PATHOGENICITY AND MOLECULAR CHARACTERISATION OF THE VP2 GENE OF INFECTIOUS BURSAL DISEASE VIRUS

MD. MAHFUZUL HOQUE

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PATHOGENICITY AND MOLECULAR CHARACTERISATION OF THE VP2 GENE OF INFECTIOUS BURSAL DISEASE VIRUS

By

MD. MAHFUZUL HOQUE

Thesis Submitted in Fulfilment of Requirement for the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine
Universiti Putra Malaysia
June 2001
DEDICATION

TO MY PARENTS (LATE MD. ABDUS SATTAR AND BEGUM MAHFUZA), UNCLE (MR. JUSTICE M. A. ROUF), WIFE (RAHIMA KHANAM) AND SONS (RIZWANUL HOQUE AND ENAMUL HOQUE)
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PATHOGENICITY AND MOLECULAR CHARACTERISATION OF THE VP2 GENE OF INFECTIOUS BURSAL DISEASE VIRUS

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June 2001

Chairman: Abdul Rahman Omar, Ph D.
Faculty: Veterinary Medicine

Pathogenicity of four infectious bursal disease virus (IBDV) isolates was studied on specific-pathogen-free (SPF) chickens. Chickens inoculated with isolates 92/04, 94/B551 and 97/61 developed severe clinical manifestations with a high mortality ranging from 70-80%, whereas the 94/273 isolate caused 10% mortality. However, regardless of the isolates, significant differences (p< 0.05) were noted in the bursal scoring lesions and bursa to body weight ratio index in the infected groups in comparison to the control groups. The isolate 94/273 had limited and comparatively less haemorrhagic lesions in the bursal tissues. However, the presence of severe haemorrhagic lesions in the bursal tissues along with the non-bursal tissues (muscles, thymus, spleen and at the junction of proventriculus and gizzard) were found only in the 92/04, 97/61 and 94/B551 isolates.
The VP2 gene (1351 bp) of the isolates (92/04, 94/273 and 94/B551) was amplified and cloned and the sequences were compared with other IBDV strains. All the isolates have the unique amino acid residues at positions P222A, V256I, and L294I as found in other vvlBDV strains. Restriction fragment length polymorphism (RFLP) and sequence analysis of the VP2 hypervariable region also indicated that all the isolates can be classified as vvlBDV based on the presence of SspI and TaqI sites at the nucleotide positions 1011 and 833, respectively. All the isolates except 94/273 also have a StyI site at nucleotide position 888. The absence of StyI site in this isolate is associated with amino acid substitution at 254 from G to S in variant strain. The 94/273 also has an amino acid substitution at 270 from A to E as found in apathogenic IBDV. Thus, this is a first report on the isolation of vvlBDV with some genotypic characteristic of variant and apathogenic IBDV strains. The 94/B551 also has one amino acid substitution at position 300 E to S, which is uncommon among other vvlBDV isolates. Based on the RFLP analysis the Malaysian (92/04, 94/273 and 97/61) and Bangladeshi (94/B551) isolates can be differentiated using the restriction enzymes PstI, Mbol and TaqI. The deduced VP2 amino acids encoded by 92/04 is identical to the vvlBDV strains from Israel, Japan and UK, whereas the other isolates (94/273 and 94/B551) have one to three amino acid substitutions, indicating that the vvlBDV is evolving. However, the phylogenetic analysis suggested that the isolates are very close to each other and all of them may have derived from same origin as the vvlBDV strains isolated from China, Japan and Europe.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Doktor Falsafah

KEPATOGENAN DAN PENCIRIAN MOLEKUL GEN VP2 VIRUS PENYAKIT BURSA BERJANGKIT

Oleh

MD. MAHFUZUL HOQUE

Jun 2001

Pengerusi: Abdul Rahman Omar, Ph D.

Fakulti: Perubatan Veterinar

Kepatogenan empat strain virus penyakit bursa berjangkit (IBDV) ke atas ayam bebas patogen khusus (SPF) telah dikaji. Ayam yang diinokulat dengan strain 92/04, 94/273 dan 97/61 menunjukkan manifestasi klinikal yang teruk dengan kadar kematian tinggi diantara 70-80%, manakala isolate 94/273 menyebabkan kematian 10%. Walau bagamanapun, tanpa mengambil kira strain, terdapat perbezaan yang ketara (p<0.05) dalam skor lesi bursa dan indek nisbah bursa kepada berat badan bagi kumpulan terjangkit berbanding dengan kumpulan kawalan. Strain 94/273 mempunyai lesi hemoraj yang kurang dan terhad dalam tisu bursa. Walau bagamanapun, kehadiran lesi hemoraj yang teruk pada tisu bursa dan tisu bukan bursa (otot, timus, limpa dan pada persimpangan proventrikulus dan humpedal) dijumpai hanya dalam strain 92/04, 97/61 dan 94/B551. Gen VP2 (1351 bp) bagi strain (92/04, 94/273 dan 94/B551) diamplifikasi dan diklonkan dan jujukan tersebut dibandingkan dengan strain IBDV yang lain. Kesemua strain mempunyai residu asid amino pada kedudukan P222A,
ACKNOWLEDGEMENTS

