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**Biological and Molecular
Characterisation and Crystallisation
of Infectious Bursal Disease Virus
and Its Major Capsid Protein**

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The bravest are surely those who have the
clearest purpose.....and go out to meet it.

Thucydides

Abstract

Infectious bursal disease virus (IBDV) is prevalent in most of the poultry producing areas worldwide and causes severe economic losses resulting not only from clinical disease and mortality, but also from the immunosuppressive effect in subclinically infected flocks. The research on IBDV in this thesis is divided into two main parts. In the first part, studies were carried out on the IBDV inadvertently introduced into New Zealand in 1993. Prior to that date, the country had been free from the virus. IBDV was successfully isolated from seven flocks of subclinically infected and/or seronegative chickens in SPF embryonating eggs, adapted to cell culture and identified by EM, immunocytochemistry and RT-PCR test. To evaluate the efficacy of the serological method used in the screening programme of the IBDV eradication scheme, a study was undertaken to compare three diagnostic methods. The study demonstrated that serological testing is not a reliable method for the detection of IBDV infection in New Zealand broiler flocks because antibodies may not have developed to detectable levels by the time of slaughter. Histological examination of affected bursae allowed the demonstration of IBD-like lesions, but these needed to be differentiated from those caused by other agents. The immunocytochemistry test was able to detect early IBDV infection and provided a rapid, definitive diagnosis.

Using the immunocytochemistry test to perform a longitudinal study of IBDV infection in a broiler and a layer farm, results showed the birds were infected as early as 6 to 7 days of age. The prevalence of IBDV infection was estimated to be 55% in the broiler flock. The results showed that the serological test had a sensitivity of 28.57% and a specificity of 73.68% for detecting the New Zealand IBDV strain infection. This indicated that there should be further evaluation of the use of serological testing as the sole method for the detection of IBDV infected farms in the current control scheme.

An *in-vivo* pathogenicity study using IBDV derived from a bursal tissue homogenate and carried out in SPF chickens demonstrated the low virulence of the virus present in

New Zealand. Molecular analysis of the hypervariable region of the VP2 gene of two IBDV isolates obtained in 1997 and 1998 showed they are more closely related to attenuated strains than other strains. In all three phylogenetic analyses, using neighbour joining, parsimony and split decomposition, the NZ isolates are closely related to attenuated strain PBG98 and Cu1 but split away from Australia 002-73, variant E, classical and very virulent strains. Both results support the hypothesis that an attenuated strain of IBDV was inadvertently introduced into the New Zealand poultry population in 1993.

In the second part of this thesis, studies on the structure of the IBDV virion and its major capsid protein were initiated by X-ray crystallography. The purification of IBDV for crystallisation and crystallisation trials are described. Several viral crystals were produced from the trials but only weak diffraction was obtained from these crystals.

With the aim of studying the structure of the major capsid protein of IBDV (VP2) and investigating the major antigenic site on this capsid protein, the *vp2* gene was cloned and expressed, the protein purified, and preliminary crystallisation trials performed. Recombinant VP2 was successfully expressed from a baculovirus expression system. The purification of the recombinant VP2 was also completed in the study and preliminary crystallisation screens determined several conditions favouring the production of crystals.

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Table of contents

Abstract	ii
Acknowledgements	iv
Table of contents	vi
List of figures	xii
List of tablesxv
Abbreviations	xvi
Abbreviations of amino acids	xix
Related publications	xx
CHAPTER 1: LITERATURE REVIEW	1
1.1 INTRODUCTION	1
1.2 VIRION PROPERTIES	3
1.2.1 Viral genome and viral proteins	3
1.2.2 Viral replication	7
1.3 HOST SUSCEPTIBILITY AND EPIZOOTIOLOGY	8
1.4 EPIDEMIOLOGY OF VERY VIRULENT AND VARIANT STRAINS OF IBDV..	10
1.5 PATHOGENESIS	11
1.5.1 Route of infection and transmission	11
1.5.2 Replication <i>in vivo</i> and organs affected	12
1.5.3 Clinical signs of IBD	13
1.5.4 Gross pathology of IBD	14
1.5.5 Histopathology of IBD	15
1.6 DIAGNOSIS OF IBDV INFECTION	16
1.7 PREVENTION AND CONTROL	19
1.8 IMMUNOSUPPRESSION AND INTERACTION WITH OTHER PATHOGENS	21
1.8.1 Immunosuppression studies of IBDV	21
1.8.2 Interaction of IBDV with other pathogens	23
1.9 ANTIGENIC AND GENETIC VARIATION OF IBDV	26
1.9.1 Various antigenic and genetic diversity studies of IBDV	27
1.9.2 IBDV classification and terminology	29
1.10 AIMS AND SCOPE OF THE THESIS	30
CHAPTER 2: ISOLATION OF IBDV AND DETECTION OF IBDV INFECTION IN CHICKENS IN NEW ZEALAND	32

