

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**RELEVANCE OF THE VARIABILITY OF THE
FELINE IMMUNODEFICIENCY VIRUS
IN REGARD TO PATHOGENICITY AND
VACCINATION IN NEW ZEALAND**

A thesis presented in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy

in

Animal Science

Massey University, Manawatū
New Zealand

Alison Louise Stickney

2018

Abstract

Cats infected with the feline immunodeficiency virus (FIV) show a range of clinical signs. Given the variability of the FIV genome, it is possible that there is variation in certain biological characteristics of FIV, such as pathogenicity. This may also be relevant to vaccination against FIV, as an effective vaccine would have to result in the generation of T cells that recognise a range of different variants in the field. The Fel-O-Vax[®] FIV vaccine has been available to veterinarians in New Zealand (NZ) for the past 12 years. Despite this, there is a paucity of studies investigating the cross-reactivity of the vaccine-induced immune response against different variants of FIV, and no studies investigating the efficacy of the vaccine in NZ.

The overall aim of the research in this thesis was to determine the relevance of the variability of FIV, in regard to pathogenicity and vaccination in NZ. Firstly, 2 separate assays were designed to assess variation in the ability of different isolates of FIV to induce apoptosis or inhibit mitogen-induced proliferation in lymphoid cells *in vitro*. Results showed that variation in FIV-apoptosis did occur, supporting the argument that FIV variants may also differ in pathogenicity. Secondly, the cross-reactivity of the vaccine-induced immune response was assessed *in vitro* and *in vivo*, by measuring antigen-specific cellular activation and a delayed type hypersensitivity (DTH) response in vaccinated cats following inoculation with NZ field isolates of FIV. Results showed that the response was at least partially cross-reactive, however quantitative differences were detected in the response to each isolate of FIV tested. Finally, efficacy of the Fel-O-Vax[®] FIV vaccine under NZ conditions was investigated by comparing the prevalence of FIV in vaccinated and unvaccinated cats in the field. Results showed that there was no effect of vaccination on FIV prevalence, suggesting poor efficacy of the Fel-O-Vax[®] FIV vaccine in NZ.

Results described in this thesis support the argument that there is variation among FIV in NZ, and that this may affect pathogenicity and vaccine efficacy in this country. The evidence presented did not support use of the Fel-O-Vax[®] FIV vaccine in NZ.

Acknowledgements

To the many people who have helped me on this journey, I am sincerely grateful. Firstly, to my supervisors; Associate Professor Nicholas Cave, Dr. Magda Dunowska and Dr. Anthony Pernthaner. Thank you Tony for taking the time to explain even the most basic concepts in flow cytometry, and for your patience in answering all my questions. I would like to express my gratitude to Magda for her friendship and support, especially in the laboratory. I have come a long way with her guidance, and appreciate all that she has taught me. I am especially grateful to Nick, who taught me to question everything and inspired me to embark on this journey. Thank you Nick for all the emotional support, your friendship and mentorship, and your understanding of the challenges that I faced throughout this adventure.

I would like to acknowledge the staff at the Hopkirk Institute, who allowed me to use their equipment and often assisted me with various assays. In particular, I would like to thank Joanna Roberts for all her assistance with the flow cytometry experiments. Thank you also to the staff at the Massey University Feline Nutrition Unit. I appreciate all of your help with sample collection and for always making me feel welcome. I am grateful to the staff in the virology laboratory who provided support along the way. In particular, to Sayani Gosh, for all her help with the samples towards the end; Kristene Gedye for helping me to understand the phylogenetic analysis, and to Laryssa Howe for taking the time to teach me how to run an ELISA. Thank you also to Jessica Hayward for her assistance in the initial phylogenetic analysis of the samples. I would like to acknowledge Zoetis, the NZ Companion Animal Society and the IVABS Postgraduate Research Fund for their financial support of this project.

Finally, I am eternally grateful to my family for their constant support throughout this process. To my beautiful children, Maryjane and Jack – I look forward to all the extra time we will now have together. And most importantly, to my husband Simon. Thank you for helping me get through this. For all the babysitting, your understanding of the time we have spent apart and constant emotional support, I will always be grateful.

