UNIVERSITI PUTRA MALAYSIA

PATHOGENICITY AND IMMUNOGENICITY OF EGG-ADAPTED VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS ISOLATED IN MALAYSIA

THUZAR THAN @ HAFIZA HJ. HASHIM

FPV 2004 15

PATHOGENICITY AND IMMUNOGENICITY OF EGG-ADAPTED VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS ISOLATED IN MALAYSIA

By

THUZAR THAN @ HAFIZA HJ. HASHIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

September 2004

DEDICATION

To My Beloved Mum and Dad

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 IMMUNOGENICITY OF EGG-ADAPTED VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS ISOLATED IN MALAYSIA

THUZAR THAN @ HAFIZA HJ. HASHIM

September 2004

Chairman: **Professor Aini Ideris, Ph.D.**

Faculty: **Veterinary Medicine**

The emergence of very virulent infectious bursal disease virus (vvIBDV) strains caused a devastating economic loss to the chicken industry in Malaysia in 1990. The recurrence of infectious bursal disease (IBD) outbreaks and vaccine failure occurred recently. It is very important to study the pathogenicity, immunogenicity and molecular characteristics of these vvIBDV isolates which are prerequisite before developing the IBD vaccine and testing it in the farms.

In this study, UPM 93273 (passage 1 to 48 in chorio-allantoic membrane) was characterized by focusing on the hypervariable region of the VP2 protein. The UPM 93273 isolate was selected among seven other isolates obtained from field outbreaks in

layer, broiler and indigenous (village) chickens in various areas in West Malaysia during 1993 to 1994. The sequence of the hv region was obtained following total RNA extraction from the wild type UPM 93273 and its different derivative passages. The hv region was amplified by RT-PCR assay and cloned into pCR-TOPO cloning vector. The right clone harboring the hv fragment was selected by *EcoR*I restriction enzyme digestion. Finally the cloned fragment was sequenced. The nucleotide and the deduced amino acid sequence alignment and analysis was carried out by using the Bio-Edit software. As a result, markers for very virulent IBDV strain were found in UPM 93273 isolate (wild type to passage 48).

The UPM 93273 was found to be very immunogenic but also highly pathogenic. The pathogenicity is reduced during the serial passages in embryonated SPF chickens at passage 31. The passage 31 (P31) of UPM 93273 isolate provided protection against the challenge virus with some degree of bursal atrophy.

An effective vaccine depends not only on the full attenuation and genetic stability of the virus strain but also on the route of vaccination. This latter was investigated to determine the immune response of the lymphoid organs against IBDV UPM 94283 strain. Ultrastructural, histopathological and immunohistochemistry studies showed that immunocompetent cells were present in the Harderian gland and were involved in the early and strong immune response against IBDV UPM strain administered via ocular route. Thus, this route can stimulate the lymphoid cells in the Harderian gland, and

support the proliferation of lymphoid cells in the bursa before the virus reaches the primary target organs. In addition to this rapid immunogenic reaction, some viruses may drain into the oral cavity when administered via ocular route. As a result, these viruses can stimulate the intestinal lymphoid aggregates along the intestinal tract.

As a conclusion, all of the serially passaged UPM 93273 isolate up to passage 48 was found to be still unstable and not sufficiently attenuated indicated by virulence markers which are still conserved. The decrease in the virulence and the pathogenicity seen in the serially passaged strain might be due to other unknown factors. Consequently the risk of reversion to the very virulent state is very high. The current numbers of passages are not sufficient and further passages are necessary in order for the virus to reach a fully attenuated and genetically stable state.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Ph.D

KEPATOGENAN DAN KEIMUNOGENAN VIRUS PENYAKIT BURSAL ISOLAT MALAYSIA BERBAHAYA YANG DI LALU-SIRIKAN

Oleh

ThuZar Than @ Hafiza Hj. Hashim

September 2004

Pengerusi: Profesor Aini Ideris, Ph.D.
Fakulti: Perubatan Veterinar

Kemunculan strain virus berbahaya dan mudah berjangkit penyebab penyakit Bursal (vvIBDV) telah menyebabkan kejatuhan ekonomi di dalam industri penternakan ayam di Malaysia pada 1990. Kemunculan kembali wabak penyakit berjangkit Bursal (IBD) dan ketidakberkesanan vaksin telah berlaku sejak akhir-akhir ini. Oleh itu, adalah penting kajian mengenai kepatogenen, keimunogenan dan cirri-ciri molekul pencilan vvIBDV ini. Ia juga prasyarat sebelum membangunkan vaksin IBD dan mengujinya di ladang.

