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LYSSAVIRUS SURVEILLANCE IN FRUIT BATS AT A LIVESTOCK BREEDING CENTRE IN PERAK, MALAYSIA

MUHAMMED MIKAIL

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By

MUHAMMED MIKAIL

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the requirement for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to my beloved Parents

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

LYSSAVIRUS SURVEILLANCE IN FRUIT BATS AT A LIVESTOCK BREEDING CENTRE IN PERAK, MALAYSIA

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Chairman **: Tengku Rinalfi Putra Bin Tengku Azizan, PhD**
Faculty **: Veterinary Medicine**

Fruit bats of the family Pteropodiformes constituted about 21 species out of 110 species of bats found in Peninsular Malaysia. These bats are known to be potential reservoirs of emerging viral zoonosis such as the Lyssavirus, Nipah virus, Tioman virus and Palau virus. Malaysian Fruit bats are threatened with habitat loss due to conversion of forest into agricultural and urban areas which subsequently result to encroachment of fruit bats into other habitat such as livestock and agricultural farms for foraging, roosting or both, hence these farms are at risk of spill over infection from wildlife due to close proximity or contact with contaminated discard fruit with other livestock or humans. All the 14 recognized Lyssaviruses with the exception of Mokola lyssavirus are known to spill over from bats to susceptible wildlife, livestock or pets and humans. The rabies lyssavirus reservoir host status of Malaysian fruit bats or any other Malaysian wildlife mammals is not known, hence the spill over risk is also not known and until 2015, when Malaysia lost its rabies free status, dogs are known to be the only reservoir host of rabies lyssavirus and responsible for rabies outbreak in Malaysia. The occurrence and transmission of bat lyssaviruses depends on several factors such as the host, host ecology, host species distribution and the particular population under study. The aim of this study is to determine the possibilities of fruit bats encroaching livestock farms that borders wildlife sanctuaries, the fruit bats species diversity in a livestock farm for possible risk of spill over infection and determine the possibility of Malaysian fruit bats, as rabies lyssavirus reservoirs host. Fifty individual fruit bats (50) were captured in a livestock area, identified to species level, and brain tissues were collected for histopathology. *Cynopterus brachyotis* was the highest captured fruit bat species, representing 40% of the total capture. The findings of Shannon index is 2.80 and Simpson index is 0.2 are suggestive of a degree of species dominance with low diversity in Lenggong livestock breeding centre. There was no report or published study about demonstration of pathognomonic Negri bodies for rabies lyssavirus in its wildlife reservoir host in Malaysian mammals such as the bats; hence a gap exists in the knowledge of the Lyssavirus reservoir host status of Malaysian fruit bats. This study, Following H & E staining, demonstrated perivascular cellular infiltrates in five (5) brain tissues of fruit bats (*Cynopterus brachyotis)* which is suggestive of viral infection and the pathognomonic rabies Negri bodies in one of the fruit bats brain tissue (*Cynopterus brachyotis),* which are suggestive of rabies lyssavirus. This study suggests that fruit bats are indeed; encroaching livestock farm that borders wildlife sanctuary, the species diversity was low and could be potential source of infection to susceptible livestock and bat rabies lyssavirus could exists in Malaysian fruit bats.