Table of Contents

CHAPTER ONE

REVIEW OF THE LITERATURE	1
1.1. INTRODUCTION	3
1.2. GENETIC DIVERSITY OF FIV	5
1.2.1. <i>The FIV genome</i>	5
1.2.2. <i>Subtype classification</i>	6
1.2.3. <i>The clinical relevance of subtype classification</i>	8
1.3. BIOLOGY OF FIV	9
1.3.1. <i>Cell tropism and the virus-host cell interaction</i>	9
1.3.2. <i>Virus assembly</i>	13
1.3.3. <i>Viral transmission</i>	14
1.3.4. <i>Viral dissemination</i>	14
1.4. FELINE ACQUIRED IMMUNODEFICIENCY SYNDROME	15
1.4.1. <i>FIV-induced immune dysfunction</i>	18
1.4.2. <i>FIV-associated neurological disease</i>	26
1.4.3. <i>FIV-associated neoplasia</i>	28
1.4.4. <i>FIV-associated gingivostomatitis</i>	29
1.5. THE IMPACT OF GENETIC DIVERSITY ON THE PATHOGENICITY OF FIV	32
1.5.1. <i>Viral evolution</i>	34
1.5.2. <i>Replication rate</i>	34
1.5.3. <i>Cell Tropism</i>	35
1.5.4. <i>Neurotoxicity</i>	35
1.5.5. <i>Lymphocyte apoptosis</i>	36
1.6. NATURAL IMMUNITY AGAINST FIV	37
1.6.1. <i>Intrinsic anti-FIV immunity</i>	37
1.6.2. <i>Innate anti-FIV immunity</i>	38
1.6.3. <i>Acquired anti-FIV immunity</i>	39
1.7. FIV VACCINATION	41
1.7.1. <i>Evidence for efficacy of the Fel-O-Vax[®] FIV vaccine</i>	42
1.7.2. <i>Vaccine-induced immunity</i>	48
1.7.3. <i>The impact of genetic diversity of FIV on the efficacy of the Fel-O-Vax[®] FIV vaccine</i>	50
1.8. FIV IN NEW ZEALAND.....	54
1.8.1. <i>Current considerations for use of the Fel-O-Vax[®] FIV vaccine in NZ</i>	55
1.9. CONCLUSION	57

CHAPTER TWO

PREPARATION OF FIV STOCK.....	59
2.1. INTRODUCTION	61
2.2. MATERIALS AND METHODS	62
2.2.1. <i>Recruitment of FIV positive cats</i>	62
2.2.2. <i>Conventional PCR</i>	63
2.2.3. <i>Sequencing of the PCR product</i>	64
2.2.4. <i>Phylogenetic analysis</i>	64
2.2.5. <i>Reverse transcriptase quantitative PCR</i>	65
2.2.6. <i>Virus isolation</i>	68
2.2.7. <i>Production of virus from infectious molecular clones</i>	70
2.2.8. <i>Concentration of virus stock</i>	72
2.2.9. <i>Quantification of virus stock</i>	72
2.3. RESULTS	75
2.3.1. <i>Recruitment of FIV positive cats</i>	75
2.3.2. <i>Conventional PCR</i>	75
2.3.3. <i>Phylogenetic analysis</i>	76

