CIRCULATING DENGUE SEROTYPES IN SANDAKAN AND KUDAT DISTRICTS OF SABAH: DURING 2016 TO 2017

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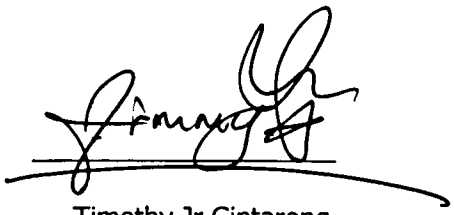

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ABSTRACT

Dengue is a mosquito-borne viral infection caused by Dengue virus (DENV). DENV is a single-stranded positive-sense RNA virus belonging to the genus *Flavivirus* of the family *Flaviviridae* with 4 serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). Subsequent infection with different serotypes causes severe dengue. A total of 200 dengue rapid test positive patients' sera were collected from Hospital Duchess of Kent Sandakan and Hospital Kudat, Sabah from June 2016 to December 2017. Dengue serotyping was done at the Department of Pathobiology and Medical Diagnostics at the Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah. The OneStep Reverse Transcriptase PCR and Nested PCR were performed using C-prM amplimers. Out of 200 serum samples tested PCR positive were 128. There are 62 patients were infected with single DENV serotype and the other 66 were co-infected with more than one serotype. All dengue serotype 1 to 4 were found circulating in Sandakan and Kudat. DENV-1 was the predominant serotype and DENV-2 was the least common serotype found in both study sites. The commonest dengue infected age groups were 21 to 30 in Sandakan, and 11 to 20 years old in Kudat. Males were affected more than females in both districts with ratio of 1.2:1. Phylogenetic analysis revealed that DENV-1, DENV-3 and DENV-4 sequences from both Sandakan and Kudat are genetically related to sequences from Asia and Southeast Asia. The DENV sequences analysis showed DENV-1 sequences are clustered to genotype IV, DENV-3 to genotype I, and DENV-4 to genotype II. DENV-2 sequences were less than 200 nucleotides, so the phylogenetic cluster was not clearly understood.

ABSTRAK

PEREDERAN SEROTIP-SEROTIP VIRUS DENGGI DI SABAH, DAERAH SANDAKAN DAN KUDAT: PADA 2016 SEHINGGA 2017

Denggi adalah jangkitan virus bawaan nyamuk yang disebabkan oleh virus denggi (DENV). DENV adalah RNA bebenang tunggal berderia positif yang terkelompok di dalam genus Flavivirus daripada family Flaviviridae yang mempunyai 4 serotip (DENV-1, DENV-2, DENV-3 dan DENV-4). Jangkitan denggi berikut daripada serotip yang berbeza-beza boleh menyebabkan denggi teruk. Sejumlah 200 sampel serum yang positif terhadap ujian pantas denggi yang masing-masing diperoleh daripada Hospital Duchess of Kent Sandakan dan Hospital Kudat, Sabah diantara Jun 2016 sehingga Disember 2017. Pengenalpastian serotip denggi telah dilakukan di Jabatan Patobiologi dan Diagnostik Perubatan, Fakulti Perubatan dan Sains Kesihatan, Universiti Malaysia Sabah. Tindak balas PCR transkriptase balik secara satu langkah dan PCR bersarang dilakukan menggunakan jujukan-jujukan amplimer daripada C-prM. Sebanyak 128 daripada 200 sampel serum adalah positif tindak balas PCR. Terdapat 62 pesakit merupakan jangkitan denggi dengan satu serotip dan 66 pesakit lagi merupakan jangkitan denggi bersama dengan lebih daripada satu serotip. Keempat-empat serotip 1 hingga 4 ditemui beredar di Sandakan dan Kudat, Sabah. DENV-1 adalah serotip predominan dan DENV-2 adalah serotip yang tidak kerap dicerap di kedua-dua tempat kajian. Kumpulan umur jangkitan denggi tertinggi di Sandakan adalah 21 hingga 30 tahun dan di Kudat adalah umur 11 hingga 20 tahun. Jantina lelaki adalah lebih tinggi terjangkit berbanding perempuan di kedua-dua daerah dengan nisbah 1.2:1. Daripada analisa filogenetik, keempat-empat jujukan DENV daripada Kudat dan Sandakan adalah secara genetik berkait rapat dengan jujukan-jujukan dari Asia dan Asia Tenggara. Analisa jujukan-jujukan mendapati jujukan serotip DENV-1 dikelaskan di dalam genotip IV, DENV-3 di dalam genotip I dan DENV-4 di dalam genotip II. Jujukan-jujukan DENV-2 mempunyai kurang daripada 200 nukleotida, oleh kerana itu kumpulan filogenetiknya adalah tidak difahami secara keseluruhan.

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LIST OF ABBREVIATIONS

-OH	Hydroxyl group
3' SL	3' stem loop
ADE	Antibody-dependant enhancement
ARSM	Agency of Remote Sensing Malaysia
AUG	Start codon
C	Capsid protein
CDC	Centers for Disease Control and Prevention
cDNA	Complimentary deoxyribonucleic acid
cHP	Capsid region hairpin
CNV	Copy number variations
C-prM	Capsid-precursor membrane
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DENV	Dengue virus
DENV-1	Dengue virus type 1
DENV-2	Dengue virus type 2
DENV-3	Dengue virus type 3
DENV-4	Dengue virus type 4
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
ddNTP	Dideoxynucleotide triphospate
DSS	Dengue shock syndrome
E	Envelope protein
ELISA	Enzyme-linked immunnoabsorbent assay
HSB	Hospital Sungai Buloh
HTAR	Hospital Tengku Ampuan Rahimah
IgG	Immunoglobulin M

IgM	Immunoglobulin G
IR	Incidence rate
JKNS	Jabatan Kesihatan Negeri Sabah@Department of Health Sabah
KKM	Kementerian Kesihatan Malaysia@Ministry of Health Malaysia
MOSTI	Ministry of Science, Technology and Innovation
NASBA	Nucleic acid sequence-based amplification
NCBI	National Center for Biotechnology Information
NIAID	National Institute of Allergy and Infectious Diseases
NS1	Non-structural protein 1
NS2a	Non-structural protein 2a
NS2b	Non-structural protein 2b
NS3	Non-structural protein 3
NS4a	Non-structural protein 4a
NS4b	Non-structural protein 4b
NS5	Non-structural protein 5
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
prM	Precursor membrane
RER	Rough endoplasmic reticulum
RDT	Rapid dengue test kit
RNA	Ribonucleic acid
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse transcription-polymerase chain reaction
RT-STNPCR	Reverse transcription single-tube nested polymerase chain reaction
SLA	Stem loop A
SLB	Stem loop B
SNP	Single-nucleotide polymorphism
SSL	Small stem loop
STNPCR	Single-tube nested polymerase chain reaction
TBE	Tris/Borate/EDTA
TT	Tourniquet test

U	Short oligo
UAR	Upstream AUG region
UTR	Untranslated region
WBC	White blood cells
WHO	World Health Organization
WHO-SEARO	World Health Organization-Southeast Asia Regional Office

LIST OF SYMBOLS

%	Percentage
°C	Degree Celcius
'	Minute
"	Second
>	Greater-than
±	Plus-minus
≤	Less-than or equal to
µ	Micro
µg	Micro-gram
µL	Micro-liter
µM	Micro-molar
cm	Centimeter
E	East
g	Gram
g/cm^3	Gram per cubic centimeter
km^2	Square kilometer
mL	Milliliter
mm	Millimeter
mM	Millimolar
mm^3	Cubic millimeter
mmHg	Millimeter of mercury
N	North
nm	Nanometer
o	Degree
pmoles/µL	Picomol/microliter
rpm	Revolutions per minute
SD	Standard deviation

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Dengue is a mosquito-borne viral infection caused by dengue virus (DENV). Dengue fever (DF) and severe dengue are the most important arthropod-borne viral diseases. The global prevalence of dengue cases has been increased in South-East Asia, Africa, the Western Pacific and the Americas (Calisher, C.H., 2005; Guzman & Kouri, 2002). DENV is a single-stranded positive-sense RNA virus that belongs to the genus *Flavivirus* of the family *Flaviviridae* with four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) (Twiddy *et al.*, 2003). Infection with one serotype does not confer protection against the other three serotypes, and subsequent infections can lead to severe dengue (CDC, 2014). Dengue is transmitted from one person to another by the bite of mosquitoes *Aedes aegypti* and *A. albopictus*, which are prevalent throughout the world (CDC, 2014).