Dalam kajian ini, UPM 93273 (laluan 1 hingga 48 di membran krio-alantoik) yang telah dicirikan dengan memfokuskan pada kawasan pembolehubah-hiper protin VP2. Pencilan UPM 93273 telah dipilih di antara 7 pencilan lain yang diperolehi daripada kawasan

wabak pada ayam ‘penelur’, ‘pedaging’ dan kampung di seluruh kawasan di Malaysia Barat pada 1993-1994. Jujukan kawasan hv didapati daripada pengekstrakan jumlah RNA daripada jenis liar UPM 93273 dan daripada laluan yang lain. Kawasan hv diamplikasikan oleh esei RT-PCR dan diklonkan kedalam vector pengklonan PCR-TOPO. Klon yang betul den mengandungi pecahan hv telah dipilih melalui penghadaman enzim terhad Eco RI. Akhirnya pecahan yang telah diklonkan itu, telah dijujukan. Analisa dan persamaan jujukan telah dijalankan ke atas nukleotida dan asid amino menggunakan perisian BIO edit. Keputusannya, penunjuk bagi stren vvIBDV telah dijujukan di pencilan UPM 93273 (jenis liar kepada laluan 48).

UPM 93273 telah ditemui sebagai amat imunogenik dan juga sangat patogenik. Kepatogenan telah dikurangkan semasa laluan jujukan ke atas ayam SPF yang telah diembriokan pada laluan 31. Pencilan laulaun 31 (P31) UPM 93273 telah memberi perlindungan terhadap virus tersebut dengan darjah atropi bursal tertentu.

Keberkesanan suatu vaksin bukan sahaja bergantung kepada attenuasi penuh dan kestabilan genetic stren virus itu tetapi juga pada aliran vaksinasi. Ia kemudiannya diselidiki untuk menentukan rangsangan imun organ limfoid menentang IBDV UPM 94283. Kajian ‘ultrastruktur’, hispatological dan kimiaimunohisto telah menunjukkan sel saingan imuno hadir di dalam kelenjar Hardain dan terlibat pada awalan dan rangsangan imuno ke atas stren IBDV UPM yang dimasukkan melalui laluan ocular. Oleh itu laluan ini boleh merangsang sel limfoid didalam kelenjar hardain dan menggalakan pertumbuhan sel limfoid di dalam bursa sebelum virus menceroboh organ tuju primer.

Selain daripada tindakbalas pantas imunogenik, sebahagian virus mungkin masuk kedalam kawasan mulut melalui laluan okular. Keputusanya, virus ini boleh merangsang agregasi limfoid usus di sepanjang talian usus.

Kesimpulannya, kesemua pencilan UPM 93273 yang dilalu-sirikan sehingga ke laluan 48 didapati masih tidak stabil dan kurang diatenuasikan ditunjukkan oleh penunjuk kebahayaan yang masih dilindungi. Penurunan tahap kebahayaan dan patogenisiti mungkin disebabkan oleh faktor yang tidak dikeahui. Sehubungan itu, risiko pembalikan kepada tahap bahayatinggi adalah besar. Bilangan laluan yang sedia ada tidak mencukupi dan laluan setrusnya adalah perlu untuk virus ini mencapai atenuasi penuh dan dalam keadaaan genetik yang stabil.

ACKNOWLEDGEMENTS

I am obliged to express my most immense gratitude to my chairperson, Professor Dr. Aini Ideris for her corrective guidance, constant moral encouragement, inexhaustible reconstruction of my streusels and giving me time out of her hectic schedules. Without her kind help, it won't be reached to finish-up stage. Again, her kind help is like reincarnation of my student life which will ever be remembered.