2.1	INTRODUCTION	32
2.2	MATERIALS AND METHODS	33
2.2.1	Collection of samples	33
2.2.2	Blood samples	34
2.2.3	Tissue organs	34
2.2.4	Serology	34
2.2.5	Macroscopic and histologic examination	36
2.2.6	Virus isolation	36
2.2.7	Electron microscopy examination	40
2.2.8	Immunocytochemistry staining of fixed infected cell	40
2.2.9	Polymerase chain reaction	41
2.3	RESULTS	44
2.3.1	Farm history and collection of samples	44
2.3.2	Serological diagnostic	44
2.3.3	Pathology	44
2.3.4	Virus isolation	46
2.3.5	Electron microscopy examination	46
2.3.6	Immunocytochemistry staining of fixed infected cell	47
2.3.7	RT-PCR detection of isolates	48
2.3.8	Sequencing of cDNA and sequence comparison	48
2.4	DISCUSSION	49
2.4.1	IBDV isolation and detection	49
2.4.2	Detection of infection with other viruses	51
2.4.3	Significance of subclinical IBDV infection	52
2.5	SUMMARY	53

CHAPTER 3: EVALUATION OF IMMUNOCYTOCHEMISTRY, SEROLOGY AND HISTOLOGICAL DIAGNOSTIC METHODS FOR THE DETECTION OF IBDV INFECTION IN BROILER FLOCKS IN NEW ZEALAND **54**

3.1	INTRODUCTION	54
3.2	MATERIALS AND METHODS	56
3.2.1	Sample collection	56
3.2.2	Serology test	57
3.2.3	Histopathology	57
3.2.4	Immunocytochemical staining on formalin-fixed tissues	58
3.3	RESULTS	59
3.3.1	Serology	59
3.3.2	Histopathology	59
3.3.3	Immunocytochemistry staining	60

3.4 DISCUSSION	63
3.4.1 Retrospective study	63
3.4.2 Field investigation	64
3.4.3 Comparison of the three diagnostic methods	65
3.5 SUMMARY	65
CHAPTER 4: LONGITUDINAL CASE STUDY OF A BROILER AND A LAYER FARM INFECTED WITH IBDV	67
4.1 INTRODUCTION	67
4.2 MATERIALS AND METHODS	68
4.2.1 Study design	68
4.2.2 Farm recording	68
4.2.3 Macroscopic and histologic examination	69
4.2.4 Serology test	69
4.2.5 Immunocytochemistry staining on formalin-fixed tissues	70
4.2.6 Disease prevalence and comparison of diagnostic test	70
4.3 RESULTS	70
4.3.1 Clinical and necropsy finding in broiler and layer flock	70
4.3.2 Serological test	71
4.3.3 Histopathology	73
4.3.4 Immunocytochemistry	73
4.4 DISCUSSION	75
4.4.1 Broiler flock	75
4.4.2 Layer flock	76
4.4.3 Disease prevalence and evaluation of diagnostic tests	78
4.5 SUMMARY	79
CHAPTER 5: CHARACTERISATION OF NEW ZEALAND ISOLATES OF IBDV	80
5.1 INTRODUCTION	80
5.1.1 Molecular variation in antigenicity and pathogenicity	80
5.1.2 <i>In vivo</i> pathogenicity study in SPF birds	83
5.2 MATERIALS AND METHODS	85
5.2.1 Genetic characterisation of IBDV	85
5.2.2 <i>In-vivo</i> pathogenicity study	86
5.3 RESULTS	89
5.3.1 Genetic characterisation of New Zealand's IBDV isolates	89
5.3.2 <i>In-vivo</i> pathogenicity study	96
5.4 DISCUSSION	97

