SEROLOGICAL AND MOLECULAR INVESTIGATION OF THE PRESENCE OF WEST NILE VIRUS (WNV) AMONG WILD BIRDS IN KUALA GULA, PERAK

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CERTIFICATION

It is hereby certified that we have read this project entitled “Serological and Molecular Investigation of the Presence of West Nile Virus (WNV) among Wild Birds in Kuala Gula, Perak”, by Fatin Amirah BintiAbas and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course of VPD 4999- Final Year Project.

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DEDICATIONS

"Animals are reliable, many full of love, true in their affections, predictable in their actions, grateful and loyal. Difficult standards for people to live up to"

-Alfred A. Montapert-

To my lovely parents,

Abas Bin Othman &AnomBintiAttan

forbeing the most supportive parents in the entire world for me.

To my family,

for the time spent to encourage me to finish up my project.

To my friends,

who always there for me.

Lastly, to all that involved in helping me to complete my project.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µL	Microliter
µM	Micromolar
CDC	Centre of Disease Control and Prevention
cDNA	Complement Deoxyribonucleic Acid
c-ELISA	Competitive Enzyme-linked Immunosorbent Assay
DdH20	Double distilled water
G	Gauge
g	Gram
HRP	Horseradish peroxidase
IACUC	Institutional Animal Care and Use Committee
IFA	Indirect immunofluorescent assay
IgG	Immunoglobulin G
JE	Japanese Encephalitis
MEGA	Molecular Evolutionary Genetic Analysis

mg	Miligram
mL	Milliliter
MVE	Murray Valley Encephalitis
NCBI	National Center for Biotechnology Information
NJ	Neighbour-joining
nm	Nanometre
NS	Non-structural
OD	Optical Density
PBS	Phosphate Buffer Solution
PERHILITAN	Department of Wildlife and National Parks Peninsular Malaysia
Pr-E	Protein E
RNA	Ribonucleic Acid
RT- PCR	Reverse Transcriptase Polymerase Chain Reaction
S/N (%)	Signal to noise ratio percentage
TAE	Tris-acetate-ethylenediaminetetraacetic acid
UPM	Universiti Putra Malaysia
US	United State
UTR	Untranslated region
WNV	West Nile Virus
x *g*	Relative centrigugal force

ABSTRAK

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

PENYIASATAN SEROLOGI DAN MOLEKULAR UNTUK KEHADIRAN VIRUS WEST NILE (WNV) ANTARA BURUNG LIAR DI KUALA GULA, PERAK.

Oleh:

Fatin Amirah Binti Abas

2018

Penyelia: Dr. Nor Yasmin Abd. Rahaman

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Virus West Nile (WNV) adalah virus bawaan artopod yang berasal dari keluarga *Flaviviridae* yang bersifat zoonotik. Virus ini kebanyakannya disebarkan oleh nyamuk dan burung liar bertindak sebagai reservoir memperkuat semulajadi manakala mamalia bertindak sebagai perumah akhir. Kajian sebelum ini berkaitan prevalens WNV dalam kalangan burung teman di Selangor menunjukkan terdapat pendedahan terhadap virus, yang menandakan bahawa vektor WNV telah wujud di Malaysia yang menyumbang kepada penyebaran virus ini. Walaubagaimanapun, masih tiada kajian yang dijalankan ke atas kejadian WNV di dalam kalangan burung liar di Malaysia. Dengan mempertimbangkan fakta bahawa burung liar adalah perumah utama virus dan sifat penghijrahan mereka dari hemis fera utara ke selatan atau sebaliknya, maka, kajian ini bertujuan untuk menyaring kehadiran antibody dan antigen WNV dalam *Ardeidae* (burung liar) di Kuala Gula, Perak. Kuala Gula dianggap kawasan berisiko tinggi memandangkan ia adalah titik untuk burung hijrah singgah sebentar dan

tempat pembiakan nyamuk disebabkan bank ekosistem sawah padi. Dua puluh ekor *Ardeidae* (burung liar) telah diperolehi melalui persampelan rawak mudah. Serum dan swab orofarinks dikumpulkan dan dijalankan dengan menggunakan masing-masing kit kompetitif ELISA (ID Screen ® West Nile competitive Multi-species) dan „one-step" rt-PCR yang menyasarkan bahagian antara kapsid dan pramembran yang sangat terabadi. Berdasarkan ujian serologi, 2/20 sampel didapati positif kepada antigen pr-E. Untuk kajian molekul, 5 sampel adalah positif untuk antigen WNV. Sampel yang positif dihantar untuk analisa penjujukan dan menunjukkan 98-100% homolog dengan strain dari Itali, Amerika Syarikat, Hungary, Rusia and Greece. Kesimpulannya, kajian ini menunjukkan bahawa burung liar daripada keluarga *Ardeidae* di Malaysia telah terdedah dan dijangkiti oleh WNV dan keberadaan WNV di perantauan ini ditunjukkan.

Kata kunci: Virus West Nile (WNV), Flaviviridae, burung liar, ELISA, rt-PCR, Perak

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine as a partial requirement for the course VPD 4999- Final Year Project.

SEROLOGICAL AND MOLECULAR INVESTIGATION OF THE PRESENCE OF WEST NILE VIRUS (WNV) AMONG WILD BIRDS IN KUALA GULA, PERAK.