Symptoms of dengue usually starts 4 to 7 days after the mosquito bite and last for 3 to 10 days (CDC, 2014). Infection by one DENV serotype produces a long-term immunity to that serotype but not to the other serotypes of the DENVs. Therefor in dengue endemic countries, people can be infected more than once over their lifetime (CDC, 2015). DENV serotypes 1 to 4 have spread throughout tropical regions worldwide. In many places, multiple DENV serotypes are circulating concurrently, which may increase the risk for the more severe form of the disease. It has been postulated that concurrent infections by multiple dengue virus serotypes may influence the clinical expressions of the disease and it is considered as a single major factor for the emergence of DHF (Loroño-Pino *et al.*, 1999).

Dengue clinical manisfestations could include non-specific signs and symptoms such as headache, nausea/vomiting, rash, malaise and abdominal pain (Kalayanarooj, S., 2011). In addition, dengue patients could also have bleeding manisfestations that include petechiae, epistaxis, gum bleeding, melena, hypermenorrhea, hemoglobinuria and hematemesis (Kalayanarooj, S., 2011). According to WHO-SEARO (2011) case definition, dengue infection is suspected in a patient with high fever with two of the following signs and symptoms such as headache, myalgia, arthralgia, rash, retro-orbital pain, bleeding manisfestations, positive tourniquet test, leukopenia (WBC ≤ 5,000 cells/mm^3), platelet count ≤ 150,000 cells/mm^3, or haematocrit (Hct) rising 5-10%.

In Malaysia, the incidence rate of dengue has increased from 72.1 per 100,000 populations in the year 2001 to 396.4 per 100,000 populations in 2015 (ARSM, MOSTI & KKM, 2017). Moreover, the incidence rate in 2016 decreased from 328.3 per 100,000 populations to 258.9 per 100,000 populations in 2017 (ARSM, MOSTI & KKM, 2017). According to the unpublished data from Vector Borne Disease Section, Ministry of Health, Malaysia, dengue cases increased from 16,368 in 2001 to 46,171 in 2010 (Cheah *et al.*, 2014). In 2017, 83,849 dengue cases reported with 177 deaths which were lower as compared to the year 2016, with 101,357 reported cases and 237 deaths (ARSM, MOSTI & KKM, 2017). According to Department of Health Sabah in

2017 and 2018, Sabah is one of the states in Malaysia that is afflicted with the spread of dengue virus with total of 3,668 cases reported in 2016 and 2,560 cases in 2017.

The diagnosis of dengue can be made serologically by detecting dengue-specific IgM and IgG antibodies, which generally appears 7 to 8 days after the onset of illness (Parida *et al.*, 2001). Virus isolation from patient serum collected early in the infection can be made in cell cultures. However, virus isolation takes time and the success rate may not be high because of presence of virus-antibody complexes (Lanciotti *et al.*, 1992). The NS1 antigen assays are useful for the early detection of dengue infection in routine surveillance and in the diagnosis of dengue in primary health care centres. A study on comparison of NS1 antigen assays and reverse transcription-polymerase chain reaction (RT-PCR) during the first three days of fever showed that, RT-PCR was more sensitive and specific than NS1 antigen (Pok *et al.*, 2010). For the control and prevention of DF, it is important to detect and type the virus in clinical samples as soon as possible. Assays based on reverse RT-PCR dengue viral RNA are rapid, sensitive, and specific for the typing of DENVs (Lanciotti *et al.*, 1992; Harris *et al.*, 1998).

According to Yang *et al.* (2014), dengue surveillance systems play an important role in monitoring the trends of distribution, spread over time in a place and detect epidemics. Nevertheless, according to Department of Health Sabah (2016b), Sandakan and Kudat are two major districts in Sabah with total dengue cases of 467 and 11 respectively in 2015, and increased to 518 and 95 cases in 2016 respectively. The informations on dengue serotype and genotype distributions in Sandakan and Kudat are scarce as a result it is hampering dengue control. Moreover, the lack of data on age and gender trends of dengue infections in both districts might disrupt on dengue cases management. Therefore, this study was done to determine the serotype and genotype distributions of dengue viruses as well as the age and gender trends of dengue cases in both districts during 2016-17 so that it will provide baseline informations for future dengue research.

REFERENCES

"Sandakan" (Online) www.wonderfulmalaysia.com/sandakan-city-malaysia.htm. Accessed on 19th September 2016.

Ab-Fatah, M., Subenthiran, S., Abdul-Rahman, P.S.A., Saat, Z. and Thayan, R. 2015. Research Note Dengue serotype surveillance among patients admitted for dengue in two major hospitals in Selangor, Malaysia. *Tropical Biomedicine* 32(1): 187-191.

AbuBakar, S., Wong, P-F. and Chan, Y-F. 2002. Emergence of dengue virus type 4 genotype IIA in Malaysia. *Journal of General Virology* 83: 2437-2442.

Ahmad Nizal, M.G., Rozita, H., Mazrura, S., Zainudin, M.A., Hidayatulfathi, O., Faridah, M.A., Noor Artika, I., Er, A.C. 2012. Dengue infections and circulating serotypes in Negeri Sembilan, Malaysia. *Malaysian Journal of Public Health Medicine* 12(1): 21-30.

Alcon-LePoder, S., Sivard, P., Drouet, M., Talarmin, A., Rice, C. and Flamand, M. 2006. Secretion of flaviviral non-structural protein NS1: from diagnosis to pathogenesis. In *Novartis Foundation Symposium* (Vol. 277, p. 233). Chichester; New York; John Wiley.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. "Basic local alignment search tool." *Journal of Molecular Biology* 215; 403-410.

Anders, K.L., Nguyet, N.M., Chau, N.V.V., Hung, N.T., Thuy, T.T., Farrar, J., Wills, B., Hien, T.T. and Simmons, C.P. 2011. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. *The American Journal of Tropical Medicine and Hygiene* 84(1): 127-134.

Anderson, R., Wang, S., Osiowy, C. and Issekutz, A.C. 1997. Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. *Journal of Virology* 71(6): 4226-4232.

Anonymous. 1986. Dengue haemorrhagic fever, diagnosis, treatment and control. World Health Organization, Geneva, Switzerland.

Arcari, P., Tapper, N. and Pfueller, S. 2007. Regional variability in relationship between climate and dengue/DHF in Indonesia. *Singapore Journal of Tropical Geography* 28: 251-271.

Avirutnan, P., Malasit, P., Seliger, B., Bhakdi, S. and Husmann, M. 1998. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *Journal of Immunology* 161(11): 6338-6346.

Ayers, M., Adachi, D., Johnson, G., Andonova, M., Drebot, M. and Tellier, R. .2006. A single tube RT-PCR assay for the detection of mosquito-borne flavivirus. *Journal of Virological Methods* 135: 235-239. DOI: 10.1016/j.jviromet.2006.03.009.

Balmaseda, A., Hammond, S.N., Perez, L., Tellez, Y., Saborio, S.I., Mercado, J.C., Cuadra, R., Rocha, J., Perez, M.A., Silva, S. and Rocha, C. 2006. Serotype-specific differences in clinical manifestations of dengue. *The American Journal of Tropical Medicine and Hygiene* 74(3): 449-456.

Balmaseda, A., Hammond, S.N., Pérez, M.A., Cuadra, R., Solano, S., Rocha, J., Idiaquez, W. and Harris, E. 2005. Assessment of the World Health Organization scheme for classification of dengue severity in Nicaragua. *The American Journal of Tropical Medicine and Hygiene* 73(6): 1059-1062.

Barnes, W.J.S. and Rosen, L. 1974. Fatal hemorrhagic disease and shock associated with primary dengue infection on a Pacific Island. *The American Journal of Tropical Medicine and Hygiene* 23: 495-506.