5.4.1	<i>In-vivo</i> pathogenicity study	97
5.4.2	Genetic characterisation of New Zealand's IBDV isolates	99
5.5	SUMMARY	102
PART II: LITERATURE REVIEW OF VIRUS AND CAPSID PROTEIN		
STRUCTURAL STUDY		103
II.1	INTRODUCTION	103
II.2	VIRUS STRUCTURAL STUDY	104
II.2.1	Electron microscope	104
II.2.2	Cryo-electron microscopy	105
II.2.3	Atomic force microscopy	105
II.2.4	X-ray crystallography	105
II.3	VIRUS QUATERNARY STRUCTURE AND SYMMETRY.....	109
II.4	CAPSID PROTEIN TERTIARY STRUCTURE	111
II.5	VIRAL CAPSID FUNCTIONS	112
II.5.1	Protection of nucleic acid genome	113
II.5.2	Protein-nucleic acid interactions	113
II.5.3	Receptor recognition	113
II.5.4	Virus-antibody interaction	115
II.5.5	Other functions of viral capsid protein	115
II.6	SUBUNIT CAPSID PROTEIN STUDY	116
II.7	THE AIMs AND SCOPE OF PART II	117
CHAPTER 6: IBDV PURIFICATION AND CRYSTALLIZATION		118
6.1	INTRODUCTION	118
6.2	MATERIALS AND METHODS	119
6.2.1	Virus sample	119
6.2.2	Virus purification	120
6.2.3	Assessment of virus concentration and purity	122
6.2.4	Polyacrylamide gel electrophoresis	122
6.2.5	Electroblotting of viral capsid protein from acrylamide gels	123
6.2.6	Detection of viral capsid protein by protein blotting and immunodetection	124
6.2.7	N-terminal sequencing of viral capsid protein	125
6.2.8	Electrospray ionisation mass spectrometry (ES-MS)	125
6.2.9	Detection of deglycosylated substrate with DIG glycan/protein double labelling kit	126
6.2.10	Virus crystallization trials	126
6.2.11	Crystal diffraction trials	127

6.3 RESULTS AND DISCUSSION	132
6.3.1 Virus purification and SDS-PAGE analysis	132
6.3.2 Purified virus concentration and EM examination	133
6.3.3 Detection of viral capsid protein by blotting and immunodetection	134
6.3.4 N-terminal sequencing and ES-MS of viral capsid protein	135
6.3.5 Detection of deglycosylated substrate with DIG glycan/protein double labelling kit	136
6.3.6 Virus crystallization an diffraction trials	137
6.4 CONCLUSION	139

CHAPTER 7: IBDV CAPSID PROTEIN EXPRESSION, PURIFICATION AND CRYSTALLIZATION

140

7.1 INTRODUCTION	140
7.2 MATERIALS AND METHODS	142
7.2.1 Insect cell culture (Sf9-cells) procedure	142
7.2.2 RT-PCR amplification of VP2 gene	144
7.2.3 Cloning of vp2 gene into pFASTBAC donor plasmid	145
7.2.4 Transposition of recombinant donor plasmid into DH10BAC cells	149
7.2.5 Extraction and screening of recombinant bacmid	150
7.2.6 Transfection of Sf9 cells with recombinant bacmid DNA	152
7.2.7 Harvest and titration of recombinant baculovirus	152
7.2.8 Determination of optimal MOI and incubation time for recombinant VP2 expression	154
7.2.9 Amplification of recombinant baculovirus working stock	155
7.2.10 Expression and purification of recombinant VP2 protein using Immobilised Metal Affinity Chromatography (IMAC)	157
7.2.11 Purification of recombinant VP2 protein by chromatographic methods..	158
7.2.12 Size exclusion chromatograph (SEC) with superdex-75	159
7.2.13 Protein quantification	161
7.2.14 Biochemical analyses of the recombinant VP2 fusion protein	161
7.2.15 Immunodiffusion test of rVP2	161
7.2.16 Circular dichroism spectroscopy	162
7.2.17 Multiangle laser light scattering (MALLS) photospectrometry	162
7.2.18 Cleavage of fusion protein with rTEV protease	163
7.2.19 Removal of rTEV protease and cleaved poly-His tag	164
7.2.20 Recombinant VP2 protein crystallisation trials	164
7.2.21 Crystal diffraction trials	165
7.3 RESULTS	166
7.3.1 RT-PCR amplification of vp2 gene	166

