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EXPERIMENTAL INTRAOCULAR INFECTION OF JAPANESE QUAILS (Coturnix coturnix japonica) WITH INFECTIOUS BURSAL DISEASE VIRUS (IBDV) INTERMEDIATE STRAIN

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Experimental Intraocular Infection of Japanese Quails (*Coturnix coturnix japonica*) with Infectious Bursal Disease (IBDV) Intermediate strain", by Siti Nor Azizah binti Mahamud and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD4999 – Project.

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DEDICATION

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

To my family,

Father

Mother

Brothers, Sisters

Awatif, Amjad, Asyraff

And to all my teachers who have committed themselves towards the noble cause of education.

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LIST OF ABBREVIATION

%	Percentage
^{0}C	Degree celcius
µl	Micrometer
ml	Mililiter
H&E	Haematoxylin and Eosin
IACUC	Institutional Animal Care and Use Committee
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
MLV	Modified-live vaccine
PBS	Phosphate buffered saline
RNA	Ribonucleic acid
rpm	Rotation per minute
RT-PCR	Reverse transcriptase polymerase chain reaction
SD	Standard deviation
$TCID_{50}$	Median tissue culture infective dose
VP	Viral protein

ABSTRAK

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

INFEKSI PERCUBAAN INTRAOKULAR KEPADA PUYUH JEPUN (*Coturnix coturnix japonica*) MENGGUNAKAN VIRUS PENYAKIT BERJANGKIT BURSAL (IBDV) STRAIN PERANTARAAN.

Oleh

Siti Nor Azizah binti Mahamud

2016

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Penemuan pertama penyakit berjangkit bursal (IBD) ialah pada tahun 1957 di Gumboro, Delaware, USA dan pertama kali diterangkan di Malaysia pada tahun 1991. IBD merupakan penyakit bawaan virus yang membawa kerugian ekonomi yang besar kepada industri poltri kerana menyebabkan imunotindasan dan kadar kematian yang tinggi. Eksperimen ini dijalankan bertujuan mencepatkan jangkitan kepada puyuh jepun menggunakan vaksin hidup dilemahkan strain perantaraan IBD

secara intraokular. Parameter yang diperolehi adalah pemerhatian tanda klinikal, lesi post-mortem, pengesanan antigen menggunakan transcriptase membalik reaksi berantai polimerase konvensional (RT-PCR) dan perubahan histopatologikal pada puyuh dari group A, B dan C. Spesifik primer telah direka untuk mensasarkan protein major luar kapsid iaitu gen protein virus 2 (VP2). Parameter ini diukur selepas vaksin diberikan dan bilangan puyuh tertentu dikorbankan pada hari ke 5, 9, dan 15 pos-infeksi dan 24 bursa telah dikumpulkan. Hasil eksperimen menunjukkan pengurangan limfoid minor pada kumpulan A, tanda klinikal yang jelas dan pengurangan limfoid sederhana pada kumpulan B dan tiada lesi post-mortem yang ketara pada semua kumpulan. Analisis transcriptase membalik reaksi berantai polimerase menunjukkan hasil negatif bagi semua sampel yang diuji. Kesimpulannya, ujian klinikal, patologikal dan molekular menunjukkan IBDV strain perantaraan tidak menghasilkan immun respons yang cukup pada puyuh untuk menjadikan meraka pembawa atau perumah.

Kata Kunci: IBDV, intraokular, puyuh jepun (*Coturnix coturnix japonica*), RT-PCR, histopathologi.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project

EXPERIMENTAL INTRAOCULAR INFECTIONS OF JAPANESE QUAILS (*Coturnix coturnix japonica*) WITH INFECTIOUS BURSAL DISEASE VIRUS (IBDV) INTERMEDIATE STRAIN.

By

Siti Nor Azizah binti Mahamud

2016

Supervisor: Dr. Mohd Hezmee bin Mohd Noor

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Infectious Bursal Disease (IBD) was first discovered in 1957 at Gumboro, Delaware, USA and was first described in Malaysia in 1991. IBD became an important viral disease in poultry industry due to its significant economic losses with high mortality and profound immunosuppression. This experiment was conducted to induce the Japanese quails with IBD modified-live vaccine intermediate strain intraocularly.

The parameters obtained were observation of clinical signs, post-mortem lesions, antigen detection using conventional reverse transcriptase PCR and histopathological changes in quails from group A, B and C respectively. Specific primer was designed to target the major outer capsid protein which is viral protein 2 gene (VP2). These parameters are measured after vaccine administration and selected number of quails from each group were euthanized at day 5, 9 and 15 post-infection and the total of 24 bursas were collected. The result reveals minor lymphoid depletion in Group A, prominent clinical signs and mild lymphoid depletion for Group B and no significant post-mortem findings in all groups. RT-PCR analysis gave negative findings in all samples tested. In conclusion, clinical, pathological and molecular results indicate that IBDV intermediate strain does not produce sufficient immune respond in quails to warrant them as carrier or host.

Keywords: IBDV, intraocular, Japanese quails (*Coturnix coturnix japonica*), RT-PCR, histopathology.

1.0 INTRODUCTION

Infectious Bursal Disease is often referred to as Gumboro disease, was discovered in 1957 in Gumboro, Delaware, USA (Khan *et al*., 2005). The outbreak of IBD was first described in Malaysia in 1991 by Hair-Bejo (1992). According to Washington Disease Diagnostic Laboratory (2015), the natural host of IBDV is the domestic fowl including chickens and turkeys and young chickens within the age of 3 to 6 weeks are the most susceptible to clinical diseases. The wild birds such as healthy ducks, guinea fowl, quail and pheasants, have been found to be naturally infected with IBDV (Washington Disease Diagnostic Laboratory, 2015). The most recent survey of international poultry specialists, conducted by *World Poultry*, highlighted continuing concern in the sector over the sanitary status of poultry. Gumboro diseases topped the list of the most serious poultry diseases (Tsukamoto *et al.*, 1999).

IBD is a highly contagious viral disease that affects mainly young chickens and is economically important to the poultry industry (Van den Berg, 2000) due to significant economic losses as it lead to high mortality and morbidity, impaired growth and profound immunosuppression. According to IDERIS (1999), IBDV causes severe inflammation of the bursa of Fabricius that leads to immunosuppression due to destruction of immature B-lymphocytes within the bursa of Fabricius and finally leads to lymphoid depletion and significant depression of the humoral antibody response.

The indirect economic impact of the disease is also considerable, due to virus-induced immunosuppression and potential interactions between IBDV and other viruses, bacteria or parasites. These indirect losses are due to secondary infections, growth retardation and condemnation of carcasses at the slaughterhouse (Van den Berg, 2000). Moreover, the increased use of antibiotics against secondary infections constitutes a growing public health concern. The birds infected with IBD became susceptible to get infection of other diseases that can result in an increase of occurrence of disease caused by opportunistic pathogens and prevents young chickens from responding optimally to vaccination (Ojeda *et al*., 1997).

The objectives of this study are:

1) To observe the clinical signs shown by challenged quails.
2) To examine the post-mortem lesions on challenged quails.
3) To examine the histopathological changes on bursa of Fabricius of challenged quails.
4) To demonstrate the presence of antigen of IBDV in challenged quails via molecular method which is RT-PCR.

Therefore, the hypotheses for this study are:

1) There are significant clinical signs shown by challenged quails.
2) There are significant post-mortem lesion on challenged quails.
3) There are significant histopathological changes on challenged quails.
4) There are presence of antigen in challenged quails upon tested with RT-PCR.

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