CHAPTER I
INTRODUCTION

Human leptospirosis is a zoonotic disease caused by bacteria of the family leptospiraceae, order Spirochaetales, genus *Leptospira.* Human gets infection through wounded, abraded, and macerated skin, or mucous membranes (*e.g.* oral, conjunctival mucosae), either directly by contact with infected/reservoir animals or their contaminated specimens, or indirectly by exposure to damp soil, mud, vegetation or fresh water seeded with the urine or carcass of the infected/reservoir animals. Human-to-human transmission is relatively rare. Human leptospirosis used to be recognized as an occupational disease with high incidence among veterinarians, abattoir workers, sewer workers and farmers. However, a number of cases were found among travelers to the disease endemic areas and individuals after various re-creational activities such as canoeing, swimming, hiking, and rafting. Bacteria in the genus *Leptospira* have been traditionally classified into two species, *i.e. L. interrogans* which included all pathogenic strains of both animals and humans, and *L. biflexa* which are non-pathogenic, free-living saprophytes. The two species are different also in their growth at different temperatures. There are more than 200 different serovars of *Leptospira* spp. based on different agglutinating antigens. The antigenically related serovars have been arbitrarily allocated into the same serogroups. Recently, molecular techniques, such as 16S rRNA analysis and multi-locus sequence typing, based on sequence alignment of genes encoding house-keeping enzymes, have been used for re-delineation of members of *Leptospira* spp. into genomospecies. *Leptospira* spp. are divided into genomospecies which do not correlate to the *L. interrogans sensu lato* and *L. biflexa sensu lato.* These genomospecies include: *L. alexanderi, L. biflexa, L. borgpetersenii, L. fainei, L. inadai, L. interrogans, L. kirschneri, L. meyeri, L. noguchii, L. parva, L. santoirasai, L. weilii, and L. wolbachii* and few other unnamed genomospecies. Members of *L. biflexa, L. parva* and *L. wolbachii* are non-pathogenic; strains of *L. inadai* and *L. meyeri* may be either pathogenic or non-pathogenic, while strains belonging to the remaining

genomospecies are pathogenic. Several antigenically related strains previously allocated in the same serogroup/serovar are now found in more than one genomospecies. For example, strains of serogroup Bataviae may belong to either *L. interrogans* or *L. santarosai*; serogroup Hardjo may be found in *L. borgpetersenii, L. interrogans* and *L. meyeri* genomospecies.

Most *Leptospira* infections in humans are subclinical which can be retrospectively recognized by the presence of serum antibodies to the bacteria. A portion of infected individuals succumb different degrees of morbidity ranging from mild, flu-like symptoms, indistinguishable from other febrile illnesses, to acute and severe disease which often leads to rapid fatality. Human leptospirosis is usually manifested in two common forms, *i.e.* anicteric and icteric or Weil's disease. Anicteric leptospirosis is usually biphasic; the initial septicemic phase starts as early as one day or as late as one month post-*Leptospira*-exposure depending upon the inoculation dose and the host immunity and is characterized by generalized leptospiremia. Symptoms including high fever, chill, cough, sudden onset of intense headache, muscular pain especially calf muscles, abdominal pain, conjunctival suffusion, blurred vision, and photophobia are common. An immune phase follows the septicemic phase about a week later and is characterized by appearance of specific antibodies and disappearance of leptospiremia although the bacteria still can be found in many organs and tissues especially the immunological privilege sites such as brain, aqueous humor and kidney tubules, where the bacteria are shed from the latter with the urine (leptospiruria). The human leptospiruria may last several months. Icteric leptospirosis is a form of severe ailment with high mortality that occurs to a fraction of clinically infected individuals. In this form of the disease, several vital organs are affected leading not only to the previously mentioned clinical manifestations, but may also include vasculitis, jaundice, hemorrhage, myocarditis, aseptic meningitis, vascular collapse and/or hepatic and renal failure.

Leptospirosis responds well to antibiotic therapy especially when started early in the course of the illness. Delayed treatment as a consequence of delayed or misdiagnosis of severe leptospirosis often leads to organ failure with exceptionally high mortality rate. Ampicillin, amoxicillin, and doxycycline have been commonly used for mild anicteric form of the disease. Patients with severe anicteric and icteric

leptospirosis are usually treated with intravenous penicillin, ampicillin, erythromycin or amoxicillin. Jarisch-Herxheimer reactions (JHR) due to bacterial toxic substances massively released from the antibiotic mediated-bacterial lysis occur in a fraction of the treated patients which may aggravate the clinical manifestations.

Recently, genomes of some serovars of *Leptospira* spp., *i.e*. Copenhageni and Lai have been completely sequenced and many attributes of the bacteria and the disease caused by them have been revealed. Nevertheless, much more is to be learned about the *Leptospira* virulence factors, their pathogenesis, leptospirosis mediated-immunopathology, and others. Currently, leptospirosis vaccine is available only for some veterinary use such as for prevention of canine leptospirosis. Although there have been some reports on the use of human leptospirosis vaccine in certain tropical countries, such as Brazil and Cuba, however, detail information about the vaccine efficacy is not readily available in English language. Most of the vaccines have been prepared from inactivated whole cell bacteria (Bacterin) which, not only the supply of the vaccine component is inadequate owing to the slow growth of the *Leptospira* spp. *in vitro*, but also the vaccines elicit limited immunity which is serovar/serogroup specific. Because of the recent upsurge of human leptospirosis in many endemic areas of which the causative serogroups or serovars vary from area to area, there is a great need of a broad spectrum leptospirosis vaccine that protects across serogroups and serovars, both for human and veterinary uses.

In this study, proteomes of both pathogenic and non-pathogenic *Leptospira* serovars of various serogroups and genomospecies obtained from an *in vitro* growth were studied using two dimensional gel electrophoresis (2DE)-based-proteomics: 2D difference gel electrophoresis (DIGE) for the separation of the bacterial proteins, protein spot picking, in gel digestion of the proteins into peptides, generation of peptide mass fingerprints by tandem mass spectrometry, and protein identification by using bioinformatics. Moreover, a plethora of *in vivo* expressed *Leptospira* antigens, *i.e.* their virulence factors, were studied by comparing the immunome of the pathogenic *Leptospira* detected by acute phase sera of patients with confirmed leptospirosis compared to the immunome revealed by convalescence phase sera of the same patients. *Leptospira* proteins reactive to antibodies in the convalescence sera but did not react or react only weakly with the acute phase sera were individually

identified. Control sera including sera of patients with other febrile illnesses and healthy counterparts were used as appropriate controls. The representative gene encoding *ompL1*, so-identified *in vivo* expressed proteins, hence *Leptospira* virulence factors which were common to all pathogenic *Leptospira* spp. was amplified using conventional PCR and oligonucleotide primers designed from the respective gene sequences in the database. The gene amplicons were cloned into a eukaryotic expression vector. The recombinant plasmid DNA was used as vaccine to immunize hamsters. The immunized animals were challenged with lethal dose of heterologous *Leptospira* spp. and the immunogenicity and protective efficacy of the DNA vaccine were evaluated. It is expected that a completion of this research will, not only provide insight information of the *Leptospira* factors which the bacteria produce within the infected host (virulence factors), but also an availability of a prototype broad spectrum leptospirosis vaccine that protects against heterologous *Leptospira* spp. infections.