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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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	*Chicken anaemia virus*
CAM	chorio-allantoic membrane
CEF	chicken embryo fibroblast
cDNA	complimentary DNA
CPE	cytopathic effect
CsCl	cesium chloride
dNTP	deoxynucleoside-5'-triphosphate
dH_2O	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	*Foot-and-Mouth Disease virus*
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	*Infectious bursal disease virus*
IBV	*Infectious bronchitis virus*

IF	immunofluorescence
IgG	immunoglobulin G
IgM	immunoglobulin M
IMAC	immobillised metal ion affinity chromatography
IP	immunoperoxidase
IPNV	*Infectious pancreatic necrosis virus*
IPTG	isopropylthio-ß-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 / actate / 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.