INTRODUCTION
1. INTRODUCTION

Till today there have been threats of new diseases emerging due to the evolution of microbes and the re-emergence of old diseases due to the development of antimicrobial resistance. Many factors have contributed to the emergence of infectious diseases are unplanned and under-planned urbanization, rapid population growth, ageing population, poverty, inadequate public health infrastructure, malnutrition and irrational antibiotics usage, increased exposure of humans to disease vectors, environmental pollution and global warming. The new megacities have overcrowding, inadequate infrastructure, poor sanitation and water supply and poverty that amplify transmission of infectious diseases. Multiple hypothesis such as human activity; seasonal variability in human immune system function; seasonal variations in vitamin D levels; seasonality of melatonin; and pathogen infectivity have been proposed that can influence the seasonal patterns of infectious diseases (Storr and Kilpatrick 2013).

Over the past 3 decades, more than two thirds of the emerging infectious diseases have had an origin in animals. These include leptospirosis, HIV infection, Ebola haemorrhagic fever, the new variant of the Creutzfeldt-Jakob disease (nvCJD) etc. Some of these diseases, especially leptospirosis, AIDS, anthrax, brucellosis, cysticercosis, neurocysticercosis, hydatid disease, rabies and Human African Trypanosomiasis (HAT) have seriously posed significant threats with millions of cases and thousands of deaths worldwide. Emerging vector-borne diseases are having an upper hand these days both in the developed and developing countries (Gargano et al., 2010).
Zoonotic Diseases

Any disease or infection that is naturally transmissible from animals to humans and vice-versa is classified as a Zoonosis. It represents the leading cause of illness and death among infectious diseases. Around 61% of human microbial pathogens and about 73% of re-emerging human pathogens were identified during the past two decades to be Zoonoses (Cascio et al., 2011). These assume a great public health importance as approximately 80% population in India lives in close contact with domesticated animals. Increased contacts with a wildlife reservoir, associated with the development of various outdoor leisure activities, such as hunting, fishing or tourism, especially ecotourism may expose humans to bacteria excreted by healthy animal carriers. In the last few decades several bacterial zoonotic diseases like leptospirosis, anthrax, brucellosis, tuberculosis, salmonellosis and campylobacteriosis have been reported to be re-emerged (Samad, 2011).

Leptospirosis

Leptospirosis a contemporary infectious disease also known as Weil’s syndrome is an emerging and re-emerging life threatening zooanthroponotic disease which affects internal organs producing multiple organ dysfunctions (MOD) to multiple organ failure (MOF). The disease occurs as two clinically recognizable syndromes, namely anicteric (atypical) leptospirosis and icteric (typical) leptospirosis, a more serious and can be potentially fatal (Weil’s syndrome) depending upon the strain and physiological condition. The disease is sometimes unnoticed due to varying clinical symptoms such as flu like illness, headache, severe myalgia and chills to renal and vascular dysfunction (Isa et al., 2014).
History

In 1886, Adolf Weil described a clinical syndrome characterized by splenomegaly, jaundice, haemorrhages and nephritis. This syndrome is usually referred to as Weil’s disease caused by *Leptospira interrogans*, serovar *icterohaemorrhagiae* or *copenhageni*. Clinical syndromes resembling Weil’s description of haemorrhagic jaundice have been known for many centuries (Vijayachari *et al.*, 2008).

In 1916, Inada and coworkers successfully transmitted the infection to guinea pigs and from the blood of the infected animals they reisolated the responsible organism. By 1940s leptospirosis in animals was recognized as an important veterinary problem as well as a source of infection to man. Rodents are the first recognized reservoirs of *Leptospira*. A large number of studies on seroprevalence and Leptospiral carrier state in rodents have been conducted in various countries (Monahan *et al.*, 2009).

The organism affects at least 160 mammalian species and has been recovered from rats, swine, dogs, cats, raccoons, cattle, and other animals. It has now become clear that almost any mammalian species including wild animals and aquatic mammals can harbor the pathogen and can act as source of infection to man (Poeppl *et al.*, 2013).

Clinical Features

Leptospirosis occurs as two clinically recognizable syndromes. The most common syndrome is anicteric leptospirosis, a self-limited illness that occurs in 85% to 90% of the cases. Icteric leptospirosis or Weil’s syndrome, is a more serious, potentially fatal, syndrome and occurs in 5% to 10% of the cases with hepato-renal involvement (Kim, 2013).
Anicteric Leptospirosis

The onset of anicteric (atypical) leptospirosis is abrupt and is characterized by fever, headache, severe myalgia, chills with rigors, prostration and sometimes, circulatory collapse. There are two different phases of anicteric leptospirosis exists namely septicemic phase and immune phase. The septicemic (or first) phase lasts 3 to 7 days. Fever is high and remitting. Headache is intense, unremitting and possibly throbbing. Anorexia, nausea, vomiting and abdominal pain occur in most patients. The most common physical finding is conjunctival suffusion in the absence of purulent discharge. Other signs include maculopapular skin rash, pharyngeal injection, lymphadenopathy, splenomegaly, hepatomegaly, and muscle tenderness. *Leptospira* can be isolated from the blood and the CSF during this phase (Tullu and Karande, 2009).

