3. REVIEW OF LITERATURE

3.1. Leptospira

*Leptospira* derives from the Greek *lepto* (thin) and Latin *spira* (coiled). Leptospires are spirochetes, about 0.1 µm in diameter by 6–20 µm in length, highly motile, slow-growing obligate aerobes that diverged early in bacterial evolution, comprising the genus *Leptospira*, which belongs to the family Leptospiraceae, order Spirochaetales (Faine et al., 1999). Leptospires are cultivated in artificial media containing 10% rabbit serum or 1% bovine serum albumin and long-chain fatty acids at pH 6·8–7·4. Optimum growth temperature is between 28-30°C. Leptospires are catalase and oxidase positive. Dark-field or phase-contrast microscopy of wet preparations is required for direct visualization of leptospires, since the bacteria stain poorly (Fig.3.1).

**Figure 3.1. Dark field photomicrograph of *Leptospira* spp.**


Growth of leptospires is often slow on primary isolation, and cultures have to be retained for about 13 weeks before being discarded. Agar may be added at low concentrations (0.1–0.2%). In such semisolid media, growth reaches a maximum density in a discrete zone beneath the surface of the medium, which becomes increasingly turbid as incubation proceeds. This growth is related to the optimum oxygen tension and is known as a Dinger’s ring or disk (Natarajaseenivasan et al., 1996). Staining methods like silver staining, immunostaining with fluorescent or immunohistochemical conjugates are used to visualize microscopically. Electron microscope structure of leptospiral cell shows it has close resemblance with Gram negative bacterial cell. Leptospires possess two flagella, one arising
at each end from an insertion through the inner membrane and lying in the periplasmic space between the peptidoglycan and the outer membrane.

3. 2. Leptospirosis

Leptospirosis is an anthropozoonotic septicaemia disease that affects human beings and animals with multisystemic involvement globally in both urban and rural areas with inestimable morbidity and mortality (Vinetz, 2001). Rodents are one of the most common carriers for spreading of *Leptospira* among all mammals and were first confirmed by isolation in Indian rats (Knowles et al., 1932). Pathogenic leptospires live in the proximal renal tubules of the kidneys of carriers, although other tissues and organs may also serve as a source of infection. From the kidneys, leptospires are excreted in urine and may then contaminate soil, surface water, streams and rivers (Fig.3.2). The bacteria survive for weeks or even months in moist soil and water after excretion in the urine. Cell aggregation and biofilm formation may contribute to the survival of leptospires outside their hosts (Treu et al., 2004; Ristow et al., 2008). Infections of animals or humans occur from direct contact with urine or indirectly from contaminated water. The carriers may be wild or domestic animals, especially rodents and small marsupials, cattle, pigs and dogs. Almost every mammal (including aquatic mammals) and marsupial worldwide has been shown to be a carrier of leptospires.

Humans almost never become chronic carriers, but suffer acute infections, sometimes with longer-term sequelae. Its severe disease form, known as Weil’s syndrome, is an acute febrile illness associated with multiorgan system complications including jaundice, renal failure, meningitis and pulmonary haemorrhage, with a mortality rate that may exceed 15% (Faine et al., 1999; Marotto et al., 1999). The clinical picture mimics the variety of other bacterial, viral and protozoal diseases. The severity of the infection depends on the age, general health of the patient, the serovar involved and the number of leptospires that entered the patient's body. Infection causes prolonged leptospiraemia until the host mounts an effective acquired immune response, which occurs one to two weeks after exposure. Leptospires can be isolated from the bloodstream within minutes after inoculation and are detected in multiple organs by the third day after infection; they may reach $10^6$–$10^7$ organisms per ml or per g in the blood and tissues of patients and infected animals (Ko et al., 2009).
Legends: The pathogens are maintained in sylvatic and domestic environments by transmission among rodent species. Leptospires can then infect livestock and domestic and wild animals and cause a range of disease manifestations and carrier states. Maintenance of leptospirosis in these populations is due to their continued exposure to rodent reservoirs or to transmission within animal herds. Leptospirosis is transmitted to humans by direct contact with reservoir animals or by exposure to environmental surface water or soil that is contaminated with their urine. Leptospires penetrate abraded skin or mucous membranes, enter the bloodstream and disseminate throughout the body tissue. Infection causes an acute febrile illness during the early ‘leptospiraemic’ phase and progresses during the late ‘immune’ phase to cause severe multisystem manifestations such as hepatic dysfunction and jaundice, acute renal failure, pulmonary haemorrhage syndrome, myocarditis and meningoencephalitis. Humans are an accidental host and do not shed sufficient numbers of leptospires to serve as reservoirs for transmission. Picture is generated at Medical Microbiology Laboratory, Bharathidasan University.

