



**Faculty of Medicine and Health Sciences**

**Vector Status and Transovarial Transmission of Chikungunya Virus (CHIKV) and Dengue Virus (DENV) in Aedes mosquitoes in Kampung Pulau Salak and Kampung Tanjong Bako, Sarawak**

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**Master of Science  
2022**

Vector Status and Transovarial Transmission of Chikungunya Virus (CHIKV)  
and Dengue Virus (DENV) in Aedes mosquitoes in Kampung Pulau Salak  
and Kampung Tanjong Bako, Sarawak

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A thesis submitted

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

(Medical Entomology)

Faculty of Medicine and Health Sciences  
UNIVERSITI MALAYSIA SARAWAK

2022

## DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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## ACKNOWLEDGEMENT

First and foremost, I would like to thank God for this opportunity to continue my study in Master of Science at UNIMAS. I would like to express my greatest gratitude to my supervisor, Assoc. Prof. Dr. Nor Aliza bt Abdul Rahim and co-supervisor, Mrs. Anna Andrew for their guidance and countless support throughout the journey in completing the research project. They have always encouraged me to do my best and making sure that I'm on the right track towards completing my study. I can't thank them enough for their support.

I would also like to thank my entomology research laboratory teammates, Marlini bt Othman, Emira Izzati Abdul Aziz and Harvie Ak Shukri for their help and support in completing this project. I am truly grateful for their assistance during field works. I feel so blessed to have you all as my laboratory teammates. I wish you all the best for your future undertakings. I am also thankful to Dr Magdline Sia Henry Sum from The Institute of Health and Community, UNIMAS for providing the positive control of chikungunya virus for this project. I would like to express my gratitude to all the staffs of the Faculty of Medicine and Health Sciences as well for their assistance throughout the process of completing my laboratory work.

I am also hugely indebted to my parents for their continuous support and encouragement throughout this journey. Thank you for always being there for me. Last but not least, I would like to thank UNIMAS for their financial support as well.

## ABSTRACT

Chikungunya virus (CHIKV) and dengue virus (DENV) are vector-borne viruses which can be transmitted through vector mosquitoes, *Ae. aegypti* and *Ae. albopictus*. Pulau Salak in Sarawak is one of those areas affected by chikungunya outbreak in 2009. Thus, this study was conducted to determine the vector status and transovarial transmission of CHIKV in *Aedes* mosquitoes on Kampung Pulau Salak, Kuching, Sarawak, since it is one of the areas which was affected due to the outbreak of CHIKV. The process of detection of DENV was carried out on the mosquitoes as well. The research was carried out at Kampung Pulau Salak and Kampung Tanjong Bako, Kuching in which Kampung Tanjong Bako act as a negative control site in this study. Mosquito larvae were collected from ovitraps and discarded receptacles before being reared in the laboratory for emergence of adults while adult mosquitoes were sampled by using bare-leg catch method (BLC) with the help of mechanical aspirators. A total of 7834 *Aedes* mosquitoes were pooled according to species, sex and sampling location. The ovitrap index recorded on Kampung Pulau Salak was 84.3%, 96.7%, and 89.3% for the first, second and third sampling respectively. On the other hand, the ovitrap index recorded in Kampung Tanjong Bako was 67.6%, 50.0%, and 87.1% for the first, second and third sampling respectively. There was no significant difference ( $p > 0.05$ ) of ovitrap indexes between Kampung Pulau Salak and Kampung Tanjong Bako. The highest number of species of mosquitoes collected in Kampung Pulau Salak was *Ae. albopictus* (90.07%) followed by *Ae. aegypti* (6.02%), *Culex* sp. (2.63%), and *Armigeres* sp. (1.28%) while the highest number of species of mosquitoes collected in Kampung Tanjong Bako was *Ae. albopictus* (99.93%) followed by *Ae. aegypti* (0.07%). RNA extraction and two-step Reverse Transcription Polymerase Chain Reaction (RT-PCR) were performed to detect CHIKV and DENV from the samples collected. There were 223 pools of *Aedes* mosquitoes