The immune (or second) stage of anicteric leptospirosis is preceded by a one to three-day asymptomatic period. The onset of the immune stage coincides with the appearance of IgM antibodies. Fever, headache and vomiting are less severe at the onset of the immune stage than during the septicemic stage. The duration of the immune stage ranges from 4 to 30 days, and the pathogen are cleared from the blood and the CSF after the first days of this stage. Leptospiruria develops and persists for 1 to 3 weeks. Aseptic meningitis is the hallmark of the immune stage. Mild pleocytosis is present, with or without meningeal signs and symptoms. The CSF cell count is <500/mm³ in most cases. Polymorphonuclear cells may predominate early in the illness, but mononuclear cells predominate later. The CSF protein levels ranges from <40mg/dl (normal) to 300 mg/dl and the CSF glucose concentration is generally normal. Uveitis, iritis, iridocyclitis and chorioretinitis may also appear during the immune stage (Vaishnavi et al., 2011).
Icteric Leptospirosis

Icteric (typical) leptospirosis or Weil’s syndrome is a form of disease characterized by symptoms of hepatic, renal and vascular dysfunction. The clinical manifestations vary in terms of severity and symptomatology. Some patients with jaundice may have no renal manifestation. Supportive therapy has reduced mortality to between 5% and 10%. Any serotype of *L. interrogans* may cause icteric leptospirosis. During the leptospiraemic phase of icteric leptospirosis, the symptoms do not suggest leptospirosis until the third to seventh day of illness, when jaundice and azotaemia develops. Hepatic dysfunction occurs, but it resolves by self limitation and it is rarely the cause of death. Jaundice appears, with hepatocellular destruction (Issa *et al.*, 2014).

**Biology of Leptospira**

*Leptospira* are tightly coiled spirochaetes, usually 0.1μm in diameter and length ranging from 6 to 20 μm, but occasional cultures may contain much longer cells. The helical amplitude is approximately 0.1 to 0.15 μm, and the wavelength is approximately 0.5 μm. The cells have pointed ends, either or both of which are usually bent into a distinctive hook. Two axial filaments (Periplasmic flagella) with polar insertions are located in the periplasmic space. The structure of the flagellar proteins is complex (Fentahun and Alemayehu, 2012). They demonstrate a characteristic ‘cork screw’ and ‘darting’ type of motility (Harvey *et al.*, 2013).

The spirochaete are obligate aerobic and microaerophilic with an optimum growth temperature of 28 to 30°C. They produce both catalase and oxidase. They are highly fastidious and their media needed to be enriched with vitamins (Vitamin B<sub>2</sub> and B<sub>12</sub> are growth factors), long chain fatty acids, and ammonium salts. The organism is a chemooorganotrophic and utilizes long chain fatty acids as the sole carbon source and is metabolized by β-oxidation (Gupta *et al.*, 2013).


**Epidemiology**

Weil's disease occurs in many countries, including India and other South-East Asian Countries, China, continental Europe and England. Leptospirosis exists in all the five inhabited continents and in a large number of countries. It occurs in tropical, subtropical and temperate zones. The disease occurs in every individual whether rich or poor, children or aged, male or female, vegetarian or non-vegetarian belonging to any religion (Tilahun *et al.*, 2013).

The disease is widespread in diverse geo-climatic zones of the world caused by infection with pathogenic *Leptospira* species. There is a sudden upsurge in the number of reported cases in past few years. Annual incidence is estimated from 0.1-1 per 100,000 in temperate climates to 10-100 per 100,000 in the humid tropics. An international survey conducted by the International Leptospirosis Society reported ≥350,000 cases of severe leptospirosis reported annually which is supported by data from an assessment of the global incidence of leptospirosis, which indicates a mean global incidence rate for leptospirosis of 5 cases/100,000 population (Agampodi *et al.*, 2014).

The disease incidence of more than 100 per 100,000 is encountered during outbreaks and in high exposure risk groups. Numerous outbreaks of leptospirosis have occurred worldwide during the past decade affecting human beings and many other species of vertebrates. A large proportion of the population depends on the agrarian way of life like intimate contact with animals, unprotected entry into waterlogged fields, and bathing in contaminated community ponds are a part of rural life and precisely these are the conditions most suitable for the survival and transmission of the pathogen (Sethi *et al.*, 2010).
The bacteria are adapted to the environment of the tropical region with plenty of rainfall and it is often difficult to avoid exposure of the people to animals or contaminated environment. Being a Zoonotic disease with a large variety of animal species acting as carriers it is difficult to eliminate and perhaps even to them control in tropical developing countries (Verma et al., 2013).

Outside India

Leptospirosis is thought to be the most wide spread zoonoses in the world. Cases are frequently reported from all continents except Antarctica and are especially prevalent in the tropics. Although leptospirosis is not a common disease, it has been reported from all regions including developed countries like United States of America. Hawaii has the highest reported annual incidence rate of leptospirosis in the United States, reaching 1.63 cases per 100,000 inhabitants (Katz et al., 2011).

An increasing proportion of the leptospirosis infections diagnosed in many European countries is imported from abroad (25% to 60% of all infections). Most countries in the South East Asian region are endemic to leptospirosis. On an average 10,000 severe cases requiring hospitalization occur world over annually. In Philippines, >1,000 people are hospitalized annually with leptospirosis. Fatality is high rate ranging from 11% to 20% this appears to be in part because people go to hospital only if severe symptoms develop because they cannot afford to pay for long stays in a hospital. Therefore, it is the urban and rural poor who are at greatest risk because of higher rates of exposure to infectious bacteria and little available income for early medical intervention (Goarant et al., 2013).
In India

Leptospirosis is a notifiable disease in India for the past three decades no accurate disease incidence figures are available. *Leptospira* has hit virtually all parts of urban, semiurban, semirural and rural India (Parasuraman et al., 2014). Taylor and Goyal (1931) first described the disease in Andaman Islands, India. Sixty cases confirmed by isolation and serological methods. There was also a severe outbreak in Mumbai in May 2000 and Kerala in August 2000, causing significant mortality. An outbreak of leptospirosis was also reported in 102 cases in Mumbai following prolonged water logging due to heavy rainfall during July, 2002. In the recent past, the incidence has been showing sudden upsurges in Kerala. The districts of Kottyam, Alleppey and Kozhikode are the worst affected. Sero-group Autumnalis is the commonest cause of infection, with Hepato-renal involvement and myocarditis as the commonest complications (Shivakumar et al., 2008).

