CHAPTER VII
CONCLUSION

Leptospirosis, a zoonosis caused by bacteria of the genus *Leptospira,* is an important emerging infectious disease worldwide. A number of severe cases with high fatality were recognized during the past two decades, not only in the rural tropics, but also in the temperate urbans. Disease eradication is difficult because there are abundance of the animal reservoirs, both wild and domestic, of *Leptospira* spp. and the long-term survival of the bacteria in the environment. Avoiding contact with the animals chronically infected with *Leptospira* spp. (reservoirs) or their environments, such as soil and water contaminated with the animal urine or carcasses, is the most effective means of the disease intervention. However, the measure is difficult to practice especially in the countries where agriculture is the foremost activity and the environmental sanitation is compromised. Vaccines to prevent against the leptospirosis prepared from inactivated whole bacterial cells or outer membrane components of pathogenic *Leptospira* spp. elicit immunity which is limited to the homologous infection. Also the vaccines failed to induce long-lasting immunity. Thus, there is a need of more effective vaccine that not only elicits immunity across the heterologous *Leptospira* spp. serovars but also induces long-lasting immunological memory, for both human and veterinary uses. Recently, the genomes of three *Leptospira* spp. serovars, *i.e.* Copenhageni, Lai, and Hardjo have been completely sequenced and the whole-genome (*in silico*) analysis has been used for identifying the broad spectrum *Leptospira* vaccine candidate genes (Adu-Bobie *et al*., 2001). However, while such approach, *i.e.* reverse vaccinology, may be relatively convenient, it is required that several genes should be studied concurrently as many of the selected candidates or their expressed proteins counterparts may turned out to be either poorly immunogenic or confer only limited immunity.

In this study, proteomes of two pathogenic *Leptospira* spp., namely *L. interrogans*, serogroup Icterohaemorrhagiae, serovar Icterohaemorrhagiae and *L. borgpetersenii*, serogroup Tarassovi, serovar Tarassovi, were revealed by using two

dimensional gel electrophoresis (2DE)-based-proteomics. Bacterial cells were disrupted in a lysis buffer containing 30 mM Tris, 2 M thiourea, 7 M urea, 4% CHAPS, 2% IPG buffer pH 3-10 and protease inhibitors and then subjected to sonication in order to solubilize as much as possible the bacterial proteins. The 2DE-separated components of both *Leptospira* homogenates were blotted individually onto membranes and antigenic components (immunomes) were revealed by probing the blots with immune serum of a mouse readily immunized with the homogenate of *L. interrogans*, serogroup Icterohaemorrhagiae, serovar Copenhageni and *l. borgpetersenii* serovar Tarassovi. The immunogenic proteins of the two pathogenic *Leptospira* spp. could be grouped into 10 groups. These are: 1) proteins involved in the bacterial transcription and translation including beta subunit transcription anti-termination protein of DNA polymerase III, elongation factors Tu and Ts, and tRNA (guanine-N1)-methyltransferase; 2) proteins functioning as enzymes for metabolisms and nutrient acquisition including acetyl-Co-A acetyltransferase, putative glutamine synthetase, glyceraldehyde-3-phospahte dehydrogenase, NifU-like protein, 3-oxoacyl-(acyl-carrier-protein) reductase, oxidoreductase, sphingomyelinase C precursor, spermidine synthase, beta subunit of succinyl-CoA synthetase, and succinate dehydrogenase iron-sulfur subunit; 3) proteins/enzymes necessary for energy and electron transfer, *i.e*., electron transfer flavoprotein, and proton-translocating transhydrogenase; 4) enzymes for degradation of misfolded proteins, *i.e.* ATP–dependent Clp protease; 5) molecular chaperone, *i.e.* 60 kDa chaperonin; 6) signal transduction system, *i.e.* response regulator; 7) protein involved in immune evasion in host, *i.e.* peroxiredoxin; 8) cell structure proteins including MreB (cytoskeletal) and flagellin/ periplasmic flagellin; 9) lipoproteins/outer membrane proteins: LipL32, LipL41, LipL45 and OmpL1; and 10) various hypothetical proteins. Many immunogenic proteins are common to both *Leptospira* spp. These proteins not only are the diagnostic targets but also have potential as candidates of a broad spectrum leptospirosis vaccine especially the surface exposed components which should be vulnerable to the host immune effector factors.

