UNIVERSITI PUTRA MALAYSIA

JAPANESE ENCEPHALITIS ANTIBODY DETECTION FROM BLOOD SAMPLES OF DOMESTIC DOGS AND CATS IN PENINSULAR MALAYSIA.

PALLIYAGE DON HESHINI ERANDIKA PERERA

FPV 2016 7

JAPANESE ENCEPHALITIS ANTIBODY DETECTION FROM BLOOD SAMPLES OF DOMESTIC DOGS AND CATS IN PENINSULAR MALAYSIA.

PALLIYAGE DON HESHINI ERANDIKA PERERA

A project paper submitted to the

Faculty of Veterinary Medicine, University Putra Malaysia

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

University Putra Malaysia,

Serdang, Selangor DarulEhsan

MARCH 2016

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Japanese Encephalitis Antibody Detection From Blood Samples of Domestic Dogs and Cats in Peninsular Malaysia", by HeshiniErandikaPerera 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 – Project.

DR. GAYATHRI THEVI SELVARAJAH
DVM (UPM), PhD. (Netherlands)
Senior lecturer
Faculty of Veterinary Medicine
University Putra Malaysia
(Supervisor)

ASSOC. PROF. DR. SITI SURI ARSHAD
DVM (UPM), M.Sc. (UPM), PhD. (London)
Deputy Dean of Academic and Student Affairs
Faculty of Veterinary Medicine
University Putra Malaysia
(Co-Supervisor)

DR. OOI PECK TOUNG
DVM (UPM), PhD. (Glasgow)
Senior lecturer
Faculty of Veterinary Medicine
University Putra Malaysia
(Co-Supervisor)

DEDICATIONS

This project paper is dedicated to my dearest family,

Grandmother

Mother

Father

Sister

Sham Pei Ni

Yong Li Hui

Tan Ying Yi

& Tai ShenRong

And to my teachers who have guided many through the path of education, including myself.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to every single person who has contributed to this project;

Firstly, to my dearest supervisors, Dr. Gayathri, Dr.SitiSuri and Dr.Ooi for their endless support and guidance.

To the shelters and University Veterinary Hospital for all their contributions, without which this project would not be possible.

To all the helping hands, Mr. Maniam, Kiven, Mira, Hidaya, Husna, Shing Wei and all other classmates who contributed to this project.

To my family without which I would not be the person I am today and to my close friends Pei Ni, Stephanie and Raquel, as well as my partner ShenRong for staying with me throughout the process.

And finally, to DVM 2011/2016.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
µl	Microliter
CSF	Cerebro-spinal Fluid
ELISA	Enzyme Linked Immunosorbent Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
JE	Japanese Encephalitis
JEV	Japanese Encephalitis Virus
MAC ELISA	Immunoglobulin M Antibody Capture Enzyme Linked Immunosorbent Assay
MVEV	Murray Valley Encephalitis Virus
nm	Nanometer
No.	Number
PCR	Polymerase Chain Reaction
^{o}C	Degree Celsius
SLEV	St. Louis Encephalitis Virus
SPSS	Statistical Package for the Social Sciences
™	Trademark

v	Version
VNT	Virus Neutralisation Test
WHO	World Health Organisation
WNV	West Nile Virus

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999- Final Year Project.

JAPANESE ENCEPHALITIS ANTIBODY DETECTION FROM BLOOD SAMPLES OF DOMESTIC DOGS AND CATS IN PENINSULAR MALAYSIA.

By

Palliyage Don HeshiniErandikaPerera

2016

Supervisor : Dr. GayathriTheviSelvarajah

Co-Supervisor: Assoc. Prof. Dr. SitiSuriArshad, Dr.Ooi Peck Toung

Introduction

Japanese Encephalitis Virus (JEV), of the *Flaviviridae* family, is a known cause of acute encephalitis in humans throughout South East Asia. It is transmitted through mosquito vector, with *Culextritaeniorhynchus*being the vector most associated with spread of the disease. It has been identified in various animals, including in cats and dogs, however, there has been no study done in Malaysia investigating JEV in cats and dogs. The purpose of this study is to identify the presence of JEV antibodies in

cats and dogs in Malaysia, using Enzyme Linked Immunosorbent Assay (ELISA).