tested for the presence of the viruses. Chikungunya virus of East Central South African genotypes were detected in one pool of male and one pool of female (n=35) emerged *Aedes albopictus* mosquitoes collected from the ovitraps in Kampung Pulau Salak. DENV of serotype 2 was detected in one pool of male and two female pools (n=35) of emerged *Aedes albopictus* collected from Kampung Pulau Salak as well. There was no presence of CHIKV and DENV detected in the emerged *Aedes* mosquitoes collected from Kampung Tanjong Bako. None of the field-caught *Aedes* mosquitoes collected from Kampung Pulau Salak and Kampung Tanjong Bako were detected with CHIKV and DENV as well. The findings of this study showed evidence of transovarial transmission of CHIKV and DENV in *Aedes* mosquitoes in Kampung Pulau Salak. This finding may contribute to the effort in controlling the vector of CHIKV and DENV in the island as the status of the vector is currently unknown.

**Keywords:** Transovarial transmission, *Aedes* mosquitoes, RT-PCR, East Central South African genotypes, chikungunya virus, dengue virus

***Status Vektor dan Transmisi Transovari Virus Chikungunya (CHIKV) dan Virus Denggi (DENV) dalam nyamuk Aedes di Kampung Pulau Salak dan Kampung Tanjong Bako, Sarawak***

**ABSTRAK**

*Virus chikungunya dan virus denggi merupakan virus bawaan vektor yang boleh disebarkan melalui vektor seperti nyamuk Ae. aegypti dan Ae. albopictus. Pulau Salak di Sarawak merupakan salah satu kawasan yang dijangkiti oleh wabak chikungunya pada tahun 2009. Oleh yang demikian, kajian ini dijalankan untuk mengesan status vektor dan penyebaran transovari virus chikungunya dalam nyamuk Aedes di Kampung Pulau Salak, Kuching, Sarawak. Proses pengesanan virus denggi juga dijalankan dalam kajian ini. Kajian dijalankan di Kampung Pulau Salak dan Kampung Tanjong Bako di mana Kampung Tanjong Bako berfungsi sebagai kawasan kawalan negatif. Larva nyamuk dikutip daripada pemasangan ovitrap dan bekas-bekas terbuang sebelum dipelihara di makmal sehingga peringkat dewasa manakala nyamuk dewasa ditangkap dengan menggunakan kaedah bare leg catching (BLC) dengan bantuan aspirator mekanikal. Sebanyak 7834 sampel nyamuk Aedes diasingkan dalam longgokan mengikut spesies, jantina, dan lokasi sampling. Indeks ovitrap dari Kampung Pulau Salak adalah sebanyak 84.3% dan 96.7% bagi pensampelan kali pertama dan kedua manakala indeks ovitrap untuk pensampelan kali ketiga adalah sebanyak 89.3%. Selain itu, indeks ovitrap dari Kampung Tanjong Bako adalah sebanyak 67.7% dan 50.0% bagi pensampelan kali pertama manakala indeks ovitrap untuk pensampelan kali ketiga adalah sebanyak 87.1%. Tiada sebarang perbezaan ketara ( $p > 0.05$ ) di antara indeks ovitrap Kampung Pulau Salak dan Kampung Tanjong Bako. Spesies nyamuk yang paling banyak dikutip daripada Kampung Pulau Salak adalah Ae. albopictus (90.07%) diikuti oleh Ae. aegypti (6.02%), Culex sp. (2.63%) dan Armigeres sp. (1.28%) manakala spesies nyamuk yang paling banyak dikutip dari Kampung Tanjong Bako adalah*

