2. OBJECTIVES

Leptospirosis is one of the most common zoonotic diseases in the world, resulting in high morbidity and mortality in humans, affecting global livestock production (Levett, 2001). It is reported that more than 500,000 cases of acute leptospirosis are reported each year, with case fatality rates exceeding 10% (WHO, 2011). Symptoms range from a mild influenza like illness to a severe infection, such as Weil’s disease or leptospirosis-associated pulmonary haemorrhage syndrome (LPHS). Infection in horses, cattle, dogs and pigs can cause abortion, stillbirth, renal failure, uveitis and can even lead to multi-organ failure (Ratnam et al., 1983; Bharti et al., 2003). Laboratory diagnosis of leptospirosis is confusing for treatment and surveillance because of its varied clinical signs and symptoms. The medical and economic losses caused by leptospirosis calls for an urgent need for developing rapid diagnostics and vaccines. The diagnosis of leptospirosis is usually based on the direct observation of leptospires, the isolation of the pathogens in culture, seropositivity for *Leptospira*-specific antibodies, and/or the demonstration of leptospiral DNA by PCR-based assays. These methods are found to be laborious and time consuming with low sensitivity and specificity. In case of vaccines currently whole cell based killed or attenuated leptospiral vaccines are available, which are hobbled by several limitations like short term immunity, LPS reactogenicity, requirement of annual boosters and lack of cross protection against the various leptospiral serovars (Raja and Natarajaseenivasan, 2015). Hence, there is an urgent need to identify the potential antigen for the development of early conclusive diagnostics and effective vaccine against leptospirosis.

Even though several leptospiral antigens was reported as highly immunogenic, none of them identified till now was proved to be the most effective immunogen to protect from leptospirosis completely or to act as potential antigen for the early conclusive diagnosis of leptospirosis. Therefore genomic library screening of a locally predominant leptospiral serovar would be an ultimate approach for the identification of immunoreactive antigen. In this outlook we used genomic DNA library screening to identify the immunoreactive proteins for the development of early conclusive diagnostics and for vaccine development.
Specific objectives of the research work

The present study was designed to meet the following objectives:

1. **Screening of genomic DNA expression library of *Leptospira interrogans* serovar Autumnalis N2 for the identification of immunogenic proteins**
   - Collection of clinical sera samples from humans
   - Genomic DNA expression library screening of Autumnalis N2 + λ Phages
   - DNA sequencing of the immunoreactive phage clones

2. **Cloning and expression analysis of immunogenic proteins encoding genes for the identification of in vivo expressed proteins**
   - Primer design and gene amplification of the immunoreactive clones
   - Molecular cloning and expression of identified genes in prokaryotic expression vector pET15b
   - Identification of in vivo expressed proteins using low passaged virulent strain (N2-MACs)

3. **Evaluation of the recombinant immunogenic proteins for the diagnosis of human leptospirosis**
   - Checkerboard ELISA for determining optimum antigen concentration
   - Development of IgM ELISA for the detection of leptospiral specific antibodies in sera samples
   - Development of combo ELISA using combination of proteins

4. **Quantitative (qPCR) based detection of leptospiral DNA in human urine using in vivo expressed *recA* and *fliD* genes**
   - qPCR primer designing and analysis for sensitivity of *recA* and *fliD*
   - Specificity of *recA* and *fliD* in leptospiral DNA detection
   - qPCR based detection of pathogenic leptospires in human urine using *recA* and *fliD* in comparison with conventional PCR

5. **Development of recombinant protein subunit / DNA / DNA prime recombinant protein subunit boost vaccination strategy for leptospirosis**
   - DNA or Protein subunit or DNA prime protein subunit vaccine immunization
   - Gene expression analysis and assessment of humoral and cell mediated immune (CMI) response
   - Challenge experiments and qPCR analysis to assess the renal clearance of leptospires