Bhatia, R., Dash, A.P. and Sunyoto, T. 2013. Changing epidemiology of dengue in South-East Asia. *WHO South-East Asia Journal of Public Health* 2(1): 23.

Bio-Rad Laboratories. 2000. Ethidium bromide solution 10 mg/ml. Alfred Nobel Drive, Hercules, California 94547. Catalog Number: 161-0433.

Blacksell, S.D., Mammen, M.P., Thongpaseuth, S., Gibbon, R.V., Jarman, R.G., Jenjaroen, K., Nisalak, A., Phetsouvanh, R., Newton, P.N. and Day, N.P. 2008. Evaluation of the Panbio dengue virus non-structural 1 antigen detection and immunoglobulin M antibody enzyme-linked immunoabsorbent assays for the diagnosis of acute dengue infections in Laos. *Diagnostic Microbiology and Infectious Diseases* 60: 43-49.

Bleijis, D.A. 2016. Dengue virus. www.denguevirusnet.com/dengue-virus.html. Accessed on 24th April 2017.

Buchy, P., Yoksan, S., Peeling, R.W. and Hunsperger, E. 2006. Laboratory tests for the diagnosis of dengue virus infection. *In WHOobotSPfRaT, Tropical Diseases, eds. Scientific Working Group, Report on Dengue*: 1-5.

Calisher, C.H. 2005. Persistent emergence of dengue. *Emerging Infectious Diseases* 11: 738–739.

Centers for Disease Control and Prevention [CDC]. 2014. Dengue Home Page Updated 9th June 2014. (Online) http://www.cdc.gov/dengue/epidemiology/ Retrieved 24 February 2015.

Centers for Disease Control and Prevention [CDC]. 2015. CDC DENV-1-4 Real-Time RT-PCR Assay for Detection and Serotype Identification of Dengue Virus: 4-5. (Online) http://www.cdc.gov/dengue/resources/rtpcr/CDCPackageInsert.pdf.

Centers for Disease Control and Prevention [CDC]. 2016. Laboratory guidance and diagnostic testing. Retrieved from https://www.cdc.gov/dengue/cliweallab/laboratory.html.

Chan, M. and Johansson, M.A. 2012. The incubation periods of dengue viruses. *PLOS One* 7(11): e50972.

Chau, T.N., Quyen, N.T., Thuy, T.T., Tuan, N.G., Hoang, D.M., Dung, N.T.P., Lien, L.B., Quy, N.T., Hieu, N.T., Hieu, L.T.M., Hien, T.T., Hung, N.T., Farrar, J. and Simmons, C.P. 2008. Dengue in Vietnamese infants-results of infection-

enhancement assays correlate with age-related disease epidemiology and cellular immune responses correlate with disease severity. *Journal of Infectious Diseases* 198: 516-524.

Cheah, W.K., Ng, K.S., Marzilawati, A-R & Lum, L.C.S. 2014. A Review of Dengue Research in Malaysia. *Medical Journal of Malaysia* 69(A): 59-67.

Chee, H-Y. and AbuBakar, S. 2003. Phylogenetic investigations of dengue virus type 2 isolated in Malaysia. *Dengue Bulletin* 27: 100-107.

Chew, M.H., Rahman, M.M. and Hussin, S. 2015. Molecular epidemiology and phylogenetic analysis of dengue virus type-1 and 2 isolated in Malaysia. *Pakistan Journal of Medical Sciences* 31(3): 615-620.

Chew, M.H., Rahman, M.M. and Salleh, S.A. 2012a. Dengue in Malaysia: An epidemiological perspective study. *Pakistan Journal of Medical Sciences* 28(4): 643-647.

Chew, M.H., Rahman, M.M., Jelip, J., Hassan, M.R. & Isahak, I. 2012b. All Serotypes of Dengue Viruses Circulating in Kuala Lumpur, Malaysia. *Current Research Journal of Biological Sciences* 4(2): 229-234.

Chien, L.J., Liao, T.L., Shu, P.Y., Huang, J.H., Gubler, D.J. & Chang, G.J.J. 2006. Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. *Journal of Clinical Microbiology* 44(4): 1295-1304.

Corwin, A.L., Larasati, R.P., Bangs, M.J., Wuryadi, S., Arjoso, S., Sukri, N., Listyaningsih, E., Hartati, S., Namursa, R., Anwar, Z., Chandra, S., Loho, B., Ahmad, H., Campbell, J.R. and Porter, K.R. 2001. Epidemic dengue transmission in southern Sumatra, Indonesia. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 95(3):257–65.

Crill, W.D. and Roehrig, J.T. 2001. Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *Journal of Virology* 75: 7769-7773.

Dash, P.K., Parida, M.M., Saxena, P., Kumar, M., Rai, A., Pasha, S.T. and Jana, A.M. 2004. Emergence and continued circulation of dengue-2 (genotype IV) virus strains in northern India. *Journal of Medical Virology* 74(2): 314-322.

Dejnirattisai, W., Jumnainsong, A., Onsimsakul, N., Fitton, P., Vasarawathana, S., Limpitikul, W., Puttikhunt, C., Edwards, C., Duangchinda, T., Supasa, S., Chawansuntati, K., Malasit, P., Mongkolsapaya, J. & Screaton, G. 2010. Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 328(5979): 745-748.

Department of Health Sabah [JKNS]. 2015. Buletin Epidemiologi Sabah: ME52.

Department of Health Sabah [JKNS]. 2016a. Buletin Epidemiologi Sabah: ME1.

Department of Health Sabah [JKNS]. 2016b. Buletin Epidemiologi Sabah: ME52.

Department of Health Sabah [JKNS]. 2017. Buletin Epidemiologi Sabah: ME42.

Department of Health Sabah [JKNS]. 2018. Buletin Epidemiologi Sabah: Jun 2018.

Department of Statistics Malaysia. 2010. Total population by ethnic group, local authority area and state, Malaysia. Malaysia: Department of Statistics Malaysia.

Destura, R.V., Petrorio, J.A.G., Vinarao, R.B. and Parungao, M.M. 2012. Idenftification of a new genotype of dengue virus 3 from Philippine isolates by analysis of the C-rM gene function. *HERDIN*: NCR-RITM-12090412084092.

Drosten, C., Gottig, S., Schilling, S., Asper, M., Panning, M. and Schmitz, H. 2002. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo haemorrhagic fever virus by real-time reverse transcription-PCR. *Journal of Clinical Microbiology* 40: 2323-2330.

Endy, T.P., Nisalak, A., Chunsuttitwat, S., Vaughn, D.W., Green, S., Ennis, F.A., Rothman, A.L. and Libraty, D.H. 2004. Relationship of pre-existing dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a

prospective cohort study of DV infection in Thailand. *The Journal of Infectious Diseases* 189(6): 990-1000.

European Bioinformatic Institute [EMBL-EBI]. 2018. Genotyping. Retrieved from https://www.ebi.ac.uk/training/online/course/functional-genomics-introduction-and-designing-e/common-study-types-functional-genomes/gen. Acessed on 30th July 2018.

Farooq, U., Latif, A., Irshad, H., Ullah, A., Zahur, A.B., Naeem, K., Khan, S.U.H., Ahmed, Z., Rodriguez, L.L. and Smoliga, G. 2015. Loop-mediated isothermal amplification (RT-LAMP): a new approach for the detection of the foot-and-mouth disease virus and its sero-types in Pakistan. *Iran Journal of Veterinary Research* 16(4): 331-334.

Figueiredo, L.T.M., Batista, W.C. and Igarashi, A. 1997. Detection and identification of dengue virus isolates from Brazil by a simplified reverse transcription – polymerase chain reaction (RT-PCR) method. *Journal of the Institute of Tropical Medicine of São Paulo* 39 (2): 1-9.

Fong, M.Y., Koh, C-L. and Lam, S-K. 1998. Molecular epidemiology in Malaysia DEN-2 viruses isolated over twenty-five years (1968-1993). *Research in Virology* 149: 457-464.

Fragnoud, R., Yugueros-Marcos, J., Pachot, A. and Bedin, F. 2012. Isotopė coded protein labeling analysis of plasma specimens from acute severe dengue fever patients. *Proteome Science* 10(1): 60.