In September 2004, there were 550 confirmed cases of leptospirosis and at least 75 deaths in South Gujarat. Subsequently there have been occasional reports of leptospirosis in Maharashtra (2005), after heavy flooding there were at least 100 deaths from leptospirosis within a two-day period. Efforts have not been taken to study the status of leptospirosis in other states of our country. However, there have been sporadic reports of leptospirosis in the North East (Barua et al., 1999), Karnataka (D’Souza et al 1990), Bihar (Jha, 1997) and Puducherry (Prabhaker et al., 1997). 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. The epidemiological pattern of disease in India has been reviewed, the seroprevalence is reported to be high (52.7%) among high-risk population of Andhraman Islands and 19.8% and 9.3% in Madras and Bangalore respectively (Sehgal, 2006).
In Tamilnadu

In Tamilnadu, leptospirosis has been recognized as an important public health problem. In 1983, in Chennai, the seroprevalence of leptospirosis in jaundiced patients was 18% and it was 24% in PUO cases. During the same year, a serological study was made among the population that consisted mainly of children in a village near Chennai, following an outbreak of disease in cattle; 35 of 75 (47%) human sera gave positive antibody titers (Ratnam et al., 1983c).

It is reported to be the predominant serogroup infecting the state and endemic to Chennai. Other serovars are also being reported as common causal factor for this disease and confirmed for the first time in India, for the serovar javanica by its isolation, identification and WHO confirmation from a human clinical case at Chennai (Saravanan et al., 1998b).

*L. autumnalis* is found very common among the rodent population in rice mills of Salem. Wet environment and unprotected workers lead to an ideal setting for an outbreak among the rice mill workers during the year 2000, and 68.3% of them were seropositive (Natarajaseenivasan, 2002). One of the major problems in reporting systems in Tamil Nadu is that the reporting is done only by hospitals and clinics run by the Government. The private sector, which serves a major portion of the population, has no participation in the system, and hence its sensitivity reported for the disease is very low (Sehgal et al., 2003).

Large number of outbreaks have been noticed during the period of October to December, every year (Prabhu et al., 2010). About 30% of pyrexia of unknown origin (PUO) cases in Chennai city during the monsoon period was found to have evidence of Leptospiral infection. There have been reports of ophthalmic involvement in the form of panuveitis and retinal vasculitis in Madurai (Sivakolundu et al., 2012).
Pediatric Leptospirosis

In India outbreaks of leptospirosis have been reported in adult patients from Chennai (Muthusethupathi et al., 1995), Kollenchery (Kuriakose et al., 1997), Port Blair (Singh et al., 1999) and Orissa (WHO, 2000) in the last decade. Nearly 232 cases have been reported from a hospital in north India between 2004 and 2008 (Sethi et al., 2010). Young children are often exposed to *Leptospira* because they are fond of water games like swimming in lakes, fishing, etc., and from petting animals and exposure to direct or indirect contact with the infected animal’s urine (Leshem et al., 2011). A sero-epidemiological study on leptospirosis among children in Salem district was carried out during 2009 to 2011. Among the suspected boys Leptospiral seropositivity was 55.65% and among the girls 44.34% were observed. *Leptospira autumnalis, icterohaemorrhagiae* and *javanica* were the predominating serogroups noted during the study (Saravananan et al., 2012a).

The precise identification, seasonal variation, role of rainfall and classification of *Leptospira* are important for epidemiological and public health surveillance especially in children. Moreover, the information available is very limited about symptomatic leptospirosis in the pediatric age group in medical literature. Urban, semi-urban, and rural incidence and prevalence of leptospirosis in Salem children is not known exactly though periodic outbreaks are known to occur in every part of our country. Hence there is a need to find out the incidence of leptospirosis among children in Salem District of Tamil Nadu.

Natural Cycle

The epidemiology of human leptospirosis reflects the ecological relationship between humans and chronically infected reservoir hosts. Humans are considered an incidental end-host from which further transmission has not been demonstrated, although individuals can excrete *Leptospira* in their urine for several weeks (Desvars et al., 2013).
There are two natural cycles of transmission of Leptospira. A sylvatic cycle exists between rodents and marsupials and a domestic cycle involves cattle, pigs, dogs and sheep. In the sylvatic cycle, leptospirosis is accidentally transmitted to farmed animals and humans from numerous infected species of rodents and marsupials. The principal means of spread and continuity of infection in rodents or marsupials is by direct transmission from the mother to the young. Humans can be infected through contact with an environment contaminated with rodent's urine. The most important sources for human infection are the various species of rodents with which humans live in domestic, agricultural or occupational association (Himani et al., 2013).

The domestic cycle of leptospirosis involves cattle, pigs, sheep, buffalo, goats and dogs. These animals can be the maintenance hosts of specific serovars for example cattle usually maintains serovars Hardjo, Pomona and Grippotyphosa; pigs harbor serovars of Pomona and Tarassovi; Sheep may harbor Pomona; and dogs may harbor Canicola (Hassanpour et al., 2012).