Methods

Two to five ml of blood was collected from shelter cats and dogs and two ml of serum was collected from diagnostic samples of cat patients to University Veterinary Hospital, with consent. Information collected for each animal included age, sex, health status, management and environment through observation and patient records. Three ELISA assays were performed, following protocol provided by the manufacturer (SunRed Biotechnology Cat JE IgG ELISA kit and MyBioSource Dog JE IgG ELISA kit). The tests were carried out with all samples in duplicate and the positive and negative samples were identified by calculating the critical value as instructed by the manufacturer.

Results

The results revealed that 15% of 40 pet cats, 17.7% of shelter cats and 80% of shelter dogs were positive for JEV antibodies, with shelter dogs being four times more likely to be seropositive than shelter cats. Fisher's Exact Test ($p < 0.05$) was used to compare results and possible factors affecting the result, from patient information, revealing that there appeared to be no significant relation between sex, health, management, age and location.

Conclusion

Dogs and cats in Malaysia are seropositive for JEV antibodies and can be used as sentinels.

Keywords:*Japanese Encephalitis, Dog, Cat, ELISA, IgG*

ABSTRAK

Abstrakkertasprojek yang dikemukakankepadaFakultiPerubatanVeterinarsebagaimemenuhisebahagiandaripada kursus VPD4999- ProjekTahunAkhir.

PENGESANAN ANTIBODI JAPANESE ENCEPHALITIS DARIPADA SAMPEL DARAH ANJING DAN KUCING DOMESTIK DI SEMENANJUNG MALAYSIA.

oleh

Palliyage Don HeshiniErandikaPerera

2016

Penyelia: Dr. GayathriTheviSelvarajah

Penyeliabersama: Prof. MadyaDr.SitiSuriArshad, Dr.Ooi Peck Toung

Pengenalan

Virus Japanese Encephalitis (JEV), keluargaFlaviviridae, adalahpunca yang diketahuiensefalitisakutpadamanusia di seluruh Asia Tenggara. Virus inidisebarkanmelaluinyamuk, danCulextritaeniorhynchusmenjadivektorutama yang dikaitkandenganpenyebaranpenyakitini.Virus initelahdikenalpastidalampelbagaijenishaiwan, termasukpadakucingdananjing; walaubagaimanapun, tiadakajian yang dilakukan di Malaysia untukmengkaji JEV padakucingdananjing.Tujuankajianiniadalahuntukmengenalpastikehadiranantibodi

JEV padakucingdananjing di Malaysia, denganmenggunakanEnzimBerkaitanimunoserapanasai (ELISA).

Kaedah

Duahinggalima ml darahtelahdikumpuldaripadakucingdaripusatperlindungandananjing, dandua ml serum dikumpulkandaripadasampeldiagnostikkucingdari Hospital VeterinarUniversiti, dengankeizinanpemilikhaiwantersebut. Maklumat yang dikumpulbagisetiaphaiwantermasukumur, jantina, status kesihatan, pengurusandanpersekitaranberdasarkanpemerhatiandanrekodpesakit.Tigaasai ELISA telahdilakukanberdasarkanprotokol yang disediakanolehpengeluar (kit SunRedBioteknologi Cat JE IgG ELISA dan kit MyBioSource Dog JE IgG ELISA). Ujiantelahdijalankanterhadapsemuasampeldalamduasalina.Sampelpositifdannegatif telahdikenalpastidenganmengiranilaikritikalseperti yang diarahkanolehpengeluar.