7.3.2	Cloning of <i>vp2</i> gene into pFASTBAC donor plasmid	166
7.3.3	Transposition of the pFBHTaVP2 into DH10BAC competent cells	167
7.3.4	Transfection of Sf9 cells with recombinant bacmid DNA, harvest and titration of recombinant baculovirus	170
7.3.5	Determination of optimal MOI and incubation time for recombinant VP2 expression	170
7.3.6	Amplification of recombinant baculovirus working stock	171
7.3.7	Purification of rVP2 protein using Immobilised Metal Affinity chromatography (IMAC)	172
7.3.8	Anion exchange chromatography (IEX) – UNO-Q column	173
7.3.9	Size exclusion chromatography (SEC) with superdex-75 (HR10/30)	174
7.3.10	Cleavage of fusion protein with rTEV protease and removal of rTEV protease	175
7.3.11	Analysis of expressed recombinant VP2 protein	176
7.3.12	Multiangle laser light scattering photospectrometry	181
7.3.13	Overall purification of rVP2 protein	182
7.3.14	Recombinant VP2 protein crystallisation trial	183
7.3.15	Crystal diffraction trials	184
7.4	DISCUSSION	184
7.4.1	Cloning and expression of rVP2 protein	184
7.4.2	Purification of rVP2 protein	187
7.4.3	Analysis of expressed rVP2 protein	188
7.4.4	Preliminary crystallisation and diffraction trial of rVP2	191
7.5	SUMMARY	192
CHAPTER 8: GENERAL DISCUSSION		193
APPENDICES		199
BIBLIOGRAPHY		208

List of figures

Figure 1.1: Schematic of the genome organisation of infectious bursal disease virus...	6
Figure 2.1: (A) Atrophied bursa from a bird in flock B (right); (B) Bursa from a bird in flock D, several haemorrhages are evident on the bursal mucosa.....	45
Figure 2.2: (A) Section of bursa from a bird in flock B showing loss of lymphocytes in the medulla of the follicles, H&E, x 40; (B) Section of bursa from a bird in flock D. There is follicular fibrosis and total depletion of lymphocytes from the bursal follicles, H&E, x 10	45
Figure 2.3: Negatively stained IBDV particles	46
Figure 2.4: Negatively stained reovirus-like particle	47
Figure 2.5: Immunocytochemistry staining of acetone-fixed Vero cell culture. (A) IBDV Antigen in infected cells stain red-brown; (B) Negative control cells.....	47
Figure 2.6: Amplification products of RT-PCR performed on Vero-cell culture supernatants and lysates.....	48
Figure 3.1: Section of bursa from bird A/11 which had a histological score of 2 showing depletion of lymphocytes in the medulla of bursal follicles, with prominent reticular endothelium between the cortex and medulla, H&E, Bar = 100µm..	61
Figure 3.2: Section of bursa from bird B/28 which had a histological score of 3, showing extensive necrosis of lymphocytes and marked pyknotic cellular debris within the medulla of a follicle and hyperplastic reticuloendothelial cells. H&E. Bar = 50 µm.....	61
Figure 3.3: IBDV antigen in lymphoid cells in the bursal follicles shown in Figure 3.1 with an immunoperoxidase score of 2. Avidin-biotin peroxidase method, DAB substrate, Mayer's heamatoxylin counterstain. Bar = 100 µm	61
Figure 3.4: Higher magnification of bursal follicle from Figure 3.3. showing IBDV antigen in lymphocytes in the cortex and medulla. Avidin-biotin peroxidase method, DAB substrate, Mayer's heamatoxylin counterstain. Bar = 50 µm	61
Figure 4.1: Box-plots showing the bursal (upper)and spleen (lower) weight of the broiler (left)and layer (right) flocks over the observation period. The rectangular box represented 50% distribution of the data within the indicated age group and 25% deviation on each side of the box.....	72
Figure 4.2: Box-plot showing IBDV ELISA titre of the broiler and layer flocks (the blue line indicated the cut-off titre value of 396)	72
Figure 5.1: Deduced amino acid sequences of VP2, from amino acid position 181-390 (numbering from the sequence of segment A of serotype 2, strain OH of IBDV) (Nagarajan and Kibenge, 1995)	84
Figure 5.2: Nucleotide sequences alignment of the hypervariable region (position 662-1157) of the VP2 gene.....	92
Figure 5.3: Deduced amino acid sequences alignment of the VP2 variable region (from position 190 to 353)	93
Figure 5.4: Splitgraph showing the phylogenetic relationship between isolates from the deduced amino acid sequences	94

