



UNIVERSITI PUTRA MALAYSIA

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

SOHAYATI ABD RAHMAN

FPV 2009 5



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

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**

February 2009





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**

By

SOHAYATI ABD RAHMAN

February 2009

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 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 recrudescence 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 seroconversion 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 arahan 'immunosuppresion'

Pteropus vampyrus dan *P. hypomelanus* boleh ditemui di hampir 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 fizikal, *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 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 'Penurunan 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 menunjukkan 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 bagaimanapun, 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.

ACKNOWLEDGEMENTS

“With the name of Allah which is the most generous and loving creator”

I would like to express my appreciation to the main supervisor Dr. Latiffah Hassan for her supervision, advice, as well as for giving me the freedom and independence to carry out my work while providing me unflinching encouragement and support in various ways. To supervisory committee member, Dr Sharifah Syed Hassan, for her continual scholarship and spirit in regard to research, also for her expertise in virology. To Dato’ Dr. Abdul Aziz Jamaluddin and Associate Professor Dr. Siti Suri Arshad for their guidance, advice, and encouragement throughout the course of study and in the preparation of this thesis.

I acknowledge Dr. Peter Daszak and Dr. Jonathon H. Epstein from the Consortium for Conservation Medicine, NY and Dr. Hume Field from Biosecurity Queensland, Department of Primary Industries and Fisheries, AUS for their crucial contribution and support on the project of ecology of Nipah virus in *Pteropus* sp. in Malaysia.

I am especially grateful to the Department of Veterinary Services Malaysia for giving me the opportunity to be involved in this project and to further my study. Many thanks to the Department of Public Services Malaysia for providing the four years scholarship to perform this study.



Special thanks to the internal fund of Veterinary Research Institute and ‘NIH/NSF-‘Ecology of Infectious Disease’ award through the Consortium for Conservation Medicine for funding this four years project.

In addition, I thank the previous and current director of Veterinary Research Institute, Department of Veterinary Services Malaysia, for granting permission to use the facilities in the institute, especially Biosafety Level 2 and 3 laboratories. Thanks are also extended to Department of Wildlife and National Parks Malaysia for their guidance and granting permission to sample bats throughout Peninsular Malaysia, and to Zoo Taiping and Night Safari for granting permission to use the facilities, especially Animals Quarantine station.

I am indebted to the field and laboratory crews for their assistance for sampling and laboratory work over the years; in particularly to M. Shamsyul Naim, Norhayati M. Noor, N., Zaini Che Mamat, Azizi Mat Yatim, Amir Nordin, Karim Abdul Hamid, and Thomas Hughes. I thank Mr. Shuhaili Abu Bakar, Roseman Abu Bakar, Adnan Rashid and Ibrahim Md. Hassim of transport unit, Veterinary Research Institute for their services in getting the crewmembers to sampling destinations and for lending an extra hand during sample collection.

I thank the bat hunters for the information of their activities and granting me permission to collect samples from their hunted bats. Thanks are also extended to the local resident in Pulau Tioman, Pulau Kapas and Pulau Perhentian for their cooperation and curiosity during the bats sampling.



My gratitude to a number of colleagues; Dr Maizan Mohamed, Mrs. Suriani M. Noor, Mrs Sharina and Miss Shamsiah of Avian virology laboratory in Veterinary Research Institute, Malaysia for their technical guidance in PCR and assistance in gene sequencing. Staff from the Monoclonal laboratory; Adam Lee, Ali A. Rahman and Fauad Tuah for technical guidance in viral isolation and IFAT technique.

I sincerely appreciate the assistance of Dr. Kim Halpin, Dr. Alex Hyatt, Dr. Chris Morrissy and Greer Mehan of the Immunology Laboratory in Australian Animals Health Laboratory, Australia for the confirmation of the SNT. My appreciation to all colleagues in Veterinary Research Institute and Henipa Ecology Research Group (HERG) for their warm friendship and kindness.