The 2DE-separated components of *Leptospira borgpetersenii* serovar Tarassovi homogenates were blotted individually onto membranes and antigenic components (*in vivo* expressed antigens) were probed with serum of a acute and convalescence phase

of leptosprirosis patients. It was found that the two pathogenic *Leptospira* spp. had several common immunogenic proteins which could be grouped into: 1) Protein involved in transcription *i.e.* DNA-directed RNA polymerase beta subunit, single-stranded DNA binding protein; 2) Protein involved translation *i.e.* elongation factor Tu; 3) Enzymes *i.e.* 3-oxoacyl-(acyl-carrier protein) reductase, acetyl-CoA acetyltransferase , ATP synthase F1, beta chain, cysteine synthase, enolase, enoyl-CoA hydratase, glutathione peroxidase, glyceraldehyde-3-phosphate dehydrogenase, probable Methylthioadenosine phosphorylase, sulfite reductase; 4) Amino acid biosynthesis *i.e.* uridylate kinase; 5) Energy Transfer and production *i.e.* Electron transfer flavoprotein beta-subunit, succinate dehydrogenase iron-sulfur subunit, NifU-like protein; 6) Chaperone *i.e.* 60 kDa chaperonin, serine protease MucD precursor, transketolase alpha subunit protein, Peroxiredoxin; 7) Protein of unknown function *i.e.* hypothetical protein LIC10314, hypothetical protein LIC10483, hypothetical protein LIC10672, hypothetical protein LIC12621, hypothetical protein LIC12821, hypothetical protein LIC12886, hypothetical protein LIC13166; 8) Cell structures *i.e.*cytoplasmic membrane protein, flagellar filament outer layer protein A, flagellin protein, Possible hook-associated protein, flagellin family9) Lipoprotein *i.e.*Lip L32 outer membrane protein AAQ98019, AAS21765 and AAZ73230, lipoprotein LipL41 AAS21807 and LipL41 BAE48275; 10) Outer membrane proteins *i.e.* OmpL1 AAS21839, AAS21839 and A40660. These proteins not only are the diagnostic targets but also have potential as candidates of a broad spectrum leptospirosis vaccine especially the surface exposed components which should be vulnerable to the host immune effector factors.

Available leptospirosis vaccines made up of inactivated bacteria or their membrane components elicit immunity which is serovar specific and unsatisfactory immunological memory. A vaccine that protects across *Leptospira* serogroups/serovars, *i.e.* broad spectrum, and induces long-lasting memory is needed for both human and veterinary uses. In this study, a plasmid DNA vaccine was constructed from cloning gene encoding a transmembrane porin protein, OmpL1, of pathogenic *Leptospira interrogans*, serogroup Icterohaemorrhagiae, serovar Copenhageni into a mammalian expression vector pcDNA3.1(+). The protective efficacy of the *ompL1*-pcDNA3.1(+) plasmid DNA vaccine was studied by

immunizing hamsters intramuscularly with three doses of the vaccine (100 μg per dose) at two week intervals. The empty pcDNA3.1(+) and PBS were used as mock as negative vaccine controls, respectively. All animals were challenged with the heterologous *Leptospira interrogans,* serogroup Pomona, serovar Pomona (10 LD50), at one week after the last vaccine booster. The *ompL1*-pcDNA3.1(+) plasmid DNA vaccine rescued some vaccinated animals from the lethal challenge and delayed death time, reduce morbidity, *e.g.* fever, and/or the numbers of *Leptospira* in the tissues of the vaccinated animals. While the results are encouraging, further studies are needed to optimize the immunization schedule, vaccine dosage and formulation in order to maximize the efficacy of the vaccine.