Fried, J.R., Gibbons, R.V., Kalayanarooj, S., Thomas, S.J., Srikiatkhachorn, A., Yoon, I.K., Jarman, R.G., Green, S., Rothman, A.L. and Cummings, D.A. 2010. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLOS Neglected Tropical Diseases* 4(3): e617.

Gomes, A.L.V., Silva, A.M., Cordeiro, M.T., Guimarães, G.F., Marques Jr, E.T.A. and Abath, F.G.C. 2007. Single-tube nested PCR using immobilized internal rimers for the identification of dengue serotypes. *Journal of Virological Methods* 145: 76-79. DOI: 10.1016/j.jviromet.2007.05.003.

Green, S. and Rothman, A. 2006. Immunopathological mechanism in dengue and dengue hemorrhagic fever. *Current Opinion in Infectious Diseases* 19(5): 429-436.

Gubler, D.J. 1997. Dengue and dengue haemorrhagic fever: its history and resurgence as a global public health problem, p. 1-22. In Gubler, D.J. and Kuno, G. (ed.), Dengue haemorrhagic fever. CAB International, London United Kingdom.

Gubler, D.J. 1998a. Dengue and dengue hemorrhagic fever. *Clinical Microbiology Reviews* 11(3): 480-496.

Gubler, D.J. 1998b. The global pandemic of dengue/dengue harmorrhagic fever: current status and prospects for the future. *Annals of the Academy of Medicine, Singapore,* in press.

Gubler, D.J. and Trent, D.W. 1994. Emergence of epidemic dengue/dengue haemorrhagic fever as a public health problem in the Americas. *Infectious Agents and Disease* 2: 383-393.

Gubler, D.J., Ooi, E.E., Vasudevan, S. and Farrar, J. 2014. *Dengue and Dengue Hemorrhagic Fever* (eds.). CABI, Oxfordshire, England.

Gubler, D.J., Suharyono, W., Tan, R., Abidin, M. and Sie, A. 1981. Viremia in patients with naturally acquired dengue infection. *Bulletin of the World Health Organization* 59: 623-630.

Gutsche, I., Coulibaly, F., Voss, J.E., Salmon, J., d'Alayer, J., Ermonval, M., Larquet, E., Charneau, P., Krey, T., Mégret, F., Guittet, E., Rey, F.A. and Flamand, M. 2011. Secreted dengue virus non-structural protein NS1 is an atypical barrel-shaped

high-density lipoprotein. *Proceedings of the National Academy of Sciences of the United States of America* 108(19): 8003-8008.

Guzman, M.G. and Harris, E. 2015. Dengue. *The Lancet* 385(9966): 453-465.

Guzman, M.G. and Kouri, G. 2002. Dengue: an update. *The Lancet Infectious Diseases* 2(1): 33-42.

Guzman, M.G. and Kouri, G. 2003. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *Journal of Clinical Virology* 27(1): 1-13.

Guzman, M.G., Buchy, P., Enria, D. and Vazquez, S. 2014. Laboratory diagnostic of dengue, p. 184-213. In Gubler *et al.* (eds.), Dengue and Dengue haemorrhagic fever. 2nd Edition. CAB International, London, United Kingdom.

Guzman, M.G., Halstead, S.B., Artsob, H., Buchy, P., Farrar, J., Gubler, D.J., Hunsperger, E., Kroeger, A., Margolis, H.S., Martínez, E. and Nathan, M.B. 2010. Dengue: a continuing global threat. *Nature Reviews Microbiology* 8: S7-S16.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Halsey, E.S., Marks, M.A., Gotuzzo, E., Fiestas, V., Suarez, L., Vargas, J., Aguayo, N., Madrid, C., Vimos, C., Kochel, T.J. and Laguna-Torres, V.A. 2012. Correlation of serotype-specific dengue virus infection with clinical manifestations. *PLOS Neglected Tropical Diseases* 6(5): e1638.

Halstead, S.B. 1992. The XXth century dengue pandemic: need for the surveillance and research. *Rapport Trimester Statistics Sanitary Monde* 45: 292-298.

Halstead, S.B. 2003. Neutralization and antibody-dependant enhancement of dengue viruses. *Advances In Virus Research* 60: 421-467.

Halstead, S.B. 2007. Dengue. *The Lancet* 370(9599): 1644-1652.

Halstead, S.B., Mahalingam, S., Marovich, M.A., Ubol, S. and Mosser, D.M. 2010. Intrinsic antibody-dependent enhancement of microbial infection in macrophages: disease regulation by immune complexes. *The Lancet Infectious Diseases* 10(10): 712-722.

Hanley, K.A. and Weaver, S.C. 2008. In Origin and Evolution of viruses (eds Domingo, E., Parrish, C.R. and Holland, J.J.): 351-392 (Elsevier, Oxford).

Harris, E., Holden, K.L., Edgil, D., Polacek, C. and Clyde, K. 2006. Molecular biology of flaviviruses. *Norvartis Foundation Symposium* 277: 23-39; discussion 40: 71-73, 251-253.

Harris, E., Roberts, T.G., Smith, L., Selle, J., Kramer, L.D., Valle, S., Sandoval, E. and Balmaseda, A. 1998. Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. *Journal of Clinical Microbiology* 36(9): 2634-2639.

Harrison, J. and Langdale, J. 2006. A step by step guide to phylogeny reconstruction. *The Plant Journal: for Cell and Molecular Biology* 45: 561-572.

Haryanto, S., Hayati, R.F., Yohan, B., Sijabat, L., Sihite, I.F., Fahri, S., Meutiawati, F., Halim, J.A.N., Halim, S.N., Soebandrio, A. and Sasmono, R.T. 2016. The molecular and clinical features of dengue during outbreak in Jambi, Indonesia in 2015. *Pathogens and Global Healths* 110(3): 119-129.

Henchal, E.A. and Putnak, J.R. 1990. The dengue viruses. *Clinical Microbiology Reviews* 3: 376-396.

Herman, R., Hartanti, D.I., Nugraha, A.A., Agustiningsih and Sembiring, M.M. 2016. Genotypes of dengue virus circulate in dengue sentinel surveillance in Indonesia. *Distribution of Dengue Genotype in Indonesia* 7(2): 69-74.

Hermann, L.L., Thaisomboonsuk, B., Poolpanichupatam, Y., Jarman, R.G., Kalayanarooj, S., Nisalak, A., Yoon, I. & Fernandez, S. 2014. Evaluation of a

dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute dengue virus infection. *PLOS Neglected Tropical Diseases* 8(10): 1-8.

Holmes, E.C. 2009. The evolution and emergence of RNA viruses. Oxford University Press.

Holmes, E.C., Tio, P-H., Perera, D., Muhi, J. and Cardosa, J. 2009. Importation and co-circulation of multiple serotypes of dengue virus in Sarawak, Malaysia. *Virus Research* 143: 1-5.

Huerta, V., Chinea, G., Fleitas, N., Sarria, M., Sanchez, J., Toledo, P. and Padron, G. 2008. Characterization of the interaction of domain III of the envelope protein of dengue virus with putative receptors from CHO cells. *Virus Research* 137: 225-234.

Iglesias, N.G., Byk, L.A. and Gamarnik, A.V. 2014. Molecular virology of dengue virus, p. 334-364. In Gubler *et al.* (eds.), Dengue and dengue haemorrhagic fever. 2nd Edition. CAB International, London, United Kingdom.

Je, S., Bae, W., Kim, J., Seok, S.H. and Hwang, E-S. 2016. Epidemiological characteristics and risk factors of dengue infection in Korean travellers. *Journal of Korean Medical Science* 31: 1863-1873.

Jumali, S., Gubler, D.J., Nalim, S., Eram, S. and Saroso, J.S. 1979. Epidemic DHF in rural Indoonesia. Part 3. *American Journal of Tropical Medicine and Hygiene* 28(4): 717-724.

Kabra, S.K., Jain, Y., Pandey, R.M., Singhal, T., Tripathi, P., Broor, S., Seth, P. and Seth, V. 1999. Dengue haemorrhagic fever in children in the 1996 Delhi epidemic. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 93(3): 294-298.

Kalayanarooj, S. 2011. Clinical manifestations and management of dengue/DHF/DSS. *Tropical Medicine and Health* 39(4): 83-87.

