UNIVERSITI PUTRA MALAYSIA

PATHOGENICITY OF A MALAYSIAN FOWL ADENOVIRUS ISOLATE IN SPECIFIC PATHOGEN FREE EMBRYONATED CHICKEN EGGS AND CHICKS

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MASTER OF VETERINARY SCIENCE

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BY

ALEMNESH WOLDEYES YIMER

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Veterinary Science

January 2009

Dedicated to

My parent

My husband, Son and siblings

For their love, prayer and encouragements

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

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ALEMNESH WOLDEYES

January, 2009

Chairman: Professor Mohd Hair Bejo, PhD

Faculty: Veterinary Medicine

The pathogenic role of fowl adenoviruses (FAdVs) in disease outbreaks remained unestablished, as some findings showed that FAdVs are the primary infectious agents whilst others showed that these viruses can co-infect with other immunosuppressive agents to cause diseases. Thus, there is a need to establish the roles and characteristics of the virus in chicken to control and prevent the disease, especially by developing vaccine and introducing vaccination programme against the disease. The objective of the study was to isolate, identify and determine the pathogenicity of Malaysian isolate of FAdV in specific pathogen free (SPF) embryonated chicken eggs and chicks.

Liver homogenate obtained from inclusion body hepatitis (IBH) field outbreaks in commercial broiler chicken was inoculated, passaged and titrated in SPF embryonated chicken eggs via chorioallantoic membrane (CAM) route and the FAdV was identified

using electron microscopy. The virus inoculum with the titre of 1x $10^{4.8}$ EID_{50}/ 0.1 ml was then inoculated into 9-day-old SPF embryonated chicken eggs as well as 9-day-old SPF chicks. Control groups were included and remained non-inoculated. They were monitored for mortality and clinical signs. The eggs and chicks were either labeled for determination of the percentage of mortality or sacrificed at days 1, 3, 5, 7, 9, 11 and 12, and days 1, 3, 5, 7, 10, 14 and 21 post inoculations (pi), respectively. On necropsy, samples of liver, CAM, yolk sac, kidney, spleen, heart and bursa of Fabricius from the embryos were collected for histological examination, whilst the liver and CAM were also examined for ultrastructural changes. Samples of liver, spleen, gizzard, proventriculus, kidney, pancreas, duodenum and bursa of Fabricius of the chicks were also examined for histological changes, whilst samples of liver were examined for ultrastructural changes as well.

The study showed 100% embryo mortality within 4 to 11 days pi of the virus in SPF embryonated chicken eggs. The gross and histological lesions of the embryo were confined in the liver at days 5, 7, 9 and 11 pi. Grossly the liver was pale with multi-focal areas of necrosis, fibrosis and hemorrhages. Histologically, moderate to severe congestion and hemorrhage, severe diffused degeneration and necrosis of the hepatocytes with intranuclear inclusion bodies (INIB), and infiltration of inflammatory cells were recorded. Hemorrhage, congestion, degeneration, necrosis and hyperplasia of the chorionic epithelium of CAM with INIB were observed at days 5, 7, 9 and 11 pi. Varying degree of congestion, hemorrhage, degeneration and necrosis were also observed in the yolk sac, kidney, spleen, heart and bursa of Fabricius. Numerous viral

particles in the nucleus of hepatocytes were recorded at days 7, 9 and 11 pi under ultrastructural examination, whereas at 5 days pi fine granular and filamentous materials of inclusion bodies were observed. The INIB in the ectoderm were either as fine granular and filamentous structures or as large viral inclusions. Neither clinical signs nor mortality and gross lesions were observed in the chicks in both groups; the FAdV inoculated and control groups. However, histologically mild to moderate degeneration with focal areas of necrosis, presence of INIB and mild infiltration of inflammatory cells in the liver and mild degeneration and necrosis in the proventriculus and pancreas were observed at days 5 to 14 pi. Ultrastructurally, fibrillar, granular and filaments particles of INIB of the hepatocytes were recorded at days 5 to 14 pi.