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

PENGAWASAN VIRUS LYSSA PADA KELAWAR BUAH MALAYSIA DI PUSAT TERNAKAN HAIWAN LENGGONG, PERAK, MALAYSIA

Oleh

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Kelawar Buah dalam keluarga Pteropodiformes terdiri daripada 21 spesis daripada 110 spesis kelawar yang dijumpai di Semenanjung Malaysia. Kelawar-kelawar ini dikenali berpotensi menjadi takungan dalam penyebaran penyakit zoonosis bawaan virus seperti Virus Lyssas, Virus Nipah, Virus Tioman dan Virus Pulau. Kelawar Buah Malaysia terancam dengan kemusnahan habitat disebabkan oleh penerokaan kawasan hutan ke kawasan pertanian dan bandar dan seterusnya menyebabkan kelawar tersebut memasuki kawasan habitat lain seperti ladang ternakan untuk mencari makanan, berteduh atau kedua-duanya, oleh itu ladang-ladang ini berisiko menyebarkan jangkitan daripada hidupan liar kerana buah-buahan daripada sisa makanan kelawar yang tercemar terdedah kepada ternakan atau manusia lain. Kesemua 14 Virus Lyssa yang dikenalpasti kecuali Virus Lyssa Mokola diketahui tersebar daripada kelawar kepada hidupan liar yang terdedah, haiwan ternakan atau haiwan peliharaan dan manusia.Status perumah Virus Lyssa Rabies daripada takungan Kelawar Buah Malaysia atau mana-mana hidupan liar mamalia di Malaysia yang lain tidak diketahui, oleh itu penyebaran ke atas risiko juga tidak diketahui dan sehingga tahun 2015, apabila status bebas rabies di Malaysia hilang, anjing dikenalpasti sebagai satu-satunya perumah takungan Virus Lyssa Rabies dan penyebab terhadap penyebaran wabak rabies di Malaysia. Kewujudan dan penyebaran Virus Lyssa Kelawar bergantung kepada beberapa faktor seperti perumah, ekologi perumah, taburan spesis perumah dan populasi tertentu masih dalam kajian. Tujuan kajian ini adalah untuk menentukan kebarangkalian Kelawar Buah memasuki ladang ternakan yang bersempadanan dengan santuari hidupan liar, kepelbagaian spesis Kelawar Buah di ladang ternakan untuk kemungkinan risiko terkena jangkitan dan menentukan kemungkinan Kelawar Buah Malaysia sebagai perumah takungan Virus Lyssa Rabies. Lima puluh (50) individu Kelawar Buah telah ditangkap di kawasan ladang ternakan, dikenalpasti spesisnya dan tisu otak diambil untuk kajian histophatologi.*Cynopterus brachyotis* adalah sepsis yang paling banyak ditangkap, mewakili 40% daripada jumlah keseluruhan tangkapan.Hasil penemuan daripada Indeks Shannon adalah 2.80 dan Indeks Simpson adalah 0.2 menunjukkan tahap dominasi spesis dengan kepelbagaian yang rendah di Pusat Ternakan Haiwan Lenggong. Tiada laporan atau kajian yang diterbitkan mengenai demonstrasi patognomonik badan Negri untuk Virus Lyssa Rabies dalam takungan perumah hidupan liar di Malaysia seperti kelawar, oleh itu, wujud kekurangan maklumat pada status takungan perumah Virus Lyssa yang disebabkan oleh Kelawar Buah Malaysia. Pada Kajian ini, daripada pewarnaan H & E, demonstrasi perivaskular selular dari lima (5) tisu otak Kelawar Buah (*Cynopterus brachyotis*) menunjukkan jangkitan virus dan patognomonik badan Negri Rabies dalam salah satu tisu otak Kelawar Buah Malaysia (*Cynopterus brachyotis*), dimana ini menunjukkan ia adalah Virus Lyssa Rabies. Kajian ini menunjukkan bahawa Kelawar Buah adalah sememangnya; memasuki ladang ternakan yang bersempadan dengan santuari hidupan liar, kepelbagaian spesis adalah rendah dan berpotensi menjadi sumber jangkitan kepada ternakan terlibat dan Virus Lyssa Rabies kelawar boleh wujud pada Kelawar Buah Malaysia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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LIST OF ABBREVIATIONS

CDC	Centre for Disease Control and Prevention
FAT	Fluorescent antibody test
H & E	Haematoxylin and Eosin
HCL	Hydrochloride
ICTV	International Committee on Taxonomy of Viruses
mM	milli Molar
Niv	Nipah Virus
OIE	Office International *des* Epizooties
PCR	Polymerase Chain Reaction
PV	Pasteur rabies virus
PuV	Pulau Virus
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
TioV	Tioman virus
WHO	World Health Organization
^{o}C	Degree Celsius
µl	Microliter

CHAPTER 1

INTRODUCTION

1.1 Introduction

Peninsular Malaysia is part of southeast Asia, dominated by a long mass that extends southward from Thailand and bordered by two sides, on the west by Indian ocean and strait of Malacca, on the east by the South China Sea (Andaya & Andaya, 2016). About 50% of the land in Southeast Asia is utilized for agriculture which resulted in high rate of deforestation in the region (Zhao et al., 2006). However conversion of forest to farming land is identified to be the major cause of this devastating effect (Corlett & Primack, 2005).

