DETECTION OF AVIAN POLYOMAVIRUS FROM PSITTACINE BIRDS IN KLANG VALLEY

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TITLE

DETECTION OF AVIAN POLYOMAVIRUS FROM PSITTACINE BIRDS IN KLANG VALLEY

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Detection of Avian Polyomavirus from Psittacine Birds in Klang Valley", by ZamirZanon 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 the Allah S.W.T., who had created me and made all things possible,

To my family,

Father

Mother

Brother, Sister

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

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LIST OF ABBREVIATIONS

%	Percent
µL	Microliter
µM	Micromolar
mm	Millimeter
°C	Degree Celsius
ATPase	Adenosine triphosphatase
bp	Base pairs
kb or kbp	Kilo base pairs
APV	Avian polyomavirus
PBFDV	Psittacine beak and feather disease virus
BFDV	Budgerigar fledging disease virus
GHP	Goose haemorrhagicpolyomavirus
BEF	Budgerigar embryonic fibroblast
CEF	Chicken embryonic fibroblast
AVMA	American Veterinary Medicine Association
DNA	Deoxyribonucleic acid
RNase	Ribonuclease
dNTP	Deoxyribonucleotide triphosphate
g	Gram
min	Minutes
ml	Milliliter
ng	Nanogram
nm	Nanometer
no.	Number
VP1	Viral protein 1
VP2	Viral protein 2
ORF	Open reading frame
PCR	Polymerase chain reaction
AGE	Agarose gel electrophoresis
TAE	Tris-acetate EDTA
V	Volt
x g	Relative centrifugal force

HYB	Hybrid
AGP	African grey parrot
SCC	Sulphur-crested cockatoo
BGM	Blue and gold macaw
SCM	Scarlet macaw
GWM	Green-winged macaw
CFM	Chesnut-fronted macaw
RFM	Red-fronted macaw
BTM	Blue-throated macaw
PQP	Pesquet's parrot
ECL	Eclectus
YCM	Yellow-collared macaw
HM	Hahn's macaw
BHP	Blue-headed parrot
RCM	Red-crowned macaw
BPC	Black palm cockatoo
HCM	Hyacinth macaw
MLC	Moluccan cockatoo
AMP	Amazon parrot
CKT	Cockatiel
BD	Budgerigar
LB	Local Budgerigar

ABSTRAK

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

PENGESANAN VIRUS POLIOMA UNGGAS DALAM KALANGAN BURUNG PSITTACI DI SEKITAR LEMBAH KLANG

Oleh

ZAMIR ZANON

2016

Penyelia: Prof. Madya Dr. Jalila Abu

Penyelia bersama:Dr. Mariatulqabtiah Abdul Razak

Virus polioma ungags boleh menjejaskan burung terutamanya anak burung dan boleh menyebabkan kematian dalam kalangan burung psittacine dan bukan burung pittaci. Burung dewasa kebiasannya rintang terhadap jangkitan; pada peringkat ini, ia akan menyimpan virus ini sehingga tempoh 90 hari dan akan bebas daripada sebarang jangkitan. Burung yang menerima jangkitan ini kebiasaannya adalah anak burung yang baru menetas, sebelum mencapai peringkat remaja dengan tanda akut seperti kelesuan, stasis tembolok, dan kematian dalam tempoh 24-48 jam.Tanda-tanda klinikal lain adalah seperti pendarahan subkutaneus, perut menggelembung dan keabnormalan bulu pelepah. Burung baji yang berumur lebih daripada tiga minggu kebiasaannya akan menunjukkan distrofi bulu pelepah (*French molt* atau hama bulu pelepah). Satu kajian telah dijalankan untuk mengesan kehadiran APV dikalangan burung psittaci terutama dalam spesies burung kakak tua di sekitar Lembah

Klang. Sejumlah 85 spesimen najis telah dipungut daripada empat tempat pembiak bakaan yang berbeza di sekitar Lembah Klang.Spesimen najis telah digabungkan mengikut jenis dan sepsis burung dan juga pembiak baka. DNA telah diasingkan daripada 85 specimen najis burung psittaci yang tidak menunjukkan apa-apa tanda berpenyakit. Kehadiran APV dianalisa dengan melakukan asai tindak balas berantai polimerase (PCR) dan elektroforesis gel agarosa (AGE) dengan menggunakan primer-primer spesifik yang menyasarkan gene VP1(5‟-CTTATGTGGGAGGCTGCAGTGTT-3‟ dan 5‟-TAC TGAAATAGCGTGGTAGGCCTC-3‟) dan APV *full length* (5‟- ACAATGCCTAACGGAACGCC-3‟, 5‟-CACCGAAGCGGCGATACTATA-3‟, 5‟-GAGGCCTACCACGCTATTTCAGTA-3‟ dan 5‟-GCACTTAGCGCCTGTCCAAT-3‟). APV telah ditemui dalam enam daripada 30 spesimen yang digabungkan (20%).Enam spesimen yang dikesan positif APV adalah dari sepsis *yellow-collared macaw, blue-headed parrot, red-crowned macaw, sulphur-crested cockatoo, blue-throated macaw* dan *Pesquet's parrot.* Sebagai kesimpulan, kehadiran APV dalam kalangan burung psittaci adalah berjaya dikesan.

