



UNIVERSITI PUTRA MALAYSIA

***EXPERIMENTAL INTRAOCULAR INFECTION OF JAPANESE QUAILS
(CORTUNIX COTURNIX JAPONICA) WITH GENOTYPE VII NEWCASTLE
DISEASE VIRUS***

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(*CORTUNIX COTURNIX JAPONICA*) WITH GENOTYPE VII NEWCASTLE
DISEASE VIRUS**

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Experimental Intraocular Infection of Japanese Quails with genotype VII Newcastle Disease Virus”, by Lizma Felisha binti Mazlan and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999– Project

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DEDICATIONS

This project paper is dedicated to Allah SWT, who had created me and made all things possible,

To my family,

Mazlan bin Amat

Haliza Hamim binti Munif

Lizma Alfasha binti Mazlan

To my supervisor,

Dr. Mohd Hezmee bin Mohd Noor

To my co-supervisors,

Prof. Dr. Abdul Rahman bin Omar

Dr. Lokman Hakim bin Idris

Dr. Tan Sheau Wei

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LIST OF ABBREVIATIONS

%	Percent
μl	Microliter
μM	Micromolar
°C	Degree Celsius
APMV-1	Avian paramyxovirus serotype-1
BHQ1	Black hole quencher
Cq	Quantification cycle
Dpi	day post-infection
ELD ₅₀	50 percent of embryo lethal dose
F	Fusion
FAM	Fluorescent reporter dye 5-carboxyfluorescein
HA	Haemagglutination
HI	Haemagglutination Inhibition
H&E	Hematoxylin and eosin
IACUC	Institutional Animal Care and Use Committee
ICPI	Intracerebral pathogenicity index
ml	Milliliter
MDT	Mean death time
n	Sample size
Nm	Nanometer
N/D	Not detected
ND	Newcastle disease
NDV	Newcastle disease virus
NTC	No template control

OD	Optical density
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
rpm	Rotation per minute
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription real-time polymerase chain reaction
SPF	Specific pathogen free
TRBC	Total red blood cell
UPM	Universiti Putra Malaysia
x	Relative concentration
x g	Relative centrifugal force



ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

EKSPERIMEN JANGKITAN INTRAOKULAR PUYUH JEPUN (*COTURNIX COTURNIX JAPONICA*) DENGAN VIRUS NEWCASTLE DISEASE GENOTYPE VII

Oleh

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Kadar prevalens kajian Newcastle disease (ND) dalam industri puyuh di Malaysia tidak direkodkan dengan terperinci untuk mengenalpasti peranan sebenar puyuh Jepun dalam epidemiologi ND. Genotype VII Newcastle disease virus (NDV) ialah virus utama yang tersebar di Malaysia dan kajian ini bertujuan untuk menentukan kecenderungan

pendedahan puyuh Jepun terhadap genotype VII NDV. Tanda-tanda klinikal, perubahan kasar patologi organ, pengesanan virus yang positif di dalam organ dan calitan kloaka dan penunjukkan titer antibodi digunakan sebagai parameter untuk mengetahui kecenderungan pendedahan puyuh Jepun terhadap genotype VII NDV. Di dalam kajian ini, sebanyak 20 ekor puyuh telah dibahagikan kepada tiga kumpulan (n= 8 untuk Kumpulan A and B; n= 4 untuk Kumpulan Kawalan). Puyuh yang berada Kumpulan A dan B, masing-masing telah dijangkitkan dengan 0.03 ml $10^{3.5}$ ELD₅₀ dan $10^{7.0}$ ELD₅₀ NDV strain IBS 002, melalui kaedah intraokular manakala kumpulan kawalan hanya menerima 1x larutan penimbal fosfat. Kemurungan serta bulu tidak kemas, rales (berderak), kelumpuhan kaki dan tortikolis telah dilihat oleh sebahagian puyuh untuk kedua-dua kumpulan jangkitan. Berpandukan analisis statistik, tiada perbezaan signifikan ($p > 0.05$) bagi tanda-tanda klinikal antara kedua-dua kumpulan jangkitan. Calitan kloaka dilakukan pada hari ketujuh selepas jangkitan untuk kesemua puyuh. Calitan itu tertakluk kepada transkripsi-berbalik reaksi realiti masa rantai polimerase (RT-qPCR) untuk pengesanan virus dan keputusannya ialah negatif untuk kesemua kumpulan puyuh. Nekropsi puyuh dijalankan pada hari ketujuh selepas jangkitan dan tiada perubahan kasar patologi organ-organ dalaman puyuh untuk kedua-dua kumpulan jangkitan. Trakea, proventrikulus, dan seka tonsil telah diambil untuk pengesanan NDV oleh RT-qPCR. Terdapat pengesanan positif virus sebahagian daripada sampel organ untuk kedua-dua kumpulan jangkitan. Ujian serologi menggunakan ujian Penghemaglutinatan-Perencatan menunjukkan peningkatan purata titer antibodi merentas masa dan kumpulan jangkitan. Analisis statistik menunjukkan

tiada perubahan signifikan ($p > 0.05$) reaksi antibodi merentas masa tetapi terdapat perubahan signifikan ($p < 0.05$) antara kumpulan-kumpulan jangkitan. Rumusnya, puyuh Jepun cenderung terdedah kepada genotype VII NDV berdasarkan parameter yang dikaji.