Keputusan

Hasilkajianmenunjukkanbahawa 15% daripada 40 kucinghaiwanpeliharaan, 17.7% daripadakucingdaripusatperlindungandan 80% daripadaanjingdaripusatperlindunganadalahpositifuntukantibodi JEV, dengananjingdaripusatperlindungan yang empat kali lebihcenderung seropositive berbandingkucingdaripusatperlindungan. Fishers' Exact Test ($p < 0.05$) digunakanuntukmembandingkankeputusandanfaktor-faktor yang mungkinmempengaruhikeputusan. Berdasarkanmaklumatpesakit,

terdapathubungkait yang signifikan di antarajantina, kesihatan, pengurusan, umurdanlokasi.

Kesimpulan

Anjingdankucing di Malaysia adalahseropositifuntukantibodi JEV danbolehdigunakansebagai sentinel.

Kata kunci:*Japanese Encephalitis, Dog, Cat, ELISA, IgG*

1.0 INTRODUCTION

1.1 Japanese Encephalitis Virus

Japanese Encephalitis Virus (JEV) of the *Flaviviridae* family, originally known as Japanese B Encephalitis, is a known cause of acute encephalitis in humans throughout South East Asia and has been discovered in various animals including racoon dogs (Ohno*et al.*, 2009), buffaloes (Mall *et al.*, 1995), pigs and water birds, with pigs acting as an amplifying host and humans being dead-end hosts. (Solomon, 2006). The virus was suspected to have come to light in the Malay Archipelago and various genotypes have evolved from it, all of which can be found in Malaysia (Solomon *et al.*, 2002), from genotype I to V and the latest genotype V that was discovered in a person originating from Muar, Malaysia in 1952 (Manal*et al.*, 2011).

The first reported cases of Japanese Encephalitis (JE) occurred in the 1870's in Japan, and the virus was isolated in the 1930's from the brain of a human (Solomon, 2006). The main vectors for this disease, mosquitoes (Culextritaeniorhyncus), are rampant within South East Asia, including Malaysia and have been effective at transmitting the virus from infected animals to humans. Thus, this became a disease of increasing importance due to its zoonotic characteristics, with infected humans showing signs of seizures and coma, especially without the existence of any effective antiviral treatment and lack of knowledge on the pathophysiological workings of the infection (Solomon *et al.*, 2002).

1.2 Japanese Encephalitis in Cats and Dogs

A study carried out in Japan (Shimoda*et al.*, 2010), explored the seroprevalence of Japanese Encephalitis within dogs and cats in the country. The purpose of the study was to identify whether the animals proved to act as good sentinals for the virus and the study concluded that dogs appeared to be so, more than cats. Another study, (Shimoda*et al.*, 2011) showed that dogs experimentally infected with the virus, did not appear to display any clinical signs. This leads us to question, whether these cats and dogs can act as silent carriers of impending infection to humans or whether they may be used in order to monitor the spread of the disease during an outbreak.

As of now there are no studies covering the seroprevalence of Japanese Encephalitis in Malaysian dogs and cats which would be an important piece of information considering the endemicity of the disease. Therefore, this study aims to detect the antibody against JEV in serum samples from domestic dogs and cats from both pet and shelter/ stray animal populations.

The hypothesis for this study is that domestic cats and dogs are seropositive for Japanese Encephalitis.

REFERENCES

Campbell, GL., Hills, SL., Fischer, M., Jacobson, JA.,Hoke, CH., Hombach, JM., Marfin, AA., Solomon, T., Tsai, TF., Tsui, VD., &Ginsburgi, AS. (2011). Estimated Global Incidence of Japanese Encephalitis: A Systematic Review. *Bull World Health Organ,* 89: 766-774

Cardosa, MJ.,Hooi, TP., &Kaur, P. (1995). Japanese Encephalitis Virus is an Important Cause of Encephalitis Among Children in Penang. *Southeast Asian J Trop Med Public Health,* 26(2):272-5

Centers for Disease Control and Prevention. (2015). JapaneseEncephaltis. Retrieved from http://www.cdc.gov/japaneseencephalitis/healthcareproviders/healthcareproviders-diagnostic.html

