UNIVERSITI PUTRA MALAYSIA

A STUDY ON TWO MALAYSIAN ISOLATES OF INFECTIOUS BRONCHITIS VIRUS

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By

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Veterinary Medicine and Animal Science Universiti Pertanian Malaysia

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Dedicated to Hafiz, Sofie, Ani and Saiful

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LIST OF ABBREVIATIONS

AGPT	Agar Gel Precipitation Test
AO	Acridine Orange
Ark	Arkansas
ATV	Antibiotic-Trypsin-Versene
BSA	Bovine Serum Albumin
BHK	Cell line Derived from Baby Hamster Kidney
°C	Degree Celcius
CEK	Chicken Embryo Kidney
CELi	Chicken Embryo Liver
CELO	Chick Embryo Lethal Orphan
CELu	Chicken Embryo Lung
CK	Chicken Kidney
cm^3	Cubic Centimeter
Conn	Connecticut
cpe	Cytopathic Effect
CRD	Chronic Respiratory Disease
CsCl	Cesium Chloride
DME	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
Dr.	Doctor
D.W	Distilled water

EID_{50}	Egg Infective Dose 50 Percent
ELISA	Enzyme-Linked Immunosorbent Assay
g	Gravity
gm	Gram
H & E	Hematoxylin-Eosin
H120	Holland 120
H52	Holland 52
HA	Hemagglutination
HI	Hemagglutination Inhibition
HRP	Horse-Radish Peroxidase
HCV	Human Coronavirus
IB	Infectious Bronchitis
IBD	Infectious Bursal Disease
IBV	Infectious Bronchitis Virus
IIF	Indirect Immunofluorescent
IgG	Immunoglobulin G
IIP	Indirect Immunoperoxidase
IP	Immunoperoxidase
IPH	Institut Penyelidikan Haiwan
kd	Kilodalton
M	Matrix
M	Molar
MDPJ	Makmal Diagnosa Petaling Jaya

M41	Massachusetts 41
mA	Miliampere
Mass	Massachusetts
MHV	Murine Hepatitis Virus
ml	Mililitre
mm	Milimeter
mol. wt.	Molecular Weight
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NI	Neutralisation Index
nm	Nanometer
N	Nucleoprotein
NTE	Sodium Tris EDTA buffer
OD	Optical Density
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffer Saline
PfU	Plaque Forming Unit
PJ	Petaling Jaya
PK 15	Cell line Derived from Pig Kidney
PTA	Phosphotungsten Acid
RNA	Ribonucleic Acid
S	Spike
SDS	Sodium dodecyl sulfate

TBS	Tween Buffer Saline
$TCID_{50}$	Tissue Culture Infective Dose 50 Percent
TGEV	Transmissible Gastroenteritis Virus
*u*g	Microgram
UK	United Kingdom
*u*l	Microliter
USA	United States of America
*u*m	Micrometer
Vero	Cell line Derived from Kidney Tissue of Green African Monkey
VN	Virus Neutralisation
v/v	Volume to volume
w/v	Weight to Volume

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SITI SURI ARSHAD

April 1993

Chairman: Prof. Abdul Latif Ibrahim

Faculty : Veterinary Medicine and Animal Science

This research was carried out to investigate whether the outbreak of Infectious Bronchitis (IB) in Malaysia is due to the vaccine virus or the emergence of a new serotype which is different from the vaccine strains. The study involves the comparison between the two local isolates (PJ41 and PJ43) and the three reference strains (H120, H52 and M41) of Massachusetts derivative.

Initial characterisation of the two isolates involved a study on their basic properties such as morphologies, hemagglutinating activity, pathogenicity in embryonated chicken eggs and their adaptibility in primary and secondary tissue cultures with the aid of Acridine Orange (AO), indirect immunoperoxidase (IIP) and indirect immunofluorescent (IIF) staining (to compare localisation of the RNA) and the Hematoxylin and Eosin staining (H & E) (to compare morphologic changes in cell culture).

Both isolates and reference strains revealed a corona-like appearance under electron microscopy and formed pathognomonic lesions of toe curling and dwarfing of the embryo. The virus strains were indistinguishable in their ability to produce cytopathic effects in both primary and secondary cell cultures. The AO staining indicated that the virus possesses a RNA. IIP and IIF tests indicated that they are located in the cytoplasm of the cell. The hemagglutinating activity however, showed that this activity is not always their properties when one of the isolates, (PJ43), and one of the reference strains, (H52), were unable to agglutinate erythrocytes.

