



Faculty of Medicine and Health Sciences

**Detection of Dengue Virus Serotypes in Aedes Mosquito Population from
Sibu and Miri Divisions of Sarawak using Reverse Transcription
Polymerase Chain Reaction (RT-PCR)**

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Detection of Dengue Virus Serotypes in Aedes Mosquito Population from
Sibu and Miri Divisions of Sarawak using Reverse Transcription Polymerase
Chain Reaction (RT-PCR)

Harvie Anak Shukri

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Medical Entomology)

Faculty of Medicine and Health Sciences
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2019

DECLARATION

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ABSTRACT

Dengue has been a public health concern for many years in Malaysia. Having knowledge on the current circulating dengue serotype and population of vector mosquito is key in controlling outbreaks and future outbreak predictions. The current study reports the first study on detecting dengue virus serotypes in the *Aedes* mosquito population in Sibul and Miri divisions of Sarawak. Mosquito samples were collected at selected localities from September 2016 to December 2017. Localities were selected randomly mainly focussing on urban residential areas. The mosquitoes collected comprised of two types namely the field-caught adults and immatures collected from artificial and natural water containers. Collected mosquitoes were identified to species level and screened for presence of dengue virus and serotyped using conventional reverse transcription polymerase chain reaction (RT-PCR) and semi-nested PCR. Dengue virus serotype 2 (DENV-2) was identified in 3 pools of field-caught female *Aedes albopictus* adults that were collected from Jalan Tong Sang, Sibul, Sibul Lake Garden, and Taman Ceria, Permyjaya, Miri, respectively. DENV-2 was also detected in one pool of adult male *Aedes albopictus* that had emerged from immatures collected from Taman Ceria, Permyjaya, Miri. The findings in this study revealed that *Aedes albopictus* was the main species colonizing the study areas, and the current circulating dengue virus serotype is DENV-2. This study also reports the first natural evidence of transovarial transmission of dengue in the natural population of *Aedes albopictus* within the study area. This latest report may aid in providing information on establishing strategies in an effective vector control and, also to control any current and future outbreaks if necessary.

Keywords: *Aedes albopictus*, dengue virus, RT-PCR, dengue virus serotype 2, field-caught adult mosquitoes, immature mosquitoes, transovarial transmission.

Pengesanan Serotip Virus Denggi dalam Populasi Nyamuk Aedes dari Bahagian Sibuan dan Miri Sarawak menggunakan Reaksi Berantai Polimerase Transkripsi-Berbalik (RT-PCR)

ABSTRAK

Denggi telah menjadi kebimbangan kesihatan awam selama bertahun-tahun di Malaysia. Pengetahuan mengenai virus denggi (DENV) yang berkitar dan populasi nyamuk vektor adalah penting dalam mengawal wabak dan ramalan tentang wabak. Kajian ini melaporkan kajian pertama mengenai pengesanan serotip virus denggi dalam populasi nyamuk Aedes di bahagian Sibuan dan Miri di Sarawak. Sampel nyamuk dikumpulkan di lokasi terpilih dari September 2016 hingga Disember 2017. Lokaliti dipilih secara rawak terutamanya pada kawasan kediaman bandar. Nyamuk yang dikumpulkan terdiri daripada dua jenis iaitu nyamuk dewasa dan jentik-jentik yang dikutip dari bekas air buatan serta bekas air semula jadi dari kawasan persampelan. Nyamuk yang terkumpul telah dikenalpasti ke tahap spesies dan disaring untuk kehadiran virus denggi menggunakan RT-PCR konvensional. Denggi serotip 2 (DENV-2) telah dikenalpasti di dalam 3 kumpulan nyamuk Ae. albopictus betina dewasa yang ditangkap dari Jalan Tong Sang, Sibuan, Taman Tasik Sibuan, dan Taman Ceria, Permyjaya, Miri. DENV-2 juga dikesan dalam satu kumpulan nyamuk jantan Ae. albopictus yang membesar dari peringkat pramatang yang dikumpul dari Taman Ceria, Permyjaya, Miri. Penemuan dalam kajian ini mendedahkan bahawa Ae. albopictus adalah spesis utama nyamuk di kawasan kajian, dan serotip virus denggi semasa ialah DENV-2. Kajian ini juga membuktikan penyebaran virus denggi semulajadi secara transovari dalam populasi Ae. albopictus di kawasan kajian. Laporan

terkini ini boleh membantu dalam membentuk strategi dalam kawalan vektor yang efektif dalam mengawal wabak denggi semasa dan dimasa akan datang.