Ding, D., Hong, Z., Zhao, S., Clemens, JD., Zhou, B., Wang, B., Huang, M., Zeng, J., Guo, Q., Liu, W., Tao, F., &Xu, ZY. (2007). Long-Term Disability from Acute Childhood Japanese Encephalitis in Shanghai, China.*Am J Trop Med Hyg,* 77(3), 528-533

Dong, SK., Jang, GC., Cha, SH., Choi, SH., Kim, HM., Kim, JH., Kang, JH., Kim, JH., Bang, J., Naimi, Z., Bouckenooghe, A., Bosch-Castells, V., &Houillon, G. (2016). Immunogenicity and Safety of a Booster Dose of a Live Attenuated Japanese Encephalitis Chimeric Vaccine Given 1 Year After Primary Immunization in Healthy Children in the Republic of Korea. *Pediatr Infect Dis J,* 35,2: 60-64

Fan, YC., Chiu, HC., Chen, LK., Chang, GJ., Chiou, SS. (2015). Formalin Inactivation of Japanese Encephalitis Virus Vaccine Alters the Antigenicity and Immunogenicity of a Neutralization Epitope in Envelope in Envelope Protein Domain III. *PLOS Neglected Tropical Diseases,* 1-25

Hsu, LC., Chen, YJ., Hsu, FK., Huang, JH., Chang, CM., Chou, P., Lin, IF., & Chang, FY. (2014). The Incidence of Japanese Encephalitis in Taiwan – A Population-Based Study. *PLoSNegl Trop Dis,* 8(7), e3030

Igarashi, A. (2002). Control of Japanese Encephalitis in Japan: Immunization of Humans and Animals, and Vector Control. *Curr Top MicrobiolImmunol,* 267:139-52

Impoinvil, DE.,Ooi, MH., Diggle, PJ., Caminades, C., Cardosa, MJ., Morse, AP., Baylis, M., Solomon, T. (2013). The Effect of Vaccination Coverage and Climate on Japanese Encephalitis in Sarawak, Malaysia.*PLoSNegl Trop Dis,* 7(8), e2334

Ismail, Z. (2014). Another Mosquito Virus: How the Japanese Encephalitis Affects Our Brain. *The Star*, Retrieved from http://www.thestar.com.my/lifestyle/health/2014/07/20/its-no-jest/

Iwasaki, Y., Zhao, JX., Yamamoto, T., Konno H. (1986). Immunohistochemical Demonstration of Viral Antigens in Japanese Encephalitis.*ActaNeuropathol,* 70(1): 79-81

Jiang, L., Chen, S., Zheng, X., Ma, S., Zhou, J., Zhang, Q., Li, X., Xiong, Y., Zhong, X., Wang, Z., & Chen, Q. (2015). Detection of Serum Antibodies Against Japanese Encephalitis Virus in Bats in Hainan and Guandong Provinces of China. *Journal of Southern Medical University,* 35(5): 720-3

Keiser, J., Maltese, MF., Erlanger, TE., Bos, R., Tanner, M., Singer, BH., Utzinger, J. (2005). Effect of Irrigated Rice Agriculture on Japanese Encephalitis, Including Challenges and Opportunities for Integrated Vector Management. *ActaTropica,* 95, 40-57

Kile, JC.,Panella, NA., Komar, N., Chow, CC., MacNeil, A., Robbins, B., & Bunning, ML. (2005). Serologic Survey of Cats and Dogs During an Epidemic of West Nile Virus Infection in Humans. *J Am Vet Med Assoc,* 15;226(8): 1349-53

King, AMQ., Adams, MJ., Carstens, EB., &Lefkowitz, EJ. (2011). Virus Taxonomy: Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses. *International Union of Microbiological Societies Virology Division,* 1003-1008.