My parents deserve special mention for their support and prayers. Words fail me to express my appreciation to my husband Mr. Zaini Che Mamat, whose dedication, love and persistent confidence in me, has taken the load off my shoulder. To my son Muhammad Nazrin Asyraf and my daughter Nur Izzah Ayuni, you are the source of my strength and perseverance.

Finally, I would like to thank everyone who has contributed to the successful realisation of this thesis, as well as expressing my apology that I could not mention them personally one by one.



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.

Members of the Examination Committee were as follows:

Saleha Abdul Aziz, PhD

Professor
Faculty of Graduate Studies
Universiti Putra Malaysia
(Chairman)

Dato’ Munn-Sann Lye, PhD

Professor
Faculty of Graduate Studies
Universiti Putra Malaysia
(Internal Examiner)

Abdul Rani Bahaman, PhD

Professor
Faculty of Graduate Studies
Universiti Putra Malaysia
(Internal Examiner)

Joanne Meer, PhD

Associate Professor
Faculty of Graduate Studies
Universiti Putra Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 29 May 2009



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:

Latiffah Hassan, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Dato' Abdul Aziz Jamaluddin, PhD

Director
Department of Veterinary Services of Malaysia
(Member)

Siti Suri Arshad, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Syarifah Syed Hassan, PhD

Associate Professor
Faculty of Medicine
University Monash (Sunway Campus)
(Member)

HASANAH MOHD. GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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.

SOHAYATI ABD RAHMAN

Date: 6 July 2009



TABLE OF CONTENTS

| | |
|------------------------------|-------------|
| DEDICATION | Page |
| ABSTRACT | iii |
| ABSTRAK | iv |
| ACKNOWLEDGEMENTS | vii |
| APPROVAL | x |
| DECLARATION | xiii |
| LIST OF TABLES | xv |
| LIST OF FIGURES | xxi |
| LIST OF ABBREVIATIONS | xxiv |
| | xxix |

CHAPTER

| | | |
|-------|--|----|
| 1 | INTRODUCTION | 1 |
| 2 | LITERATURE REVIEW | |
| 2.1 | History Of Nipah Virus Infection | 6 |
| 2.1.1 | The Emergence of Nipah virus in Malaysia | 6 |
| 2.1.2 | Clinical Signs and Lesions in Pigs Naturally Infected with Nipah virus | 7 |
| 2.1.3 | Control And Eradication of Nipah virus in Malaysia | 9 |
| 2.1.4 | Retrospective Studies of Archival Sample for Nipah virus Infection | 11 |
| 2.2 | Nipah virus | 11 |
| 2.2.1 | Biological Characteristics of Nipah virus | 11 |
| 2.2.2 | Molecular Characteristics of Nipah virus | 13 |
| 2.3 | Diagnosis of Nipah virus | 19 |
| 2.3.1 | Virus Isolation | 19 |
| 2.3.2 | Reverse Transcriptase-Polymerase Chain Reaction | 20 |
| 2.3.3 | Serum Neutralisation Test | 20 |
| 2.3.4 | ELISA | 21 |
| 2.3.5 | Immunohistochemistry | 23 |
| 2.4 | Methodology for Investigating Wildlife Reservoir of Disease | 23 |
| 2.5 | The Role of Bats in the Epidemiology of Nipah virus Infection | 24 |
| 2.5.1 | Global Distribution of <i>Pteropus</i> Bats | 25 |
| 2.5.2 | Malaysian Pteropid Bats | 25 |
| 2.5.3 | <i>Pteropus hypomelanus</i> (<i>P. hypomelanus</i>) | 26 |
| 2.5.4 | <i>Pteropus vampyrus</i> (<i>P. vampyrus</i>) | 28 |