3.3. Genetics of Leptospira

Leptospira genome has possessed two circular chromosomes. The largest replicon, with sizes that vary between 3.6 and 4.3 Mb in length, exhibits characteristic features of a bacterial chromosome. It carries genes for the replication proteins dnaA, dnaN, gyrA, and gyrB, as well as most of the essential housekeeping genes of the bacterium. The smaller chromosome, 277- to 350-kb in size, contains a plasmid-like origin of replication. It carries essential genes such as genes involved in the biosynthesis of amino acids. The L. interrogans and L. borgpetersenii genomes contain approximately 3,400 and 2,800 predicted coding regions (excluding transposases and pseudogenes), respectively, of which 656 are pathogen specific.
and not found in the saprophyte *L. biflexa*. The functions of most (59%) of these genes are unknown, suggesting the existence of pathogenic mechanisms that are unique to *Leptospira* spp. The saprophyte *L. biflexa*, which survives exclusively in the external environment, has many more genes encoding environment-sensing and metabolic proteins than the pathogenic leptospires (Picarudeau et al., 2008).

3.4. Virulence factors

*Leptospira* possess two membranes where the outer membrane is not attached to the peptidoglycan layer and the periplasmic space contains the flagellum. The outer membrane contains lipopolysaccharides (LPS), lipoproteins, transmembrane proteins and peripheral OMPs. Inner membrane contains several transport systems and proteins, lipoprotein signal peptidase (SPase I and SPase II), Lol Pathway, LipL31 and penicillin binding proteins (PBPs) (Fig.3.3).

**Figure 3.3. The architecture of leptospiral outer membrane**


LPS is the major lipid part consists of three regions, a hydrophobic lipid-A, hydrophilic core oligosaccharide and an outer polysaccharide present in the outer leaflet of the outer membrane. LPS is responsible for the antigenic diversity of *Leptospira* and immunity is serovar specific, which exclude the consideration of LPS from the diagnostic and vaccine investigation of leptospirosis and makes researchers to focus on the virulent proteins of *Leptospira*. The virulence factors that have been identified to date are primarily membrane
proteins, which are thought to mediate the interaction between the bacterium and the host tissues. Although several proteins are secreted by *Leptospira* spp., including degradative enzymes, there is no evidence for a dedicated protein secretion pathway similar to the type III and type IV secretion machinery that is used by Gram-negative bacteria to inject proteins into host cells. The *Leptospira* genomes contain at least 79 motility-associated genes, including orthologs for gliding motility. Similar to other spirochetes, *Leptospira* uses FlaA sheath protein and FlaB core protein as essential components of its endoflagellar filament (Nascimento et al., 2004). Motility and chemotaxis encoding genes of *Leptospira, Treponema pallidum* and *Borrelia burgdorferi* are well conserved among 42 genes. Genomic analysis indicated that the chemotaxis system of *Leptospira* is more complex than that of either *T. pallidum* or *B. burgdorferi*. The reason for the greater number of motility-associated genes in *Leptospira* than in other spirochetes is not clear.