*Ae. albopictus* (99.93%) dan diikuti oleh *Ae. aegypti* (0.07%). Pengekstrakan RNA dan tindak balas rantaian polimerase dengan menggunakan transkripsi berbalik (RT-PCR) secara dua langkah telah dilaksanakan untuk mengesan virus chikungunya dan virus denggi dalam sampel yang dikutip. Sebanyak 223 longgokan nyamuk *Aedes* telah dikaji pengesanan virus chikungunya dan denggi. Virus chikungunya dari genotip East/ Central/ South/ African telah dikesan dalam satu longgokan nyamuk jantan dan satu longgokan nyamuk betina *Ae. albopictus* (n=35) yang dikutip dari Kampung Pulau Salak. Virus denggi serotip-2 juga telah dikesan dalam satu longgokan nyamuk jantan dan dua longgokan nyamuk betina (n=35) *Ae. albopictus* dari Kampung Pulau Salak. Tiada virus chikungunya dan denggi dikesan dari nyamuk *Aedes* yang dibesarkan dan dikutip dari Kampung Tanjong Bako. Tiada virus chikungunya dan denggi juga dikesan dari nyamuk dewasa yang ditangkap di lapangan dari kedua-dua buah kampung tersebut. Penemuan kajian ini menunjukkan bahawa berlakunya penyebaran transovari dalam nyamuk *Aedes* di Kampung Pulau Salak. Kajian ini boleh menyumbang kepada usaha untuk mengawal vektor virus chikungunya dan denggi di Kampung Pulau Salak memandangkan status vektor yang terkini di kawasan tersebut masih belum dikenalpasti.

**Kata kunci:** Penyebaran transovari, nyamuk *Aedes*, RT-PCR, genotip East Central South African, virus chikungunya dan denggi



## TABLE OF CONTENTS

	<b>Page</b>
<b>DECLARATION</b>	i
<b>ACKNOWLEDGEMENT</b>	ii
<b>ABSTRACT</b>	iii
<b><i>ABSTRAK</i></b>	v
<b>TABLE OF CONTENTS</b>	vii
<b>LIST OF TABLES</b>	xii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF ABBREVIATIONS</b>	xvi
<b>CHAPTER 1: INTRODUCTION</b>	1
1.1 Study Background	1
1.2 Problem Statement	3
1.3 Aims	4
1.4 Research Objectives	4
<b>CHAPTER 2: LITERATURE REVIEW</b>	6
2.1 Vector-borne Diseases	6
2.2 Pathogens of Vector-borne Diseases	8
2.3 Modes of Transmission of Vector-borne Diseases	10
2.4 Chikungunya	12

2.4.1	Epidemiology of Chikungunya	12
2.4.2	Chikungunya Fever	17
2.4.3	Chikungunya Virus and its Genotypes	17
2.5	Dengue	18
2.5.1	Epidemiology of Dengue	18
2.5.2	Dengue Fever	23
2.5.3	Dengue Virus and its Serotypes	24
2.6	Vector Mosquitoes	25
2.7	Life cycle of Mosquitoes	27
2.7.1	<i>Aedes albopictus</i>	29
2.7.2	<i>Aedes aegypti</i>	30
2.8	Control of Aedes Mosquitoes	32
2.9	Virus Maintenance and Transovarial Transmission	33
2.9.1	Virus Maintenance and Transovarial Transmission of CHIKV	33
2.9.2	Virus Maintenance and Transovarial Transmission of DENV	38
2.10	Minimum Infection Rate	40
<b>CHAPTER 3: MATERIALS AND METHODS</b>		41
3.1	Study Area	41
3.2	Mosquito Sample Collection	42
3.2.1	Field Collection of Immatures	42