2.3.4. Reverse transcriptase quantitative PCR	82
2.3.5. Virus isolation	90
2.3.6. Production of virus from infectious molecular clones	93
2.3.7. Quantification of virus stock.....	96
2.4. DISCUSSION	102
2.5. CONCLUSION	110
CHAPTER THREE	
COMPARISON OF THE IN VITRO PATHOGENICITY OF NZ ISOLATES OF FIV	111
3.1. INTRODUCTION	113
3.2. MATERIALS AND METHODS	116
3.2.1. Development and optimisation of a flow-cytometric apoptosis assay	116
3.2.2. Demonstration of FIV-induced apoptosis.....	120
3.2.3. Comparison of apoptosis and necrosis of MYA-1 cells, induced by different isolates of FIV	121
3.2.4. Effect of FIV infection on MYA-1 cell concentration in culture	122
3.2.5. Inhibition of mitogen-induced lymphocyte proliferation by different isolates of FIV.....	122
3.2.6. Statistical analysis	122
3.3. RESULTS	124
3.3.1. Development and optimisation of a flow-cytometric apoptosis assay	124
3.3.2. Comparison of apoptosis and necrosis of MYA-1 cells induced by different isolates of FIV	132
3.3.3. Effect of FIV infection on MYA-1 cell concentration in culture	138
3.3.4. Inhibition of mitogen-induced lymphocyte proliferation by different isolates of FIV.....	140
3.4. DISCUSSION	141
3.5. CONCLUSION	150
CHAPTER FOUR	
IN VITRO CROSS-REACTIVITY OF THE FEL-O-VAX® FIV VACCINE-INDUCED IMMUNE RESPONSE AGAINST NZ ISOLATES OF FIV	153
4.1. INTRODUCTION	155
4.2. MATERIALS AND METHODS	158
4.2.1. Animals.....	158
4.2.2. Preparation of viral antigen	159
4.2.3. CD25 assay	160
4.2.4. DTH response	163
4.2.5. Statistical analysis	164
4.3. RESULTS	167
4.3.1. CD25 assay	167
4.3.2. DTH response	178
4.4. DISCUSSION	181
4.5. CONCLUSION	188
CHAPTER FIVE	
FIELD EFFICACY OF THE FEL-O-VAX® FIV VACCINE IN NZ	189
5.1. INTRODUCTION	191
5.2. MATERIALS AND METHODS	193
5.2.1. The effect of RNA stabilisation solution on detection of FIV provirus	193
5.2.2. The effect of RNA stabilisation solution at high dilutions on detection of FIV provirus	194
5.2.3. Detection of FIV provirus in a buccal swab.....	194
5.2.4. The prevalence of FIV in vaccinated and unvaccinated cats in the field	195
5.2.5. Statistical analysis	199
5.3. RESULTS	199
5.3.1. The effect of RNA stabilisation solution on detection of FIV provirus	199
5.3.2. The effect of RNA stabilisation solution at high dilutions on detection of FIV provirus	200
5.3.3. Detection of FIV provirus in a buccal swab.....	201
5.3.4. The prevalence of FIV in vaccinated versus unvaccinated cats in the field	203

5.4. DISCUSSION.....	211
5.5. CONCLUSION	217
CHAPTER SIX	
CONCLUDING REMARKS.....	219
BIBLIOGRAPHY.....	229
APPENDIX 1	
SUBMISSION FORM FOR RECRUITMENT OF FIV POSITIVE CATS.....	261
APPENDIX 2	
SUMMARY OF RESULTS FROM FIV SEROPOSITIVE CATS RECRUITED FROM VARIOUS VETERINARY PRACTICES	263
APPENDIX 3	
SUMMARY OF TROUBLESHOOTING PROCESS FOR REAL-TIME GAG PCR REACTION	265
APPENDIX 4	
CHAPTER 2 RAW DATA.....	269
<i>Endpoint dilution assay results</i>	<i>269</i>
APPENDIX 5	
CHAPTER 3 RAW DATA.....	271
<i>Comparison of apoptosis and necrosis induced by different isolates of FIV</i>	<i>271</i>
<i>Effect of FIV infection on MYA-1 cell concentration in culture.....</i>	<i>273</i>
<i>Inhibition of mitogen-induced lymphocyte proliferation by different isolates of FIV</i>	<i>276</i>
APPENDIX 6	
CHAPTER 3 STATISTICS.....	279
<i>Comparison of apoptosis and necrosis induced by different variants of FIV</i>	<i>279</i>
<i>Effect of FIV infection on MYA-1 cell concentration in culture.....</i>	<i>283</i>
<i>FIV-induced inhibition of mitogen-induced lymphocyte proliferation</i>	<i>285</i>
APPENDIX 7	
CHAPTER 4 RAW DATA.....	287
<i>Cross-reactivity of Fel-O-Vax® FIV vaccine-induced antigen-specific cellular activation</i>	<i>287</i>
<i>Cross-reactivity of the Fel-O-Vax® FIV vaccine-induced DTH response</i>	<i>297</i>
APPENDIX 8	
CHAPTER 4 STATISTICS.....	299
<i>Cross-reactivity of Fel-O-Vax FIV vaccine-induced antigen-specific cellular activation</i>	<i>299</i>
<i>Cross-reactivity of the Fel-O-Vax FIV vaccine-induced DTH response.....</i>	<i>303</i>
APPENDIX 9	
SUBMISSION FORM FOR RECRUITMENT OF VACCINATED AND UNVACCINATED CATS	305
APPENDIX 10	
CHAPTER 5 RAW DATA.....	307
<i>Summary of PCR results in vaccinated and unvaccinated cats.....</i>	<i>307</i>
APPENDIX 11	
CHAPTER 5 STATISTICS.....	317
<i>The effect of vaccination on FIV status in all cats.....</i>	<i>317</i>
<i>The effect of vaccination on FIV status in cats tested prior to vaccination</i>	<i>318</i>