Immunoprecipitation studies have reported OmpL1, a 31kDa protein with 320 amino acid residues is the heat-modifiable porin, present in the outer membrane in small amounts. It is the first transmembrane OMP to be described from a pathogenic spirochete (Haake et al. 1993). Proteins similar to OmpL1 has been reported in other spirochetes also including Borrelia Oms28, P13, BBA01, P66, Oms38, and BesC and Treponema major outer sheath protein (Msp). It exists in a typical trimeric form and allows passage of small molecules (<1000 Daltons) (Haake et al. 1993; Shang et al. 1995). In silico study using PRED-TMBB or TMBETA-NET program has put forth a growing list of OmpL proteins namely OmpL36, OmpL37, OmpL47 and OmpL54 (Pinne and Haake 2009). A recent report highlights OmpL37 protein to have an ability to bind human skin and other host factors (Pinne et al., 2010). Earlier a group of outer membrane proteins called Mammalian cell entry proteins (Mce) was identified in *Mycobacterium tuberculosis* which plays a major role in mammalian cell invasion. Similar gene pattern was also found to be present in pathogenic *Leptospira* species. Sequence analysis showed the gene to have a small intracellular region, a short transmembrane sequence, and a large extracellular region containing a domain called RGD motif. Studies on the functional role of *mce* gene showed an up regulation of the protein during infection and found to bind with integrins of the host cells which completely differ from other leptosiral adhesins such as LipL32, LenA, OmpL37, LigA, and LigB that bind to extracellular matrix. Leptosiral *mce* contains an RGD motif which was recognized by
integrins of the host cell that trigger intracellular signalling and causes microbial internalization (Zhang et al., 2012).

Iron is an essential nutrient for pathogenic leptospires and the transport of heme or other iron-containing molecules by Gram negative bacteria often relies on the active transport through TonB dependent outer membrane receptors (Louvel et al., 2006; Asuthkar et al., 2007). After binding, transport of the nutrient across the outer membrane into the periplasm is an energy-dependent step requiring interaction of the TonB-dependent OMP with TonB. Till date only few TonB dependant OMPs have been studied and many are yet to be identified. One of the TonB dependants OMP is FecA that is identified by random insertional mutagenesis in *L. biflexa* (Louvel et al., 2005). The mechanism of *fec* transport in *E.coli* is found to be different from *Leptospira* and absence of *fecBCDE* homologues, suggest a novel alternative pathway is involved in ferric dicitrate transport. Another group reported HbpA, iron regulated TonB - dependant outer membrane protein in *Leptospira* (Asuthkar et al., 2007) and its protein fold is identical to the ferric siderophore receptors FepA, FhuA and FecA of *Escherichia coli*.

Although many experiments have characterized lot of immunodominant lipoproteins little is known about their biological role, structural information, lipobox sequence determinants, lipoprotein export signals, and types of lipoproteins expressed during infection of the mammalian host. A detailed study using liquid chromatography tandem mass spectrometry (LC-MS/MS) and cryo-electron tomography (cryoET) identified proteome map for pathogenic *Leptospira* species that is quantitation of protein per cell and its accuracy was also determined (Malmstrom et al., 2009). Some of the known lipoproteins and their biological roles are described herewith in this section.

