UNIVERSITI PUTRA MALAYSIA

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

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FPV 2001 14

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By

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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

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**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 vvIBDV strains. Restriction fragment length polymorphism (RFLP) and sequence analysis of the VP2 hypervariable region also indicated that all the isolates can be classified as vvIBDV based on the presence of *Ssp*I and *Taq*I sites at the nucleotide positions 1011 and 833, respectively. All the isolates except 94/273 also have a *Sty*I site at nucleotide position 888. The absence of *Sty*I 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 vvIBDV 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 vvIBDV 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 *Pst*I, *Mbo*I and *Taq*I. The deduced VP2 amino acids encoded by 92/04 is identical to the vvIBDV 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 vvIBDV 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 vvIBDV 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**

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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 hempedal) dijumpai hanya dalam strain 92/04, 97/61 dan 94/B551. Gen VP2 (1351 bp) bagi strain (92/04, 94/273 dan 94/B551) diamplifasi dan diklonkan dan jujukan tersebut dibandingkan dengan strain IBDV yang lain. Kesemua strain mempunyai residu asid amino pada kedudukkan P222A,

V256I dan L294I sebagaimana yang dijumpai dalam strain vvIBDV lain. Analisa fragmen pembatasan polimorfoma (RFLP) dan jujukan bagi kawasan hiper boleh ubah VP2 juga menunjukkan bahawa kesemua strain boleh dikelaskan sebagai vvIBDV berdasarkan kehadiran tapak *Ssp*I dan *Taq*I pada kedudukan nukleotid 1011 dan 833. Kesemua strain kecuali 94/273 mempunyai tapak *Sty*I pada kedudukan nukleotid 888. Ketiadaan tapak *Sty*I dalam strain ini dikaitkan dengan penggantian asid amino pada 254 daripada G kepada S dalam strain varian. Strain 94/273 juga mempunyai penggantian asid amino pada 270 daripada A kepada E sebagaimana yang dijumpai dalam IBDV bukan patogen. Oleh itu, ini merupakan laporan pertama ke atas pemencilan vvIBDV dengan sebahagian ciri genotip bagi strain IBDV varian dan bukan patogen. Strain 94/B551 juga mempunyai satu penggantian asid amino pada kedudukkan 300 E kepada S, yang mana jarang didapati di kalangan strain vvIBDV lain. Berdasarkan analisa RFLP, strain Malaysia (92/04, 94/273 dan 97/61) dan strain Bangladesh (94/B551) boleh dibezakan dengan menggunakan enzim pembatas *Pst*I, *Mbo*I dan *Taq*I. Asid amino VP2 yang dikodkan oleh 92/4 adalah sama dengan strain vvIBDV dari Israel, Jepun dan UK, manakala strain-strain lain (94/273 dan 94/B551) mempunyai satu hingga tiga penggantian asid amino, menunjukkan bahawa vvIBDV terlibat sedang berubah. Walan bagaimanapun analisa filogenesis mencadangkan bahawa strain-strain tersebut adalah sangat hampir antara satu sama lain dan kesemua strain mungkin datang dari asal yang sama seperti strain vvIBDV yang dipencilkan dari China, Jepun dan Eropah.

## 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.

My sincere thanks and appreciation are for Professor Dr. Aini Ideris, a member of the supervisory committee for her constructive suggestion, proper guidance and encouragement throughout my study period and her social support during my study period. I would like to extend my sincere gratitude and appreciation to Associate Professor Dr. Mohd. Hair Bejo, who always accepted me with a smiling face and provided valuable suggestion, assistance and brilliant comments to accomplish my PhD project.

I am also grateful to Professor Dato' Dr. Sheikh Omar Abdul Rahman, the Dean of the Faculty and Professor Dr. Mohamed Shariff Mohamed Din, the Deputy Dean for Research and Postgraduate Studies for allowing me to use the facilities in the Faculty and being helpful whenever I ran into difficulties. I shall never forget the kindness of Professor Dr. Mohamed Shariff Mohamed Din who came forward to save my experimental chicks in a stormy night by restoring electricity at the chicken house.

I am also grateful to the staff members of the Biologics Laboratory, Puan Rodiah Husin and Mr. Adam Ahmad for always being so willing to

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 thanks 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.

My heartfelt appreciation also goes to my mother (Begum Mahfuza), brothers (Abu Raihan, Lutfor Rahman and Mustafizur Rahman), sisters (Nazma, Farida and Lipy), uncle (Mr. Justice M.A. Rouf) and other relatives and friends, who always encouraged and supported me during my study period. My special thanks and appreciation are for my wife and sons for their patience, spontaneous support and understanding during the period of my study. My special thanks goes to my friends Dr. Kamaruddin, Mr. Ajhar, Mr. Ziqrul, Mr. Ershad, Mr. Jahangir, Dr. Asad, Dr. Urrme and Mr Mollah for their cooperation during my study period. Special thanks to Dr. Kazi M.Emdadul Huque, Director General of BLRI as well as colleagues and friends of the same institute for their cooperation and moral support.

I would like to acknowledge the funding authority for bearing all the expenses of my study through Agricultural Research Management Project (ARMP), BLRI part of the IDA credit 2815-BD.

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| | |
|---|---|
| AAF | Allantoamnionic fluid |
| AC | Antigen capture |
| AGDP | Agar gel diffusion precipitin |
| B | Bursa |
| B/B | Bursa/body weight |
| BGM | Baby grivet monkey kidney |
| BHK | Baby Hamster kidney |
| BLRI | Bangladesh Livestock Research Institute |
| bp | Basepair |
| C | Cytosine |
| Ca | Calcium |
| CAM | Chorioallantoic membrane |
| cDNA | Complementary deoxyribonucleic acid |
| CEB | Chick embryo bursa |
| CEF | Chicken embryo fibroblast |
| CEK | Chicken embryo kidney |
| cm | Centimetres |
| CMGF | Chicken myelomonocytic growth factor |
| CsCl | Caesium chloride |
| CEP | Cytopathic effect |
| ºC | Degree Celsius |
| d- | Deoxy |
| DAS-ELISA | Double antibody sandwich |
| DI | Defective interfering |
| dd | Dideoxy |
| DNA | Deoxyribonucleic acid |
| ds | Double stranded |
| DTT | Dithiothreitol |
| *E. coli* | *Escherichia coli* |
| EDTA | Ethylene diamine tetra acetic acid |
| $EID_{50}$ | Embryo infective dose fifty |
| ELISA | Enzyme-linked immunosorbent assay |
| Fig | Figure |
| FMDV | Foot and mouth disease virus |
| HCl | Hydrochloric acid |
| HIV | Human immunodeficiency virus |
| hv | Hypervariable |
| IBD | Infectious bursal disease |
| IBDV | Infectious bursal disease virus |
| IFN | Interferon |
| kb | kilobase |
| kDa | kilodalton |
| KS | Karplus-Schulz |

| | |
|---|---|
| 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 | Phytohemmagglutinin |
| 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 |

| | |
|---|---|
| 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', -tetramethyllenediamine |
| 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 |
| vv | Very virulent |
| VSA | Vesicular stomatitis virus |
| (w/v) | Weight/volume |
| X-gal | 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside |
| > | Greater than |
| ~ | Approximately |

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

# 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