3.2.2	Field-caught <i>Aedes albopictus</i>	43
3.3	RNA Extraction/ Virus /Isolation	43
3.4	Reverse Transcriptionpolymerase Chain Reaction of CHIKV	49
3.4.1	Reverse transcription (RT) of CHIKV	49
3.4.2	Polymerase Chain Reaction (PCR) of CHIKV	51
3.4.3	Visualization of PCR Products in Agarose Gel Electrophoresis of CHIKV	52
3.5	Reverse-transcription Polymerase Chain Reaction (RT-PCR) of DENV	53
3.5.1	Reverse Transcription (RT) of DENV	53
3.5.2	Polymerase chain reaction (PCR) of DENV	55
3.5.3	Visualization of PCR Products in Agarose Gel Electrophoresis of DENV	56
3.6	Data Analysis	57
3.6.1	Calculation of Ovitrap Index and Identification of Mosquitoes	57
3.6.2	Calculation of Minimum Infection Rate	58
3.6.3	Phylogenetic and Statistical Analysis	58
	<b>CHAPTER 4: RESULTS</b>	60
4.1	Species of Mosquitoes Collected	60
4.1.1	Immatures and Adult Mosquitoes Collected from Kampung Pulau Salak	60
4.1.2	Immatures and Adult Mosquitoes Collected from Kampung Tanjong Bako	61
4.2	Detection of CHIKV	62
4.2.1	Detection of CHIKV in Mosquitoes Collected from Kampung Pulau Salak	62

4.2.2	Detection of CHIKV in mosquitoes collected from Kampung Tanjong Bako	63
4.3	Detection of DENV	64
4.3.1	Detection of DENV in mosquitoes collected from Kampung Pulau Salak	64
4.3.2	Detection of DENV in mosquitoes collected from Kampung Tanjong Bako	65
4.4	Temperature and pH Recorded in Ovitrap and Discarded Receptacles	66
4.4.1	Temperature and pH Recorded in Ovitrap and Discarded Receptacles in Kampung Pulau Salak	66
4.4.2	Temperature and pH Recorded in Ovitrap and Discarded Receptacles in Kampung Tanjong Bako	67
<b>CHAPTER 5: DISCUSSION</b>		68
5.1	The Diversity of Mosquitoes Found at Kampung Pulau Salak and Kampung Tanjong Bako	68
5.2	CHIKV in Aedes Mosquitoes	70
5.3	DENV in Aedes Mosquitoes	73
5.4	Minimum Infection Rate of CHIKV and DENV in Aedes Mosquitoes	74
5.4.1	Minimum Infection Rate of CHIKV in Aedes Mosquitoes	74
5.4.2	Minimum Infection Rate of DENV in Aedes Mosquitoes	75
5.5	Temperature and pH of the Medium of Growth for Immatures of Aedes Mosquitoes	75
<b>CHAPTER 6: CONCLUSION AND RECOMMENDATIONS</b>		77
6.1	Conclusion	77

6.2	Limitations of Study	78
6.3	Recommendations	78
	<b>REFERENCES</b>	80
	<b>APPENDICES</b>	100

## LIST OF TABLES

		<b>Page</b>
Table 2.1	Vector-borne diseases and their vectors	7
Table 2.2	Countries and territories in which chikungunya cases have been reported (up to date until 17 September, 2019) (Center for Disease Control and Prevention, 2019)	15
Table 2.3	The number of chikungunya cases reported in each state within Malaysia from 2015 until 18 <sup>th</sup> July 2020 (MOH, 2019; MOH, 2020)	16
Table 3.1	Sequences of the primers (CHIKF and CHIKR) used for detection of chikungunya virus (Dayakar et al., 2015)	49
Table 3.2	Reagents used for the preparation of master mix for the reverse transcription of CHIKV	50
Table 3.3	Thermal cycler (Applied Biosystems® Veriti® Thermal Cycler) programmed for the RT process of CHIKV	50
Table 3.4	Reagents used for the preparation of the master mix for polymerase chain reaction for detection of CHIKV	51
Table 3.5	Thermal Cycler (Applied Biosystems® Veriti® Thermal Cycler) programmed for PCR of CHIKV	52
Table 3.6	Sequences of the primers (D1 and D2) used for detection of dengue virus (Lanciotti et al.1992)	53
Table 3.7	Reagents used for the preparation of the master mix for reverse transcription of DENV	54

