UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERISATION OF INFECTIOUS BURSAL DISEASE VIRUS AND EXPRESSION OF VP2 PROTEIN FOR THE DEVELOPMENT OF DIAGNOSTIC KIT AND RECOMBINANT VACCINE

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By

NURULFIZA BINTI MAT ISA

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

September 2008

UPM

DEDICATED WITH LOVE AND GRATITUDE
TO:

MY HUSBAND MOHD AZRIRUDIN MOHMAD RAZALI, MUMMY (HAZEZAH YEOP ISMAIL), UNCLE (BAHARI YEOP ISMAIL), MOTHER IN LAW (AZIZAH ABDUL AZIZ) AND SON (MUHAMMAD AQIL MUHAIMIN)

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

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Chairman: **Professor Dr. Mohd Hair Bin Bejo, Ph.D.**

Faculty: **Veterinary Medicine**

Outbreak of infectious bursal disease (IBD) in chickens due to highly pathogenic strain of IBD virus (vvIBDV) was first reported in Europe in late 1980's and in Malaysia in 1991. The disease caused significant economic losses, estimated more than RM72 million per year in Malaysia alone due to high mortality and immunosuppression. Treatment of IBD is of no value and the disease can only be controlled and prevented by proper vaccination programme and biosecurity. It was the objectives of the study to determine the molecular characteristic of Malaysian field isolates of IBDV and expression of the VP2 gene of the isolate for the development of diagnostic kit and recombinant vaccine. Three IBDV isolates identified as UPM04178, UPM04190 and UPM04238 were characterised. Based on their pathogenicity and sequence characteristic, the highest similarity (98%) concerning both nucleotide and amino acid sequences, the IBDV isolates were characterized as vvIBDV strains. Evolutionary relatedness of the isolates to vvIBDV strains was demonstrated by three phylogenetic methods: bootstrap values of 100%, 95%

and 90% for nucleotide sequences and those of 58%, 86% and 96% for amino acid sequences were obtained by the distance, maximum parsimony and maximum likehood methods, respectively. Phylogenetic analysis revealed clustering of the isolates with vvIBDV strains of serotype 1, which originate from a common ancestor of IBDV strains present in Malaysia.

Using informative characteristics of the isolate, both diagnostic kit and recombinant vaccine were successfully developed using a new wild-type field vvIBDV strain of UPM04190 isolate. A safe and effective recombinant IBD vaccine was developed base on the construction of recombinant VP2 gene of the isolate cloned into an *Escherichia coli* expression system. The VP2 gene was inserted into pRSET B vector as a fusion protein with histidine tag, which can be easily purified. The recombinant VP2 protein bands were expressed to their expected sizes of ~50 kDa from cell lysate. The pRSET vectors are pUC-derived expression vectors and expression of the gene of interest from pRSET is controlled by the strong phage T7 promoter that drives expression of gene 10 (Φ10) which provides protein stability and help to maintain the original structure of the protein. High-level production (3 mg/ml) of soluble product of VP2 recombinant protein was achieved with modified techniques of expression conditions and approaches. Efficacy test demonstrated that the recombinant vaccine of various fractions could provide protection ranging from 75% to 100% in highly susceptible chickens (specific pathogen free chickens) when challenged with vvIBDV (B00/81) at $10^{4.25}$ EID_{50}/ml per chicken following vaccination. One-step-immunostrip kit which is highly specific and sensitive was developed using whole virus as capture antigen and high-affinity polyclonal

IBD antibodies coated with gold particles. Rapid detection of IBD antibody can be achieved as fast as two minutes in a clinical or field environment. The kit is highly sensitive as it can detect as low as 250 ELISA units compared to commercial ELISA kit that only goes to 391 ELISA units for positive samples. The specificity of the kit was evaluated against antibody of other chicken viruses. No signal of reactivity or cross react exists among the antibodies tested. Thus, it was highly specific to IBDV. It was concluded that the local IBDV isolates were proven to be vvIBDV strain, the constructed recombinant vaccine provide a safe and effective protection and, the developed one-step-immunostrip kit is rapid, specific, sensitive, safe and economic in detection of IBDV infection and monitoring immune status of chicken against IBD.

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

PENCIRIAN SECARA MOLEKUL BAGI VIRUS PENYAKIT BERJANGKIT BURSAL DAN PENGEKSPRESAN PROTEIN VP2 UNTUK PEMBANGUNAN KIT DIAGNOSIS DAN VAKSIN REKOMBINAN