I would like to extend my heartiest gratitude and appreciation to Dr. Abdul Rahman Omar, chairman of the supervisory committee for providing invaluable advice, untiring assistance, encouragement and motivation that enabled me to accomplish the PhD programme smoothly and efficiently.

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render assistance throughout the course of the study. Special thanks also goes to Mr. Fauzi Che Yusof and Mr. Ho Ooi Kaun for their assistance to develop the photograph in this study. I would also like to thank and appreciate Mr. Lee Weng Way for helping me to use various software programmes. I would also like to extend my thanks to all staff members of the Faculty of Veterinary Medicine and Graduate School Office for helping me in one way or another, toward the completion of my study.

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I certify that an Examination Committee met on 18th June 2001 to conduct the final examination of Md. Mahfuzul Hoque on his Doctor of Philosophy thesis entitled “Pathogenicity and Molecular Characterisation of the VP2 Gene of Infectious Bursal Disease Virus” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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I hereby declare that the thesis is based on my original work except for quotations and citations, which have duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

MD. MAHFUZUL HOQUE

Date: 25 June 2001
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<tr>
<td>AAF</td>
<td>Allantoamnionic fluid</td>
</tr>
<tr>
<td>AC</td>
<td>Antigen capture</td>
</tr>
<tr>
<td>AGDP</td>
<td>Agar gel diffusion precipitin</td>
</tr>
<tr>
<td>B</td>
<td>Bursa</td>
</tr>
<tr>
<td>B/B</td>
<td>Bursa/body weight</td>
</tr>
<tr>
<td>BGM</td>
<td>Baby grivet monkey kidney</td>
</tr>
<tr>
<td>BHK</td>
<td>Baby Hamster kidney</td>
</tr>
<tr>
<td>BLRI</td>
<td>Bangladesh Livestock Research Institute</td>
</tr>
<tr>
<td>bp</td>
<td>Basepair</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CAM</td>
<td>Chorioallantoic membrane</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CEB</td>
<td>Chick embryo bursa</td>
</tr>
<tr>
<td>CEF</td>
<td>Chicken embryo fibroblast</td>
</tr>
<tr>
<td>CEK</td>
<td>Chicken embryo kidney</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetres</td>
</tr>
<tr>
<td>CMGF</td>
<td>Chicken myelomonocytic growth factor</td>
</tr>
<tr>
<td>CsCl</td>
<td>Caesium chloride</td>
</tr>
<tr>
<td>CEP</td>
<td>Cytopathic effect</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>d-</td>
<td>Deoxy</td>
</tr>
<tr>
<td>DAS-ELISA</td>
<td>Double antibody sandwich</td>
</tr>
<tr>
<td>DI</td>
<td>Defective interfering</td>
</tr>
<tr>
<td>dd</td>
<td>Dideoxy</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ds</td>
<td>Double stranded</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
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<tr>
<td><em>E. coli</em></td>
<td>Escherichia coli</td>
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<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>EID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Embryo infective dose fifty</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>FMDV</td>
<td>Foot and mouth disease virus</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>hv</td>
<td>Hypervariable</td>
</tr>
<tr>
<td>IBD</td>
<td>Infectious bursal disease</td>
</tr>
<tr>
<td>IBDV</td>
<td>Infectious bursal disease virus</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobase</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
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<tr>
<td>KS</td>
<td>Karplus-Schulz</td>
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kV  kilovolt
LB  Luria-Bertani
LS  Least Square
M  Molar
Mab  Monoclonal antibody
MD  Maryland
ME  Minimum evolution
Mg  Magnesium
MK  Monkey kidney
ML  Maximum likelihood
MP  Maximum Parsimony
ml  Millilitre
SPF  Specific pathogen free
MVP  Malaysian Vaccine Pharmaceutical
mM  Millimolar
μm  Micrometre
μg  Microgramme
NaCl  Sodium chloride
NCR  Non coding region
ng  Nanogramme
NJ  Neighbour-joining
nm  Nanometre
NO2  Nitrogen dioxide
OK  Ovine kidney
ORF  Open reading frame
OsO4  Osmium tetra-oxide
P2  Passage two
P3  Passage three
PBS  Phosphate buffered saline
PBL  Peripheral blood lymphocytes
PCR  Polymerase chain reaction
PHA  Phytohemagglutinin
PHYLIP  Phylogenetic interference package
p.i  Post inoculation
QGDPT  Quantitative gel diffusion precipitin test
RE  Restriction endonuclease
RFLP  Restriction fragment length polymorphism
RK  Rabbit kidney
RT  Reverse-transcriptase
RNA  Ribonucleic acid
RdRp  RNA dependent -RNA polymerase
rpm  Revolution per minute
S  Spleen
S/B  Spleen/Bursa
SD  Standard deviation
SDS  Sodium dodecyl sulphate
SN  Serum neutralisation test