| | | |
|----------|--|----|
| 2.6 | Nipah virus Reservoir | 29 |
| 2.6.1 | Serological and Prevalence of Nipah virus Infection in Bats of Malaysia, Cambodia, Thailand and Indonesia | 30 |
| 2.6.2 | Clinical Signs and Lesions in Bats Experimentally Infected with Nipah virus | 32 |
| 3 | GENERAL MATERIALS AND METHODS | |
| 3.1 | Target and Study Population | 34 |
| 3.2 | Estimating Population Size | 35 |
| 3.2.1 | Mark and Capture | 35 |
| 3.2.2 | Head Count Method | 37 |
| 3.3 | Sample Size | 38 |
| 3.4 | Bats Sampling | 38 |
| 3.4.1 | Hunt Method | 39 |
| 3.4.2 | Trap Method | 40 |
| 3.4.3 | Bat Immobilisation | 50 |
| 3.5 | Biological Sampling | 53 |
| 3.5.1 | Sampling of Live Bats | 53 |
| 3.5.2 | Sampling of Dead Bats | 56 |
| 3.6 | Data Collection | 59 |
| 3.6.1 | Global Positioning | 59 |
| 3.6.2 | Identification Number | 59 |
| 3.6.3 | Commutation/Interview with Hunter and Local Residents | 59 |
| 3.6.4 | Bats Biological Data | 60 |
| 3.7 | Transportation and Storage of Samples | 64 |
| 3.8 | Laboratory Analysis | 65 |
| 3.8.1 | Viral Isolation | 65 |
| 3.8.2 | Detection of Specific Antibody to Nipah virus using Serum Neutralization Test (SNT) | 67 |
| 3.8.3 | Detection of Viral RNA using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) | 73 |
| 3.9 | Data Analysis | 76 |
| 3.9.1 | Descriptive Data | 76 |
| 3.9.2 | Seroprevalence | 77 |
| 3.9.3 | Odds Ratio of Seropositivity | 77 |
| 3.9.4 | Confidence Interval | 78 |
| 3.9.5 | Incidence Rate | 79 |
| 4 | POPULATION CHARACTERISTICS OF <i>PTEROPUS VAMPYRUS</i> AND <i>PTEROPUS HYPOMELANUS</i> IN PENINSULAR MALAYSIA | |
| 4.1 | Introduction | 80 |



| | | |
|----------|---|-----|
| 4.2 | Materials and Methods | 81 |
| 4.2.1 | Definitions on Terms | 81 |
| 4.2.2 | Data Collection | 81 |
| 4.2.3 | Data Analysis | 81 |
| 4.3 | Results | 82 |
| 4.3.1 | <i>P. hypomelanus</i> | 82 |
| 4.3.2 | <i>P. vampyrus</i> | 93 |
| 4.4 | Discussion | 111 |
| 4.5 | Conclusion | 114 |
| 5 | SPATIAL DISTRIBUTION OF NIPAH VIRUS INFECTION IN <i>PTEROPUS</i> SPECIES IN PENINSULAR MALAYSIA | |
| 5.1 | Introduction | 115 |
| 5.2 | Materials and Methods | 116 |
| 5.2.1 | Study design | 116 |
| 5.2.2 | Bats Sampling | 116 |
| 5.2.3 | Data Collection | 117 |
| 5.2.4 | Samples Collection | 117 |
| 5.2.5 | Samples Transportation and Storage | 117 |
| 5.2.6 | Laboratory Analysis | 117 |
| 5.2.7 | Data Analysis | 118 |
| 5.3 | Results | 119 |
| 5.3.1 | Viral Isolation and Seroprevalence of Nipah virus in <i>P. hypomelanus</i> between Regions | 119 |
| 5.3.2 | Viral Isolation and Seroprevalence of Nipah virus in <i>P. vampyrus</i> between Regions | 120 |
| 5.3.3 | Comparison of Seroprevalence between <i>P. hypomelanus</i> and <i>P. vampyrus</i> | 121 |
| 5.4 | Discussion | 123 |
| 5.5 | Conclusion | 125 |
| 6 | TEMPORAL DISTRIBUTION AND RISK FACTORS OF NIPAH VIRUS INFECTION IN A COLONY OF <i>PTEROPUS HYPOMELANUS</i> IN PULAU TIOMAN, PAHANG | |
| 6.1 | Introduction | 126 |
| 6.2 | Materials and Methods | 127 |
| 6.2.1 | Study Design | 127 |
| 6.2.2 | Bats Sampling | 127 |
| 6.2.3 | Data Collection | 128 |
| 6.2.4 | Samples Collection | 128 |
| 6.2.5 | Samples Transportation and Storage | 128 |
| 6.2.6 | Laboratory Analysis | 128 |
| 6.2.7 | Data Analysis | 129 |
| 6.3 | Results | 130 |
| 6.3.1 | Passive Immunity of the Young <i>Pteropus</i> | 131 |