Oleh

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September 2008

Pengerusi: **Profesor Dr. Mohd Hair Bin Bejo, Ph.D.**

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Wabak penyakit bursa berjangkit (IBD) pada ayam disebabkan oleh strain virus IBD (IBDV) yang sangat patogenik buat pertama kali dilaporkan di Eropah lewat 1980-an dan di Malaysia pada 1991. Penyakit ini menyebabkan kerugian ekonomi secara signifikan, jangkaan kerugian disebabkan kematian dan depresi yang tinggi di Malaysia sahaja melebihi RM72 juta setahun. Rawatan terhadap IBD adalah sia-sia dan penyakit tersebut hanya boleh dikawal dan dicegah melalui program vaksinasi yang betul dan keselamatan secara biologi. Oleh itu, matlamat pengajian kini adalah untuk mengenalpasti ciri-ciri isolat IBDV daripada lading ayam di Malaysia dan pengekspresan protin VP2 bagi isolat tersebut untuk digunakan dalam pembangunan kit analisa dan vaksin rekombinan. Tiga isolate virus yang dikenalpasti sebagai UPM04178, UPM04190 dan UPM04238 telah dicirikan. Berdasarkan kepatogenan dan ciri-ciri jujukan, kesamaan tertinggi (98%) yang memberi tumpuan kepada kedua-dua jujukan nukleotida dan asid-asid amino adalah dicirikan sebagai strain sangat virulen IBDV (vvIBDV). Hubungan secara evolusi bagi

ketiga-tiga isolat terhadap strain vvIBDV ditunjukkan oleh tiga kaedah filogenetik iaitu nilai bootsrap 100%, 95% dan 90% bagi jujukan nukleotida dan; 58%, 86% dan 96% bagi jujukan asid amino yang didapati daripada kaedah "distance", "maximum parsimony" dan "maximum likelihood" setiap satunya. Analisis filogenetik menyimpulkan bahawa ketiga-tiga isolat adalah dikelaskan kepada strain vvIBDV serotaip 1, dimana ianya berasal daripada keturunan strain IBDV yang biasa didapati di Malaysia.

Berbekalkan ciri-ciri berguna bagi isolat tersebut, kedua-dua kit analisa dan vaksin rekombinan telah berjaya dibangunkan menggunakan strain virulen isolat UPM04190 IBDV liar. Vaksin rekombinan IBD yang selamat dan efektif telah dibangunkan berasaskan pembinaan gen VP2 rekombinan bagi isolat tersebut yang diklonkan ke dalam system pengekspresan *Escherichia coli*. Gen VP2 bagi isolat ini dimasukkan ke dalam vektor pRSET B sebagai protein fusion bersama-sama tag histidin, di mana ianya mudah dibersihkan. Pengekspresan jalur protein VP2 rekombinan adalah pada saiz yang dijangkakan iaitu ~50 kDa daripada lysate sel. Vektor-vektor pRSET adalah merupakan vektor pengekspresan hasilan-pUC dan pengekspresan gen yang diminati daripada vektor pRSET dikawal oleh promoter T7 faj yang kuat yang mengaturkan pengekspresan gen 10 (Φ10) dimana ianya membekalkan kestabilan protein dan membantu mengekalkan struktur asal protein. Penghasilan produk terlarut yang tinggi (3 mg/ml) bagi protein VP2 rekombinan dicapai melalui teknik-teknik pembaharuan bagi kepelbagaian keadaan pengekspresan dan juga permintaan. Ujian keberkesanan menunjukkan bahawa vaksin rekombinan daripada pelbagai pecahan mampu memberi perlindungan pada had 75% sehingga 100% pada ayam yang sangat berpotensi (ayam bebas pathogen tertentu)

apabila dicabar dengan $10^{4.25}$ EID_{50}/ml vvIBDV (B00/81) setiap ayam sejurus vaksinasi. Kit "one-step-immunostrip" yang sangat spesifik dan sensitif telah dibangunkan menggunakan keseluruhan virus sebagai antigen penangkapan dan poliklonal antibodi IBD berkeafinitian tinggi yang dilapisi dengan partikel emas. Pengenalpastian yang pantas bagi antibodi IBD boleh dicapai sepantas dua minit dalam persembahan klinikal atau ladang. Kit tersebut sangat sensitif hingga mampu mengesan serendah 250 unit ELISA berbanding kit ELISA komersial yang hanya mampu mengesan 391 unit ELISA bagi sampel positif. Ketepatan sistem jalur ujian berasas pepejal dinilai bersandarkan virus-virus ayam. Antibodi virus ayam tersebut yang duji terhadap IBDV yang terperangkap tidak menunjukkan sebarang isyarat dan tindak balas. Oleh itu, ianya sangat spesifik terhadap IBDV. Kesimpulannya, keputusan yang didapati menunjukkan isolat-isolat tempatan adalah merupakan strain vvIBDV, vaksin rekombinan yang dibina adalah selamat dan berkesan dalam memberi perlindungan dan, pembangunan kit "one-step-immunostrip" adalah pantas, spesifik, sensitif, selamat dan ekonomik dalam pengenalpastian jangkitan IBDV, dan pengawalan status imun IBD pada ayam.

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I certify that an Examination Committee has met on 22nd September 2008 to conduct the final examination of Nurulfiza Mat Isa on her Doctor of Philosophy thesis entitled "Molecular Characterisation of Infectious Bursal Disease Virus and the Expression of VP2 Protein for the Development of Diagnostic Kit and Recombinant Vaccine" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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Date: 19 December 2008

DECLARATION

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

_____

NURULFIZA MAT ISA

Date:

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