark field microscopy or amplification of specific fragment of leptospiral DNA) and indirect evidences (detection of antibodies to leptospires). Alternatively, the different methods used in the laboratory are categorized in to bacteriological, microscopic, serological and molecular. Isolation of organism from clinical specimens is central for confirmatory diagnosis. Alternative tests for confirmation of diagnosis include MAT on paired sera collected during acute and convalescent phases of the disease to establish sero-conversion or rise in titre or amplification of specific fragment of leptospiral DNA using PCR. Sehgal *et al* (1999) reported that MAT has a sensitivity of 41% during the first week and rose to 82% during second to fourth week and 96% beyond the fourth week of illness. Some of the patients who had negative MAT results during the second to fourth week become positive when another sample is obtained 30 days after the onset of the illness. This indicates that to rule out the diagnosis a third sample obtained after one month is essential, which is not practicable in clinical practice. Considering these limitations, MAT is being used on single sample for presumptive diagnosis. However cut off titre for single MAT would vary from one geographical area to the other. The protean manifestations of the disease make it difficult to arrive at a clinical diagnosis.

Lack of facilities for isolation, performing MAT or PCR, necessitates institutions or hospitals in developing countries to adopt simple and rapid diagnostics *viz*., IgM ELISA, Micro capsule agglutination test (MCAT), LEPTO Dipstick, Macroscopic slide agglutination test (Macroscopic SAT), LEPTO Lateral flow, Indirect hemagglutination assay (IHA) and LEPTO Dri Dot (Sehgal *et al* 1999; Sehgal *et al* 2003; Vijayachari *et al* 2002).

12. Indian scenario

In the recent past, the incidence has been showing sudden upsurges in Kerala. The districts of Kottyam, Alleppey and Kozhikode are the worst affected serogroup Autumnalis is the commonest cause of infection, with Hepato-renal involvement and myocarditis as the commonest complications (Kuriakose *et al* 1997). In Tamil Nadu, leptospirosis has been recognized as an important public health problem. It is a major cause of renal failure in Chennai (Muthusethupathi *et al* 1994). There have been reports of ophthalmic involvement in the form of panuveitis and retinal vasculitis in Madurai (Rathinamsivakumar *et al* 1996; Rathnam *et al* 1997). About 30% of pyrexia of unknown origin (PUO) cases in Chennai city during the monsoon period were found to have evidence of leptospiral infection. Leptospirosis has been reported from Mumbai quite early (Dalal 1960). Subsequently there have been occasional reports of Leptospirosis in Maharashtra but most of them were based on clinical grounds (Sahni *et al* 1995). There was a case report of suspected case of Leptospirosis in Orissa state in 1945 (Mohanty 1945). However, after this nothing was known about the status of leptospirosis in the state. An outbreak of febrile illness with haemorrhagic manifestations, particularly pulmonary haemorrhage, in November 1999 following a cyclone and flooding. Leptospirosis has been reported from Gujarat since 1994. In 1997 a major outbreak occurred in Surat and Valsad. These upsurges during the July – September period repeated in 1998 also. Not many efforts have been taken to study the status of Leptospirosis in other states. However, there have been sporadic reports of leptospirosis in many other states. There was a suspected outbreak in Delhi. This infection has been reported in the North East (Baruah *et al* 1999), Karnataka (Madhusudhana and Bhargawa 1988; D'Souza *et al* 1990), Bihar (Jha 1997) and Puducherry (Prabhakar *et al* 1997). It has been suspected in Kashmir in the early part of the 20th century. Although the diagnosis in several of these reports was not based on authentic diagnostic technique, these show the overall situation in the country regarding the existence of this infection.

13. Treatment

Antibiotic treatment is effective within 7 to 10 days of infection and it should be given immediately on diagnosis or suspicion. The antibiotic of choice is benzyl penicillin by injection in doses of 5 million units per day for five days. Patients who are hypersensitive to penicillin may be given erythromycin 250 mg 4 times daily for 5 days. Doxycycline 100 mg twice daily for 10 days is also recommended. Tetracyclines are also effective but contraindicated in patients with renal insufficiency, in children and in pregnant women.

14. Prevention and control

Prevention of leptospirosis is essentially by identifying the source and interrupting the transmission (Faine 1994). In

different epidemiological settings, different animal species could be the primary source of infection. Risk factor analysis highlight, significant association of leptospirosis with possible carrier animals viz., cats (Bovet *et al* 1999) and rats (Sarkar *et al* 2002). While in the Andaman archipelago of the Andaman & Nicobar islands, cattle were found to be associated with leptospiral seropositivity (Murhekar *et al* 1998). Instances were there is absence of statistical evidence of association between contact with an animal species and human leptospirosis, but if significant carrier state is detected in animals, it could be taken as a potential source of infection to humans. However, as seropositivity is not a reliable indicator of carrier state (Faine 1994), demonstration of excretion of leptospires in the urine by bacteriological or molecular tools are required.

Several strategies have been described to reduce the load of carrier domestic animals. Total herd immunization controlled leptospirosis in cattle in a trial (Little *et al* 1992). A proposed clean herd policy in UK, which categorized cattle into different levels based on infection and immunization status and imposing restrictions on pasture, restocking herds etc. was later abandoned as it could not eradicate infection (Faine 1994). An alternate 'test and slaughter' strategy can be considered if no alternatives exists (Faine 1982).