Livestock, wildlife and their ecosystem affects the health and wellbeing of the humans. This also includes disease transmission risk that emerges at the human, animal and ecosystem interface. More than 75% of zoonotic pathogens are known to originate from wildlife and they have significant impact on the health of the human, livestock and wildlife respectively (Takayama-Ito *et al.,* 2006). Infectious diseases are known to occur within balanced host-agent equilibrium and within their natural ecological zones, but some wildlife pathogens had spill over and emerge in livestock population (Patz *et al*., 2005).

The emergence, re-emergence and pathogen transmission occur as a result of many factors some of which includes; ecological dynamics, land use for agricultural practises and deforestation which create a contact of man or livestock to wildlife and changes the existing ecology of infectious agents and their reservoir host (Bengis *et al.,* 2004).

Livestock farming which is a subsector of agriculture constitute about 37% of the agricultural gross domestic product worldwide(Thornton, 2010)and disease agent causes both direct losses to the sector through decreased productivity and increased mortality of the livestock at the same time indirect losses related to the cost of control, market values and insecurity and many more (Dehove*et al.,* 2012). Mortality are usually due to diseases which most of them are shared between livestock and wildlife, and this create a burden to the ecosystem, thereby affecting biodiversity, the animal populations as well as making the species at the stage of going extinct (Daszak *et al.,* 2000). The role of wildlife at wildlife-livestock interfaces have been characterized as spill over or spill back, maintenance or dead end hosts (Bengis *et al.,* 2002).

Wildlife continues to play an important role in the emergence of diseases of livestock and human as a result of multiple changes taking place within the wildlife-livestock interface (Jones *et al.,* 2008). Present day livestock productions continue to increasealong with encroachment into area of wildlife through land alteration (Daszak *et al.,* 2000). The population demography of wildlife resulted in increased contact rate and infectious agent transmission at the wildlife-livestock interface.

Bats of the order Chiroptera are the only mammals known to have sustained powered flight which are classified into two suborders; The Yinpterochiroptera or the Pteropodiformes that comprises a taxa known before as Megabats and five Microbats families namely; Craseonycteridae, Hipposideridae, Rhinopomatidae, Rhinolophidae and Megadermatidae. The Yangochiroptera (Vespertilioniformes) are made up of mostly Microbats with the exception of the above listed in Yinpterochiroptera (Tsagkogeorga *et al.,* 2013). Tsagkogeorga *et al.* (2013) also reports that based on evidence of fossil records, bats exist for more than 65million years ago and have undergone a rapid diversification during this period. The actual bat diversity in Malaysia may be under estimated as recently identified species remained previously unknown due to their cryptic morphology (Murray *et al.,* 2012).

About 110 species of bats exist in Peninsular Malaysia, out of which 105 have known locality records (Lim *et al.,* 2017). Bats are known to be associated with many viral pathogens. They have been implicated in numerous emerging infectious disease events and are continuously recognized as an important reservoir host for viruses that cross the species barrier to infect humans, livestock and wildlife. There are about 66 different species of viruses been isolated from bats or being detected within their tissues and there are serological evidences for many(Shi, 2013).

Bat lyssavirus infection occurs across the globe, although different virus species are present in different region and tend to infect particular bat species. Bat rabies lyssavirus are found only in bats of the new world, with no report of related lyssavirus in bats of the Americas. Related lyssaviruses are reported only in bat population across Africa, Eurasia and Australia (Banyard *et al.,* 2011).

1.2 Statement of the problem

1. Dogs are known to be the primary reservoir host of rabies lyssavirus and there is no known wildlife reservoir host of rabies in Malaysia (Shamsuddin, 2001).
2. Malaysia is known to be a rabies free country, until 2015 when it lost its status following an outbreak of canine rabies (Kamaruddin, 2015).
3. Malaysia was classified as a high risk rabies lyssavirus country due to lack of routine lyssavirus surveillance, as a result of which the status of rabies lyssavirus reservoir host of Malaysian bats is not known (www.gov.uk/government/publications/rabies-risk-by country/rabies-risk-in terrestrial-animals-by-country) Accessed on 10/11/2017

1.3 Justification of the research

The home range of wildlife can sometimes contribute to the emergence of disease agent at the interface between wildlife and livestock and wildlife and human. In most instances, it is usually not always a direct physical interaction or even sharing of the same space at the same time. An example of this is the indirect contact through the soil, food and water and has been in contact with infected animal or the body excretions such as the faces, urineand saliva ocular or nasal discharges or through shared insect vectors or intermediate host (Bengis *et al.,* 2002).