Kata kunci: *Ae. albopictus, virus denggi, RT-PCR, virus denggi serotip 2, nyamuk dewasa tangkapan dari lapangan, nyamuk pramatang, penyebaran secara transovari*

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LIST OF ABBREVIATIONS

%	percentage
°C	degree Celsius
μL	microliter
A.D	<i>Anno Domini</i>
<i>Ae.</i>	<i>Aedes</i>
AMCA	American Mosquito Control Association
bp	basepair
BSC	biosafety cabinet
CDC	Center for Disease Control
c-DNA	complementary deoxyribonucleic acid
C/prM	capsid and pre-membrane
DENV	dengue virus
DF	dengue fever
DHF	dengue haemorrhagic fever
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DSS	dengue shock syndrome
ELISA	enzyme-linked immunosorbent assay
Ig	immunoglobulin
kb	kilobase
mg	milligram
MgCl ₂	magnesium chloride

min	minutes
MIR	minimum infection rate
M-MLV	Moloney Murine Leukemia Virus
mm	millimetre
nm	nanomolar
PAP	Peroxidase-antiperoxidase
PCR	polymerase chain reaction
RNA	ribonucleic acid
Rnasin	rnase inhibitor
rpm	revolutions per minute
RT	reverse transcription
<i>sp.</i>	species
TBE	tris-borate-edta
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Dengue has been one of the most important public health concerns worldwide and has caused significant number of deaths every year. According to the World Health Organization (2017), approximately half of the global population are at risk of dengue infection in over 125 endemic countries. Dengue is an acute systemic form of a viral disease which has established itself both epidemically and endemically which can lead to a range of clinical manifestations from mild fevers to severely fatal cases (Bhatt et al., 2013). Dengue manifest in two forms: dengue fever (DF) and dengue haemorrhagic fever (DHF). The World Health Organization estimates that 50 to 100 million DF cases occur worldwide, including 500,000 DHF cases yearly. DF is characterized by an acute and sudden onset of symptoms including fever, body aches, and skin rashes (CDC, 2012). DHF/DSS is a much more severe and chronic form of dengue manifestation. It is characterized by onset of symptoms including severe pain, bleeding from nose and gums, and vomiting blood. (CDC, 2012). Dengue infection is caused by the dengue virus (DENV), a single-stranded RNA virus belonging to family and genus of *Flaviviridae* and *Flavivirus* respectively, similar to yellow fever virus, West Nile virus, chikungunya and Zika virus. There are four serotypes of DENV; DENV-1, -2, -3, and -4. All of the serotypes can be found in all dengue endemic and epidemic areas around the world. Malaysia has all four serotypes of DENV making it hyperendemic for dengue (Cheah et al., 2014; Mohd-Zaki et al., 2014).

DENV is a vector-borne virus. The main vector for DENV is the *Aedes* mosquitoes primarily

Ae. aegypti and *Ae. albopictus*. Both mosquitoes can be found worldwide in most tropical and sub-tropical areas. These mosquitoes transmit dengue from humans to humans through blood-feeding, therefore being the vector of the virus. This mode of transmission is called the horizontal transmission. Transmission of dengue can also occur in the mosquito population itself. This mode of transmission is called the vertical or transovarial transmission, where the virus is inherited by the progeny from the DENV infected female *Aedes* mosquito (Martins et al., 2012). Furthermore, vertical transmission of DENV does not only occur in mosquitoes but also in human beings. There were evidences of DENV being transmitted to infants from their mother during late stages of pregnancy (Chotigeat et al., 2000; Phongsamart et al., 2008; Yin et al., 2016).

