



UNIVERSITI PUTRA MALAYSIA

**EPIDEMIOLOGICAL INVESTIGATION OF EQUINE INFLUENZA
VIRUS INFECTION IN PENINSULAR MALAYSIA**

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Abdul Rahman D. Abdul Hadi

**MASTER OF VETERINARY SCIENCE
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By

Abdul Rahman D. Abdul Hadi

**Thesis Submitted to the School Of Graduate Studies, Universiti Putra
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Veterinary Science**

April 2009



DEDICATED

To my late father “may Allah bless him with his supreme benevolence”.

**To my caring, lovely mother and father who have shown me the way to the
right path.**

**To my wife, brother, sisters, and all those who passed away in struggle for
sovereignty of my fatherland.**

To my patient and bleeding country, may Allah grant you peace.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia In fulfillment of the requirement for the Degree of Master of Veterinary Science

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Chairman: Assoc. Prof. Dr. Bashir Ahmad Fateh Mohamed, PhD

Faculty: Veterinary Medicine

A cross-sectional study was conducted in Peninsular Malaysia with the objectives of determining the serological prevalence, molecular evidence and risk factors of equine influenza virus (EIV) distribution among different geographical regions. A total of 435 serum samples and 172 nasopharyngeal swabs were collected during July 2007- July 2008. Our study showed that the prevalence of antibodies against EIV was recorded in 215 of the 435 sera (49.4%). The prevalence of circulating antibodies against equine influenza virus in relation to states were recorded as; Selangor 127 out of 170 sera (74.7%) from vaccinated horses, and 9 out of 12 sera (75%) from unvaccinated horses, in Kelantan 37 out



of 165 sera (22.4%) are unvaccinated horses, in Melaka 11 out of 25 sera (44%) are unvaccinated horses, in Negeri Sembilan 2 out of 23 sera (8.7%) are unvaccinated horses, in Johor 16 out of 17 sera (94.1%) are vaccinated horses, in Kedah 11 out of 14 sera (78.6%) are unvaccinated horses and in Pahang 2 out of 9 sera (22.2%) are vaccinated horses. The viral nucleic acid was detected in 77 of the 172 nasopharyngeal swabs (44.7%). The prevalence of positive nasopharyngeal swabs among vaccination status, were also recorded as, 44 out of 102 (43.1%) from vaccinated horses and 33 out of 70 (47.1%) nasopharyngeal swabs from non-vaccinated horses.

The association between several putative risk factors from vaccinated and unvaccinated groups on the seroconversion of equine influenza virus using binary logistic regression was recorded as; Age factor showed to be not significant factor in vaccinated groups against possible EIV infection as compared to unvaccinated groups which is recorded as 5.5 times chances to seroconversion. Thoroughbred groups showed a significant risk above unity whereas the pony groups breed showed a significant low risks. All others group of horses had non-significant, low risks. Sex did not contribute significantly to the epidemiology of the infection since there were no significant risk differences between sexes.



This moderate antibody level detected from horses might indicate exposure of these animals to the virus or evidence of recent infection. The horses that were detected positive for equine influenza might be shedding the virus among naïve population, and likely represent an important role in the epidemiology of respiratory disease outbreaks. In conclusion, the data presented in this study revealed that the EIV circulates among vaccinated and non-vaccinated horses in Malaysia and the incidence rate of EIV is relatively high. The periodic movement of sub-clinically infected horses at the international level provides the potential for interaction with susceptible populations and may serve as a crucial factor in transmission of infections among horse population. Absence of mandatory of vaccination program against EIV in Malaysia most probably contributed to the spread of the disease between provinces. Therefore, it is advisable to update equine influenza vaccine regularly.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah sarjana Veterinar Sains

KAJIAN EPIDEMIOLOGI JANGKITAN VIRUS INFLUENZA EKUIN DI SEMENANJUNG MALAYSIA

Oleh

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April 2009

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Kajian rentasan telah dijalankan bagi menentukan prevalen serologi, bukti molekular dan faktor risiko penyebaran virus influenza ekuin di antara wilayah geografi yang berlainan di Semenanjung Malaysia. Sebanyak 435 sampel serum dan 172 sampel calit nasofar nks telah dikutip sepanjang Julai 2007 hingga Julai 2008. Kajian ini telah menunjukkan bahawa prevalen antibodi terhadap EIV telah direkodkan pada 215 sampel daripada 435 sampel keseluruhannya (49.4%). Prevalen ataran antibodi melawan virus ekuin influenza mengikut negeri telah direkodkan seperti; di Selangor daripada 170 sera sebanyak 127 sera (74.7%) adalah kuda yang telah divaksinakan, dan 9 daripada 12 sera (75%) adalah daripada kuda yang belum divaksinakan, di Kelantan sebanyak 37 daripada 165



sera (22.4%) adalah kuda yang belum divaksin, di Melaka 11 daripada 25 sera (44%) adalah kuda yang belum divaksin, di Negeri Sembilan sebanyak 2 daripada 23 sera (8.7%), di Johor sebanyak 16 daripada 17 sera (94.1%) adalah kuda yang belum divaksin, di Kedah sebanyak 11 daripada 14 sera (78.6%) adalah kuda yang belum divaksin, dan di Pahang 2 daripada 9 sera (22.2%) adalah yang belum divaksin. Virus asid nukleik telah dikesan dalam 77 sampel daripada 172 sampel calit nasofarinks (44.7%). Prevalen sampel calit nasofarinks yang positif bagi status pemvaksin, juga telah direkodkan iaitu sebanyak 44 daripada 102 (43.1%) sampel kuda yang telah disuntik vaksin dan 33 daripada 70 (47.1%) sampel calit nasofarinks daripada kuda yang tidak disuntik vaksin.