Wildlife-livestock interface refers to an area where wildlife meets with livestock. It can either be dynamic, bidirectional, multidimensional and is usually located at a physical contact point that they all meet to access the natural resources such as farm land or range land(Siembieda *et al.,* 2011). Rabies lyssavirus mode of transmission is a bidirectional with direct contact between wildlife and livestock and occasional spill over to humans. However, in some areas, majority of rabies cases are from domestic dog and cattle while in Africa and North America different wildlife species are reported to play a role in the maintenance and spread of rabies lyssavirus (Bengis *et al.,* 2002).

Livestock farms at the border of wildlife sanctuaries, causes expansion in grazing land for livestock and sharing of habitat with the wildlife. Fruit bats are known to excrete infectious agent through urine, faces and saliva in the partially eaten fruit in areas around the trees or orchard where they feed or roost. If this particular roosting site is located within an animal farm, the livestock are at risk of being exposed to the pathogens through different means such as consumption of contaminated discarded fruit pulp from bats by horses resulting to Hendra virus outbreak(Edson *et al.,* 2015).

However in the tropics, early morning or afternoon grazing of livestock is common and the high ambient temperature causes the livestock to seek protection under the tree shade. Bats are known to forage from sunset to sunrise and roost on trees, where the livestock concentrate for wallowing, hence these areas act as an interface for disease transmission from bats to the livestock through faecal excretion or consumption of discarded fruit contaminated by bats saliva (Ayele *et al.,* 2004).

In Malaysia, similar example of spill over occurred in the transmission of Nipah virus from bats to pigs and eventually to humans(Plowright *et al.,* 2015). The emergence of bat-related zoonotic viral diseases has been attributed to the loss of natural habitats of bats which were destroyed by human activities as a result of which bats faces challenges of acquiring food. This in turn will result in a weaker immune system, leading to an increase in virus load, that will eventually be secreted into the environment through their urine and saliva(Plowright *et al.,* 2015).

Livestock rabies has been documented in Malaysia and most outbreak of rabies occurs within the immune zone of Peninsular Malaysia (Shamsuddin, 2001). Rabies immune zone refers to an area that borders rabies endemic areas, which carried out intense control measures in order to control its spread. In Malaysia, it was set in 1995 in the Peninsula which comprises the northern states of Perlis, Kedah, Kelantan and Perak and it usually covers 30km to 80km from the Thailand (www.nst.com.my/option/establish rabies immune belt border).

While wildlife rabies has not been documented, upon considering the relative abundance of fruit bats which are known reservoir host of zoonotic viruses (Chua *et al.*, 2002), the possibilities of fruit bats encroaching into livestock farms that borders wildlife sanctuaries, the fruit bat species diversity in a livestock farm and the lyssavirus reservoir host status of Malaysian fruit bat need to be investigated.

However, the relationship of Malaysian fruit bats and lyssavirus is not known, hence we begin with the null hypothesis that;

1. fruit bats do not encroached livestock farm area that borders wildlife sanctuaries
2. the species diversity of fruit bats in a livestock farm was low
3. Malaysian fruit bats are not rabies lyssavirus reservoir host

1.4 Main aim of the research:

The main aim of this research is to determine the ecology and rabies reservoir host status of Malaysian fruit bats in the altered ecosystem of livestock farming.

1.5 Objectives of the research

The objectives of this research are;

1. to determine the possibilities of fruit bats encroaching into livestock farm that borders wildlife sanctuaries.
2. to determine the species diversity of Malaysian fruit bats in a livestock farm area.
3. to determine the rabies lyssavirus reservoir host status of Malaysian fruit bats.

REFERENCES

Andaya, B. W., & Andaya, L. Y. (2016). *A History of Malaysia*: Palgrave Macmillan.

Anderson, A., Shwiff, S., Gebhardt, K., Ramírez, A. J., Shwiff, S., Kohler, D., & Lecuona, L. (2014). Economic Evaluation of Vampire Bat (Desmodus rotundus) Rabies Prevention in Mexico. *Transboundary and Emerging Diseases, 61*(2), 140-146. doi: 10.1111/tbed.12007.