1.2 Problem statement

Most of the published studies regarding the detection of DENV serotypes in *Aedes* mosquito populations were conducted in Peninsular Malaysia but to date, no such study had been published from the State of Sarawak. Furthermore, studies conducted in Sarawak mainly focuses on the distribution of *Aedes* mosquito population where some of them were conducted more than 30 years ago (Chang & Jute, 1982). Recently, an ovitrap survey study was also conducted in Sarawak, where only one species of *Aedes* mosquito was identified, which is *Aedes albopictus* (Lau et al., 2017). Studies regarding the circulation of DENV have been conducted but focuses only on clinical samples rather than on the entomological aspect (Holmes et al., 2009). Amidst the increase of dengue cases in Sarawak in recent

years, especially in Sibul and Miri, it is appropriate that this study be conducted. The outcome would greatly assist in establishing effective strategies in monitoring, controlling and preventing dengue outbreaks. It will not only benefit the community in Sarawak but may also be beneficial for the whole community in Malaysia.

1.3 Aim

This study aims to identify the current circulating dengue virus serotype in the natural population of *Aedes* mosquitoes in Sibul and Miri divisions of Sarawak.

1.4 Objectives

The objectives of this study are:

- i. To identify the species of *Aedes* mosquitoes in selected localities in Sibul and Miri divisions of Sarawak.
- ii. To detect the presence of DENV in the natural population of *Aedes* mosquitoes in the study areas.
- iii. To determine the circulating DENV serotypes in the study areas.
- iv. To determine the presence of transovarial transmission of DENV in *Aedes* mosquito population.

CHAPTER 2

LITERATURE REVIEW

2.1 Epidemiology of dengue

2.1.1 Global epidemiology of dengue

Dengue is one of the world's most infectious disease that affects approximately 400 million people worldwide, which the incidence has grown dramatically over the past decade causing half of the global population to be at risk in over 125 endemic countries (World Health Organization, 2017). Dengue is an acute systemic form of a viral disease which has established itself both epidemically and endemically leading to a range of clinical manifestations from mild fevers to severely fatal cases (Bhatt et al., 2013). The global epidemiology trends of dengue have been changing due to several factors. One of the factor is changes in demography such as population growth, economic trends in tropical countries and patterns of land usage. Another important factor is the rise in population size and density from rural to urban migration (Ferreira, 2012). This may be due to increased job opportunities making more people from rural areas moving into the urban area. Furthermore, modernisation of transportation has also contributed to changes in dengue epidemiology due to increased movement of human, disease vectors and pathogens. Constant development in public health policies and infrastructures also play a part in changing the trends of dengue epidemiology (Ferreira, 2012).

The first recorded incidence of a disease similar to dengue dated back 1800 years ago during the Chinese Chin Dynasty (265-420 A.D). The Chinese assumed that the disease was similar to the water poison disease associated with flying insects and water (Gubler, 1998). In addition, there were also reports on an illness outbreak in the French West Indies and Panama during the middle and end of the 17th century, which was likely to be dengue. Therefore, dengue could have a wide geographic distribution prior to the 18th century. Dengue only became a public health burden in Southeast Asia right after the Second World War during the late 1940s and early 1950s (Ooi & Gubler, 2009). Due to the ecological and demographic changes, it created a suitable environment and conditions for the transmission of this vector-borne disease. This was also heightened by the movement of equipment and people during the war, therefore increasing the transportation of the dengue virus vector primarily the *Aedes aegypti* mosquito.

According to the World Health Organization (WHO) (2017), only 9 countries were affected by dengue epidemics prior to the 1970s. Currently, the number of countries affected have increased significantly and at present more than 100 countries are affected by dengue epidemics (Cheah et al., 2014). These countries are mostly located in most of the globes major continents such as, South-East Asia, the Western Pacific, the Middle East, Africa, America, Europe, Australia and New Zealand (Wilder-Smith et al., 2010). Furthermore, people in the South-East Asia region and the Western Pacific region bear closely to 75% of the dengue global disease burden and the Latin America region in recent years showed an increase in amplitude and frequency in dengue epidemics (Ferreira, 2012). Figure 2.1 shows the current global dengue affected areas from the 1960's until today.