1. INTRODUCTION

Leptospirosis is a neglected life-threatening bacterial disease that occurs in diverse epidemiological settings with a higher incidence in low-income, tropical countries and imparts its greatest burden on resource-poor populations (Bharti et al., 2003; Lau et al., 2010; Costa et al., 2015). The causative agents are a unique group of spirochetes divided into 17 pathogenic *Leptospira* species and >300 serovars (Bourhy et al., 2014; Adler, 2015). Descriptions of leptospirosis-like syndromes were reported in the scripts of ancient civilizations, but the first modern clinical description of leptospirosis was published by Weil in 1886. In a landmark study in 1916, Inada et al. isolated leptospires, identified the organism as the causal agent of leptospirosis and determined that rats are reservoir for transmission to humans. Leptospires were subsequently isolated from a wide range of animal reservoir species and classified in to serogroups and serovars as a function of their antigenic determinants. Leptospirosis is a major public health issue in many countries, especially in regions of South and Southeast Asia, Oceania, Caribbean, Andean, Central, and Tropical Latin America, and East Sub-Saharan Africa (WHO, 1999 & 2011; Natarajaseenivasan et al., 2011a; Costa et al., 2015). Recently it is reported that annually 1.03 million cases (95% CI 434,000 – 1,750,000) and 58,900 deaths (95% CI 23,800–95,900) due to leptospirosis worldwide. A large proportion of cases (48%, 95% CI 40–61%) and deaths (42%, 95% CI 34–53%) were estimated to occur in adult males with age of 20–49 years (Costa et al., 2015).

Leptospirosis affects risk groups that are exposed to animal reservoirs or contaminated environments, such as abattoir and sewage workers, military personnel, blue metal mine workers, and individuals partaking in water sports and recreation (Sejvar et al., 2003; Lau et al., 2010; Agampodi et al., 2014; Prabhakaran et al., 2014; Asha Parveen et al., 2016). However, leptospirosis has a broader health impact as a disease of impoverished subsistence farmers, cash croppers, and pastoralists (McBride et al., 2005; Sethi et al., 2010; Crump et al., 2013) from tropical regions. Furthermore, leptospirosis has emerged as a health threat in new settings due to the influence of globalization and climate. The emergence of leptospirosis in India, Thailand and Sri Lanka (Wuthiekanun et al., 2007; Agampodi et al., 2009; Sugunan et al., 2009; Costa et al., 2015) highlights the potential of the pathogen to rapidly spread and cause large unexplained nationwide outbreaks. Urban epidemics are reported in cities throughout the developing world and will likely intensify as the world’s slum population doubles to two billion by 2030 (United Nations Human Settlements Programme, 2003).
Pathogenic leptospires are responsible for a worldwide zoonosis, leptospirosis, in which humans are occasional hosts in a cycle involving wild and domestic animals. The disease has a broad geographical distribution due to the large spectrum of mammalian hosts that harbor and excrete the spirochete agent from their renal tubules (Pappas et al., 2008; Ko et al., 2009). The animal reservoir includes mostly rodents; they excrete leptospires in their urine and thus contaminate hydric environment, transmitting the disease to other animals or to humans. *Leptospira* can cause two distinctly different disease manifestations depending on the mammalian host and infecting serovar. Infection leads to either a chronic, nearly asymptomatic infection or an acute, potentially life-threatening disease. The most severe form, classic Weil’s disease, or acute leptospirosis, occurs most commonly in accidental hosts, including humans, with a wide range of disease manifestations. In contrast, infection of a normal maintenance host will typically result in a chronic infection with little outward sign of infection (Faine et al., 1999). Maintenance hosts most commonly show evidence of infection during pregnancy, manifested by the appearance of reproductive failure (infertility, abortions, stillbirths, or birth of weak offspring). It is important to note that the same bacterial strain can often cause both acute and chronic infections, depending largely on the mammalian species that is infected. Development of chronic or acute infection is dependent upon poorly understood factors that pair specific mammalian host species with selected *Leptospira* serovars. Presumably, the interplay between the host immune system and infecting strain of bacteria is critical in directing the outcome of *Leptospira* infection.

Laboratory diagnosis of leptospirosis is confusing for treatment and surveillance because of its varied clinical signs and symptoms. Misdiagnosis of leptospirosis has become a significant problem and it is often misdiagnosed as influenza, aseptic meningitis, encephalitis, dengue fever, hepatitis or gastroenteritis. As per WHO recommendations, the standard reference method for diagnosis of leptospirosis is microscopic agglutination test (MAT) (Faine, 1982) but it does not permit early diagnosis because it relies on detection of antibodies to leptospiral antigens and cannot detect infection until 5–7 days after exposure. The limitations of MAT in the diagnosis of leptospirosis have prompted to develop several other diagnostic methods. Presently available laboratory diagnostic methods for leptospirosis are isolation of the causative organism from body fluids and observation of the leptospires under dark field microscopy (DFM) or to determine elevation of IgM and IgG in the serum, staining techniques, microscopic agglutination test (MAT) and whole cell killed leptospiral
antigen based tests like macroscopic slide agglutination test (MSAT), microcapsule agglutination test (MCAT), enzyme linked immunosorbent assay (ELISA), lepto dipstick, lepto dridot and polymerase chain reaction (PCR). Most of these assays employ antigens from nonpathogenic *Leptospira biflexa* strain Patoc I or detection by cross reactivity with repeating disaccharides of lipopolysaccharide and all these techniques were found to have low sensitivity during the acute stage of the disease and laborious to be performed in all laboratories. Therefore researchers are focusing on development of recombinant protein based assays that would be more sensitive during the early stage of the disease.