**Risk Factors**

Risk factors for infection vary significantly between countries, and depend on many cultural, environmental, and ecological variables. Occupational and recreational exposures account for 30-50% of leptospirosis cases. Previously, most cases were due to occupational exposure. However, more cases are occurring due to recreational exposure; although, occupational exposure is still of a significant concern (Fang et al., 2013).

**Occupational Hazard**

The disease has been known by a variety of names that reflect the method (usually occupational) by which the disease was contracted. Alternative names include Rice field fever (in Indonesia), Seven-day fever (in Japan), Cane cutter's disease (in Australia), Swineherd's disease and Schlammfieber or Mud fever (in Europe), Dairy farm fever, Swamp fever and Fort Bragg fever (in the United States). Thus, leptospirosis had various names.
in different parts of the world that denoted seasonal association, symptoms, duration or occupations that were thought to be associated with the disease (Visweswaran and Sreelatha, 2012). Occupational groups include pet shop owners, veterinarians, veterinary technicians, agricultural workers, abattoir workers, plumbers, meat handlers, meat inspectors, hunters, laboratory staff, butchers, herders, meat carriers, coal miners, construction workers, fish industry workers, military personnel, civil emergency personnel, sewer workers, and garbage collectors (Dreyfus et al., 2014).

Recreational Hazard

High-risk recreational exposures for leptospirosis include adventure travelling to the tropics, cave explorers, camping, hiking and riding trail-bikes through contaminated puddles, swimming, canoeing and kayaking, fishing, water skiing and windsurfing, and other outdoor sports played in infected water. Recreational activities such as water sports and adventure travel are emerging as an important risk factor for leptospirosis. Large outbreaks occur when these recreational activities are organized as a part of competitions (Visweswaran and Sreelatha, 2012).

Sources of Infection

Infection is acquired through contact with contaminated water, directly or indirectly due to urine of rodents, carrier or diseased animals. Infected animals urine is thus the chief vehicle of the infection. Direct transmission of the disease is rarely reported. The sources of infection vary in different regions. The disease has traditionally been considered mainly as a disease of farmers in places where cattle and pigs are raised. These animals are often chronically colonized by pathogenic *Leptospira*. It is a serious contagious disease commonly transmitted by not only the urine of rats but also it spreads through flood waters, garbage, wet ground and contaminated plants (Calvo-Cano et al., 2014).
Mode of Transmission

The modes of transmission can be either direct or indirect. Direct transmission occurs from chronically infected animals to other susceptible animals through animal’s urine. Indirect transmission occurs when animals or humans acquire infection by Leptospira from the environment through the conjunctivae, the oral mucosa, and respiratory tract mucous membrane or cuts and abrasion in the skin (Loffler et al., 2014).

Pathogenesis

Leptospira are presumed to enter via small abrasions or other breaches of the surface integument. The incubation period for leptospirosis is usually 7 to 12 days, but it can range from 2 to 20 days. They may also enter directly into the bloodstream or lymphatic system via the conjunctiva, the genital tract in some animals, the nasopharyngeal mucosa, possibly through a cribiform plate, the lungs following inhalation of aerosols or through an invasion of the placenta from the mother to the foetus at any stage of pregnancy in mammals (Zhang et al., 2012).

Pathogen can be isolated from the bloodstream within minutes after inoculation and detected in multiple organs by the third day after infection. They may reach 106 -107 organisms per ml or g in the blood and tissue of patients and infected animals. Therefore, they evade the host innate immune response during the initial stages of infection mainly through clearance by phagocytosis and killing by complement (Zhang et al., 2012).

The adhesion of pathogen to host tissue components is thought as an initial and necessary step for infection and pathogenesis. Attachment to host cells and ECM are likely to be necessary for the ability of them to penetrate, disseminate and persist in mammalian host tissues. In-silico analysis and experimental techniques employed to identify leptospiral surface-exposed proteins that might have potential roles in Leptospira adhesion and pathogenesis (Pinne and Haake, 2009).
It is known that they can alter their biosynthetic mechanism for the production of the LPS in their outer membrane thus allowing them to adapt to new host (Saravananan et al., 2013). Virulent pathogens bind to endothelial, fibroblast, kidney epithelial and monocyte/macrophage cell lines when cultured in vitro. Though they are not intracellular parasites, they efficiently enter host cells in vitro and rapidly translocate across polarized cell monolayers without altering the transepithelial electrical resistance. They reside only transiently within these cells and it affects internal organs producing multiple organ dysfunctions (MOD) to multiple organ failure (MOF). The clinical manifestations vary in terms of severity and symptoms from fever, headache, severe myalgia and chills to renal and vascular dysfunction (Forbes et al., 2012).

Virulence Factors

Leptospiral virulence factors such as hemolysin, lipopolysaccharide, glycoprotein, peptidoglycan, heat shock proteins, flagellin and many others may contribute to the pathogenesis. The production of toxins by pathogenic Leptospira in vivo was inferred by Arean. Endotoxic activity has been reported in several serovars. Leptospiral LPS preparations exhibit activity in biological assays for endotoxin, but of much lower potencies. Haemolysins play an important role in the toxic response and several genes coding for predicted haemolysins were identified in the genome sequencing of L. interrogans (Wang et al., 2012).