Kalayanarooj, S., Vaughn, D.W., Nimmannitya, S., Green, S., Suntayakorn, S., Kunentrasai, N., Viramitrachai, W., Ratanachu-Eke, S., Kiatpolpoj, S., Innis, B.L. and Rothman, A.L. 1997. Early clinical and laboratory indicators of acute dengue illness. *Journal of Infectious Diseases* 176(2): 313-321.

Kao, C.L., King, C.C., Chao, D.Y., Wu, H.L. and Chang, G.J. 2005. Laboratory diagnosis of dengue virus infection: current and future perspective in clinical diagnosis and public health. *Journal of Microbiology, Immunology and Infection* 38(1): 5-16

Katzelnick, L.C., Gresh, L., Halloran, M.E., Mercado, J.C., Kuan, G., Gordon, A., Balmaseda, A. and Harris, E. 2017. Antibody-dependent enhancement of severe dengue disease in humans. *Science*. 10.1126/science.aan6836.

Kelley, J.F., Kaufusi, P.H., and Nerurkar, V.R. 2012. Dengue hemorrhagic fever-associated immunomediators induced via maturation of dengue virus nonstructural 4B protein in monocytes modulate endothelial cell adhesion molecules and human microvascular endothelial cells permeability. *Journal of Virology* 322(2): 326-337.

Kiam, C.E. 2004. Borneo's tropical eden: Sabah. Singapore: Simply Green.

King, C-C., Chao, D-Y., Chien, L-J., Chang, G-J.J., Lin, T-H., Wu, Y-C. and Huang, J-H. 2008. Comparative analysis of full genomic sequences among different genotypes of dengue virus type 3. *Virology Journal* 5(1): 63.

Koh, B.K, Ng, L.C., Kita, Y., Tang, C.S., Ang, L.W., Wong, K.Y., James, L. and Goh, K.T. 2008. The 2005 dengue epidemic in Singapore: epidemiology, prevention and control. *Annuals Academy of Medicine Singapore* 37(7): 538-545.

Koraka, P., Burghoorn-Maas, C.P., Falconar, A., Setiati, T.E., Djamiatun, K., Groen, J. and Osterhaus, A.D. 2003. Detection of immune-complex-dissociated nonstructural-1 antigen in patients with acute dengue virus infections. *Journal of Clinical Microbiology* 41(9): 4154-4159.

Kosasih, H., Alisjahbana, B., de Mast, Q., Rudiman, I.F., Widjaja, S., Antonjaya, U., Novriani, H., Susanto, N.H., Jusuf, H., van der Ven, A., Beckett, C.G., Blair, P.J., Burgess, T.H., Williams, M. and Porter, K.R. 2016. The epidemiology, virology and clinical findings of dengue virus infections in a cohort of Indonesian adults in Western Java. *PLOS Neglected Tropical Diseases* 10(2): e0004390.

Kotaki, T., Yamanaka, A., Mulyatno, K.C., Labigah, A., Sucipto, T.H., Churrotin, S., Soegijanto, S., Konishi, E. and Kameoka, M. 2014. Phylogenetic analysis of dengue virus type 3 strains primarily psolated in 2013 from Surabaya, Indonesia. *Japanese Journal of Infectious Diseases* 67(3): 227-9.

Kouri, G.Á., Guzman, M.G. and Bravo, J.R. 1987. Why dengue haemorrhagic fever in Cuba? An integral analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 81(5): 821-823.

Kouri, G.P., Guzmán, M.G., Bravo, J.R. and Triana, C. 1989. Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. *Bulletin of the World Health Organization* 67(4): 375.

Kumar, S., Stecher, G. and Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870-1874.

Kumaria, R. 2010. Correlation of disease spectrum among four dengue serotypes: a five years hospital based study from India. *Brazilian Journal of Infectious Diseases* 14(2): 141-146.

Kurane, I., Innis, B.L., Nimmannitya, S., Nisalak, A., Meager, A., Janus, J. and Ennis, F.A. 1991. Activation of T lymphocytes in dengue virus infections. High levels of soluble interleukin 2 receptor, soluble CD4, soluble CD8, interleukin 2, and interferon-gamma in sera of children with dengue. *Journal of Clinical Investigations* 88(5): 1473-1480.

Kurane, I. 2007. Dengue hemorrhagic fever with special emphasis on immunopathogenesis. *Comparative Immunology, Microbiology & Infectious Diseases* 30(56): 329-340.

Laiq, S. & Haider, K. 2015. Immunofluorescence technique and one step real time RT PCR technique for diagnosis and eliminating dengue infection. *IOSR Journal of Humanities and Social Sciences* 4(20): 77-82.

Lanciotti, R.S., Calisher, C.H., Gubler, D.J., Chang, G.J. and Vorndam, A.V. 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *Journal of Clinical Microbiology* 30(3): 545-551.

Lee, K.S., Lai, Y.L., Lo, S., Barkham, T., Aw, P., Ooi, P.L., Tai. J.C., Hibberd, M., Johansson, P., Khoo, S.P. and Ng, L.C. 2010. Dengue virus surveillance for early warning, Singapore. *Emerging Infectious Diseases* 16(5): 847-849.

Lee, K-S., Lo, S., Tan, S.S-Y., Chua, R., Tan, L-K., Xu, H. and Ng, L-C. 2012. Dengue virus surveillance in Singapore reveals high viral diversity through multiple introductions and in situ evolution. *Infection, Genetics and Evolution* 12: 77-85.

Leitmeyer, K. C., Vaughn, D. W., Watts, D. M., Salas, R., Villalobos, I., Ramos, C., & Rico-Hesse, R. 1999. Dengue virus structural differences that correlate with pathogenesis. *Journal of Virology* 73(6): 4738-4747.

Lestari, C.S.W., Yohan, B., Yunita, A., Meutiawati, F., Hayati, R.F., Trimarsanto, H. and Sasmono, R.T. 2017. Phylogenetic and evolutionary analyses of dengue viruses isolated in Jakarta, Indonesia. *Virus Genes*. DOI: 10.1007/s11262-017-1474-7.

Libraty, D.H., Young, P.R., Pickering, D., Endy, T.P., Kalayanarooj, S., Green, S., Vaughn, D.W., Nisalak, A., Ennis, F.A. and Rothman, A.L. 2002. High circulating levels of the dengue virus non-structural protein NS1 early in dengue illness correlate with the development of dengue haemorrhagic fever. *Journal of Infectious Diseases* 186(8): 1165-1168.

Lodeiro, M.F., Filomatori, C.V. and Gamarnik, A.V. 2009. Structural and functional studies of the promoter element for dengue virus RNA replication. *Journal of Virology* 83: 993-1008.

Loroño-Pino, M.A., Cropp, C.B., Farfán, J.A., Vorndam, A.V., Rodriguez-Angulo, E.M., Rosado-Paredes, E.P., Flores-Flores, L.F., Beaty, B.J. & Gubler, D.J. 1999. Common occurrence of concurrent infections by multiple dengue virus serotypes. *The American Journal of Tropical Medicine and Hygiene* 61(5): 725-730.

Mahadi, S.A.R.S. 2014. Indonesian labour migration to Sabah: changes, trends and impacts. Australia: University of Adelaide.

Malavige, G.N. and Ogg, G.S. 2015. Role of skin homing T cells in acute dengue Infection. *Annals of Translational Medicine* 3(17): 252.

Malavige, G.N., Fernando, S., Fernando, D.J. and Seneviratne, S.L. 2004. Dengue viral infections. *Postgraduate Medical Journal* 80(948): 588-601.

Malaysia Meteorological Department (Online) www.met.gov.my/en/web/metmalaysia/climate. Accessed on 19th September 2017.

Malaysian Remote Sensing Agency [ARSM], Kementerian Sains, Teknologi & Inovasi [MOSTI] & Disease Control Unit of Ministry of Health Malaysia [KKM]. 2016. Malaysia dengue incidence rate and case fatality rate for year 2000-2016 (Online) https://www.idengue.remotesensing.gov.my/idengue. Accessed on 5th June 2017.