xxi
SPSS  Statistical package for social science
SS   Single stranded
STC  Standard Challenge strain
TAE  Tris-acetate-EDTA
TBE  Tris-borate-EDTA
TCVN Tissue culture virus neutralisation
TE   Tris-EDTA
TEM  Transmission electron microscopy
TEMED N,N,N',N',-tetramethylenediamine
Tm   Melting Temperature
Tris 2-amino-2-(hydroxymethyl)-1, 3 propandiol
U    Uracyl
UPGMA Unweighted pair group with arithmetic mean
UPM  Universiti Putra Malaysia
VRI  Veterinary Research Institute
vw   Very virulent
VSA  Vesicular stomatitis virus
(w/v) Weight/volume
X-gal 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
>   Greater than
~   Approximately

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CHAPTER I

INTRODUCTION

Infectious bursal disease virus (IBDV) is the aetiological agent of infectious bursal disease (IBD) or Gumboro disease that causes significant losses to the poultry industries either by causing high mortality in an acute disease or as a consequence of immunosuppression in young chickens (3-6 weeks old) (Lukert and Saif, 1991; Van Den Verg, 2000). Infection by IBDV causes destruction of lymphoid organs, especially B-lymphocytes in the bursa of Fabricius (Hirai et al., 1974; Saif, 1991). Two distinct serotypes of IBDV, designated serotype 1 and 2 have been identified (Jackwood and Saif, 1987). The serotype 1 strains are pathogenic to chickens and vary in their virulence, whereas serotype 2 strains, isolated from turkeys, are apathogenic for both turkeys and chickens (Ismail et al., 1988; Jackwood et al., 1982 and McFerran et al., 1980). Serotype 1 can be divided on the basis of virulence and antigenic variation into classical virulent strain, very virulent (vv) strain and antigenic variant strain (Brown et al., 1994; Lasher and Shane, 1994; Snyder, 1990 and Zierenberg et al., 2000).

Infectious bursal disease is considered to be among the most costly infectious diseases affecting commercial poultry producers (Kibenge et al., 1988c; Lasher and Shane, 1994 and Shane et al., 1994). Economic losses
from IBD arise from direct mortality, a reduction in the performance of clinically ill birds, cost of control measures and increased carcass downgrades and condemnation due to gangrenous dermatitis, colisepticemia and air-sacculitis (Lasher and Shane, 1994; Lukert and Saif, 1997). Virus-induced immunosuppression adds to these costs, in the form of vaccination failures and increased incidence or severity of bacterial, viral, and parasitic diseases (Anderson et al., 1977; Lasher and Shane, 1994).

IBDVs are non-enveloped, icosahedral particles with a genome consisting of two segments (A and B) of double-stranded (ds) RNA that are packed inside a single-shelled capsid of about 60 nm in diameter. Nucleotide sequence analysis shows that segment A (~3.3 kb) has a long open reading frame (ORF) of 3036 bp in length and a short ORF of 435 bp (VP5), which overlaps with the 5' end of the long ORF (Kibenge et al., 1990). The long ORF encodes the VP2-VP4-VP3 polyprotein (110-kDa) which is cleaved by auto-proteolysis into individual viral proteins (VP2 and VP3) (Azad et al., 1985; Hudson et al., 1986). The shorter ORF has been shown to encode a small cystine-rich 17-kDa protein (Mundt et al., 1995). Of the three viral proteins, VP2 and VP3 are the major viral structural proteins, whereas VP4 is a minor protein involved in the processing of the precursor polyprotein (Dobos et al., 1979; Jagadish et al., 1988). The VP2 protein is exposed on the surface of the virion and contains strain-specific epitopes. In vitro expression of VP2 and its utilisation as an antigen revealed that VP2 is the