The results showed that the local isolates have many of the properties of IBV reference strains. The isolates and the reference strains were then serotyped and their proteins were analysed. Neutralisation of the isolates and the reference strain were seen with the homologous antisera and not with the heterologous antisera in the embryonated chicken egg. In contrast, staining of the viral protein was seen with both homologous and heterologous antisera in both the IIP test as well as the Western Blot analyses. The latter test showed that IBV possesses three structural proteins whereby the matrix (M) being a conserved protein.

In conclusion, this is the first detailed study conducted on local isolates of IBV in Malaysia. The study shows that they are related to the Massachusetts strain and hence the hypothesis stated for this study is proven true. However, it cannot be differentiated either from vaccine strain or the field strain unless a detail study on the amino acid content on the spike protein could be carry out. The study has also provided more information on the characteristics of local isolates and the

relationship of these isolates with the Massachusetts field strain and the vaccine strain. Such an information is very important in the planning of any control program. The success of any vaccination lies on the ability to fully characterise the isolate and to select an appropriate vaccine strain.

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KAJIAN KE ATAS DUA ISOLAT VIRUS BRONKITIS BERJANGKIT MALAYSIA

Oleh

SITI SURI BT ARSHAD

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Penyelidikan ini dijalankan untuk menyiasat samada wabak Bronkitis Berjangkit (IB) di Malaysia disebabkan oleh vaksin virus atau kehadiran serotip baru yang berbeza dari strain vaksin. Kajian ini melibatkan perbandingan di antara dua isolat tempatan (PJ41 dan PJ43) dengan tiga strain rujukan (H120, H52 dan M41) yang berasal dari rentetan Massachusetts.

Pencirian terawal dua isolat ini melibatkan kajian ke atas sifat khas seperti morfologi, aktiviti pengaglutinatan, kepatogenan dalam telur ayam berembrio dan penyesuaiannya dalam kultur tisu primer dan sekunder dengan bantuan pewarnaan akridina jingga, imunoperoksidase tak langsung (IIP) dan imunopendarfluor tak langsung (IIF) (untuk perbandingan kedudukan RNA) dan pewarnaan Hematoksilin-Eosin (H&E) (untuk perbandingan perubahan morfologi dalam kultur sel).

Kedua-dua isolat dan strain rujukan menunjukkan rupa bak korona di bawah mikroskopi elektron dan lesi yang patognomonik iaitu lentikan jari kaki dan kerencatan embrio. Virus-virus tersebut tidak dapat dibezakan melalui keupayaan

dalam menghasilkan kesan sitopati pada kedua-dua sel primer dan sekunder. Pewarnaan akridina jingga membuktikan bahawa virus tersebut adalah RNA dan ujian IIP dan IIF menunjukkan kedudukan virus dalam sitoplasma sel. Sifat pengaglutinatan bagaimanapun menunjukkan bahawa ianya tidak semestinya sifat khas apabila salah satu isolat (PJ43) dan strain rujukan (H52) tidak berupaya untuk mengaglutinat eritrosit.

Keputusan kajian menunjukkan bahawa isolat tempatan mempunyai banyak sifat khas strain rujukan IBV. Isolat dan strain rujukan kemudiannya diserotip dan proteinnya dianalisis. Peneutralan ke atas isolat dan strain rujukan dapat dilihat dengan antiserum homologus dan tidak dapat dilihat dengan antiserum heterologus dalam telur ayam berembrio. Sebaliknya, pewarnaan protein virus dapat dilihat dengan kedua-dua antiserum homologus dan heterologus dalam ujian IIP dan analisis Western Blot. Analisis Western Blot menunjukkan IBV mempunyai tiga protein struktur di mana matriknya (M) adalah protein kekal.

Kesimpulannya, kajian ini merupakan satu kajian terperinci yang pertama yang pernah dilaksanakan di Malaysia terhadap isolat IBV tempatan. Pengkajian menunjukkan bahawa isolat tersebut adalah berkaitan dengan strain Massachusetts dan dengan itu membuktikan bahawa hipotesis bagi kajian ini adalah benar. Walaubagaimanapun, ianya tidak dapat dibezakan samada dari strain vaksin atau strain lapangan sehingga kajian terperinci keatas kandungan acid amino dalam protin spikanya dapat dijalankan. Pengkajian ini juga memberi lebih maklumat tentang ciri isolat tempatan dan perkaitannya dengan strain lapangan Massachusetts dan strain vaksin. Maklumat seumpama ini sangat berguna dalam

perancangan sebarang program pengawalan. Kejayaan sesuatu pemvaksinan bergantung kepada kebolehan dalam mencirikan keseluruhan isolat dan dalam memilih strain vaksin yang bersesuaian.