Martina, B.E., Koraka, P. and Osterhaus, A.D. 2009. Dengue virus pathogenesis: an integrated view. *Clinical Microbiology Reviews* 22(4): 564-581.

Martinez-Torrez, E., Polanco-Anaya, A.C. and Pleitez-Sandoval, E.B. 2008. Why and how children with dengue die? *Revista Cubana de Medicina Tropical* 60(1): 40-47.

Mattingley, P.F. 1957. Genetical aspects of the *Aedes aegypti* problem: taxonomy and bionomics. *Annals of Tropical Medicine and Parasitology* 51: 392-408.

McBride, W. 2009. Evaluation of dengue NS1 test kits for the diagnosis of dengue fever. *Diagnostic Microbiology and Infectious Diseases* 64: 31-36.

Meftahuddin, T., Anisah, A.B. and Mohd Faizal, A. 2004. Outbreak of dengue in Bandar Baru Bangi. *Malaysian Journal of Public Health Medicine* 4(1): 22-28.

Megawati, D., Masyeni, S., Yohanm B., Lestarini, A., Hayati, R.F., Metiuwati, F., Suryana, K., Widarsa, T., Budiyasa, D.G., Budiyasa, N., Myint, K.S.A. and Sasmono, R.T. 2017. Dengue in Bali: clinical characteristics and genetic diversity of circulating dengue virus. *PLOS Neglected Tropical Diseases*: 1-5.

Ministry of Health [MOH], Malaysia. 2010. Annual Report 2010.

Moi, M.I., Takasaki, T., Saijo, M. and Kurane, I. 2013. Dengue virus infection enhancing activity of undiluted sera obtained from patients with secondary dengue virus infection. *Transaction of The Royal Society of Tropical Medicine and Hygiene* 107: 51-58.

Monath, T.P. 1995. Dengue: the risk to developed and developing countries. In Roizman, B. (*ed.*) *Infectious Diseases In an Age of Change: The Impact of Human Ecology and Behaviour on Disease Transmission*: 43-58. National Academy Press, Washincton, DC.

Mongkolsapaya, J., Dejnirattisai, W., Xu, X.N., Vasanawathana, S., Tangthawornchaikul, N., Chairunsri, A., Sawasdivorn, S., Duangchinda, T., Dong, T., Rowland-Jones, S., Yenchitsomanus, P.T., McMichael, A. Malasit, P. and Screaton, G. 2003. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nature Medicine* 9(7): 921-921

Morens, D.M., Halstead, S.B. and Larsen, L.K. 1985. Comparison of dengue virus plaque reduction neutralization by macro and "semi-micro" methods in LLC-MK2 cells. *Microbiology and Immunology* 29: 1197-1205.

Mudin, R.N. 2015. Dengue incidence and the prevention and control program in Malaysia. *The International Medical Journal of Malaysia* 14(1): 5-10.

Muller, D.A. & Young, P.R. 2013. The flavivirus NS1 protein: molecular and structural biology, immunology, role in pathogenesis and application as diagnostic biomarker. *Antiviral Research* 98(2): 192-208.

Myat, T.W., Thu, H.M., Win, H.M., Than, K.S., Tun, Z.T., Aye, K.M., Zaw, N., Aye, K.S. and Thant, K.Z. 2017. Clinical profile and circulating dengue virus serotype among adults admitted to Yangon General Hospital during the 2015 dengue outbreak. *OSIR Journal* 10(2): 8-13.

Najri, N.I., Mazlan, Z., Jaimin, J.J., Mohammad, R., Mohd, T.A.A., Hameed, A.A., Kumar, V. and Hoque, M.Z. 2017. AB804. The circulating serotypes of dengue in Sabah, Malaysian Borneo. *Annals of Translational Medicine* 5(Suppl 2).

Nathanson, C.M., Enria, D.A., Gubler, D.J., Hunsperger, E., Margolis, H.S., Artsob, H., Farrar, J., Pelegrino, J.L., Guzman, M.G., Cardosa, M.J., Rizzo, N., Buchy, P., Peeling, R.W., Halstead, S.B., Devi, S., Vázquez, S., Kliks, S., Yoksan, S. and Nguyen, V.C. 2010. Evaluation of diagnostic tests: dengue. *Nature Reviews Microbiology* 8(12supp): S30.

National Center for Biotechnology Information [NCBI] (Internet). Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. 1988. https://www.ncbi.nlm.nih.gov/. Accessed on 16th March 2017.

Nature Education. 2011. Dengue virus structure. Retrieved from https://www.nature.com/scitable/content/dengue-virus-structure-22401481. Accessed on 21st July 2018.

Nature Education. 2014. Host response to the dengue virus. www.nature.com/scitable/topicpage/host-response-to-the-dengue-virus-22402106/. Accessed on 12th April 2017.

Navarro-Sanchez, E., Altmeyer, R., Amara, A., Schwartz, O., Fieschi, F., Virelizier, J.L., Arenzana-Seisdedos, F. and Desprès, P. 2003. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Reports* 4(7): 723-728.

Ng, L.C., Chem, Y.K., Koo, C., Mudin, R.N., Amin, F.M., Lee, K.S. & Kheong, C.C. 2015. 2013 dengue outbreaks in Singapore and Malaysia caused by different viral strains. *The American Journal of Tropical Medicine and Hygiene* 92(6): 1150-1155.

Nizal, M.A., Rozita, H., Mazrura, S., Zainudin, M.A., Hidayatulfathi, O., Faridah, M.A., Artika, I.N. and Er, A.C. 2012. Dengue infections and circulating serotypes in Negeri Sembilan, Malaysia. *Malaysian Journal of Public Health Medicine* 12(1): 21-30.

Noor Hisham Abdullah. 2016. Situasi semasa demam denggi di Malaysia bagi minggu 1/2016 (3 hingga 9 Januari 2016). Kenyataan akhbar Ketua Kementerian Kesihatan. 11 January 2016.

Nusa, R., Prasetyowati, H., Meutiawati, F., Yohan, B., Trimasanto, H., Setianingsih, T.Y. & Sasmono, R.T. 2014. Molecular surveillance of dengue in Sukabumi, West Java province, Indonesia. *Journal of Infection in Developing Countries* 8(6): 733-741.

Osman, O., Fong, M.Y. and Devi, S. 2007a. A preliminary study of dengue infection in Brunei. *Japanese Journal of Infectious Diseases* 60(4): 205-208.

Osman, O., Fong, M.Y. and Devi, S. 2007b. Molecular characterization of dengue virus type 1 and 2 isolated during the 2005-2006 dengue outbreak in Brunei. *12th Bilogical Science Graduate Congress*: University of Malaya.

Pancharoen, C., Kulwichit, W., Tantawichien, T., Thisyakorn, U. and Thisyakorn, C. 2002. Dengue infection: a global concern. *Journal of the Medical Association of Thailand* 85: S25-33.

Pang, J., Salim, A., Lee, V.J., Hibberd, M.L., Chia, K.S., Leo, Y.S. and Lye, D.C. 2012. Diabetes with hypertension as risk factors for adult dengue haemorrhagic fever in a predominantly dengue serotype 2 epidemic: a case control study. *PLOS Neglected Tropical Diseases* 6(5): e16141.

Parida, M., Harioke, K., Ishida, H., Dash, P.K., Saxena, P., Jana, A.M., Islam, M.A., Inoue, S., Hosaka, N. and Morita, K. 2005. Rapid detection and differentiation of dengue virus serotype by a real time reverse transcription-loop mediated isothermal amplification assay. *Journal of Clinical Microbiology* 43: 2895-2903.

Parida, M., Upadhyay, C., Saxena, P., Dash, P.K., Jana, A.M. & Seth, P. 2001. Evalution of a dipstick ELISA and a rapid immunochromatographic test for diagnosis of dengue virus infection. *Acta Virologica* 45: 299-304.