Baby, J., Mani, R. S., Abraham, S. S., Thankappan, A. T., Pillai, P. M., Anand, A. M., Sreekumar, S. (2015). Natural Rabies Infection in a Domestic Fowl (Gallus domesticus): A Report from India. *PLoS Negl Trop Dis, 9*(7), e0003942. doi: 10.1371/journal.pntd.0003942.

Badrane, H., & Tordo, N. (2001). Host Switching in Lyssavirus History from the Chiroptera to the Carnivora Orders. *J Virol, 75*(17), 8096-8104. doi: 10.1128/jvi.75.17.8096-8104.2001.

Bengis, R. G., Leighton, F. A., Fischer, J. R., Artois, M., Morner, T., & Tate, C. M. (2004). The role of wildlife in emerging and re-emerging zoonoses. *Rev Sci Tech, 23*(2), 497-511.

Botvinkin, A. D., Poleschuk, E. M., Kuzmin, I. V., Borisova, T. I., Gazaryan, S. V., Yager, P., & Rupprecht, C. E. (2003). Novel lyssaviruses isolated from bats in Russia. *Emerg Infect Dis, 9*(12), 1623-1625. doi: 10.3201/eid0912.030374.

Bibi., Fehmeeda., & Zulfiqar. (2013). Measurement of avian communities at Taunsa Barrage Wildlife Sanctuary, Pakistan. *J. of Anim & Plant Sci*. 23: 469-474.

Centers for Disease, Control, & Prevention. (1999). Update: outbreak of Nipah virus--Malaysia and Singapore, 1999. *MMWR Morb Mortal Wkly Rep, 48*(16), 335-337.

Chua, K. B. (2003). Nipah virus outbreak in Malaysia. *J Clin Virol, 26*(3), 265-275. doi: 10.1016/s1386-6532(02)00268-8

Chua, K. B., Bellini, W. J., Rota, P. A., Harcourt, B. H., Tamin, A., Lam, S. K., Mahy, B. W. J. (2000). Nipah Virus: A Recently Emergent Deadly Paramyxovirus. *Science, 288*(5470), 1432.

Chua, K. B., Koh, C. L., Hooi, P. S., Wee, K. F., Khong, J. H., Chua, B. H., Lam, S. K. (2002). Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect, 4*(2), 145-151.

Cliquet, F., Freuling, C., Smreczak, M., Van der Poel, W., Horton, D., Fooks, A., Müller, T. (2010). Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union. *EFSA Supporting Publications, 7*(7), 67E. doi: doi:10.2903/sp.efsa.2010.EN-67.

Constantine, D. G., & Blehert, D. S. (2009). Bat Rabies and Other Lyssavirus Infections *Circular*. Reston, VA.

Dacheux, L., Reynes, J.-M., Buchy, P., Sivuth, O., Diop, B. M., Rousset, D., Bourhy, H. (2008). A Reliable Diagnosis of Human Rabies Based on Analysis of Skin Biopsy Specimens. *Clinical Infectious Diseases, 47*(11), 1410-1417. doi: 10.1086/592969.

David, D., Hughes, G. J., Yakobson, B. A., Davidson, I., Un, H., Aylan, O., Rupprecht, C. E. (2007). Identification of novel canine rabies virus clades in the Middle East and North Africa. *Journal of General Virology, 88*(3), 967-980. doi: doi:10.1099/vir.0.82352-0.

de Wit, E., & Munster, V. J. (2015). Animal models of disease shed light on Nipah virus pathogenesis and transmission. *J Pathol, 235*(2), 196-205. doi: 10.1002/path.4444.

Di Quinzio, M., & McCarthy, A. (2008). Rabies risk among travellers. *CMAJ : Canadian Medical Association Journal, 178*(5), 567-567. doi: 10.1503/cmaj.071443.

Dietzschold, B., Gore, M., Casali, P., Ueki, Y., Rupprecht, C. E., Notkins, A. L., & Koprowski, H. (1990). Biological characterization of human monoclonal antibodies to rabies virus. *J Virol, 64*(6), 3087-3090.

Durr, S., Naissengar, S., Mindekem, R., Diguimbye, C., Niezgoda, M., Kuzmin, I., Zinsstag, J. (2008). Rabies diagnosis for developing countries. *PLoS Negl Trop Dis, 2*(3), e206. doi: 10.1371/journal.pntd.0000206.

