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SEROPREVALENCE OF JAPANESE ENCEPHALITIS VIRUS (JEV) IN BIRDS IN MALAYSIA

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SEROPREVALENCE OF JAPANESE ENCEPHALITIS VIRUS (JEV) IN BIRDS IN MALAYSIA

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It is hereby certified that we have read this project paper entitled "Seroprevalence of Japanese Encephalitis Virus (JEV) in Birds in Malaysia", by Anisah Binti Abdul Rasid and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

"Animals are such agreeable friends.
They ask no questions, they pass no criticism"
-George Eliot-

To my lovely parents,
Abdul Rasid Bin Bakar & Rasiahwati Binti Sanudin
for always believe in me and for the endless motivation and support.

To my family,
for the love and care.

To my dearest cats,
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LIST OF ABBREVIATIONS

JEV	Japanese Encephalitis Virus
JE	Japanese Encephalitis
RNA	Ribonucleic Acid
WHO	World Health Organization
ELISA	Enzyme-linked Immunosorbent Assay
IACUC	Institutional Animal Care and Use Committee
G	Gauge
OD	Optical Density
Ag	Antigen
Ab	Antibody
HRP	Horseradish Peroxidase
°C	Degree Celcius
nm	Nanometer
-ve	Negative
+ve	Positive
%	Percentage
n	Total
C.I	Confidence Interval
g	Gravity
mL	Millilitre

ABSTRACT

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

PREVALEN ANTIBODI VIRUS JAPANESE ENCEPHALITIS PADA BURUNG DI MALAYSIA

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Japanese Encephalitis (JE) merupakan salah satu daripada penyakit zoonotik yang disebabkan oleh virus Japanese Encephalitis yang berasal daripada famili *Flaviviridae* dan genus *Flavivirus.* Virus ini disebar melalui nyamuk *Culex*

terutamanya nyamuk *Culex tritaeniorhynchus* dengan burung ardeid sebagai perumah takungan. Babi dan burung memainkan peranan yang penting sebagai perumah pemangkin dengan manusia dan kuda sebagai perumah terakhir. Empat puluh lima burung terdiri daripada ayam kampung, ayam hutan kacukan dan burung air di sampel di Tanjung Piandang, Perak, Jenderam Hulu, Sepang, Selangor dan di Taman Wetland Putrajaya. Sampel darah diproses dan serum diuji dengan menggunakan ELISA sandwic antigen-berganda untuk mengenalpasti antibodi terhadap virus JE (MyBiosource®). Daripada empat puluh lima sampel, 28.89% (13/45) didapati positif terhadap antibodi virus JE. Berdasarkan lokasi, Jenderam Hulu mempunyai prevalen antibodi tertinggi (50%) diikuti oleh Tanjung Piandang (21.74%) dan akhir sekali Taman Wetland Putrajaya (20%). Berdasarkan faktor umur, burung muda menunjukkan prevalen antibodi tertinggi (35.71%) berbanding burung dewasa (25.81%). Berdasarkan kepada spesis, kedua- dua spesis ayam hutan kacukan dan flamingo America mempunyai prevalen antibodi (50%) diikuti dengan ayam kampung (21.74%). Burung jantan menunjukkan prevalen antibodi tertinngi dengan (50%) diikuti burung betina (28.13%). Analisis chi square menunjukkan tiada hubung kait antara faktor risiko dan prevalen antibodi terhadap virus JE. Secara kesimpulannya, terdapat antibodi yang dapat dikesan terhadap virus JE dalam burung di Malaysia dan kesemua burung mempunyai risiko yang sama kepada jangkitan virus JE dalam kondisi umur, spesis dan lokasi. Kajian

selanjutnya perlu dilakukan untuk mengenalpasti genotip virus yang berada di dalam populasi burung melalui kajian molekul.

Kata kunci: Burung, Japanese Encephalitis, Malaysia, prevalen, antibodi, faktor risiko, ELISA sandwic antigen-berganda

ABSTRACT

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

SEROPREVALENCE OF JAPANESE ENCEPHALITIS VIRUS (JEV) IN BIRDS IN MALAYSIA

By

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2016

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Japanese Encephalitis is one of the most important zoonotic diseases caused by Japanese Encephalitis virus from family *Flaviviridae* and genus *Flavivirus*. The virus is transmitted through *Culex* mosquito primarily by *Culex tritaeniorhynchus* with ardeid birds as reservoir. Pigs and birds play an important role as the main vertebrate amplifier with human and horse as the dead-end host. Forty-five birds

consisted of village chicken, jungle fowl cross and waterbirds were sampled in Tanjung Piandang, Perak, Jenderam Hulu, Sepang, Selangor and Putrajaya Wetland, respectively. Blood samples were processed and the serum were subjected to double-antigen sandwich ELISA for detection of antibody against Japanese Encephalitis virus (MyBiosource®). Out of forty-five sample, 28.89% (13/45) were positive for JEV antibodies. Based on location, Jenderam Hulu has the highest seroconversion (50%) followed by Tanjung Piandang (21.74%) and Putrajaya Wetland (20%). Based on age, young birds showed higher seroconversion (35.71%) than adult (25.81%). According to species, both jungle fowl cross and American flamingo has 50% seroconversion followed by village chicken with 21.74%. Male showed highest seroconversion (50%) followed by female (28.13%). Chi square test analysis revealed that there were no association between the risk factors and seroprevalence of JEV. In conclusion, there was presence of seroconversion against JEV in birds in Malaysia and all birds have similar risk to JEV infection in terms of age, sex, species and location. Further work should examine the genotype of the virus circulating in the birds' population by molecular studies.