Measures targeting human beings include chemo-prophylaxis and vaccination. Chemoprophylaxis with doxycycline has been tried in soldiers from non-endemic areas visiting endemic areas and was found to be almost 100% effective (Takafuji *et al* 1984), while it gave only 54% protection (Sehgal *et al* 2000). Thus, chemoprophylaxis can be used in outbreak situations or in travellers. A good understanding of the ecological, epidemiological and cultural characteristics of a community that faces the problem of leptospirosis is the essential prerequisite for devising an effective and acceptable intervention strategy (figure 4).

Vaccines have been developed for use in man (Torten *et al* 1973). Existence of a large number of serovars makes it difficult to develop a universally effective vaccine. Leptiospires have evolved ways to evade host immune response (Zhijun *et al* 2007a). Because of the ability of pathogenic leptospires to translocate between mammalian cells quickly (Barochi *et al* 2002) and reach blood stream and their ability to enter and initiate programmed cell

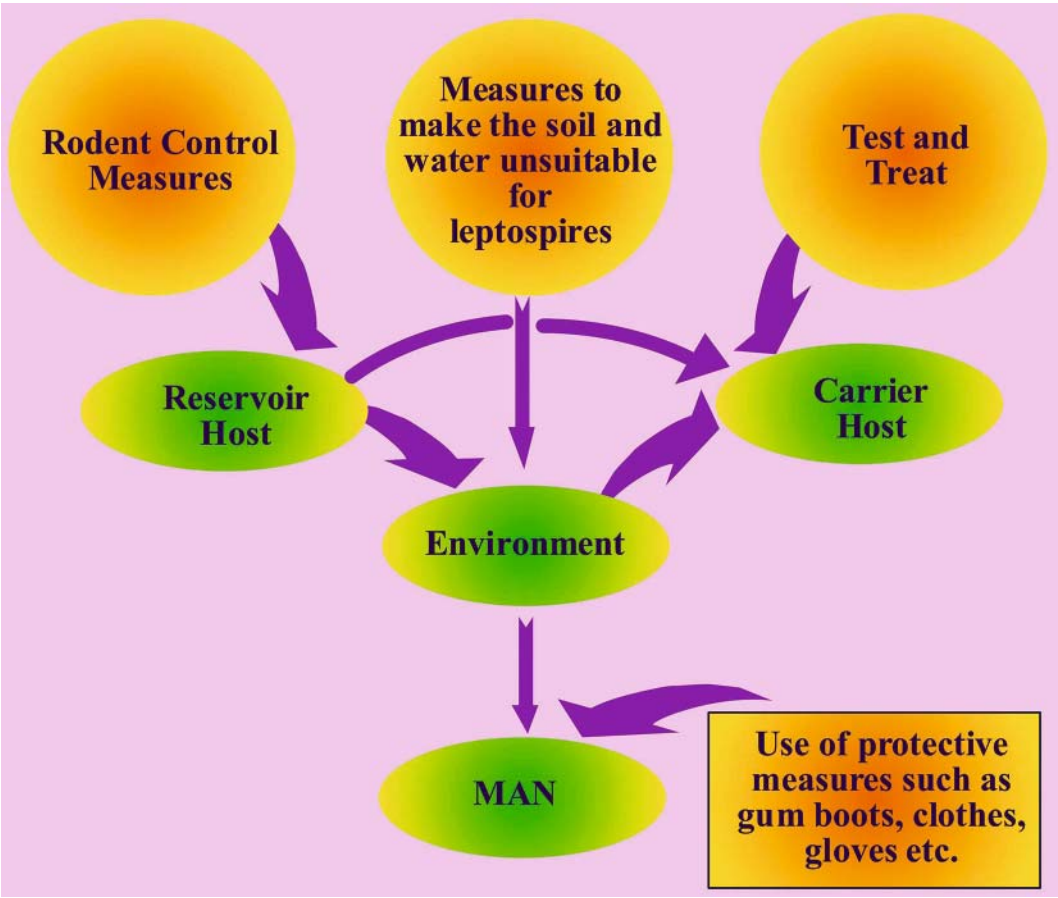

Figure 4. Different control strategies for leptospirosis.

death in kidney fibroblasts rapidly (Merien *et al* 1997), it is difficult for the immunologists to develop effective and safe leptospirosis vaccines. Currently the molecular and cellular studies on leptospirosis vaccines have been focused on bacterial motility, lipopolysaccharides (LPSs), lipoproteins, outer-membrane proteins (OMPs) and potential virulence factors (Zhijun *et al* 2007b).

Various attempts for development of leptospirosis vaccines are summarized in the web site 'Leptospirosis Vaccine Ontology Database' (Zhijun *et al* 2007b). Various recombinant leptospiral proteins have been constructed and tested for their usefulness as vaccine candidates. Amongst these, OMPs, lipoproteins and virulence factors have generated considerable interest. Leptospira external membrane protein LipL32 cloned and expressed in mycobacterial vectors have been found to be protective to hamsters (Seixas *et al* 2007). In 2002 a 130 kDa immunoreactive leptospiral immunoglobulin-like protein (LigA) was described (Palaniappan *et al* 2002). LigA as well as another immunoglobulin-like protein LigB were found to be immunogenic. It has been shown that LigA DNA vaccine gives significant protection against leptospirosis in hamsters. Inactivated and attenuated vaccines for leptospirosis have been described for more than 50 years. These vaccines have been tested in cattle and some of these are currently being used. Some of these vaccines have been tested in humans as well. At present, the only leptospirosis vaccine licensed for use in humans is being produced in Cuba. However, there is still an interest in inactivated and attenuated vaccines. Recently the focus is on newer vaccines such as DNA vaccines. At least two DNA vaccines, one encoding haemolysis-associated protein (Hap1) and the other endoflagella gene (flaB2) have been tested. These DNA vaccines require more extensive investigations.

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