

Siriwadee Phromnoi 2010: Expression of Recombinant VP2 Protein of Canine Parvovirus in *Escherichia coli*. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Theerapol Sirinarumitr, Ph.D. 79 pages.

Canine parvovirus (CPV) appears to be endemic in almost all populations of wild and domesticated dogs. It causes serious contagious enteric disease especially in puppies. The VP2 of CPV is a major capsid protein and plays an important role in the host immune response. Twenty six isolates of CPV were obtained from infected dogs and full-length VP2 gene was amplified by PCR. Its sequences were analyzed. Nineteen isolates were characterized as CPV type 2a variants and the rest of the isolates were characterized as CPV type 2b. These results indicated that both types are currently prevalent field CPV circulating in Thailand and type 2a is the predominant genotype. Neither CPV type 2 nor type 2c was observed in this study.

In the present study, the recombinant VP2 (rVP2) protein of CPV was expressed and tested with rabbit antibody against rVP2. The whole VP2 gene was amplified by PCR using specific primers. The size of PCR product has about 1,700 bp and was ligated with plasmid pBAD202/D-TOPO to transform into *E. coli* strain TOP10. The optimum concentration of arabinose and time course for expression was 0.002% and 8 hours. By SDS-PAGE analysis, the rVP2 protein band was about 80 kDa. By Western blot analysis, the rVP2 proteins were specifically interact with rabbit anti-CPV hyperimmune serum. Each rabbit group was immunized with PBS, 300 µg of rVP2, 500 µg of rVP2 and CPV commercial vaccine. By ELISA, all immunized rabbits generated antibodies against rVP2 at the 2<sup>nd</sup> week of immunization and reached its peak within the 7<sup>th</sup> week and were stable until the end of the experiment. By ANOVA, there were significantly differences between the control group and the immunized group from the 4<sup>th</sup> week of immunization through the end of the experiment. However, there was no significantly different between the antibody titer from rabbits immunized with both recombinant protein concentrations.

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Thesis Advisor's signature