Haemolysin

The pathogenic bacteria cause diverse damage in human as a consequence of virulence factors produced by them and the haemolysins are the most important one. Virulent strains of the organism exhibit higher hemolysin activity than avirulent strains, and strains cultured for a long period under laboratory condition lose virulence and hemolysin production simultaneously (Carvalho et al., 2010).
Haemolysin (Sphingomyelinases) *sphH* gene has been reported in a number of leptospiral serovars. Several putative leptospiral haemolysins have been identified with the completion of *Leptospira* genome sequencing. Earlier studies performed in Tamilnadu, based on isolation and serology among human beings had shown that *L. autumnalis* was the dominant infecting serogroup. *Leptospira autumnalis* is a pathogenic bacteria cause diverse damage in human as a consequence of virulence factors produced by them among the various hemolysins and outer membrane proteins are the most important ones. Hemolysin *sphH* (Sphingomyelinases) gene has been reported in a number of leptospiral serovars (Saravanan et al., 2012b).

Pathogenic *Leptospira* requires ions for their growth and these spirochetes probably use their haemolysins such as the sphingomyelinases to obtain ions from host red blood cells during infection which results in erythrocyte lysis in host. The ability of haemolysins to lyse erythrocytes and other cell membranes makes them potential virulence factors (NarayanaVARI et al., 2012). The role of leptospiral sphingomyelinases and sphingomyelinase-like proteins in leptospiral pathogenesis is obscure several molecular and proteomic studies are needed to understand their activity.

**Outer Membrane Proteins (OMPs)**

The outer membrane of *Leptospira* contains LPS and several lipoproteins (Outer membrane proteins [OMPs]). The LPS is highly immunogenic and is responsible for serovar specificity. Leptospiral OMPs are generally well conserved and would have the potential advantage of inducing comprehensive immunity and play a role in virulence. Only few transmembrane OMPs have been described: the first is the OmpL1 protein potentially acting as a porin; OmpL36, OmpL37, OmpL47 and OmpL54 have been recently described as novel membrane spanning proteins, whose role has yet to be investigated (Pinne and Haake, 2009).
Loa22, the first genetically described virulence factor in *Leptospira*. It is upregulated during acute infection and highly conserved among pathogenic *Leptospira*, supporting a role in pathogenesis (Tung et al., 2010). LenA, LenB, Len C, Len D, Len E and Len F are other proteins evolved in invasion and colonization; lenA was firstly described as LfhA (Barbosa et al., 2006), and was found to bind human factor H, FHR-1 and laminin. Recent studies have revealed that this protein binds to human plasminogen, which may aid bacterial dissemination through host tissue (Verma et al., 2010). Identification and characterization of outer membrane components of *Leptospira* species is complex. Many of these proteins virulent factors are not well studied (Patricia et al., 2014).

**Laboratory Diagnosis**

Leptospirosis mimics many other diseases, with a wide variety of clinical manifestations and may be easily confused with many other diseases that are endemic and epidemic in the same areas and conditions. Clinically therefore, diagnosing leptospirosis is not possible. Laboratory abnormalities include anemia, thrombocytopenia, leucocytosis with neutrophilia and an increase in the level of creatinine phosphokinase are not useful in diagnosing leptospirosis at an early stage. In the recent years, leptospirosis, either as outbreaks or as sporadic cases, has been occurring in increasing frequency, both in developing as well as in the developed world because of poor diagnosis facility for this disease. Specific diagnostic tools though available most of them are not useful for local small clinical laboratory conditions. Laboratory diagnosis of leptospirosis is an area ill-understood by many of the workers involved in diagnosis and surveillance. Selection of the right specimens, right diagnostic method and the correct interpretation of test results are important in order to provide better patient care. Three line diagnostic tools and techniques are available for the diagnosis of leptospirosis they are namely Direct, indirect and molecular (Advanced) methods (Canal et al., 2013).
Direct Detection Methods

Direct visualization of *Leptospira* in blood or urine by dark field microscopic examination has been used for diagnosis. However, artefacts are commonly mistaken for *Leptospira*, and the method has both low sensitivity and specificity. A range of staining methods has been applied to direct detection, including immunofluorescence staining, immunoperoxidase staining and silver staining. These methods are not widely used because of the lack of commercially available reagents and their relatively low sensitivity (Zimmermann *et al.*, 2013).

Isolation and Identification

Though considered as the gold standard of diagnosis it is very difficult to cultivate *Leptospira* in artificial media. The infecting strains can be isolated from blood, CSF, and peritoneal dialysate fluids during the first 10 days of illness. Specimens should be collected while the patient is febrile and before antibiotic therapy is initiated and transferred aseptically into sterile containers without preservatives. It must be processed within a short time of collection; best results are obtained when the delay is less than 1 hour, because they do not survive well in acidic environments (Tilahun *et al.*, 2013).

Cultures are grown in albumin-polysorbate media such as Ellinghausen-McCullough-Johnson-Harris medium (EMJH), which is available commercially. Older media contained serum. Primary cultures are performed in semisolid medium, to which 5-fluorouracil is usually added as a selective agent. Cultures are incubated at 30° C for several weeks, because initial growth may be very slow. Isolated *Leptospira* are identified to serovar level by traditional serologic methods or by molecular methods, such as pulse field gel electrophoresis (Galloway and Levett, 2008).
These techniques are limited in availability to a few reference laboratories. Though cultural technique was regarded as Gold standard of diagnosis isolation rate of the causative organism from clinical specimens is very low due to prior indiscriminate use of antibiotics, highly fastidious nature of the pathogen and also it involves expensive culture media. Therefore, currently serological (indirect detection) techniques have become the cornerstone of the diagnosis (Shekatkar et al., 2010).