Pearson, W., Artimo, P., Jonnalagedda, M., Arnold, K., Baratin, D., Csardi, G., de Castro, E., Duvaud, S., Flegel, V., Fortier, A., Gasteiger, E., Grosdidier, A., Hernandez, C., Ioannidis, V., Kuznetsov, D., Liechti, R., Moretti, S., Mostaguir, K., Redaschi, N., Rossier, G., Xenarios, I., and Stockinger, H. 2012. ExPASy: SIB bioinformatics resource portal – LALIGN programme, *Nucleic Acids Research*, 40(W1):W597-W603. Online accessed at: https://embnet.vital-it.ch/software/LALIGN_form.html

Phuong, C.X.T., Nhan, N.T., Wills, B., Kneen, R., Ha, N.T.T., Mai, T.T.T., Huynh, T.T.T., Lien, D.T.K., Solomon, T., Simpson, J.A. and White, N.J. 2002. Evaluation of the World Health Organization standard tourniquet test and a modified tourniquet test in the diagnosis of dengue infection in Viet Nam. *Tropical Medicine & International Health* 7(2): 125-132.

Pickett, B.E., Sadat, E.L., Zhang, Y., Noronha, J.M., Squires, R.B., Hunt, V., Liu, M., Kumar, S., Zaremba, S., Gu, Z., Zhou, L., Larson, C.N., Dietrich, J., Klem, E.B. & Scheuermann, R.H. 2012. ViPR: an open bioinformatics database and analysis resource for virology research. *Nucleic Acids Research* 40(Database issue): D593–D598. http://doi.org/10.1093/nar/gkr859

Pinheiro, F.P. 1989. Dengue in the Americas, 1980-1987. *Epidemiology Bulletin* 10: 1.

Pinheiro, F.P. and Corber, S.J. 1997. Global situation of dengue and dengue haemorrhagic fever and its emergence in the Americas. *World Health Statistics Quaterly* 50: 161-169.

Pok, K-Y., Lai, Y-L., Sng, J., & Ng, L-C. 2010. Evaluation of nonstructural 1 antigen assays for the diagnosis and surveillance of dengue in Singapore. *Vector Borne Zoonotic Diseases* 10(10): 1009–1016.

Pongsiri, P., Themboonlers, A. and Poovorawan, Y. 2012. Changing pattern of denfue virus serotypes in Thailand between 2004 and 2010. *Journal of Health, Population and Nutrition* 30(3): 366-370.

Rabe, I.B., Staples, J.E., Villanueva, J., Hummel, K.B., Johnson, J.A., Rose, L., Hills, S., Wasley, A., Fischer, M. and Powers, A.M. 2016. Interim guide for interpretation of Zika virus antibody test results. *Morbidity and Mortality Weekly Report* 65. DOI: http://dx.doi.org/10.15585/mmwr.mm6521e1

Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V. and Jackson, R.B. 2011. Dideoxy chain termination method for sequencing DNA. In Campbell Biology (10th Ed., p. 410) San Francisco, CA: Pearson.

Rice, C.M. 1990. Overview of flavivirus molecular biology and future vaccine development via recombinant DNA. *Southeast Asian Journal of Tropical Medicine and Public Health* 21: 670-677.

Rice, C.M. 1996. Flaviviridae: the viruses and their replication. In: Fields, B.M. (ed.) *Virology.* Lippincott-Raven, Philadelphia, Pennsylvania, pp. 931-960.

Rigau-Peréz, J.G. 1998. The early use of breakbone fever (quebranta huesos, 1771) and dengue (1801) in Spanish. *The American Journal of Tropical Medicine and Hygiene* 59: 272-274.

Rigau-Peréz, J.G. 2006. Sever dengue: the need for new case definitions. *Lancet Infection* 6: 297-302.

Rigau-Pérez, J.G., Clark, G.G., Gubler, D.J., Reiter, P., Sanders, E.J. and Vorndam, A.V. 1998. Dengue and dengue haemorrhagic fever. *The Lancet* 352(9132): 971-977.

Rodenhuis-Zybert, I.A., Wilschut, J. and Smit, J.M. 2010. Dengue virus life cycle: viral and host factors modulating infectivity. *Cellular and Molecular Life Sciences* 67(16): 2773-2786.

Rodhain, F. and Rosen, L. 1997. Mosquito vectors and dengue virus-vector relationships. In Gubler, D.J. and Kuno, G. (*eds.*) *Dengue and Dengue Hemorrhage Fever*: 45-60. CAB International, London.

Rodier, G., Gubler, D.J., Cope, S.E., Bercion, R., Cropp, C.B., Soliman, A.K., Boulomie, J., Piccolo, J.-J., Polycarpe, D., Abdourhaman, J.A., Delmarie, P., Bonnet, J.-P., Parra, J.-P., Gray, G.G. and Fryauff, D.J. 1995. Epidemic dengue 2 in the city of Djibouti, Horn of Africa, 1991-1992. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 90: 237-240.

Rothman, A. L. 2011. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nature Reviews Immunology* 11(8): 532.

Rudnick, A. 1965. Studies of the ecology of dengue in Malaysia: a preliminary report. *Journal of Medical Entomology* 2: 203-208.

Rudnick, A. 1986. Dengue virus ecology in Malaysia. *Institute for Medical Research Malaysia Bulletin* 23: 51-152..

Rye, C., Wise, R., Jurukovski, V., DeSaix, J., Choi, J. and Avissar, Y. 2013. Whole-genome sequencing. In Biology (p. 453-455). Houston, TX: OpenStax.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4): 406-425.

Scott, T.W., Naksathit, A., Day, J.F., Kittayapong, O. and Edman, J.D. 1997. A fitness advantage for *Aedes aegypti* and the viruses it transmits when females feed

only on human blood. *The American Journal of Tropical Medicine and Hygiene* 57: 235-239.

Screaton, G. and Mongkolsapaya, J. 2006. T cell responses and dengue haemorrhagic fever. *Novartis Foundation Symposium* 277: 164-171.

Sekaran, S.D., Lan, E.C., Mahesawarappa, K.B., Apanna, R. and Subramaniam, G. 2007. Evaluation of a dengue NS1 cature ELISA assay for the rapid detection of dengue. *Journal of infection in Developing Countries* 1: 182-188.

Seok, M.W. and Sekaran, S.D. 2010. Early diagnosis of dengue infection using a commercial dengue duo rapid test kit for the detection of NS1, IgM, and IgG. *The American Journal of Tropical Medicine and Hygiene* 83(3): 690-695.

Shu, P.Y., Chang, S.F., Kuo, Y.C., Yueh, Y.Y., Chuen, L.J., Sue, C.L., Lin, T.H. and Huang, J.H. 2003. Development of group and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR for dengue virus. *Journal of Clinical Microbiology* 41: 2408-2416.

Sittivicharpinyo, T., Wonnapinij, P. and Surat, W. 2018. Phylogenetic analyses of DENV-3 isolated from field-caught mosquitoes in Thailland. *Virus Research* 244: 27-35.

Sjatha, F., Takizawa, Y., Yamanaka, A. and Konishi, E. 2012. Phylogenetic analysis of dengue virus types 1 and 3 isolated in Jakarta, Indonesia in 1988. *Infection, Genetics and Evolution* 12(8): 1938-1943.

Skae, F.M.T. 1902. Dengue fever in Penang. *British Medical Journal* 2(2185): 1581.

Smith, C.E. 1958. The distribution of antibodies to Japanese encephalitis, dengue and yellow fever viruses in five rural communities in Malaya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 52: 237-252.

Srikiatkhachorn, A., Krautrachue, A., Ratanaprakarn, W., Wongtapradit, L., Nithipanya, N., Kalayanarooj, S., Nisalak, A., Thomas, S.J., Gibbons, R.V., Mammen Jr, M.P. and Libraty, D.H. 2007. Natural history of plasma leakage in dengue

hemorrhagic fever: a serial ultrasonographic study. *The Paediatric Infectious Disease Journal* 26(4): 283-290.

Statistics Solutions (2018) Chi-Square test of independence (Online) Retrieved from: http://www.statisticssolutions.com/non-parametric-analysis-chi-square/. Acessed on 5th September 2018.

Stephenson, J.R. 2005. Understanding dengue pathogenesis implications for vaccine design. *Bulletin of World Health Organization* 83(4): 308-314.

Suwandono, A., Kosasih, H., Nurhayati, Kusriastuti, R., Harun, S., Ma'roef, C., Wuryadi, S., Herianto, B., Yuwono, D., Porter, K.R., Beckett, C.G. and Blair, P.J. 2006. Four dengue virus serotypes found circulating during an outbreak of dengue fever and dengue hemorrhagic fever in Jakarta, Indonesia, during 2004. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 100: 855-862.