Sincere thanks are also conveyed to my supervisory committee member, Prof. Dr. Abdul Rani Bahaman for his advice, helpful discussion and comments.

Special thanks deserve to Assoc. Professor Dr. Noordin M. Mustapha to his thorough review, restorative reconstruction of the pathology respects of my thesis, teaching me his ways of analysis, invaluable comments and inch by inch guidance. With his kind and patient help, my Ph.D. thesis became materialized to this stage.

I owed special thanks to Assoc. Prof. Dr. Abdul Rahman Omar for his ever willing help, guidance, constructive and invaluable advises which kept me moving right way.

I would like to acknowledge my memorable thanks to Tan Sri Prof. (retired) Dr. Syed Jalalludin, for his kind encouragement and help to salvage my study life during the crisis.

Deep appreciation and gratitude deserve to Dato' Prof. Sheik Omar Abdul Rahman for his invaluable help whenever in urgency, moral support and encouragement. All of his help will never be forgotten.

My special thanks also go to chairperson of the examination committee, Prof. Dr. Mohd. Zamri Saad, Dean of the Faculty of Veterinary Medicine, the internal examiners

Asso. Prof. Dr. Jasni Sabri and Asso. Prof. Dr. Siti Suri Arshad for their detail examination reports, thorough corrections and comments.

My gratitude also owes to Prof. Dr. Jimmy Kwong, National University of Singapore for his valuable comments and suggestions for my thesis in 2-week-examination period.

Indeed, my deep appreciation and thanks to my husband, Ghrici Mohamed for his immeasurable help throughout my Ph.D. study especially on Molecular Virology. For his patience, sacrifices, encouragement and support are exceptional.

I would like to express my thanks to all teachers who have taught me since my primary school till now. My thanks also go to ex-supervisor Assoc. Prof. Dr. Mohd. Hair Bejo for his help in the early part of my study and providing the IBDV isolate.

My last but not least thanks to my beloved parents U Than and Daw Khin Yee Soe, my brothers: Dr. Ko Ko Gyi, Dr. Than Oo and Dr. Nay Aung Kyaw; my sisters: Thida Than, Theingi Than and Min Min Than and my cousin Zaw Zaw for their patience, love, support and understanding me during this lengthy period of study.

My sincere appreciation goes to my husband and my two little kids, Leena and Lounas for their love, patience and understanding. Special thanks to Thi Thi and Win; their helps are unforgettable.

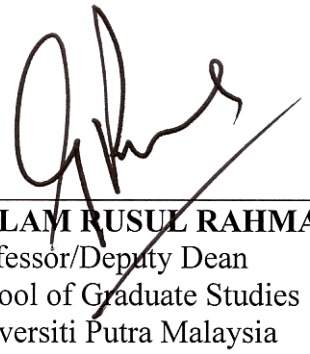
I certify that an Examination Committee met on 8th September 2004 to conduct the final examination of ThuZar Than @ Hafiza Hj. Hashim on her Doctor of Philosophy thesis entitled "Pathogenicity and Immunogenicity of Egg-Adapted Very Virulent Infectious Bursal Disease Virus Isolated in Malaysia" in accordance 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:

Mohd. Zamri Saad, Ph.D.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia (Chairman)

Jasni Sabri, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia (Member)

Siti Suri Arshad, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia (Member)

Jimmy Kwang, Ph.D.
Professor
Animal Biotechnology Health
Temasek Life Sciences Laboratory
National University of Singapore
(Independent Examiner)



GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 20 DEC 2004

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Aini Ideris , Ph.D.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairperson)

Abdul Rani Bahaman, Ph.D.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Noordin Mustapha, Ph.D.
Associate Profesor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Abdul Rahman Omar, Ph.D.
Associate Profesor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS , Ph.D.
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 November 2004

DECLARATION

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

_____

THUZAR THAN @ HAFIZA HJ HASHIM

Date: 28 Nov 2004

TABLE OF CONTENTS

LISTS OF TABLES

LISTS OF FIGURES

LISTS OF PLATES