Kono, R., & Kim, KH. (1969). Comparative Epidemiological Features of Japanese Encephalitis in the Republic of Korea, China (Taiwan) and Japan.*Bull Org MondSante Bull WldHlth Org,* 40, 263-277

Lacey, LA., & Lacey, CM. (1990).The Medical Importance of Riceland Mosquitoes and Their Control Using Alternatives to Chemical Insecticides.*J Am Mosq Control AssocSuppl*, 2:1-93

Lindahl, J., Chirico, J., Boqvist, S., Thu, HTV.,& Magnusson, U. (2012). Occurrence of Japanese Encephalitis Virus Mosquito Vectors in Relation to Urban Pig Holdings.*Am J Trop Med Hyg,* 87(6), 1076-1082

Lopez, AL., Aldaba, JG., Roque, VG Jr., Tandoc, AO III., Sy, AK., Espino, FE., DeQuiro-Castro, M., Jee, Y., Ducusin, MJ., Fox, KK. (2015). Epidemiology of Japanese Encephalitis in the Philippines: A Systematic Review. *PLoSNegl Trop Dis,* 9(3): e0003630

Mall, MP., Kumar, A., & Malik, SV. (1995). Sero-positivity of Domestic Animals Against Japanese Encephalitis in Bareilly Area, U.P. *J Commun Dis.*, 27(4): 242-6

Manal, A.F., Galbraith, S.E., Radford, A.D., Dove, W., Takasaki, T., Kurane, I., & Solomon, T. (2011). Molecular Phylogenetic and Evolutionary Analyses of Muar Strain of Japanese Encephalitis Virus Reveal it is the Missing Fifth Genotype. *Infection, Genetics and Evolution,* 11, 855-862

Mitchell, C.J., Chen, P.S., &Boreham, P.F.L. (1973).Host-feeding Patterns and Behaviour of 4 Culex Species in an Endemic Area of Japanese Encephalitis. *Bull Org MondSante Bull WldHlth Org,* 49, 293-299

Mwandawiro, C., Tuno, N., Suwonkerd, W., Tsuda, Y., Yanagi, T., & Takagi, M. (1999). Host Preference of Japanese Encephalitis Vectors in Chiangmai, Northern Thailand.*Med EntomolZool,* 50;4, 323-333

Nyari, N., Singh, D., Kakkar, K., Sharma, S., Pandey, SN., Dhole, TN. (2015). Entomological and Serological Investigation of Japanese Encephalitis in Endemic Area of Eastern Uttar Pradesh, India.*J Vector Borne Dis*, 52, 321-328

Ohno, Y., Sato, H., Suzuki, K., Yokoyama, M., Uni, S., Shibasaki, T., Sashika, M., Inokuma, H., Kai, K., & Maeda, K. (2009).Detection of Antibodies against Japanese Encephalitis Virus in Raccoons, Raccoon Dogs and Wild Boars in Japan.*J. Vet. Med. Sci.,* 71(8): 1035–1039

Paterson, PY., Ley, HR Jr., Wisserman, CL Jr., eta/. (1952). Japanese Encephalitis in Malaya. I. Isolation of Virus and Serologic Evidence of Human and Equine Infections. *Am J Hyg,* 56: 32D-30

Rodrigues, FM., Guttikar, SN.,& Pinto, BD. (1981). Prevalence of Antibodies to Japanese Encephalitis and West Nile Viruses Among Wild Birds in the

Krishna-Godavari Delta, Andhra Pradesh, India. *Trans R Soc Trop Med Hyg,* 75(2): 258-62

Saxena, V., & Dhole, TN. (2008).Preventive Strategies for Frequent Outbreaks of Japanese Encephalitis in Northern India.*J Biosci,* 33, 505-514

Self, LS., Ree, HI., Lofgren, CS., Shim, JC., Chow, CY., Shin, HK., & Kim, KH. (1973). Aerial Applications of Ultra-Low-Volume Insecticides to Control the Vector of Japanese Encephalitis in Korea.*Bull Org MondSante Bull WldHlth Org,* 49, 353-357

