UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY, TRANSMISSION AND ISOLATION OF NIPAH VIRUS IN LARGE FRUIT BATS (*PTEROPUS* SPECIES) IN PENINSULAR MALAYSIA

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By

SOHAYATI ABD RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

Dedicated with love and greatest gratitude to my parent, Abdul Rahman M. Diah and Rokiah Othman, my husband, Zaini Che Mamat, my children, M. Nazrin Asyraf and Nur Izzah Ayuni

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EPIDEMIOLOGY, TRANSMISSION AND ISOLATION OF NIPAH VIRUS IN LARGE FRUIT BATS (*PTEROPUS* SPECIES) IN PENINSULAR MALAYSIA

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Chairman: Latiffah Hassan, PhD

Faculty: Faculty of Veterinary Medicine

Bats of the genus *Pteropus* are considered the natural reservoir hosts for NiV and other henipaviruses. The present study was carried out to investigate the epidemiology of NiV in *Pteropus* sp. in Malaysia. The specific objectives of this study are to describe the geographical distribution and population characteristics of *Pteropus* spp. in the peninsular, describe the geographical extent of NiV antibody in pteropid bats in the peninsular, identify the risk factors associated with the infection, determine the natural route of NiV excretion, transmission and serological patterns of the infection in captured *Pteropus*, estimate the seroprevalence and incidence rate of NiV seroconversion in the bats and investigate the possibility of viral recrudescence in naturally infected bats and in experimentally NiV immuno-suppressed seropositive bats

P. vampyrus and *P. hypomelanus* were found throughout Peninsular Malaysia. *P. hypomelanus* inhabits the islands surrounding the peninsular while *P. vampyrus* were found on the mainland. *P. vampyrus* was extremely sensitive even to low-level human activities. Physically, *P. vampyrus* was significantly bigger and heavier than *P. hypomelanus*. The physical characteristics of bats of both species differ significantly given age and sex. Both species had similar breeding pattern throughout the year.

The seroprevalence of NiV in *P. hypomelanus* and *P. vampyrus* were 11% and 32.5%, respectively. The odds ratio of seropositive for NiV was higher in *P. vampyrus* compared to *P. hypomelanus*. A repeated cross-sectional study show that NiV seroprevalence in a single population of *P. hypomelanus* ranged between 1% and 20%. The seroprevalence was found associated with time and the reproductive status of female bats. The bats that were either pregnant, lactating, carrying or nursing a pup were at a significantly higher risk to be seropositive when compared to dry bats.

A prospective study on the bats revealed at least 5 basic serological patterns: i) High Static Positive, ii) Low Static Positive, iii) Waned-off, iv) Waned-off and Rising and v) Static Negative. Passive immunity to NiV of pup born to seropositive dam was detected for a period of up to a year. This suggests that the maternal antibody against NiV may last up to a year in captive bats.

The isolation of the virus from a bat's urine from 'Waned-off and Rising' antibody pattern provides for the first time, the objective evidence of the possible viral recrudescent in *Pteropus* bats. The virus was excreted in very low concentration and in a

very short time period. This indicates that a very narrow window exist where NiV is shed by bats in the wild. The seroconvertion of another two bats within a month after the virus isolation suggests the possibility of horizontal transmission within the colony. The NiV incidence rate for seroconversion was 486 per 1000 bat-year.

Stress in seropositive bats induced chemically resulted in an increased neutrophil and decrease in lymphocytes count. However, no virus was discovered from samples collected during the experiment and from organs at the end of the study.

The findings from the study have contributed significantly to the understanding on the distribution of NiV among healthy *Pteropus* bats, transmission and persistency of the virus within the colony, and the basic bat immune response due to NiV infection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

EPIDEMIOLOGI, PENYEBARAN DAN PENGASINGAN VIRUS NIPAH DALAM KELUANG (SPESIS *PTEROPUS*) DI SEMENANJUNG MALAYSIA

Oleh

SOHAYATI ABD RAHMAN

Februari 2009

Pengerusi: Latiffah Hassan, PhD

Fakulti: Perubatan Veterinar

Keluang dari genus *Pteropus* seringkali dikaitkan sebagai perumah reservoir semulajadi untuk NiV dan virus henipavirus lain. Kajian ini dijalankan untuk mengkaji dengan lebih terperinci mengenai epidemiologi NiV dalam *Pteropus* sp. di Semenanjung Malaysia. Objektif khusus untuk kajian ini adalah untuk menerangkan taburan geografi dan ciri-ciri populasi kedua-dua jenis spesis *Pteropus* dalam semenanjung, menerangkan taburan geografi antibodi NiV dalam keluang di semenanjung, mengenal pasti faktor-faktor risiko yang berkaitan dengan jangkitan pada keluang, menentukan laluan perkumuhan semulajadi NiV dari badan keluang termasuk cara ia disebarkan serta pola serologi jangkitan NiV dalam keluang, menganggarkan kadar seroprevalen dan jangkitan (seroconversion) NiV dalam keluang dan juga untuk mengkaji kemungkinan kemunculan kembali NiV (dari jangkitan pendam) dalam keluang yang terjangkit secara semulajadi