*LipL32*, the most abundant antigen that accounts for 75% of the outer membrane proteome is the field of intense research for the past two decades. Similar to the abundance of LipL32 in *Leptospira* other lipoproteins in spirochetes have been reported including OspA of *Borrelia burgdorferi*, the Vmp of the relapsing fever *Borreliae* species, SmpA of *Brachyspira hydysenteriae* and Tpp47 of *Treponema pallidum*. The analysis of crystal structure of LipL32 (Vivian et al., 2009) showed several adhesion sites of which calcium binding property of LipL32 was found to be noteworthy. The binding of calcium to LipL32 increased the thermal stability of protein (Hauk et al., 2009) and also it’s binding to
fibronectin (Tung et al., 2010) plays an important role in the pathogenesis of *Leptospira*. The results of phage display showed LipL32 to interact with chloride channel accessory 2, glycoprotein VI, scavenger receptor expressed by endothelial cell isoform I (SREC-I), coronin 2A, laminin alpha 5, collagen XX, and prostaglandin receptor EP1 of the host cells (Chaemchuen et al., 2011). Though large number of studies provided strong evidence for LipL32 in the role of pathogenesis, the unexpected results of Murray et al. (2009) revealed by transposon mutagenesis studies in *Leptospira interrogans* denied the fact of LipL32 in pathogenesis. Screening of PhoA fusion library with convalescent mouse sera lead to the identification of the second most abundant protein, Loa22. Loa22 have an OmpA domain located in the outer membrane, whereas small portion is exposed on the cell surface (93% periplasmic space, 25% outer membrane) (Koizumi and Watanabe, 2003). Since it has ompA domain, the major function of this protein is to play a role in structural integrity (Picardeau et al., 2008). Similar to the location of Loa22 on the outer membrane of *Leptospira* species, *Borrelia burgdorferi* have OspA and OspC on its outer membrane. The random transposon mutagenesis in Loa22 by Himar1 insertion led to a complete loss of virulence in the guinea pig disease model. This proved to be a real breakthrough of identifying Loa22 as a virulence determinant, which satisfied Koch’s molecular postulates (Ristow et al., 2007). However, later studies on comparative genome sequence analysis of the saprophyte *L. biflexa* revealed that pathogenic loa22 gene has an ortholog with 73% similarity in *L. biflexa* and its presence in the saprophytic species suggests that it is involved in survival rather than being a direct virulence factor.

Phage library screening of lambda ZAP II vector containing *Eco*RI fragments of *L. kirschneri* DNA lead to the identification of LipL41, a surface exposed lipoprotein which is antigenically conserved among pathogenic *Leptospira* species (Shang et al., 1996). It is considered to be third most abundant protein with copy number of 10,000 per cell. LipL41 has many essential features which favor the indispensable role played in the pathogenicity, first of all it has no orthologue with saprophytes, it has been reported to have protective vaccine efficacy when co-administrated with OmpL1 gene which binds hemin (Asuthkar et al., 2007). Earlier LipL21 was considered as a second major outer membrane protein in *Leptospira* but later studies showed Loa22 to be the second most abundant protein where the copy number is found to be approximately 30,000. Surface biotinylation studies and recognition of the protein by immune sera from humans and hamsters infected with
Leptospira showed the protein to be surface exposed and immunogenic. LipL21 is reported to be highly conserved among pathogenic Leptospira spp. (Cullen et al., 2003), however an ortholog in L. biflexa with 50% similarity has been reported recently (Picardeau et al., 2008). But interestingly LipL21 DNA vaccine protects guinea pigs from infection in the challenge experiments (He et al., 2008).

LipL36 is found to be rich in alanine similar to that of TmpB of T. phagedenis, T. pallidum and several lipoproteins of Neisseria species (Yelton et al., 1991). LipL36 protein is found to be abundant during the early log phase and thereafter it decreases in quantity during the mid-log phase and does not appear to occur when the bacteria grows in vivo. LipL36 reported to be down regulated during mammalian infection, as like observed in OspA of B. burgdorferi. LipL40, a 40-kDa leptospiral outer membrane lipoprotein (Gamberini et al., 2005) was reported to interact with plasminogen (PLG) and generate plasmin, in the presence of activator (Vieira et al., 2010). The identification of this protein showed the ability of Leptospira to bind PLG, which may have a role in pathogenesis. Characterization and sequencing of pL45 by MALDI-TOF lead to the identification of a 46 kDa protein LipL46 (Cullen et al., 2002). The LipL46 FSISC lipobox sequence was found to be similar to the LSISC lipobox sequence of B. burgdorferi OspD and FFISC lipobox of LruA (Verma et al., 2005). LipL46 was found to have an ortholog with 54% identity in L. biflexa. LipL53 is a surface exposed leptospiral adhesin that mediate the binding to several extracellular matrix components (Oliveira et al., 2010). Immunization studies with hamster model showed only partial protection against leptospiral infection but reacted well with the antibodies from patient’s serum.