List of figures

FIGURE 1-1 PHYLOGENETIC CLASSIFICATION OF FIV SUBTYPES.	7
FIGURE 1-2 THE FIV-HOST INTERACTION.	12
FIGURE 1-3 APOPTOTIC PATHWAYS AND FIV-INDUCED MECHANISMS OF APOPTOSIS.	23
FIGURE 2-1 GEL ELECTROPHORESIS SHOWING CONVENTIONAL FIV PCR RESULTS.	76
FIGURE 2-2 PHYLOGENETIC ANALYSIS OF NZ FIV ISOLATES.	79
FIGURE 2-3 NUCLEOTIDE ALIGNMENT OF THE SELECTED FIV ISOLATES USED IN SUBSEQUENT CHAPTERS OF THIS THESIS.	80
FIGURE 2-4 RECOMBINANT ANALYSIS OF NZ RVC001 ISOLATE.	81
FIGURE 2-5 OPTIMISATION OF THE FIV qRT-PCR.	84
FIGURE 2-6 GEL ELECTROPHORESIS SHOWING PRIMER-DIMER FORMATION WITH THE FIV qRT-PCR.	84
FIGURE 2-7 OLIGO 7 OUTPUT SHOWING POTENTIAL FOR DUPLEX FORMATION BETWEEN PRIMERS.	85
FIGURE 2-8 MELT CURVE RESULTS FROM THE FIV RT-qPCR.	86
FIGURE 2-9 NUCLEOTIDE ALIGNMENT OF THE SEQUENCED FIV RT-qPCR PRODUCT.	86
FIGURE 2-10 STANDARD CURVES GENERATED USING THE OPTIMISED FIV RT-qPCR.	89
FIGURE 2-11 STANDARD CURVE GENERATED FOR ABSOLUTE QUANTIFICATION OF FIV.	90
FIGURE 2-12 VIRUS ISOLATION FROM FIV-INFECTED PBMC CO-CULTURED WITH DONOR PBMC.	91
FIGURE 2-13 VIRUS ISOLATION FROM FIV-INFECTED PBMC CO-CULTURED WITH MYA-1 CELLS.	92
FIGURE 2-14 QUANTIFICATION OF VIRAL RNA IN CULTURE OF MYA-1 CELLS FOLLOWING PASSAGE OF FIV ISOLATES.	93
FIGURE 2-15 VIRAL REPLICATION FOLLOWING TRANSFECTION OF CRFK CELLS WITH PETF14 CLONES.	94
FIGURE 2-16 MELT CURVE ANALYSIS OF FIV DNA FROM TRANSFECTED CRFK CELLS.	94
FIGURE 2-17 VIRAL RNA SAMPLED FROM TRANSFECTED CRFK CELLS AND INFECTED MYA-1 CELLS.	95
FIGURE 2-18 AMPLIFICATION PLOTS OF FIV DNA AND cDNA IN VIRUS STOCKS.	96
FIGURE 2-19 MELT CURVE ANALYSIS FOLLOWING FIV RT-qPCR ON VIRUS STOCK.	97
FIGURE 2-20 COMPARISON OF DNA EXTRACTION METHODS.	98
FIGURE 2-21 AMPLIFICATION OF LIVE VERSUS INACTIVATED FIV.	99
FIGURE 2-22 STANDARD CURVE USED TO CALCULATE THE p24 CONCENTRATION OF VIRUS STOCKS.	101
FIGURE 3-1 FLOW CYTOMETRY GRAPH OF PBMC SEPARATION.	124
FIGURE 3-2 FLOW CYTOMETRY GRAPHS DEMONSTRATING CAMPTOTHECIN-INDUCED APOPTOSIS.	125
FIGURE 3-3 THE EFFECT OF TIME AND CONCENTRATION ON CAMPTOTHECIN-INDUCED APOPTOSIS.	126
FIGURE 3-4 THE EFFECT OF MEDIA COMPOSITION ON CELL VIABILITY OVER TIME.	127
FIGURE 3-5 THE EFFECT OF CONA ON CELL VIABILITY AND CELLULAR ACTIVATION.	128
FIGURE 3-6 THE EFFECT OF CONA AND FIV INFECTION ON APOPTOSIS AND NECROSIS OF FELINE PBMC.	129
FIGURE 3-7 FLOW CYTOMETRY GRAPHS DEMONSTRATING CAMPTOTHECIN-INDUCED APOPTOSIS IN MYA-1 CELLS.	130
FIGURE 3-8 THE EFFECT OF TIME ON CAMPTOTHECIN-INDUCED APOPTOSIS OF MYA-1 CELLS.	131
FIGURE 3-9 FLOW CYTOMETRY GRAPHS DEMONSTRATING THE EFFECT OF FIV INFECTION ON APOPTOSIS IN MYA-1 CELLS.	132
FIGURE 3-10 THE EFFECT OF CONTROL CONDITIONS ON MYA-1 CELL VIABILITY.	133
FIGURE 3-11 FLOW CYTOMETRY GRAPHS DEMONSTRATING THE EFFECT OF FIV INFECTION ON MYA-1 CELL APOPTOSIS AT DAY 10.	134
FIGURE 3-12 OVERLAY HISTOGRAM DEMONSTRATING FIV-INDUCED APOPTOSIS AT DAY 10.	134
FIGURE 3-13 COMPARISON OF MYA-1 CELL VIABILITY IN CULTURES INFECTED WITH DIFFERENT ISOLATES OF FIV.	136
FIGURE 3-14 COMPARISON OF THE PERCENTAGE OF MYA-1 CELLS UNDERGOING APOPTOSIS OVER TIME IN CULTURES INFECTED WITH THE RVC009 AND CVK001 ISOLATES OF FIV.	137
FIGURE 3-15 COMPARISON OF THE PERCENTAGE OF MYA-1 CELLS UNDERGOING NECROSIS AT DAY 10 IN CULTURES INFECTED WITH DIFFERENT ISOLATES OF FIV. THE RVC009 AND CVK001 ISOLATES OF FIV.	137
FIGURE 3-16 THE EFFECT OF FIV INFECTION ON MYA-1 CELL CONCENTRATION.	139
FIGURE 3-17 THE EFFECT OF FIV INFECTION ON MITOGEN-INDUCED LYMPHOCYTE PROLIFERATION.	141
FIGURE 4-1 FLOW CHART DEPICTING THE ALLOCATION OF CATS TO EXPERIMENTAL GROUPS.	159
FIGURE 4-2 OVERLAY HISTOGRAMS SHOWING THE EFFECT OF VACCINATION ON CD25 EXPRESSION.	168
FIGURE 4-3 THE EFFECT OF VACCINATION ON ACTIVATION OF FIV-SPECIFIC LYMPHOCYTES.	168
FIGURE 4-4 RESULTS FROM ANTI-CD25 ANTIBODY TITRATION.	170
FIGURE 4-5 RESULTS FROM ANTI-CD4 ANTIBODY TITRATION.	171
FIGURE 4-6 RESULTS FROM ANTI-CD8 ANTIBODY TITRATION.	172
FIGURE 4-7 FOUR-COLOUR FLOW CYTOMETRY ON CONA ACTIVATED PBMC.	173
FIGURE 4-8 THE EFFECT OF FIV ON CD25 EXPRESSION IN PBMC FROM UNVACCINATED CATS.	174