Table 3.8	Thermal cycler (Applied Biosystems® Veriti® Thermal Cycler) programmed for the RT step of DENV	55
Table 3.9	Reagents used for the preparation of the master mix for polymerase chain reaction for detection of DENV	55
Table 3.10	Thermal Cycler (Applied Biosystems®Veriti® Thermal Cycler) programmed for PCR of DENV	56
Table 4.1	Mosquitoes species collected from Kampung Pulau Salak, Kuching	60
Table 4.2	Mosquitoes species collected from Kampung Tanjong Bako, Kuching	61
Table 4.3	Range of temperature and pH recorded from discarded receptacles found around Kampung Pulau Salak	66
Table 4.4	Range of temperature and pH recorded from discarded receptacles found around Kampung Tanjong Bako	67

## LIST OF FIGURES

		<b>Page</b>
Figure 2.1	Map showing the countries and territories in which chikungunya cases have been reported (as of 17 September, 2019) (Centers for Disease Control and Prevention, 2019)	14
Figure 2.2	Genome structure of CHIKV (An et al., 2017)	18
Figure 2.3	Dengue cases reported worldwide up to December 2019 (European Centre for Disease Prevention and Control, Jan 2020)	21
Figure 2.4	Number of dengue cases reported and the rate of incidence in Malaysia from the year 2000 until 2019 (Ministry of Health Malaysia, n.d.)	22
Figure 2.5	Structure and genome of dengue virus (Hottz et al., 2011)	25
Figure 2.6	Life cycle of mosquitoes (The American Mosquito Control Association, n.d.)	28
Figure 2.7	Male <i>Aedes albopictus</i> with magnification (Ellis, n.d.)	29
Figure 2.8	Female <i>Aedes albopictus</i> (40x magnification)	29
Figure 2.9	Male <i>Aedes aegypti</i> with magnification (Entomology Today, 2018)	31
Figure 2.10	Female <i>Aedes aegypti</i> (40x magnification)	31
Figure 2.11	Transmission cycles of CHIKV (Adapted from Horwood et al., 2015)	36
Figure 2.12	Urban cycle of CHIKV (Adapted from Whitehead, 2007)	37
Figure 2.13	Process of replication of CHIKV (Monteiro et al., 2019)	37



Figure 2.14	Dissemination of CHIKV to different tissues and organs (Caglioti et al., 2013)	38
Figure 3.1	Map of Pulau Salak from UNIMAS (Photo courtesy from Google Map)	45
Figure 3.2	Map of Kampung Pulau Salak (Photo courtesy from Google Map)	46
Figure 3.3	Map of Kampung Tanjong Bako from UNIMAS (Photo courtesy from Google Map)	47
Figure 3.4	Map of Kampung Tanjong Bako (Photo courtesy from Google Map)	48
Figure 4.1	Maximum likelihood tree of 24 gene sequences of ECSA genotype of CHIKV created by using MEGA 7 (2 from this study, 22 from GenBank)	63
Figure 4.2	Maximum likelihood tree of 30 gene sequences of DENV-2 created by using MEGA 7 (3 from this study, 27 from GenBank)	65

## LIST OF ABBREVIATIONS

%	Percentage
°C	Degree celcius
µL	Microliter
<i>Ae.</i>	<i>Aedes</i>
c-DNA	Complementary deoxyribonucleic acid
Bp	Base pair
CHIKV	Chikungunya virus
DENV	Dengue virus
DHF	Dengue haemorrhagic fever
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleic phosphate
MgCl <sub>2</sub>	Magnesium chloride
MIR	Minimum infection rate
MOH	Ministry of Health
ml	Milliliter
Mm	Millimeter
NCBI	National Center for Biotechnology Information
ORF	Open reading frame
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
Rnasin	Rnase inhibitor
Rpm	Revolutions per minute

