



UNIVERSITI PUTRA MALAYSIA

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

THUZAR THAN @ HAFIZA HJ. HASHIM

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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

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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 *EcoRI* 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**

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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 ciri-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 immuno hadir di dalam kelenjar Hardain dan terlibat pada awalan dan rangsangan immuno 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 mencero boh 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 bahaya yang masih dilindungi. Penurunan tahap bahaya dan patogenisiti mungkin disebabkan oleh faktor yang tidak dikehui. Sehubungan itu, risiko pembalikan kepada tahap bahayatinggi adalah besar. Bilangan laluan yang sedia ada tidak mencukupi dan laluan seterusnya adalah perlu untuk virus ini mencapai atenuasi penuh dan dalam keadaan genetik yang stabil.



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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:

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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



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