Tajima, S., Nakayama, E., Kotaki, A., Moi, M.L., Ikeda, M., Yagasaki, K., Saito, Y., Shibasaki, K., Saijo, M. and Takasaki, T. 2017. Whole genome sequencing-based molecular epidemiologic analysis of autochthonous dengue virus type 1 strains circulating in Japan in 2014. *Japan Journal of Infectious Diseases* 70: 45-49.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725-2729.

Tazeen, A., Afreen, N., Abdullah, M., Deeba, F., Haider, S.H., Kazim, S.N., Ali, S., Naqvi, I.H., Broor, S., Ahmed, A. and Parveen, S. 2017. Occurrence of co-infection with dengue viruses during 2014 in New Delhi, India. *Epidemiology and Infection* 145: 67-77.

Teoh, B.T., Sam, S.S., Tan, K.K., Johari, J., Shu, M.H., Danlani, M.B., Abd-Jamil, J., MatRahim, N., Mahadi, N.M. and AbuBakar, S. 2013. Dengue virus type 1 clade

replacement in recurring homotypic outbreaks. *BMC Evolutionary Biology* 13: 213.

Thayan, R., Sinniah, M., Satwant, S. and Mohaman Taha, A. 2001. The role of virological surveillance of dengue serotype for the prediction of dengue outbreak. *Tropical Biomedicine* 18(2): 109-116

ThermoFisher Scientific. 2018. What is Genotyping? Retrieved from https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-learning-center/genotyping-analysis-real-time-pcr-information/what-is-genotyping.html. Acessed on 30th July 2018.

Tomashek, K.M. 2011. Dengue fever. CDC Health Information for International Travel 2012: The Yellow Book. USA: Oxford University Press. 156-161.

Tsai, J.J., Chan, K.S., Chang, J.S., Chang, K., Lin, C.C., Huang, J.H., Lin, W.R., Chen, T.C., Hsieh, H.C., Lin, S.H. and Lin, J.C. 2009. Effect of serotypes on clinical manifestations of dengue fever in adults. *Journal of Microbiology, Immunology, and Infection* 42(6): 471-478.

Twiddy S.S., Pybus O.G. & Holmes E.C. 2003. Comparative population dynamics of mosquito borne flavivirus. *Infection, Genetics and Evolution* 3: 87-95.

Twiddy, S.S., Farrar, J.J., Chau, N.V., Wills, B., Gould, E.A., Gritsun, T., Lloyd, G. and Holmes E.C. 2002. Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. *Virology* 298: 63-72.

van der Schaar, H.M., Rust, M.J., Chen, C., van der Ende-Metselaar, H., Wilschut, J., Zhuang, X. and Smit, J.M. 2008. Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. *PLOS Pathogens* 4(12): e1000244.

Vasilakis, N., Cardosa, J., Hanley, K.A., Holmes, E.C. and Weaver, S.C. 2011. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nature Reviews Microbiology* 9: 532-541.

Villordo, S.M. and Gamarnik, A.V. 2009. Genome cyclization as strategy for flavivirus RNA replication. *Virus Research* 139: 230-239.

Vinomarlini, G., Rogayah, T.G., Saraswathy, T.S., Thayan, R., Apandi, M., Fauziah, M.K. & Saat, Z. 2011. Molecular typing of dengue viruses circulating on the East Coast of Peninsular Malaysia from 2005 to 2009. *The Southeast Asian Journal of Tropical Medicine and Public Health* 42(1): 94-99.

Vorndam, V. and Kuno, G. 1997. Laboratory diagnosis of dengue virus infection. In: Gubler, D.J. and Kuno, G., eds. Dengue and dengue haemorrhagic fever. CAB International: 313-333.

Wallace, H.G., Lim, T.W., Rudnick, A., Knudsen, A.B., Cheong, W.H. and Chew, V. 1980. Dengue hemorrhagic fever in Malaysia: the 1973 epidemic. *The Southeast Asian Journal of Tropical Medicine and Public Health* 11(1): 1-13.

Wang, B., Li, Y., Feng, Y., Zhou, H., Liang, Y., Dai, J., Qin, W., Hu, Y., Wang, Y., Zhang, L., Baloch, Z., Yang, H. and Xia, X. 2015. Phylogenetic analysis of dengue virus reveals the high relatedness between imported and local strains during 2013 dengue outbreak in Yunnan, China: a retrospective analysis. BMC Infectious Diseases 15: 142-148.

Wang, Q.Y., Patel, S.J., Vangrevelinghe, E., Xu, H.Y., Rao, R., Jaber, D., Schul, W., Gu, F., Heudi, O., Ma, N.L. and Poh, M.K. 2009. A small-molecule dengue virus entry inhibitor. *Antimicrobial Agents and Chemotherapy* 53(5): 1823-1831.

Wang, W.K., Sung, T.L., Tsai, Y.C., Kao, C.L., Chang, S.M. and King, C.C. 2002. Detection of dengue virus replication in peripheral blood mononuclear cells from dengue virus type 2-infected patients by a reverse transcription real-time PCR assay. *Journal of Clinical Microbiology* 40: 4472-4478.

Warillow, D., Nothill, J.A. and Pyke, A.T. 2012. Sources of dengue viruses imported into Queensland, Australia, 2002-2010. *Emerging Infectious Diseases* 18(11): 1850-1857.

Welsh, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C.K.E., Walther, P., Fuller, S.D., Antony, C., Krijne-Locker, J. & Bartenschlager, R. 2009. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host & Microbe* 5(4): 365-375.

Whitehead, S.S., Blaney, J.E., Durbin, A.P. and Murphy, B.R. (2007) Prospects for a dengue virus vaccine. *Nature Review Microbiology* 5(7): 518-528.

World Health Organization [WHO], Western Pacific Region. 2015. Emerging disease Surveillance and Report, Dengue Situation Updates. (Online) http://www.wpro.who.int/emergingdiseases/DengueSituationUpdates/en/. Retrieved 29 May 2016.

World Weather & Climate Information. 2016. Average monthly weather in Sandakan Malaysia (Online) https://weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine. Sandakan, Malaysia. Accessed on 21st September 2016.

World Health Organization [WHO]. 2005. Dengue, dengue haemorrhagic fever and dengue shock syndrome in the context of the integrated management of childhood illness. Geneva: WHO.

World Health Organization [WHO]. 2009. Dengue guidelines for diagnosis, treatment, prevention and control. France: WHO.

World Health Organization-Regional Office for South-East Asia [WHO-SEARO]. 2011. Comprehensives guidelines for prevention and control of dengue and dengue haemorrhagic. Revised and expanded edition. India: WHO.

Yacoub, S., Mongkolsapaya, J. and Screaton, G. 2013. The pathogenesis of dengue. *Current Opinion in Infectious Diseases* 26(3): 284-289.

Yang C-F., Su C-L., Hsu T-C., Chen L-Y. & Shu P-Y. 2014. Surveillance and molecular characterization on dengue viruses in Taiwan, 2013. *Taiwan Epidemiology Bulletin* 30(9): 157-170.

Young, P.R., Hildritch, P.A., Bletchly, C. & Halloran, W. 2000. An antigen capture enzyme-linked immunoabsorbent assay reveals high levels of the dengue virus protein NS1 in the sera of the infected patients. *Journal of Clinical Microbiology* 28(3): 1053-1057.

Yung, C.F., Lee, K.S., Thein, T.L., Tan, L.K., Gan, V.C., Wong, J.G., Lye, D.C., Ng, L.C. and Leo, Y.S. 2015. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. *The American Journal of Tropical Medicine and Hygiene* 92(5): 999-1005.

Wu, S.J., Lee, E.M., Putvatana, R., Shurtlift, R.N., Porter, K.R., Suharyono, W., Watts, D.M., King, C.C., Murphy, G.S., Hayes, C.G. and Romano, J.W. 2001. Detection of dengue viral RNA using a nucleic acid sequence-based amplification assay. *Journal of Clinical Microbiology* 39: 2794-2798.