It was concluded that the FAdV of Malaysian isolate is highly pathogenic to SPF embryonated chicken eggs, but low pathogenic to the chicks. The embryonic liver is the best organ to be used for adaptation and passaging of the virus since severe lesions and numerous viral particles were observed in the liver, the target organ of the virus. The failure of the virus particles to develop in the hepatocytes could result in the failure of disease development in the FAdV infection in the chicks. It appears that the FAdV of Malaysian isolate has high potential to be used as a viral vector to produce recombinant vaccine against poultry diseases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PATOGENISITI SATU ISOLAT VIRUS ADENO UNGGAS MALAYSIA TERHADAP TELUR BEREMBRIO DAN ANAK AYAM BEBAS PENYAKIT KHUSUS

Oleh

ALEMNESH WOLDEYES

Januari, 2009

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Peranan patogenik virus adeno unggas (FAdVs) dalam kes wabak penyakit masih tidak dapat ditentukan kerana sesetengah hasil penemuan menunjukkan bahawa FAdVs adalah agen berjangkit primari sedangkan yang lain menunjukkan bahawa FAdVs adalah agen sampingan kepada agen penyebab kehilangan daya tahan lain yang menjadi penyebab penyakit. Oleh itu adalah perlu untuk menentukan peranan dan ciri-ciri virus ini di dalam ayam untuk pengawalan dan pencegahan penyakit, terutamanya dalam penghasilan vaksin dan pengenalan program vaksinasi untuk menangani penyakit ini. Adalah menjadi objektif kajian ini untuk mengasingkan, mengenalpasti dan menentukan patogenisiti isolat FAdV Malaysia di dalam telur ayam berembrio bebas penyakit khusus (SPF) dan anak ayam. Homogenat hepar dari kes wabak 'inclusion body hepatitis (IBH)' di ladang komersil ayam pedaging diinokulasi, pasage, dan dititrasi ke dalam telur SPF melalui membran korioalantoic (CAM) dan isolat FAdV dikenalpasti di bawah mikroskop elektron. Virus inokulum dengan titer 1x 10 $^{4.8}$ EID_{50}/ 0.1 ml kemudiannya

diinokulasi ke dalam telur berembrio SPF berumur 9 hari dan anak ayam SPF berumur 9 hari. Kumpulan kawalan disertakan dan tidak diinokulasi. Pemerhatian ke atas kematian dan tanda klinikal dijalankan. Telur dan anak ayam dilabel untuk penentuan peratusan kematian atau dikorbankan masing-masing pada 1, 3, 5, 7, 9, 11 dan 12 hari, dan 1, 3, 5, 7, 10, 14 dan 21 hari selepas inokulasi (pi). Semasa nekropsi, sampel hati, CAM, kantung yok, buah pinggang, limpa, jantung dan bursa Fabricius daripada embrio dikumpulkan untuk pemeriksaan histologi, di mana hati dan CAM turut diperiksa untuk perubahan ultrastruktur di bawah elektron mikroscop. Sampel hati, limpa, hempadal, proventrikulus, buah pinggang, pankreas, duodenum dan bursa Fabricius daripada anak ayam diperiksa untuk perubahan histologi, di mana sampel hati turut diperiksa untuk perubahan ultrastruktur.

Kajian ini menunjukkan 100% kematian embrio dalam 4 dan 11 hari pi virus di dalam telur ayam berembrio SPF. Lesi mata kasar dan histologi embrio dapat dilihat pada hati pada 5, 7, 9 dan 11 hari pi. Secara mata kasarnya, hati pucat dengan terdapat banyak kawasan fokal nekrosis, fibrosis dan hemoraj. Secara histologi, konjasi dan hemoraj yang sederhana ke teruk, degenerasi yang teruk dan nekrosis sel-sel hati dengan inclusion bodi dalam nuklear dan menyeluruh, dan infiltrasi sel-sel inflamatori direkodkan. Hemoraj, konjasi, degenerasi, nekrosis dan hiperplasia pada epitelia CAM dengan INIB diperhati pada 5, 7, 9 dan 11 hari pi. Pelbagai darjah konjasi, hemoraj, degenerasi dan nekrosis juga diperhati dalam kantung yok, buah pinggang, limpa, jantung dan bursa Fabricius. Partikel virus di dalam nukleus sel-sel hati juga direkodkan pada 7, 9 dan 11 hari pi di bawah pemeriksaan ultrastruktur, manakala pada 5 hari pi