Lig proteins are the first leptospiral proteins described to be expressed only during infection (Palaniappan et al., 2002). Three classes of leptospiral Lig proteins have been described until now namely LigA, LigB and LigC. Immunoelectron and immunofluorescence microscopy studies showed Lig proteins to be anchored on the surface of leptospiral outer membrane and contain 12 to 13 tandem bacterial immunoglobulin-like repeat domains (LigB 12 Ig-like imperfect tandem repeats, LigA and LigC have 13 Ig-like tandem repeats). The organization of LigC is similar to that of LigB but contains mutations that disrupt the reading frame, so called as pseudogene. Lig proteins are reported to have binding affinity with multiple host proteins and its expression, extracellular release and surface exposure are
highly influenced by osmolarity, additionally it has no ortholog with the saprophytes (Matsunaga et al., 2005). These features authenticated its role during infection. *Leptospira interrogans* is the most reported agent for causing severity during leptospirosis and possesses large gene content on comparison with *L. borgpetersenii*. In this case it is reported that genome of *L. interrogans* contain both *LigA* and *LigB* genes, whereas *L. borgpetersenii* genome has only the *LigB* gene. This may predict the fact that *LigA* which is not having an ortholog between these two species may be the reason for the severity of the disease. Mutational studies with *LigB* showed that the *LigB* mutant by allelic exchange does not protect hamster from challenge experiment (Croda et al., 2008). Recently it was reported that recombinant LipL32 and *LigA* are unable to produce immunity in hamster models (Lucas et al., 2011).

*Lsa24* was identified as leptospiral laminin binding outer membrane protein (Barbosa et al., 2006). The genes of this protein showed similarity to mammalian endostatin so, later renamed as *LenA* (leptospiral endostatin-like protein A) (Stevenson et al., 2007). Subsequent studies reported that *Len* family consist of 6 types of proteins namely *LenA*, *LenB*, *LenC*, *LenD*, *LenE* and *LenF*. The essential features of pathogenic microbes are serum resistance where leptospiral serum resistance is mediated, at least in part, by *LenA*, which helps against complement mediated killing. These proteins share structural and functional characteristics with mammalian endostatins, fragments of collagens XVIII and XV. *LenA* is able to bind with host factor H, FHR-1, laminin and does not bind factor H-like protein 1 (FHL-1), which lacks heparin binding domain (Verma et al., 2006), where *LenB* was found to bind human factor H. Other *Len* proteins including *LenB*, *LenC*, *LenD*, *LenE* and *LenF* have strong affinities for fibronectin (Stevenson et al., 2007).

Screening of genomic DNA expression library of *L. interrogans* against uveitic horses eye fluids identified a novel protein *LruC* (Verma et al., 2012). It is reported to be present in inner leaflet of the leptospiral outer membrane and reported to be present in pathogenic *Leptospira* species. Antibodies against *LruC* were found to be higher in eye fluids and sera of uveitic horses than healthy horses. *P31*LipL45 is the first described peripheral membrane protein. LipL45, an inner membrane protein has been found associated at its C terminus to a 31-kDa peripheral membrane protein, *P31*LipL45. Membrane fractionation studies showed *P31*LipL45 to be associated both with the inner and outer membranes (Matsunaga et al.,
2002). The exact mechanism of its transport and its role in infection is not well understood. A group of leptospiral surface adhesins was found to be present in surface of *Leptospira* species namely Lsa21, Lsa24, Lsa27, Lsa30, Lsa63, and Lsa66 where Lsa24 was renamed as Len proteins whereas role of other leptospiral surface adhesins remains to be elucidated. Other than this many outer membrane leptospiral proteins like Lp29, Lp30, Lp49, MPL17, MPL21, MPL36 and Omp52 was reported however its exact function in pathogenesis is remains to be elucidated.

### 3.5. Survival of leptospires in vivo

Pathogenic *Leptospira* resists phagocytosis by macrophages and neutrophils unless specific antibodies are present (McGrath et al., 1984). However, studies have suggested that pathogenic leptospires are able to survive inside macrophages and to subsequently escape by inducing apoptosis (Jin et al., 2009) this ability was correlated with virulence. Virulent leptospires were also found in the cytoplasm of non-phagocytic Vero cells, with evidence of receptor-mediated endocytosis (Merien et al., 1997). On the other hand, Barocchi et al. (2002) suggested that leptospires could transcytose cultured MDCK cell monolayers, but were rarely found intracellularly, leading the researchers to label *Leptospira* a non-intracellular pathogen. However, this conclusion must be viewed in the light of findings that intracellular invasion/uptake was lost extremely rapidly upon *in vitro* passage, with substantial reduction observed after only a single laboratory subculture (Merien et al., 1997). A further proviso is that studies have used different cell types, making direct comparison difficult.