Shimoda, H., Inthong, N., Noguchi, K., Terada, Y., Nagao, Y., Shimojima, M., Takasaki, T., Rerkamnuaychoke, W., Maeda, K. (2013).Development and Application of an Indirect Enzyme-Linked Immunosorbent Assay for Serological Survey of Japanese Encephalitis Virus Infection in Dogs.*J Virol Methods,* 187(1): 85-9

Shimoda, H., Ohno, Y., Mochizuki, M., Iwata, H., Okuda, M., & Maeda, K. (2010). Dog as Sentinels for Human Infection with Japanese Encephalitis Virus. *Emerging Infectious Diseases,* 16:7, 1137-1139

Shimoda, H., Tamaru, S., Morimoto, M., Hayashi, T., Sjimojima, M., & Maeda, K. (2011). Experimental Infection of Japanese Encephalitis Virus in Dogs.*J. Vet. Med. Sci.*, 73(9): 1241-1242

Simpson, DI., Smith, CE., Marshall, TF., Platt, GS., Way, HJ., Bowen, ET., Bright, WF., Day, J., McMahon, DA., Hill, MN., Bendell, PJ., &Heathcote, OH. (1976). Arbovirus Infections in Sarawak: the Role of the Domestic Pig. *Trans R Soc Trop Med Hyg,* 70(1): 66-72

Solomon, T. (2006).Control of Japanese Encephalitis – Within Our Grasp?*N Engl J Med.*, 355: 869-871

Solomon, T., Ni, H., Beasley, D., Ekkelenkamp, M., Cardosa, MJ.,& Barrett, A. (2002). Origin and Evolution of Japanese Encephalitis Virus in Southeast Asia. *Journal of Virology,* 77, 3091-3098

Solomon, T., Thao, LTT., Minh Dung, N., Kneen, R., The Hung, N., Nisalak, A., Vaughn, DW., Farrar, J., Hien, TT., White, NJ., &Cardosa, MJ. (1998). Rapid Diagnosis of Japanese Encephalitis by Using an Immunoglobulin M Dot Enzyme Immunoassay.*Journal of Clinical Microbiology*, 36,7: 2030-2034

Takken, W., &Verhulst, NO.(2013). Host Preferences of Blood-feeding Mosquitoes. *Annu Rev Entomol,* 58: 433-53

Van Den Hurk, AF., Ritchie, SA., &Mackenzies, JS. (2009). Ecology and Geographical Expansion of Japanese Encephalitis Virus.*Annu Rev Entomol,* 54: 17-35

Vashishtha, VM.,&Ramachandran, VG. (2015). Vaccination Policy for Japanese Encephalitis in India: Tread with Caution! *Indian Pediatrics,* 52, 837-839

Vaughn, DW.,&Hoke, CH. (1992). The Epidemiology of Japanese Encephalitis: Prospects for Prevention. *Epidemiologic Reviews*, 14, 197-221

Vythilingam, I., Oda, K., Mahadevan, S., Abdullah, G., Thim, CS., Hong, CC., Vijayamalar, B., Sinniah, M., & Igarashi, A. (1997). Abundance, Parity, and Japanese Encephalitis Virus Infection of Mosquitoes (Diptera: Culicidae) in Sepang District, Malaysia. *J Med Entomol,* 34(3): 257-262

World Health Organisation. (1999). 1999- Epidemic Encephalitis in Malaysia. Retrieved from http://www.who.int/csr/don/1999_03_26/en/

World Health Organisation. (2015). 1999- Japanese Encephalitis Fact Sheet No 386. Retrieved from http://www.who.int/mediacentre/factsheets/fs386/en/

Yang, DK., Oh, YI., Kim, HR., Lee, YJ., Moon, OK et al. (2011). Serosurveillance for Japanese Encephalitis Virus in Wild Birds Captured in Korea.*Journal of Veterinary Science,* 12(4)