granul halus dan bahan filamen inclusion bodi diperhatikan. INIB dalam ectoderm adalah semada berbentuk granul halus dan berstruktur filemen atau berbentuk inclusion virus yang besar. Tiada tanda klinikal atau kematian diperhati dalam anak ayam daripada kedua-dua kumpulan; kumpulan FAdV inokulat dan kawalan. Walaubagaimanapun, perubahan histologi manunjukkan sedikit hingga sederhana degenerasi dengan kawasan nekrosis yang fokal, terdapat INB dan infiltrasi sel-sel inflamatori yang sedikit dalam hati dan degenerasi dan nekrosis yang sedikit pada proventrikulus dan pankreas diperhati pada 5 sehingga 14 hari pi. Pemeriksaan ultrastruktur merekodkan INIB berpartikel fibril, granul dan filamen dalam sel hati pada 5 sehingga 14 hari pi.

Kesimpulannya, FAdV isolat Malaysia adalah sangat patogenik ke atas telur ayam berembrio tetapi berpatogenik rendah terhadap anak ayam. Hati embrio merupakan organ paling sesuai untuk penyesuaian dan passage virus kerana lesi teruk dan banyak partikel virus diperhatikan di dalam hati, organ target FAdV. Kegagalan partikel virus untuk mengembang dalam sel-sel hati mungkin menyebabkan kegagalan perkembangan penyakit daripada jangkitan FAdV dalam anak ayam. FAdV isolat Malaysia mempunyai potensi tinggi untuk digunakan sebagai vektor virus untuk menghasilkan vaksin rekombinan terhadap penyakit-penyakit ayam.

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I certify that a Thesis Examination Committee has met on 19 January 2009 to conduct the final examination of Alemnesh Woldeyes on her thesis entitled "Pathogenicity of a Malaysian Fowl Adenovirus Isolate in Specific Pathogen Free Embryonated Chicken Eggs and Chicks" in accordance with the Universities and University colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. The members of the Supervisory Committee were 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.

_____

Alemnesh Woldeyes Yimer

Date:

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LIST OF ABBREVIATIONS

CAV	Chicken anemia virus
CEL	Chicken embryo liver
CK	Chicken kidney
CAM	Chorioallantoic membrane
CELO	Chicken embryo lethal orphan
DNA	Deoxynucleic acid
ddH_2O	Deionized double distilled water
EID_{50}	Embryo infective dose
ELISA	Enzyme linked immunosorbent assay
FAdV	Fowl adenovirus
HAdV	Human adenovirus
HE	Hematoxylin and eosin
HPS	Hydropericardium syndrome
KCl	Potassium chloride
KH_2PO_4	Potassium hydrophosphate
IBD	Infectious bursal disease
IBH	Inclusion body hepatitis
INIB	Intranuclear inclusion body
ITRs	Inverted terminal repeats
$Na\ Cl_2$	Sodium chloride
Na_2HPO_4	Sodium hydrophosphate

PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pi	Post inoculation
SD	Standard deviation
SPF	Specific pathogen free
TAV	Turkey adenovirus
TEM	Transmission electron microscopy
UPM	Universiti Putra Malaysia
W/V	Weight/volume

CHAPTER 1

1. INTRODUCTION

In many countries poultry production is ahead of all other livestock production. It is the most highly developed segment of food animal production globally. Broiler chickens in particular are the largest segment of the industry. Nevertheless, the rapid spread of infectious diseases and the emergence of virulent strains of virus create challenges in the yearly production of large population of broilers.

Adenoviruses are medium-sized of about 70-90nm in diameter, non enveloped, double-stranded DNA viruses commonly infecting humans, a wide variety of wild and domestic mammals and birds (Russell and Benkö, 1999). The family Adenoviridae is categorized into four genera: *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus* and *Siadenovirus* (Benkö *et al*., 2000).

The first avian adenovirus isolated from a distinct clinical condition in birds was from a fatal outbreak of respiratory disease of quail in 1949 in West Virginia (Olson, 1950). Since then the adenoviruses have been widespread throughout avian species and been isolated from chickens, turkeys, geese, duck, guinea fowl, pigeon and ostrich (McFerran and Adair, 2003)