FIGURE 4-9 THE EFFECT OF VACCINATION ON CD25 EXPRESSION IN PBMC STIMULATED WITH FIV.....	175
FIGURE 4-10 THE EFFECT OF VACCINATION ON CD25 EXPRESSION IN T CELLS.....	175
FIGURE 4-11 THE EFFECT OF VIRUS ON CD25 EXPRESSION IN LYMPHOCYTE SUBSETS.....	177
FIGURE 4-12 THE EFFECT OF TIMING OF VACCINATION ON CD25 EXPRESSION IN LYMPHOCYTES.....	178
FIGURE 4-13 THE EFFECT OF VACCINATION ON THE VACCINE-INDUCED DTH RESPONSE IN A SINGLE CAT.	179
FIGURE 4-14 COMPARISON OF THE DTH ELICITED BY EACH FIV ISOLATE.....	180
FIGURE 4-15 THE DTH RESPONSE FOR EACH CAT AT DAY 3.....	181
FIGURE 5-1 FLOW CHART DESCRIBING THE PROTOCOL FOR SAMPLE TESTING.....	198
FIGURE 5-2 EFFECT OF SAMPLE DILUTION ON FIV QPCR RESULTS.	200
FIGURE 5-3 THE EFFECT OF HIGH SAMPLE DILUTION ON FIV QPCR RESULTS.	201
FIGURE 5-4 QUANTIFICATION OF FIV PROVIRUS IN BUCCAL SWAB SAMPLES.	202
FIGURE 5-5 GEOGRAPHICAL DISTRIBUTION OF SAMPLES COLLECTED FROM DIFFERENT REGIONS OF NZ.....	204
FIGURE 5-6 REPRESENTATIVE MELT CURVE ANALYSIS FROM THE SCREENING FIV QPCR ASSAY.	205
FIGURE 5-7 GEL ELECTROPHORESIS OF PCR PRODUCTS AMPLIFIED FROM SUSPECT FIV POSITIVE SAMPLES.	206
FIGURE 5-8 NUCLEOTIDE ALIGNMENT OF THE PCR PRODUCTS.....	207
FIGURE 5-9 REPRESENTATIVE MELT CURVE ANALYSIS FROM CONFIRMATORY FIV QPCR.....	208
FIGURE 5-10 REPRESENTATIVE MELT CURVE ANALYSIS FOR THE HOUSEKEEPING GENE QPCR ASSAY.....	209
FIGURE 5-11 FLOW CHART SHOWING THE RESULTS FOLLOWING TESTING OF ALL BUCCAL SWAB SAMPLES.....	210