Figure 5.5: Neighbour Joining tree showing the phylogenetic relationship of the NZ IBDV isolates with overseas IBDV strains	95
Figure 5.6: Branch and Bound parsimony tree showing the phylogenetic relationship of the 8 taxa	95
Figure II.1: Diagram of a regular icosahedron showing the 2-fold, 3-fold and 5-fold rotational symmetry axes	110
Figure II.2: Example of the virus capsid protein in T unit	111
Figure II.3: The jellyroll-fold consisting of 8 β -strands (B through I) forming 2 antiparallel sheets	112
Figure 6.1: SDS-PAGE analysis of the (A) Ammonium sulfate (A/S) precipitation and (B) PEG 6000 precipitaion of IBDV	132
Figure 6.2: SDS-PAGE analysis of the direct centrifugation method	133
Figure 6.3: EM examination of the purified IBDV from direct ultracentrifugation method . (A) Low magnification of 15, 300 x, bar = 500 nm; (B) Higher magnification of the virus at 31, 800 x, bar = 500 nm	134
Figure 6.4: (A) SDS-PAGE analysis of purified IBDV. (B) Western immunodetection of IBDV capsid proteins	135
Figure 6.5: The glycan/protein detection using DIG glycan/protein double labelling kit. A blue-green precipitation indicates glycans present and a brown precipitation denotes protein detected	136
Figure 6.6: IBDV crystals grown using the conditions described in section 6.3.6	138
Figure 7.1: Map of pFASTBAC HTa donor plasmid showing the <i>Ehe I</i> restriction site for cloning and the primer set used for the amplification of the <i>vp2</i> insert gene in RT-PCR	147
Figure 7.2: Schematic diagram showing predicted PCR products using various primer pairs on the sucessfully and correctly ligated plasmid pFBHTaVP2	149
Figure 7.3: Schematic diagram showing predicted PCR products using the pUC/M13 forward (F1) and pUC/M13 reverse (R1) directed on either side of the mini-attTn7 of the bacmid in the verification of transposition.....	151
Figure 7.4: Overview of generation of recombinant baculovirus and rVP2 expression with BAC-TO-BAC Expression System	156
Figure 7.5: A summary of the purification scheme of recombinant VP2 protein	160
Figure 7.6: RT-PCR amplification of the VP2 insert gene	166
Figure 7.7: Results from colony screening of the donor plasmid pFASTBAC HTa for correct insertion of <i>vp2</i> gene	167
Figure 7.8: Amplification products from PCR amplification of bacmid DNA using pUC/M13 primers	168
Figure 7.9: Amplification products of the PCR reaction carried out using recombinant bacmid from colonies 2, 4, 14, 15 and 31 as template, (A)with pUC/M13 primers, expected product size 3786 bp; (B) with pUC/M13 forward and <i>vp2</i> insert gene reverse primers, expected product size 3006 bp	169
Figure 7.10: Agarose gel electrophoresis of mini-prep bacmid DNA	170
Figure 7.11: A plaque titration well showing clear plaques in the overlaid gel against the russet red background.....	170

Figure 7.12: Western immunodetection of rVP2 from cultures of different MOI and incubation times using rabbit anti-IBDV polyclonal antibody.....	171
Figure 7.13: SDS-PAGE analysis of fractions from Ni ²⁺ charged IMAC column purification	172
Figure 7.14: Chromatogram of the elution from UnoQ column of the rVP2 protein	173
Figure 7.15: SDS-PAGE analysis of fractions from UnoQ column purification	173
Figure 7.16: Chromatogram of elution from Superdex 75 HR10/30. The major peak eluted in the void volume was analysed by SDS-PAGE in lane 1	174
Figure 7.17: Cleavage of rVP2 protein with rTEV protease, incubated at 25°C for 24, 48, 72 and 120 hours as indicated in each lane	175
Figure 7.18: SDS-PAGE and western immunoblotting of recombinant VP2 protein and native IBDV proteins	176
Figure 7.19: Immunodiffusion test of rVP2 against mouse monoclonal antibody R63 ...	177
Figure 7.20: Results of the ES-Mass spectrometry of the purified rVP2 protein	178
Figure 7.21: Result of the circular dichroism spectroscopy	178
Figure 7.22: Concentration of rVP2 protein resulted in aggregation of the protein	179
Figure 7.23: Characterisation of the self-assembly of rVP2 by negative-staining EM. (A) EM showing the spherical forms of the rVP2, approximately in 12 to 13 nm diameter. (B) EM showing of the association of the rVP2 into tubular structures of various diameter	180
Figure 7.24: SEC-DRI elution profile (—) of the predominant elution peak of the rVP2 proteins. The elution profile is overlaid with the calculated molar mass (◆)	181
Figure 7.25: SDS-PAGE of purified and concentrated rVP2, (A) after concentration; (B) after concentration and stored at 4 °C >24 hours	182
Figure 7.26: Crystals grown in condition of 12% PEG 20,000, 0.1 M MES, pH 6.5 at 4°C	183

