MOLECULAR CHARACTERISATION OF INFECTIOUS BURSAL DISEASE VIRUS ISOLATED IN IRAN FOR DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY BASED ON RECOMBINANT VPX PROTEIN

BY

SEYED DAVOOD HOSSEINI

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

December 2005

DEDICATED with love and gratitude

to:

My dearest Father, Mother, Wife, Sisters , two lovely Daughters (Mohadeseh and Fatemeh) and son (Mahdi).

Who have always given me strength and courage with their co-operation,

patience and prayer to carry-out this research.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

MOLECULAR CHARACTERISATION OF INFECTIOUS BURSAL DISEASE VIRUS ISOLATED IN IRAN FOR DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY BASED ON RECOMBINANT VPX PROTEIN

By

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December 2005

Chairman: Professor Aini Ideris, PhD

Faculty : Veterinary Medicine

The acute form of infectious bursal disease (IBD) is considered as an economically significant disease among the poultry diseases reported in Iran. It was first reported as being caused by very virulent IBD virus (IBDV) based on conventional methods. Infectious bursal disease outbreaks are still being reported frequently, in spite of vaccination and sanitation measures which are the routine practices for the control of IBD in the country. Since there was no report available on the molecular characteristics of IBDV based on segment A in Iran, which is necessary for the establishment of proper control measures, it was important to characterise the antigenic and virulent properties of the strains prevalent in Iran.

Infected bursa of Fabricius were collected from chicken obtained from an unvaccinated farm in Iran. The chickens showed clinical signs of depression, anorexia, ruffled feathers, trembling, whitish or watery diarrhea and mortality.

Virus isolation was carried out in embryonated eggs and the isolated virus showed 96% mortality in 4 weeks-old specific pathogen free (SPF) chickens, which was typical of very virulent IBDV. The complete nucleotide sequences of segment A of the isolate, which code for the viral proteins (VPs), VP2, VP4, VP3, and VP5, was amplified by reverse transcriptase-polymerase chain reaction method, sequenced and compared with some published IBDV sequences. A total of 9 common amino acid substitutions, 3 at VP2, (222 Ala, 256 Ile and 294 Ile), 3 at VP4 (685 Asn/Ser, 715 Ser and 751 Asp), 2 at VP3 (990 Val and 1005 Ala) and 1 at VP5 (49 Arg) were found in the isolate as well as in other very virulent (vv) IBDV isolates. However, the Iranian isolate also demonstrated 8 unique amino acid substitutions of which 2 each were in VP2 and VP4, respectively, 3 in VP3 and 1 in VP5. Phylogenetic analysis indicated that the Iran isolate was closely related to vvIBDV isolates from Asian countries, however, it likely shares a common origin as other vv strains isolated from other parts of the world.

The characterised IBDV isolate (designated as SDH1) was subjected to expression in prokaryotic system. The VP2 and VPX genes were expressed in *Escherichia coli* system as a fusion protein with six-histidine tag. Protein bands with the expected molecular weight of 48KD and 51KD were detected by direct protein staining and Western blotting. Since most of the neutralizing epitopes are located on VP2 and VPX, the expressed VPX protein was considered as a suitable candidate antigen for the development of a serological test. However, instead of using whole virion as an antigen, this study focused on the use of recombinant VPX as the antigen for the

development of ELISA for the detection of IBDV specific antibody. The results showed that sera obtained from vaccinated broiler chickens reacted specifically using the developed ELISA, suggesting that the recombinant VPX protein is properly folded and expressed the neutralizing epitopes. In addition, when the developed ELISA technique was compared to a commercial ELISA kit from IDEXX, USA, which uses whole virus preparations as test antigen, it showed an excellent correlation value of R^2=0.972.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

Pencirian Molekul Virus Penyakit Bursa Berjangkit yang Diasingkan di Iran untuk Pembangunan Asai Imunoerap Berkaitan Enzim Berdasarkan Protein Rekombinan VPX

Oleh

Seyed Davood Hosseini

December 2005

Pengerusi: Profesor Aini Ideris, PhD

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Penyakit bursa berjangkit (IBD) akut dianggap antara penyakit ayam yang menjejaskan ekonomi pernah dilaporkan di Iran. Ianya pertama kali dilaporkan akibat jangkitan virus IBD (IBDV) sangat virulen berdasarkan kaedah konvensional. Walaupun pemvaksinan dan langkah kebersihan adalah amalan rutin untuk mengawal IBD dalam negara, wabak IBD kerap dilaporkan. Dengan ketiadaan laporan yang berlandaskan pencirian molekul segmen A IBDV di Iran, yang mana diperlukan bagi membangun langkah pergawalan, adalah sangat penting untuk mencirikan ciri antigenik dan virulen strain yang wujud di Iran.