Edson, D., Field, H., McMichael, L., Vidgen, M., Goldspink, L., Broos, A., Smith, C. (2015). Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One, 10*(10), e0140670. doi: 10.1371/journal.pone.0140670.

Epstein, J. H., Olival, K. J., Pulliam, J. R. C., Smith, C., Westrum, J., Hughes, T., Daszak, P. (2009). Pteropus vampyrus, a hunted migratory species with a multinational home-range and a need for regional management. *Journal of Applied Ecology, 46*(5), 991-1002. doi: 10.1111/j.1365-2664.2009.01699.

Fooks, A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C., McElhinney, L. M., Müller, T. (2009). Emerging Technologies for the Detection of Rabies Virus: Challenges and Hopes in the 21st Century. *PLoS Negl Trop Dis, 3*(9), e530. doi: 10.1371/journal.pntd.0000530.

Fu, Z. F., Dietzschold, B., Schumacher, C. L., Wunner, W. H., Ertl, H. C., & Koprowski, H. (1991). Rabies virus nucleoprotein expressed in and purified from insect cells is efficacious as a vaccine. *Proceedings of the National Academy of Sciences, 88*(5), 2001.

Gaudin, Y. (2000). Rabies Virus-Induced Membrane Fusion Pathway. *The Journal of Cell Biology, 150*(3), 601-612.

George, D. B., Webb, C. T., Farnsworth, M. L., O'Shea, T. J., Bowen, R. A., Smith, D. L., . . . Rupprecht, C. E. (2011). Host and viral ecology determine bat rabies seasonality and maintenance. *Proc Natl Acad Sci U S A, 108*(25), 10208-10213. doi: 10.1073/pnas.1010875108.

Gilbert, A. T., McCracken, G. F., Sheeler, L. L., Muller, L. I., O'Rourke, D., Kelch, W. J., & John C. New, J. (2015). Rabies Surveillance among Bats In Tennessee, USA, 1996–2010. *J Wildl Dis, 51*(4), 821-832. doi: 10.7589/2014-12-277.

Goh, K. J., Tan, C. T., Chew, N. K., Tan, P. S. K., Kamarulzaman, A., Sarji, S. A.,Lam, S. K. (2000). Clinical Features of Nipah Virus Encephalitis among Pig Farmers in Malaysia. *New England Journal of Medicine, 342*(17), 1229-1235. doi: 10.1056/NEJM200004273421701.

Gongal, G., & Wright, A. E. (2011). Human Rabies in the WHO Southeast Asia Region: Forward Steps for Elimination. *Adv Prev Med, 2011*, 383870. doi: 10.4061/2011/383870.

Gunawardena, P. S., Marston, D. A., Ellis, R. J., Wise, E. L., Karawita, A. C., Breed, A. C., Fooks, A. R. (2016). Lyssavirus in Indian Flying Foxes, Sri Lanka. *Emerg Infect Dis, 22*(8), 1456-1459. doi: 10.3201/eid2208.151986.

Horton, D. L., Banyard, A. C., Marston, D. A., Wise, E., Selden, D., Nunez, A., Fooks, A. R. (2014). Antigenic and genetic characterization of a divergent African virus, Ikoma lyssavirus. *J Gen Virol, 95*(Pt 5), 1025-1032. doi: 10.1099/vir.0.061952-0.

Mani, R. S., Dovih, D. P., Ashwini, M. A., Chattopadhyay, B., Harsha, P. K., Garg, K. M., Madhusudana, S. N. (2017). Serological Evidence of Lyssavirus Infection among Bats in Nagaland, a North-Eastern State in India. *Epidemiol Infect, 145*(8), 1635-1641. doi: 10.1017/S0950268817000310.

Mani, R. S., & Madhusudana, S. N. (2013). Laboratory diagnosis of human rabies: recent advances. *ScientificWorldJournal, 2013*, 569712. doi: 10.1155/2013/569712.

Markotter, W., Kuzmin, I., Rupprecht, C. E., Randles, J., Sabeta, C. T., Wandeler, A. I., & Nel, L. H. (2006). Isolation of Lagos Bat Virus from Water Mongoose. *Emerg Infect Dis, 12*(12), 1913-1918. doi: 10.3201/eid1212.060514.