Key words: Birds, Japanese Encephalitis, Malaysia, seroconversion, antibody, risk factors, double-antigen sandwich ELISA

1.0 INTRODUCTION

Japanese Encephalitis virus (JEV) is a mosquito-borne *Flavivirus* in the family of *Flaviviridae.* It is the most important cause of viral encephalitis in both humans and animals in Asia (Chen *et al*., 1990). JEV also has been deemed the most important cause of epidemic encephalitis worldwide and is the leading recognized cause of childhood encephalitis in Asia (Nemeth *et al.*, 2010). *Flavivirus* is a spherical, enveloped, single stranded linear RNA virus measuring about 40-50µm in diameter. The capsid of the virus has an icosahedral symmetry and the lipid bilayer of the *Flavivirus* is featuring as a glycoprotein spikes. Three structural protein present in a *Flavivirus* is nucleocapsid protein, a membrane like protein and an envelope glycoprotein (Himani *et al*., 2014).

Transmission of JEV involves *Culex tritaeniorhynchus* mosquito and its similar species that lay eggs in rice paddies and other open water source, with pigs and aquatic birds as the major vertebrate amplifying host (Halsted *et al*., 2008). According to Tsuchie *et al*. (1994) pigs and some avian species are important amplifier because of its significant viraemia following infection, large number of population, high turnover rate and preferential feeding by vectors. Two JEV transmission has been observed and in general, Japanese encephalitis is epidemic in temperate regions of Asia and endemic in tropical regions. The reasons for this

two pattern is unknown but the best explanation is because there is differences in virulence among JEV strain (Burke and Leake, 1988).

Most of the monsoon areas in Asian countries which includes Malaysia have climate condition of sufficiently high temperature during summer and precipitation during rainy season, allowing for rice cultivation in watered paddy fields (Tsuchie *et al*., 1994) which eventually will become a place for the mosquito to lay eggs. Chicken rearing is quite common in Malaysia especially in village where it is nearby the paddy fields, swamp area or river. Both of this condition help in maintaining the JEV in nature. Other than that, according to Chen *et al*. (1990) JEV might reintroduced annually by migrating birds from tropical region of Asia. Chickens and ducklings have been considered to pay the best minor role in outbreaks because of their low or absent viremia. In endemic region which includes the tropical region, large number of free-ranging chickens and ducks lived and breed nearby humans, providing an abundant pool of potential amplifying hosts of JE virus. Domestic poultry also produce high enough viraemia for an extended period of time to serve as a possible alternative source of JEV infection and transmission in humans (Cleton *et al*., 2014). As a viral reservoir or amplifying hosts, birds do not develop clinical symptoms (Yang *et al*., 2011).

In Malaysia, the first JE case was reported in 1952 (Erlanger *et al*., 2009) and several outbreaks had occur years after that. In 1974, outbreaks had occur in

Langkawi with 10 cases causing 2 deaths (Fang *et al*., 1980), 1988 in Penang with 9 cases and 4 deaths (Cardosa *et al.*, 1995) and in 1992 in Serian district of Sarawak with 9 cases and 4 deaths (WHO, March 1999). In Malaysia, there are about 9 to 91 cases of JE were reported each year (WHO, March 1999) and recently until June 2014, there are 16 reported cases of JE that causing 4 deaths. This disease occurs in almost every state in Malaysia, with greater number of cases in Penang, Perak, Selangor and Johor in West Malaysia and Sarawak in East Malaysia (Tsuchie *et al*., 1994).

JE is an important and endemic zoonotic disease in many country including Malaysia, Thailand, Philippines and other developing countries. However, currently there is no report on the JE in birds in Malaysia and the status of this species involvement in maintaining the virus in the environment is not known despite them act as a potential amplifier (Tsuchie *et al*., 1994; Halsted *et al.*, 2008; Cleton *et al*., 2014). Therefore, this study will give some a clear view on the serological prevalence of JE in birds in Malaysia. This study will hope to give path and served as a foundation for future epidemiological research on JE virus in Malaysia. In this preliminary study, the aim is to document the serological prevalence of JEV in birds as it is the important potential amplifying host for JEV based on previous study. The antibodies will be detected using MyBiosource® Chicken Japanese Encephalitis IgG Antibody enzyme-linked immunosorbent assay (ELISA) kit.

Thus, the objective of this study includes:

1. To determine the serological prevalence of JEV in birds in Malaysia.

2. To determine the association between seropositivity against JEV and potential risk factors.

The hypothesis for this study is:

1. There is presence of seroconversion against JEV in birds in Malaysia

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