# CHAPTER 1

## INTRODUCTION

### 1.1 Study Background

Vector-borne diseases are diseases that are caused by pathogens such as parasites, viruses, and bacteria, which are transmitted by living organisms classified as vector. Most of these vectors are blood-sucking insects such as mosquitoes, blackflies, and ticks (WHO, 2020). Vector-borne diseases are responsible for about 17% of the estimated global burden of infectious diseases and it had affected the poorer populations as well. The emergence of vector-borne diseases will hinder economic development through direct medical costs and indirect costs, which include loss of productivity and tourism (WHO, 2017). Vector-borne diseases such as malaria, leishmaniasis and dengue had caused great health risks and fatalities widely (Wilson et al., 2020).

Malaysia is one of those countries that are affected by vector-borne diseases. Some of the diseases that are commonly found in the country include dengue, Zika, malaria and Japanese encephalitis, which are transmitted by vector mosquitoes. Two known vector mosquitoes in Malaysia that are responsible for the transmission of dengue virus (DENV) and chikungunya virus (CHIKV) are *Ae. albopictus* and *Ae. aegypti* (Tham., 1993; Lee et al., 2015). *Ae. aegypti* is the primary vector of both diseases while *Ae. albopictus* acts as the secondary vector (Lee et al., 2015). Although *Ae. albopictus* is classified a secondary vector, it has been reported that this species of mosquito is an efficient vector for DENV and CHIKV (Scholte et al., 2007). In addition, the population of *Ae. albopictus* is more widespread in Sarawak compared to *Ae. albopictus* (Chang & Jute, 1982). Both species of mosquitoes have

different preferences of habitat as *Ae. aegypti* prefers indoor environment while *Ae. albopictus* prefers outdoor environment (Camara, 2010; Kweka et al., 2019).

Dengue fever and chikungunya are vector-borne diseases that spread widely and are imposing public health treats to both industrialized and developing nations worldwide. It is expected that mosquito-borne diseases such as DENV and CHIKV will invade new areas of the world including the United States since the *Aedes* vectors had spread worldwide (Fredericks & Fernandez-Sesma, 2014). Both diseases can cause morbidity and are endemic in several areas in the tropics (Azeredo et al., 2015; Dhenni et al., 2021).

Various control methods through chemical, physical, biological or an integrical approach have been adopted to handle the impact of *Aedes*-borne diseases. The control of vector-borne diseases is one of the hardest challenges on the agenda of global health. One of the first chemical approach that had been used to control adult dengue vectors is by using Dichlorodiphenyl trichloroethane (DDT) which led to the introduction of second and third generation insecticides (Sarimin et al., 2020).

On the other hand, an example of the biological approach is through the release of *Wolbachia*-infected *Ae. aegypti*. The infected mosquitoes will have a shorter life span and it cannot support the development of dengue virus. Another control innovation that were created to handle these mosquito-borne diseases includes outdoor residual spraying, insecticidal paint, ovitrap, autocidal adult and larva trap (Lee et al., 2015). Integrated vector management (IVM) approach, which involved both chemical and non-chemical method, had also been carried out in Malaysia in order to control *Aedes* vectors (Sarimin et al., 2020).

Both CHIKV and DENV could be transmitted by *Aedes* vectors through vertical transmission and venereal transmission (Sánchez-Vargaz et al., 2018; Monteiro et al., 2019).

Vertical transmission took place due to infection and dissemination of the virus within the female from ovarian cells to the eggs during the process of fertilization while venereal transmission occurs during mating (Pan American Health Organization, 2011; Sánchez-Vargas et al., 2018). Both viruses could be maintained through enzootic and epidemic cycle/urban cycle. Enzootic cycle involved the natural transmission of the virus between wild animals and *Aedes* vectors while epidemic cycle is maintained between human and *Aedes* mosquitoes (WHO, 2011; Go et al., 2014).