Marston, D. A., Horton, D. L., Ngeleja, C., Hampson, K., McElhinney, L. M., Banyard, A. C., Lembo, T. (2012). Ikoma lyssavirus, highly divergent novel lyssavirus in an African civet. *Emerg Infect Dis, 18*(4), 664-667. doi: 10.3201/eid1804.111553.

Mavrakis, M., Méhouas, S., Réal, E., Iseni, F., Blondel, D., Tordo, N., & Ruigrok, R. W. H. (2006). Rabies virus chaperone: Identification of the phosphoprotein peptide that keeps nucleoprotein soluble and free from non-specific RNA. *Virology, 349*(2), 422-429. doi: https://doi.org/10.1016/j.virol.2006.01.030.

Meng, S. L., Yan, J. X., Xu, G. L., Nadin-Davis, S. A., Ming, P. G., Liu, S. Y., Yang, X. M. (2007). A molecular epidemiological study targeting the glycoprotein gene of rabies virus isolates from China. *Virus Res, 124*(1-2), 125-138. doi: 10.1016/j.virusres.2006.10.011.

Montgomery, J. M., Hossain, M. J., Gurley, E., Carroll, D. S., Croisier, A., Bertherat, E., Breiman, R. F. (2008). Risk Factors for Nipah Virus Encephalitis in Bangladesh. *Emerg Infect Dis, 14*(10), 1526-1532. doi: 10.3201/eid1410.060507.

Muller, T., Cox, J., Peter, W., Schafer, R., Johnson, N., McElhinney, L. M., Fooks, A. R. (2004). Spill-over of European bat lyssavirus type 1 into a stone marten (Martes foina) in Germany. *J Vet Med B Infect Dis Vet Public Health, 51*(2), 49-54. doi: 10.1111/j.1439-0450.2003.00725.

Murray, S. W., Campbell, P., Kingston, T., Zubaid, A., Francis, C. M., & Kunz, T. H. (2012). Molecular phylogeny of hipposiderid bats from Southeast Asia and evidence of cryptic diversity. *Molecular Phylogenetics and Evolution, 62*(2), 597-611. doi: https://doi.org/10.1016/j.ympev.2011.10.021.

Nahar, N., Mondal, U. K., Sultana, R., Hossain, M. J., Khan, M. S., Gurley, E. S., Luby, S. P. (2013). Piloting the use of indigenous methods to prevent Nipah virus infection by interrupting bats' access to date palm sap in Bangladesh. *Health Promot Int, 28*(3), 378-386. doi: 10.1093/heapro/das020.

Nanayakkara, S., Smith, J. S., & Rupprecht, C. E. (2003). Rabies in Sri Lanka: Splendid isolation. *Emerg Infect Dis, 9*(3), 368-371. doi: 10.3201/eid0903.020545.

Pant, G. R., Horton, D. L., Dahal, M., Rai, J. N., Ide, S., Leech, S., Fooks, A. R. (2011). Characterization of rabies virus from a human case in Nepal. *Arch Virol, 156*(4), 681-684. doi: 10.1007/s00705-010-0868-9.

Parashar, U. D., Sunn, L. M., Ong, F., Mounts, A. W., Arif, M. T., Ksiazek, T. G., Anderson, L. J. (2000). Case-Control Study of Risk Factors for Human Infection with a New Zoonotic Paramyxovirus, Nipah Virus, during a 1998–1999 Outbreak of Severe Encephalitis in Malaysia. *The Journal of Infectious Diseases, 181*(5), 1755-1759. doi: 10.1086/315457.

Peixoto, Z. M. P., Cunha, E. M. S., Sacramento, D. R. V., Souza, M. C. A. M., Silva, L. H. Q. d., Germano, P. L., Kotait, I. (2000). Rabies laboratory diagnosis: peculiar features of samples from equine origin. *Brazilian Journal of Microbiology, 31*, 72-75.

Peet., R. K. (1974).The Measurement of Species diversity. *Annu Rev of Ecol & Systs*. 5: 285-307.

Plowright, R. K., Eby, P., Hudson, P. J., Smith, I. L., Westcott, D., Bryden, W. L., McCallum, H. (2015). Ecological dynamics of emerging bat virus spillover. *Proceedings of the Royal Society B: Biological Sciences, 282*(1798), 20142124. doi: 10.1098/rspb.2014.2124.