| | | | |
|----------|--|---|-----|
| | | (Pup) | |
| | 6.3.2 | Prevalence (%) of Nipah virus and the Distribution of Nipah virus Positive Titre | 131 |
| | 6.3.3 | Logistic Regression Analysis | 134 |
| 6.4 | Discussion | | 136 |
| | 6.4.1 | Sampling Time | 136 |
| | 6.4.2 | Age | 137 |
| | 6.4.3 | Sex | 138 |
| | 6.4.4 | Reproductive Status | 138 |
| | 6.4.5 | Viral Isolation | 140 |
| 6.5 | Conclusion | | 141 |
| 7 | ISOLATION OF NIPAH VIRUS FROM <i>PTEROPUS VAMPYRUS</i> IN CAPTIVITY | | |
| | 7.1 | Introduction | 142 |
| | 7.2 | Materials and Methods | 142 |
| | 7.2.1 | Study Population | 142 |
| | 7.2.2 | Study Design | 143 |
| | 7.2.3 | Study Location and Sample Size | 143 |
| | 7.2.4 | Data Collection | 144 |
| | 7.2.5 | Samples Collection | 144 |
| | 7.2.6 | Samples Transportation and Storage | 145 |
| | 7.2.7 | Laboratory Analysis | 145 |
| | 7.2.8 | Data Analysis | 145 |
| | 7.3 | Results | 146 |
| | 7.3.1 | Overall Seroprevalence | 146 |
| | 7.3.2 | Seroprevalence in Dams with Pup (at Entry Point) and Dams that gave Birth in the Captive Colony | 148 |
| | 7.3.3 | Seroprevalence in Pups Carried by Dams (on Entry) and Newborn in the Captive Colony | 148 |
| | 7.3.4 | Serological Patterns or Profiles | 152 |
| | 7.3.5 | Incidence Rate | 158 |
| | 7.3.6 | Virus Isolation and Detection | 159 |
| | 7.4 | Discussion | 162 |
| | 7.4.1 | Serological Profiles | 162 |
| | 7.4.2 | Seroprevalence in Newborns, Pups and Dams | 166 |
| | 7.4.3 | Nipah virus Isolation and Detection | 167 |
| | 7.4.4 | Viral Recrudescence | 168 |
| | 7.4.5 | Horizontal Transmission | 170 |
| | 7.5 | Conclusion | 171 |



| | | |
|----------|---|------------|
| 8 | EFFECTS OF DEXAMETHASONE-INDUCED STRESS ON NIPAH VIRUS-SEROPOSITIVE <i>PTEROPUS VAMPYRUS</i> | |
| 8.1 | Introduction | 172 |
| 8.2 | Material and Methods | 173 |
| 8.2.1 | Bats | 173 |
| 8.2.2 | Experimental Design | 174 |
| 8.2.3 | Laboratory Analysis | 174 |
| 8.2.4 | Data Analysis | 175 |
| 8.3 | Result | 176 |
| 8.3.1 | Hematological Responses to Dexamethasone-induced Stress | 176 |
| 8.3.2 | Viral Isolation | 176 |
| 8.4 | Discussion | 177 |
| 8.5 | Conclusion | 183 |
| 9 | SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH | 184 |
| | REFERENCES | 196 |
| | APPENDICES | 207 |
| | BIODATA OF STUDENT | 210 |
| | LIST OF PUBLICATIONS | 211 |