By:

Fatin Amirah BintiAbas

2018

Supervisor: Dr. Nor Yasmin BintiAbd. Rahaman

Co- Supervisor: En. Ahmad Khusaini Bin Mohd. Kharip Shah

West Nile Virus (WNV) is an arthropod borne virus derived from *Flaviviridae* family which is zoonotic in nature. The virus is transmitted mostly by mosquitoes and wild birds act as the natural amplifying reservoir while mammals served as dead-end host. Previous study on the prevalence of WNV among companion birds in Selangor showed

there was an exposure towards the virus which signified the vector of WNV is presence in Malaysia that contribute to the transmission of the virus. However, there is still no study conducted on the occurrence of WNV among wild birds in Malaysia. By considering the facts that wild bird is the main reservoir of the virus and their migration nature from north to south hemisphere or vice versa, therefore, this study aims to screen the presence of WNV antibody and antigen in *Ardeidae* (wild birds) in Kuala Gula Bird Sanctuary, Perak. Kuala Gula is considered as high risk area since it is one of the spot for the migratory bird to stop by temporarily and mosquitoes breeding area due to abundance of rice paddies fields ecosystem. Twenty *Ardeidae* juvenile and adult birds were obtained through convenience sampling. Serum and oropharyngeal swabs were collected and subjected to competitive ELISA (ID Screen ® West Nile Competitive Multi-species) and one step rt-PCR targeting highly conserved gene in between the capsid and pre-membrane protein, respectively. Based on the serological test, 2/20 samples were positive to the pr-E antigen. For molecular study, 5 samples were positive for WNV antigen. The positive results were sent for sequencing analysis and revealed 98-100% homologous to WNV strain from Italy, United State, Hungary, Russia and Greece. Interestingly, this region are located within the migratory bird flyways. As a conclusion, this study showed that *Ardeidae* family of wild birds in Malaysia were exposed and infected to WNV and evidence of WNV is circulating in the region is demonstrated.

Keywords: West Nile Virus (WNV), Flaviviridae, wild birds, ELISA, rt-PCR, Perak

1.0 INTRODUCTION

West Nile Virus (WNV) is a single-stranded RNA virus that can cause encephalitis in animals and human. WNV was derived from the West Nile region of Uganda, where the first isolation and detection of WNV in a women was demonstrated. It is the most widespread arthropod-borne virus, transmitted by mosquitoes (Rappole et al., 2000). WNV is considered a member of the Japanese Encephalitis (JE) virus serocomplex, including JE, St. Louis encephalitis and Murray Valley encephalitis (MVE) viruses after hemagglutination inhibition and cross-neutralization data were analyzed (Lanciotti et al., 2002). The virus population is maintained in the enzootic cycle, in between mosquitoes and wild birds, where wild birds are considered as an important amplifying reservoir that developed high viraemia that able to infect mosquitoes to transmit the virus (Chancey et al., 2015). The most common species of mosquitoes that are infected with this virus are from Culex spp. mosquitoes, however, in Israel, Aedes spp. are also considered as a potent vector for this virus (Orshan et al., 2008). Other mammals such as horse and human are incidental host that are actually a dead end host, they have very low viraemia and unable to transmit the virus through mosquitoes. Based on Centre of Disease Control and Prevention (CDC), 70-80% of people infected with WNV are asymptomatic, and only less than 1% of infected people had neuroinvasive symptoms such as seizures, mental status changes, focal neurologic deficits, or movement disorders. The non-neuroinvasive manifested as mild flu-like symptoms with malaise, eye pain, headache, myalgia, gastrointestinal discomfort and rash (Lim et al., 2011). The severity of the disease also depends on the risk factors such as old age and immunocompromised host (Gyure, 2009). A number of studies stated that this

virus has tropism toward basal ganglia, thalamus, hippocampus, cerebellum, midbrain, and pons (Penn et al., 2006; Gamino et al., 2013). The virus already existed in United States, and distributed to Middle East, Europe, Southern and Eastern Asia and North America (Chancey et al., 2015).

In Malaysia, according to Rais et al. (2011) and Marlina et al. (2014), about 4.21% (3/68) and 1.21% (9/742) of captive birds in Selangor Orang Asli in Negeri Sembilan and Pahang are seropositive for WNV antibodies, which means there were evidence of exposure of WNV among the captive birds and Orang Asli in Malaysia. Apart from that, abundance of mosquitoes as vectors and availability of birds as amplifying reservoir in Malaysia, increase the risk of WNV transmission in Malaysia. Furthermore, there is no study conducted for the WNV status among wild birds in Malaysia. The hypothesis for this study, it will showed evidence of exposure and infection for WNV among Ardeidae (wild birds) in Kuala Gula, Perak through serological and molecular study using competitive-ELISA and RT-PCR, respectively.

Therefore, this study was conducted to investigate the WNV status among wild birds in Malaysia.

The objectives of the study were:

1. To detect the presence of WNV antibodies among Ardeidae family (wild birds) in Perak serologically by using competitive-ELISA.
2. To detect the presence of WNV antigen among Ardeidae family (wild birds) in Perak through molecular study by performing reverse transcriptase-PCR.

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