List of tables

Table 2.1: Summary of results in serological, virus isolation, EM and RT-PCR Tests.....	48
Table 3.1: Flock details, age, histological lesion score, immunoperoxidase staining score and serological results of chickens in the study	62
Table 4.1: Summary of B/BW, S/BW ratio, ELISA, IP and Histological scoring of broiler flock	74
Table 4.2: Summary of B/BW, S/BW ratio, ELISA, IP and histological scoring of layer flock	74
Table 5.1: Serological results, bursa and spleen to body weight ratios, and histological lesion scores of IBDV-inoculated and uninoculated groups of birds	97
Table II.1: Summary of viruses that have been studied by X-ray crystallography	108
Table 6.1: IBDV crystallisation screen # 1	128
Table 6.2: IBDV crystallisation screen # 2	129
Table 6.3: IBDV crystallisation screen # 3	130
Table 6.4: IBDV crystallisation screen # 4	131
Table 7.1: Summary of the purification of recombinant VP2 protein from 500mL of culture.....	183

Abbreviations

aa	amino acid
A	adenine
AGDP	agar gel immunodiffusion precipitaion
ATP	adenosine-5'-triphosphate
ATV	Antibiotic / trypsin / versene
bp	base pair
BLAST	basic local alignment search tool
BSA	bovine serum albumin
C	cytosine
CAA	<i>Chicken anaemia virus</i>
CAM	chorio-allantoic membrane
CEF	chicken embryo fibroblast
cDNA	complimentary DNA
CPE	cytopathic effect
CsCl	cesium chloride
dNTP	deoxynucleoside-5'-triphosphate
dH ₂ O	distilled water
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
ds	double-strand
DTT	dithiothreitol
EDTA	ethylenediamine tetra-acetic acid
EID ₅₀	mean embryo infective dose 50%
ELISA	enzyme linked immunosorbent assay
EM	electron microscopy
ES-MS	electrospray mass spectrometry
FBS	feotal bovine serum
FMDV	<i>Foot-and-Mouth Disease virus</i>
G	guanine
GM	growth medium
H & E	haematoxylin and eosin
HEPES	N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)
HPLC	high-performance liquid chromatography
IBDV	<i>Infectious bursal disease virus</i>
IBV	<i>Infectious bronchitis virus</i>

IF	immunofluorescence
IgG	immunoglobulin G
IgM	immunoglobulin M
IMAC	immobilised metal ion affinity chromatography
IP	immunoperoxidase
IPNV	<i>Infectious pancreatic necrosis virus</i>
IPTG	isopropylthio- β -D-galactoside
kbp / kb	kilobase pair
kDa	kilo daltons
LB	luria broth
MAb / MCA	monoclonal antibody
MEM + n	minimal essential medium + non-essential amino acids
MM	maintenance medium
MOI	multiplicity of infection
MW	molecular weight
MWCO	molecular weight cut-off
NZ	New Zealand
ORF	open reading frame(s)
PAGE	polyacrylamide gel electrophoresis
PAUP	phylogenetic analysis using parsimony
PBS	phosphate buffered saline, pH 7.0
PCR	polymerase chain reaction
PEG	polyethylene glycol
p.i.	post-infection
PMSF	phenylmethylsulfonyl fluoride
PSK	penicillin / streptomycin / kanamycin
PVDF	polyvinylidene difluoride
RE	restriction endonuclease
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcriptase-PCR
SDS	sodium dodecyl sulphate
SFM	serum-free medium
SNT	serum neutralisation test
SPF	specific pathogen free
T	thymine

TAE	tris / acetate / EDTA
TBE	tris / borate / EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	tris (hydroxymethyl)-aminomethane
UV	ultraviolet
Vero	African green monkey cells
VN	virus neutralisation
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactoside

Abbreviations for Amino Acids

Amino Acid	Three Letter Symbol	One Letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Asparagine or Aspartic Acid	Asx	B
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glutamine or Glutamic Acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Related Publications

The results presented in chapters three and five have been published in peer-reviewed journals.

Y. F. Chai, J. Meers and N. H. Christensen (1999) Evaluation of serological, histological and immunocytochemical methods for the detection of infectious bursal disease virus infection in broiler flocks in New Zealand. *New Zealand Veterinary Journal*, 47:175-179.

Y. F. Chai, N. H. Christensen, C. R. Wilks and J. Meers (2001) Characterisation of New Zealand isolates of infectious bursal disease virus. *Archives of Virology*, 146:1-10.