LIST OF TABLES

| Table | | Page |
|-------|--|------|
| 3.1 | Analysis of VRI SNT (various antibody titre) Based on Geelong SNT at seropositive antibody titre at ≥ 5 | 72 |
| 3.2 | Cross-tabulation Between SNT Results from AAHL and VRI | 72 |
| 3.3 | Sequence, Location and Characterization of the Primers used in RT-PCR of Nipah virus | 75 |
| 3.4 | The 2 x 2 Contingency Table for Measurement of Association | 78 |
| 4.1 | Distribution of <i>P. hypomelanus</i> in Pulau Tioman from Repeated Cross-sectional Study Based on Sex, Reproductive Stage and Sampling Time | 83 |
| 4.2 | Distribution of <i>P. hypomelanus</i> from Other Islands in Cross-sectional Study Based on Sex, Reproductive Status and Sampling Time | 84 |
| 4.3 | Measurements of Body Weight Lengths of Forearm, Body and Head of <i>P. hypomelanus</i> in Pulau Tioman | 89 |
| 4.4 | Measurements of Body Weight, Lengths of Forearm, Body and Head of Adult Male <i>P. hypomelanus</i> from Other Islands | 93 |
| 4.5 | Measurements of Body Weight, Lengths of Forearm, Body and Head of Adult Female <i>P. hypomelanus</i> from Other Islands | 93 |
| 4.6 | Distribution of <i>P. vampyrus</i> Captured in Perak Based on Sex, Reproductive Status and Sampling Time | 97 |
| 4.7 | Distribution of <i>P. vampyrus</i> Captured in Pahang Based on Sex, Reproductive Status and Sampling Time | 100 |
| 4.8 | Distribution of <i>P. vampyrus</i> Captured in Johor Based on Sex, Reproductive Status and Sampling Time | 103 |
| 4.9 | Measurements of Body Weight, Length of Forearms, Body and Head of <i>P. vampyrus</i> in Peninsular Malaysia | 105 |
| 4.10 | Measurements of Body Weight, Lengths of Forearm, Body and Head of Adult <i>P. hypomelanus</i> and <i>P. vampyrus</i> | 108 |



| | | |
|-----|--|-----|
| 5.1 | Seroprevalence of 119 <i>P. hypomelanus</i> Sampled at Multiple Locations (Islands) Surrounding Peninsular Malaysia between 2004 and 2006 for Evidence of Nipah virus Infection | 120 |
| 5.2 | Seroprevalence, OR and 95% CI of 252 <i>P. vampyrus</i> Sampled at Multiple Locations in Peninsular Malaysia between 2004 and 2006 for Evidence of Nipah virus Infection | 121 |
| 5.3 | Antibody Titer Range to Nipah virus in <i>P. vampyrus</i> Based on Regions in Peninsular Malaysia | 121 |
| 5.4 | Seroprevalence of 367 <i>Pteropus sp.</i> Sampled at Multiple Locations in Peninsular Malaysia between 2004 and 2006 for Evidence of Nipah virus Infection | 122 |
| 5.5 | Antibody Titer Range to Nipah virus between <i>Pteropus sp.</i> in Peninsular Malaysia | 122 |
| 6.1 | Univariate Association between Independent Variables and Nipah virus Serostatus of 632 <i>P. hypomelanus</i> Surveyed in Pulau Tioman, from January 2004 to October 2006 | 132 |
| 6.2 | The Binary Logistic Regression of Risk Factors for Nipah virus Infection Based on Nipah virus Serostatus in Non-randomly Sampled <i>P. hypomelanus</i> Surveyed in Pulau Tioman, from January 2004 to October 2006 | 135 |
| 7.1 | The IDs and Characteristics of 19 <i>P. vampyrus</i> Bats at Entry Point into the Study | 147 |
| 7.2 | Nipah virus Antibody Titer Range in <i>P. vampyrus</i> Based on Sex and Age Groups | 147 |
| 7.3 | Serial Antibody Titre of Two Pairs of Dams with Pups Carried at the Entry Point of the Study between June 2004 and June 2005 | 149 |
| 7.4 | Serial Antibody Titre of Two Pairs of Dams with Newborn Pups at the Entry Point of the Study between June 2004 and June 2005 | 150 |
| 7.5 | Correlation Between Antibody Titre of Dam and Pup | 150 |
| 7.6 | ‘Static High Positive’ Serological Profile: Serial antibody titres of two bats with two-fourfold or greater fluctuations over a minimum 6 months period | 154 |