Kata kunci: Virus polioma unggas, burung psittaci, PCR

ABSTRACT

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

DETECTION OF AVIAN

POLYOMAVIRUS FROM PSITTACINE BIRDS IN KLANG VALLEY

By

ZAMIR ZANON

2016

Supervisor: Assoc. Prof. Dr. Jalila Abu

Co-supervisor: Dr. Mariatulqabtiah Abdul Razak

Avian polyomavirus (APV) primarily affects young birds and can cause mortality in wide range of psittacine and non-psittacine birds.Adult birds typically are resistant to infection; they will seroconvert and shed the virus for up to 90 days, then clear the infection. The typical presentation of APV-infected birds is a well-fleshed juvenile, just before fledgling age, with acute onset of lethargy, crop stasis, and death within 24–48 hour. Other clinical signs are cutaneous hemorrhage, abdominal distention, and feather abnormalities. Surviving budgerigars with age more than three week-old often exhibit feather dystrophy (French molt or feather dusters). This is the first study to detect the presence of APV in Malaysia. Therefore, a preliminary study was conducted to detect the presence of avian polyomavirus among psittacine birds especially in parrot species in Klang Valley by using polymerase chain reaction (PCR) technique. A total of 85 faecal samples were collected from psittacines‟ species of individual pet owners and parrot breeders. DNA was isolated from feces of 85 symptom-free psittacine birds taken from four different breeders from Klang Valley. The

presence of APV was analyzed by performing polymerase chain reaction assays (PCR) and agarose gel electrophoresis (AGE) using specific primers targeting VP1 (5‟-CTTATGTGGGAGGCTGCAGTGTT-3‟ and 5‟-TACTGAAATAGCGTGGTAGGCCTC-3‟) and APV full length (5‟- ACAATGCCTAACGGAACGCC-3‟, 5‟-CACCGAAGCGGCGATACTATA-3‟, 5‟-GAGGCCTACCACGCTATTTCAGTA-3‟ and 5‟-GCACTTAGCGCCTGTCCAAT-3‟) genes. Positive results were detected in six out of 30 pooled samples (20%) which were from yellow-collared macaw, blue-headed parrot, red-crowned macaw, sulphur-crested cockatoo, blue-throated macaw and Pesquet‟s parrot.As a conclusion, presence of APV in psittacine birds has been successfully detected.

Keywords: avian polyomavirus, psittacine birds, PCR

1.0 INTRODUCTION

1.1 Polyomavirus

The family *Polyomaviridae* contains a single genus, *Polyomavirus*, which includes viruses in: humans; non-human primates (African green monkey, baboon, stump-tail and rhesus macaque); rodents, including mice (polyomavirus and K virus), hamsters (hamster polyomavirus) and rats (rat polyomavirus); rabbits (rabbit kidney vacuolating agent); birds (avian polyomaviruses, including budgerigar fledging disease polyomavirus); cattle (bovine polyomavirus); equine (equine polyomavirus). The avian polyomaviruses segregate independently of the mammalian ones on the basis of genome sequence analyses, indicating long-standing evolutionary divergence that may result in their being included in different genera in the future. Polyomaviruses of veterinary importance occur in laboratory animals and birds (Parrish, 2011).

1.2 Avian Polyomavirus

Avian polyomavirus have been subclinically identified in numerous species of birds (Parrish, 2011). Disease syndromes associated with polyomavirus infections in birds include increased mortality in a variety of young captive psittacine birds (e.g. lovebirds, macaws, conures, ring-necked parakeets, caiques, Eclectus parrots, Amazon parrots and cockatoos) (Parrish, 2011). Avian polyomavirus is also associated with budgerigar fledging disease (also known as "French molt, a milder disease of budgerigars that results in chronic disorder of feather formation) (Parrish, 2011). Other birds species are also get affected with this virus. Subclinical polyomavirus infection has been described in European raptors, zebra finches, Ross's turaco and a kookaburra. It is believed that only sulphur-crested cockatoo has been infected in natural setting in Australia (Parrish, 2011).

The virus can be shed in the feces for up to six months (Parrish, 2011). Sudden death of the affected birds usually associated with minimal clinical warning, but they briefly manifest weakness, pallor, subcutaneous hemorrhages, anorexia, dehydration and crop stasis (Parrish, 2011).

Currently, the information or status of Avian Polyomavirus (APV) is lacking in Malaysia. No study has been done on the disease status as well as virus detection among psittacine species in Malaysia. Many birds are subclinically infected and shed the virus in respiratory secretions, crop secretions, feather dust and droppings during times of stress such as during the breeding season and juvenile stage in life.

Despite the significant contribution of exotic birds breeding to the Malaysian pet birds industry, no study has been carried out to determine the presence of APV in Malaysia. Hence, this study was undertaken to fulfill the following objective:

i. To detect the presence of avian polyomavirus among psittacine birds especially in parrots species.

For this research, the following hypothesis was proposed; avian polyomavirus might present in psittacine birds in Klang Valley.

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