Hubung kaitan di antara beberapa faktor risiko anggapan dari kelompok yang telah disuntik vaksin dan tidak disuntik vaksin pada penukaran sero kuda dari virus influenza menggunakan binari logistik regresi mencatat sebagai, usia merupakan faktor yang tidak signifikan dalam kelompok yang disuntik vaksin terhadap berkemungkinan jangkitan EIV jika dibandingkan dengan kelompok yang tidak disuntik vaksin yang mana telah direkodkan sebanyak 5.5 kali lebih baik. Baka kuda Throughbred menunjukkan risiko yang signifikan melebihi kesatuan sedangkan baka kuda padi menunjukkan risiko signifikan yang

rendah. Semua baka kuda yang lain telah menunjukkan faktor tidak signifikan yang rendah. Jantina tidak menyumbang secara signifikan kepada jangkitan epidemiologi kerana tiadanya risiko perbezaan yang signifikan di antara jantina.

Paras antibodi yang sederhana yang telah dikesan pada kuda tersebut telah menunjukkan bahawa haiwan tersebut telah terdedah kepada virus atau jangkitan terkini. Kuda yang telah dikenal pasti positif terhadap influenza ekuin berkemungkinan boleh menyebarkan virus tersebut di kalangan populasi yang lemah, dan berkemungkinan memainkan peranan yang penting dalam epidemiologi wabak penyakit pernafasan. Kesimpulannya, data yang ditunjukkan dalam kajian ini menyatakan bahawa virus influenza ekuin berlegar di kalangan kuda yang telah disuntik vaksin dan yang tidak disuntik vaksin di Malaysia. Dan kadar insidens EIV secara relatifnya adalah tinggi. Kekurangan pemantauan terhadap program vaksinasi terhadap EIV di Malaysia mungkin turut menyumbang terhadap penyebaran penyakit. Oleh itu, adalah disyorkan vaksin EIV dikemas kini dengan lebih kerap.

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Life is a challenge to most people and success is measured in many ways. I have always believed that you should challenge yourself everyday and strive to achieve success, or at least satisfaction, through attacking each challenge with a balance of knowledge and ability while maintaining sanity. The tools needed to attack each challenge have been gained through the help, advice, and leadership of many people. This belief was imposed upon me, not in words but by actions, first by my parents and secondly by counselors and instructors.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

Abdul Rahman D. Abdul Hadi

Date: 20 – 4 - 2009



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LIST OF ABBREVIATIONS

µg	microgram
µM	micromolar
bp	base pair
CI	confidence intervals
cDNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EI	equine influenza
EIA	enzyme immunoassay
EIV	equine influenza virus
ELISA	Enzyme-linked immunosorbent assay
HA	haemagglutinin activity
HN	haemagglutinin neuraminidase
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IM	intramuscular
IN	intranasal
M	matrix protein

MEM	minimal essential medium
MDCK	Madin-Darby Canine kidney
min	minutes
ml	milliliter
mM	millimolar
N	nasopharyngeal
NA	neuraminidase
NP	nucleoprotein
NS	nonstructural
°C	degree Celsius
OD	optical density
OR	Odds ratio
PCR	polymerase chain reaction
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcriptase-polymerase chain reaction
SRH	single radial hemolysis
<i>Taq</i>	<i>Thermus aquaticus</i>
TB	Thoroughbred
TMB	Tetramethylbenzidine



UK	United Kingdom
v/v	volume/volume
VTM	virus transport medium
w/v	weight/volume
WHO	World Health Organization



CHAPTER 1

INTRODUCTION

1.1 GENERAL BACKGROUND

Influenza is Italian for "influence" (Crosby, 1993), Latin: influential. It used to be thought that the disease was caused by a bad influence from the heavens. In the Middle Ages influenza was also called "Knock-me-down-fever". Ancient descriptions have suggested the possibility of influenza as a cause of respiratory disease, which was the case where Hippocrates described an outbreak of flu-like illness in 412 BC (Sovinova *et al.*, 1958).

Equine influenza virus is a species-type A influenza virus from the *Orthomyxoviridae* family, and is comprise of eight segments of RNA. These RNA segment are coated by nucleoprotein (NP), which along with a complex of polymerase enzymes, is responsible for transcription and replication of the virus within the nucleus of the host cell. The segments are surrounded by matrix protein (MP) and the entire structure is enclosed within a lipid bilayer called the virion envelope, two major surface glycoprotein's, hemagglutinin and

