Anchalee La-ard 2008: Molecular and Immunogenicity Characterizations of *flaB* and *lig* and Their Applications for Leptospirosis Detection. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Thavajchai Sukpuaram, Ph.D. 166 pages.

In this experiment was to test the ability of the specific pair of primers (*rrs*, *flaB* and *lig*) to amplify the DNAs of *Leptospira* and other bacteria and to determine and evaluate the use of nearly full-length 749-bp *flaB* sequencing from well-characterized type strains for species identification of *Leptospira* spp. The result demonstrated that, the primers which were used in this PCR are specific for *Leptospira*. All recognized and unknown species of leptospira can be identified by using standardized *flaB* gene sequencing. Furthermore cloning and expression of target antigen genes from *Leptospira* derived recombinant protein may be useful for developing improved test for rapid diagnosis of leptospirosis. In this study, *L. interrogans* serovar Canicola strain Hond Utrecht IV was chosen to be a target for cloning *flaB.* Recombinant FlaB (His.FlaB) protein was expressed as an antigen for the production of polyclonal antibodies. The specificity of polyclonal antibodies was confirmed by immunoblot analysis and indirect immunofluorescent antibody technique using purified recombinant FlaB protein and whole-cell antigen preparation from pathogenic and non-pathogenic leptospires and other bacteria as antigens. The result demonstrated that polyclonal antibodies were a potential tool for *Leptospira* spp. detection. *L. kirschneri* serovar Grippotyposa strain Moskva V was chosen to be a target for cloning the conserved region of *lig*. The specificity of antibodies against recombinant Lig protein was determined by using immunoblot analysis. The recombinant Lig antigen (GST.Lig) was developed and used as an ELISA antigen for the detection of the anti-leptospiral antibodies. This assay was evaluated with canine sera (n=91) that were microscopic agglutination test (MAT)-negative (at < 1:100 dilution) and sera (n=103) that were MAT-positive (at $\geq$ 1:100 dilution) using 24 serovars. The ELISA showed a relative sensitivity as compared to MAT was 84.5% whereas the specificity was 76.9%.

In conclusion, the primers which were used in the PCR reaction are specific for *Leptospira* and *flaB* sequence has become an accurate tool for discrimination and identification of leptospires. The polyclonal antibodies against recombinant FlaB proteins were a potential tool for *Leptospira* spp. detection. The recombinant Lig protein based ELISA could be used as a screening test for serodiagnosis of canine leptospirosis. The ELISA described here is suitable for large scale serological examination, routine diagnostics, epidemiology surveys and follow-up investigations for outbreaks. It would be particularly useful in canine with full consideration of clinical picture and vaccination history of animals.

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