The limited success of conventional antibiotic therapy in the treatment of leptospirosis underlines the need for the development of an effective vaccine. However, the existence of over 300 pathogenic serovars, with their associated antigen variation, makes the development of an efficacious broad-spectrum vaccine problematic. Currently, there is no human vaccine against leptospirosis. Most veterinarians use commercially available heat-killed or formalin-killed leptospires, which may produce incomplete, short-term immunity and have drawbacks in conferring protection against heterologous serovars. Efforts to develop recombinant vaccines against leptospirosis have focused on conserved immunogenic leptospiral proteins that represent potential targets for immunological defense mechanisms. Several leptospiral proteins have been evaluated as vaccine candidates; however, to date, no broadly conserved antigen has been able to induce sterilizing and long-term protective immunity. As such, there is a distinct need to characterize new targets. The main disadvantages in vaccine development for leptospirosis are, it is still in its infancy and under trial and it need larger evaluation for transforming from animal model to humans. The available leptospiral vaccines are regarded as being effective at controlling clinical disease and preventing mortality but only a few claim to reduce infection or renal excretion following challenge; an important property in reducing the spread of this zoonotic disease. This issue has raised a concern about whether vaccine immunity persists or whether more frequent re-vaccination is necessary (Sonrier et al., 2000; Haake, 2000). The tribulations with existing vaccines and the projected increased incidence of leptospirosis focus an urgent need for development of novel, low-cost strategies for the prevention of leptospirosis.

DNA and subunit vaccines represent potential intervention strategies for leptospirosis and have been extensively evaluated (Branger et al., 2005; Faisal et al., 2008; Feng et al.,
Both are able to induce a humoral immune response, and DNA vaccines stimulate cell-mediated immunity. DNA-vaccines provide a potentially attractive immunization strategy. These vaccines induce antigen-specific immune responses following the direct injection of non-replicating plasmids into a host target tissue. Once injected, plasmids drive the synthesis of specific foreign proteins within the immunized host and, as such, mimic natural infection in terms of inducing immune responses. The host provides post-translational modifications to antigen that typically and accurately reproduces native conformations. These host-synthesized proteins then become a target for immune surveillance via both the MHC class-I and class-II pathways. These processes lead to elicitation of protective immunity against an infectious agent. Over the past decade, studies have shown that prime-boost immunizations can be given with unmatched vaccine delivery methods while using the same antigen, in a “heterologous” prime-boost format. The most interesting and unexpected finding is that, in many cases, heterologous prime-boost is more effective than the “homologous” prime-boost approach. The rapid progress of novel vaccination approaches, such as DNA vaccines and subunit vaccines, has certainly further expanded the scope of heterologous prime-boost vaccination (Lu, 2009).

The clinical aspects and progression of disease in humans and domestic animals are well understood (Ellis, 2014; Haake and Levett, 2014). However, knowledge of specific virulence mechanisms and host factors that determine the outcome of infection is limited. When considering the pathogenesis of leptospirosis at the cellular and molecular level, our understanding of the processes and interactions between bacteria and host lags some way behind what is known for most other bacterial species and indeed for infections caused by other spirochete genera (Adler et al., 2011; Ko et al., 2009). After leptospiral infection, the host produces antibodies to a myriad of bacterial antigens expressed constitutively or expressed in vivo. The antigens that are expressed in vivo interact with the immune system and prime an immune response to eliminate leptospires. Hypothetically, proteins specifically induced during in vivo growth but not expressed by in vitro cultures have enhanced value because of their potential involvement in pathogenesis and participation in acquired immune protective responses (Guerreiro et al., 2001). Examples of such proteins that have gained prominence as diagnostic reagents and vaccine candidates are LigA and LigB immunoglobulin-like proteins and HbpA expressed strongly in vivo and found only on freshly
isolated pathogenic *Leptospira* species (Palaniappan et al., 2002; Matsunaga et al., 2003; Asuthkar et al., 2007). The diagnostic and vaccine efficacy of these *in vivo* proteins and its immunogenic epitopes has been studied extensively (Palaniappan et al., 2002, 2004, 2006; Chang et al., 2007; Velineni et al., 2008; Faisal et al., 2008; Forster et al., 2013; Kanagavel et al., 2014). Thus, studies on proteins increased in abundance *in vivo* not only to contribute the understanding of host-pathogen interactions but also to help in a great detail of designing the novel diagnostics and potential vaccine development.