**Indirect Detection Methods**

Reliable serological diagnosis is now within the capacity of most general duty laboratories. Serological tests can be a guide to the infecting serum and this information is useful for prognosis and epidemiology. Persistent antibodies allow retrospective diagnosis but seroconversion or a 4 fold or grater rise in titer in paired serum samples in the presence of a compatible clinical illness is an important criterium for the definitive diagnosis of leptospirosis.

**Microscopic Agglutination Test (MAT)**

The WHO reference standard assay is the microscopic agglutination test (MAT), in which live antigens representing different serogroups of *Leptospira* are reacted with serum samples and then examined by dark field microscopy for agglutination. MAT has many pitfalls it is slow, tedious, potentially bio hazardous, painstaking and subjective preparations for MAT require meticulous culture of a collection of the strains used alive as antigen suspensions in the tests, their regular subculture and quality control for authenticity, purity agglutination and needs skilled and educated personnel (Isa et al., 2014).
For these reasons, MAT is not frequently employed for diagnosis other than research. Therefore, an alternate tool with good sensitivity and specificity in par with MAT is needed with added advantages such as rapid, economic and simple for its diagnosis. A variety of serological tests other than MAT have been developed for the easy diagnosis of leptospirosis. Among them are the complement fixation test, the macroscopic slide agglutination test (MSAT), the microcapsule agglutination test (MCAT), the indirect hemagglutination assay (IHA), several enzyme-linked immunosorbent assay (ELISA), the dipstick assay etc. None of the above technique is reliable for the definitive diagnosis of leptospirosis. Therefore, there is a need for an alternate diagnostic tool to serve this purpose.

**Macroscopic Slide Agglutination Test**

Macroscopic Slide Agglutination Test (MSAT) is a rapid test which can be used to screen human and animal serum samples. These tests are carried out with a dense suspension of *Leptospira* which agglutinate into clumps visible to the naked eye. Though it helps in making a provisional diagnosis of acute leptospirosis within a few minutes, it is not suitable for retrospective or survey work. Positive reactions should be confirmed by Complement Fixation Test (CFT) or MAT (Levett, 2005).

**Microcapsule Agglutination Test**

Microcapsule Agglutination Test (MCAT) test was developed in 1982 for serodiagnosis of leptospirosis, based on the passive agglutination of synthetic polymer carriers, sensitized with mixed antigens of sonicated *Leptospira*, by leptospiral antibody. It could not detect antibodies against some serovars, e.g. *sejroe* or the *australis* serogroup in Slovakia, and it may not detect antibodies in sera collected more than 1 to 2 months after the onset of disease (Vijayachari and Sehgal 2006).
Indirect Hemagglutination Assay (IHA)

IHA is a rapid and easily performed method of diagnosis commonly used for diagnosis of Dengue fever it is based on genus-specific antibodies (Peeling et al., 2010). It has a very limited scope in diagnosing Leptospira infection before 8 days. However, contrasting results have been obtained through various studies done to find the sensitivity and specificity of IHA in early infections (Budihal and Perwez, 2014).

ELISA

Several attempts have been made to develop serotype-specific ELISA tests with a variety of extracted antigens. The test is based on boiled whole cell antigens tend to be genus specific but those based on ultrasound disintegrated or phenol-extracted preparations show considerable serotype specificity. A single serum sample taken during an acute febrile illness with symptoms of leptospirosis is a presumptive evidence of infection, and therefore requires confirmation by further testing. The test is reliable only when used in combination with MAT (Shekatkar et al., 2010).

Dipstick ELISA

Dipstick assay for the detection of Leptospira-specific IgM antibodies in human sera was evaluated in 1997. The assay revealed cross reactivity with sera from patients with HIV, Hanta virus, Toxoplasma infection, Lyme borreliosis, malaria, meningococcal meningitis and hepatitis A infection. The performance of the dipstick assay is useful for single serum specimens, but it is not recommended for use with paired serum samples. Moreover false positivity and false negativity are common among these assays (Pavli and Maltezou, 2008).
Counter immuno-electrophoresis

Counter immuno-electrophoresis (CIE) is a popular method used for the diagnosis of numerous infectious diseases. Highest Cysticercosis positive cases were detected in infected pigs by CIE than in ELISA (Sreedevi, 2013). CIE is safe, fast, easy to perform, inexpensive and ideal for analysis of large number of samples. The test gives result within 10-15 minutes. The test system with proper standardization has got the potentiality to be deployed for the accurate diagnosis of leptospirosis (Saravanan et al., 2014).

Molecular Detection Methods
DNA Restriction Enzyme Analysis (DNA REA)

DNA REA involves the extraction of DNA from a homogenous population of organisms, digestion of the DNA with a restriction endonuclease and electrophoresis of the digested DNA in an agarose gel. The application of REA for the identification of Leptospira was first proposed by Marshall and coworkers in 1981. This technique has its own limitation like cell free DNA is required (Rao et al., 2003).