Pritchard, L. I., Chua, K. B., Cummins, D., Hyatt, A., Crameri, G., Eaton, B. T., & Wang, L. F. (2006). Pulau virus; a new member of the Nelson Bay orthoreovirus species isolated from fruit bats in Malaysia. *Arch Virol, 151*(2), 229-239. doi: 10.1007/s00705-005-0644-4.

Rahman, S. A., Hassan, L., Epstein, J. H., Mamat, Z. C., Yatim, A. M., Hassan, S. S., Henipavirus Ecology Research, G. (2013). Risk Factors for Nipah virus infection among pteropid bats, Peninsular Malaysia. *Emerg Infect Dis, 19*(1), 51-60. doi: 10.3201/eid1901.120221.

Reynes, J. M., Molia, S., Audry, L., Hout, S., Ngin, S., Walston, J., & Bourhy, H. (2004). Serologic evidence of lyssavirus infection in bats, Cambodia. *Emerg Infect Dis, 10*(12), 2231-2234. doi: 10.3201/eid1012.040459.

Ruf, T., & Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biological Reviews, 90*(3), 891-926. doi: 10.1111/brv.12137.

Rupprecht, C., Kuzmin, I., & Meslin, F. (2017). *Lyssaviruses and rabies: current conundrums, concerns, contradictions and controversies [version 1; referees: 2 approved]* (Vol. 6).

Sazzad, H. M. S., Hossain, M. J., Gurley, E. S., Ameen, K. M. H., Parveen, S., Islam, M. S., Luby, S. P. (2013). Nipah Virus Infection Outbreak with Nosocomial and Corpse-to-Human Transmission, Bangladesh. *Emerg Infect Dis, 19*(2), 210-217. doi: 10.3201/eid1902.120971.

Sendow, I., Ratnawati, A., Taylor, T., Adjid, R. M., Saepulloh, M., Barr, J., Field, H. (2013). Nipah virus in the fruit bat Pteropus vampyrus in Sumatera, Indonesia. *PLoS One, 8*(7), e69544. doi: 10.1371/journal.pone.0069544.

Shi, Z. (2013). Emerging infectious diseases associated with bat viruses. *Science China Life Sciences, 56*(8), 678-682. doi: 10.1007/s11427-013-4517.

Stein, L., Rech, R., Harrison, L., & Brown, C. (2010). Immunohistochemical study of rabies virus within the central nervous system of domestic and wildlife species. *Vet Pathol, 47*. doi: 10.1177/0300985810370013.

Takayama-Ito, M., Inoue, K.-i., Shoji, Y., Inoue, S., Iijima, T., Sakai, T., Morimoto, K. (2006). A highly attenuated rabies virus HEP-Flury strain reverts to virulent by single amino acid substitution to arginine at position 333 in glycoprotein. *Virus Res, 119*(2), 208-215. doi: https://doi.org/10.1016/j.virusres.2006.01.014.

Tao, X. Y., Niezgoda, M., Du, J. L., Li, H., Wang, X. G., Huang, Y., Liang, G. D. (2008). [The primary application of direct rapid immunohistochemical test to rabies diagnosis in China]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi, 22*(3), 168-170.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philos Trans R Soc Lond B Biol Sci, 365*(1554), 2853-2867. doi: 10.1098/rstb.2010.0134.

Yaiw, K. C., Crameri, G., Wang, L., Chong, H. T., Chua, K. B., Tan, C. T., Wong, K. T. (2007). Serological Evidence of Possible Human Infection with Tioman virus, a Newly Described Paramyxovirus of Bat Origin. *The Journal of Infectious Diseases, 196*(6), 884-886. doi: 10.1086/520817.

Yamagata, J., Ahmed, K., Khawplod, P., Mannen, K., Xuyen, D. K., Loi, H. H., Nishizono, A. (2007). Molecular epidemiology of rabies in Vietnam. *Microbiol Immunol, 51*(9), 833-840. doi: 10.1111/j.1348-0421.2007.tb03979.

Yob, J. M., Field, H., Rashdi, A. M., Morrissy, C., van der Heide, B., Rota, P., Ksiazek, T. (2001). Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis, 7*(3), 439-441.

Zhao, S., Peng, C., Jiang, H., Tian, D., Lei, X., & Zhou, X. (2006). Land use change in Asia and the ecological consequences. *Ecological Research, 21*(6), 890-896. doi: 10.1007/s11284-006-0048-2.