Kata kunci: Virus Newcastle disease genotype VII, RT-qPCR, Ujian Pengehemaglutinatan-Perencatan, kecenderungan pendedahan

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 - Project

EXPERIMENTAL INTRAOCULAR INFECTION OF JAPANESE QUAILS (*CORTUNIX COTURNIX JAPONICA*) WITH GENOTYPE VII NEWCASTLE DISEASE VIRUS

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2016

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The prevalence of Newcastle disease (ND) in quail industry in Malaysia is not well documented in order to clarify the actual role played by Japanese quails in the epidemiology of ND. Genotype VII Newcastle disease virus (NDV) is the most predominant virus circulating in Malaysia, thus this study was aimed to determine the susceptibility of Japanese quails towards genotype VII NDV. Clinical signs, gross

pathological lesions of organs, positive detection of virus in organs and cloacal swabs as well as the expression of the antibody titer were used as parameters to assess the susceptibility of Japanese quails following infection of genotype VII NDV. In this experimental study, 20 quails were divided into three groups (n= 8 for Group A and B; n= 4 for Control Group). The quails in the Group A and B were infected via intraocular route with 0.03 ml of $10^{3.5}$ ELD₅₀ and $10^{7.0}$ ELD₅₀ of NDV strain IBS 002, respectively, while the control group received 1x phosphate-buffered saline (PBS). Depression and ruffled feathers, trachea rales, leg paralysis and torticollis were shown in some of the quails in both infected groups. Based on statistical analysis, there was no significant difference ($p > 0.05$) in clinical signs between the infected groups. Cloacal swabs that were taken on day seven post-infection for all quails were subjected to one-step reverse transcription real time polymerase chain reaction (RT-qPCR) for detection of virus and the results were found to be negative for all groups. Necropsy was conducted on day seven post-infection and there were no gross pathological lesions of organs observed for quails in both infected groups. Trachea, proventriculus, and caecal tonsil were taken for the detection of NDV by RT- qPCR, and some of the organ samples showed positive detections of virus in both infected groups. Haemagglutination inhibition (HI) assay showed an increase in mean titers of antibody across time and between infected groups but statistical analysis revealed no significant difference ($p > 0.05$) of antibody reaction across time, but significantly difference ($p < 0.05$) between infected groups. In summary, Japanese quails are susceptible to genotype VII NDV based on parameters assessed.

Key words: Genotype VII Newcastle disease virus, RT-qPCR, HI assay, susceptibility



1.0 INTRODUCTION

Newcastle disease virus (NDV) or avian paramyxovirus type 1 (APMV-1) is a non-segmented, single-stranded, negative-sense RNA virus which is a member of the genus Avulavirus of the family Paramyxoviridae. The virus widespread among wild and domestic birds with all bird species and some other vertebrates, including humans (transitory conjunctivitis) are susceptible to be infected (Leighton and Heckert, 2007).

Generally, strains of NDV are classified as highly (velogenic), moderately (mesogenic), and weakly pathogenic (lentogenic) pathotypes (Alexander, 1988). NDV strains also grouped into different genotypes based on the sequence and phylogenetic analysis of the F gene. NDV strains have been classified into ten genotypes (I-X), with genotypes VI and VII being further divided into seven (VIa–VIg) and five (VIIa–VIIe) sub-genotypes, respectively (Liu *et al.*, 2003; Tsai *et al.*, 2004). Recently, Shohaimi *et al.* (2015) reported the emergence of new subgenotype known as subgenotype VIIh, showing that many NDV isolates recovered during 2010-2011 were caused by subgenotype VIIh. Nowadays, genotype VII NDV is the predominant virus circulating in Asia including Malaysia and numerous geographical regions, such as Europe, China, Middle East and South Africa were also reported about the virus circulation in those regions since 1990s (Roohani *et al.*, 2015).

The prevalence of Newcastle disease (ND) in quail industry in Malaysia is not well documented in order to clarify the actual role played by the quails (*Coturnix coturnix japonica*) in the epidemiology of ND, particularly genotype VII NDV.

This study was undertaken to determine the susceptibility of Japanese quails towards velogenic genotype VII Newcastle disease virus by observing clinical signs and gross pathological lesions of organs, presence of viruses in organs and cloacal swabs as well as expression of antibody titer.

For this research, the proposed hypothesis was Japanese quails is susceptible towards genotype VII NDV with the expectations of:

- i) Clinical signs of ND will be observed in quails following the infection of genotype VII NDV
- ii) Gross pathological lesions of organs will be seen following infection of genotype VII NDV
- iii) NDV will be detected in organ samples of quails by one-step reverse transcription real-time polymerase chain reaction (RT-qPCR)
- iv) NDV will be detected in cloacal swab specimens of quails by one-step reverse transcription real-time polymerase chain reaction (RT-qPCR)
- v) Expression of antibody against NDV will be detected in quails following infection of genotype VII NDV

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