Polymerase Chain Reaction (PCR)

PCR involves in-vitro enzymatic amplification of a target DNA sequence through a series of polymerizations carried out by a thermo stable DNA polymerase, primed with a pair of the short DNA fragments, which bind specifically to the sequence of interest. PCR was first developed for the detection of Leptospira in urine samples of infected cattle. As little as 10-1 pg of purified DNA and as few as 10-1 leptospires/ml can be identified by this method (Patil et al., 2014). Molecular approaches have improved the diagnosis of leptospirosis through their advantages of speed, sensitivity and specificity. The main limitations of these methods as a routine diagnostic procedures depends on the need of special equipment, the relative high cost of the reagents and the absence of automated and standardized procedures allowing the testing of large sets of samples, particularly in tropical countries where the disease is endemic (Caraguél et al., 2011).
Among the various methods utilized for leptospirosis diagnosis each technique has several limitations and could not be used as a routine diagnostic method. Conventional methods are not reliable and are too slow in diagnosis. Molecular methods are expensive, need an expertise person with sophisticated laboratory for diagnosis. There is a need for standardization of a technique with good sensitivity, specificity, reliable, rapid, non-hazardous, less expensive and not involving highly skilled labor.

Prevention and Control

Leptospirosis is a preventable disease. Thus control measures should include risk communications, improvement in sanitation and living conditions, and rodent control, as well as both prophylactic and therapeutic medical and veterinary interventions.

Social control measures

Social control measures are an essential component for the success of prevention and control of leptospirosis. It has typically been considered an occupational disease and thus social control measures directed towards agriculture and other at-risk workers. Moreover, awareness and education are necessary among administrative, education and health professionals in human and veterinary medicine including primary health care workers, wild life and conservation scientists and infrastructure and urban planners (Victoriano et al., 2009).

Rodent control

Rodents are recognized as the most important reservoirs in the transmission of leptospirosis, especially of more severe forms. Rodent-vector control activities like use of rodenticides, entrapment of animals, and improved sanitation have been shown to successfully diminish the risk of leptospirosis transmission. In India, the timing of rodent control was shown to be a vital consideration in the prevention of disease transmission (Mendoza, 2010).
Prophylaxis

On-going research into numerous vaccine preparations for humans and animals includes the use of inactivated and attenuated vaccines, recombinant protein or lipoprotein vaccines, LPS vaccines and DNA vaccines. The local variability in serovars of endemic leptospiral strains complicates the development of a vaccine that could be used worldwide. Current bacterial vaccines elicit immunity that is generally restricted to serovars with related agglutinating LPS antigens (Victoriano et al., 2009). A killed vaccine is available in China, Japan, and Vietnam to prevent leptospirosis in humans. However, human vaccines for leptospirosis are serovar-specific and require yearly boosters. In addition, there can be painful swelling after revaccinations. The vax-SPIRAL vaccine was developed in Cuba for the control of leptospirosis in human populations but the vaccine is for at risk populations and no proper human vaccine is available (Verma et al., 2013).

Therapy

Antibiotic therapy should be initiated as early in the course of the disease as suspicion allows. Therapeutic benefits of antibiotics may be difficult to demonstrate in populations in which patients present for medical care with late and/ or severe disease. Several studies have suggested that chemoprophylaxis with Doxycycline may be an effective treatment for leptospirosis. Doxycycline 100 mg, oral single dose, for five to seven days is the first-line treatment for leptospirosis in the community setting. Amoxicillin 500 mg, three times daily, for five to seven days is an alternative. Treatment is most effective if antibiotics are initiated within five days of symptom onset, after which the efficacy of antibiotic treatment is less certain (Murtagh and Rosenblatt, 2011).
Leptospirosis in humans has traditionally been treated with intra venous penicillin in case of severe disease and oral Doxycline for mild disease. Penicillin, cephems, tetracyclines and macrolides have been widely used in the treatment of human leptospirosis. However, when these antimicrobial agents are used for the treatment, long-term therapy with large doses may be required from the early stage of the disease until the appearance of antibodies (Saravanan et al., 2013).

**Adverse effects**

Patients receiving penicillin should be monitored because of the increased morbidity and mortality of such reactions. Serious and occasionally fatal hypersensitivity reactions have been reported in patients receiving penicillin therapy. Although anaphylaxis is more frequent following parenteral therapy, it has occurred in patients receiving oral penicillin. The most common manifestations of hypersensitivity are: skin eruptions (from mild rash to exfoliative dermatitis) with an overall incidence of approximately 2%, urticaria, chills, fever, edema, eosinophilia and anaphylaxis (Bratzler et al., 2013).

Initiation of chemotherapy in spirochetal diseases may precipitate a febrile inflammatory reaction known as the Jarisch–Herxheimer reaction (JHR) characterized by an acute inflammatory response associated with the release of large amounts of cytokines, resulting from clearance of spirochetes from the circulation leading to fever, tachycardia, rigors and hypotension. Patients receiving penicillin or other antibiotics for the management of leptospirosis have to be monitored immediately after initiation of treatment to prevent any detrimental effects of a potential JHR (Guerrier and Ortenzio, 2013).
Multi-Drug resistance

Multi-drug resistance is one of the major public health problems especially in developing countries where relatively easy availability and higher consumption of medicines have lead to disproportionately higher incidence of inappropriate use of antibiotics and greater levels of resistance compared to developed countries. In India the infectious disease burden is highest in the world and recent report showed the inappropriate and irrational use of antimicrobial agents against these diseases, which led to increase in development of antimicrobial resistance. The production of extended spectrum β-lactamases (ESBL) in multi drug resistant enterobacteriaceae has become very common in India. In addition, various studies in South India highlighted the drug resistance pattern like multidrug resistant Extended-Spectrum β-Lactamase producing *Klebsiella pneumoniae*, emergence of vancomycin-intermediate *Staphylococci*, fluoroquinolone resistance among *Salmonella* enteric serovar *Paratyphi A*. Moreover, emergence of multiple drug resistant strains of microorganisms including *Leptospira* occurs due to indiscriminate use of antibiotics (Kumar *et al.*, 2013).