In recent years there has been increased investigation of *Leptospira* with the host innate immune system. It is suggested that the tissue damage observed in animal species susceptible to severe, acute leptospirosis, such as hamsters (and by inference humans), is mediated at least in part by the increased production of pro-inflammatory cytokines and chemokines, compared with resistant animal species such as mice (Matsui et al., 2011). Indeed, as well as lesser production of pro-inflammatory immune mediators, mice showed a more rapid production of the anti-inflammatory cytokine IL-10. It has long been known that pathogenic leptospires are resistant to the bactericidal activity of complement while saprophytic *Leptospira* spp. are highly susceptible (Johnson and Muschel, 1966), but it is only recently that potential mechanisms have been hypothesized.
Proteins of *Leptospira* are the potential key players during infection and has diverse roles in pathogenesis like iron uptake, toxin production, adhesion, invasion, serum resistance, hemolysins, maintaining the bacterial cell structure, attachment to various substrates, importing nutrients, exporting bactericidal and toxic agents. Leptospiral pathogenesis comprises of several steps like dissemination in the host (by adhesion, escape from phagocytosis and complement pathway), persistent colonization, disease manifestations and tissue damage. The mechanism of pathogenic *Leptospira* to enter the host is not well understood, however after infection it evades the immune response and affinity towards host extracellular matrix molecules is the key factor for studying the role of virulent proteins in stage-specific interactions with the host. Several leptospiral proteins are reported to have binding capacity with ECM components like fibronectin, fibrinogen, collagen, laminin, elastin, etc. Most of the immunogenic proteins are found to interact with multiple ECM components and at the same time multiple proteins are found to interact with the same ECM components for example almost many leptospiral proteins reported till now are found to interact with laminin. However some surface proteins like LigA, LigB, LipL53 and Lsa21 are used to interact only in certain physiological osmolarity conditions with respect of temperature and pH. They also found to be up regulated under these conditions. Even though many leptospiral proteins were reported to bind with various ECM components the major limitations behind this is, all binding experiments were carried out *in vitro* and *in vivo* confirmation of these interactions will be of major impact for pathogenesis.

Finding the immunogenic proteins of pathogenic leptospires which facilitate it to escape from phagocytosis and complement system of the host will pave way for the identification of pathogenic mechanisms because escape from phagocytosis and complement system is the basis for first level of infection and moreover the saprophytes are killed in minutes by the normal human serum *in vitro*. Till now only certain virulent proteins like Lig &Len family proteins, LcpA were reported to interact with Host factor H (HF.H), Factor H related protein 1(FHR-1), Factor H-like protein 1(FHL-1), Human complement regulator C4 binding protein (C4BP) and complement proteins (C3b & C4b). Considering that leptospires are highly invasive microorganisms, there may be several other leptospiral immunogenic proteins, which may play a role in invasion and evading the immune system. Identification of these receptors and bacterial ligand is of great relevance, since they may represent as targets for immune interference.
3.6. Diagnosis of leptospirosis

Confirmation of a clinically suspected leptospirosis case in the laboratory also has many bottlenecks. Standard tests, such as culturing and the microscopic agglutination test (MAT) are tedious, laborious and require well-equipped laboratories with experienced staff and, therefore, are restricted to a few ‘expert’ centres. Because MAT is the reference test in serodiagnosis, an international proficiency testing scheme has been developed to standardize its performance level at a global scale (Chappel et al., 2004). However, novel or adapted simplified diagnostic tests for diagnosis in both humans and animals are badly needed. Several rapid tests for human use are currently available. Recombinant protein based diagnosis was evaluated for most of the leptospiral proteins like OmpL1, HbpA, LipL32, LipL21, LipL36, LipL41, Loa22, LigA, LigB, MPL17 and MPL21 using different diagnostic methods like ELISA, PCR, latex agglutination test, dipstick assay, dot blot, immunoblot assay and flow through based immunoblot assay. However none of the methods was proved to be universally effective for early diagnosis of leptospirosis.

