# **TABLE OF CONTENTS**

	Page	
TABLE OF CONTENT		
LIST OF FIGURES		
LIST OF ABBREVIATIONS		
INTRODUCTION	1	
LITRURATURE REVIEWS	3	
Overview of Canine Parvovirus	3	
Baculovirus as Expression Vectors for		
Recombinant Protein Production	12	
MATERIALS AND METHODS		
Cell Line and Virus	14	
Polymerase Chain Reaction for Diagnostic of CPV	14	
Polymerase Chain Reaction for Cloning VP2 Gene	15	
Construction of Transfer Vector	16	
Insect Cell Transfection and Recombinant Virus Amplification	17	
Recombinant Protein Expression and Detection	18	
Preparation of Polyclonal Antibody against CPV	18	
Immunodot Analysis	18	
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis		
(SDS-PAGE) and Western Blot Analysis	19	
RESULTS	20	
DISCUSSION		
CONCLUSION	42	
LITURATURE CITED		
APPENDIX	48	
Appendix A	49	
Appendix B	54	

# LIST OF FIGURES

Figure

# Page

1	(a)The Non infected A-72 cell line	20		
	(b)The Cytopathic effect performance	20		
2	Analysis of PCR products of 400 bp partial fragment of VP2 gene	22		
3	The schematic review of the cloning steps of CPV VP2 gene	24		
4	Analysis of PCR products of 700 bp fragment of VP2 gene			
5	Analysis of PCR products of VP2 gene 2			
6	Analysis of Restriction enzyme digestion of recombinant			
	pFastBac <sup>TM</sup> Htb –VP2 and recombinant pFastBac <sup>TM</sup> Htb 1 kb	27		
7	Sequence alignment of VP2 gene of canine parvovirus	28		
8	Sequence alignment of VP2 protein amino acid	30		
9	(a) Dot blot analysis of extracted crude protein using			
	rabbit hyper immune serum against canine parvovirus virus	32		
	(b) Dot blot analysis of extracted crude protein using			
	monoclonal antibody against histidine	32		
10	10% SDS-PAGE analysis of crude protein	33		
11	Western blot analysis of crude protein using			
	monoclonal antibody against histidine	34		
12	Western blot analysis of crude protein using			
	rabbit hyperimmune serum against canine parvovirus	35		

### **Appendix Figure**

1	The pGEMT-Easy cloning vector circle map	61
2	The pFastBac <sup>TM</sup> HTB plasmid circle map	62
3	The multiple cloning sequence of pFastBac <sup>TM</sup> HTB plasmid	63
4	Diagram of canine parvovirus genome	64

# LIST OF ABBREVIATIONS

μl	=	microlitre
ml	=	millilitre
μg	=	microgram
mg	=	milligram
bp	=	base pair
kDa	=	kilodalton
pmol	=	picomole
mM	=	millimolar
Μ	=	molar
U	=	unit
DNA	=	deoxyribonucleic acid
dNTPs	=	deoxynucleotide tri-phosphate
PCR	=	polymerase chain reaction
EtBr	=	ethidium bromide
UV	=	ultraviolet
SDS-PAGE	=	sodium dodecyl sulfate polyacrylamide gel -
		electrophoresis
rpm	=	round per minute
°C	=	degree celsius
h.p.i.	=	hour post infection
MOI	=	multiplicity of infection
<u>E. coli</u>	=	Escherichia coli