List of tables

TABLE 1-1 SUMMARY OF VIRAL NOMENCLATURE.....	4
TABLE 1-2 CELL TROPISM OF FIV.	13
TABLE 1-3 SUMMARY OF THE DUAL-SUBTYPE FIV VACCINE EFFICACY STUDIES.	43
TABLE 2-1 PCR PRIMERS USED FOR AMPLIFICATION OF THE <i>ENV</i> GENE IN CONVENTIONAL PCR.....	64
TABLE 2-2 REAL-TIME PCR PRIMERS FOR AMPLIFICATION OF THE <i>GAG</i> GENE.....	66
TABLE 2-3 ADDITIONAL PRIMERS USED TO DETERMINE SENSITIVITY OF THE FIV RT-QPCR.	67
TABLE 2-4 CLINICAL CHARACTERISTICS OF CATS INFECTED WITH SELECTED NZ FIELD ISOLATES.	75
TABLE 2-5 PAIRWISE COMPARISON OF NZ FIV ISOLATES TO REFERENCE SEQUENCES.....	77
TABLE 2-6 PAIRWISE COMPARISON OF SELECTED NZ FIV ISOLATES.	77
TABLE 2-7 FINAL AMPLIFICATION CONDITIONS FOR THE OPTIMISED FIV RT-QPCR.	87
TABLE 2-8 REPRESENTATIVE RESULTS FROM ENDPOINT DILUTION ASSAY.....	100
TABLE 2-9 THE P24 CONCENTRATION OF EACH VIRUS STOCK SOLUTION.	101
TABLE 2-10 SUMMARY OF QUANTIFICATION RESULTS FOR EACH VIRUS USING 3 DIFFERENT METHODS.....	102
TABLE 3-1 MEDIA FORMULATIONS USED FOR OPTIMISATION OF PBMC CULTURE.	119
TABLE 4-1 AN EXAMPLE TO DEMONSTRATE THE GROUPING OF CELLS FROM UNVACCINATED CATS FOR STATISTICAL ANALYSIS.....	165
TABLE 4-2 THE METHOD USED TO POOL RESULTS FOR STATISTICAL ANALYSIS OF CD25 EXPRESSION.	166
TABLE 5-1 HOUSEKEEPING GENE PCR PRIMERS.	197
TABLE 5-2 SUMMARY OF RESULTS FROM ALL VACCINATED AND UNVACCINATED CATS.	211
TABLE 5-3 SUMMARY OF RESULTS FROM FIV-TESTED VACCINATED CATS AND UNVACCINATED CATS.....	211

List of abbreviations

2-ME	2-Mercaptoethanol
7AAD	7 amino actinomycin D
AB	Annexin binding buffer
AICD	Activation induced cell death
AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
CD	Cluster of differentiation
cDNA	Complementary DNA
CFGS	Chronic feline gingivostomatitis syndrome
ConA	Concanavalin A
CPM	Counts per minute
CPT	Cell preparation tube
Cq	Quantification cycle
CRD1	First cysteine rich domain of the CD134 molecule
CRD2	Second cysteine rich domain of the CD134 molecule
CRFK	Crandell-Reese Feline Kidney
CTLA-4	Cytotoxic T lymphocyte antigen 4 (CD152)
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
DTH	Delayed-type hypersensitivity
EDTA	Ethylenediaminetetraacetic acid
env	Envelope
FBS	Foetal bovine serum
FHV-1	Feline herpesvirus 1
FITC	Fluorescein isothiocyanate
FIV	Feline immunodeficiency virus
FMO	Fluorescence minus one
FSC	Forward scatter
gag	Group-specific antigen
GM	Growth medium
gp100	Surface unit of the FIV envelope glycoprotein
gp35	Transmembrane unit of the FIV envelope glycoprotein
HIV	Human immunodeficiency virus
IFN	Interferon
IL	Interleukin
MEM	Minimum essential medium
MFI	Mean fluorescence intensity (geometric)
MHC	Major histocompatibility complex
MOI	Multiplicity of infection
mRNA	Messenger RNA
MUAEC	Massey university animal ethics committee
MUFNU	Massey university feline nutrition unit
MUVTH	Massey university veterinary teaching hospital
NaCl	Sodium chloride

NEAA	Non-essential amino acids
NK	Natural killer
NZ	New Zealand
OD	Optical density
ORF	Open reading frame
p24	Capsid peptide of the FIV gag protein
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline (pH 7.2)
PCR	Polymerase chain reaction
PD-1	Programmed death receptor 1
PF	Preventable fraction
PI	Post inoculation
pol	Polymerase
PS	Phosphatidylserine
qPCR	Quantitative PCR
RNA	Ribonucleic acid
RPMI	Roswell park memorial institute (medium)
rRNA	Ribosomal RNA
RT	Reverse transcriptase
SI	Stimulation index
SSC	Side scatter
SU	Surface unit of the FIV envelope glycoprotein (gp100)
TCID ₅₀	Median tissue culture infectious dose
TH1/2	T helper 1/2 cells
TM	Transmembrane unit of the FIV envelope glycoprotein (gp35)
TNF	Tumour necrosis factor
UV	Ultraviolet
V1-V9	Hypervariable regions (1-9) of the FIV envelope gene
vif	Viral infectivity factor
VNA	Virus neutralising antibodies