| | | |
|------|--|-----|
| 7.7 | `Static Low Positive' Serological Profile: Serial antibody titres of four with two-fold or less fluctuation over a minimum 6 months period | 155 |
| 7.8 | `Waned-off' Serological Profile: Serial antibody titres of four bats those were seropositive on entry and later waned-off to become seronegative over a minimum 6 months period | 156 |
| 7.9 | `Waned-off and Rising' Serological Profile: Serial antibody titres of three bats that were seropositive on entry and later waned off to become seronegative and later reise to a four fold increase over over a minimum 6-month period | 157 |
| 7.10 | The IDs and Weeks at Risk to Nipah virus of 19 Captive <i>P. vampyrus</i> Bats between June 2004 and June 2005 | 158 |
| 7.11 | Number of Samples from the 19 Bats in the Captive Colony that was Examined for Nipah virus in June 2004 and June 2005 | 159 |
| 8.1 | Total leucocytes (WBC) and various Types of White Blood Cells (WBC) Counts in Captive <i>P. vampyrus</i> (bat ID 33 & 26) following Dexamethasone-induced Immuno-Selepression | 180 |
| 8.2 | Serial Antibody Titre to Nipah virus of Two Seropositive <i>P. vampyrus</i> following Dexamethasone-induced Immuno-Selepression | 181 |



LIST OF FIGURES

| Figure | | Page |
|--------|--|------|
| 2.1 | The Spread of Nipah Virus from Tambun to the North and South of the Country | 8 |
| 2.2 | Timeline of the Emergence of Henipavirus | 10 |
| 2.3 | Structure of Henipavirus | 14 |
| 2.4 | The Henipavirus Genome | 15 |
| 2.5 | A Phylogenic Tree Based on the Deduced Amino Acid Sequences of the Matrix Protein of Member of the Family <i>Paramxoviridae</i> | 16 |
| 2.6 | The Phylogenetic Relationship Between the N Gene Sequences of the 4 Human Nipah virus Isolates from the Bangladesh Outbreak in 2004 and the N Gene Sequences from Pig and Human Nipah virus Isolates from Malaysia | 18 |
| 2.7 | The Phylogenetic Tree of Partial M-Gene Nucleotide Sequences of Siliguri (India) Nipah virus Isolates from the Bangladesh and Malaysia Isolates | 18 |
| 2.8 | The Global Distribution of Megachiroptera and <i>Pteropus</i> Species | 25 |
| 2.9 | Geographic Distributions of <i>P. hypomelanus</i> in Southeast Asia | 27 |
| 2.10 | Geographic Distributions of <i>P. vampyrus</i> in Southeast Asia | 29 |
| 3.1 | Sampling Locations <i>P. vampyrus</i> and <i>P. hypomelanus</i> in Peninsular Malaysia | 36 |
| 3.2 | A Hunter Aiming at the Bats | 40 |
| 3.3 | Bats Shot Down During Hunting Activity | 41 |
| 3.4 | Erecting the Pole from the Tip at Pulau Tioman | 44 |
| 3.5 | Second (Bottom) Pulley Mounted using Nylon Rope | 45 |