Due to the emergence of drug resistance and side effects produced by chemotherapy today, people are showing greater interest towards alternate therapeutic methods especially of herbal medicines. It is safer than synthetic medicines because of the phytochemicals in the plant extract target the biochemical pathways. To overcome the side effects and other deleterious effects produced by synthetic drugs, herbal based therapeutics are only recently attempted in treating leptospirosis though herbal medicines have been in use for many diseases from time immemorable days (Chakraborty *et al.*, 2010). However, detailed studies pertaining to the active principles of these herbal drugs are very limited. Herbal extracts’ of hepatoprotective and renoprotective phytochemicals from important medicinal plants are need of this hour.
Indian Medicine

Today, people around the globe are giving more preference to herbal medicine than other alternative medicines such as ayurvedha, naturopathy and homeopathy. It is now evident that according to WHO traditional medicines are relied upon by 80% of the World's population for their primary health care needs (Policepatel and Manikroa, 2013).

According to WHO, medicinal plants are the best source to obtain a variety of drugs to combat serious diseases and it advocates that countries should venture into other aspects of traditional medicine. This should be with a view of identifying safe and effective remedies for ailments of microbial diseases (Singh and Kumar, 2013). There has been an increasing interest worldwide on therapeutic values of natural products. It is believed that the cure of any debilitating human ailments and diseases may be found among the world’s flora in nature’s pharmacy and there are multitudes of potential useful bioactive substances to be derived from plants. One way of preventing antibiotic resistance of pathogenic species is development of newer components from several medicinal plants and should not be based on existing synthetic antimicrobial agents (Ofokansi et al., 2012).

Herbal based therapeutics for liver and renal disorders has been in use in India for a long time and has been popularized worldwide by leading pharmaceuticals. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver and renal disease models by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy (Katiyar et al., 2013).
A large number of plants and their formulations have been claimed to have hepatoprotective and renoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver and renal protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi-ingredient plant formulations. *Annona squamosa*, *Silybum marianum*, *Chamomile capitula*, *Coccinia grandis*, *Wedelia calendulaeae*, *Aegle marmelos*, *Phyllanthus amarus*, *Ficus carica*, *Lepidium sativum*, *Sargassum polycystum*, *Solanum nigrum*, *Cassia roxburghii*, *Phyllanthus amarus*, *Eclipta alba*, etc., were commonly used against any liver and renal related diseases (Saleem et al., 2010).

**New Possibilities**

*Phyllanthus amarus* (Keezhaneli in Tamil) and *Eclipta alba* (Karishalenganni in Tamil) are locally available plants which has various medicinal properties and used for treating liver and renal diseases both in Chinese and Indian Traditional system of medicine (Sharmila and Aparupa, 2014). Traditionally, these plants are ayurvedic herb used in Southern India for the treatment of liver and renal diseases (Saravanan et al., 2013).

These plants showed significant antimicrobial activity against multi-drug resistant pathogen like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa* (Antara and Amla, 2012). However, studies on these plants on anti-leptospiral activities are very limited. Hence there is a need for these herbal extracts impact on virulence factors of *Leptospira* especially the most important virulent factor haemolysin and its *sphH* gene which are obscure, to establish the usefulness of these herbal extracts.
Plant based medicines have been used as crude formulations such as infusions, tinctures and extracts, essential oils, powders, poultices and other herbal preparations. Crude plant extracts may contain hundreds, or even thousands of different chemical constituents that interact in complex ways. Often it is not known how an extract works, even when its therapeutic benefit is well established (Cock, 2011). The current trend is to isolate and characterize the individual phytochemical components with the aim of producing an analogue of increased bioactivity/bioavailability. In recent years, the major secondary plant metabolites (phytochemicals) are of potential medicinal interest that has been extensively investigated as a source of medicinal agents in drug discovery (Singh and Kumar, 2013).

The recent approaches of applying hyphenated chromatography and spectrometry such as high-performance liquid chromatography-diode array detection (HPLC–DAD), gas chromatography–mass spectroscopy (GC–MS), HPLC–MS and HPLC–NMR, could provide the additional spectral information like massive databases of genomic, proteomic and chemical data which in combination with efficient separation methods and powerful spectrometric methods for identification and structure elucidation of active compounds. A powerful and deep biological approach that integrates such large and diverse sources of information together is actually needed to fully understand the pharmacological effects of these plants extracts' (Rasheed et al., 2013).

Thus when we see the current status of leptospirosis in Salem district we can see the following lacunae such as

i. need for an epidemiological study on pediatric cases in and around Salem,

ii. need for an alternate diagnostic tool with easy, economic, non-hazardous, non-cumbersome and not involving highly skilled labor for the routine leptospirosis diagnosis,
iii. need for a molecular study in establishing the usefulness of Indian medicine with some popular herbal extracts for the leptospirosis management,

iv. need to identify the active principle behind the important herbal extracts by way of characterizing the active molecules present in the extracts to contrive the candidate drug which has the ability to work not only against *Leptospira* but also to protect our vital organs. Keeping all the lacunae and the possible solutions in mind the present research work was designed and entitled "A MAT study on suspected pediatric leptospirosis cases of Salem, Leptospiral PCR study on *sphH* gene and SDS – PAGE study on its protein before and after treatment with a couple of medicinal plant extracts".