Meaningful multicentre comparisons have not been carried out between these tests, using sera from adequately defined cases, to ascertain their relative merits as diagnostic tools. Evaluation studies should include the collection of sera from both the leptospirosis suspected patients with different clinical manifestations and non-leptospirosis patients and analyze together the sera of other diseased person will help to provide sensitivity and specificity of the test results. Also, these tests are for screening purposes only, and results must be confirmed by standard tests. In addition, they are applicable only at a later stage of disease when effective treatment with antibiotics is likely to fail. Moreover, Leptospira related to reproductive failure in animals is a chronic condition associated with declining antibody titres or the absence of detectable antibody titres. There is an urgent need for robust and easy to use diagnostics, particularly during acute infection among humans and domestic animals. In addition, it must be remembered that human and veterinary diagnostic requirements differ in some respects, as follows. In human leptospirosis the first requirement is to get a diagnosis and genus specific tests are suitable, whereas in animals the individual is less important than the population from which it comes. Therefore, it is important to get an early diagnosis of the infecting serovar as control measures such as vaccination are serovar dependant. Improved diagnostics will contribute to an improved case detection and a consequent increased
awareness and control of leptospirosis and, hence, be beneficial for veterinary public health care and (national) economies.

3.7. Vaccine for leptospirosis

The traditional approach to vaccine preparation follows the principles established by Pasteur, which consist of using inactivated whole pathogens, extracts from them, or an attenuated live form of the pathogen to immunize the host. The vaccine search for leptospirosis started after the first demonstration of immunization with killed leptospires, which protected against experimental infection in inoculated guinea pigs (Ido et al., 1916). Development of a common vaccine is urgently needed because currently available vaccines have disadvantages, including short duration of immunity, adverse reactions, and serovar specific effectiveness. Therefore due to limitation of whole organism vaccines, research focus enters to second generation vaccines or subunit vaccine. Subunit vaccines consisting of recombinant leptospiral lipoproteins are an area of intense investigation. The first evidence that immunization with a recombinant protein confers protection against experimental leptospirosis is proved using leptospiral outer membrane proteins OmpL1 and LipL41 (Haake et al., 1999), which exhibit synergistic immunoprotection among the animal model. Most of the outer membrane protein antigens was used as subunit vaccines for protection of host among which Lig proteins are the most promising subunit vaccine candidates showing high-level protection approaching 100% in mice (Koizumi and Watanabe, 2004) and hamsters (Palaniappan et al., 2006; Silva et al., 2007; Yan et al., 2009). LipL36 failed to express in vivo and hence found not suitable for leptospirosis vaccine. Vaccination using an adenovirus vector encoding the LipL32/hap-1 gene induced cross-protection in the gerbil model of leptospirosis (Branger et al., 2001). The multi component recombinant protein immune strategy found to provide better immune responses against Leptospira than single-component OMPs or single DNA or protein immunization (Feng et al., 2009).

The most innovative areas in the vaccine as on today are the DNA vaccines for Leptospira. Until now few leptospiral proteins (OmpL1, OmpL37, LemA, LipL32, LipL45, LipL21, LigA and LigB) was evaluated for their DNA vaccine efficacy. We need more and more information regarding vaccine studies because, animal models cannot provide full guarantee due to one’s own immune system make up. Hence the application of animal tested vaccine in humans is still questionable and further study is needed to test the prophylactic and
immunotherapeutic efficiency of these vaccines. The research community is going on searching for potential virulence factors for developing a universal vaccine continues until a major attention is paid on studying the biological and immunological role played by the virulent components of this pathogen. The search for novel potential virulence factors for developing a universal vaccine candidate and studying the biological and immunological role will greatly be appreciated.