Bursa Fabricius yang dijangkiti dikumpul dari ayam yang diperolehi dari ladang di Iran yang tidak mempraktikkan suntikan vaksin. Ayam tersebut menunjukkan tanda klinikal seperti tekanan, anoreksia, pelepah bulu kusut, menggigil, cirit birit cair atau berwarna putih, dan kematian. Pemencilan virus

dijalankan dalam telur berembrio dan virus yang diasingkan menunjukkan kadar kematian 96% dalam ayam bebas patogen spesifik (SPF) yang berumur 4 minggu, di mana ianya khusus untuk IBDV sangat virulen. Jujukan nukleotida lengkap segmen A yang mengkodkan protein virus (VPs), VP2, VP4, VP3 dan VP5 isolat tersebut diperbanyakkan melalui kaedah transkriptase membalik-tindak balas rantai polimerase, dijujuk dan dibandingkan dengan beberapa jujukan IBDV yang pernah dilaporkan. Sejumlah 9 asid amino yang biasa digantikan, 3 di VP2 (222 Ala, 256 Ile dan 294 IIe), 3 di VP4 (685 Asn/Ser, 715 Ser dan 751 Asp), 2 di VP3 (990 Val dan 1005 Ala) dan 1 at VP5 (49 Arg) dijumpai pada isolat tersebut dan juga isolat IBDV sangat virulen (vvIBDV) yang lain. Walau bagaimanapun, isolat Iran juga menunjukkan 8 penggantian asid amino yang unik di mana 2 di VP2 dan VP4, masing-masing, 3 di VP3 dan 1 di VP5. Analisis filogenetik menunjukkan isolat Iran berhubung rapat dengan isolat vvIBDV dari negara Asia, namun ia berkongsi sumber yang umum sepertimana strain vv yang pernah diasingkan dari bahagain lain di dunia.

Isolat IBDV yang dicirikan (dinamakan sebagai SDH1) digunakan bagi tujuan ekspresi dalam sistem prokariot. Gen VP2 dan VPX diekspres di sistem *Escherichia coli* sebagai protein gabungan dengan *tag six-histidine*. Jalur protein dengan berat molekul yang terjangka di antara 48kDa dan 51kDA dapat dkesan dengan pewarnaan protein terus dan sap Western. Oleh kerana kebanyakan epitop peneutralan terdapat pada VP2 dan VPX, protein VPX yang terekspres dianggap sebagai calon antigen yang sesuai untuk pembangunan satu ujian serologi. Walau bagaimanapun, kajian ini memberi

tumpuan terhadap penggunaan rekombinan VPX dan bukan mengenai penggunaan virion penuh sebagai antigen bagi membangunkan ELISA untuk mengesan antibodi khusus IBDV. Keputusan menunjukkan sera yang diambil daripada ayam pedaging yang disuntik vaksin bertindak secara khusus dengan ELISA yang dibangunkan, mencadangkan protein VPX rekombinan tersebut terbentuk dengan betul dan mengekspres epitop peneutralan. Tambahan pula, bila teknik ELISA yang dibangunkan tersebut dibandingkan dengan satu kit komersial ELISA daripada IDEXX, USA, yang menggunakan seluruh virus sebagai antigen ujian, ia menunjukkan korelasi yang cemerlang dengan nilai R^2=0.972.

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I certify that an Examination Committee met on 24th December 2005 to conduct the final examination of Seyed Davood Hosseini on his Doctor of Philosophy thesis entitled "Molecular Characterisation of Infectious Bursal Disease Virus Isolated in Iran for Development of an Enzyme-Linked Immunosorbent Assay Based on Recombinant VPX Protein " 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 follow:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements 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.

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SEYED DAVOOD HOSSEINI

Date :

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