Pulau Salak, Kuching was one of the areas affected due to the outbreak of CHIKV in 2009 (Nor Aliza, 2012). There were no published data regarding the vector status of CHIKV and DENV that have been circulating in Pulau Salak. Thus, this study served as a reference in case of future CHIKV and DENV outbreaks. The *Aedes* mosquitoes collected in the villages were also tested for the presence of DENV. Kampung Tanjong Bako, Kuching was chosen as a control site for this research in which the *Aedes* mosquitoes collected from the area were also tested for the presence of CHIKV and DENV.

## **1.2 Problem Statement**

It was observed that the number of dengue and chikungunya cases have been increasing in Malaysia based on the data reported by the Ministry of Health, Malaysia from year 2015 to 2018 (MOH, 2019; MOH 2020; MOH n.d.). However, there are not many studies done regarding the vector status and transovarial transmission of CHIKV and DENV in Sarawak. Nor Aliza et al. (2019) have reported the first natural evidence of transovarial transmission of DENV in the natural population of *Ae. albopictus* in selected areas in Kuching and Kota Samarahan divisions and the presence of dengue virus serotype 2 was detected in the study. On the other hand, the number of chikungunya cases recorded it peaked

in 2009 in Sarawak and there were no chikungunya cases reported by the end of November 2011 (Chua, 2010; MOH 2019). The presence of chikungunya cases, however resurfaced in Sarawak in the year 2017 and have been showing an increasing trend in Malaysia since 2015 (MOH, 2019; MOH, 2020).

There are no published data regarding the vector status and transovarial transmission of CHIKV and DENV on Kampung Pulau Salak, Kuching despite the increasing trend in the number of cases of chikungunya and dengue in Sarawak. This study could serve as a reference in case of CHIKV and DENV outbreaks in the area and could be beneficial to the effort of handling the outbreaks in the future.

### **1.3 Aims**

This study aims to provide information regarding the vector status, to determine the occurrence of transovarial transmission of CHIKV and DENV among the mosquito, and to determine the parameters of immature mosquitoes breeding containers in Kampung Pulau Salak and Kampung Tanjong Bako.

### **1.4 Research Objectives**

The objectives of this study are:

- i. To document the container breeding and field-caught mosquitoes on Kampung Pulau Salak and Kampung Tanjong Bako.
- ii. To investigate CHIKV and DENV status of the container breeding and field-caught mosquitoes at the two locations.

- iii. To determine the occurrence of transovarial transmission and the minimum infection rate of both CHIKV and DENV in mosquitoes.
- iv. To document the environmental parameters of immature mosquitoes breeding site

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Vector-borne Diseases

Vectors can be defined as living organisms that are responsible for the transmission of infectious diseases between humans or from animals to humans. Most of these vectors are insects, which blood feed from infected host and transfer disease-producing microorganisms into another host during their subsequent blood meal (World Health Organization, 2017).

Vector-borne diseases (VBDs) are illnesses that are caused by pathogens such as parasites, viruses, and bacteria that are transmitted by a vector such as mosquitoes, blackflies, sandflies, triatomine bugs, ticks, tsetse flies, mites, lice, and snails (Table 2.1). According to WHO (2017), VBDs such as malaria, dengue, Human African trypanosomiasis, Chagas disease, schistosomiasis, leishmaniasis, Japanese encephalitis, Yellow fever, and onchocerciasis have caused more than 700 000 deaths annually around the world and that the most common vector of VBDs are mosquitoes. It was first discovered in 1877 that mosquitoes could transmit diseases such as filariasis from human to human followed by the discovery of the fact that they could be the vector of malaria, Yellow fever and dengue from the year 1878 to 1903 (Gubler, 1998).

Generally, arthropods are accountable for hundreds of millions of cases of vector-borne diseases in humans and animals every year. The process of reversing the emergence of vector-borne diseases would be a great challenge and some available vaccines for VBD are not widely used as vaccine prospects for major vector-borne diseases are not progressing well.