dan dalam keluang berstatus seropositif kepada NiV dibawah aruhan `immunosuppresion'

Pteropus vampyrus dan *P. hypomelanus* boleh ditemui dihampir keseluruhan Semenanjung Malaysia. *Pteropus hypomelanus* boleh ditemui di pulau-pulau sekitar semenanjung sementara *P. vampyrus* boleh ditemui di tanah besar semenanjung. *Pteropus vampyrus* didapati sangat sensitif dengan aktiviti manusia walau pada tahap yang rendah. Secara fisikal, *P. vampyrus* didapati lebih besar dan berat dari *P. hypomelanus*. Ciri-ciri fizikal kedua-dua spesis keluang memiliki perbezaan yang bererti diantara umur dan jantina. Kedua-dua spesis keluang ini memiliki pola pembiakan yang hampir serupa untuk sepanjang tahun.

Seroprevalens NiV dalam *P. hypomelanus* dan *P. vampyrus* adalah 11%. dan 32.5%, setiapnya. Risiko untuk menjadi seropositif kepada NiV adalah lebih tinggi dalam *P. vampyrus* berbanding *P. hypomelanus*. Dalam kajian rentas berulang didapati seroprevalens terhadap NiV pada salah salah satu koloni *P. hypomelanus* adalah diantara 1% hingga 20%. Seroprevalens ini didapati berkait rapat dengan masa dan status pembiakan keluang betina. Keluang yang samaada sedang mengandung, membawa atau menyusukan anak didapati memiliki risiko yang lebih tinggi untuk menjadi seropositif berbanding keluang betina yang tidak aktif dalam pembiakan.

Dari kajian prospektif yang dijalankan didapati sekurang-kurangnya 5 pola serologi asas NiV dalam keluang: i) Positif Statik Tinggi ii) Positif Statik Rendah iii) Penurunan iv) Penurunan dan Peningkatan dan v) Negatif Statik. Immuniti pasif NiV dalam anak

keluang yang lahir dari ibu yang berstatus seropositif telah dikesan untuk jangkamasa yang menghampiri setahun. Ini mencadangkan bahawa yang antibodi terhadap NiV yang diperolehi dari ibu mungkin dapat bertahan selama setahun dalam anak keluang yang dikurung bersama ibu.

Pengasingan virus dari air kencing salah seekor keluang dari pola serologi `Penurun dan Peningkatan' merupakan bukti kepada pengaktifan semula jangkitan dari jenis pendam atau `latent' NiV dalam keluang. Virus telah dirembeskan pada kadar kepekatan yang sangat rendah dan dalam masa yang sangat singkat. Ini menunjukan bahawa penyebaran virus yang berlaku dalam keadaan semulajadi adalah sangat terhad. Kadar jangkitan berdasarkan 'seroconversion' dalam kajian ini adalah 486 per 1000 tahun keluang.

Tekanan (stress) dibawah aruhan bahan kimia pada keluang seropositif telah menyebabkan peningkatan kiraan sel neutrofil dan penurunan sel leukosit. Walau bagaiamana pun, tiada virus ditemui dari sampel yang diambil semasa kajian dan dari organ-organ keluang berkenaan diakhir kajian.

Penemuan dari kajian ini telah menyumbang kepada pemahaman dan pengetahuan terhadap taburan NiV dikalangan keluang yang sihat, cara penyebaran dan bagaimana virus boleh terus kekal dalam koloni keluang, serta asas kepada pengetahuan terhadap tindak balas immuniti keluang terhadap jangkitan NiV.

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I certify that a Thesis Examination Committee has met on 29 May 2009 to conduct the final examination of Sohayati binti Abd Rahman on her thesis entitled "Epidemiology, Transmission and Isolation of Nipah virus in Large Fruit Bats (*Pteropus* Species) in Peninsular Malaysia" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date: 8 June 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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SOHAYATI ABD RAHMAN

Date: 6 July 2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES