COMBINATION OF GRAVID OVIPOSITING STICKY TRAP AND NS1 ANTIGEN TEST: NEW PARADIGM FOR DENGUE VECTOR SURVEILLANCE IN SELANGOR MALAYSIA

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ABSTRACT

Dengue fever is a serious public health problem in tropical countries and has increased 37 folds in Malaysia compared to decades ago. Selangor, the most developed and populated state in Malaysia has contributed about 50% cases in the country. Vector control has been the hallmark for surveillance and control of dengue. However, there is no correlation between *Aedes* index and dengue cases. Thus, new proactive paradigms are necessary for vector surveillance which would help in the prevention of dengue epidemics in the country. This two-year study was conducted in dengue epidemic urban area of Selangor; where GOS trap (Gravid Mosquito Ovipositing in Sticky Trap) was used to capture gravid *Aedes* mosquitoes. All *Aedes* mosquitoes were tested with NS1 rapid antigen test kit. All dengue cases from the study site reported to the Ministry of Health were recorded. Microclimatic data such as rainfall, temperature and humidity were recorded weekly. *Aedes aegypti* was the predominant mosquito (95.6%) caught in GOS traps, 23% (43/187) pools of mosquitoes were positive for virus dengue using the NS1 antigen kit. Confirmed cases were observed with a lag of one week after positive *Ae. aegypti* were detected. *Aedes aegypti* density as analyzed by distributed lag non-linear models, will increase lag of 2-3 weeks for temperature increase from 28 to 30^{o}C; and lag of three weeks for increased rainfall. In conclusion, the combined use of GOS trap and NS1 antigen kit to detect dengue virus in mosquitoes can be used as a new tool for dengue vector surveillance. It seems to be a proactive method where control action can be activated when positive mosquitoes are obtained. However, a randomized control trial needs to be conducted to prove that this paradigm will indeed reduce dengue epidemics.

Keywords: *Aedes*, mosquitoes, dengue, sticky trap, Selangor

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ABSTRAK

Demam denggi merupakan satu masalah kesihatan awam yang serius di negara-negara tropika dan telah meningkat sebanyak 37 kali ganda di Malaysia berbanding dengan sedekad dahulu. Selangor, merupakan negeri yang paling membangun dan padat dengan penduduk di Malaysia, telah menyumbangkan lebih kurang sebanyak 50% kes dalam negara. Kawalan vektor telah menjadi kaedah utama untuk surveilen dan kawalan denggi. Walau bagaimanapun, didapati tiada perhubungan kait antara indeks *Aedes* dan kes denggi. Sehubungan itu, paradigma proaktif baru amat diperlukan untuk surveilen vektor yang boleh membantu dalam pencegahan epidemik denggi dalam negara. Kajian selama dua tahun telah dijalankan di kawasan epidemik denggi di Selangor, di mana perangkap GOS (*Gravid Mosquito Ovipositing in Sticky Trap*) digunakan untuk memerangkap nyamuk *Aedes* yang bertelur (*gravid*). Semua nyamuk *Aedes* diuji dengan NS1 *rapid test kit*. Semua kes denggi dari tapak kajian yang dilaporkan ke Kementerian Kesihatan Malaysia adalah direkod. Data mikro-iklim seperti taburan hujan, suhu dan kelembapan direkod secara mingguan. *Aedes aegypti* merupakan nyamuk pre-dominan (95.6%) diperangkap dengan perangkap GOS, sebanyak 23% (43/187) kelompok nyamuk yang diuji dengan menggunakan NS1 *antigen kit* adalah didapati positif dengan virus denggi. Kes denggi yang sah diperhatikan berlaku sebanyak selang satu minggu selepas *Ae. aegypti* positif dikesan. Densiti *Ae. aegypti* yang dianalisa dengan menggunakan model *distributed lag non-linear*, didapati akan meningkat selang 2-3 minggu bagi peningkatan suhu dari 28 ke 30^{o}C; dan sebanyak selang tiga minggu bagi peningkatan untuk taburan hujan. Secara kesimpulan, gabungan penggunaan perangkap GOS dan NS1 *antigen kit* untuk mengesan virus denggi dalam nyamuk boleh digunakan sebagai alat baru untuk

surveilen vektor denggi. Ia merupakan sesuatu kaedah proaktif yang membolehkan tindakan kawalan boleh diaktifkan apabila positif nyamuk telah didapati. Walau bagaimanapun, percubaan kawalan secara rawak (*randomized control trial*) perlu dilakukan untuk membuktikan paradigma ini sebetulnya akan mengurangkan epidemik denggi.

Kata kunci: *Aedes*, nyamuk, denggi, perangkap sticky, Selangor

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LIST OF SYMBOLS AND ABBREVIATIONS

-	:	Negative
%	:	Percentage
&	:	And
+	:	Positive
<	:	Less than
=	:	Equal to
>	:	More than
µM	:	Micrometer
ABC-PRO	:	American Biophysics Corporation Standard Professional
AD	:	anno Domini
Ae.	:	*Aedes*
Ag	:	Antigen
Ag-ELISA	:	Antigen-detection enzyme-linked immunosorbent assay
AGO-B	:	Autocidal Gravid Ovitrap
AI	:	*Aedes* index
AIC	:	Akaike Information Criterion
ANOVA	:	Analysis of Variance
BG-Sentinel	:	Biogents-Sentinel
BI	:	Breteau indices
Bti	:	*Bacillus thuringiensis israelensis*
CBT	:	Catch Basin Trap
CDC	:	Centers for Disease Control and Prevention
CDC-AGO trap	:	Centers for Disease Control and Prevention autocidal gravid ovitrap

CFR	:	Case Fatality Rate
CHIK	:	Chikungunya
CHIKV	:	Chikungunya virus
CI	:	Container indices
CI	:	Confidence Interval
CO2	:	Carbon dioxide
COMBI	:	Communication-for-behavioural-impact
Cx	:	*Culex*
DDBIA	:	Destruction of Disease Bearing Insect Act
DENV	:	Dengue virus
DF	:	Dengue Fever
df	:	Degree of Freedom
DHF	:	Dengue-Haemorrhagic Fever
DLNM	:	Distributed Lag non-Linear Models
DSS	:	Dengue Shock Syndrome
DST	:	Double Sticky Trap
ECDPC	:	European Centre for Disease Prevention and Control
ELISA		Enzyme-linked immunosorbent assay
EMEM	:	Eagle's minimum essential medium
et al.	:	*et alia* (others)
EVS trap	:	Heavy Duty Encephalitis Vector Survey trap
GAT	:	Gravid *Aedes* Trap
GEOHIVE	:	A website with geopolitical data, statistics on human populaion
GF	:	Ground Floor
GIS	:	Geographic Information System

GLMM	:	Generalized Linear Mixed Model
GM	:	Genetically Modified
GOS	:	Gravid Mosquito Ovipositing in Sticky Trap
HCGT	:	Harris County Gravid Trap
HI	:	House indices
HLC	:	Human Landing Catch
HSD	:	Honest Significant Difference
IgG	:	Immunoglobulin G.
IgM		Immunoglobulin M.
IMFA	:	Mean Index of *Aedes* Females
IR	:	Incidence Rate
IR	:	Infection Rate
IVM	:	Integrated Vector Management
Kb	:	Kilobase pairs
KKM	:	Kementerian Kesihatan Malaysia
MBPJ	:	Majlis Bandaraya Petaling Jaya
MET	:	Mosquito Emerging Trap
MET	:	Mean Egg Counts Per Trap
$MgCl_2$	:	Magnesium chloride
MIR	:	Minimum Infection Rate
ml	:	Milliliter
MLTD	:	Mosquito Larvae Trapping Device
mM	:	Millimeter
MOH	:	Ministry of Health
MQT	:	MosquitoTRAP
Ms	:	Microsoft

NMRR	:	National Institutes of Health
NS1	:	Nonstructural Protein 1
^{o}C	:	Degree Centigrade
ODFP	:	Omnidirectional Fay-Prince trap
P	:	Level of significance
PBS	:	Phosphate Buffer Solution
PCR	:	Polymerase Chain Reaction
PI	:	Post-infection
POI	:	Positive Ovitrap Indices
r	:	Correlaton coefficient
rd	:	Ordinal number is used with numbers ending in 3
RIDL	:	Release of Insects Carrying a Dominant Lethal
RNA	:	Ribonucleic acid
RT-PCR	:	Reverse transcription polymerase chain reaction
SIT	:	Sterile Insect Technique
Sq.	:	Square
th	:	Ordinal number is used for all other numbers except numbers ending in 1, 2 and 3
ULV	:	Ultra-Low Volume
USD	:	United States Dollar
VBDCP	:	Vector-Borne Diseases Control Programme
WHO	:	World Health Organization
WNV	:	West Nile Virus
YFV	:	Yellow Fever Virus
ZIKV	:	Zika virus

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background

Dengue is an important mosquito-borne viral disease and about 390 million dengue infections are reported globally per year (Murray et al., 2013). It is estimated that 3.97 billion people from 128 countries are at risk for dengue infection (Brady et al., 2012; WHO, 2016a). Dengue occurs in urban and semi-urban areas in most tropical and sub-tropical countries worldwide such as the Americas, South-East Asia, Africa, the Eastern Mediterranean and the Western Pacific, which is shown in the Figure 1.1 (WHO, 2014), and there has been a 30-fold increase over the past 50 years (CDC, 2016; WHO, 2016d).

Figure 1.1: Countries at risk of dengue transmission in 2013 (Source: WHO, 2014)

Based on officially reported surveillance data, dengue continued to show high levels in the Western Pacific Region (Arima et al., 2013), and still continues its increasing trend. The World Health Organization (WHO) in the Western Pacific Region (WHO,

2016b) reported that a few years after the large dengue outbreaks in 1998, countries in the Western Pacific started to report an increased number of dengue cases, from 150,000-170,000 cases annually during the period 2003 – 2006. However, from 2007 cases have increased to 200,000 per year.

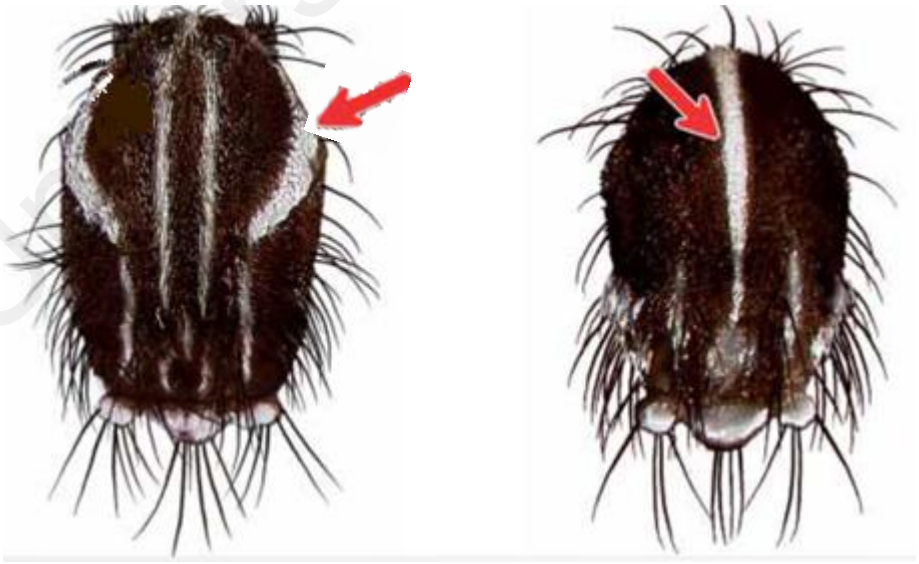
Malaysia which is in the Western Pacific Regions was characterized by the World Health Organization as having large dengue outbreaks in the year 2015 (WHO, 2016a). More than 111,000 suspected dengue cases were reported which was an increase of 59.5% compared to the previous year (WHO, 2016a). At the same time, there was also an increase of 336.4% in the number of dengue deaths in 2015 compared to the previous year (KKM, 2015; KKM, 2016b). Mohd-Zaki et al. (2014) showed that the epidemiology of dengue cases in Malaysia was characterized by a non-linear increase in the number of reported cases, from 7,103 in 2000 to 46,171 in 2010. Selangor which is the most heavily populated and urbanized state in Malaysia contributed about 52 – 55% of the dengue cases yearly in Malaysia (KKM, 2015; KKM, 2016b). Petaling District in the state of Selangor contributed about 23% of dengue cases and 13% dengue death in Malaysia, while it accounted for 42% of dengue cases and 31% dengue death in Selangor (KKM, 2014a).

Aedes aegypti, is the primary vector of dengue virus in the urban setting (Chen et al., 2006; Higa, 2011; Higa et al., 2010), while *Aedes albopicus* is the secondary vector (Smith, 1956). However, *Ae. albopictus* is the principal vector in the transmission of Chikungunya virus (CHIKV) in Malaysia (Sam et al., 2012) and in several countries bordering the Indian Ocean, Central Africa and Europe (Paupy et al., 2009). *Aedes aegypti* is also a secondary vector of Chikungunya virus in Malaysia (Rohani et al., 2005; Vega-Rúa et al., 2014). Recently, *Ae. aegypti* and *Ae. albopictus* have been incriminated as potential vectors to transmit Zika virus (ZIKV) (Li et al., 2012; Wong et al., 2013). Zika

virus was first isolated *from Ae. aegypti* in Bentong, Pahang, Malaysia in 1965 (Marchette et al., 1969). In 2016, local transmission of the Zika virus was reported in Singapore and Malaysia (Ho et al., 2017; WHO, 2016f). *Aedes aegypti* is also known as the primary vector to transmit Yellow Fever virus in West and Centre Africa, South and Central America (Harper, 2004), and it can also transmit diseases such as Murray Valley encephalitis and Ross River virus (Lee at al., 1987). In addition, *Ae. aegypti* has also been documented with parasitic infections such as *Wuchereria bancrofti*, *Dirofilaria immitis* and *Plasmodium gallinaceum* (Munstermann, 2007).

Aedes aegypti which is known as yellow fever mosquito, belongs to the scutellaris group of genera *Stegomyia*. It can be identified by conspicuous white lyre shape marking on the upper surface of the thorax (Figure 1.2) and white banded legs (Munstermann, 2007). In contrast, *Ae. albopictus* has a single longitudinal silvery dorsal stripe in the middle of the thorax (Figure 1.2) (Leopoldo, 2004).

Thorax of adults



Aedes aegypti ***Aedes albopictus***

Figure 1.2: Key characters of *Aedes aegypti* and *Aedes albopictus*. *Aedes aegypti* showed with white lyre-shaped markings, while *Aedes albopictus* showed with a narrow median-longitudinal white stripe (Source: Leopoldo, 2004)

1.2 Problem Statement and Justification

Dengue is on the rise and in the absence of drugs and vaccine (MOH, 2015), vector control is still the leading tool for the prevention. Strategies for control in Malaysia are larval surveys, source reduction, health education, chemical control, such as fogging and Ultra-Low Volume (ULV) (Lam, 1993; Mudin, 2014). Integrated Vector Management as proposed by WHO is also carried out where possible (KKM, 2009). In Malaysia there is the enforcement of the Destruction of Disease-Bearing Insect Act (DDBIA 1975) (Lam, 1993) and inter-agency collaboration (Teng & Singh, 2001) was enforced throughout the country in Malaysia. Fogging and Ultra-Low Volume (ULV) are conducted when cases are reported or when the *Aedes* house index is high (Lam, 1993; Mudin, 2014). However, all these approaches were not able to decrease the number of dengue cases in the country.

Therefore, advanced strategies have been developed recently for more effective dengue control such as Geographic Information System (GIS) (Carbajo et al., 2006; Honorio et al., 2009a; Honorio et al., 2003; Koenraadt et al., 2008; Lee et al., 2013), Release of Insects Carrying a Dominant Lethal (RIDL) (de Valdez et al., 2011; Eisen & Lozano-Fuentes, 2009; Lacroix et al., 2012), Sterile Insect Technique (Alphey et al., 2010; Esteva & Yang, 2006; Oliva et al., 2012), *Wolbachia*-infected mosquitoes to control mosquito population or reduce dengue transmission (Iturbe-Ormaetxe et al., 2011; Lambrechts, 2015; Lambrechts et al., 2015), Outdoor Residual Spraying (Lee et al., 2015; Rozilawati et al., 2005), lethal ovitraps (Ritchie et al., 2009), sticky ovitraps (Facchinelli et al., 2008; Lee et al., 2013; Ritchie et al., 2004), autocidal adult and larva traps (Lee et al., 2015), auto-dissemination of insect control agents using ovitraps (Caputo et al., 2012), insecticidal paint (Lee et al., 2015) and dengue vaccine (Bhamarapravati & Sutee, 2000; Halstead, 2012). Although most of these new tools look encouraging, unfortunately randomized control trials or large-scale trials have not been carried out (Achee et al., 2015a). Thus, there is an urgent need to introduce new proactive methods for the

surveillance of dengue vectors. What is required is an early warning that can trigger the health authorities to take action before an epidemic occurs (Runge-Ranzinger et al., 2016).

In Malaysia, there are some limitations to reduce *Aedes* mosquito population significantly. Control methods such as fogging and ULV are becoming more challenging due to the rapid development and mushrooming of houses and unplanned urbanization. Also, indiscriminate use of insecticides can produce insecticide resistance (Ishak et al., 2015; Othman et al., 2013; Rong et al., 2012). House to house larval surveys are being advocated for the surveillance and control of dengue. However, due to recent rapid urbanization in Malaysia, this method has become less effective as the outcome is dependent on the ability of the field worker to find the breeding grounds, it is also time consuming and very labour intensive. Studies showed that *Aedes* larval surveys have no correlation to dengue cases (de Melo et al., 2012). Similarly, one of the most important steps to improve further the efficacy of *Ae. aegypti* borne disease control programme is to target the adult mosquito for surveillance and control (Achee et al., 2015a; Lee et al., 2013; Steffler et al., 2011 In this study, the new paradigm will target the adult mosquitoes and enable detection of dengue virus in an area so as to prevent epidemics.

Since the current methods are all reactive and cases of dengue are on the increase it is timely to introduce new methods which will be more proactive so that control measure can be conducted before epidemics occur. Since *Ae. aegypti* are now breeding in cryptic sites and larval surveys are labour intensive it would be more effective to target the adult mosquito.

This study was carried out to evaluate the use of the GOS trap (Gravid Mosquito Ovipositing in Sticky Trap), detection of dengue virus from the mosquitoes and the association of the climate data at micro level to assist in the surveillance of dengue

vectors. Strategy for vector control for dengue has remained static for the past 40 years. House to house larval surveys have been the hallmark of the dengue control programme in Malaysia and neighbouring countries (Hapuarachchi et al., 2016; Kumarasamy, 2006; Lam, 1993; Lee et al., 2015; Mudin, 2015; Song, 2016; Vythilingam et al., 2016). However, this has been effective in the past because *Aedes* house index has decreased compared to many years ago (Hapuarachchi et al., 2016; Shah & Sani, 2011; Tham, 1993; Vythilingam et al., 1992). This new methodology will enable the detection of dengue in an area before an epidemic takes place. Thus, the results of this study will be valuable for surveillance and control of dengue.

1.3 Objective of The Study

1.3.1 General objective

The general objective of this dissertation was to develop a new proactive paradigm for vector surveillance which would help in the prevention of dengue epidemics in hotspot areas in the state of Selangor.

1.3.2 Specific objective

Specific objectives are as follows:

1) To determine the sensitivity of GOS trap in detecting *Aedes* vectors in the study area (Chapter 3).

2) To determine the optimum number of traps to be used in high rise apartments for dengue surveillance (Chapter 3).

3) To evaluate the efficacy of GOS trap and NS1 antigen test as a new paradigm for vector surveillance (Chapter 4).

4) To study the effect of rainfall, temperature, and humidity on *Aedes* density and dengue cases at micro-level (Chapter 5).

5) To determine virus serotype by RT-PCR from *Aedes* mosquitoes that were positive by NS1 (Chapter 4).

6) To correlate the relationship between dengue cases based on climate factors and infected mosquitoes (Chapter 5).

These objectives will be discussed in separate chapters in this dissertation. Objectives one and two will be discussed in Chapter 3 while objectives three and five will be discussed in Chapter 4; and objective four and six will be discussed in Chapter 5.

1.4 Conceptual Framework

The conceptual framework in Figure 1.3 presented the interplay between the independent and dependent variable in the study. Characteristics and behavior of Vector Borne Disease typically vary across space and time; besides they are influenced by multiple direct and indirect factors forcing complex interactions with the environment, pathogen and host (Parham et al., 2015).

The same conception framework presented in Figure 1.4 in this study shows the interplay between the main variables that contributes to the dengue epidemics such as climate factors, pathogen and the vectors.

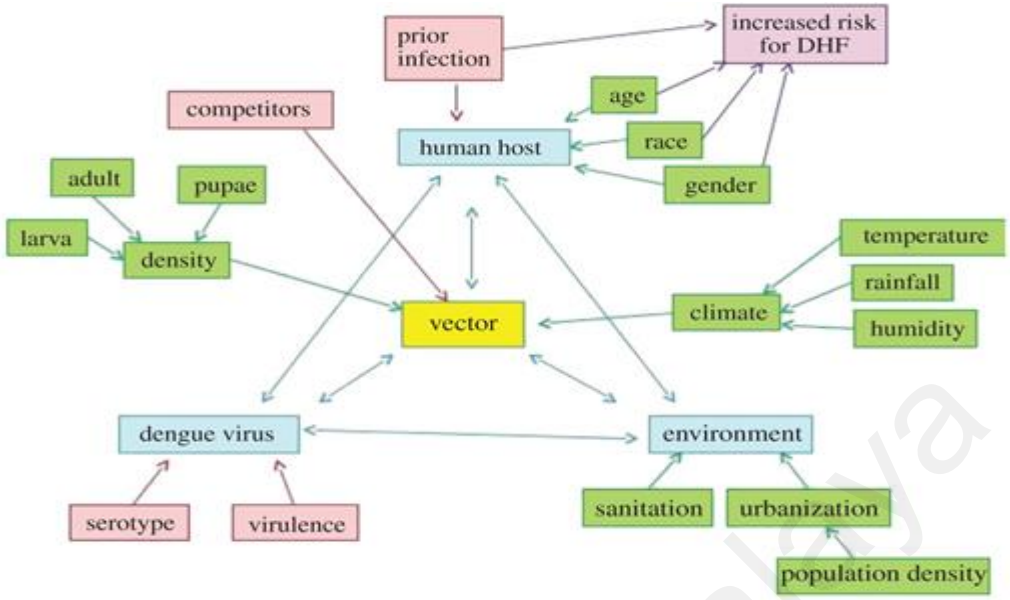


Figure 1.3: System diagram showing the key requirements for understanding the risk of dengue virus transmission in humans (pink), and the linkages between drivers, hosts (blue) and potential indicators (green) for monitoring (Source: Parham et al., 2015)

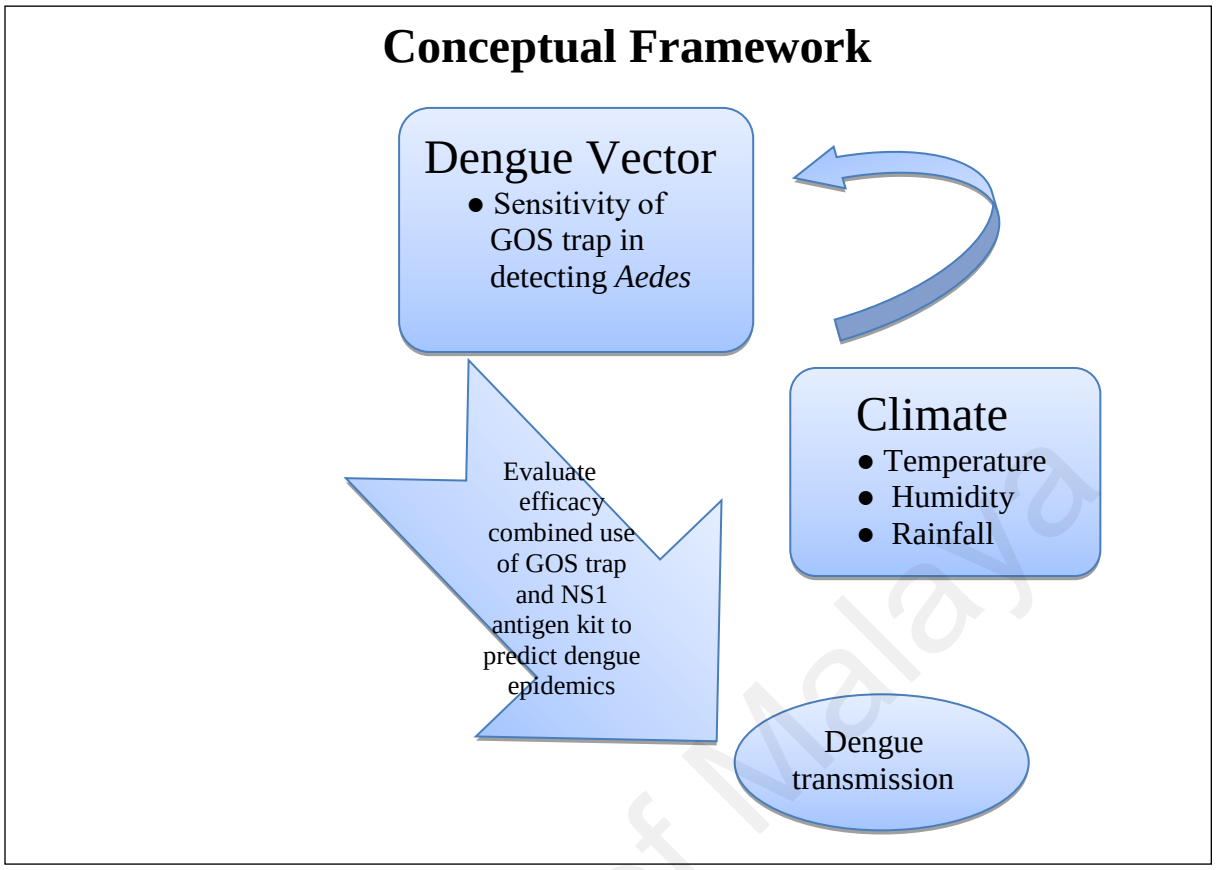


Figure 1.4: Conceptual framework to show the interaction between pathogen-vector together with climate factors on the dengue transmission

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Dengue, a mosquito borne viral disease is well known to cause life threatening infections and is found in tropical and sub-tropical regions worldwide, typically in urban and semi-urban areas. In spite of numerous studies on dengue, it still remains as a serious public threat. However, until today, there is still no treatment for dengue and only lately there is availability of a licensed vaccine (Aguiar et al., 2016; Scott, 2016). Thus, disease surveillance and vector population control remain the mainstay of dengue prevention. A new paradigm for control should include intensive surveillance and approaches that kill adult mosquitoes, development and testing of products that appeal to the consumer. This would make the national programs more cost effective and economical (Morrison et al., 2008). Therefore, a new paradigm is needed in order to control dengue epidemics more effectively.

2.2 Dengue Background

"Dengue" may be traced to the Swahili word for the disease "ki-dingapepo". However, the earliest description of "dengue" can be traced in Spanish written records from 1800. The term "denga", or "dyenga" had also been used to designate the disease throughout outbreaks in East Africa and West India during the early 19th century. The word "dengue" came into general use only after the 1828 outbreak in Cuba (Carey et al., 1971). Dengue fever was first documented as clinically compatible disease in a Chinese medical encyclopaedia in 992 (Gubler, 2006) and recorded during the Jin Dynasty (265-420 AD) in China (Cecilia, 2014; Gubler, 1998; Murray et al., 2013). The disease was

entitled water poison and thought to be somehow linked with flying insects associated with water by the Chinese. The first record of the outbreaks of illness in the French West Indies in 1635 and in Panama in 1699 could have been dengue. Thus, dengue could have had a wide geographic distribution earlier than the 18th century before the first known pandemic of dengue began (Gubler, 1998).

2.2.1 History of dengue epidemics

Soon after the identification of dengue fever in 1779, dengue epidemics occurred almost simultaneously in Asia, Africa, and North America in the 1970s (Cecilia, 2014; Gubler, 1998; Rodrigues et al., 2012). The expansion of the global shipping industry in the 18th and 19th centuries, created the spread of the principal mosquito vector, *Ae. aegypti*. After World War II, rapid urbanization in Southeast Asia led to increased transmission and hyperendemicity of dengue (Gubler, 2006). A pandemic began in Southeast Asia in the 1950s (Gubler, 2012). Severe dengue was known as Dengue Haemorrhagic Fever (DHF), which was first recognized during the dengue epidemics in the Thailand from 1950 and Philippines from 1953 (Gubler, 1997; Halstead, 2008a; WHO, 2016a). Dengue has spread very fast from only 9 countries having severe dengue epidemics before 1970 to currently more than 100 countries in the WHO regions of Africa, the Americas, the Eastern Mediterranean, the Western Pacific and South-East Asia (WHO, 2016d). Most seriously affected countries are the America, South-East Asia and Western Pacific regions (WHO, 2016a). Severe haemorrhagic disease evolved in Southeast Asia in the 1960s and 1970s (Gubler, 1997). While in 1980s, a dramatic geographical expansion of endemic dengue haemorrhagic fever (DHF) occurred in Asia, followed by the resurgence of the disease in Singapore through the 1990s. In 1997, dengue fever or DHF has become the most important arboviral diseases of humans, with

estimated 50 to 100 million cases occurring each year (Murray et al., 2013). Since then, dengue has become one of the most significant resurgent tropical diseases in the past 17 years with expanding geographical distribution of both the viruses and mosquito vectors. Besides, the circulation of multiple virus serotypes and increased frequency of epidemics and emerging of DHF in new areas have created serious public health threats (Gubler, 1997). While, Bhatt et al. (2013) estimated dengue infection per year currently to be 390 million, with 96 million manifests apparently. The total infection is more than three times the dengue burden estimate of the World Health Organization.

2.2.2 Dengue at global level

In 2008, dengue case exceeded 1.2 million and in 2013, there was 3 million across the regions of America, South-East Asia and Western Pacific (WHO, 2016a). It is estimated that about 2.5 billion people live in dengue endemic areas (WHO, 2011), with 50 million dengue infections occurring worldwide annually and 2.5% of the 500,000 people affected with DHF require hospitalization or die (WHO, 2011). Based on the global spatial limits of dengue virus transmission by evidence-based consensus in 2012 that estimated population at risk with an upper bound of 3.97 billion people (Brady et al., 2012). However, in 2015, the dengue situation has deteriorated worldwide, not only was the number of cases increasing but the disease had spread to new areas and explosive outbreaks have occurred (WHO, 2016a).

In 2015, large dengue outbreaks occurred worldwide, in which Americas reported 2.35 million dengue cases with 1181 deaths of which Brazil reported more than 1.5 million cases, which was approximately three-fold higher than in 2014. Countries in South-East Asia such as Philippines reported more than 169,000 cases and Malaysia

recorded 111,000 suspected cases of dengue, which represent 59.5% and 16% increase compared to previous year respectively (WHO, 2016a).

2.2.3 Dengue in Malaysia

Dengue was first reported in Malaysia in 1902 (Singh, 2000; Skae, 1902), while emergence of dengue haemorrhagic fever (DHF) was recorded in 1962 in Penang Island (Lee, 2000; Rudnick et al., 1965; Singh, 2000). Subsequently, dengue has become endemic throughout the country. In 1973, there was a major outbreak of DHF. Consequently, dengue was made legally notifiable under the Infectious Diseases Act in 1974 and the Destruction of Disease Bearing Insect Act (DDBIA 1975) was introduced in 1975 (KKM, 2006; Singh, 2000). In Malaysia, the Dengue Control Programme was established in 1973 under the Epidemiology Unit, Ministry of Health, Malaysia. However, in 1981, the programme was integrated with other vector borne diseases to establish Vector-Borne Diseases Control Programme (VBDCP) (KKM, 2006; Singh, 2000). In 1994, the Vector-Borne Diseases Control Programme (VBDCP) was integrated into the Disease Control Division in the Ministry of Health, Malaysia (MOH) (KKM, 2006).

There was a dengue outbreak in 1974 and 1982, and a major outbreak in 1988 with 27,381 cases reported. Meanwhile, dengue steadily increased from 14,255 cases per year in 1996 (Teng & Singh, 2001) (Figure 2.1) up to 120,836 cases in 2015 (KKM, 2016a), which has been eight-fold increase in the past 19 years. Figure 2.2 shows the number of dengue cases from 2000 until 2014 (Mudin, 2015). In 2015, the incidence rate (IR) increased from 72 cases in 100,000 population (Mudin, 2015) to 396 cases in 2015 (KKM, 2016a). Dengue deaths amplified tremendously from 42 cases in 2000 (Mudin, 2015) up to 336 cases in 2015 (KKM, 2016a), however the case fatality rate (CFR)

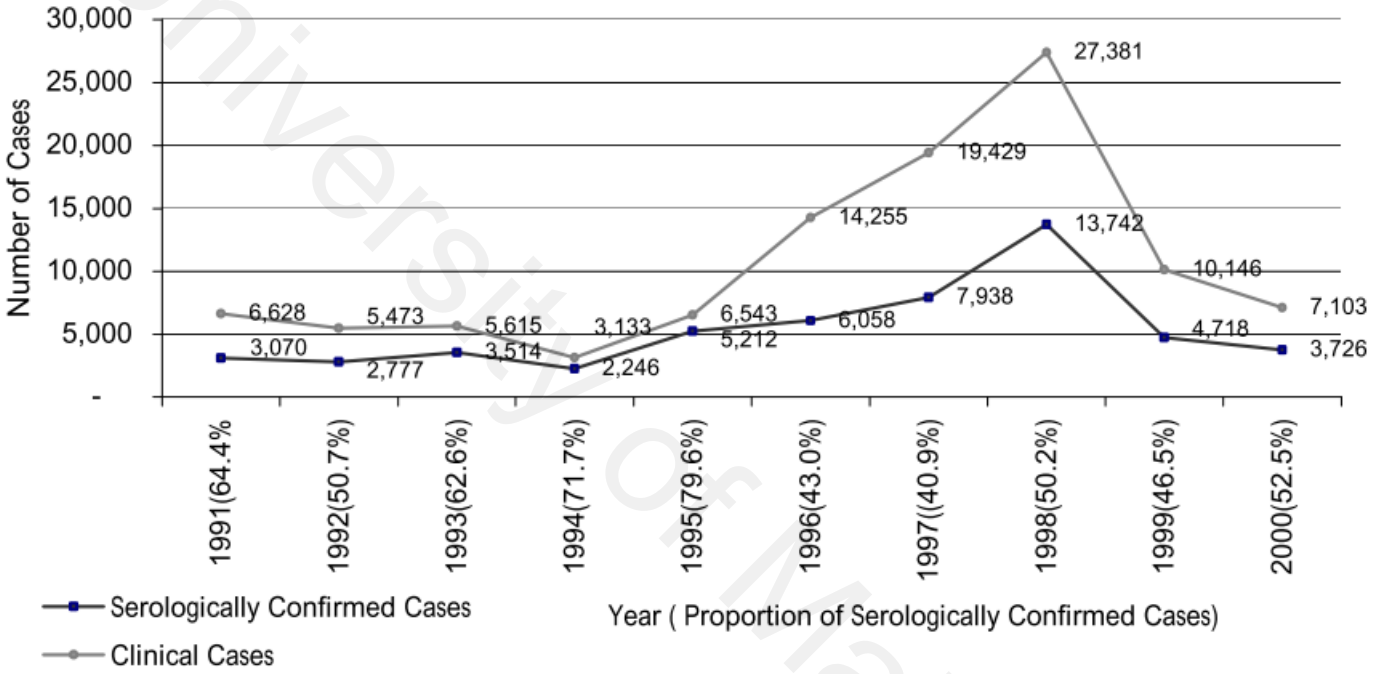


Figure 2.1: Distribution of clinically-diagnosed and serologically-confirmed cases per 100 000 population, 1991-2000

(Source: Teng & Singh., 2001)

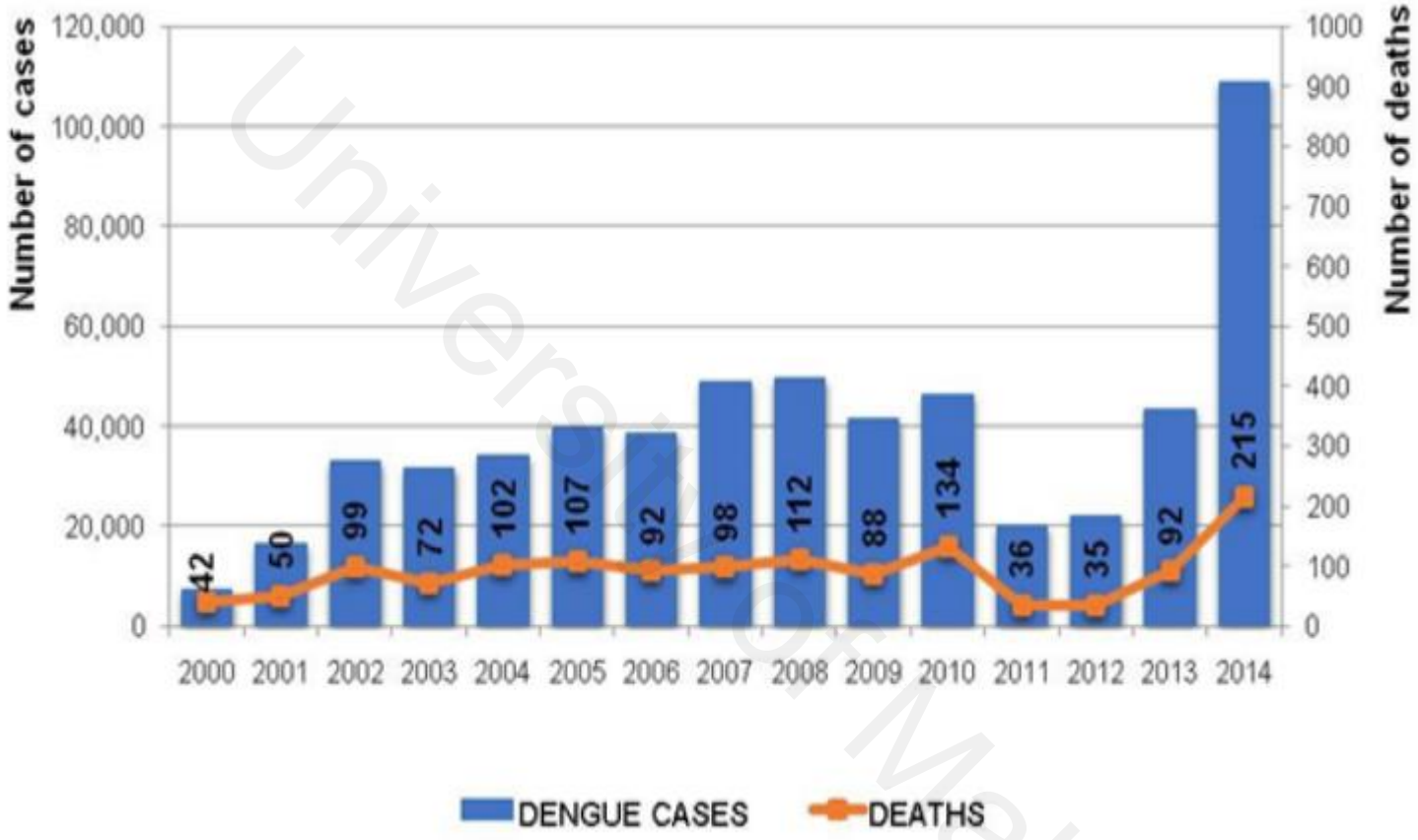


Figure 2.2: Number of dengue cases and deaths for Malaysia, 2000 – 2014 (Source: Mudin, 2015)

remained constant at 0.2 – 0.3%, with 0.63% reported for the year 2000 and 0.28% for year 2015 (KKM, 2016a; Mohd-Zaki et al., 2014). The dengue virus surveillance system has been established in Malaysia since 1990s. All four dengue serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) are found in Malaysia, while the dominant DENV serotypes changed from year to year, from DENV-2 in 2000, DENV-3 in 2001-2002, DENV-1 in 2003-2005, DENV-2 in 2006-2009, DENV-1 in 2010-2011, heterogeneous distribution of DENV in 2012, DENV-2 in 2013-2015 (Mohd-Zaki et al., 2014; Mudin, 2015)

2.2.4 Dengue in Selangor

Selangor state which is about 8,104 sq. km in area, is located along the west coast of Peninsular Malaysia. It is the most developed state and has the prime population in Malaysia with 5,411,324 in 2010 (GEOHIVE, 2016) which increased to 5,874,100 in 2015 (MCMM, 2016). Selangor contributes about 12 – 20% of the population in Malaysia from 1991 – 2010 (GEOHIVE, 2016), and also contributed to the highest number of dengue cases in Malaysia, which ranged from 46% to 52.3% (KKM, 2016b; Mudin, 2015). Study by Latif & Mohamad (2015) found that highest cases in the year are at the same locations in Selangor, and the high-risk areas detected were Ampang, Damansara, Kapar, Kajang, Klang, Semenyih, Sungai Buloh and Petaling. These areas were also having high population densities and high rainfall (Latif & Mohamad, 2015). Ministry of Health identified problems contributing to high number of dengue case in the country, especially in Selangor, were as follows: poor environmental sanitation, poor garbage disposal, poor community behavior, high density population, rapid movement of people and rapid urbanization (KKM, 2016b). It was revealed that although the level of knowledge of people from Selangor on *Aedes* mosquitoes, dengue disease and preventive

measures ranges from fair to good, even attitude towards the measures was high, however, frequent level of personal practices of larval control was low (Mohamad et al., 2014).

2.2.5 Current Situation and Other

Globally, the number of dengue cases has increased dramatically almost two-fold in the past 10 years and the indigenous dengue transmission also occurred in more than 100 countries in South-East Asia, Western Pacific, Africa, the Americas and the eastern Mediterranean (WHO, 2016d). Malaysia recorded large dengue outbreaks in 2015 and dengue case were constantly very high in following years, which reported about 101,357 cases for the year 2016 (KKM, 2017a) and 81,790 cases up to week 50 for year 2017 (KKM, 2017b). ZIKV is transmitted by the same vector which is *Aedes* mosquito, mainly *Ae. aegypti* in tropical regions. Record until October 2016 showed that 72 countries and territories have described evidence of mosquito-borne Zika virus transmission (ECDPC, 2016). Zika, the disease linked with microcephaly and Guillain-Barré syndrome was originally discovered in humans in 1952 and the first outbreak outside Africa and Southeast Asia was in Yap Island in 2007 (Hayes, 2009; Roth et al., 2014; WHO, 2016e). Singapore has reported Zika outbreak since August 2016, while first locally acquired mosquito-borne. Zika infection in Malaysia occurred in September 2016 (ECDPC, 2016; WHO, 2016f). In Malaysia, ZIKV may be overlooked due to large outbreaks of dengue and CHIKV (Jamal et al., 2016).

2.3 Dengue Virus

Dengue virus which is the causative agent of dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and is an acute mosquito-borne infection. Dengue is an enveloped virus with size 40-60 nm and is an arbovirus

from Family *Flaviviridae* and genus *Flavivirus* (Paranjape & Harris, 2010). Dengue is transmitted to humans through the infected *Ae. aegypti* or *Ae. albopictus*, and after intrinsic incubation of 5-8 days they cause infected human to develop the symptoms such as fever, influenza type symptoms, rash, arthralgias, myalgias and the febrile period lasts for 2 to 10 days. It can cause death if the patients do not receive proper treatment (Rico-Hesse, 2009).

2.3.1 Dengue virus serotypes

Dengue virus is a positive-sense RNA virus with a ~10.7 kb genome that exists as four serotypes which is Dengue 1-4. It is related to other flaviviruses including Japanese encephalitis, yellow fever viruses and West Nile (Paranjape & Harris, 2010). Moreover, fifth serotype was announced in 2013. The emergence of new serotype could be genetic recombination, natural selection and genetic bottlenecks. This serotype follows the sylvatic cycle unlike the other four serotypes which follow the human cycle (Normile, 2013). Although the four serotypes were antigenically distinct but depicts the same epidemiology and cause similar illness in humans (Gubler, 2002). DENV2 appeared to be more commonly associated with fatal cases (Gubler, 1997).

2.3.2 Dengue viral infection in mosquito

Both *Ae. aegypti* and *Ae. albopictus* which belong to subgenus *Stegomyia* are recognized as the primary vectors of dengue virus (Gubler, 2002; Gubler et al., 1979). Dengue virus in *Ae. aegypti* (Garcia-Rejon et al., 2008, Rohani et al., 1997; Arya & Agarwal, 2014; Sylvestre et al., 2014) and in *Ae. albopictus* (Rohani et al., 1997) were detected in many studies using various methods. Since the dengue virus has been detected

from larvae of *Ae. aegypti* and *Ae. albopictus*, the authors suggest the possibility of the occurrence of transovarial transmission of dengue virus in *Aedes* mosquitoes (Rohani et al., 1997; Edillo et al., 2015; Giarola Cecílio et al., 2015).

2.3.3 Methods for detection of dengue virus in mosquitoes

Various methods have been used by researchers to detect dengue virus in mosquitoes such as Platelia Dengue NS1 Ag-ELISA (Arya & Agarwal, 2014; Sylvestre et al., 2014), RT-PCR (Rohani et al., 1997; Chow et al., 1998; Garcia-Rejon et al., 2008; Gurukumar et al., 2009), virus isolation through C6/36 clone (Rohani et al., 1997; Mulyatno et al., 2012) and detection of dengue virus by the peroxidase anti-peroxidase staining (Rohani et al., 1997). *Aedes* mosquito adults and larvae sampled from Terengganu, Penang and Johor were positive by virus isolation through C6/36 clone and RT-PCR in 1993 – 1995 (Rohani et al., 1997).

A study showed that Dengue NS1 Ag Strip® can be used for detection of dengue virus (DENV) in *Ae. aegypti*, and sensitivity of the test kit was comparable to that of real-time reverse transcriptase-polymerase chain reaction. The kit was able to detect all DENV four serotypes (DENV1, DENV2, DENV3 and DENV4) in infected dengue vectors. The sensitivity of the kit to test *Aedes* mosquito was 95.8% (Tan et al., 2011). However, the test was unable to detect the low level of DENV in field caught mosquito pools (Ekiriyagala, 2013). Whereas the sensitivity and specificity of the SD Duo NS1/IgM in diagnosis of acute dengue infection in human gave a comparable detection rate by either serology or RT-PCT, which gave the sensitivity of 88.65% and specificity of 98.75% (Wang & Sekaran, 2010). The Platelia Dengue NS1 Ag kit ELISA was found to have a sensitivity (Arya & Agarwal, 2014) of about 98% for the detection of DENV in mosquito pool (Voge et al., 2013), and was more effective than RT-PCR if used for very large pools

of mosquitoes (Voge et al., 2013). It was also more effective than virus isolation especially in 7 days old dead *Ae. aegypti* (Sylvestre et al., 2014). Besides, a dry-format PCR assay on advanced PCR platform was claimed can test for DENV in vector and human samples in field environments (Pal et al., 2015).

2.3.4 Relationship between infected mosquitoes and dengue cases

Studies in Colombia showed that the infection rate (IR) in mosquitoes and the influence of temperature was a better predictor of dengue cases compared to *Aedes* indices (Peña-García et al., 2016). However, other studies showed that the positivity and average number of *Ae. aegypti* females per household and egg average showed the association with dengue transmission but not with egg positivity (Dibo et al., 2008). Although many studies were carried out to determine the association between vector densities and dengue transmission, there was little evidence to quantify the association for outbreak prediction (Shamsul et al., 2016).

There were fewer studies done on the lag time analysis to predict dengue epidemics from the detection of infected mosquitoes. Studies showed that there was a lag of one to two weeks between the females *Ae. aegypti* average curve to the dengue incidence curve (Dibo et al., 2008). However, a study in Singapore showed that infected *Ae. aegypti* were detected by using RT-PCR technique as early as six weeks before the start of dengue outbreaks in 1995 – 1996 (Chow et al., 1998).

2.4 Mosquito Vectors

Aedes (*Stegomyia*) *aegypti* (Linnaeus, 1762) is the primary vector for dengue worldwide (Black et al., 2002; Carrington & Simmons, 2014; Gubler, 1997; WHO, 2011).

While, *Ae. albopictus* (Skuse, 1894) known as the Asian tiger mosquito is the secondary vector for dengue (Paupy et al., 2009). Also, *Aedes* mosquitoes are considered as vectors of globally important arboviruses such as yellow fever virus, chikungunya virus (Kraemer et al., 2015) and Zika virus (Li et al., 2012; Wong et al., 2013). *Aedes aegypti* is found within of the house, whereas *Ae. albopictus* occupies natural and disposable breeding grounds, in sites farther away from peridomiciliary premises (Serpa et al., 2013).

2.4.1 Life cycle

Over 950 species of *Aedes* mosquitoes occur worldwide (Rozendaal, 1997). *Aedes* mosquitoes like all other mosquitoes go through a complex metamorphosis cycle which includes stages of egg, larvae, pupae and adult. Once, the female mosquitoes take blood, the digestion of a blood-meal and development of eggs takes about 2-3 days in the tropics. The gravid females lay between 30 and 300 eggs at a time just above the water or on wet mud. However, *Ae. aegypti* is a highly domesticated mosquito that prefers to lay its eggs in artificial water-containers commonly found in urban areas of the tropics, such as used car tyres, tin cans, roof gutters and bottles, flower vases and plastic containers (Dom et al., 2013; Gubler, 1997; Thavara et al., 2001). These breeding habitats, naturally contain relatively clean water. However, *Ae. albopictus* breeds more often outdoors in temporary and natural containers such as leaf axils, tree holes, ground pools, discarded bottles, tins and tyres. *Aedes albopictus* is still the dominant outdoor breeder in Malaysia as it prefers outdoor conditions with more vegetation (Dhang et al., 2005; Dom et al., 2013).

The eggs hatch when they are flooded by water (Rozendaal, 1997), however it can resist desiccation for 6 months (Luz et al., 2008). Eggs take about 2-3 days to hatch and

the larval period last about 4-7 days. However, pupal period last for 1-3 days. Therefore, the complete life cycle from egg to adult will take about 7 – 13 days under favourable conditions (Rozendaal, 1997) (Figure 2.3). However, it often takes much longer due to competition for food in containers (Jazzmin & Roberto, 2004).

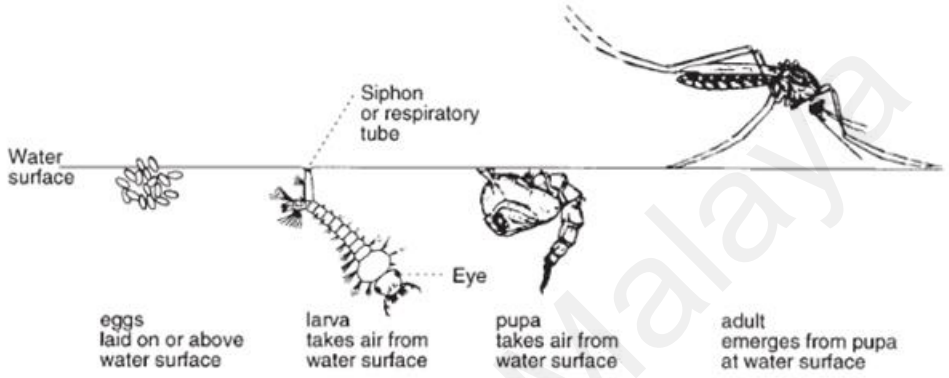


Figure 2.3: Life cycle of mosquito [Source: WHO (Rozendaal, 1997)]

2.4.2 Mechanism of disease transmission

The adult mosquitoes are rarely noticed, preferably rest indoors and bite human in an unobtrusive and undetected way (Gubler, 1997). *Aedes* is also known to bite mainly in the mornings or evening (Rozendaal, 1997). Dengue virus spread through the bite of an infected *Aedes* mosquitoes which obtains the virus from a viremic person. Individuals infected with viruses do not show signs and symptoms during the incubation period, that last for an average 4 to 6 days before the person experience an acute onset of fever accompanied by a variety of non-specific signs and symptoms (Gubler, 1997). However, studies have shown that symptom free people are more infectious to mosquitoes than clinically symptomatic patients (Duong et al., 2015).

When the mosquito bites an infected person, the virus enters the mosquito midgut and binds on the cellular surface of the midgut epithelium. Mosquito will be infected after

the virus is successfully shed into the hemocoel and subsequently disseminate and infect secondary tissues which include the salivary glands. The virus may be transmitted to a new host via saliva of the infected mosquito when it has the next feeding event (Carrington & Simmons, 2014; Goindin et al., 2015). The extrinsic incubation period takes about 8 to 12 days (Gubler, 1997; WHO, 2011). *Aedes aegypti* is a more competent vector of dengue virus and smaller-sized females *Ae*. *aegypti* are more likely to become infected and disseminate the virus (Alto et al., 2008)

It has been estimated that about 43-46% of engorged mosquitoes can bite more than one person within each gonotrophic cycle, thus making the mosquitoes efficient to transmit dengue viruses, causing rapid spread of dengue virus and making dengue prevention more difficult (Harrington et al., 2014; Scott et al., 1993). The increase in the biting rate of *Ae. aegypti* also results in dengue outbreak with greater numbers of primary and secondary infections, causing severe biennial epidemic (Luz et al., 2011).

2.4.3 Mosquito distribution

Aedes mosquitoes prefer to breed in clear water, has flight range of 200m (Lee, 2000) and are distributed worldwide. *Aedes aegypti* survives in the tropics and sub-tropics, primarily in northern Brazil and Southeast Asia, while distribution of *Ae. albopictus* extends into southern Europe, northern China, southern Brazil, northern United States and Japan as the species has the ability to tolerate lower temperatures (Kraemer et al., 2015). Due to global transportation, the density of *Ae. aegypti* increased and expanded its distribution (Shope, 1991; Soper, 1967; Surtees, 1967). Ecological changes, population growth and unprecedented urbanization in Southeast Asia during War World II has enabled *Ae. aegypti* to adapt to this part of the world (Gubler, 1997).

2.5 Vector Control and Prevention

In the absence of efficient licensed vaccine and effective antiviral drugs, vector control remains an essential component to reduce dengue transmission. Vector control which was recommended by the WHO to combat mosquito through Integrated Vector Management (IVM) includes advocacy, social mobilization and legislation, collaboration within the health sector and other sectors, integrated approach including non-chemical and chemical vector control methods, evidence-based decision-making and capacity-building (Lam, 2013; WHO, 2009).

2.5.1 Dengue control and prevention strategies

2.5.1.1 Larval survey

Traditional household larval survey is still the most widely adopted mosquito surveillance method in programs based on periodic household inspection for the presence of larvae-bearing containers. Results from larval surveys will trigger control strategies, as larval surveys provide measures of infestation in the form of House indices (HI), Breteau indices (BI) and Container indices (CI) (Codeço et al., 2015). However, it requires laborious surveys to locate individual larval habitats (Resende et al., 2013; Tun-Lin et al., 1996). Besides, traditional larval indices are known to exhibit poor relationship with the risk of dengue transmission (de Melo et al., 2012; Shah & Sani, 2011). It is also unreliable and inefficient for estimating the density of adult mosquitoes responsible for transmission and also do not reflect the human exposure risk (Focks, 2004). Larval survey also fails to detect cryptic breeding sites, thus the larval index obtained would not reflect the true situation (Codeço et al., 2015).

2.5.1.2 Law enforcement

Law enforcement uses the judicial system to enforce sanitary legislation and regulations which fines contractor or house owner that fail to prevent mosquito breeding on their premises (WHO, 2009; Tham, 2001). However, law enforcement alone is not a mainstay strategy used in effective and sustained dengue vector control (Bhumiratana et al., 2014; Ooi et al., 2006). It is more effective if the community understand through communication regarding the importance of preventing mosquito breeding within their premises and assist them to have a proper system to do so. However, working with various agencies, can achieve better long-term cooperation and result than through law enforcement (Boo, 2001).

2.5.1.3 Chemical control

Current dengue vector control relied greatly on chemical approach such as space treatment either thermal or ULV fogging, while larviciding is used to treat household drinking water containers with insecticide which has low, relative toxicity and is safe for humans. The failure of the chemical control approaches might be due to several factors such as technical problem of the fogger, timing of treatment, environmental factors, insecticide effectiveness or resistance and depending on the community to apply the larvicides regularly (Chang et al., 2011; Ong, 2016). However, excessive use of chemical insecticides and the lack of supervision on the dosages used for control have led to widespread resistance in *Aedes* mosquitoes in several countries of America, Asia and Africa. Safer alternative chemical options are also not available for vector control in different countries (Manjarres-Suarez & Olivero-Verbel, 2013). Besides the use of insecticides in spatial application for some years is also being criticized due to its negative impacts on environmental and human health (Lima et al., 2015). Chua et al. (2005) study showed that immature *Aedes* mosquitoes collected in the immediate post-fogging period

was more than that in the immediate pre-fogging period, besides fogging can affect the natural predators of *Aedes* mosquitoes.

2.5.1.4 Health promotion and social mobilization

Health promotion is one of the essential practice in any vector control program as it involves removal of possible breeding sites of larvae. It targets on promoting health education and public awareness among the community to improve the control of dengue mosquito vectors (Al-Shami et al., 2014). Wide range of strategies are used to provide health education to community through radio, television, billboards, banners, flipcharts, poster and leaflets (Andrade, 2007). However, health promotion efforts will be in vain if people do not change their behavior. Therefore, social mobilization is used to bring together all feasible and practical solutions to raise people's awareness on knowledge and to change their behaviour towards dengue prevention and control (Park et al., 2004; WHO, 2009). In 2004, WHO published the guidelines to use the COMBI (Communication-for-behavioural-impact) planning methodology to focus on communication and mobilization efforts in promoting and measuring changes in behaviour, and not just changes in knowledge and attitudes (Chang et al., 2011; WHO, 2009). However, there was insufficient evaluation of the sustainability of behavioural changes or the impact of vector control and dengue transmission. Besides people may be reluctant to take appropriate dengue prevention measures despite the advocacy of community participation except during a dengue outbreak (Chang et al., 2011).

2.5.1.5 Source reduction

Control of dengue vectors has mainly been through source reduction which eliminate the containers that are favorable sites for oviposition and development of the aquatics stages (WHO, 2012). Community based source reduction was found effective to

control dengue outbreak through entomological surveillance rather than relying on chemical control (Basker et al., 2013; Vanlerberghe et al., 2009).

2.5.1.6 Biological control

Biological control is based on the introduction of organisms that prey upon, parasitize, compete with or otherwise reduce populations of the target species (WHO, 2009). Biological control measures such as the use of *Mesocyclops* by the community-based vector control programme in Vietnam was highly effective (Nam et al., 1997), whereas the study in French Polynesia which released *Mesocyclops* and the larvivorous fishes to control larvae of *Ae. aegypti* demonstrated that the biting rate of adult *Ae. aegypti* was not reduced by biological control of larvae and thus was unsuccessful as a means of vector control (Lardeux, 1992).

Bacillus thuringiensis israelensis (*Bti*) is a microbial control agent that effectively kills the larval stage of *Aedes* mosquitoes and it is effective when used as a larviciding agent against *Aedes* larvae (Lee et al., 2008; Lee et al., 2015). There is very limited evidence that dengue morbidity can be reduced through the *Bti* alone although it can reduce the number of immature *Aedes* in treated containers in the short term (Boyce et al., 2013). However, the limitation for the *Bti* to be a potent biolarvicide, is due to its short residual activity, thus requiring frequent application (Poopathi & Tyagi, 2006). *Bti* also does not grow or reproduce well outside host organism and might remain in an inactive state in the absence of a host (Shannon et al., 1989).

2.5.1.7 Other new vector control tools

a. *Wolbachia*-infected *Aedes* mosquitoes

Wolbachia is an endosymbiotic bacterium which is found in most insects but not in *Ae. aegypti* (Coon et al., 2016). It has now been introduced into *Ae. aegypti* and thus can reduce adult lifespan, affect mosquito reproduction and interfere with pathogen replication as indicated by reduced susceptibility of *Wolbachia* infected *Ae. aegypti* to dengue virus (Iturbe-Ormaetxe et al., 2011; Lambrechts, 2015). Release of *Wolbachia*-infected *Aedes aegypti* mosquitoes was used as additional weapons against mosquitoes so as to reduce the transmission of dengue virus (Lambrechts, 2015). It has the benefit of being more environmentally benign than insecticide-based approaches and potentially more cost effective (Iturbe-Ormaetxe et al., 2011). However, stable trans infection of *Wolbachia* into heterologous mosquitoes hosts clearly produces antiviral effects against arboviruses including DENV (Dengue Virus), WNV (West Nile Virus), YFV (Yellow Fever Virus) and CHIKV (Chikungunya Virus) (Johnson, 2015). Field trials to assess the epidemiologic impact of *Wolbachia*-infected *Ae. aegypti* on dengue virus transmission has just began recently (Achee et al., 2015a).

b. Pyriproxyfen as auto-dissemination

Pyriproxyfen is a juvenile hormone mimic and inhibit metamorphosis to prevent emergence of adults from pupae (Mbare et al., 2014; Sihuincha et al., 2005). Its effectiveness to control mosquito larvae can persist for up to four months in variety of aquatic habitats (Vythilingam et al., 2005). “Auto-Dissemination” approach which is based on the possibility that the wild adult females exposed to containers treated with pyriproxyfen, can disseminate it to other larval habitats and thus interfere with adult mosquito emergence (Snetselaar et al., 2014). A study showed that “Auto-

Disesemination" approach was feasible to control *Ae. albopictus* in urban areas (Caputo et al., 2012).

c. Lethal ovitrap

Lethal ovitrap is an ovitrap incorporated with insecticides on the oviposition substrate which allow oviposition but prevents adult emergence. Study showed that mass trapping using lethal ovitrap was not rejected by the public and was effective in reducing the *Aedes* mosquito density (Ritchie et al., 2009), and thus can be considered as an effective component of a dengue control strategy (Rapley et al., 2009). Lethal ovitraps can be in many forms such as biodegradable lethal ovitrap which was made from a starch-based plastic (Ritchie et al., 2008), modified trap design (AGO-B) (Mackay et al., 2013) and sticky surface covering the interior (CDC-AGO trap) (Barrera et al., 2014; Nurulhusna et al., 2011). Sticky trap was also used as a tool to reduce the vector population through attraction and then killing female mosquitoes as they lay eggs (Degener et al., 2015).

d. Release of insects carrying a dominant lethal (RIDL)

Release of insects carrying a dominant lethal (RIDL), is a genetically modified technology able to suppress the *Ae. aegypti* population without any adverse effects (de Valdez et al., 2011; Lacroix et al., 2012). However, this require continuous releases of mosquitoes lasting about one year and followed by intermittent releases (Franz et al., 2014). However, RIDL has faced the difficulties to be implemented due to accusation from public of incomplete risk assessment procedures, lack of transparency regarding results and political agendas (Borame et al., 2016). The major issue to implement RIDL strategy is the high cost for the production and release of GM *Ae. aegypti* (Ong, 2016).

e. Sterile Insect Technique (SIT)

Releasing sterile insects in large numbers which is a Sterile Insect Technique (SIT) using gamma radiation is widely being studied to be used as a tool to control dengue in the future (Alphey et al., 2010; Oliva et al., 2012). Although SIT has been used successfully for suppressing or eliminating a number of agricultural pests (Dyck et al., 2005), there are limited large-scale SIT programs in operation against any mosquito species although some trials were conducted in recent years (Alphey et al., 2011).

f. Insecticidal paint

Insecticidal paint is an emulsion paint formulation impregnated with an insecticide for the purpose to control and eliminate insect pests. Insecticidal paint has been suggested for vector control since year 1940s, however it was only commercially available a few years ago, mainly in Europe and North America. It was promoted against nuisance pests that dwell on walls and ceilings. Recently, insecticidal paint is receiving renewed interest for their potential use against disease vectors (Ong, 2016). Insecticidal paint which contained deltamethrin was tested in a small kitchen and showed to be effective for 3 years against cockroaches, housefly, ants and lizards (Lee et al., 2015). However, field testing of the said insecticidal paint against dengue has not been conducted (Lee et al., 2015).

g. Indoor/Outdoor Residual Spraying

Indoor residual spraying which mostly applied to malaria control also has been carried out on a few occasions for dengue vector control. Studies indicated that indoor residual spraying when used appropriately can reduces adult mosquitoes (Ritchie et al., 2004) and significantly reduce dengue virus transmission (Vazquez-Prokopec et al., 2010). However, outdoor residual spraying of deltamethrin study in Kuala Lumpur

showed that it was not very effective against *Aedes* (Rozilawati et al., 2005), while Lee et al. (2015) showed that Polyzon used for outdoor residual spraying was effective to reduce mosquito density and control dengue case for high rise building.

2.5.2 Strategies for dengue control and prevention in Malaysia

Strategies for vector control programme in Malaysia includes chemical control, house to house *Aedes* larval surveys, source reduction (Lam, 1993), health promotion, community participation, Inter-agency collaboration, law enforcement (Teng & Singh, 2001; Tham, 2001), Integrated Vector Management, community mobilization and Communication For Behavioural Impact (COMBI) (KKM, 2009). Many problems have been identified in carrying out these control activities, such as illegal dumping of household refuse and unusual breeding sites which hamper source reduction efforts. The unusual breeding sites are cocoa pods, rubber tyres, septic tanks, vacant land, abandoned housing projects, roof gutters, refrigerator trays and cemeteries (Tham, 1993). Problem encountered in house inspection was that coverage and frequency of visits to houses were not up to expectation due to shortage of manpower (Lam, 1993; Mudin, 2015). Enforcing the DDBIA Act was still a problem. The support and participation from public in source reduction measures and fogging activities were poor as house-owners tend to close the doors and windows, thus not achieving total coverage of all houses. In addition, private pest control operators also conduct fogging without adequate supervision (Lam, 1993).

2.5.3 Challenges of vector control and prevention

Currently there are limited tools for the effective management of vectors and insecticides remain as the main strategy. However, the use of insecticides face challenges such as insecticide resistance, toxicity concerns, biosafety issues, community acceptance, long-term sustainability (Chang et al., 2011; Lam, 2013), as well as cost and delivery

(Chang et al., 2011). Chemical control target on adult stages of mosquitoes have its limitation due to its toxicity, difficulty of achieving total coverage of all houses (Lam, 1993) and can develop insecticide resistance if usage of insecticide is beyond 2 years (Lam, 2013), thus these insecticide-based approaches can lead to the increase the size of future epidemics (Luz et al., 2011). Due to the extensive application of insecticides, resistance to organophosphate (temephos) and pyrethroids has been reported widespread in *Ae. aegypti* (Lima et al., 2011), and resistance has also been reported to *Ae. albopictus* (Chan & Zairi, 2013), these includes *Ae. aegypti* and *Ae. albopictus* from Malaysia which has shown resistance to both groups of insecticide such as organophosphate and pyrethroids (Ishak et al., 2015).

Larvicides are used widely, however tree holes, leaf axils and deep wells are inaccessible to their application (Lam, 2013). Studies showed that environmental management drives the reduction of infestation while insecticides do not improve environmental vector control (Favier et al., 2006). The observation showed that the chemical controls alone showed the worst performance, while the integrated strategy showed the best (Lima et al., 2015).

2.6 Vector Surveillance

Vector surveillance is an entomological surveillance which is used to determine the distribution and density of vector, evaluate control activities and the information is used for decision making regarding interventions.

2.6.1 Type of vector surveillance

There are number of methods used to detect or monitor immature and adult population. Type of method selected depends on the objective of surveillance, available funding, accuracy of the outcome, levels of infestation and skills of personnel. Methods are derived to describe the population of *Aedes* based on their life cycle stages such as larvae, pupae and adult.

2.6.1.1 Larval surveys

Larval surveys have been the hallmark of the dengue control programmes in many countries. From the larval surveys, *Aedes* house index, Breteau index and container index can be calculated. House index is the percentage of houses infested with larvae or pupae, while the Breteau index is the number of positive containers per 1000 houses inspected and container index is the percentage of containers positive with larvae or pupae (WHO, 2016c). The larval survey has been very useful decades ago when the *Aedes* house index was high. There has been a reduction in *Ae. aegypti* population in the 1990's compared to the 1980's, perhaps due to vector control programmes and provision of piped water. In the 1980's the *Aedes* house index ranged from 4.7 to 58.8% (Ho & Vythilingam, 1980), whereas in the 1990's the index ranged from 0.1 to 6.9% (Sulaiman et al., 1996). A more recent report stated that the index ranged from 1.5 to 2% (Mudin, 2015), although the number of premises was inspected has increased about 1.5 times (1997: 4,239,489 premises; 2015: 6,261,089 premises) (KKM, 2016a; Tham, 2001) and dengue cases increase about 6.2 times more (1997: 19,429 cases; 2015; 120,836 cases) (KKM, 2016a; Teng & Singh, 2001). However, currently the number of houses has increased while the health staff remains static. Thus, larval surveys have become labour intensive and plagued by difficulties to access houses particularly in urban areas (Sivagnaname & Gunasekaran, 2012). Limitation of the larval surveys could be due to cryptic breeding sites which make

the surveys more labour intensive. Studies showed that there was no evidence of a relationship between larva infestation and dengue occurrence (Barbosa et al., 2010; de Melo et al., 2012). Larval survey has been claimed as a weak indicator of dengue vector populations and does not provide information needed to tailor vector control operation. Furthermore, thresholds for Breteau, House and Container Indices are not realistic to explain the risk of transmission and do not represent an adult vector population (Azil et al., 2011; Focks, 2004).

However, classical measures using immature stages densities still remain the most usual way to quantify mosquito infestation due to economic viability, easy to operate, knows the distribution of immature stages for source reduction purposes despite the lack of unequivocal relationships with adult population or dengue epidemic risk (Focks, 2004). Larval survey is useful in identifying new infestation areas. It can be initiated immediately on case notifications besides surveys can be done simultaneously while performing source reduction activities and health education. Larval survey can also be used to identify key containers and premises for targeted control interventions (Azil et al., 2011). However, it is known that currently it is not useful to forestall a dengue epidemic (Focks et al., 2007).

2.6.1.2 Ovitrap

Due to the limitation of the *Aedes* house index, ovitrap was used as a complementary surveillance method. The ovitrap index was a more sensitive technique when the larval surveys indicated low infestation and have proved especially useful for the early detection of new infestations in an area (Morato et al., 2005). Ovitrap indices reveal greater power of detection of positivity of mosquito compared to Breteau and House Indices and proved to be an economical and operationally viable method (Braga et al., 2000; Morato et al., 2005).

Ovitrap was also used to indirectly estimate the female population. It has a low operating cost and is a sensitive tool to detect the presence of vector (Dibo et al., 2008). It proved to be more sensitive than MosquiTRAP (Honório et al., 2009a). However, it failed to detect the period of dengue transmission for adopting ideal control measures when it has high egg positivity (Dibo et al., 2008) as *Ae. aegypti* can distribute small numbers of eggs among many sites, and this "skip oviposition" is a driver for dispersal (Reiter, 2007).

An ovitrap can become a breeding site if it is not checked and monitored. Thus, some people have modified the ovitrap to be an autocidal ovitrap (Lok et al., 1977; Zeichner & Debboun, 2011). Autocidal ovitrap was made to prevent the escape of any adults and was first tested in Singapore and found to be effective for the control and possible eradication of *Ae. aegypti* from some areas (Lok et al., 1977). Another, example is the Mosquito Larvae Trapping Device (MLTD) which was treated with *Bacillus thuringiensis israelensis* (*Bti*) was used as surveillance and control tool in dengue hotspots in Kuala Lumpur. MLTD is made from plastic and sprayed with black paint. The trap was primarily maintained by staff from Kuala Lumpur City Hall and was used to trap mosquitoes and fly (Azil et al., 2011). Some have used an ovitrap as a lethal device by treating the oviposition strip with an insecticide so it becomes lethal to *Ae. aegypti* adult and larvae (Rapley et al., 2009; Ritchie et al., 2008; Ritchie et al., 2009; Zeichner & Perich, 1999). The same technique also was applied in Brazil where *Bacillus thuringiensis israelensis* (*Bti*) was added in ovitraps to prevent the survival of the larvae while the ovitrap was used for detecting *Ae. aegypti* population and preventing dengue outbreaks (Mackay et al., 2013; Regis et al., 2008).

2.6.1.3 Pupae surveys

Due to the limitation of the larval indices, pupae indices were developed to better reflect the risk of transmission (Focks et al., 2000). Pupae survey provides more realistic results as they closely resemble the adult population (Focks & Chadee, 1997; Focks et al., 2000). The ratio of pupae per person was found more appropriate for assessing risk and directing control operations because it was possible to be counted in absolute number, has low mortality and can be more accurate to predict the threat of dengue transmission compared to larva index (Focks et al., 2000). Pupae per person threshold was developed as range 0.5 – 1.5 was used for assessing the risk of transmission in some countries such as Cuba and Singapore (Focks et al., 2000). Study in Thailand showed that pupal survey can be good for assessing dengue transmission risk based on the strength of correlations between pupal and adult populations (Koenraadt et al., 2008), and it also showed no correspondence with the House, Container, and Breteau indices (Focks & Chadee, 1997). Direct pupal counts were found most suitable for the productive types of containers compared to the index related about the presence of immature forms (Barrera et al., 2006b). However, collecting individual pupae is time-consuming, labour intensive (Focks et al., 2007; Focks, 2003) and difficulty in locating breeding sites, especially the cryptic breeding sites (Pilger et al., 2011).

2.6.1.4 Adult surveys

Adult survey was carried out to assess the abundance of adult mosquitoes using either the landing rate or the indoor resting density during the collection time. However, the old methods used such as landing or biting collections on humans (Human Landing Catch) (HLC) although is sensitive, but labour-intensive means to detect low-level infestations (WHO, 2016c). However, HLC is not recommended for dengue vector since there are no drugs for treatment and is unethical to expose people to mosquito bites.

However, resting collection using backpack aspirators or sweep nets can be used (Achee et al., 2015b). Densities are recorded as the number of mosquitoes per house or the number of adult mosquitoes collected per unit of time (WHO, 2016c). An indoor resting collection of *Aedes* adult usually yields a less number and estimated about 50 percent caught of the exiting vectors (Sivagnaname & Gunasekaran, 2012). Collection of adults using these techniques is also labour intensive and intrusive. It also depends on the person carrying out the collections.

Studies found a significant and positive association between density of larvae and pupae of *Ae. aegypti* but negative relationship between larval and emerging females as larva were influenced by resources limitation or competition (Barrera et al., 2006a), however studies in Mexico showed that there was an association between the presence of adults with pupal presence at the household level and also with ovitrap positivity (Manrique-saide et al., 2014) but not associated with larval or immature numbers (Tun-Lin et al., 1996). Entomological sampling indicators which were reviewed by WHO also mentioned that the traditional *Stegomyia* indices (the House, Container, and Breteau Indices) are of some operational value, but not proxies for adult vector abundance and neither are they useful for assessing transmission risk (Focks, 2004).

Reliable and highly useful indices such as adult index is warranted as despite the low immature indices, the re-emergence of dengue disease still occurred in many countries. Relation of immature *Ae. aegypti* density to the transmission risk was weak compared to the adult mosquitoes (Sivagnaname & Gunasekaran, 2012). Adult mosquito collection can best inform the quantity of adult mosquitoes per area or inhabitant or as main predictor of dengue occurrence (Dibo et al., 2008).

2.6.2 Methods to collect adult mosquitoes

Currently, many different methods are used to collect and obtain sufficient number of adult mosquitoes in order to understand the dengue transmission risk, so that appropriate control strategies can be instituted accordingly. Various methods such as BG-Sentinel traps, sticky traps, (Sivagnaname & Gunasekaran, 2012), Resting Boxes (Kittayapong et al., 1997) and Omnidirectional Fay-Prince trap (ODFP) (Jones & Sithiprasasna, 2003) have been used to collect adult *Aedes* mosquitoes.

2.6.2.1 Types of traps and equipment

Different types of traps were invented to collect adult mosquitoes. Sticky traps are currently widely used as the most effective adult trap (Chadee & Ritchie 2010a; Facchinelli et al., 2007; Lee et al., 2013) and sweep nets was the conventional method to collect adult mosquito samples (Rohani et al., 1997).

Backpack aspirator was found to collect all gonotrophic stages of females but it is labour-intensive and not suitable for routine use because the operational need for diligence, skill, consistency of effort and able to access to all the areas (Chadee & Ritchie, 2010a).

BG-Sentinel traps which are suction traps that use BG-Lure human skin odors to attract host seeking mosquito, are capable of collecting mostly unfed females of *Ae. aegypti* and *Ae. albopictus* but not the gravid mosquitoes. The study showed no significant difference between human landing rates and the capture rates of BG-Sentinel traps (Krockel et al., 2006). The BG-Sentienl trap was also found to collect more *Ae. aegypti* females than a backpack aspirator (Chadee & Ritchie, 2010a; Maciel-de-Freitas et al., 2006), sticky trap (Krockel et al., 2006), CDC light trap (Dhimal et al., 2014) and EVS

trap while CDC Backpack Aspirator collected more blood fed *Ae. aegypti* (Williams et al., 2006). However, BG-Sentinel trap is too expensive, require daily mosquito collection and thus not very useful in dengue endemic countries for routine surveillance.

BG-Sentinel can capture *Ae. aegypti*, *Cx. quinquefasciatus* (Barrera et al., 2013), and *Ae. albopictus* (Crepeau et al., 2013; Farajollahi et al., 2009; Unlu & & Farajollahi, 2014). It was used as a strategy to reduce indoor biting by *Ae. aegypti* (Salazar et al., 2012) and claimed to be a reliable tool in *Ae. aegypti* surveillance with consistent sampling outcome (Ball & Ritchie, 2010; Degener et al., 2014). It was also found to be more effective and caught a wide range of mosquito species, the highest being *Culex* mosquitoes compared to traps such as Heavy Duty Encephalitis Vector Survey trap (EVS trap), Centres for Disease Control miniature light trap (CDC trap and Mosquito Magnet Pariot Mosquito trap (MM trap) (Luhken et al., 2014). Although BG-Sentinel trap has been attempted in monitoring *Ae. aegypti*, their utility is limited due to various setbacks mentioned above for entomological and epidemiological studies (Sigvagnaname & Gunasekaran, 2012).

MosquiTRAP was shown to be an effective and reliable device for trapping gravid *Ae. aegypti*, however, these traps need to be evaluated through a longer time series (Steffler et al., 2011). Although MosquiTrap was able to collect more female *Ae. aegypti* than AdultTrap which was a kind of trap for capturing gravid *Ae. aegypti* females during oviposition and consist of three chambers, however MosquiTRAP can act as a breeding site for dengue vector (Sivagnaname & Gunasekaran, 2012).

It was verified that ovitrap and MosquiTRAP were better detection methods for predicting dengue occurrence compared to larval survey, both spatially and temporally, and was more accurate to signal dengue transmission risks both geographically and temporally (de Melo et al., 2012). MosquitoTRAP and Adultrap which were tested in Rio

de Jenerio seem to be efficient, reliable in collecting gravid *Ae. aegypti* females (Maciel-de-Freitas et al., 2008), but mass trapping using MosquiTRAP did not reduce adult *Ae. aegypti* abundance (Degener et al., 2015) .

Whereas the other traps that have been studied and reported such as Harris County Gravid Trap (HCGT) which is a motor operated trap recorded more *Cx. quinquefasciatus* and *Ae. albopictus* in the field (Dennett et al., 2007). Mouse-baited BG-Sentinel was claimed useful for in-depth field studies and evaluation of control methods (Lacroix et al., 2009). Propane-powered commercial traps collected more *Ae. albopictus* than CDC-light trap and *Aedes*-specific traps (Hoel et al., 2009). Mosquito Magnet Liberty which use burning propane to release carbon dioxide and moisture was found to reduce the abundance of nuisance mosquitoes (Jackson et al., 2012) and collected the most *Ae. albopictus* (Hoel et al., 2009). While tent trap which consist of two rectangular tents that use human bait was tested and found more *Ae. aegypti* males than females were caught, while with *Ae. albopictus,* it was opposite (Casas et al., 2013). Centers for Disease Control and Prevention autocidal gravid ovitrap (CDC-AGO Trap) which was tested in Puerto Rico showed that it was useful and inexpensive mosquito surveillance device (Barrera, R. et al., 2014). Whiles, GAT, which is a mosquito trap and relies on visual and olfactory cues to lure gravid *Ae. aegypti* and the chamber impregnated with a pyrethroid insecticide was claimed more efficient to capture *Ae. aegypti* compared to other sticky traps (Eiras et al., 2014). GAT collected more female *Ae. aegypti* than MosquiTRAP and double sticky trap, but less than the BG-Sentinel trap (Ritchie et al., 2014).

Although many types of traps have been developed and all perform better than the House Index in the measuring the seasonal variation in mosquito abundance, the choice between traps are dependent on the behavior of the trap indices, cost, ease-of-use and sensitivity (Codeço et al., 2015). It was found that battery-powered traps with contrasting

color schemes and movement worked considerably better than stationary CDC miniatures without color or movement (Dennett et al., 2004). However, landing/biting collections at human bait still behave as the best trap to provide large samples as compared to other different types of trap (Russell, 2004). This also showed that none of the trap devices such as American Biophysics Corporation Standard Professional (ABC-PRO) light trap, the Omni-Directional Fay-Prince trap (with and without CO2), and the Centers for Disease Control and Prevention Wilton trap evaluated in the study was better than backpack aspirator or human-landing collections for monitoring population of adult mosquitoes (Schoeler et al., 2004).

2.6.2.2 Attractant to trap adult mosquitoes

Attractants are used in order to make the trap more attractive to mosquitoes as compared to the surrounding man-made containers. Mosquitoes are attracted to the CO_2 released from a person's lungs and chemical odours produced by human skin. Studies showed that synthetic blend of chemicals comprising volatiles released by the human body was effective in attracting *Ae. aegypti* females under controlled laboratory conditions (Silva et al., 2005).

Compound and light sensitive simple eyes are used to spot host movement particularly during daytime, while maxillary palpus is heat sensitive and helps to locate warm-blooded host and pinpoint capillaries. These facts are meticulously considered as an attractant to develop a more efficient adult trap (Chadee & Ritchie, 2010a). Higher pupal productivity was observed in unattended containers in the backyards, and significantly positively associated with the number of trees per premise, water volume and lower water temperature. This association was due to presence of shade, lower evaporation rates, lower water temperatures and trees can contribute organic matter and nutrients for the aquatic community (Barrera et al., 2006a).

The Centres for Disease Control and Prevention (CDC) light trap was made attractive by using dry ice-baited and white light which suspended around 1.5 m above the ground and capturing mosquitoes with the down draft produced by a motor and fan (Mcelly, 1989). However, other baited such as olfactory attractant 1-octen-3- olalone was combined with carbon dioxide revealed species-specific responses to olfactory attractants (Shone et al., 2006). Another study showed octenol bait 1-octen-3-ol significantly enhances the collections of *Ae. albopictus* in urban environments (Qualls & Mullen, 2007). However, significantly more *Ae. albopictus* were captured in traps baited with octanol + L-lactic acid (Lurex^{TM}) than in traps baited only with octenol (Hoel et al., 2007). Besides attractants are present in human skin volatiles can attract *Ae. aegypti* (Owino et al., 2014), entrained and eluted host odor can also be used to attract *Ae. aegypti* (McCall et al., 1996).

Studies found that more mosquitoes were collected using CO_2 traps than any other method of trap (de Azara et al., 2013; L'Ambert et al., 2012). Dry ice baited trap was proved to be more efficacious over yeast generated CO_2 trap (Oli et al., 2005), while another study showed that yeast-containing tablet was the most attractive odor lure to mosquitoes (Snetselaar et al., 2014). However, a combination of at least three factors such as a visual cue, CO_2 and a chemical cue can have more value for trapping and estimating the relative adult population sizes of *Ae. aegypti* and *Ae. albopictus* (Kawada et al., 2007).

The study showed a synthetic mixture of an oviposition-stimulating kairomone can attract more *Ae. aegypti* egg-laying (Barbosa et al., 2010). The other attractants source was used for ovipositing female mosquitoes were larval water (Vartak et al., 1995) and aqueous infusion from wood inhabiting fungus (*Polyporus* sp.) were applied in the water (Sivagnaname et al., 2001).

Gravitrap with hay infusion was shown to be highly attractive to *Cx. quinquefasciatus*, and not *Ae. albopictus* (Burkett-Cadena & Mullen, 2007), however, it was used for enhancing the oviposition response of gravid females *Ae. albopictus* by using a hay infusion of *Pennisetum* grass and rice straw (Gopalakrishnan et al., 2012). Increasing the size of the trap entrance, altering the color of the trap, components and increasing the volume or surface area of the aqueous increased 3.7-fold of *Ae. aegypti* capture in Puerto Rico (Mackay et al., 2013). However, using Bermuda grass as attractant can attract a greater number of the mosquitoes as compared to others grass species such as oak leaves, acacia leaves, rabbit chow (alfalfa pellets) and green algae (McPhatter et al., 2009).

2.6.2.3 Sticky trap

The earliest type of sticky trap was the use of sticky pipe trap with an adhesive paper on the underside of service manholes to record the entry and exit of adult mosquitoes through the keyhole openings. It was tried in north Queensland, Australia in dry seasons of 1996-97 showed both males and predominantly nulliparous females for 5 species, mainly *Aedes tremulus* group and *Ae. aegypti* were collected (Kay et al., 2000).

Sticky trap was first used to sample female *Ae. aegypti* (L.) in Cairns, Queensland, Australia in 2003 to show sticky ovitrap index (mean number of female *Ae. aegupti* per trap per week) could be useful in gauging the risk of dengue transmission (Ritchie et al., 2004).

Surveillance adult trap was found to be an attractive alternative to the traditional labour-intensive household survey due to its low cost, species exclusivity, ease of distribution, indecency from electric power and consistent sampling profile

(Sivagnaname & Gunasekaran, 2012). Sticky trap collected significantly more *Ae. aegypti* and *Ae. albopcitus* female than backpack aspirators from outdoor (Chadee & Ritchie, 2010a; Facchinelli et al., 2008) and standard oviposition trap. It also trapped more *Ae. albopictus* females than other Culicidae species representing >90% of the total catches (Facchinelli et al., 2007). The study also showed the percentage of sticky trap positives was double for *Ae. aegypti* and almost 20 times higher for *Ae. albopictus* (Facchinelli et al., 2008). Sticky trap has more advantage as it is an inexpensive method and does not need any electricity and can be left unattended for up to seven days (Chadee & Ritchie, 2010a). A study carried out in a dengue-endemic village in Thailand showed that sticky traps collected significantly more *Ae. aegypti* and *Ae. albopictus* females than did backpack aspirator (Marini et al., 2010). However, sticky traps still have its limitation as it targets only ovipositing females rather than host-seeking mosquitoes and its efficacy may be compromised by nearby natural oviposition sites (Chadee & Ritchie, 2010a). Although sticky ovitrap can be used to estimate dengue transmission, however it requires additional personnel-time to be spent to process the sticky ovitrap after fieldwork (Azil et al., 2011).

Sticky trap, MosquiTRAP (MQT) which was tested in Brazil showed that it did not reduce adult *Ae. aegypti* abundance and mass treatment did not affect the DENV, lgM seropositivity (Degener et al., 2015). The trap revealed significant correlations of moderate strength between larval survey, ovitrap and MosquiTRAP measurements. It observed positive relationship between temperature, adult capture measurements and egg collections, whereas exhibited a negative relationship with precipitation and frequency of rainy days (Resende et al., 2013). However, another study showed that temperature and rainfall did not affect the adult density but seems to have affected the larvae indices. Although the MosquiTRAP caught a low number of *Aedes* mosquitoes, it was more sensitive than the larval survey to detect the presence of *Aedes* mosquitoes (Gama et al.,

2007). Sensitivities of MosquiTRAP and manual aspirations to detect the presence of *Ae. aegypti f*emales were similar but were lower compared to oviposition traps (Fávaro et al., 2008).

Double sticky trap (DST) which was made of two sticky traps were fully assembled with holding clips and panels was tried out in east-central Trinidad collected significantly more adults than single sticky trap (STs), however both can collect both adult and immature stages (Chadee at al., 2010a). Another type of trap which was *Aedes*Trap was made of disposable plastic soda bottle coated inside with colophony resin, results showed that they were capable to capture *Ae. aegypti* and other culicidae mosquitoes, it was able to collect three times more outdoors versus indoors (de Santos et al., 2012).

However, Singapore also used gravitrap as a dengue cluster management to trap *Aedes* mosquitoes and mosquitoes tested positive for dengue virus (Lee et al., 2013). Test carried out to compare different types of sticky traps showed that large Gravid *Aedes* Trap (GAT) using 9.2-liter bucket outperformed a smaller 1.2-liters GAT and collected more *Ae. aegypti* than the MosquitoTRAP and sticky ovitrap respectively (Ritchie et al., 2014). New adhesive traps which were Mosquito Emerging Trap (MET) and Catch Basin Trap (CBT) were tested on the campus of the University of Rome to monitor urban mosquito adult abundance and seasonal dynamics and to assess the efficacy of control measures (Caputo et al., 2015).

2.7 Relationship of Mosquitoes and Climate

2.7.1 Relationship between climate variables and density of mosquitoes

Some studies showed that *Ae. aegypti* population dynamics are influenced by climate variability. However, the relative effect of these variations depends on local ecology and social context (Stewart et al., 2013).

The study showed that both temperature and rainfall were significantly related to *Ae. aegypti* (Soper, 1967) indices at a short (1 week) time lag in Rio De Janeiro, Brazil (Honório et al., 2009a; Lana et al., 2014). Study in Cairns, Australia showed that *Ae. aegypti* density was associated with temperature and rainfall with short (0-6 weeks) and long (0-30 weeks) lag periods (Duncombe et al., 2013). However, the study conducted in 2 apartments in Kuala Lumpur showed that rainfall and relative humidity had significant relationship with the number of *Aedes* larvae collected but not with temperature (Roslan et al., 2013). However, population of *Aedes* larvae was not correlated with climatic factors, but depends on food supplies (Surtees, 1967). Studies in Thailand showed that larval abundance coincided with the periods of greater rainfall because availability of water sources and these also correspond to the time of year with the greatest dengue transmission (Strickman & Kittayapong, 2002).

Besides, weekly temperature above 22 – 24^{o}C is associated with abundance of *Ae. aegypti*, thus increasing the risk of dengue transmission (Honório et al., 2009a). Another study also showed high temperature having an added effect of enhancing vector competence (Chepkorir et al., 2014). It also can increase the epidemic potential of dengue-carrying mosquitoes, given viral introduction, especially to the susceptible human populations bordering endemic zones (Patz et al., 1998). Besides, the effect of the higher temperature also increased the female average and positivity and egg average, which also followed the rainfall pattern with a time lag (Dibo et al., 2008). It is known that higher

temperature can enhance virus transmission due to the shortening of the incubation period in the mosquito, causing wider distribution of *Ae. aegypti*, faster mosquito metamorphosis and more rapid development cycle of mosquito (Shope, 1991; Watts et al., 1986). Higher temperature also cause optimizing biting and parity of female mosquitoes, thus can increase the speeds of epidemic spread. However, the best daily survival was found at 27^{o}C and lowest survival was found at the highest temperature of 30^{o}C (Goindin et al., 2015). However, studies in the Petaling district in Malaysia showed a moderate increase in temperature does not necessarily lead to a greater dengue incidence (Williams et al., 2015).

2.7.2 Climate variation effect on dengue transmission related to density of mosquitoes

Challenges are faced when need to describe and predict the impacts of climate variability and change on the transmission of vector-borne diseases, as it involves the complexity of other factors such as multitude of epidemiological, ecological and socio-economics that drive vector-borne diseases transmission (Parham et al., 2015). Water Budgeting Technique was used as dengue forecasting model in the Puerto Rico showed that dengue incidence was significantly influenced by climate over at least an 8 weeks period (Schreiber, 2001). While, study in Taiwan using Autoregressive Integrated Moving Average Models showed that there was two months lag for an association of dengue incidence with temperature and relative humidity but was not in the case of rainfall as most of the containers filled with water was man made (Lana et al., 2014; Wu et al., 2007). Favier et al. (2006) also mentioned the nature of the link between climate and larval population should be investigated in larger-scale studies before being used in forecasting models.

However, the climatic variations alone do not explain the *Ae. aegypti* and dengue transmission, factors such as the abundance of the breeding sites, how they are filled with water, the domestic behavior of the vector, a degree of immunity of the population and many other factors should be considered in the design of the explanatory epidemiological model of dengue occurrence (Dibo et al., 2008). Studies also showed an increased risk of *Ae. aegypti* range expansion was not directly due to climate change, but rather to human activities such as installation of large domestic water storing containers (Barrera et al., 2011; Kearney et al., 2009), human movement (Honorio et al., 2009b; Reiner et al., 2014; Ritchie et al., 2013), domestic environment (Jansen & Beebe, 2010), human behavioral adaption (Padmanabha et al., 2010) and social risk factors (Stewart et al., 2013). Dense population has the effect for higher infestation level (Honório et al., 2009b).

Nevertheless, understanding the relationship between climate and dengue transmission is difficult because no-linear relationship exists between the survivals of *Ae. aegypti*, the extrinsic incubation period (EIP) of the virus, temperature and humidity (Beebe et al., 2009). Based on a study in Singapore, population immunity factor was also important when quantifying the threshold of density of female mosquitoes for vector control in dengue-endemic areas (Oki & Yamamoto, 2012). However, usefulness of models to predict mosquito population dynamics depends on the reliability of their predictions, which can be affected by different sources of uncertainty, including the model parameter estimation, model structure, measurement errors in the data, individual variability and stochasticity in the environment (Xu et al., 2010).

Mostly forecasting model was used to predict the effect of climate variation such as Descriptive and Regional Model based on satellite image and climate variable in Argentina using multiple linear regression found a correlation between mosquito density with mean temperature and precipitation with a time lag of a month (Estallo et al., 2008),

Biophysical Model of energy and mass transfer in Australia to predict climatic impacts on the potential range of *Ae. aegypti* showed that the potential direct impact of climate on the distribution and abundance of *Ae. aegypti* is minor when compared to the potential effect of change water-storage behaviour (Kearney et al., 2009) and stochastic dynamical model describes disease dynamics triggered by the arrival of infected people in a city and size of epidemic outbreaks seasonal depended on seasonal climatic variations (Otero & Solari, 2010). Above all these, the integration of epidemiological, virological, entomological and meteorological data to develop sensitive dengue risk indicators to trigger vector control is required (Azil et al., 2011). The spatial stimulation model showed warmer weather and increased human movement had only a small effect on the spread of the virus, while a shorter virus strain-specific extrinsic incubation time can cause explosive outbreaks (Karl et al., 2014).

Studies showed that mosquitoes lived longer and have higher DENV transmission season under large temperature fluctuations, while low DENV transmission for the short-term temperature variations (Brady et al., 2013; Carrington et al., 2013a; Lambrechts et al., 2011). However, temperature fluctuations in the laboratory-based experiments do not fully reflect what is happening in nature. This complexity may in turn reduce the accuracy of population dynamic modelling and downstream applications for mosquito surveillance and disease prevention (Carrington et al., 2013a). Warmer climate predicts the increase of *Ae. aegypti* and the rate of viral replication within the vector and extrinsic incubation period (Morin et al., 2013).

Climate-based multivariate non-linear model study in Noumea, New Coledonia showed that the epidemic peak lagged the warmest temperature by 1-2 months and was in phase with maximum precipitations, relative humidity and entomological indices (Descloux et al., 2012). Study in Brazil showed that both temperature and rainfall have

the effect on *Ae. aegypti* indices at a short 1-week lag (Honório et al., 2009a), this was also true for humidity (Simoes et al., 2013). However, based on the study in Australia, for the longer effect, temperature have 4 – 6 months effect on the abundance of adult during the wet season. Humidity rather than rainfall was found to be a strong predictor of *Ae. aegypti* abundance in either longer or shorter-term models (Azil et al., 2010). Studies in Cairns, Australia showed that density of *Ae. aegypti* was associated with temperature and rainfall with the lag periods between short (0-6weeks) and long (0-30 weeks) (Duncombe et al., 2013).

Simulation study of the spread of dengue fever in a dense community in Brazil showed that house index values from field data were incorrect since the circulation of the virus was found even in situations where house index was below 3% (de Castro et al., 2011). Study in São Paulo, Brazil showed that entomological indicators such as egg, larva-pupa and adult stages were not associated with the incidence of dengue in a mid-size city (Barbosa et al., 2014). Besides, land use factors were also associated with dengue cases, the study showed that the most important land used factors are human settlements (39.2%), followed by water bodies (16.1%), mixed horticulture (8.7%), open land (7.5%) and neglected grassland (6.7%) (Cheong et al., 2014).

2.7.3 Temporal variation for *Aedes*

Temporal variation study for *Ae. aegypti* showed complex and association with temperature and rainfall (Duncombe et al., 2013). Evolution of the environmental and entomological indices was markedly seasonal with higher values in the rainy seasons but the entomological values were not null in the dry season (Favier et al., 2006). Rainfall was climatic determinant of the evolution of the potential breeding sites and temperature played a role on the productivity of positive containers (Favier et al., 2006). Seasonal

transmission was attributed to the effect of climate on mosquito abundance and within host virus dynamics (Lana et al., 2014). Mosquito seasonality was associated preferentially with temperature than with precipitation even in areas where temperature variation was small (Codeço et al., 2015).

CHAPTER 3: EVALUATION OF NEW TOOL FOR *AEDES* SURVEILLANCE

3.1 INTRODUCTION

Various mosquito traps have been created in most of the countries for the purpose of trapping mosquitoes for surveillance and research. Effectiveness of mosquito trap depends on the attractant used. An ovitrap was first described in 1966 to be used for monitoring *Aedes* population (Amador, 1995). In Malaysia, it was firstly used in the study for the abundance and distribution of *Aedes* species in Penang Island (Yap, 1975). It is a sensitive tool and is good for using in the areas of low infestation rates (Braga et al., 2000; Dibo et al., 2008; Morato et al., 2005), however it is not good for predicting dengue transmission, as it has high egg positivity due to the "skip oviposition" habit of *Ae. aegypti* (Reiter, 2007). Hence, difference types of adult traps were invented to collect adult mosquitoes which can be used for the direct assessment of the transmission risk in certain localities. Sticky traps are currently widely being used as the most effective adult trap (Chadee & Ritchie, 2010a; Facchinelli et al., 2007). It was first used for sampling female *Ae. aegypti* in Australia in 2003 (Ritchie et al., 2004). In this study, the gravid mosquito ovipositing in sticky (GOS) trap was evaluated for its efficacy to trap mosquitoes in a dengue endemic locality in Selangor.

3.1.1 Objectives of the study

3.1.1.1 General objectives

To evaluate the efficacy of trap as a tool for vector surveillance in a dengue endemic locality in Petaling district in the state of Selangor.

3.1.1.2 Specific objectives

1) To determine the sensitivity of GOS trap in detecting *Aedes* vector in the study area.

2) To determine the optimum number of trap to be set in high rise apartments for dengue surveillance.

3) To test the effectiveness of the NS1 antigen kit

3.1.2 Research hypotheses

1) H_o: The GOS trap is not efficient in collecting *Aedes* mosquitoes in the field. This hypothesis would like to evaluate the ability of GOS trap to capture *Aedes* mosquitoes in the field and get the optimum number of traps that need to be set.

2) H_o: There is no significant difference between Ovitrap index and GOS trap index. In this hypothesis, the sensitivities of sticky trap and traditional surveillance methodologies were compared.

3) H_o: There was no significant correlation between the densities of *Ae. aegypti* and the egg density per trap.

4) H_o: There was no statistical difference in the index value between the blocks, between the floors and between locations.

3.1.3 Significance of the study

1) Data collection in this study will enable us to determine the efficacy of the GOS trap, which can be used to further study the relationship between vector, dengue cases and climate.

2) This study will also provide valuable information about vector status in the chosen study site, whether there is a difference in vector density between floors and blocks. This study will also assess the suitability of the selected site.

3) From this study, the optimum number of traps necessary for the second phase of the study can be determined. This can also be applied to other similar type of high rise building.

3.2 Materials and Methods

3.2.1 Ethical approval

This study protocol was approved by the National Institutes of Health, Ministry of Health (MOH) Malaysia with reference no. is NMRR-13-1725-15193 (IIR).

3.2.2 Study site

The study site is located at Petaling district in Selangor state which is the most problematic district and state for dengue in Malaysia. Selangor's geographical position is in the center of Peninsular Malaysia (Figure 3.1). It is considered as Malaysia's transportation and industrial hub, is also the most populated state, contributing 19.6% of the population in Malaysia (GEOHIVE, 2016). Selangor consists of nine districts, of which Petaling was chosen due to the highest number of dengue cases (26.7 – 40.25% of

the total cases) and is also the most populated district in Selangor state (comprising 33% of total population in Selangor) (Table 3.1). Mentari Court Apartment was selected as the study site based on high number of cases every year from 2011 until 2013 (28 in 2011, 30 in 2012 and 17 from January to May 2013) (Table 3.2). Cases may be contracted elsewhere due to the mobility of people and spread to the study site. It is located at the prime location of Bandar Sunway with coordinate 3º4.916'N Latitude and 101º36.593'E Longitude, which is a populated town in Petaling Jaya City Council (MBPJ) area. The Mentari Court Apartment with 7.5 hectares land comprises of 7 blocks with 17 floors in each block and a total of 3,272 premises (Figure 3.2). There are car parks, 24 shop lots, two recreation parks and five refuse storage areas. Area per unit is 770 – 773 square leg. The population is about 12,000 people. Almost 40% of the residents are immigrants from Africa, Bangladesh, India, Middle East, Mongolia and Vietnam.

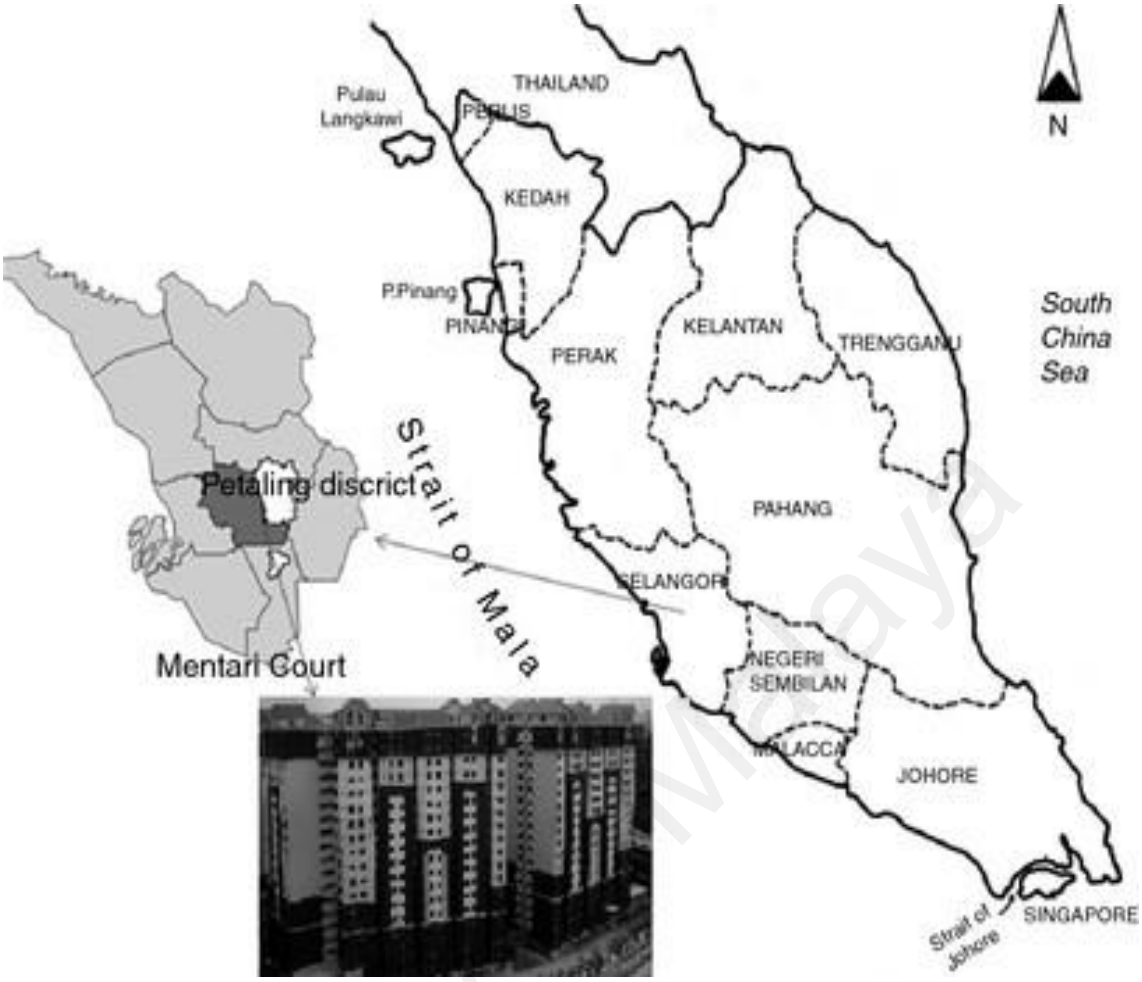


Figure 3.1: Map of Peninsular Malaysia showing the different states. Insert is the map of Selangor, showing all districts. Study site which known as Mentari Courts apartments is situated in Petaling district.

Table 3.1: Total population and dengue cases by districts in Selangor for year 2011 -2013 (Source: population data from Census of population and housing Malaysia 2010, Department of Statistic Malaysia)

District	Population			Total of cases		
	2011	2012	2013	2011	2012	2013
Petaling	1,862,100	1,895,300	1,928,900	2,074	2,554	9,601
Hulu Langat	1,171,700	1,182,700	1,193,800	1,995	2,242	6,371
Gombak	690,600	695,700	700,900	1,468	970	3,325
Klang	879,200	889,100	899,200	1,366	2,291	2,645
Sepang	223,600	233,200	242,900	89	214	663
Hulu Selangor	202,100	203,900	205,800	260	261	480
Kuala Langat	229,800	231,600	233,400	165	189	288
Kuala Selangor	212,500	214,000	215,500	259	272	304
Sabak Bernam	105,900	105,400	104,800	93	120	175
Total	**5,577,500**	**5,650,900**	**5,725,200**	**7,769**	**9,113**	**23,852**

(Source of data: ● Population data - Census of population and housing Malaysia 2010, Department of Statistic Malaysia, ● Dengue case - eDengue system, Ministry of Health)

Table 3.2: Number of dengue cases in the Mentari Court apartment by blocks and floors from 2012 until May 2013 (Source: eDengue, Ministry of Health)

Block	By floor			Total of cases		
	2011	2012	2013	2011	2012	2013
A	4 (1), 15 (1), 2 (1)	11 (1), 15 (1), 17 (1)		3	3	0
B	10 (1), 17 (1), 16 (1), 2 (2), 12 (1)	1 (1), 3 (1), 6 (1), 8 (1)	2 (1), 3 (1), 4 (1), 5 (1)	6	4	4
C	0	3 (2), 4 (1), 5 (1), 13 (1), 15 (1)	3 (3), 7 (1), 13 (1), 15 (1)	0	6	6
D	8 (1), 11 (2), 4 (1), 8 (1)	1 (1), 2 (1), 15 (1), 17 (1)	8 (1), 9 (1), 12 (1), 16 (1), 17 (1)	5	4	5
E	1 (2), 6 (1), 3 (1)	1 (1), 4 (1), 6 (1), 8 (1), 10 (1), 14 (1)	8 (1)	4	6	1
F	1 (1), 7 (1), 11 (1)	G (1), 5 (1)	0	3	2	0
G	2 (2), 3 (1), 6 (1), 9 (1), 11 (1), 17 (1)	6 (1), 2 (1), 10 (1), 17 (2)	15 (1)	7	5	1
		Total		**28**	**30**	**17**

Note: Figure - floor number, in bracket ( ) is number of dengue cases.

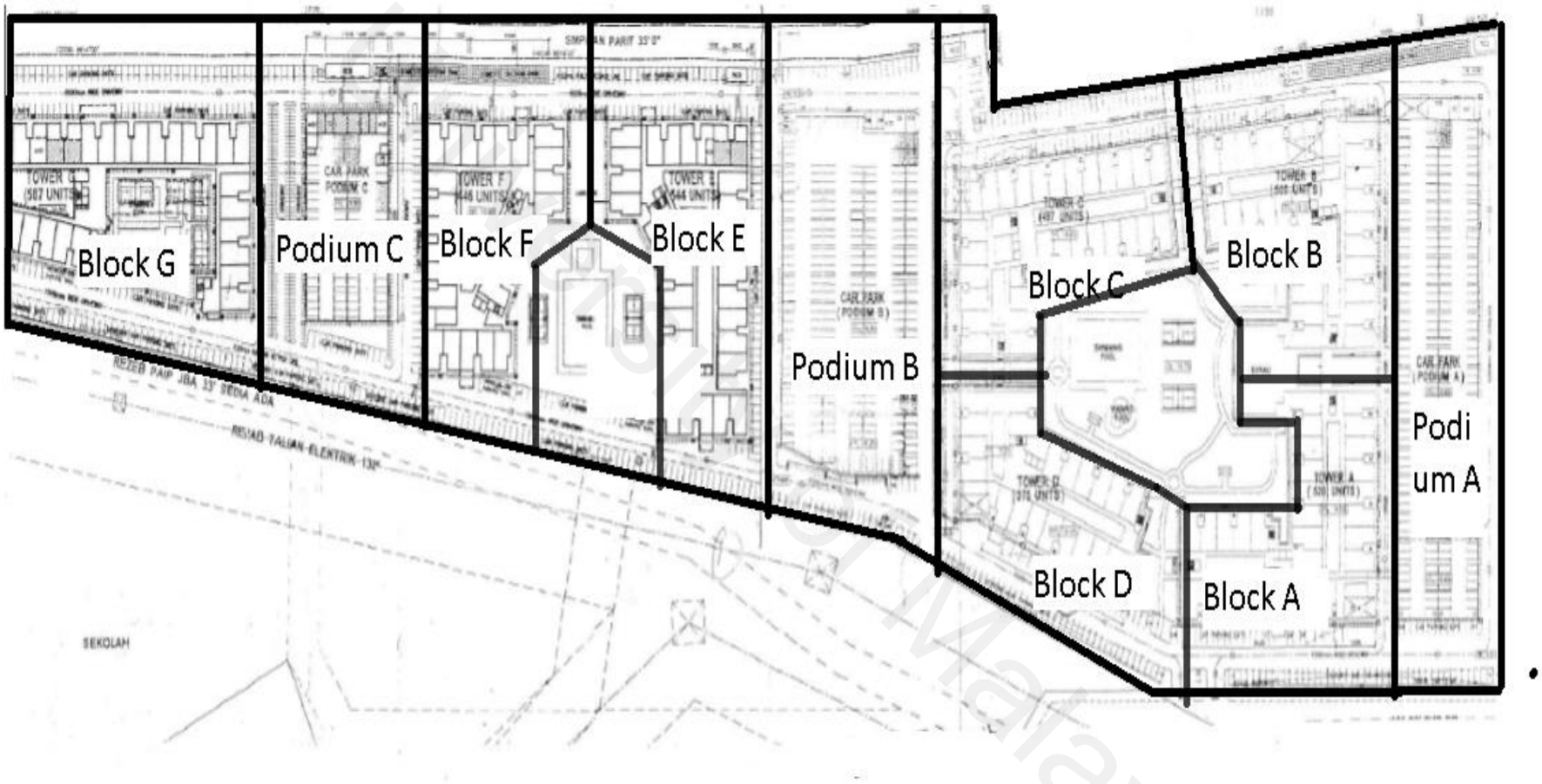


Figure 3.2: Layout plan for Mentari Court apartment which consists of 7 blocks and 3 podium car park

3.2.3 Baseline Survey

Larval surveys were carried out randomly on 23 May and 30 May 2013 to obtain baseline data as to provide the base information about the chosen study site such as relative populations of *Ae. aegypti* before the subsequent study activities were carried out. One to two team involved each time and each team covered an average of 25 premises. At the same times, about 250 conventional ovitraps were set on all 17 floors in Block E on 10 – 14 April 2013, whereas, a total of 608 sticky traps were set on all 17 floors plus outside the block with 4 traps per floor and in all 7 blocks on 7 – 14/5/2013.

3.2.4 GOS trap

GOS trap which stands for gravid mosquito oviposition in sticky trap, is used to attract the gravid *Aedes* mosquitoes to lay eggs in the traps. About 10% seven-day old hay infusion water was used in the GOS traps so that these traps will be more attractive to the mosquitoes compared to other containers. The GOS trap consisted of two plastic containers which were sprayed black as shown in Figure 3.3. The bigger container was 11.5 cm in diameter and 10cm in height while the smaller container was 11.5 cm in diameter and 7 cm in height. The smaller container had netting at the bottom. The sides of the containers were lined with brown disposable paper sprayed with sticky insert Cather®. This Cather consists of synthetic solid rubber (53%), solvent (46.6%) and yellow dye (0.4%) and is produced by SR Megah Chemicals (Taman Klang Perdana, Klang, Malaysia). The larger container was filled with 10% hay infusion water. The smaller container containing the sticky surface was placed inside the larger container is to trap the ovipositing mosquitoes on the sticky surface. The netting at the base of the container is to prevent emerging adults from escaping if a mosquito sits on the netting to lay eggs.

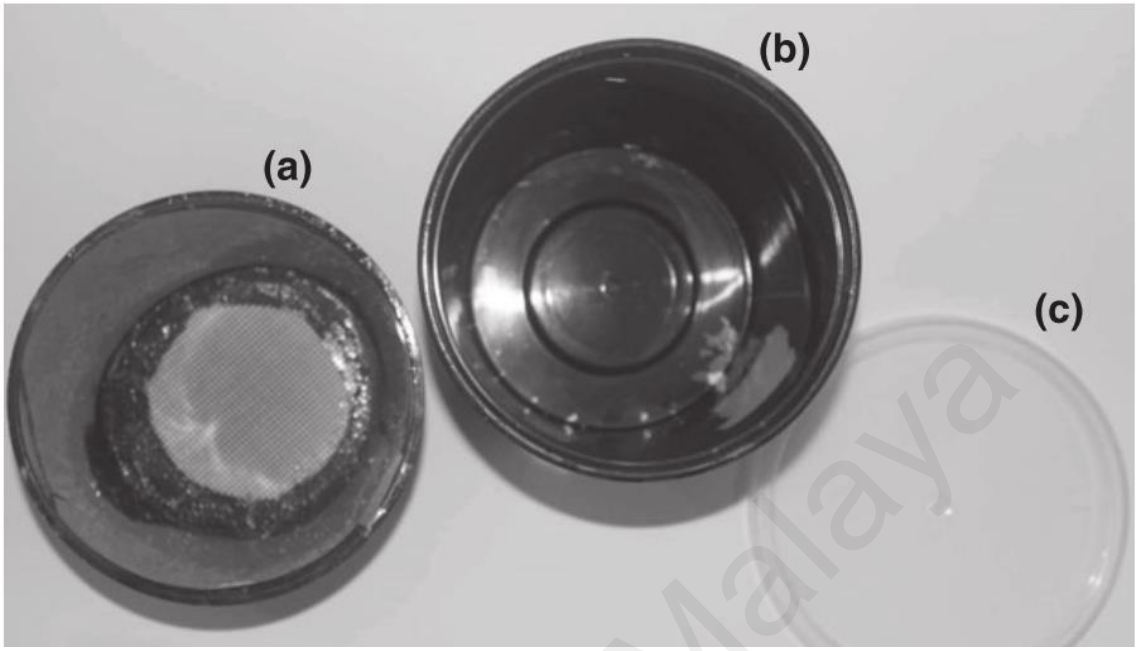


Figure 3.3: Picture of the sticky trap.

(a) The small container which has the sticky surface and netting at the bottom. (b) The large container containing the hay infusion water and the small container will be placed inside this larger container (c) Cover which is used when the containers are transported to the field and laboratory.

3.2.5 Field sampling

3.2.5.1 Phase 1: Trial 1

Phase 1 was conducted to test the efficacy of the GOS trap to collect *Aedes* mosquitoes and suitability of the site for subsequent studies. The initial study was conducted from 6 June 2013 until 30 September 2013. Block C and D were chosen for first trial study based on the previous 3 years case, since most cases occurred from these 2 blocks (Table 3.2). A total of 62 sticky traps were set in block C and D, on floors: ground floor (GF), 3rd, 6th, 9th, 12th and 15th. In Block C, the number of sticky traps set on the respective floors starting from the ground floor (GF) was 1, 2, 4, 6, 8 and 10, respectively, while in Block D, it was the reverse. Thus, in block D, the ground floor had the most traps. Different number of traps were set for each floor is to determine the

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entomological indices when different trap densities are used. Figure 3.4 shows how the traps were set in Block C and D. All traps were labelled accordingly and all sticky traps were examined twice a week. If no insects were stuck on the surface, the GOS trap paper was changed once a month or as needed when it became dirty. The hay infusion water was replaced weekly.

The GOS traps were set inside the house or outside, under the roof to prevent direct sunlight and rain. The sticky trap index was calculated as the percentage of traps positive for *Aedes*. The *Aedes* density was calculated as the total number of *Aedes* divided by the number of inspected trap.

From July to September 2013, two ovitraps per floor per block were set on each floor with GOS trap to monitor the presence of *Aedes*. All ovitraps contained hay infusion water and were serviced twice a week. The ovitrap index was the percentage of ovitrap positive, while the egg density was calculated as the total number of eggs divided by the number of inspected traps.

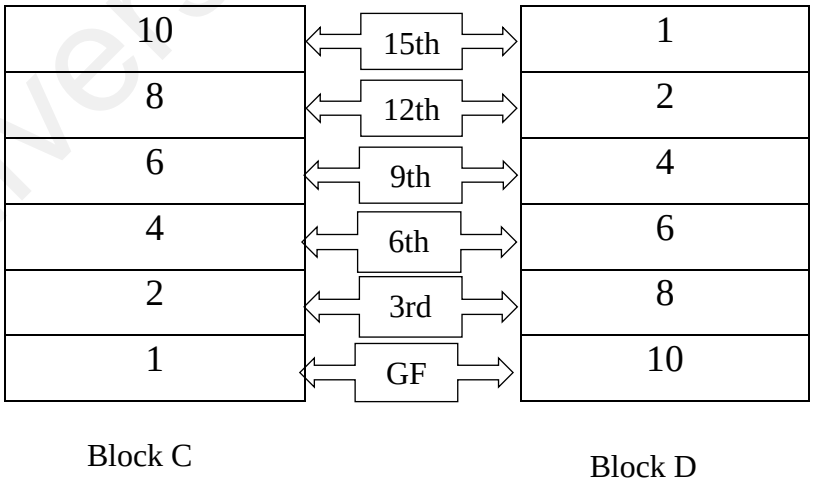

Figure 3.4: Number of GOS trap set per floor for Block C and D

3.2.5.2 Phase 1: Trial 2

The second trial was conducted to determine the optimum number of traps that would be needed for surveillance. The trial was carried out for 5 weeks from 1 October 2013 to 6 November 2013. The traps were set in all seven blocks during the trial. The GOS traps were set on several floors such as GF, 3rd, 6th, 9th, 15th and 17th starting with three traps for the first week, five traps on the second week, seven traps on the third week and nine traps on 4th and 5th week, respectively. Thus, the total number of sticky traps ranged from 147 to 441. At the same time, one ovitrap was placed on each of the floors mentioned above. All traps were examined and serviced weekly.

3.2.6 Identification and processing of mosquitoes

All sticky papers with insects were examined under stereomicroscope in the laboratory. Mosquitoes were identified up to species level. Only the *Ae. aegypti* and *Ae. albopictus* were processed for the detection of virus. The abdomens of the mosquitoes were pooled into five in a pool, while the head and thorax of each mosquito was kept individually in Eppendorf tubes at -20°C until real-time RT-PCR processing.

3.2.7 Detection of dengue viral antigen in abdomen of mosquitoes

To each pool of mosquito abdomens, 50 µl of Phosphate Buffer Solution (PBS) was added and homogenized lightly using a pestle and hand-held homogenizer (Kontes Thompson Scientific). The tube was centrifuged for 3 min at 1006 g. The SD Bioline®NS1 Ag kit (Standards Diagnostic Korea) was used for testing the dengue antigen in mosquito following the manufacturer's protocol. Briefly, the content from each tube

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was pipetted using the pipet provided onto the well of the device (test kit). After 10-15 min, the reading was taken. If the sample was positive, two bands will be seen. If negative, only the control band was seen. For all the pooled abdomens that were positive, the individual head and thorax of the respective mosquito was tested for dengue virus by the real-time RT-PCR.

3.2.8 Positive mosquito serotyping using Real time RT-PCR

3.2.8.1 RNA extraction

Individual mosquitoes were ground in pre-chilled Eppendorf tubes with 0.25 ml of a growth medium (Eagle's minimum essential medium, EMEM). The mosquito suspensions were then centrifuged at 21000 g for 15 min at 4^{o}C. RNA extraction was carried out with High Pure Viral RNA Isolation Kit (Roche Applied Science) according to the manufacturer's protocol. The homogenate (200 µl) was mixed with 400 µl of binding buffer and centrifuged at 8000 g for 15 s. The RNA was then washed twice with washing buffer and centrifuged at 8000 g for 1 min. A total of 30 µl of viral RNA were eluted from the sample using elution buffer. The extracted RNA was collected and stored at -80^{o}C for viral detection through real-time RT-PCR.

3.2.8.2 One-step TaqMan real-time RT-PCR

The one-step TaqMan real-time RT-PCR was carried out in a CFX96 Thermocycler (Bio-Rad) (Kong et al., 2006). Briefly, 5 µl of the sample RNA, 0.5 µM of each primer, four TaqMan probes (0.25 µM) and 5.0 mM of $MgCl_2$ were used in a 25 µl reaction volume containing the one-step RT-PCR premix (BioNeer). The thermal cycling

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profile of this assay consisted of an initial RT step at 50°C for 30 min, and Taq polymerase activation at 95°C for 15 min, followed by 40 cycles of PCR with the following conditions: denaturation at 95°C for 30 s, annealing/extension at 60°C for 1 minute.

3.2.9 Statistical analysis

All statistical analyses were performed using R (R Development Core Team, 2008) programming language for statistical analysis (version 3.1) and Excel 2010. Data were subjected to analysis of variance (ANOVA), t-test, nonparametric tests (Pearson's χ^2 test), nonlinear regression (Box-Lucas) and general linearized modelling. The minimum infection rate (MIR) was calculated by maximum likelihood estimation method (Chiang & Reeves, 1962) based on 45 pools of 5 mosquitoes.

3.3 Results

3.3.1 Baseline Survey

Result of baseline survey showed that only 25 of 46 premises (54.3%) were inspected during the first visit on 23 May 2013 and 40 premises on the 30 May 2013. During the first visit, one bucket at the balcony of the case house, was found positive breeding of *Ae*. *aegypti* with *Aedes* index (AI) 4%, Breteau index (BI) 4 and container index (CI) 2%. During the second visit, no positive breeding container was found, however there were many potential breeding places all around such as gully traps, sand traps, bucket, toilet flush cistern, astro dish, water tank, bucket and perimeter drain.

Result of the ovitrap showed that there was high ovitrap index for the block E, about 44.0% with highest ovitrap positive rate was at 8th floor (75%) and followed by ground floor (60%) and 9th floor (60%). However, sticky ovitrap results showed that adult

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Aedes mosquitoes were present in all floors and blocks except 8th and 13rd floor, the highest number of mosquitoes were caught from ground floor (GF) and 4th floors (7 mosquitoes for each). Details of result shown in Appendix A and B. This result provides a guide to set GOS trap at any floors and blocks for the subsequent trial study.

3.3.2 Phase 1: Trial 1

3.3.2.1 Efficacy of trap to capture *Aedes* mosquitoes

(a) Collection by mosquito species

A total of 223 female and 19 male *Ae. aegypti*, 7 females and 1 male *Ae. albopictus*, 190 females and 7 male *Cx. quinquefasciatus* and 3 female *Cx gelidus* were obtained from the two blocks during 18 weeks of the first trial as shown in Table 3.3. Other arthropod and reptile species were also trapped such as Phoridae (*Megaselia* sp.) (6,827), Psychodidae (1,604), Ceratophoganidae (805), ants (278), *Musca domestica* (215), Chironomid sp. (173), lizards (88), bees (86), cockroach (44), spiders (64) and other insects (19) during the investigation. Besides, a total of 55 traps (2.3% of the total traps) were spoilt or lost during the study, either being thrown or lost the sticky paper and rubbish were dumped inside the trap.

Table 3.3: Mosquito-species-collected in GOS trap in Mentari Court for trial 1 from 6 June to 30 September 2013

	Aedes aegypti		***Aedes albopictus***		***Culex quinquefasciatus.***		***Culex gelidus***	
	Females	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**
Total	223	19	7	1	190	7	3	0
Mean	12.39	1.05	7.00	1.00	10.56	7.00	1.50	0.00
Range	2 - 29	0 - 4	0 - 2	0 - 1	1 - 25	0 - 2	0 - 1	0
Standard error	1.98	0.34	0.14	0.06	1.81	0.14	0.17	0.00
Upper limit (95% CI)	16.27	1.72	0.67	0.16	14.10	7.28	1.83	0
Lower limit (95% CI)	4.52	0.77	0.33	0.13	4.13	0.33	0.38	0.00

Note:
Mean - total number of mosquitoes caught per week.
Total number of trap, n=1116
Total week trapping – 18 weeks

(b) Temporal Distribution of Aedes mosquitoes in relation to dengue cases

The phase 1 study showed that *Ae. aegypti* was the predominant mosquito (54%) obtained in the study block, followed by *Cx. quinquefasciatus* (43.7%). *Aedes albopictus* only comprised of 1.78% of the collection. The number of *Ae. aegypti* collected per week ranged from 2 - 29 and *Ae. albopictus* from 1 – 2 (Table 3.3).

Figure 3.5 shows the distribution of the *Aedes* mosquitoes, dengue cases and the positive mosquitoes from 2 blocks and 6 floors throughout the 18 weeks study period. The first positive mosquito pool was detected in the first week's collection from 6 to 10 June 2013 before the first case was reported on 8 June 2013. The date of onset of the case was on 6 June 2013. The second case was reported on the 3rd week and a positive mosquito was also obtained.

Distribution of cases recorded among the 17 floors throughout 18 weeks during the study period is shown in Table 3.4, analysis demonstrated that the cases occurred independently of block and floor (Pearson's $\chi 2=112.22$, df=102, *P*-value > 0.05).

The results of Pearson correlation analyses were found not statistically significant between number of cases and number of *Ae. aegypti* and *Ae. albopictus*, r(16)=+0.295, *P* >0.05, two tailed and *Ae. albopictus* (r(16)=-0.146, *P* >0.05, two tailed respectively. Further correlation analysis on lag time (2, 3 and 4 weeks) of occurrence of cases and number of *Aedes* caught did not show significant relationship between the two variables. However, the relationship between the number of cases and *Aedes* caught yielded significant relationship using general linearized model (GLM). The relationship can be described with the equation $y = 1.1517 + 0.0404x$ ($F_{1,20} = 3.95$, $P < 0.001$) as shown Figure 3.6.

Table 3.4: Distribution of cases of dengue by block and floor in Mentari Court from June to November 2013

Block	Cases	Floor	Cases	Floor	Cases
A	23	GF	4	9	4
B	39	1	8	10	7
C	20	2	9	11	5
D	16	3	7	12	9
E	18	4	7	13	4
F	16	5	7	14	12
G	14	6	14	15	9
		7	9	16	7
		8	8	17	16
Total	146		73		73

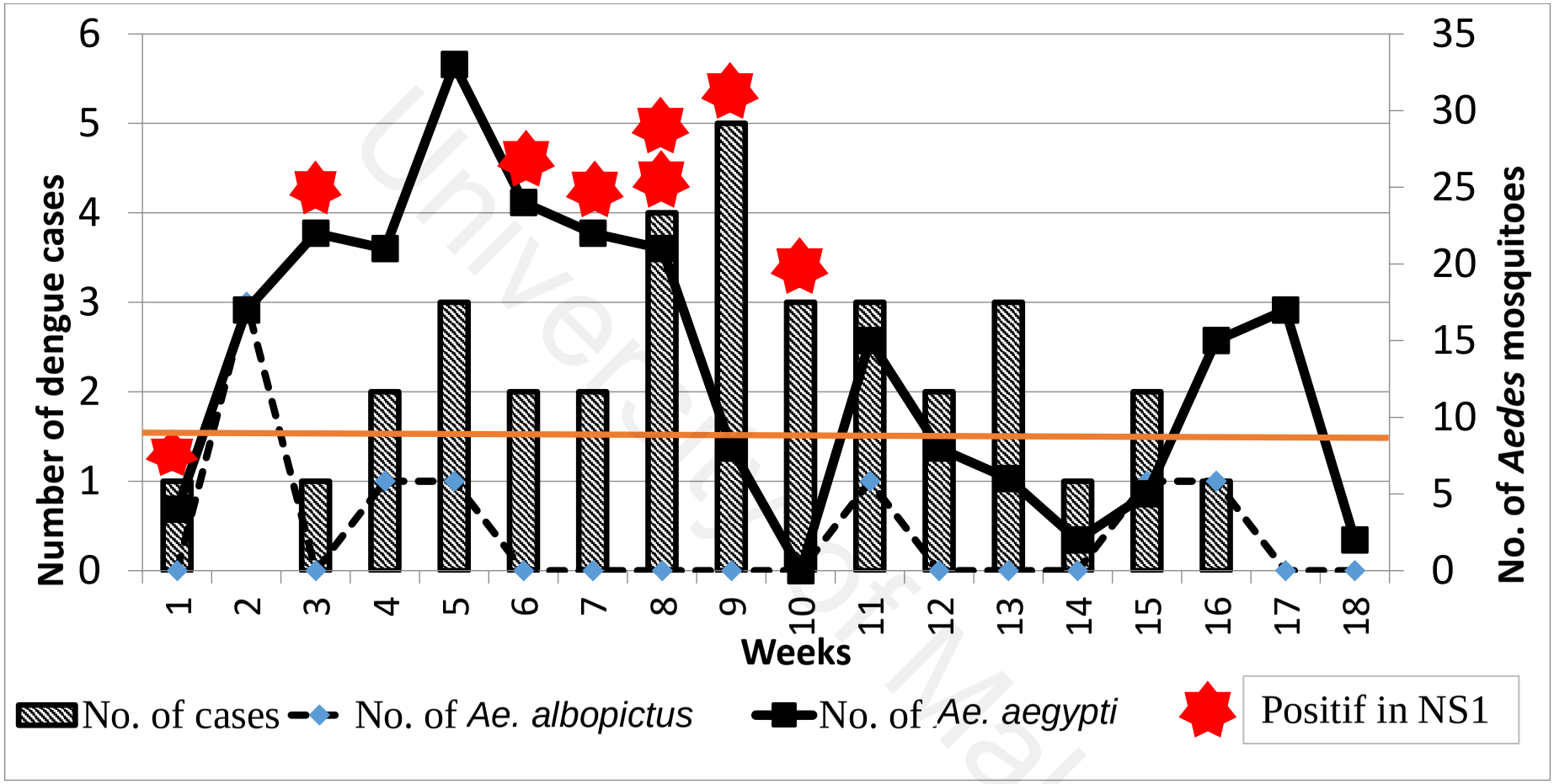

Figure 3.5: Total of *Ae. aegypti*, *Ae. albopictus*, total number of cases and pooled positive mosquitoes by NS1 test. Data for the above are combined data for blocks C and D. Denotes pools of positive *Ae. aegypti*. Week 8 had two pools of mosquitoes positive. Horizontal graph line denotes median number of *Ae. aegypti*

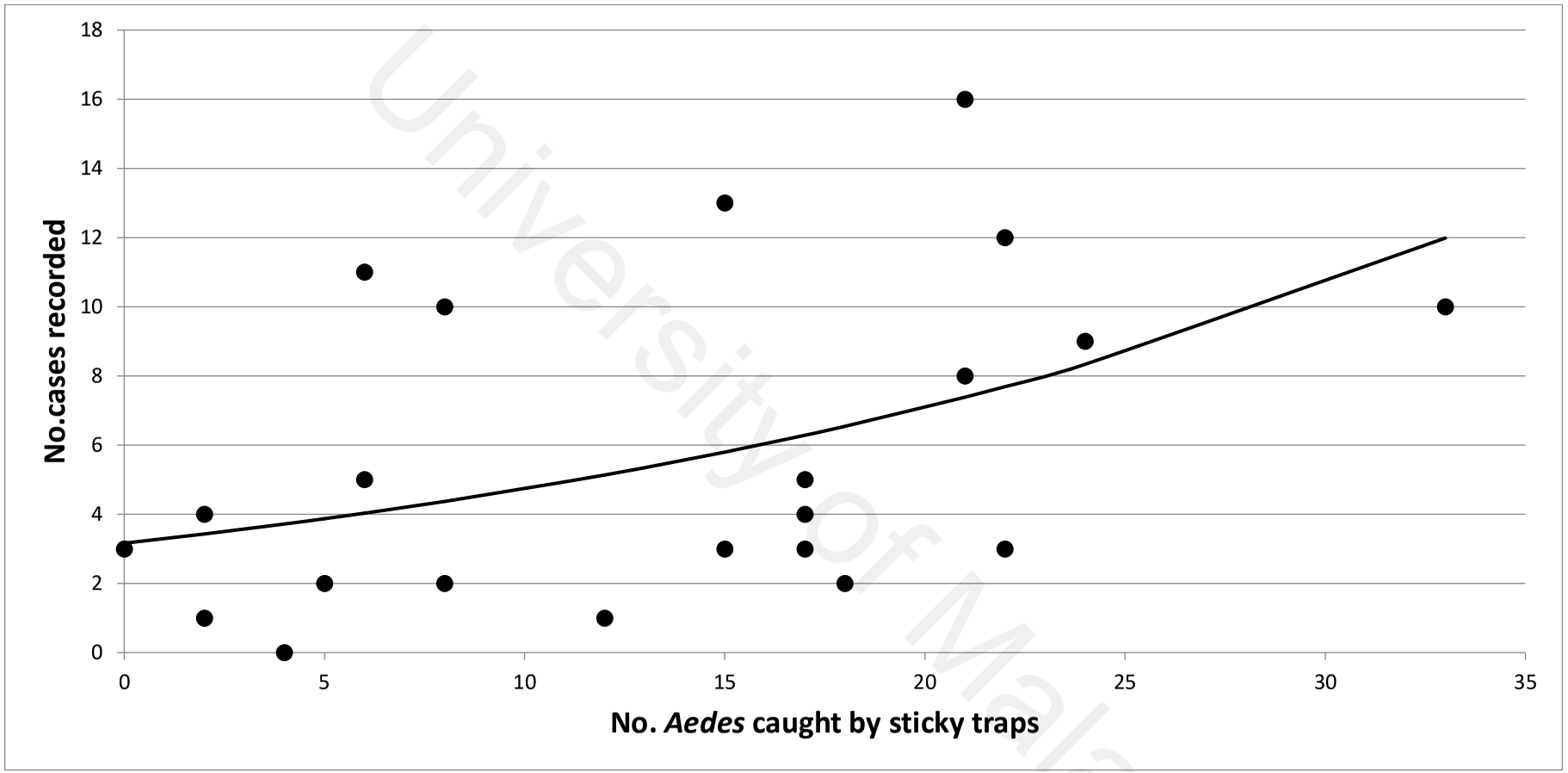

Figure 3.6. General linearized model for cases against *Ae. aegypti* caught with equation described as y=1.1517+0.0404x, *P*<0.001

3.3.2.2 Comparison between GOS trap and traditional ovitrap

(a) Percentage positive of traps

Figure 3.7 shows the GOS trap index and ovitrap index. The percentage of GOS trap positive was lower than ovitrap. Percentage of GOS trap positive ranged from 0.00 – 30.65, while ovitrap ranged from 33.33 to 93.10. The ovitrap index seemed to follow the same trend as the GOS trap index. However, the ovitrap index was higher than GOS trap which was expected because a single mosquito can lay eggs in many ovitraps (Reiter, 2007). The results of Pearson correlation test indicated that there was no statistically significant relationship between the percentage of GOS positive and ovitrap positive, r(11)=+0.544, P >.05, two tailed.

(b) Density of Ae. aegypti and eggs per trap

Density of *Ae. aegypti* and density of eggs per trap is shown in Figure 3.8. Both show the same trend and there was no statistically significant relationship between the densities of *Ae. aegypti* and eggs per trap, r(11)=+0.491, P >.05, two tailed. ANOVA indicated that there was no difference in egg density per trap between blocks ($F_{6,216}$ = 1.70, P > .05) nor between weeks ($F_{4,216}$ = 1.66, P > .05). Similarly, there was no difference between blocks ($F_{7,39}$ = 1.52, P > .05) but a significant difference existed between weeks ($F_{4,39}$ = 5.82, P < 0.001) in case of positive GOS traps. As for the number of eggs, there was significant difference between floors ($F_{5,336}$ = 6.66, P < 0.001), between the locations of the traps ($F_{23,336}$ = 4.90, P < 0.001) and weeks ($F_{12,336}$ = 3.86, P < 0.001).

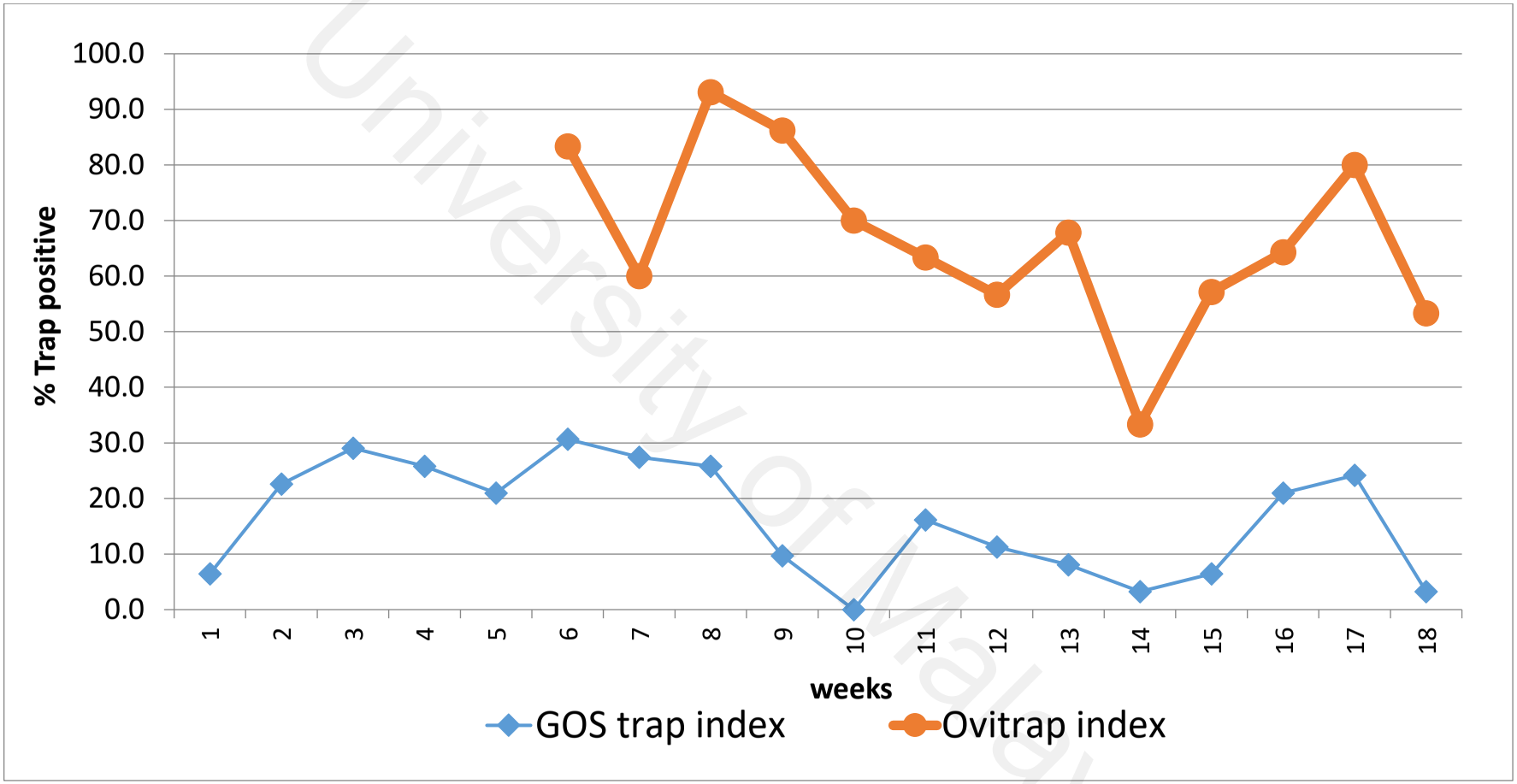

Figure 3.7: GOS trap index and ovitrap index (percentage positive) for the 18 weeks.

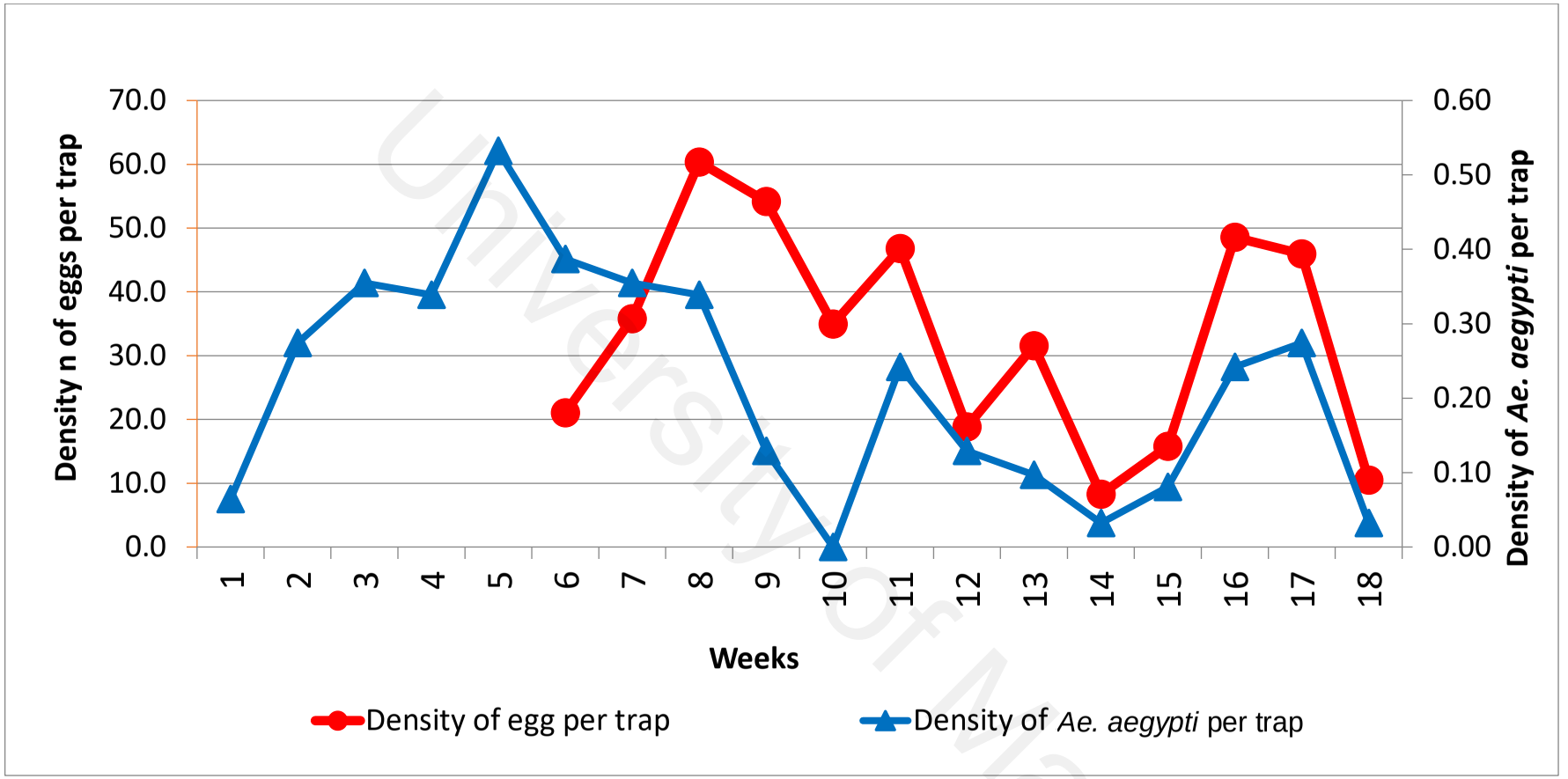

Figure 3.8: Density of *Ae. aegypti* and density of eggs per trap for 18 weeks.

Figure 3.9 shows the correlation between density of *Aedes* (*Aedes* per trap) and sticky trap (trap positivity) with r^2=0.73, df=33, *P*<0.001. Figure 3.10 shows the trend of the number of eggs which was the same as ovitrap index. The number of eggs collected per week ranged from 248 to 1750 eggs and the number of eggs per trap ranged from 10 – 60 eggs per traps. However, the density of *Ae. aegypti* per trap was ranged from 0.03 to 0.53. In this trial study, an average 38 eggs were collected per *Aedes* mosquito.

3.3.2.3 Vector status information for the study site

(a) Percentage of positive traps between blocks

The result shows that Block D trapped 52% more mosquitoes compared to Block C. Blocks D caught about 155 *Ae. aegypti*, 7 *Ae. albopictus* and 141 *Culex quinquefasciatus* while Block C, it was 86 *Ae. aegypti*, 1 *Ae. albopictus* and 57 *Culex quinquefasciatus*. The ANOVA analyses result as in Table 3.5 indicated that there was no statistical differences in the GOS index values between the blocks (*P* > 0.05), while Table 3.6 also shows no statistical differences for the ovitrap index (*P* > 0.05) as well.

Table 3.5: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage GOS trap positive between block C and D

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between blocks	1	0.0001	0.000117	(-0.08352385, 0.07631274)	0.008	0.927
Residuals	34	0.4732	0.013918			
Total	35	0.4733				

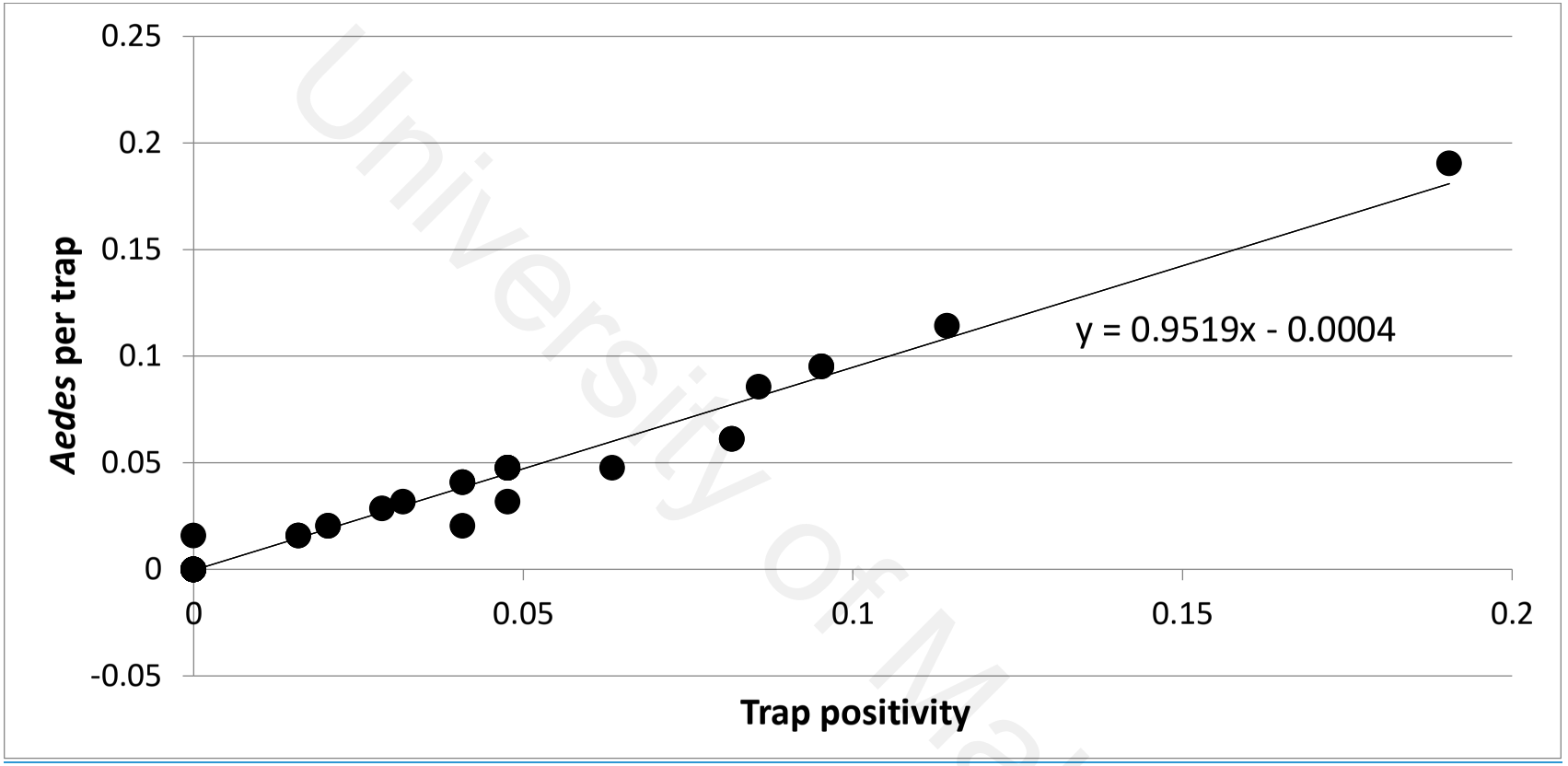

Figure 3.1. Correlation between density of *Aedes* (*Aedes* per trap) and sticky trap (trap positivity), r2=0.73, df=33, *P*<0.001.

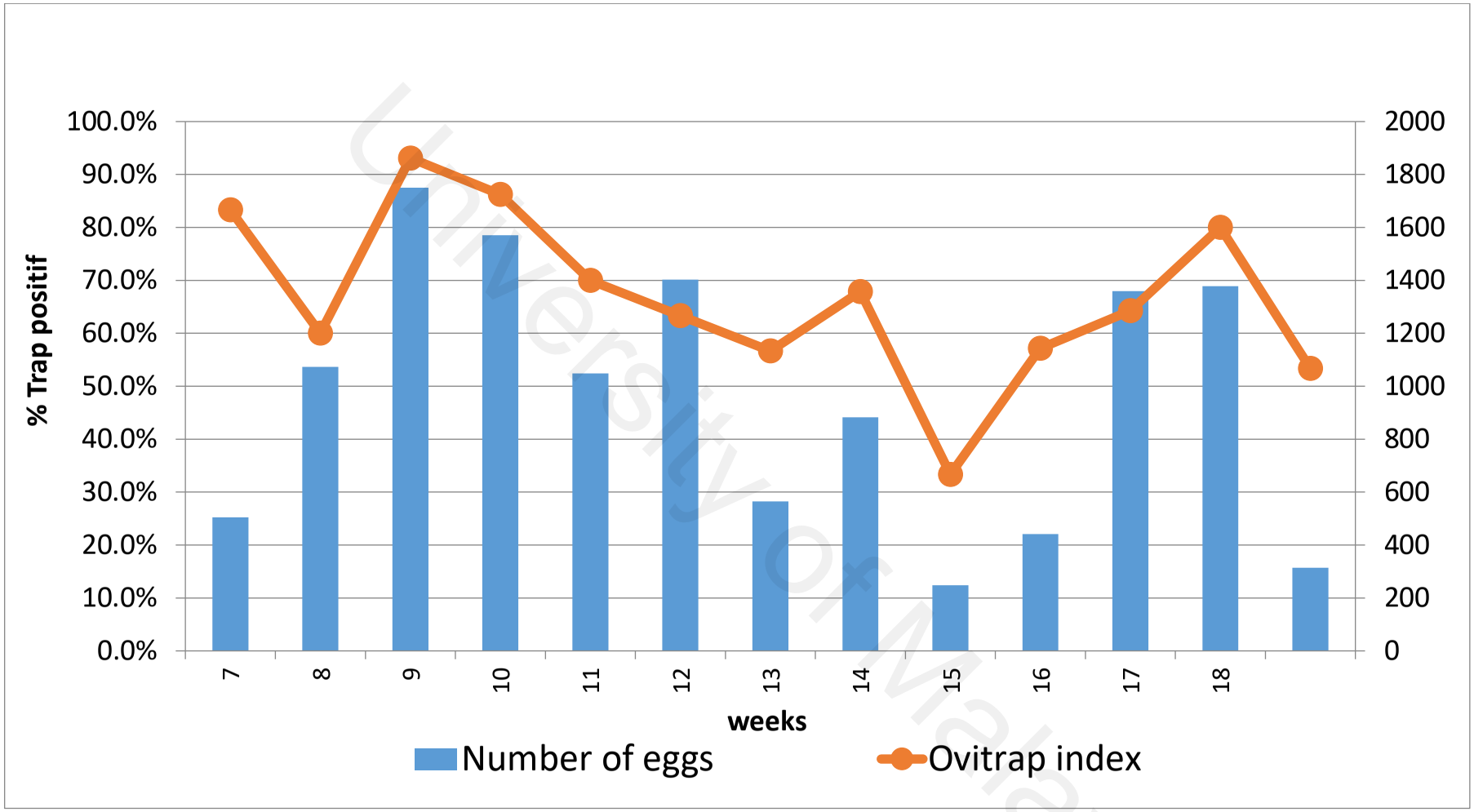

Figure 3.10: Number of *Aedes* eggs and ovitrap index per week (total of ovitrap=376) for 12 weeks

Table 3.6: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage ovitrap positive between block C and D

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between blocks	1	0.0019	0.001862	(-0.126137, 0.1599832)	0.06	0.809
Residuals	24	0.7495	0.03123			
Total	25	0.7514				

(b) Percentage positive of traps between locations

Results of the ANOVA analysis for the comparison of the GOS index between GOS trap location is shown in Table 3.7, demonstrated statistical differences (P<0.05). Three traps tagged as D-GF-2 (Block D, Ground Floor), D-GF-3 (Block D, Ground Floor) and D-6-1 (Block D-6th Floor) were significantly different from other traps. It was noted that these 3 traps had the highest GOS index with 48.48%, 39.39% and 33.33% respectively compared to the other traps. Trap no. D-GF-2 trapped the highest number of mosquitoes with 23 *Ae. aegypti*, 2 *Ae. albopictus* and 27 *Cx. quinquefasciatus*. The highest number of *Ae. aegypti* per trap was 4 mosquitoes by trap. No. C-GF-1 (Block C, Ground Floor) in week 7 (June) and week 12 (July), 2013. It was noticed that attraction for the mosquitoes was not influenced by nearby potted plants as higher percentage trap positive with mosquitoes were the traps set under the staircase (18.2%) and next to water pipe (10.79%) as compared to potted plant (8.48%). The ANOVA analyses in Table 3. shows no statistical differences for the ovitrap index (P> 0.05).

Table 3.7: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage GOS trap positive between GOS trap

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between GOS trap	61	9.58	0.15697	(-0.639584, 0.695139)	3.789	<2e-16 ***
Residuals	1054	43.67	0.04143			

Significant codes: '***' for p< 0.001

Table 3.8: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage ovitrap positive between ovitrap location

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between Ovitrap	29	0.526	0.01814	(-0.3586629, 0.3586629)	0.448	0.995
Residuals	390	15.786	0.04048			

(c) Percentage of positive traps between floors

The ANOVA analyses in Table 3.9 indicated statistical differences in the GOS index values between floors (*P <0.05*). The highest percentage of *Aedes* mosquitoes (41.9%) was obtained from ground floor which was also similar for mosquito eggs (46.2%). Although there was significantly higher number of eggs was recorded on the ground floor ($P < 0.001$), however ANOVA analyses show in Table 3.10 that there was no statistical differences between floors for ovitrap index.

Table 3.9: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage GOS positive between floors

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between floor	5	0.8406	0.16811		12.99	6.82e-11 ***
Residuals	192	2.4849	0.01294			
15th-12th				(-0.08244141, 0.07880505)		0.9999998
6th-12th				(-0.07759293, 0.08365354)		0.9999979
9th-12th				(-0.11698687, 0.04425960)		0.7856585
GF-12th				(0.08180101, 0.24304748)		**0.0000004**
3rd-15th				(-0.08789596, 0.07335051)		0.9998378
6th-15th				(-0.07577475, 0.08547172)		0.9999782
9th-15th				(-0.11516869, 0.04607778)		0.8199088
GF-15th				(0.08361919, 0.24486566)		**0.0000003**
6th-3rd				(-0.06850202, 0.09274444)		0.9980501
9th-3rd				(-0.10789596, 0.05335051)		0.9257380
GF-3rd				(0.09089192, 0.25213838)		**0.0000001**
9th-6th				(-0.12001717, 0.04122929)		0.7230323
GF-6th				(0.07877071, 0.24001717)		**0.0000007**
GF-9th				(0.11816465, 0.27941111)		**0.0000000**

Significant codes: '***' for *P*< 0.001

Table 3.10: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage ovitrap positive between floors

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between floor	5	0.126	0.02524		0.646	0.665
Residuals	414	16.186	0.03910			
15-12				-0.10698580 0.10698580		1.0000000
3-12				-0.08912865 0.12484294		0.9968994
6-12				-0.07127151 0.14270008		0.9314111
9-12				-0.08912865 0.12484294		0.9968994
GF-12				-0.10379645 0.07522502		0.9974947
3-15				-0.08912865 0.12484294		0.9968994
6-15				-0.07127151 0.14270008		0.9314111
9-15				-0.08912865 0.12484294		0.9968994
GF-15				-0.10379645 0.07522502		0.9974947
6-3				-0.08912865 0.12484294		0.9968994
9-3				-0.10698580 0.10698580		1.0000000
GF-3				-0.12165360 0.05736788		0.9083412
9-6				-0.12484294 0.08912865		0.9968994
GF-6				-0.13951074 0.03951074		0.5994723
GF-9				-0.12165360 0.05736788		0.9083412

Significant codes: '***' for *P*< 0.001

The distribution of *Ae aegypti* among the various floors is shown in Figure 3.11. In Block C, the highest percentage of *Ae. aegypti* was obtained on the 15th floor, while in Block D, it was on the ground floor. These were the floors that had the highest number of traps.

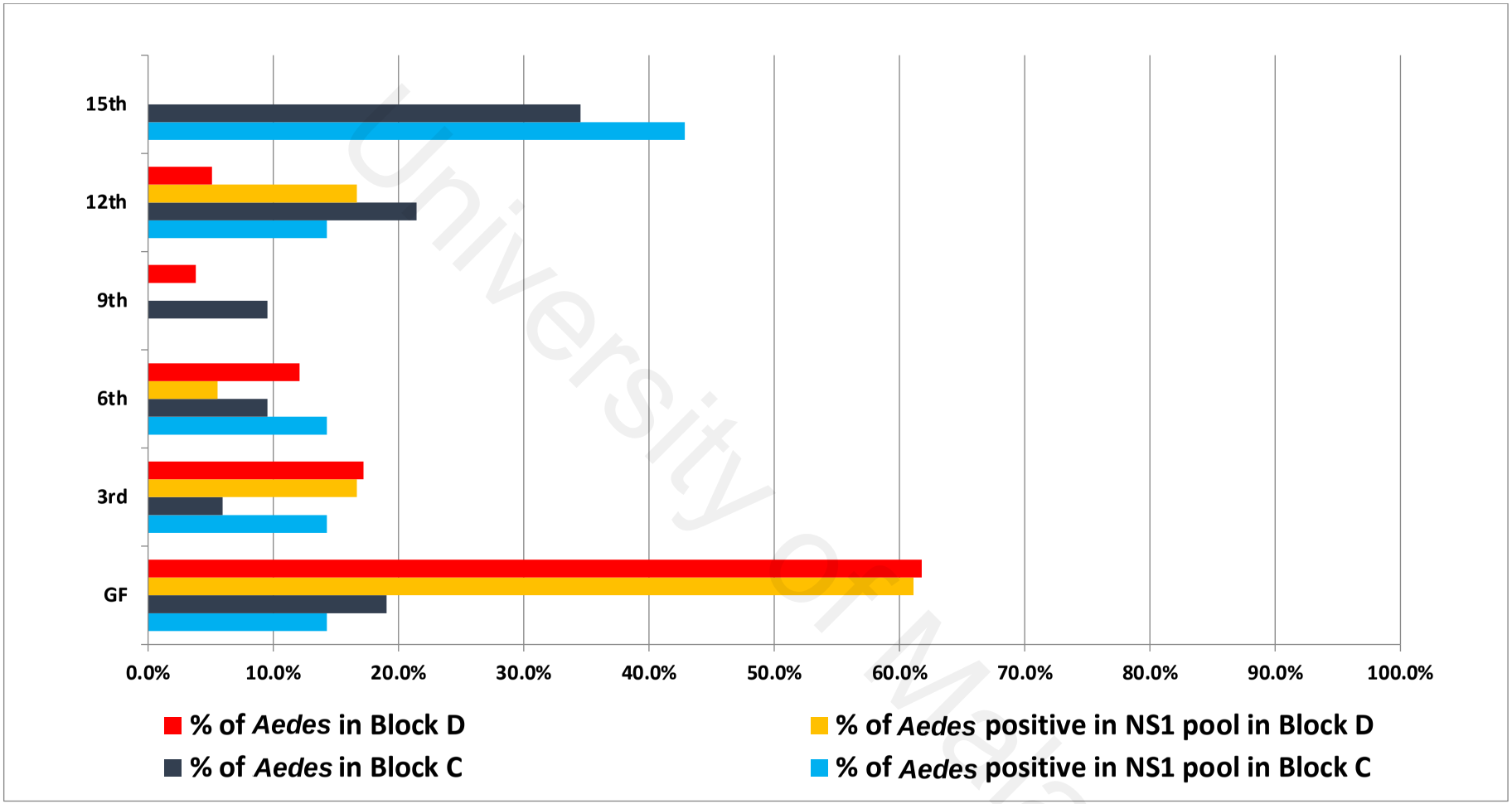

Figure 3.11: Percentage of *Ae. aegypti* caught as well as the percent of positive *Ae. aegypti* in NS1 pool test on each floor based on the *Ae. aegypti* captured in each block

3.3.3 Phase 1: Trial 2

The second trial was carried out for 5 weeks from 1 October 2013 until 6 November 2013. In this study, the total number of traps were increased by week, starting with three traps per floor for the first week (total 147), five traps on the second week (total 245), seven traps in the third week (total 343) and nine traps on 4th and 5th week (total 441). The following analysis was conducted to determine the optimum number of traps to be set in high risk apartments for dengue surveillance.

3.3.3.1 Percentage of GOS positive and *Ae. aegypti* density

In trial 2, total of 50 female and 11 male *Ae. aegypti*, 20 female *Cx. quinquefasciatus* and 3 *Cx. gelidus* were obtained from 7 blocks. Figure 3.12 shows the percentage of GOS trap positive and the density of *Ae. aegypti* per trap for 5 weeks. It showed that percentage of positive traps and density of *Ae. aegypti* per trap were reduced although the number of traps set were increased per week. The highest percentage of GOS positive was recorded in first week (8.84%) and the density of *Ae. aegypti* per trap was 0.12.

3.3.3.2 Percentage of ovitrap positive and egg density

Figure 3.13 shows the percentage of ovitrap positive and the density of eggs per trap for 5 weeks. It also shows the similar trend as GOS trap index where the percentage of ovitrap positive and density of eggs reduced by week. The highest percentage of ovitrap positive was recorded in week 1 (61.22%) and the number of eggs was 1,408 with 28.73 eggs per trap.

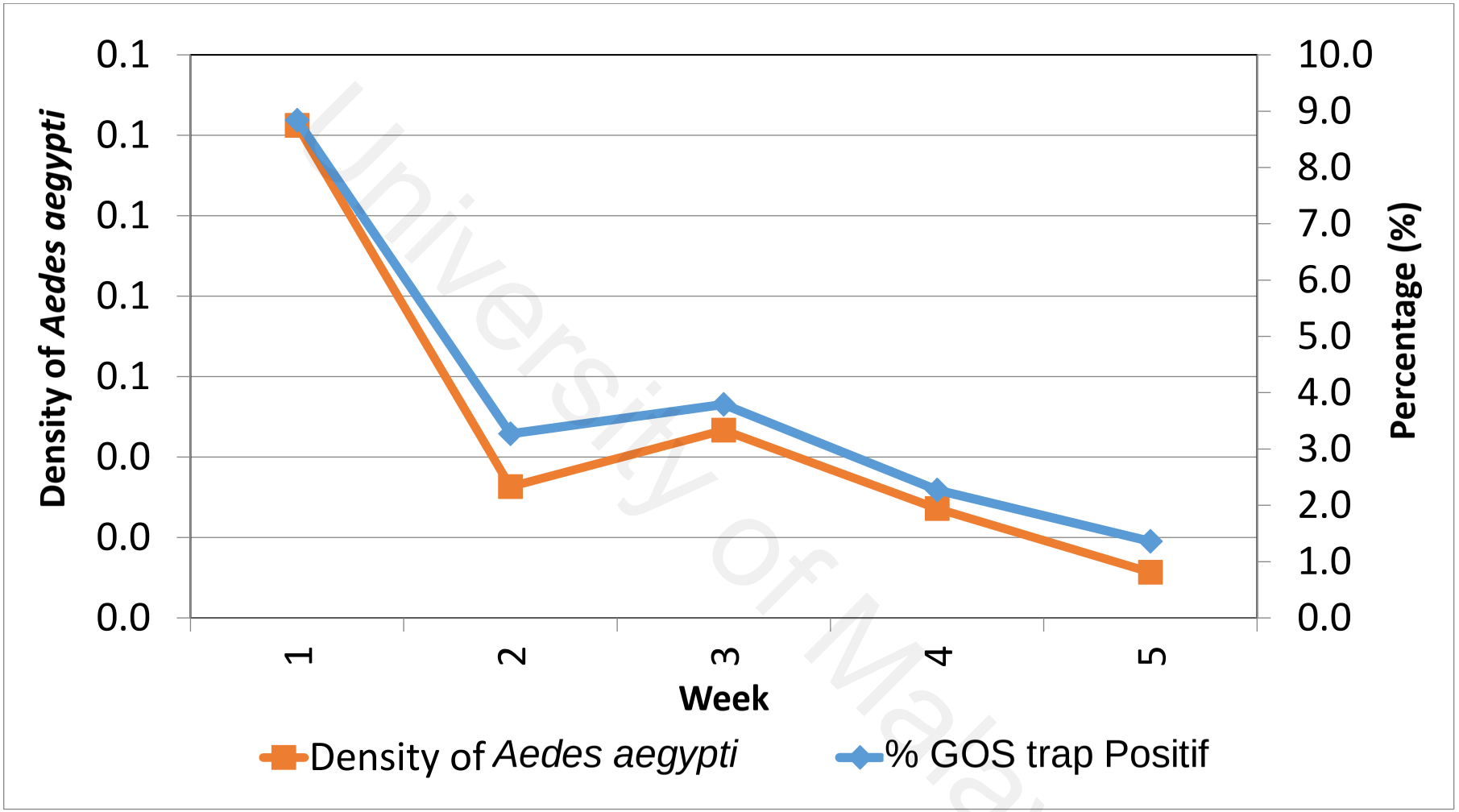

Figure 3.12: Percentage (%) of GOS trap and *Aedes aegypti* density for 7 blocks from 1 – 30 October 2013

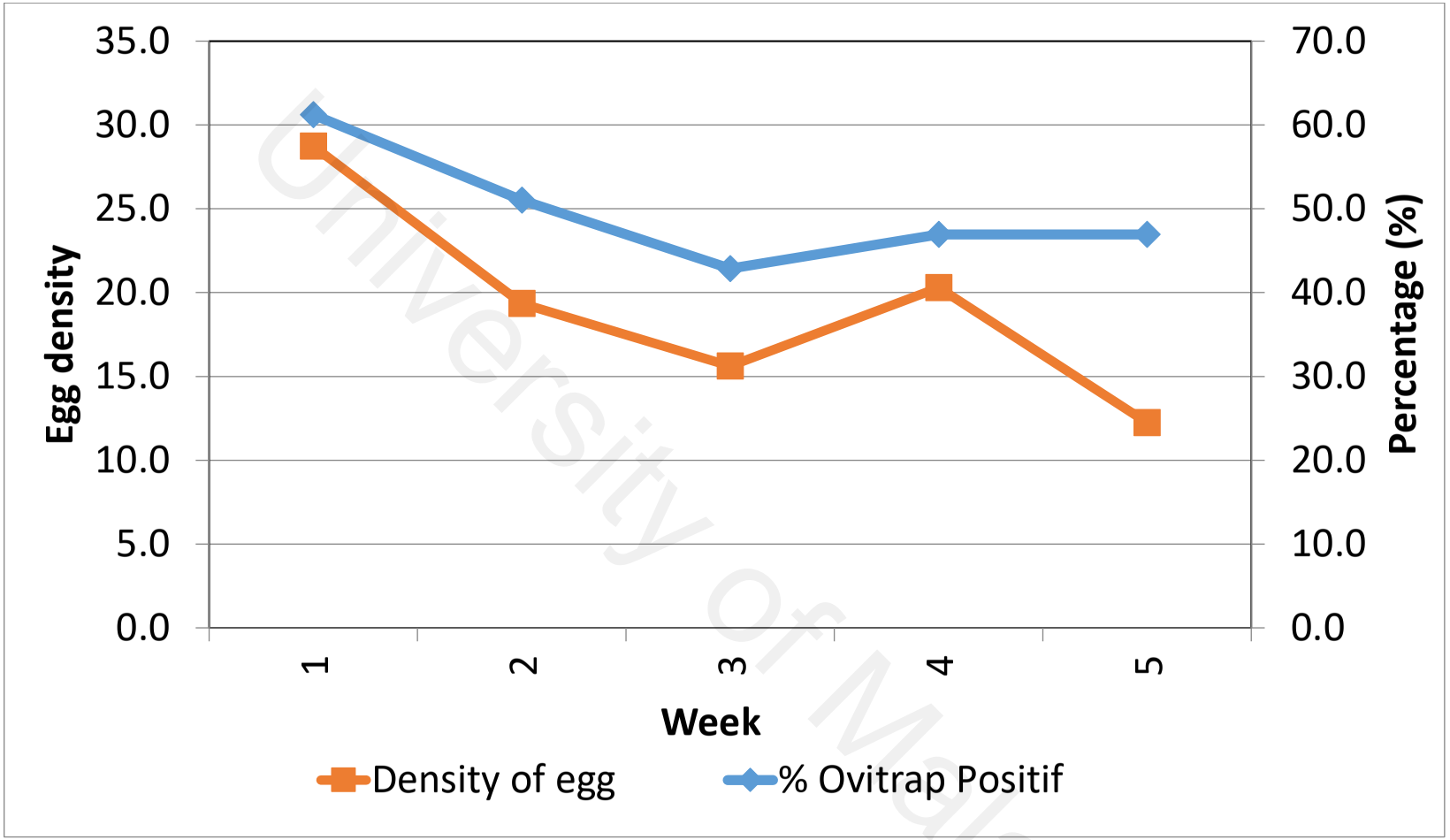

Figure 3.13: Percentage (%) of ovitrap positive and egg density for 7 blocks in Mentari Court from 1-30 October 2013

Pearson correlation analysis shows that there was a significant relationship between GOS positive with *Ae. aegypti* and the proportion of positive ovitrap with *Aedes* eggs ($r^2 = 0.43$, df = 17, $P < 0.01$).

3.3.3.3 Determine the optimum number of trap to be set

The relationship between *Ae. aegypti* caught (y) and number of traps (x) is best described by a nonlinear model (Box–Lucas 1959). The equation obtained is y = 19.92 (1-exp(-0.27x)($P < .001$) which is shown in Figure 3.14. The equation is asymptotic at around 20 suggesting that 20 traps per block would be sufficient to be deployed for monitoring *Aedes* population.

3.3.4 Detection of dengue virus

Mosquitoes which were caught by sticky paper was further processed for virus detection using NS1 rapid test kits on the pooled abdomen, while head and thorax of the mosquitoes were tested by RT-PCR. Table 3.11 showed that total of eight pool of *Ae. aegypti* (17.78%) were positive for dengue virus using the NS1 antigen detection kit, and the minimum infection rate per 1000 mosquitoes (MIR) was 38.02 (18.00 – 71.18). About 40 mosquitoes (head and thorax) were tested individually using real-time RT-PCR, among them 15 were positive by giving an infectious rate of 6.02. Of these, 10 had dual infection of DENV2 and DENV3 (two were positive for DENV3, and one was positive for DENV2), and two were positive for DENV1.

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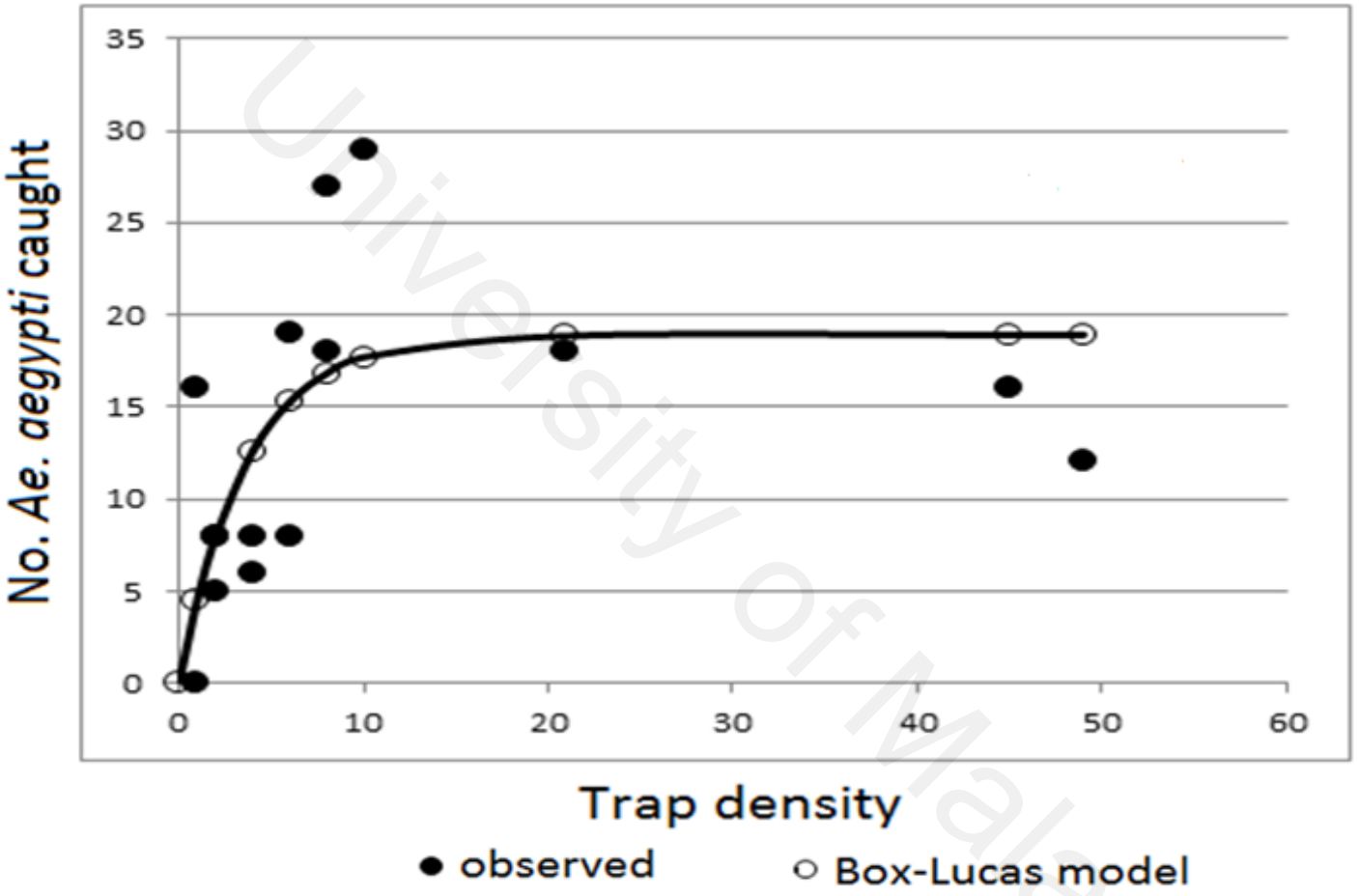

Figure 3.14: Total number of *Ae. aegypti* captured using different densities of GOS trap over 5 weeks. The equation for Box-Lucas function is y=19.92 (1-exp(-0.27x) (*P*<.001)

Table 3.11: Percentage of NS1 pool and number of *Aedes* mosquitoes pooled in test were positive with NS1 rapid test

Study trial	Duration of study	NS1 Antigen Test					
		Aedes aegypti			*Aedes albopictus*		
		Total pools (mosquitoes tested)	Total pools positive (number of mosquitoes)	% Positive pools	Total pools (mosquitoes tested)	Total pools positive (number of mosquitoes)	% Positive pools
1	6/6/2013 - 30/9/2013	45 (223)	8(40)	17.78	2 (7)	0 (0)	0.00
2	1/10/2013 - 6/11/2013	10 (50)	0 (0)	0.00	0(0)	0 (0)	NA
	Total:	55 (273)	8 (40)	8.42	2 (7)	0 (0)	0.00

Note: NA – Not available

3.4 DISCUSSION

The GOS trap had the ability to capture *Ae. aegypti* which was the main vector species in the study site which had longest dengue outbreak period in Malaysia in 2013 for about 195 days and reported up to 129 cases (KKM, 2014b).

In this study site, dengue cases occurred independently of blocks and floors and was also the same for the past 3 years (2011 – 2013). However, compared to the distribution of *Aedes* mosquitoes, there was no significant difference in the GOS index and ovitrap index per block. However, significantly higher GOS index and number of eggs were obtained from ground floors, but not for ovitrap index. Comparison by trap location demonstrated that the GOS index was significant difference for three traps which were set on the ground floor and sixth floor in Block D, while ovitrap index showed no statistical difference. This trial showed that *Ae. aegypti* were caught on every floor up to 17^{th} floor with the highest percentage trapped at ground floor (41.6%). Similar result was also revealed that *Aedes* mosquitoes could be found from the ground floor to highest floor (Lau et al., 2013; Roslan et al., 2013), including the roof-top of a sixteen-story building (flats) in an urban area in Kuala Lumpur. Another study showed that 97.5 eggs per eggs per ovitrap per week was found on the second floor compared to 3.4 eggs per ovitrap per week on the ground floor (Sulaiman S. et al., 1993). The finding of the experiment in Singapore exhibit that *Ae. aegypti* prefer to breed near ground level with higher percentage (64.91%) of mosquitoes were trapped on floors 2 – 6^{th} (Lee et al., 2013). While, the highest number of larvae were obtained from the sixth floor in high-rise buildings in Selangor and Wilayah Kuala Lumpur (Wan-Norafikah et al., 2010).

The result of baseline survey showed that health teams were only able to survey 25 to 40 premises per day. It also demonstrated that although *Aedes* index (AI) and Breteau index (BI) obtained were very low (4% and 0% respectively) but the ovitrap index (44.03%) and stick trap index (10.22%) were high. The larval survey has the limitation as data can be underestimated. It depended on vector control technicians to follow the standardize procedures and whether able to capture the temporal variability of the entomologic indices between the inspection interval (Sanchez et al., 2006). Besides, collection of larval indices is more labour intensive and plagued by difficulties of access particularly in urban settings (Sivagnaname & Gunasekaran, 2012). However, ovitrap index is a more sensitive technique to detect mosquitoes in an area compared to the House Index (Braga et al., 2000) and sticky trap (Honório et al., 2009a) but because *Ae. aegypti* exhibits skip oviposition (Harrington & Edman, 2001; Reiter, 2007), the ovitrap index may be overestimated of gravid female populations. The sticky trap was found to be more useful compared to the classical larval indices because it is a better proxy of measuring adult densities (Sivagnaname & Gunasekaran, 2012).

Although ovitrap index was higher than GOS trap, however there was no statistically significant relationship between these two indices and the correlation coefficients was 0.544. The similar result was also observed for the density of *Ae. aegypti* and eggs density per trap, both show the same trend but there was no statistically significant relationship between them. However, in Brazil, a significant correlation was observed among the larval, oviposition and adult trap indices. The correlation coefficient between the MosquiTrap positive index and ovitrap positive index was 0.7846 which was higher than the correlation coefficient of the present study (Resende et al., 2013). In Italy, high correlation (r=0.96) was found between the number of females *Ae. albopictus* and the number of eggs collected by the traps (Facchinelli et al., 2007). A poor correlation was also detected between the ovitraps and mosquiTrap (Gama et al., 2007). However, a

longer period will be needed to confirm this and the results of long term studies will provide more reliable results.

Dengue virus was detected in the mosquitoes before a case was reported the following week, while outbreak occurred after the second case was reported 10 days later. However, others have shown that the peak of entomological inoculation seems to precede the human dengue cases by several weeks to a month (Garcia-Rejon et al., 2008). In Colombia, there were weak associations between *Aedes* index and dengue incidence, on the other hand, the association was more evident between DENV infection in female mosquitoes (IR) and dengue cases (Peña-García et al., 2016). It has also been indicated that abundance of larvae or pupae was not predictive of an abundance of *Ae. aegypti* females (Morrison et al., 2008). The relationship between vector abundance and dengue transmission needs to be elucidated (Bowman et al., 2014), to introduce adult mosquito sampling as a routine and current indice like Breteau are not reliable universal dengue transmission threshold. In Thailand, Yoon et al. (2012) demonstrated a positive association between infected *Ae. aegypti* and dengue infected children in the same and neighbouring houses. The positive mosquito was obtained before the index case was reported.

Identification of dengue virus in mosquitoes using molecular technique has been proposed as a useful tool for epidemiological surveillance and identification of serotypes circulating in field (Guedes et al., 2010; Liotta et al., 2005; Victor, 2009). Various types of techniques were developed for better detection of virus in mosquitoes. The virus isolation using mice inoculation is time consuming and requires many passages, while immunofluorescent assay using serotype specific monoclonal antibodies is labour intensive and this method is not practical to screen a large number of field specimens (Victor, 2009). Although the detection of dengue virus in mosquitoes using RT-PCR

showed 99.52% accuracy (Liotta et al., 2005), it would not be practical for use by dengue control personnel.

Although adult mosquitoes can be used for estimating dengue transmission risk (Ritchie et al., 2004) and for dengue surveillance, it is not being implemented as a surveillance tool in most of the dengue-endemic countries including Malaysia. This is due to the use of RT-PCR for the detection of dengue virus in mosquitoes requires expertise and laboratory support and would be expensive. In this study, ten mosquitoes carried two serotypes of dengue virus, all serotypes except DENV-4 was present. A study conducted in Brazil showed only one serotypes presented in one mosquito and also absence of DENV-4 (Guedes et al., 2010). According to Mohd-Zaki et al. (2014), DENV-4 was the least prevalent of all serotypes and it formed <20% of all serotypes detected between 2000-2012 in Malaysia. Dengue virus has been found in field-collected mosquitoes in Mexico (Garcia-Rejon et al., 2008), South-East Asia (Chow et al., 1998; Chung & Pang, 2002) and India (Tewari et al., 2004). Thus, it shows that using GOS traps plus NS1 dengue antigen test kit could be more cost-effective and suitable for providing an early warning before large epidemics. Besides, NS1 dengue antigen test kit is a simple test where results can be obtained within 20 minutes and large number of mosquitoes can be easily tested. Hence, both GOS trap and NS1 dengue antigen test kit is simple procedure that can easily be carried out by health staff at the ground level. Sticky trap was also shown to be a more suitable tool for collecting adult mosquitoes for subsequent test and was suitable as an alternative *Ae. aegypti* surveillance tool (Chadee & & Ritchie, 2010a; Facchinelli et al., 2007). In Singapore as well, it has been shown that the antigen detection NS1 kit was useful in detecting the dengue viral antigen in field-collected mosquitoes (Lee et al., 2013; Tan et al., 2011). Thus, using GOS trap for surveillance would be more cost-effective and could provide warning before large epidemics.

Due to the mushrooming of houses and apartments in the urban areas as well as lack of health personal, there is shortage of manpower to carry out *Aedes* surveys. The *Aedes* survey which has been considered as the hallmark of the surveillance programme for decades (Azil et al., 2011) has its limitation (Tun-Lin et al., 1996) and is currently not sustainable. In most urban areas, people are at work place during the day and accessibility to houses for larva surveys is a major problem. Therefore, the new paradigms for dengue surveillances is needed. An inexpensive and effective *Ae. aegypti* specific adult trap would be a significant surveillance breakthrough and could allow quick virus testing (Resende et al., 2013). Virus detection in mosquitoes can be an additional benefit to take necessary control measures to break the chain of transmission especially in the areas where the source of infection of dengue is not detected. Besides, the actual incidence of the disease in Malaysia may be underestimated due to the use of passive reporting system and low levels of reporting from private sector (Beatty et al., 2010). This can be a more proactive measure for a control programme. Thus, GOS trap and NS1 antigen diagnostic kit which has been tested in this trial can serve as a useful tool for surveillance of dengue. However, further testing for longer periods is required.

Although similar studies have been conducted in different countries (Chadee & Ritchie, 2010a; de Santos et al., 2012; Gama et al., 2007; Honório et al., 2009a; Resende et al., 2013; Ritchie et al., 2004) for showing the effectiveness of the sticky trap in collecting the *Aedes* mosquitoes and its importance in a surveillance programme, it has not been implemented in any control programme in South-East Asia (Sivagnaname & Gunasekaran, 2012) with the exception of Singapore where it is used for dengue cluster management (Lee et al., 2013). However, in Brazil, besides using larval survey, some municipalities are using Intelligent Dengue Monitoring (MI Dengue) which consists of using MosquiTRAP (a sticky trap with a synthetic attractant), palmtops/cell phones and GIS software (Geo-Dengue). The adult indices are used for larviciding and source

reduction (Eiras & Resende, 2009). In the baseline study, it was observed that the larval survey could cover only for an average of 20 premises per day and not many positive breeding places were found compared to positive GOS trap and ovitrap. Another important aspect of this trap is that a female *Ae. aegypti* will have to lay eggs after a blood meal (with or without virus), and the sticky trap will catch it. When it searches for containers to lay eggs, the possibility it may select the sticky trap for its oviposition and thus will not be able to transmit the virus. The number of infected mosquitoes obtained in the study was high and the survival of these old age females was important because they have to survive at least 6.5-15 days (extrinsic incubation period) (Chan & Johansson, 2012) after feeding on an infected blood meal in order to transmit dengue virus to human. Indirectly the sticky trap prevented the human-vector contact, which would reduce the infective bites and also eliminate all mosquito progeny.

However, the major limitation of the sticky trap is that it targets only gravid females seeking ovipositing sites rather than host-seeking ones and its efficacy could be reduced by the presence of nearby natural oviposition sites (Sivagnaname & Gunasekaran, 2012). For this reason, the hay infusion water was used to make the sticky trap containers more attractive than the surrounding containers. Ideally, attractant would be used instead of preparing hay infusion every week. These GOS trap also have the advantage over other traps that were used for collection for adult mosquitoes as they do not need to be serviced daily. It would not be practical for a control programme to use a tool that has to be serviced daily. Commercial trap is also very expensive. Although BG-Sentinel trap is a favored method for field workers in Cairns (Australia) because of its user-friendliness, but is not as cost-efficient as the sticky trap (Azil et al., 2014). Advantage of this trap is that the netting is placed at the bottom of the inner container so as to prevent escaping of the adult mosquitoes during death stress and allow oviposition of female *Ae. aegypti* by shooting out eggs directly into the water when caught on the

sticky surface (Chadee & Ritchie, 2010a). Whereas, in other studies suggested that larvicides could be applied to kill the emerging larvae, such as methoprene (Ritchie et al., 2009) and Bti can be added to water (Rapley et al., 2009).

This second trial have also shown that it is unnecessary to set a large number of traps. From the number of GOS traps which ranged from 147 to 441 have been tried in this experiment, showed that setting three traps in each floor or about 20 traps per block supported by the Box-Lucas equation, were sufficient to collect and monitor the adult *Ae. aegypti* population for control programmes purposes. Similar study was conducted in Brazil and showed that setting as many as four traps in their study area was sufficient (Resende et al., 2012). However, before the introduction of GOS trap, it will be necessary to determine the number of trap needed for each house based on type and location. This trial also showed that the number of *Aedes* was decreasing with the increasing number of traps and egg density decreased over time. However, the egg density decreased was not as much as to the adult mosquitoes as *Aedes* mosquito performs skip oviposition (Reiter, 2007).

CHAPTER 4: SURVEILLANCE OF ADULT *AEDES* MOSQUITOES USING GOS TRAP AND NS1 ANTIGEN KIT

4.1 INTRODUCTION

Evaluation of the suitability of GOS trap (Gravid Mosquito Oviposition in The Sticky Trap) for capturing *Aedes aegypti* in Phase 1 (Chapter 3) showed that it could be used as a tool for vector surveillance in a dengue endemic locality in Selangor. The GOS trap uses sticky papers which attract and trap the gravid mosquitoes when they come to lay eggs in the trap. The similar concept of using sticky traps has been experimented previously in dengue-endemic areas in some countries (de Santos et al., 2012; Facchinelli et al., 2008; Lee et al., 2013; Ritchie et al., 2004). It was claimed that sticky ovitraps, which sampled female *Ae. aegypti* weekly in Queensland, Australia could gauge the risk of dengue transmission (Ritchie et al., 2004). The gravitrap were deployed in dengue cluster areas in Singapore to manage dengue cases (Lee et al., 2013). The MosquiTRAP, a type of sticky trap was used to assess the risk classification of dengue fever based on the number of *Ae. aegypti* captured at an area in Brazil (Steffler et al., 2011).

Mentari Court apartment was observed to be a suitable study site for dengue surveillance based on the number of dengue cases and dengue vectors, with *Ae. aegypti* (54% of total mosquitoes caught) being the main vector followed by *Ae. albopictus* (1.78%). The other mosquitoes were *Culex quinquefasciatus* (43.77%) and *Culex gelidus* (0.67%). The pilot study revealed that *Aedes* mosquitoes were trapped mostly from the ground floor, with three traps set per floor or about 20 traps per block were sufficient to monitor the adult *Ae. aegypti* population for the subsequent two years study. A similar type of study was conducted in Brazil, where four traps were found sufficient for their

study area (Resende et al., 2012), and another study used one trap per block to access the risk classification of dengue fever (Steffler et al., 2011), whereas the minimum number of sample units necessary for maintaining a fixed level of precision or sensitivity depends upon the mean density of the population to be sampled (Facchinelli et al., 2007). In Singapore, a total of 4-6 gravitrap were placed in each apartment block with reported dengue cases (Lee et al., 2013). It was noted that weekly servicing of traps was more appropriate than monthly servicing during favorable climatic conditions due to rapid larval development (Chadee & & Ritchie, 2010a; Facchinelli et al., 2007; Ritchie et al., 2004). However, gravitraps in Singapore were checked and serviced in every 3 – 4 days (Lee et al., 2013).

In most parts of Southesast Asia, vector control has been the hallmark of the dengue control programme (Chang et al., 2011). However, house to house larval surveys, source reduction, fogging and ULV which were effective in the 1970s and 1980s (Vythilingam & Panart, 1991, Ooi et al., 2006) are no longer sustainable nor cost effective as studies have shown there is no correlation between larval indicies and dengue cases (Morrison et al., 2008). Besides, resistance of *Aedes* to pyrethroids and temephos insecticides (Chen et al., 2013, Rong et al., 2012, Ishak et al., 2015) also hampers the control programme. Therefore, obtaining the adult female *Ae. aegypti* indices is considered the most direct measure of exposure to dengue transmission (Focks, 2004). Although various novel sampling devices were used to sample adult female *Ae. aegypti* (Maciel-de-Freitas et al., 2008; Mackay et al., 2013; Ritchie et al., 2014), studies on infection of the mosquitoes were lacking. Routine sampling of *Ae. aegypti* adults were deployed to identify high-risk localities which were then targeted for vector control (Mammen Jr et al., 2008; Pepin et al., 2013) and dengue prevention (Eiras & Resende, 2009).

Adult *Aedes* sp. infestation rates in Belo Horizonte had moderate significant correlation with the number of dengue cases (r=0.67) compared to House Indices (HI) (r=0.10 - 0.25) (Corrêa et al., 2005). While other studies showed significant relationship between adult *Ae. aegypti* with the dengue cases (Alshehri, 2013; Chan et al., 1971; Dibo et al., 2008; Lien et al., 2015). However, some studies showed no correlation between the numbers of adult females *Ae. aegypti* and incidence of dengue (Barrera et al., 2002; Romero-Vivas & Falconar, 2005).

Since human DENV infections are commonly asymptomatic (Gubler, 1988, Kyle & Harris, 2008), it was felt that perhaps detection of dengue virus in mosquitoes would serve as proactive tool for the control programme. This chapter will elaborate the efficacy of the GOS trap and NS1 antigen kit over a period of two years for dengue vector surveillance. It is a tool for early detection of dengue outbreaks which would perhaps replace the labour intensive house to house larval surveys.

4.1.1 Objectives of the study

4.1.1.1 General objectives

To determine the efficacy of the combined used of GOS trap and NS1 antigen kit to detect dengue virus in mosquitoes as a new paradigm for dengue vector surveillance.

4.1.1.2 Specific objectives

1) To capture *Aedes* mosquitoes using GOS trap in a two-year study.

2) To evaluate the efficacy of the combined use of GOS trap and NS1 antigen test as a new paradigm for vector surveillance.

3) To study the efficacy of GOS trap and ovitrap for surveillance of dengue.

4) To determine dengue infection rate in *Aedes* mosquitoes.

5) To determine virus serotype by RT-PCR in *Aedes* mosquitoes positive by NS1.

4.1.2 Research hypotheses

1. H_o: There is no correlation between the number of *Aedes* obtained and the number of dengue cases in the study area.
2. H_o: There is no significant difference between the ovitrap index and the GOS trap index. In this hypothesis, the sensitivity of sticky trap and traditional surveillance methodologies will be compared.
3. H_o: There is no significant correlation between the densities of *Ae. aegypti* and the egg density per trap.
4. H_o: There is no statistical difference in the index value between the blocks, floors and locations.
5. H_o: There is no correlation between the number pool of *Aedes* tested positive with NS1 Antigen test kit and the number of dengue cases in the study site. This hypothesis would like to test the effectiveness of NS1 antigen test kit to pick up dengue virus from the mosquito population, thus can predict dengue epidemics.
6. H_o: There is no correlation between the number pool of *Aedes* tested positive with NS1 Antigen test kit and the mosquito density in the study site. This hypothesis would like to test whether there is a relationship between the number pool of *Aedes* positive with DENV and the density of mosquitoes obtained in a locality.

4.1.3 Significance of the study

1) The two-year data will enable us to determine the relationship between the infected vector and dengue cases in the study area. It can help to determine whether infected mosquito information can be a better indicator to access the dengue risk.

2) A longer period of data collection (2 years) can provide more valuable information on the efficacy of the combined used of GOS trap and NS1 antigen kit as a new paradigm tool for vector surveillance.

3) This study enables to determine the relationship between density of mosquitoes and number of pool of *Aedes* tested positive with DENV.

4) This study will help to determine dengue infection rate in *Aedes* mosquitoes in a dengue endemic locality in Selangor.

5) From this study, virus serotype by RT-PCR in *Aedes* mosquitoes positive by NS1 will determined. The result also can determine the accuracy of NS1 antigen test as compared with RT-PCR test.

6) This study will also provide valuable information about the vector status in the chosen study site (difference in the vector density between floors and blocks). The longer period of data collection will provide more accurate information.

4.2 Materials and Methods

4.2.1 Study site

The two-year study was conducted in Mentari Court apartment which is a dengue endemic locality, situated in the Petaling district. Based on the result of the Phase 1 study,

it was determined as a suitable experimental site due to high dengue cases and *Aedes* mosquito density. Details information on the study site has been described in **Chapter 3**.

4.2.2 GOS trap

The GOS trap which was examined in Phase 1 showed that it could capture *Aedes* mosquito and it could be used as a tool for vector surveillance. Detail information about the GOS trap has been described in **Chapter 3**.

4.2.3 Field sampling

Phase 2

The study was conducted for two years from 14th November 2013 (week 47) until 4th December 2015 (week 47). Three traps per floor were deployed in each block as determined from the Phase 1 study. Three traps were set on the ground floor (GF), 3rd, 4th, 9th, 12th, 15th and 17th floor. Traps were set along the common corridor, 50 – 100 m apart and placed near the potted plants (if available). All traps were filled with seven-day-old hay infusion water. The traps were checked weekly, and the water was changed during the inspection. One ovitrap per floor was also set on the same floor where the GOS traps were set mainly for the purpose of checking for the presence of the *Aedes* mosquitoes. Figure 4.1 shows the distribution of traps for all seven blocks (Block A, B, C, D, E, F, and G). Two teams consisting of two men each checked the traps weekly. The traps were inspected carefully, and those traps with mosquitoes on the sticky surface were covered with a lid, placed inside a big plastic container and brought back to the laboratory for

further processing. If there were no mosquitoes trapped the sticky sheets, were changed monthly or as required if they were dirty.

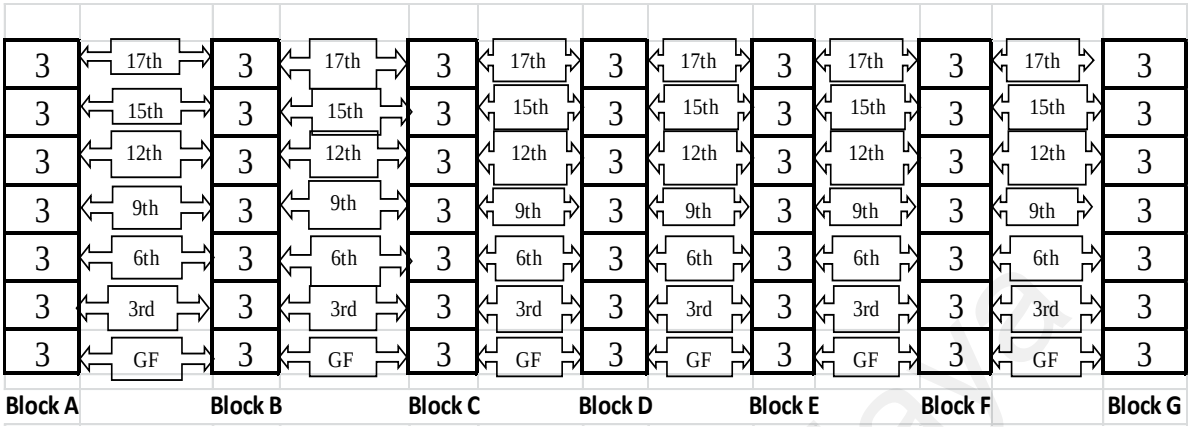

Figure 4.1: Number of GOS traps set per floor in the seven blocks (Blok A, B, C, D, E, F, and G).

4.2.4 Identification and processing of mosquitoes

In the laboratory, the mosquitoes were identified morphologically to the species level. A pair of heat sterilized forceps was used to remove the mosquitoes from the sticky surface to prevent cross-contamination. Details of processing of the specimens have been described in **Chapter 3**.

4.2.5 Detection of dengue viral antigen in abdomen of mosquitoes

In the laboratory, the mosquitoes were identified morphologically to species. The mosquitoes were then removed from the sticky surface of paper using heat sterilized forceps to prevent cross contamination. All the abdomens of the *Ae. aegypti* and *Ae. albopictus* were pooled in five for viral antigen detection tests (The SD Bioline®NS1 Ag kit was used for the test). The head and thorax were individually stored in Eppendorf

tubes at -80^{o}C until processed by RT-PCR for determining dengue virus serotypes. The details of the NS1 antigen test is provided in **Chapter 3**.

4.2.6 RNA extraction and multiplex RT-PCR

Individual mosquitoes (head and thorax) was homogenized in pre-chilled Eppendorf tubes with 0.2 ml of growth medium (Minumum Essential Medium, MEM: Biowest, Missouri, USA). The homogenate was then centrifuged at 21,000x g for 15 min at 4^{o}C. RNA extraction was carried out using Cardo pathogen extraction kit (Qiagen Hiden, Germany) and the kit's protocol was strictly followed. The extracted samples were then subjected to one step multiplex RT-PCR using AccuPower RT-PCR PreMix (Bioneer, Seoul, South Korea) using the protocol of (Yong et al., 2007). Briefly, this was a premix in a lyophilized form and was contained in 0.2 ml tubes. Thus, 15 µl of primer mix was added to each tube followed by 5 µl of the RNA template, vortexed and briefly spun. RT-PCR was performed in a Bio-RAD (Hercules, California, USA) PCR machine. The steps for this assay consisted of a 30-min RT step at 50 °C, 15 min of Taq polymerase activation at 95°C, followed by 40 cycles of PCR at 95 °C denaturation for 30 s, 60°C of annealing for 30 s and 72 °C extension for 1 min. Final extension was 72 °C for 10 min. Five µl of the PCR product was then analyzed by gel electrophoresis.

4.2.7 Dengue case data from Mentari Court Apartment

Data of dengue cases confirmed serologically either by NS1 or IgM/IgG from the seven residential blocks was obtained from the Ministry of Health, Malaysia. In Malaysia,

it is mandatory for all hospitals and private practitioners to report dengue cases to the Ministry of Health. The date of onset of each dengue case was used for all data analyses.

4.2.8 Statistical analysis

All statistical analyses were performed using R programming language (version 3.1) (R Development Core Team, 2008) and MS Excel 2010 program. This analysis used weekly data collected such as *Aedes* mosquitoes caught, confirmed dengue cases, positive-NS1- mosquito pools and *Aedes* eggs from all 7 blocks (21 traps per block). Preliminary analysis of simple linear and nonlinear correlation analysis indicated a lack of relationship between NS1-positive mosquito pools and dengue cases, due to lag effect. Subsequently, the family of distributed lag non-linear models (DLNM) (DLNM package version 2.20) (Gasparrini, 2016), which can simultaneously analyze non-linear factor-response dependencies and delayed effects and provides an estimate of the overall effect in the presence of delayed contributions (Gasparrini et al., 2010) was used for the investigation. The effect of the positive mosquito pool to the dengue cases was investigated using the model: glm (case ~ cb.total_aegypti +cb.ns1positive + ns(time, 3) + woy, family = quasipoisson, data) where woy = week of the year. The correlation was analyzed using Person correlation in R programming language.

Data were also subjected to analysis of variance (ANOVA), t-test, nonparametric tests (Pearson's χ^2 test), nonlinear regression (Box-Lucas) and general linearized modeling. The minimum infection rate (MIR) was calculated by maximum likelihood estimation method (Chiang & Reeves, 1962) based on 45 pools of 5 mosquitoes. Both the *Ae. aegypti* trapped per week and the dengue cases per week at each floor were analyzed separately using generalized linear mixed model (GLMM). In GLMM, the block and floor

were considered as fixed factors and the week as a random factor. Besides, zero inflation and Poisson distribution were incorporated in the analysis. In addition, differences in the numbers of *Ae. aegypti* and the dengue cases between blocks, floors and trap locations were tested with Tukey's method contrasts at P=0.05.

4.3 Results

4.3.1 Collection of mosquito species

The study site was predominantly considered as an *Ae. aegypti* (95.6%) area where 840 females (85%) and 148 males (15%) *Ae. aegypti* were caught as compared with 37 females (80%) and nine males (20%) *Ae. albopictus*. Other mosquitoes caught during this study were as follows: 53 males and 485 female *Culex quinquefasciatus*, 10 female *Cx gelidus* and 5 female *Coquilettidia crassipes*. Details of the mosquito species collected from all seven blocks during the two years study are shown in Table 4.1. A total of 166 traps (0.84% of the total traps) were spoilt or lost during the study.

4.3.2 Temporal distribution of *Aedes* mosquitoes in relation to dengue cases

Aedes aegypti which was the predominant species recorded had the highest density in 2013 with a median number of 9; this subsequently reduced to 8 in 2014 and to 7 in 2015 (Figure 4.2). The total number of *Ae. aegypti* trapped per week was the highest in January 2014. Followed by a regular increase in a six-monthly pattern by the spline graph (June-July 2014, January 2015, and June-July 2015, and the end of 2015) as presented in Figure 4.2a. However, for the number of dengue cases, there were three peaks in January 2014, March 2015, and August-September 2015 (Figure 4.2b). It was noted

Table 4.1. Mosquito species collected by the GOS trap in Mentari Court during Phase 2 experiment from 14 November 2013 to 4 December 2015

	Aedes aegypti		*Aedes albopictus*		*Culex quinquefasciatus.*		*Culex gelidus*		*Coquilettidia crassipes*	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
Total	840	148	37	9	485	53	10	0	5	0
Mean	7.85	1.38	0.35	0.08	4.58	1.47	1.00	0.00	1.00	0.00
Range	0 - 42	0 - 6	0 - 4	0 - 1	0 - 28	0 - 8	0 - 1	0	0 - 1	0
Standard error	0.63	0.15	0.07	0.04	0.47	0.31	0.00	0.00	0.00	0.00
Upper limit (95% CI)	9.09	1.67	0.48	0.15	5.49	2.08	1.00	0	1.00	0
Lower limit (95% CI)	5.31	1.22	0.58	0.30	3.89	1.26	0.00	0.00	0.00	0.00

Note:
Mean - total number of mosquitoes caught per week.
Total number of trap, n=19,902 (186 traps per week)
Total week trapping – 107 weeks

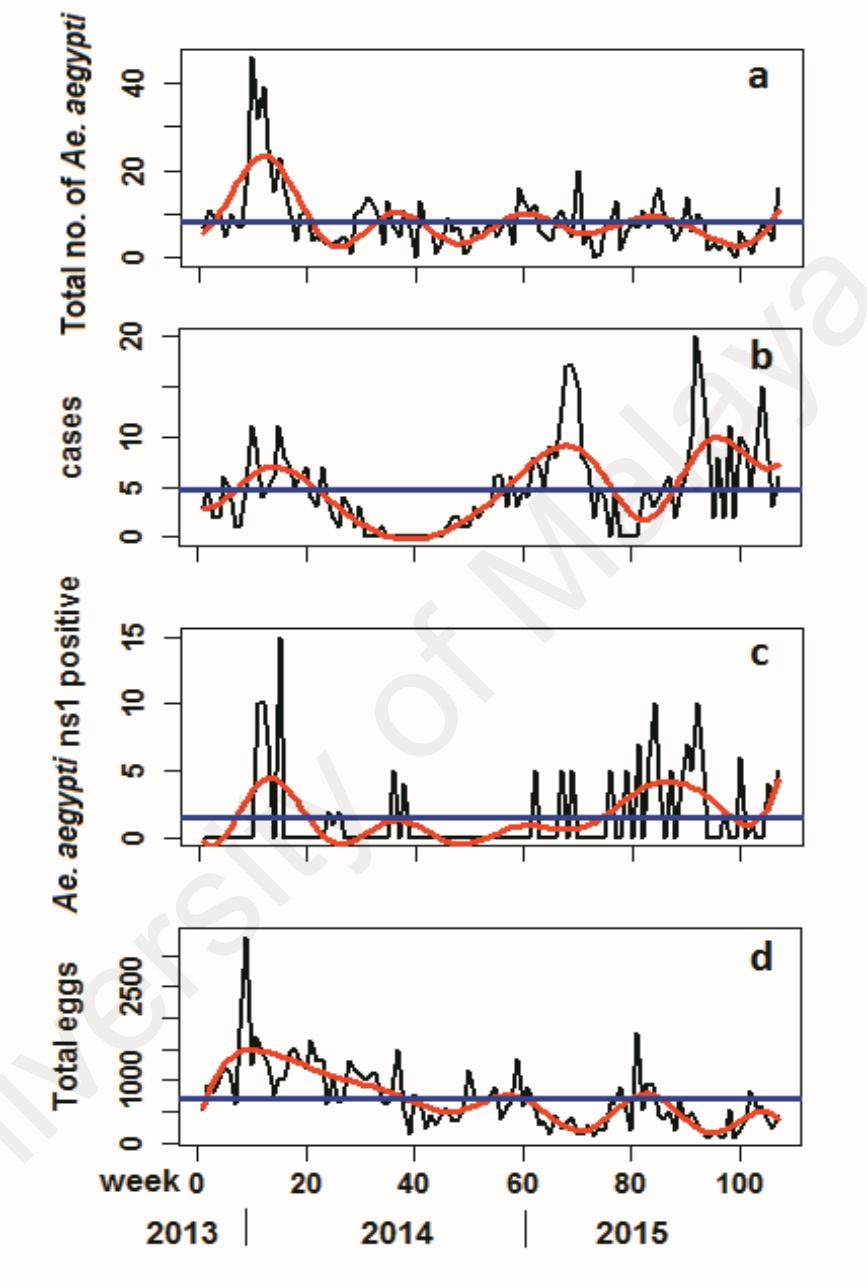

Figure 4.2: Time series of the total number *Ae. aegypti* trapped per week (a), a total number of dengue cases (b), the number of *Ae. aegypti* testing positive (c), a total number of eggs collected from the ovitraps (d) from November 2013 to December 2015, in Subang Jaya, Selangor, Malaysia. The solid red curve is a natural cubic smoothing spline, and the horizontal blue line indicates the overall mean value. The total number represents the sum of data from seven blocks with 21 traps in each block.

that only the trend of the number of NS1 mosquito pools found positive followed the trend of dengue case which as showed in Figure 4.2c. The number of eggs followed the same pattern as the total number of Aedes but the peaks appeared to decrease with time (Figure 4.2d).

The number of *Ae. aegypti* collected per week ranged from 1 to 42 and *Ae. albopictus* from 1 to 4 (Table 4.1), the maximum number of mosquitoes caught per week was higher than the Phase 1 study (Table 3.3).

Figure 4.3 shows the distribution of the *Aedes* mosquitoes and the dengue case throughout the 2-years study period. Pearson correlation analyses revealed no statistically significant correlations between the number of dengue cases and the number of *Ae. aegypti* [r(104)=+ 0.188, P>0.05, two tailed] or *Ae. albopictus* [r(104)=+ 0.132, *P*>0.05, two tailed] respectively. Further correlation analysis of the lag time (2, 3 and 4 weeks) of occurrence of dengue cases and the number of *Aedes* caught revealed non-significant relationship between the two variables. The same result was also obtained in Phase 1 study. However, the relationship between the number of dengue cases and *Aedes* caught demonstrated significant relationship using the general linearized model (GLM). The relationship can be described with the equation y = 1.35379 + 0.01996x ($F_{1,105}$ = 28.68, P < 0.001) as shown in Figure 4.4.

4.3.3 Number of NS1 mosquito pools in relation to dengue cases and mosquito density

Figure 4.5 shows the distribution of the pooled positive mosquito and the dengue case throughout the 2-years study period. Maximum number of *Ae. aegypti* pools detected positive per week were 3 pools, which occurred in week 4 of 2014. The peak of pooled

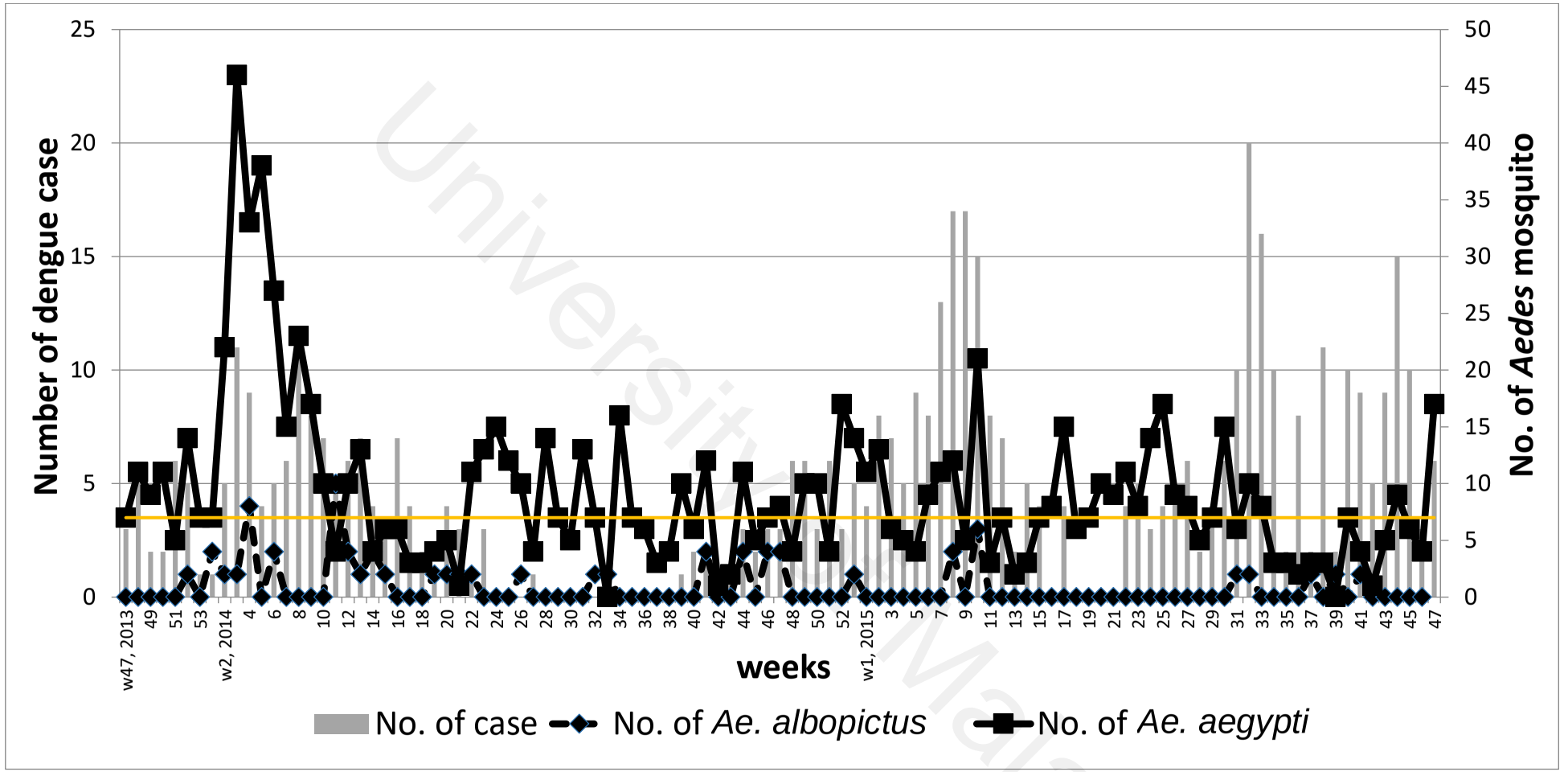

Figure 4.3: Total number of *Ae. aegypti*, *Ae. albopictus* and a total number of dengue cases. Data represent combined data for all seven blocks (Block A, B, C, D, E, F, and G), and three traps per floor were set. Horizontal graph lines denote the median number of *Ae. aegypti.*

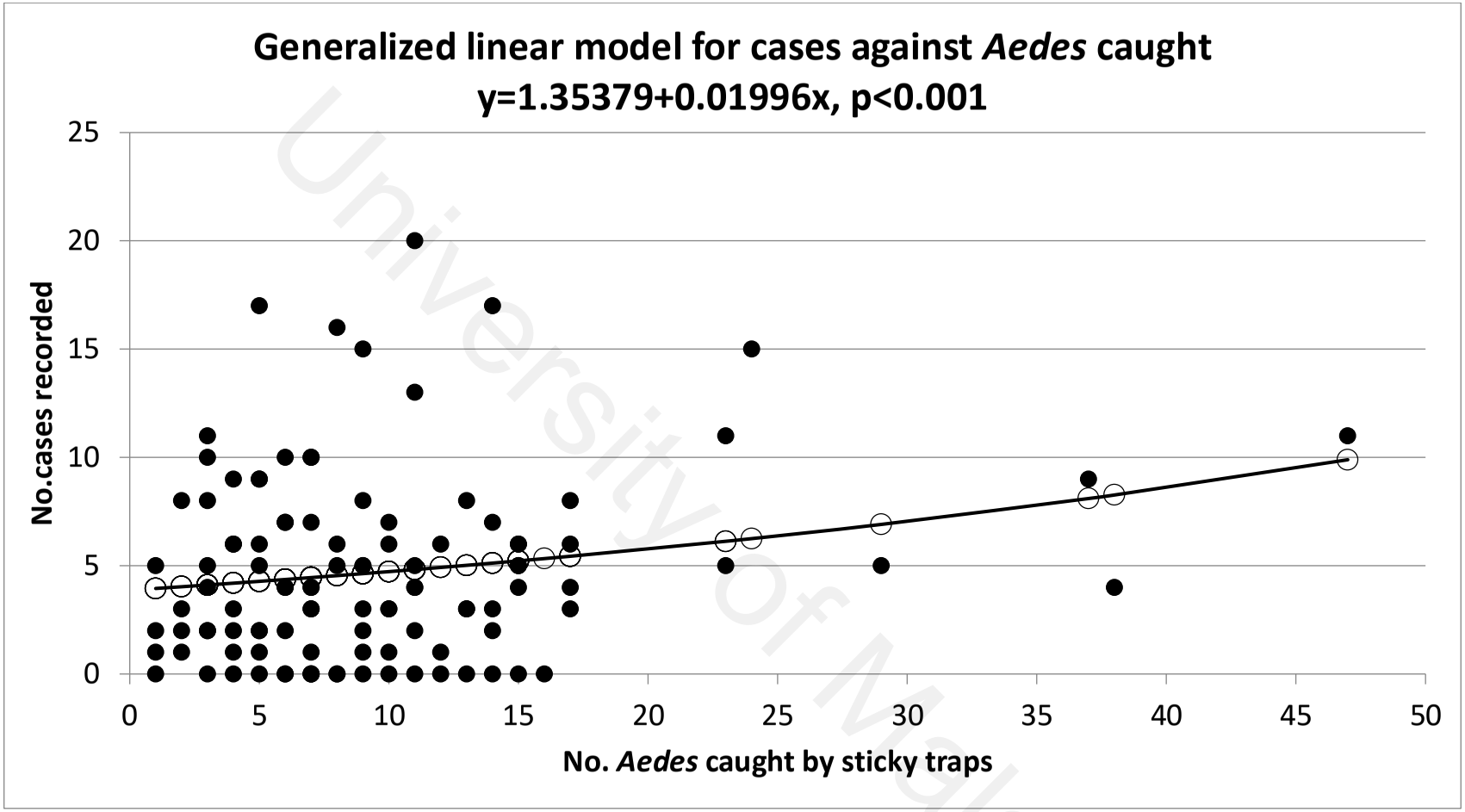

Figure 4.4: Generalized linear model for the number of cases against *Ae. aegypti* trapped, described as y=1.35379+0.01996, p<0.001

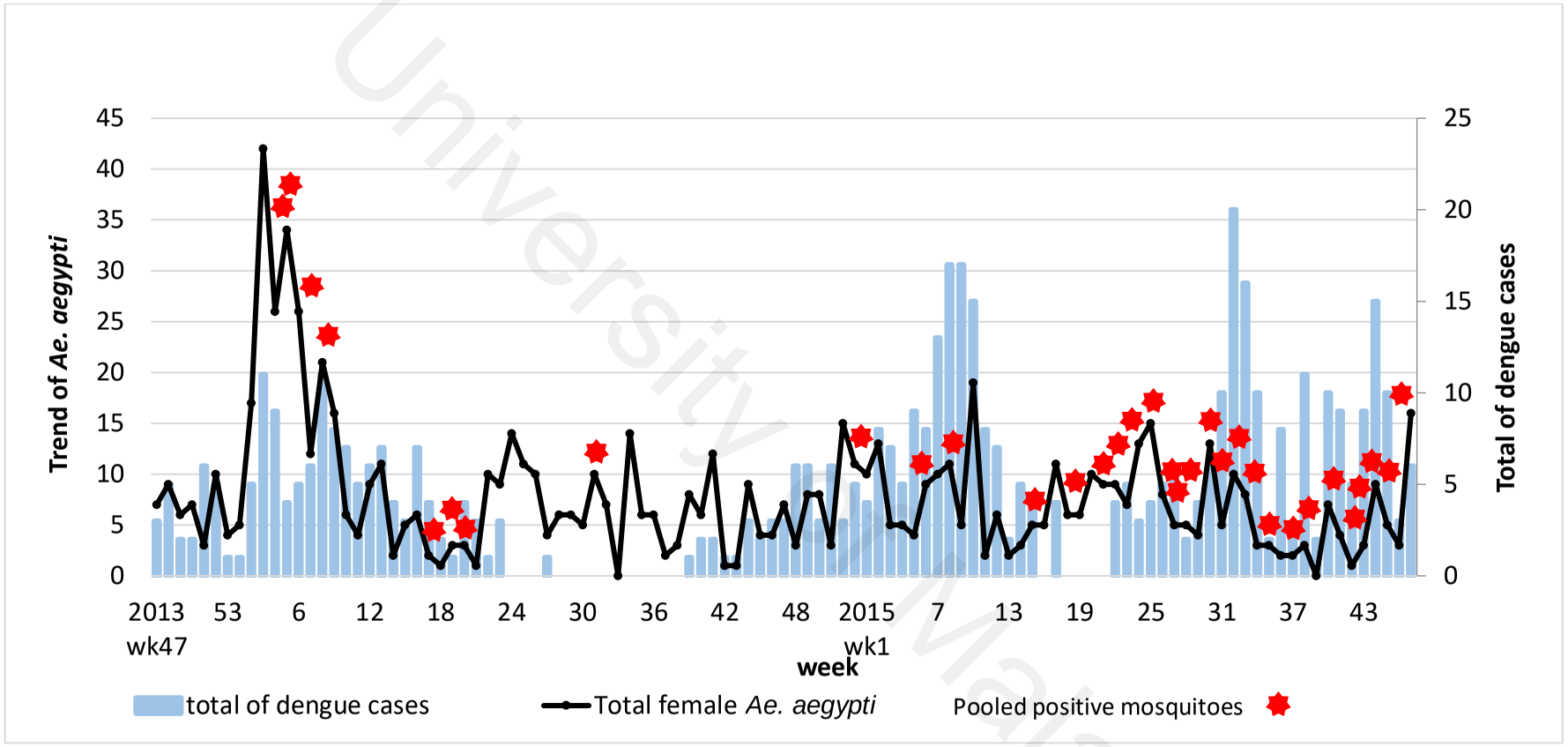

Figure 4.5: Total number of *Ae. aegypti*, total number of case and pooled positive mosquito. Denotes pools of *Ae. aegypti*. Data represent combined data for all seven blocks (Block A, B, C, D, E, F, and G), and three traps per floor were set.

positive mosquito was detected during the weeks 4 – 8 in 2014 (from January to February), it consistently showed positive from the weeks 17 until 27 in 2015 (from April to November 2015). Dengue cases also showed the same trend with 1 peak in early of year 2014 and 2 peaks in the middle and end of the year 2015, whereas the density of mosquitoes and the number of mosquito eggs did not show increasing trend in the year of 2015. However, only 3 pools out of 15 pools of *Ae. albopictus* were positive in the week of 6 and 33 in 2014 and week 32 in 2015.

Further analysis with Pearson correlation analyses revealed that there were statistically significant correlations between the numbers of pooled mosquitoes positive and the number of dengue case [r(104)=+ 0.289, $P<0.05$, two tailed] and also for the number of *Ae. aegypti* [r(104)=+ 0.319, $P<0.05$, two tailed]. However, a non-significant relationship existed between the number of mosquitoes and the number of dengue case as was shown earlier. The relationship of the number of dengue cases with both the number of NS1 positive mosquito pools and lag is depicted in Figure 4.6. Dengue cases occurred after a lag of one week after NS1-positive mosquito pool was detected but peaked at 2 weeks lag. The plot of lag-response curves Figure 4.7 for the different number of NS1-positive mosquito pools indicated that the dengue cases would be highest at 2-3 weeks lag.

4.3.4 Positivity of *Aedes* mosquitoes in NS1 rapid test and PCR test

Table 4.2 showed that a total of forty-three pool of *Ae. aegypti* (22.99%) were positive for dengue virus using the NS1 antigen detection kit, and the minimum infection rate per 1000 mosquitoes (MIR) was 51.2. Only three *Ae. albopictus* pools were positive by NS1 but none of the heads and thoraces were positive by RT-PCR. About 128

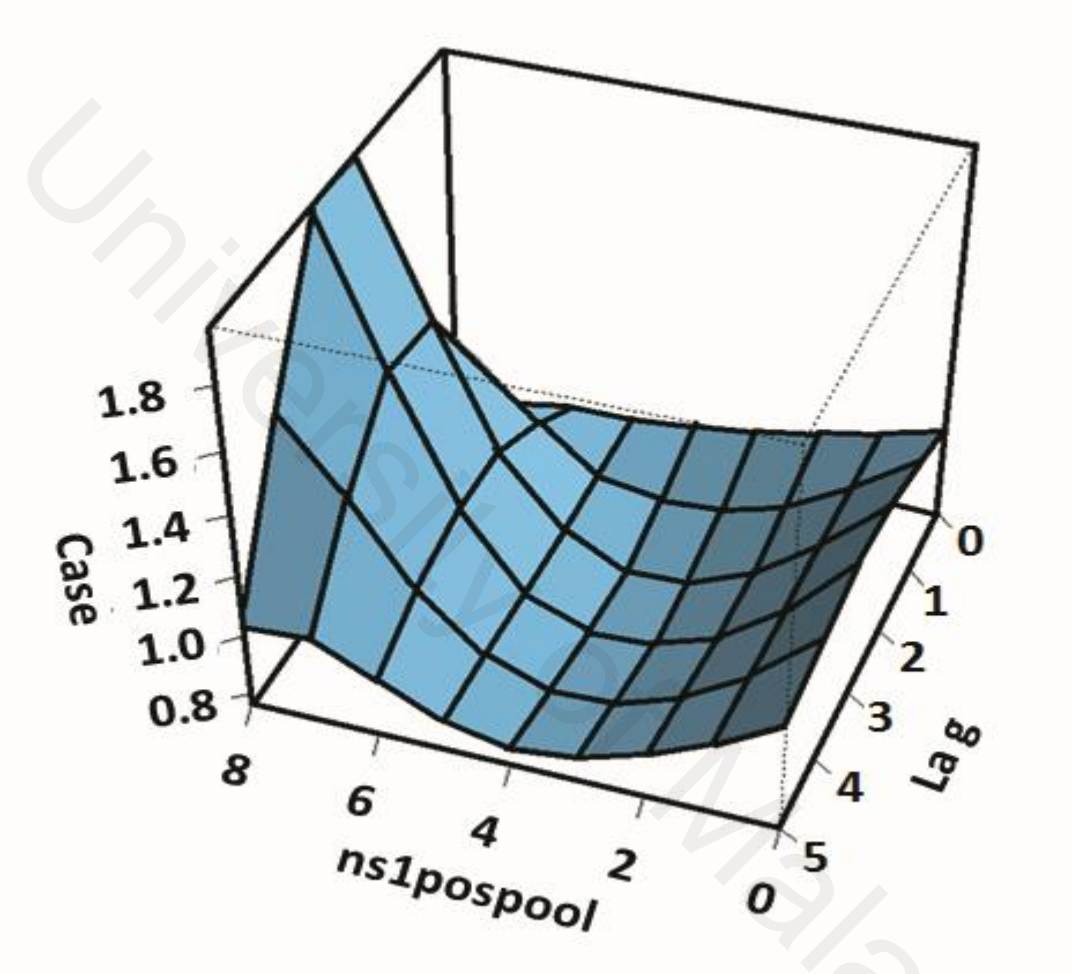

Figure 4.6: Three-dimensional plot of cases along NS1-positive mosquitoes and lags, with reference to none NS1-positive detected

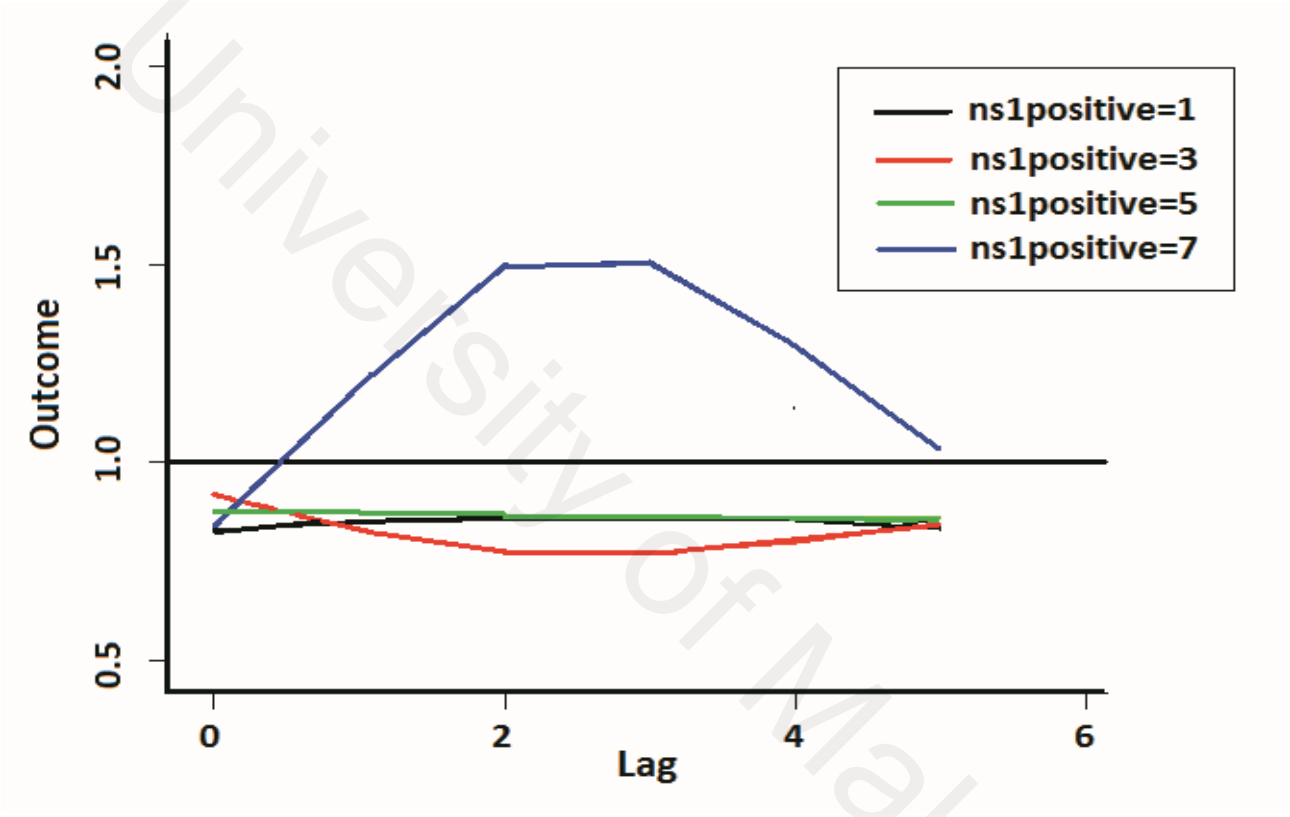

Figure 4.7. Plot of lag-response curves for different NS1-positive mosquitoes on dengue cases with reference line in NS1 positive (line at 1.0)

Table 4.2: Total pools and number of mosquitoes positive by weeks using NS1 Rapid Test Kit

Year	Week	NS1 Antigen Test					
		Aedes aegypti			*Aedes albopictus*		
		Total pools (mosquitoes tested)	Total pools positive (number of mosquitoes)	% Positive pools	Total pools (mosquitoes tested)	Total pools positive (number of mosquitoes)	% Positive pools
2013	wk47 - wk53 (7 week)	9 (46)	0 (0)	0.00	1 (1)	0 (0)	0.00
2014	wk1 - wk53 (53 week)	105 (475)	13 (56)	12.38	12 (30)	2 (2)	16.67
2015	wk1 - wk47 (47 week)	73 (319)	30 (135)	41.10	2 (6)	1 (1)	50.00
	Total:	187 (840)	43 (191)	22.99	15 (37)	3 (3)	20.00

mosquitoes Ae. aegypti (head and thorax) were tested individually using real-time RT-PCR, among them 35 were positive as follows: DENV1: 3; DENV2: 1; DENV3: 27; DENV2/DENV3: 3; DENV1/DENV3: 1 (Table 4.3). Three pools of mosquito (head and thorax) were negative. This negative phenomenon might be due to the fact that the virus was still only incubating in the midgut and had not been disseminated to the salivary glands, or due to degradation of RNA in the mosquitoes. Head and thoraces of mosquitoes from four negative pools were tested and shown to be negative by RT-PCR.

4.3.5 Comparison of the number of dengue cases and mosquito density by block

The total number of dengue cases distributed among the seven blocks is shown in Table 4.4. Figure 4.8 shows that the highest number of mosquitoes were obtained from block F (18.05% of the total) followed by block E (16.70%) and G (12.16%). However, the highest number of dengue cases were reported from block E (22.90%) followed by block G (21.02%) and F (13.34%). These three blocks (E, F, and G) are the newer phase which was constructed 1 year later in the year 2008 and located separately about 60 m from the other four blocks (A, B, C, and D).

ANOVA revealed that the dengue cases were significantly different between the blocks ($P < 0.05$) (Table 4.5), however further analysis using generalized linear mixed model (GLM) indicated that there was no statistical difference between the blocks (Table 4.6). However, for the distribution of mosquitoes using both the analysis ANOVA (Table 4.5) and GLM (Table 4.7) showed that there was a statistically significantly difference between the blocks. Block E showed a significantly higher number of mosquitoes compared to other blocks, while block B had the least mosquitoes.

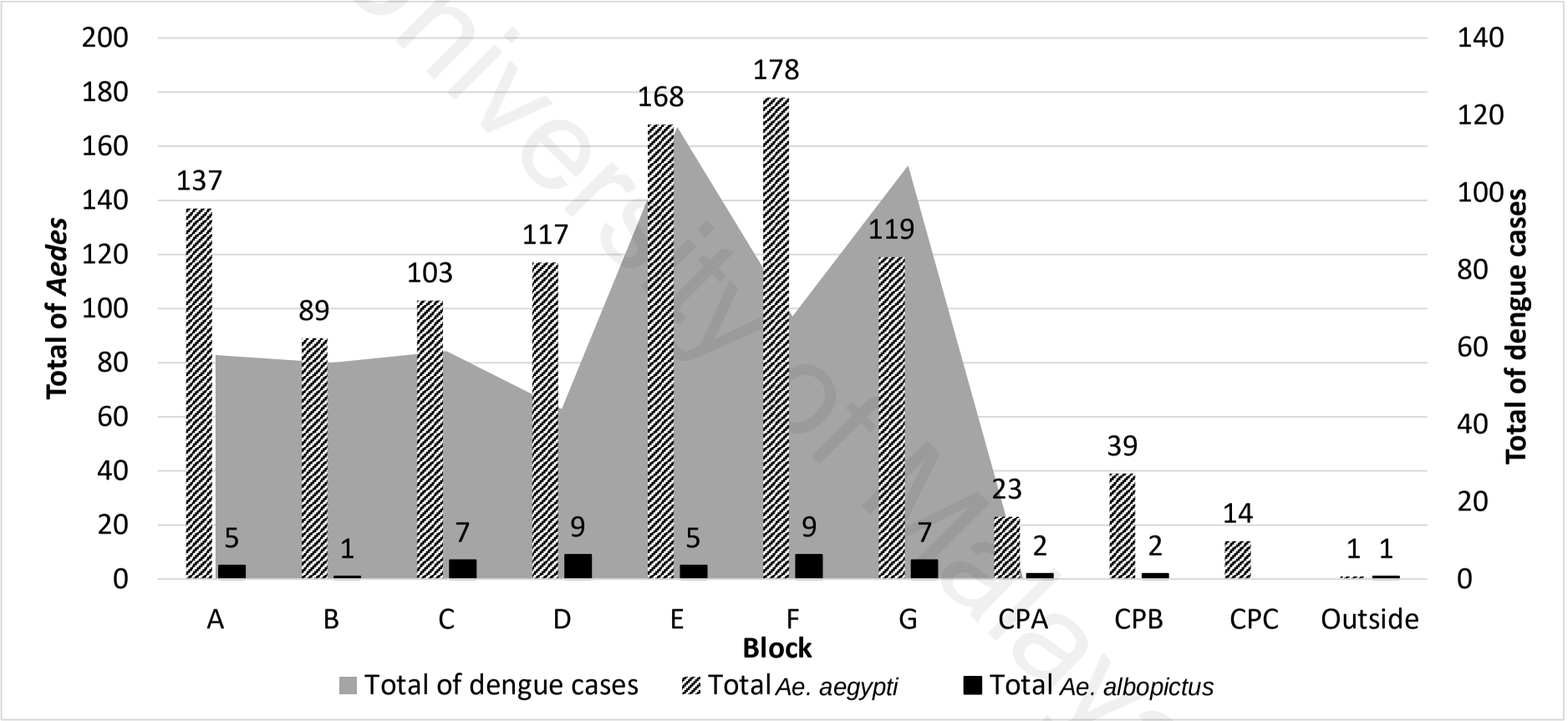

Figure 4.8. Distribution of dengue cases and mosquito density by blocks (A, B, C, D, E, F, and G) for 2 years, and 186 traps per week were set

Table 4.3: Mosquito pools tested by NS1 and RT-PCR

Pool	Not-tested	Positive in RT-PCR (at least one mosquito positive in the NS1 pool)	Negative in RT-PCR	Total
Positive NS1 pool	5	35	3	43
Negative NS1 pool	140	0	4	144
Total	145	35	7	187

Table 4.4: Cases of dengue in seven blocks in Mentari Court week 47, 2013 until week 47, 2015

Block	Cases	Floor	Cases	Floor	Cases
A	58	GF	26	9	30
B	56	1	35	10	24
C	59	2	29	11	29
D	44	3	37	12	28
E	117	4	28	13	22
F	68	5	34	14	27
G	107	6	31	15	21
		7	27	16	26
		8	23	17	32
Total	509		73		509

Table 4.5: One-way ANOVA with post-hoc Tukey HSD and generalized linear mixed model test for the comparison of dengue cases and mosquito density between blocks (A, B, C, D, E, F & G).

	DF	Sum Sq.	Mean Sq.	F value	Pr (>F)
Comparisons of dengue cases					
Difference between blocks	6	0.79	0.13205	3.004	0.00624
Residuals	5236	230.19	0.04396		
Total	5242	230.98			
Comparisons of mosquito density					
Difference between blocks	6	2.9	0.4812	4.643	0.000101***
Residuals	15722	1629.2	0.1036		
Total	15728	1632.1			

Significant codes: '***' for *P*< 0.001

Further analysis using generalized linear mixed model, result as follows. The model used is of the form "glmm < −glmmadmb (cases ~ block + floor+ (1|year), zero Inflation = T, data = data, family = Poisson)" (95% CI)

	Dengue cases		**Mosquito density**	
Block	estimate	*P*. value	estimate	*P*. value
A-B	-0.08407244	1.0000	0.251490221	0.6578
A-C	0.18365296	0.9972	0.171319657	0.9017
A-D	0.23590839	0.9899	0.065731983	0.9981
A-E	-0.65220731	0.1732	-0.382555246	0.1353
A-F	-0.28644346	0.9509	-0.335688856	0.1348
A-G	-0.45230953	0.6471	-0.009357604	1.0000
B-C	0.26772540	0.9960	-0.080170565	0.9998
B-D	0.31998083	0.9890	-0.185758238	0.9798
B-E	-0.56813487	0.7574	-0.634045467	**0.0495**
B-F	-0.20237102	0.9990	-0.587179077	0.0617
B-G	-0.36823709	0.9708	-0.260847825	0.8999
C-D	0.05225543	1.0000	-0.105587674	0.9993
C-E	-0.83586027	0.4324	-0.553874902	0.1319
C-F	-0.47009642	0.9160	-0.507008512	0.1189
C-G	-0.63596249	0.7235	-0.180677260	0.9733
D-E	-0.88811570	0.3680	-0.448287229	0.3317
D-F	-0.52235185	0.8709	-0.401420838	0.3096
D-G	-0.68821792	0.6505	-0.075089586	0.9995
E-F	0.36576385	0.9598	0.046866390	1.0000
E-G	0.19989778	0.9985	0.373197642	0.5669
F-G	-0.16586607	0.9995	0.326331252	0.5607

Table 4.6: Generalized linear mixed model fitted for the dengue cases data for 2013-2015

Block	No. of case per week	Floor	No. of case per week
A	0.0709[a]	Ground floor	0.0742[a]
B	0.0771[a]	3th floor	0.1057[a]
C	0.0590[a]	6th floor	0.0869[a]
D	0.0560[a]	9th floor	0.0856[a]
E	0.1361[a]	12th floor	0.0797[a]
F	0.0944[a]	15th floor	0.0611[a]
G	0.1115[a]	17th floor	0.0913[a]

The model used is of the form "glmm < −glmmadmb (cases ~ block + floor + (1|year), zero Inflation = T, data = data, family = Poisson)". Akaike Information Criterion (AIC) = 1727.95. Block means with different superscript letters indicate significant difference at $P < 0.05$ (5% level).

Table 4.7: Mean value of *Ae. aegypti* trapped per week from each block and each floor as predicted by the generalized linear mixed model

Block	No. of *Ae. aegypti* trapped per week	Floor	No. of *Ae. aegypti* trapped per week
A	0.1528[ab]	**Ground floor**	**0.4554[d]**
B	**0.1188[a]**	3th floor	0.1997[c]
C	0.1287[ab]	6th floor	0.1669[bc]
D	0.1431[ab]	9th floor	0.0940[ab]
E	**0.2240[b]**	12th floor	0.0946[a]
F	0.2138[ab]	15th floor	0.1030[abc]
G	0.1542[ab]	17th floor	0.1776[bc]

Different letters within the column indicate the means are significantly different at $P < 0.05$ by Tukey's test

4.3.6 Comparison of the number of the dengue cases and mosquito density by floor

The total number of dengue cases distributed by floors are shown in Table 4.4 and Figure 4.9. The ANOVA analyses shown in Table 4.8 indicated that there were no statistical differences in the total number of dengue cases between floors ($P < 0.05$) and the values ranged from 23 (floor 8) to 37 (floor 3) while floor 17 had 32 cases (Table 4.4) (Figure 4.9). Analysis using the generalized linear mixed model (GLM) also indicated that there was no significant difference for dengue cases between the floors (Table 4.6). In contrast, the ANOVA analyses shown in Table 4.8 indicated that the mean density of *Aedes* mosquitoes was statistically different between floors ($P < 0.05$) with the highest percentage of *Aedes* mosquitoes about 41.2% of *Ae. aegypti* and 61.36% of *Ae. albopictus* recorded from the ground floor. Highest percent *Ae. aegypti* mosquitoes positive with the virus also were caught from the ground floor (Figure 4.10). The generalized linear mixed model (GLM) analysis also showed that the number of mosquitoes caught was significantly different between floors (Table 4.7).

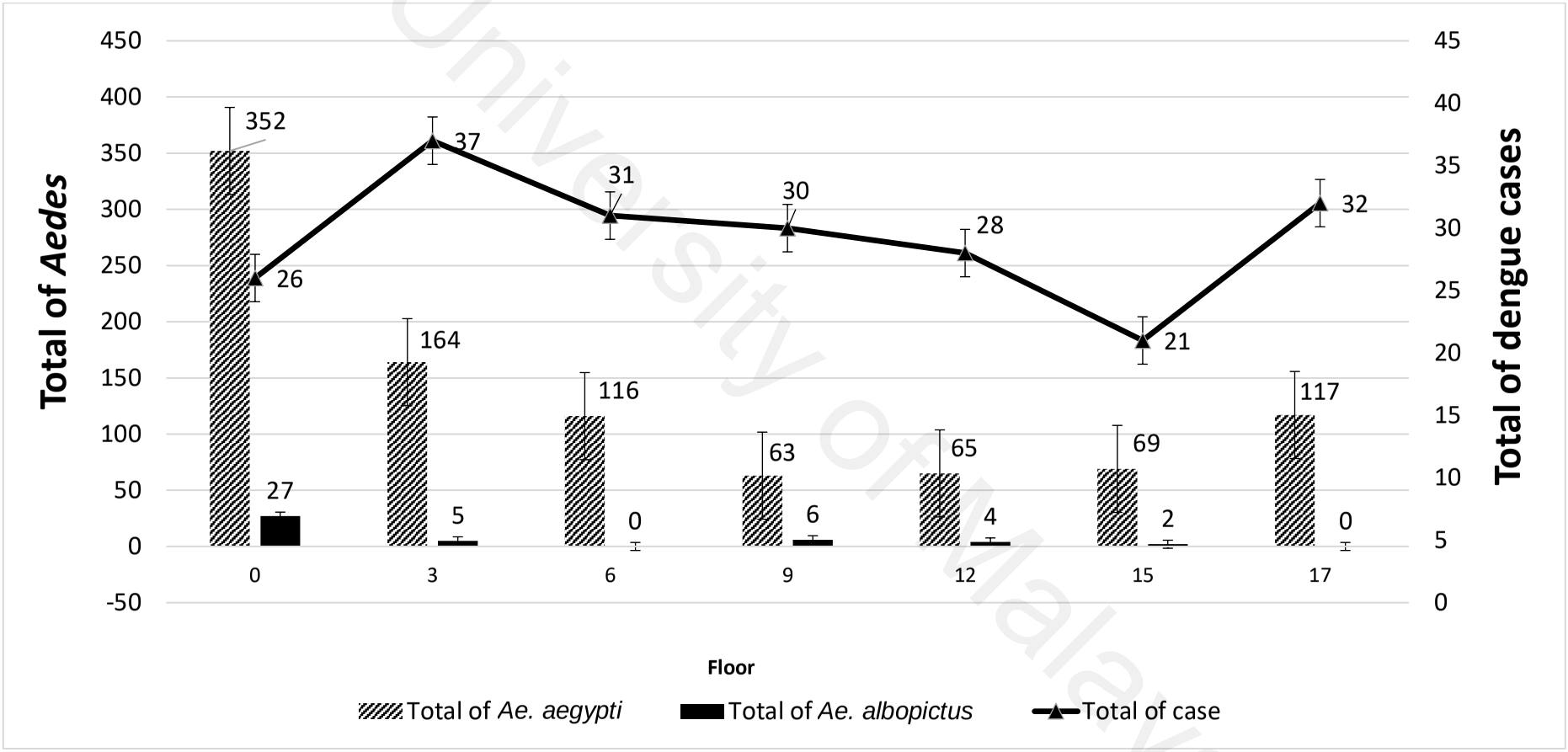

Figure 4.9: Distribution of dengue cases and mosquito density by floor (GF, 1st, 3rd, 6th, 9th, 12th, 15th and 17th)

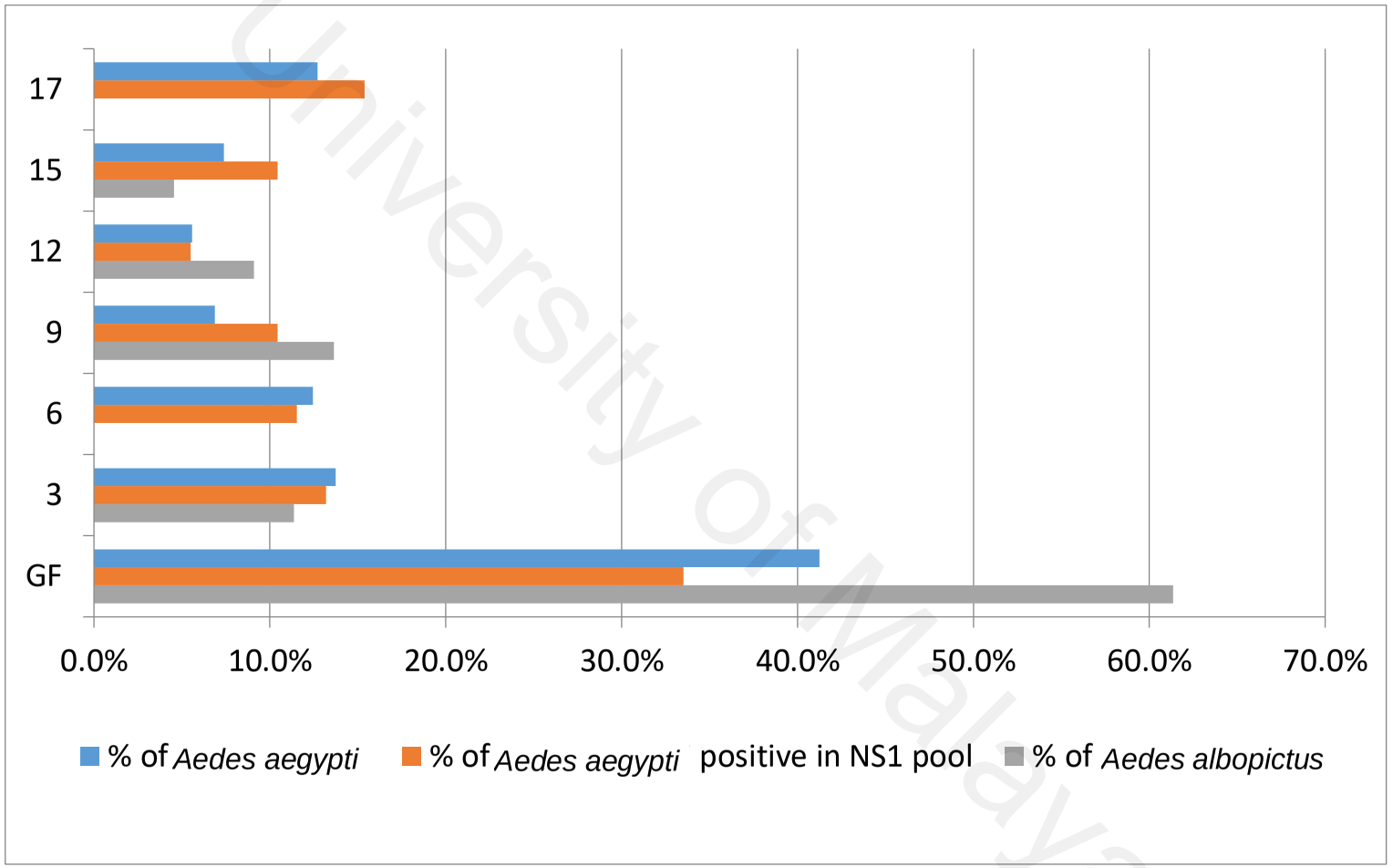

Figure 4.10: Percentage of female *Ae. aegypti* and *Ae. albopictus* caught as well as the percent of positive *Ae. aegypti* in NS1 pool test on each floor. This is calculated based on the total number of female mosquito captured for all seven blocks (A, B, C, D, E, F, and G).

Table 4.8: One-way ANOVA with post-hoc Tukey HSD and the generalized linear mixed model test for the comparison of dengue cases and mosquito density between floors (GF, 1st, 3rd, 6th, 9th, 12th, 15th and 17th)

	DF	Sum Sq.	Mean Sq.	F value	Pr (>F)
Comparisons of dengue cases					
Difference between floors	6	0.2	0.03370	0.764	0.598
Residuals	5236	230.8	0.04408		
Total	5242	231.0			
Comparisons of mosquito density					
Difference between floors	6	27	4.502	44.09	<2e-16 ***
Residuals	15722	1605	0.102		
Total	17728	1632			

Significant codes: '***' for $P< 0.001$

Further analysis using generalized linear mixed model, result as follows. The model used is of the form "glmm < −glmmadmb (cases ~ block + floor+ (1|year), zero Inflation = T, data = data, family = Poisson)" (95% CI)

	Dengue cases		**Mosquito density**	
Floors	estimation	*P*. value	estimation	*P*. value
12th-15th	0.26653176	0.9749	-0.085004571	0.9993
12th-17th	-0.13537281	0.9989	-0.629768718	**0.0029**
12th- 3rd	-0.28230339	0.9372	-0.747014435	**0.0001**
12th- 6th	-0.08568103	0.9999	-0.567800385	**0.0108**
12th- 9th	-0.07084963	1.0000	0.006474767	1.0000
12th-GF	0.07181487	1.0000	-1.571369152	**<.0001**
15th-17th	-0.40190458	0.9559	-0.544764147	0.2915
15th-3rd	-0.54883515	0.8217	-0.662009864	0.1105
15th-6th	-0.35221279	0.9782	-0.482795814	0.4500
15th-9th	-0.33738139	0.9824	0.091479338	0.9999
15th-GF	-0.19471689	0.9992	-1.486364581	**<.0001**
17th-3rd	-0.14693057	0.9997	-0.117245717	0.9988
17th-6th	0.04969178	1.0000	0.061968333	1.0000
17th-9th	0.06452318	1.0000	0.636243485	0.1317
17th-GF	0.20718769	0.9984	-0.941600435	**0.0007**
3th-6rd	0.19662235	0.9985	0.179214050	0.9858
3th-9rd	0.21145375	0.9979	0.753489202	**0.0313**
3th- GF	0.35411826	0.9698	-0.824354718	**0.0055**
6th- 9th	0.01483140	1.0000	0.574275152	0.2153
6th - GF	0.15749590	0.9997	-1.003568768	**0.0003**
9th - GF	0.14266451	0.9998	-1.577843919	**<.0001**

4.3.7 Percentage positive of traps between locations

Results of the ANOVA analysis for the comparison of the GOS index and ovitrap index between GOS trap location is shown in Table 4.9 and Table 4.10 which demonstrated statistically significant difference ($P < 0.05$) for both indexes. The ANOVA analysis showed that about 84.6% of the GOS and 95.2% of the ovitraps were significantly different from each other. It was noted that the highest number of *Ae. aegypti* caught per GOS trap for two years was from A-GF-1 (Block A, Ground Floor) and F-GF-1 (Block F, Ground Floor) which contributed about 3.95% (39 mosquitoes) of the total *Ae. aegypti* caught. The highest number of *Aedes* eggs collected from ovitrap number MC 7 (Block A, Ground Floor) contributed about 5.34% (3,864 eggs) of total eggs collected. The GOS number D-GF-1 (Block D, Ground Floor) trapped the highest number of mosquitoes per collection with 8 *Ae. aegypti* in week 10 of the year 2015 (collection on 17 Mac 2015). However, the highest frequency of GOS trapped mosquitoes was obtained from the F-GF-1 (Block F, Ground Floor) with 16 times positive collection (Appendix C, a-c). Figure 4.11 and Figure 4.12 shows the pattern of traps on the ground floor as well as the number of *Aedes* mosquitoes and mosquito eggs. Nevertheless, it was found that the GOS trap from 17th floor showed the second highest number of mosquito caught and eggs collected.

Table 4.9: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage GOS trap positive between GOS traps

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between GOS trap	185	32.8	0.17739	(-0.3652052, 0.3652052)	4.625	<2e-16 ***
Residuals	19716	756.1	0.03835			

Significant codes: '***' for *P*< 0.001

Table 4.10: One-way ANOVA with post-hoc Tukey HSD test for the comparison of percentage ovitrap positive between ovitraps

	DF	Sum Sq.	Mean Sq.	95% CI	F value	Pr (>F)
Difference between ovitraps	61	208.1	3.411	(-0.9271607, 0.9195960)	15.62	<2e-16 ***
Residuals	6644	1450.4	0.218			

Significant codes: '***' for *P*< 0.001

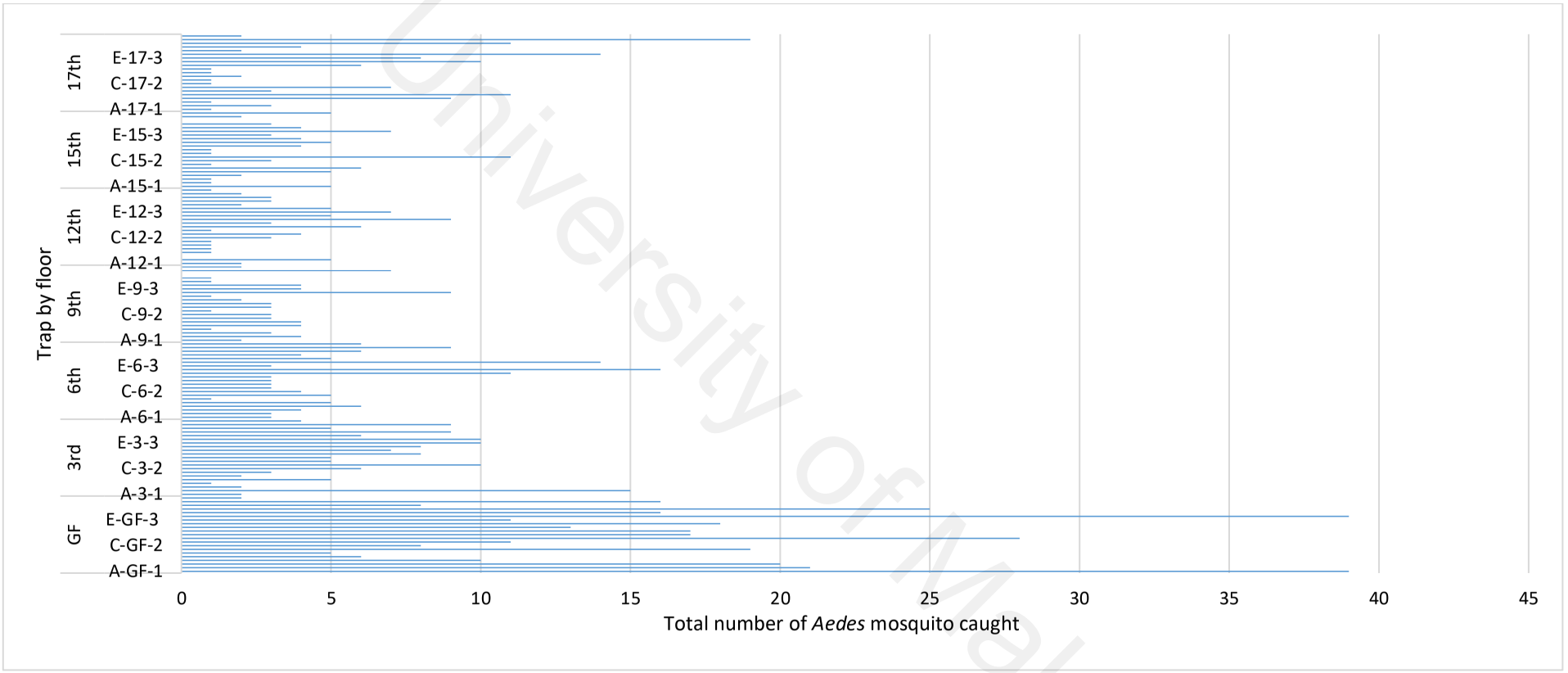

Figure 4.11: Total number of *Aedes* mosquitoes caught by using GOS traps set on seven floors (GF, 1st, 3rd, 6th, 12th, 15th and 17th) for seven blocks (A, B, C, D, E, F, and G)

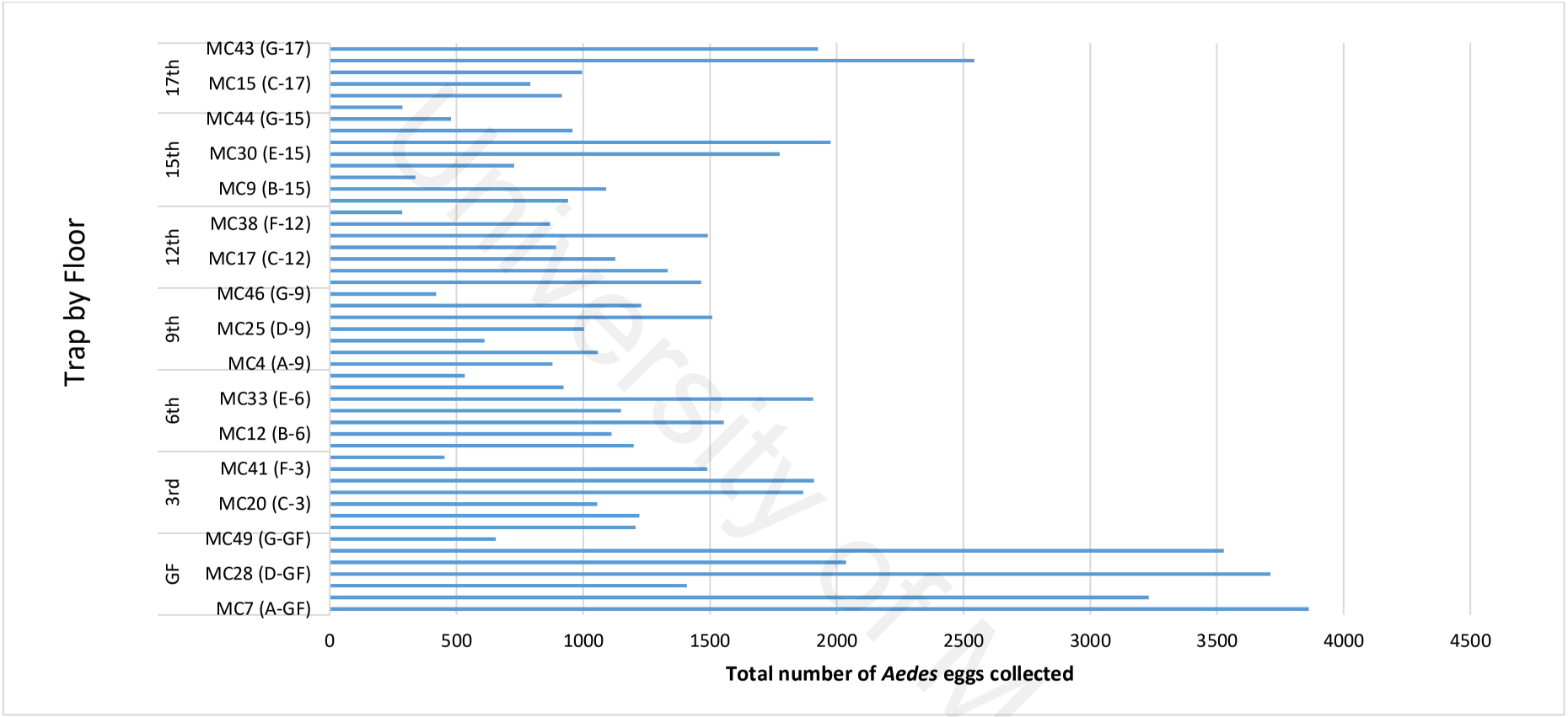

Figure 4.12: Total number of *Aedes* eggs collected using ovitraps set on seven floors (GF, 1st, 3rd, 6th, 12th, 15th and 17th) for seven blocks (A, B, C, D, E, F, and G)

4.3.8 Comparison of GOS trap and traditional ovitrap

(a) Percentage positive of traps

Figure 4.13 shows the GOS trap and ovitrap indices. The percentage of GOS traps positive was lower than ovitraps index because a single mosquito can lay eggs in many ovitraps. The percentage of GOS traps positive ranged from 0.54 to 13.44% while that of ovitrap ranged from 12.9 to 86.21%. Pearson correlation analysis indicated a statistically significant relationship between the percentage of positive GOS traps and ovitrap, [r(105)=+0.476, P <.05, 95% CI 0.3149804 – 0.6109560].

(b) Density of Ae. aegypti and eggs per trap

The density of *Ae. aegypti* and density of eggs per trap is shown in Figure 4.14. Both densities showed the same trend with the significant relationship between density of eggs per trap and density *Aedes* per trap given as r=0.445397, df = 105, p < 0.05, 95% CI 0.2335243 – 0.5527235. The number of eggs collected per week ranged from 94 to 3,522 eggs (total number of eggs=83,976), and the number of eggs per trap ranged from 1.52 to 56.81 eggs per trap. However, the number of *Aedes* collected per week per 186 traps set ranged from 1 to 47 mosquitoes with the density of *Ae. aegypti* per trap ranging from 0.01 to 0.25. In this study, 81 eggs on average were collected per *Aedes* mosquito. A total of 173 female *Aedes* were randomly checked for their gravid status and about 32.95% were gravid, 5.78% had eggs formed in the abdomen, 5.20% had blood in the abdomen and mostly (about 56.07%) were non-blood fed *Aedes*. However, Chadee & Ritchee (2010b) showed that most of the females collected by sticky trap were parous (99%) with many older females collected. It could display "death stress oviposition" behavior when trapped in glue.

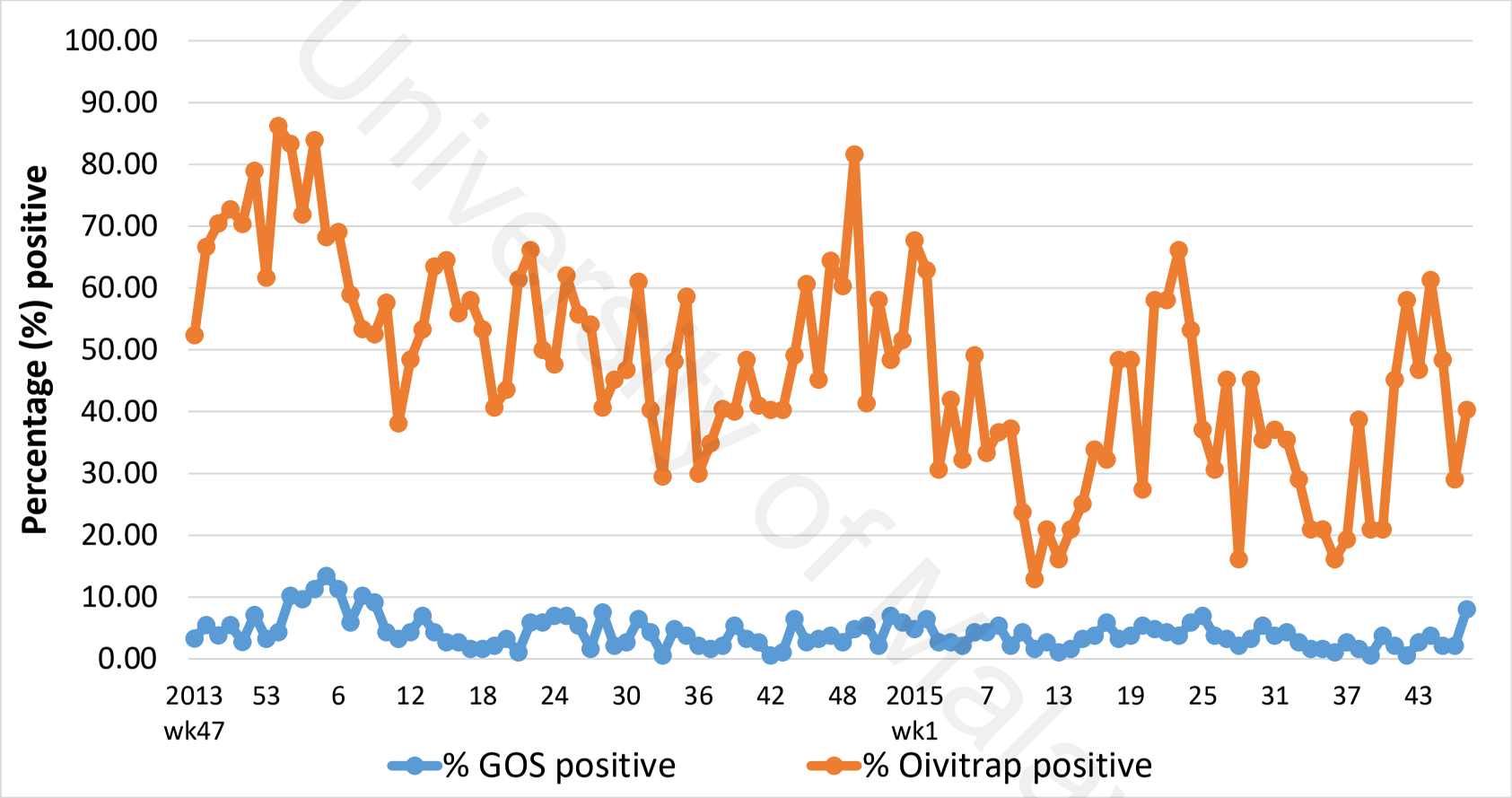

Figure 4.13: GOS trap index and ovitrap index (percentage positive) over the 2 years of the study

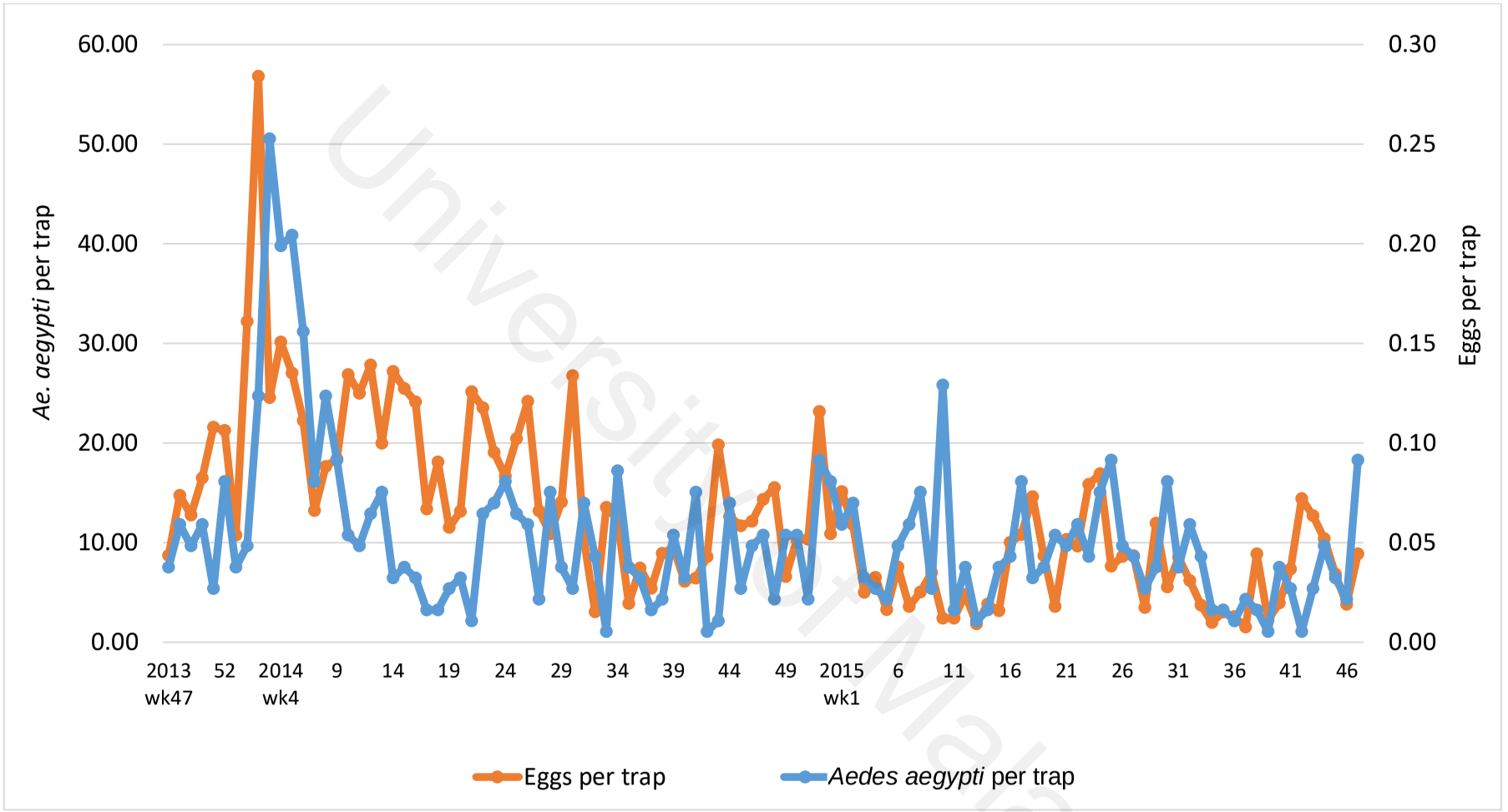

Figure 4.14: *Ae. aegypti* per trap and eggs per trap over the 2 years of the study

4.4 Discussion

Dengue has become a serious public health problem and it is obvious that the current surveillance and control measures being instituted are no longer effective (Morrison et al., 2008, Reiter et al., 1997, Chang et al., 2011; Ong, 2016). There is an urgent need to switch from larval surveys and focus on adult mosquitoes for surveillance. This two years study showed that there was no significant relationship between the number of dengue cases and *Aedes* caught or the correlation between the lag time (2, 3 and 4 weeks). In Colombia, there was lack of association between the *Aedes* index or mosquito density and dengue incidence (Peña-García et al., 2016). However, some studies did show a positive relationship between adult mosquito density and dengue fever cases in Jeddah using light traps (Alshehri, 2013), Belo Horizonte in Brazil using MosquiTRAPs (de Melo et al., 2012), São Paulo, Brazil using manual aspirators (Dibo et al., 2008) and in Puerto Rico using BG trap (Barrera et al., 2011). However, as stated by Barrera and colleagues, trend for peaks of mosquito density may not necessarily be associated with a large increase in dengue incidence (Barrera et al., 2011). In this study, it was observed that during certain peaks of dengue incidence in December 2014 – February 2015, and July 2015 – September 2015, there was a low density of *Ae. aegypti*. It has also been shown larger number of dengue cases occurred after 80 days of high *Aedes* density from MosquiTRAP, and for ovitrap index was after about 200 days (de Melo et al., 2012).

In this dengue hotspot locality in Selangor the dengue cases ranged from 165 – 320 cases and the number of *Ae. aegypti* per trap using GOS trap in the dengue hotspot locality ranged from 0.01 – 0.25 (total *Ae. aegypti* female=840) and the number of eggs per trap per week ranged from 1.52 – 56.81 (total eggs=83,976). The highest number of mosquitoes caught per trap was 39 for the two years study. This range is almost similar

to the results shown in Singapore which used Gravitrap in dengue cluster areas and can captured about 0.022 – 0.167 *Ae. aegypti* females per trap per week (Lee et al., 2013). This shows that even a small number of *Ae. aegypti* is sufficient to cause outbreaks since one infected mosquito takes blood from many people during blood feeding as it is easily disturbed and flies from one host to another host (Carrington & Simmons, 2014). In other countries like Brazil 0.21 *Ae aegypti* females per trap per week collected using MosquiTRAP (MQT) during low dengue transmission period (Degener et al., 2015). In essence although many studies have been carried out using different traps to capture *Aedes* mosquitoes it was difficult to predict dengue outbreaks based on just adult mosquitoes (Barrera et al., 2011, de Santos et al., 2012, Barrera et al., 2014, de Melo et al., 2012). The lack of correlation between mosquito population and dengue could be due to underestimation of incidence data during epidemics (Zeidler et al., 2008).

However, some are using *Aedes* index based on *Ae. aegypti* females in MosquitTrap as surveillance tool to access for the risk of dengue (Eiras & Resende, 2009). An index of < 0.2 indicated risk free areas, between 0.2 – 0.4 indicates areas on alert, and > 0.4 indicates areas at risk (Eiras & Resende, 2009).

Ritchie et al. (2004) proposed the uses of a sticky ovitrap index (mean number of female *A. aegypti* per trap per week) where more than one female per trap per week represents an increase in dengue transmission and less than one female per trap per week represents a decrease in transmission. Barrera et al. (2011) reported that the levels of *Ae. aegypti* females per BG trap or the number of eggs per ovitrap should be reduced below two and ten respectively to prevent dengue transmission. While according to Mogi et al. (1988), the number of eggs in ovitraps two or less can cease dengue hemorrhagic fever cases in Chiang Mai. However, the present study, Mentari Court apartment has reported

dengue cases throughout the years with the number of *Ae. aegypti* per trap per week ranging from 0.01 to 0.25 could be proposed as being at risk for dengue transmission.

Thus, although effort has been made to rely on adult indices instead of larval indices, it still does not serve as a good surveillance tool where action can be taken before epidemics occur. Since adult *Ae. aegypti* can be easily trapped using simple cheap traps, it is essential to test the mosquitoes for dengue virus using NS1 kit as it is easy and quick. There is a relationship between the number of pooled positive mosquitoes and the number of dengue cases, with 1-week lag effect and the highest at 2-3 weeks lag as shown by this study. Thus, further analysis using data from infected mosquitoes improved prediction accuracy of the incidence of dengue showing there was a relationship between both variables. The staff of health department should take the necessary action to inform the people in the surrounding area to take action to clean up the surrounding areas and also to seek treatment if they fall ill. Peña-García et al. (2016) also reported that the density of mosquitoes was not a good predictor of the incidence of dengue owing to the weak association between the density of mosquitoes and their infection with DENV. Studies have been shown that *Ae. aegypti* can pick up dengue virus when biting asymptomatic or oligosymptomatic subjects (Nguyen et al., 2013) resulting in silent transmission from humans to mosquitoes. This might explain why dengue epidemics are on the rise. An important finding by Lien et al. (2015) in Vietnam showed that *Ae. aegypti* formed 95% of the mosquitoes in houses of dengue patients and were also positive by RT-PCR. Thus, the virus infection in mosquito can be considered as an index to determine dengue epidemic. Several reports demonstrated the relationship between dengue outbreak and virus infection in *Ae. aegypti* mosquitoes. This correlation seems to be more practical and effective tool to predict dengue for planning dengue control (Chompoosri et al., 20012, Kittichai et al., 2015, Thavara et al., 2006).

The present study indicated that the detection of dengue positive mosquito will give rise to dengue cases after a lag of one week. This observation leads credence to our hypothesis that one way forward for dengue surveillance is the use of GOS trap coupled with the use of NS1 antigen kit for the detection of the virus in mosquitoes. The sensitivity of NS1 antigen kit on mosquitoes containing the virus has been established to be high (95%) (Tan et al., 2011). According to Sylvester et al. (2014) the NS1 antigen kit has higher sensitivity compared to the qRT-PCR and virus isolation on dried *Aedes* mosquitoes. Similar result by Voge et al. (2013), which showed NS1 antigen kit (Platelia Dengue NS2 Ag) detected 98% infected mosquitoes compared to 79% by RT-PCR and 29% by virus isolation.

In the 1980s and 1990s, DENV was detected in individual or pooled mosquitoes by immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) for viral antigens, by reverse transcription –polymerase chain reaction (RT-PCR) for virus or by isolation of infectious virus (Samuel & Tyagi, 2006). However, surveillance DENV in mosquito by using these diagnostic techniques can be prohibitively expensive, may require special reagents, laboratory facilities or equipment or extensive training of personnel, and may be laborious and time consuming. Tests such as virus isolation and RT-PCR can become more complicated for pathogen detection if the sample contains particulates and environmental contaminants. Besides, field-relevant conditions such as mosquito traps are only inspected for an extended period and in the remote locations, mosquito samples subjected to cycles of freezing and thawing during identification, pooling, processing and assaying can result in infectious virus inactivation or destruction of viral analysis (Van den Hurk et al., 2012). RT-PCR was widely used for detection of arboviruses including DENV (Garcia-Rejon et al., 2008), it can detect dengue virus RNA in mosquitoes captured over a period of 28 days on sticky lure traps (Bangs et al., 2001) and detect one infected mosquito in pool of up to 59 negative mosquito head (Chow et

al., 1998). Although RT-PCR is an excellent test, however, it is expensive and requires trained personal, specialized equipment and laboratory facilities (Samuel & Tyagi, 2006). However, now the ideal method for DENV surveillance in vectors is available which is simple to perform, rapid, cost-effective, specific and capable of detecting the pathogen under field conditions.

Tan et al. (2011) were the first to demonstrate that antigen detection kits (Dengue NS1 Ag strip®) designed to detect DENV nonstructural protein 1 (NS1) in human serum also could be used in laboratory-infected *Ae. aegypti* and in the wild-caught mosquito population in Singpore. Dengue virus NS1 antigen was detected in mosquitoes 10 days after infection in the laboratory with DENV serotypes 1, 2, 3 or 4, as well as in field-collected DENV-infected mosquitoes. The test was as sensitive as real-time RT-PCR in detecting DENV infected mosquitoes. Thereafter, several types of NS1 test kits were tested, e.g. Panbio Dengue Early ELISA from Australia proved to be sensitive and can detect DENV in pools of up to 50 mosquitoes at Days 0, 5 and 15 post infection (PI) (Muller et al., 2012).

In the present study, there was a significant association between the ovitrap index and the GOS trap index, as well as between the densities of *Ae. aegypti* and the egg density of trap (Phase 2 study) conversely, there was no-statistically significant association in the Phase 1 study. Perhaps, the longer study period and larger sampling size provided better results. The percentage of GOS trap positive (13.44%) was always lower than that of the ovitrap (86.21%). The high ovitrap index was observed because *Aedes* mosquitoes exhibit "skip oviposition", they lay eggs from a single gonotrophic cycle at several sites (Harrington & Edman, 2001; Williams et al., 2008b; Apostol et al., 1994; Nazni et al., 2016). Varied results have been obtained in studies comparing ovitraps and sticky traps. Some have shown that both traps provide similar positivity rates (Fávaro et al., 2006;

Ritchie et al., 2003). However, other studies obtained similar results as this study where positivity of ovitrap was higher than sticky trap (Chadee & & Ritchie, 2010a; de Santos et al., 2012; Fávaro et al., 2008). Most also found there was a correlation between the number of eggs in the ovitraps and *Aedes* females captured by traps (Barrera, 2011; de Santos et al., 2012), however in Trinidad that was not the case (Chadee & Ritchie, 2010a). Although previous studies showed ovitraps were useful indicators for the presence of *Aedes* mosquitoes (Dhang et al., 2005; Dibo et al., 2008; Fávaro et al., 2008; Focks, 2003), the association between ovitraps and dengue cases has not been established. Therefore, the ovitrap index is not a useful indictor for surveillance. Besides, ovitrap provide an infected mosquito a place to lay eggs as well as to continue infecting people. The present study also shows that the GOS trap was as effective as the standard ovitrap in detecting *Ae. aegypti* with both showing a significant association. Thus, GOS traps could be used as vector surveillance tool. On the other hand, the advantages of the GOS trap are that it traps the gravid mosquito which can then be used for virus detection and also the infected mosquito will be captured and not able to transmit the virus, thus breaking the chain of transmission.

It was found that the dengue cases still occur although the GOS trap index was as low as 1.0%, conversely the ovitrap index was always above 10.0% which is the risk threshold set by the Ministry of Health Malaysia (KKM, 1986). This study revealed that there was no relationship between the number of dengue cases and the number of trapped *Aedes*. Outbreaks of dengue occurred during March 2015 and August-September 2015, although density of *Aedes* was low.

House to house *Aedes* larval surveys followed by source reduction and larviciding remain as the main tools for dengue control in most of the countries in Southeast Asia including Malaysia (Chang et al., 2011). These main control strategies for dengue have

not changed since their inception in the 1970s (Chang et al., 2011). In Phase 1, two teams of the health department staff were only able to inspect 40 premises (larval surveys) per day. It has been documented that these methods are not effective but they are still being used (Bowman et al., 2014; de Melo et al., 2012; Sulaiman et al., 1996). Gama et al. (2007) found that approximately 10% MosquitTRAPS were positive whereas the House Index was negative. Indices based on immature forms of the vector were found to be inadequate for the prediction of virus transmission (Focks, 2003). Similar observation by Coelho et al. (2008) revealed that it was not a reliable predictor of the incidence of dengue.

Although sticky trap can be applied as an index to initiate traditional control, but it can also be used as a good and cheap alternative to trap *Ae. aegypti*, however their ability to suppress *Aedes* population is variable. In Brazil (Degener et al., 2014) no reduction in the *Aedes* population was detected in the treated areas while in Puerto Rico they managed to suppress the *Ae. aegypti* population (Barrera et al., 2014). However, a comparative study in the parts of Brazil using various traps and comparing them to regular house surveys found that the traps produced better results compared to *Aedes* house index (Codeço et al., 2015). Thus, it is more important in dengue-prone areas to test the mosquitoes for dengue virus and institute control measures when positive mosquitoes are obtained. It would be more cost-effective to setup the GOS traps and monitor the adult population for dengue virus. As suggested one way forward is a package of proactive measures that aim to prevent, diminish or eliminate dengue transmission (Achee et al., 2015a). The study in Thailand using RT-PCR to detect the dengue virus in mosquitoes also showed a positive association between infected *Ae. aegypti* and dengue-infected children (Yoon et al., 2012). Their study demonstrated the occurrence of an infected mosquito prior to the reporting of the index case (s). It has been stated recently that dengue virus transmission varies from year to year and place to place making vector control interventions difficult (Reiner et al., 2016), thus it is the time for new measures to

be introduced for dengue control instead of relying on reactive tools. The GOS traps could at least be introduced in the hotspot areas where dengue outbreaks occur. This GOS trap could also be used in public places such as transportation hubs (train station, bus stops, schools etc.) recreation areas and commercial areas as viral-positive *Aedes* have also been obtained from these areas (JKNS, 2016).

The study site was the most problematic dengue hotspot in Malaysia, predominated by *Ae. aegypti* (95.6%), which is recognized as a primary vector (Chen et al., 2006; Higa et al., 2010); it can also be a major vector for transmission of Zika virus (Manzoor et al., 2017). There was a significant difference between the blocks for *Aedes* mosquito density but not for the number of dengue cases. In this study, more mosquitoes were obtained from Block E, F, and G, which were built in a later phase. The abundance of *Ae. aegypti* females in certain location are associated with the heterogeneity of the availability of human blood meals and containers for laying eggs. Dispersal of female mosquitoes is reduced in the areas with geographical barriers that limit their flight from 50 to 300 meters over their entire lives, hence they would not often migrate beyond the block where they initiated their activities (Harrington et al., 2005). This study also revealed that the spatial density of the mosquito population can significantly contribute to higher incidence of dengue, therefore the target blocks could be identified by the local health authorities for taking concerted effort to reduce and eradicate mosquitoes in these blocks. Besides, it was found that the GOS traps set nearby stagnant water were more attractive to mosquitoes for laying eggs. Although, Aziz et al. (2014) observed that the types of land use did not influence the population of mosquito within six zones in Kuala Lumpur area, while water (r=0.246, *P*=0.016) had higher correlation with the spatial density of mosquito as compared to the Built-up area (r=0.16, *P* =0.118), cleared area (r

= -0.107, *P* = 0.304), dense vegetation (r=-0.206, *P* = 0.046), or sparse vegetation sparse (r=0.023, *P* = 0.823).

In this study, mosquitoes could be obtained from all floors up to the highest 17th floor, but the significantly higher *Aedes* mosquitoes were caught from the ground floor (41.2% *Ae. aegypti* and 61.36% *Ae. albopictus*). However, there was no significant difference of dengue case distribution by floors. A study by Lau et al. (2013) in Selangor and Kuala Lumpur in Malaysia also showed that the *Aedes* mosquitoes could be found from the ground floor to the highest floor of a multiple storied building; where no significant difference in density was observed between floors. Nevertheless, a study in a high-rise apartment in Putrajaya Malaysia showed that *Ae. aegypti* were mostly obtained from level 6 and were only observed up to the 10th floor, while *Ae. albopictus* was found only up to the 6th floor (Wan-Norafikah et al., 2010). A gravitrap study in Singapore found a higher percentage (64.91%) of mosquitoes trapped on floors 2-6 than floors 7-13 (35.09%) (Lee et al., 2013). In the present experiment, the *Ae. aegypti* were also found breeding in the water tanks on the roof top which could explain the higher number of *Ae. aegypti* on floor 17. However, the dengue infection could occur in any of the floors.

This chapter describe the relationship between vectors, infected vectors and dengue cases in the endemic dengue site. In addition, it showed that the GOS trap could be used effectively for trapping mosquitoes. Infected mosquitoes, instead of the density of mosquito and ovitrap index could play a better role in the development of risk modelling for predicting dengue cases. Thus, this present study suggests one way forward as a package of proactive measures that aim to prevent, diminish or eliminate dengue transmission.

CHAPTER 5: ADULT *AEDES AEGYPTI* AND DENGUE CASES IN RELATION TO ENVIRONMENTAL FACTORS

5.1 INTRODUCTION

The *Aedes* mosquitoes are highly sensitive to the environmental conditions. The environmental condition such as temperature, humidity, and precipitation are the critical issues for mosquito survival, reproduction and development. The environmental or meteorological condition can influence the presence and density of adult mosquitoes. Hence, the effect of the environmental condition on the surveillance of *Aedes* mosquito will be discussed in this study (Chapter 5).

Several studies showed that warmer climate leads to a large mosquito population and increase in dengue transmission (Dibo et al., 2008; Estallo et al., 2008; Paul & Tham, 2015; Walton & Reisen, 2014). Higher temperature affects mosquito parity rate and longevity (Goindin et al., 2015), by decreasing the development time and size of the adults (Alto & Juliano, 2001; Tun-Lin et al., 2000). While, high humidity and rainfall can increase the productivity of the environment owing to the increasing number of potential of breeding sites (Favier et al., 2006). High relative humidity along with high temperature and heavy rainfall also have positive influence on the survival rate besides increasing the breeding places (Hales et al., 2002). Whereas some studies showed that rainfall was not a strong predictive indicator of *Ae. aegypti* abundance compared to other variables (Azil et al., 2010; Wu et al., 2007). This may be attributed to the manually filled containers (e.g. pot plants saucers) in local *Ae. aegypti* population dynamics (Barrera et al., 2011; Beebe et al., 2009; Williams et al., 2008a). Nevertheless, some studies also showed the

significant effects of rainfall on entomological indices and dengue incidence (Barrera et al., 2011; Chadee et al., 2007; Moore et al., 1978; Sirisena et al., 2017).

Climate factors also interfere with the efficiency of vector in transmitting dengue, for example increase in ambient temperature can increase virus transmission in vector population (Bangs et al., 2006), reducing the extrinsic incubation period, increasing the replication rate of the virus (Watts et al., 1986), increasing the number of blood meals during a gonotrophic cycle (Dibo et al., 2005) and faster dissemination rate (Parham et al., 2015). Temperature was found as a strong dependent variable for outbreak of dengue epidemics (Liu-Helmersson et al., 2014). However, a large diurnal temperature range of 18.6°C to a 26°C mean resulted in low dengue virus transmission in northwestern Thailand, due to reduced midgut infection rates and extended virus extrinsic incubation period (Carrington et al., 2013b). A similar result also showed large temperature fluctuation also reduced the probability of vector survival through extrinsic incubation period and expectation of infectious life (Lambrechts et al., 2011) However, high humidity was found to contribute to increase virus replication (Focks et al., 1993).

Studies on relationship between climatic conditions, dengue cases and vectors have produced varied results, thus limiting its use for dengue vector surveillance. Hence in this study it was attempted to determine the effect of environmental conditions on *Aedes* density, on infected mosquitoes and dengue cases at the microhabitat.

5.1.1 Objectives of the study

5.1.1.1 General objectives

To assess the influence of meteorological variables on the abundance of adult *Aedes* mosquito and dengue cases.

5.1.1.2 Specific objectives

1) To study the effect of rainfall, temperature, and humidity on *Aedes* density (adult and eggs) and dengue cases at the micro-level.

2) To determine correlation of dengue cases in relation to meteorological factors and infected mosquitoes.

5.1.2 Research hypotheses

1) Ho: Meteorological parameters such as temperature, humidity, and rainfall do not affect the density of *Aedes* mosquitoes.
2) Ho: Meteorological parameters such as temperature, humidity, and rainfall do not affect the number of reported dengue cases.
3) Ho: Meteorological parameters such as temperature, humidity, and rainfall do not affect the infectivity of *Aedes* mosquitoes.
4) Ho: Meteorological parameters such as temperature, humidity, and rainfall do not affect the number of *Ae. aegypti* eggs/ovitrap and ovitrap index.

5.1.3 Significance of the study

1) A two-year data study will enable us to determine the relationship among the vectors, dengue cases and climatic condition in the endemic dengue site.
2) This experiment will be helpful in determining if climatic conditions can be used as a surveillance tool for dengue vector control.
3) This study will enable us to know whether climatic conditions can increase the number of infected mosquitoes.

5.2 Materials and Methods

5.2.1 Study site

Detail information on the study site has been described in **Chapter 3**.

5.2.2 GOS trap

The GOS trap (gravid mosquito oviposition in the sticky trap), a type of mosquito trap used in this study has also been described in **Chapter 3**.

5.2.3 Field sampling

Phase 2

Study of the relationship between climatic factors with the density of mosquitoes and dengue cases was conducted in Phase 2 study. Phase 2 study was conducted for 2 years, from 14 November 2013 to 4 December 2015. A total of 186 GOS trap were set on the selected 7 floors (GF), 3rd, 4th, 9th, 12th, 15th and 17th floor) of all 7 blocks (Block A, B, C, D, E, F and G) description of the field sampling methods in phase 2 has to be referred to **Chapter 4**.

5.2.4 Identification and processing of the mosquitoes

In the laboratory, the mosquitoes were identified morphologically up to the species level. A pair of heat sterilized forceps was used to remove the mosquitoes from the sticky surface to prevent cross contamination. Detail of the processing of the

specimens has been described in Chapter 3. The procedure for the subsequent test to detect dengue viral antigen by using SD Bioline NS1 antigen kit and RT-PCR in the mosquito were described in Chapter 3, and the RT-PCR of the mosquito has been described in Chapter 4. However, mosquito eggs were counted from the paddles after ovitraps were collected from the field and the paddle was dried at room temperature for 2 days. The stereo microscope was used for checking and count the eggs.

5.2.5 Data of dengue case in the Mentari Court Apartment

Methods to collect the data of dengue cases have been described in **Chapter 4**.

5.2.6 Meteorological data

Data of weekly rainfall was obtained using rain gauge RGR126 (Oregon Scientific Inc., Oregon, USA) in the study site. The maximum and minimum measures of temperature and humidity were obtained from the nearest meteorological station (Section 9, Petaling Jaya) located 5 km from the study site.

5.2.7 Statistical analysis

Statistical analyses were carried out using weekly data and R programming language for statistical analysis (version 3.2.4) (R Development Core Team, 2008) and Excel 2010. This analysis used a weekly number of *Aedes* mosquitoes caught, the number of positive pool mosquitoes, confirmed dengue cases and *Aedes* eggs collected summed over the seven residential blocks, and the weekly environmental parameters as well.

Firstly, the correlation among rainfall, temperature, and humidity with the total *Aedes* was analyzed. When the preliminary simple linear and nonlinear correlation analysis indicated a lack of relationship between the environmental factors and the total numbers of *Aedes* trapped due to lag effect, then the distributed lag non-linear models (DLNM) was used in the present analysis. The family of distributed lag non-linear models (DLNM) (Distributed Lag Non-linear Models, 2016) can simultaneously analyze non-linear factor-response dependencies and delayed effects which would provide an estimate of the overall effect in the presence of delayed contributions (Gasparrini et al., 2010). The DLNM is developed based on a cross-basis which is a bi-dimensional space of functions. Besides, the DLNM describes the shape of the relationship between the space of the predictor and the lag dimension of its occurrence. Thus, this method allows representation of the time-course of the predictor-response relationship in a 3-D graph.

In the DLNM method, various combinations of the relationship (linear, non-linear natural spline, quadratic B spline) could be tested up to five lags and quasi-poisson distribution constructing could be constructed on the cross-basis. However, the final model could be chosen based on the analysis of variance of different models.

For analyzing the effect of rainfall and temperature on the total number of *Aedes* trapped, the effect of rain was assumed to be null up to 20mm of rain per week and non-linear relationship with quadratic B-spline along with 4 degrees of freedom was used for the temperature. The Bi-dimensional perspective was adopted to represent the associations which vary non-linearly along the space of the predictor and lags. The model which was used in the present experiment can be represented as:

Model < - glm (Aedes~cb.temp+cb.rain, family=quasi-poisson(), data); where cb = cross basis.

This exploration revealed how the predictor can be used to forecast the occurrence of a predicted event, when distributed over a specific period using several parameters to explain one to five-week lags which can be used to forecast the occurrence of an event.

5.3 Results

5.3.1 Total number of mosquitoes: relationship to climate factors

(a) Temperature

The weekly mean temperature fluctuated within a range of 27.6 – 31^{o}C (Figure 5.1), and there was no discernible trend in the relationship between the temperature and the total number of trapped *Aedes*. The plot of lag-response curves (Figure 5.2) for different temperatures indicated that the number of trapped *Aedes* would be higher at 29 to 31^{o}C during 2-3 weeks lag.

(b) Rainfall

The weekly mean rainfall ranged from 0.00 and 310.13 mm (Figure 5.1) with the high rainfall in March – August 2014, September – December 2014, and October – December 2015. It appears to have some relationship, albeit lagged. Rainfall appeared to have a direct negative effect on the number of trapped *Aedes*, but a positive effect was observed after the third week (Figure 5.3), indicated that the number of *Aedes* would be higher by a 3-week lag.

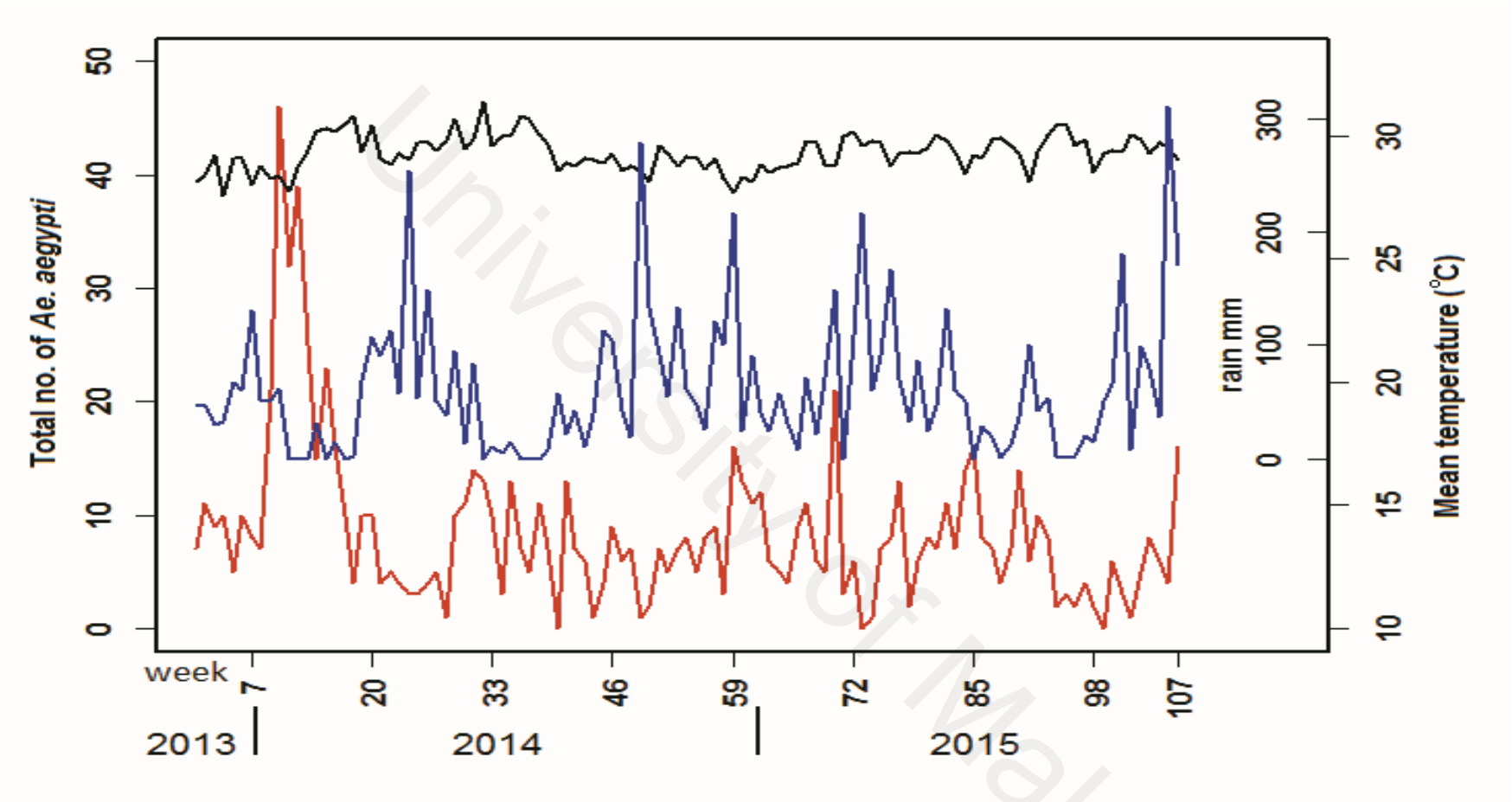

Figure 5.1: Plot of rainfall, mean temperature and total *Aedes aegypti* trapped per week related to time.

Key: red: *Ae. aegypti* trapped, blue:rain, black: temperature (°C)

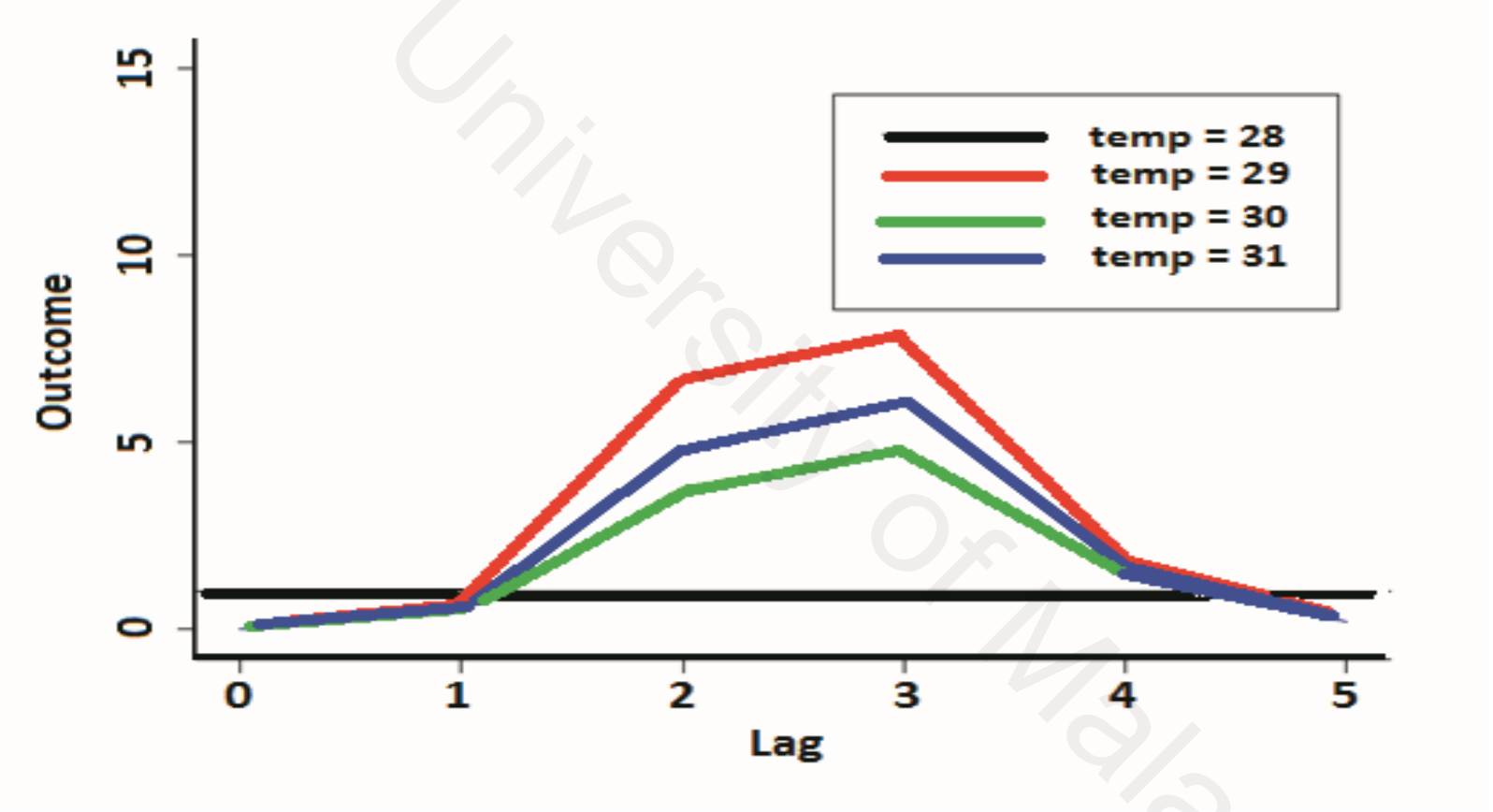

Figure 5.2: Lag-response curves of temperature on weekly total numbers of *Aedes aegypti* trapped, with reference levels at 28°C

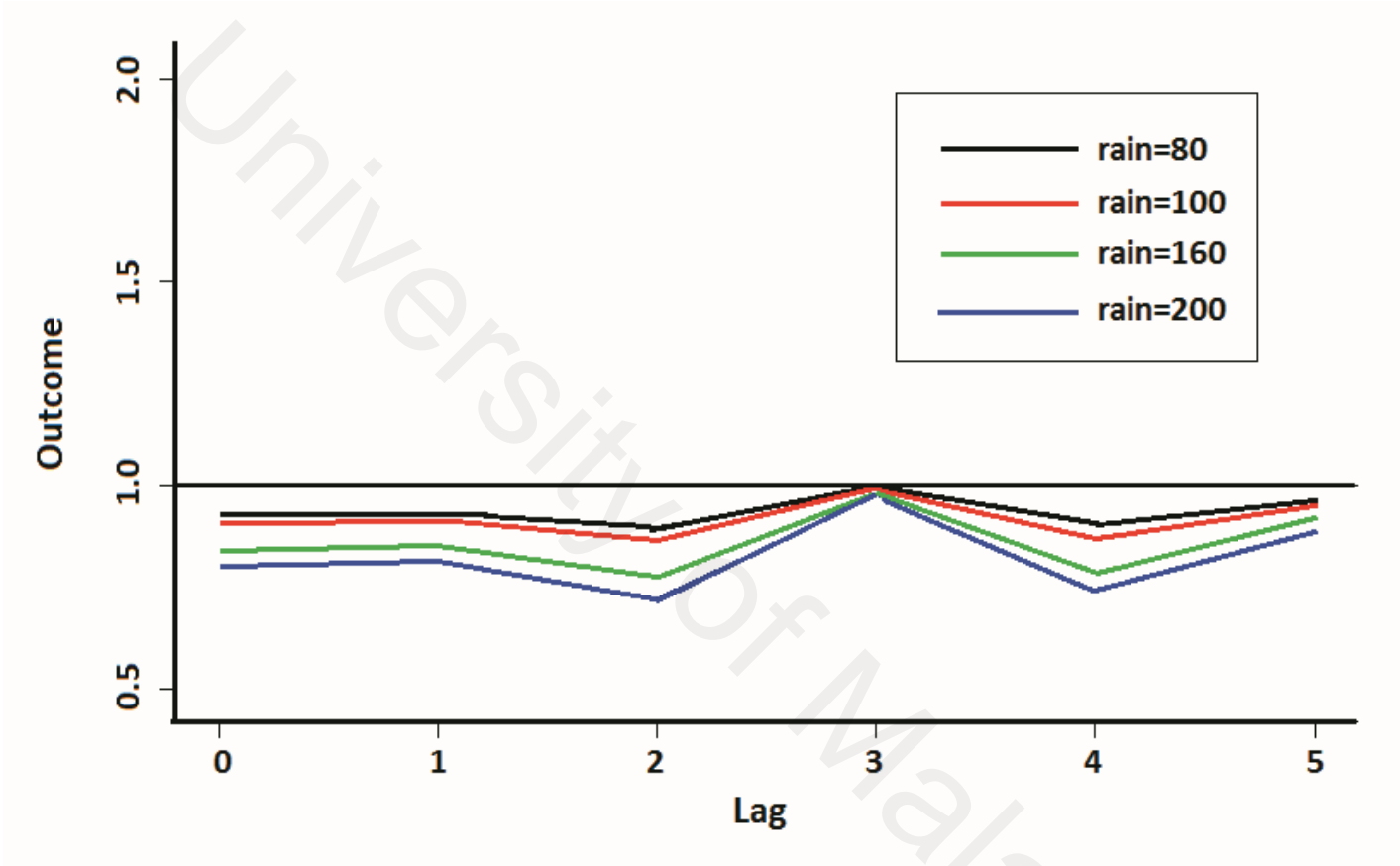

Figure 5.3: Lag-response curves of weekly rainfall on the total numbers of *Aedes aegypti* trapped, with reference levels at 20 mm rainfall

(c) Humidity

The weekly mean humidity ranged from 34% to 94% (Figure 5.1), and it was higher at the end of the year or during the high rainfall season. Humidity also had a direct negative effect on the number of trapped *Aedes*, but a positive effect was observed from the third week, while a significant relationship was noted only for fifth and sixth weeks, indicated that the number of *Aedes* would be higher by a 5-6 week lag (Figure 5.4).

5.3.2 Relationship between the number of dengue cases and climate factors

(a) Temperature

There was also no discernible trend in the relationship between temperature and the total number of dengue cases. Analysis with Pearson's correlation test indicated there was no significant relationship between temperature and the number of dengue case ($P > 0.05$) also for lag time (2,3,4,5 and 6 weeks) analysis. Nevertheless, the plot of lag-response curves (Figures 5.5) for different temperature also indicated that the number of dengue cases was negatively related to the temperature.

(b) Rainfall

Although the weekly rainfall showed same three peaks as the dengue cases throughout the 2-year study period, the Pearson's correlation analysis exhibited statistically non-significant relationship between rainfall and dengue cases. Further correlation analysis on lag time (2, 3, 4, 5 and 6 weeks) of the occurrence of dengue cases and rainfall also did not reveal a significant relationship between two variables ($P > 0.05$). However, for the plot of lag-response curves, it appeared that rainfall had the positive effect on the dengue case by 1-week lag (Figure 5.6).

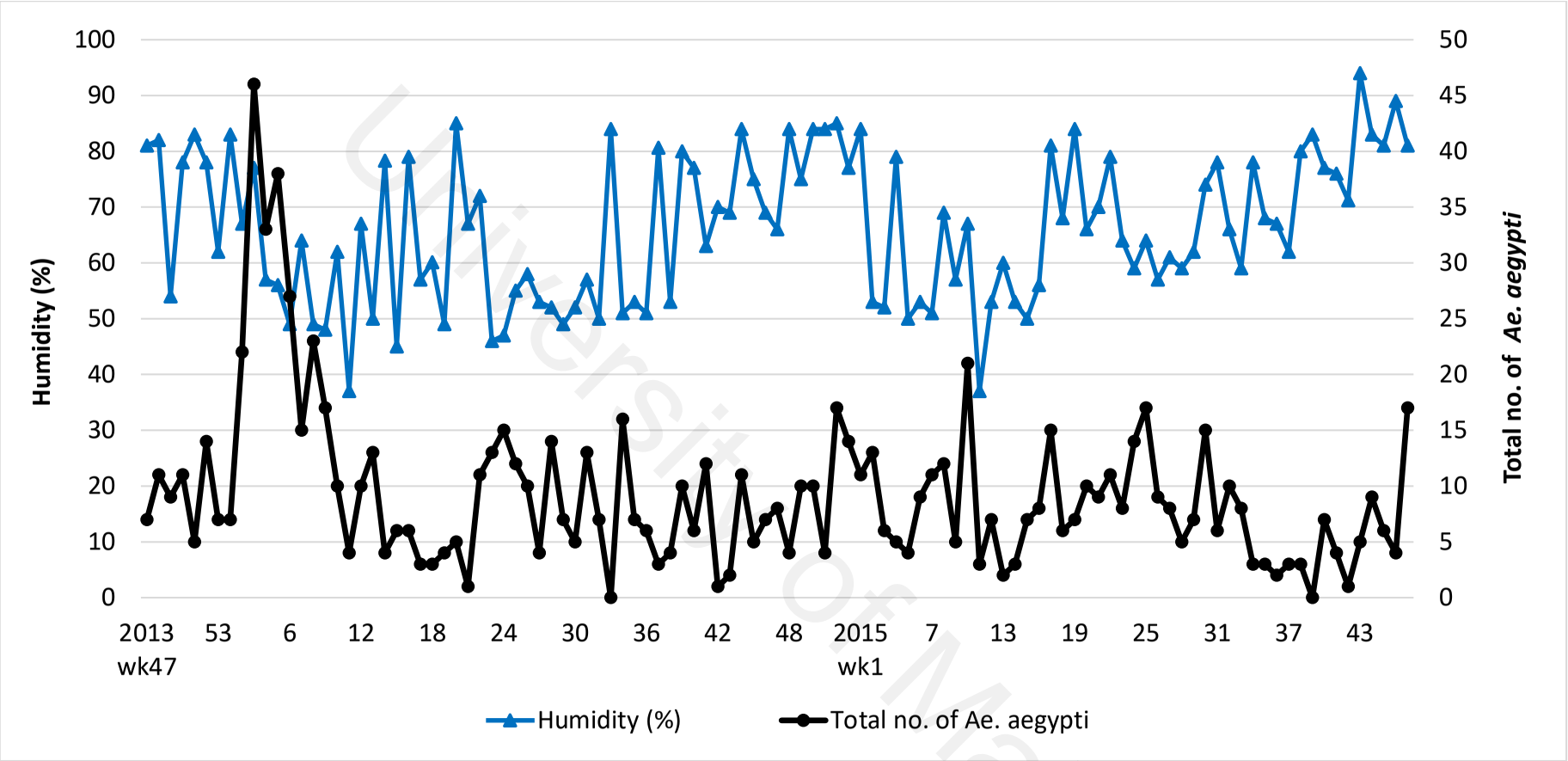

Figure 5.4: Comparison between humidity (%) and the total number of *Ae. aegypti* caught with lag time analysis (Lag time 0, 1, 2, 3, 4, 5, 6 week) using Pearson's product-moment correlation as Lag time 0 week: r=-0.1466 (*P* > 0.05), Lag time 1 week: r=-0.001 (*P* > 0.05), Lag time 2 week: r=-0.0054 (*P* > 0.05), Lag time 3 week, r=0.0920 (*P* > 0.05), Lag time 4 week, r=0.1674 (*P* > 0.05), Lag time 5 week, r=0.2351 (*P* < 0.05) and Lag time 6 week, r=0.4009 (*P* < 0.05)

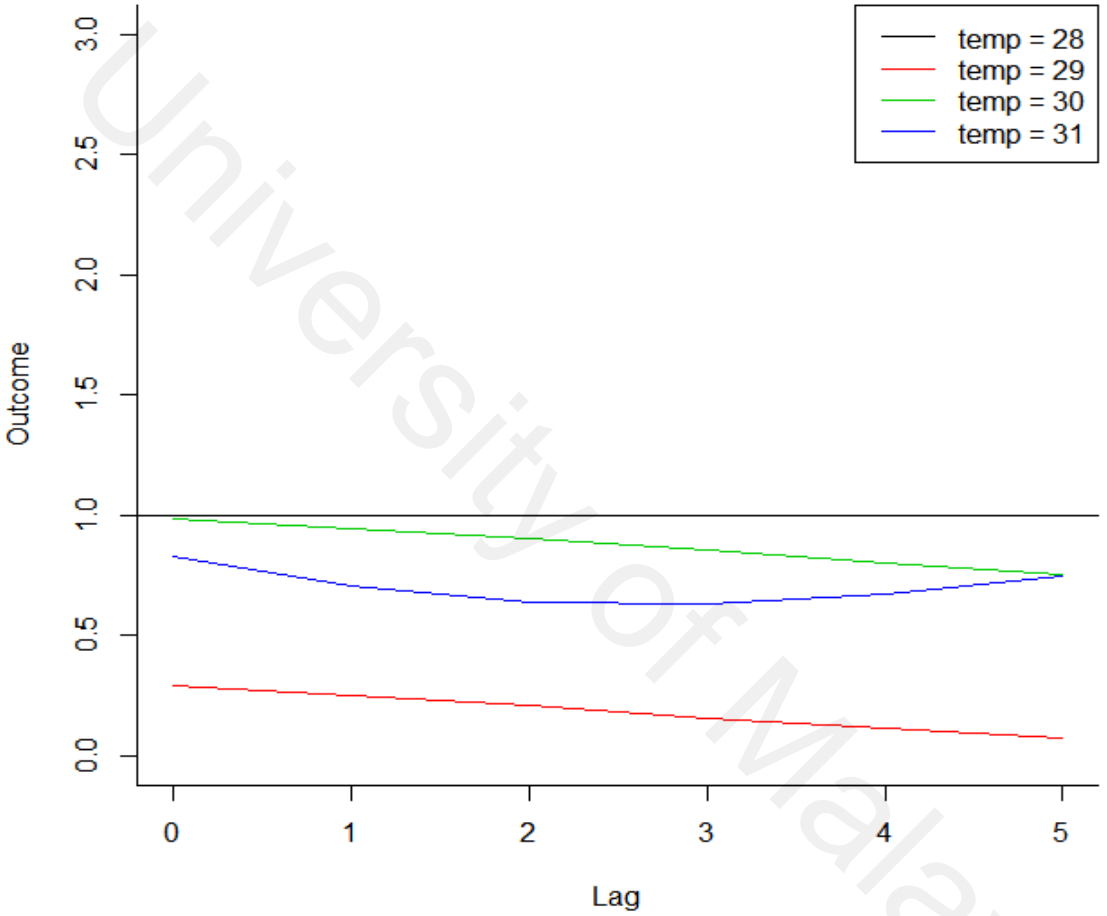

Figure 5.5: Lag-response curves of temperature on weekly total numbers of dengue cases, with reference levels at 28°C

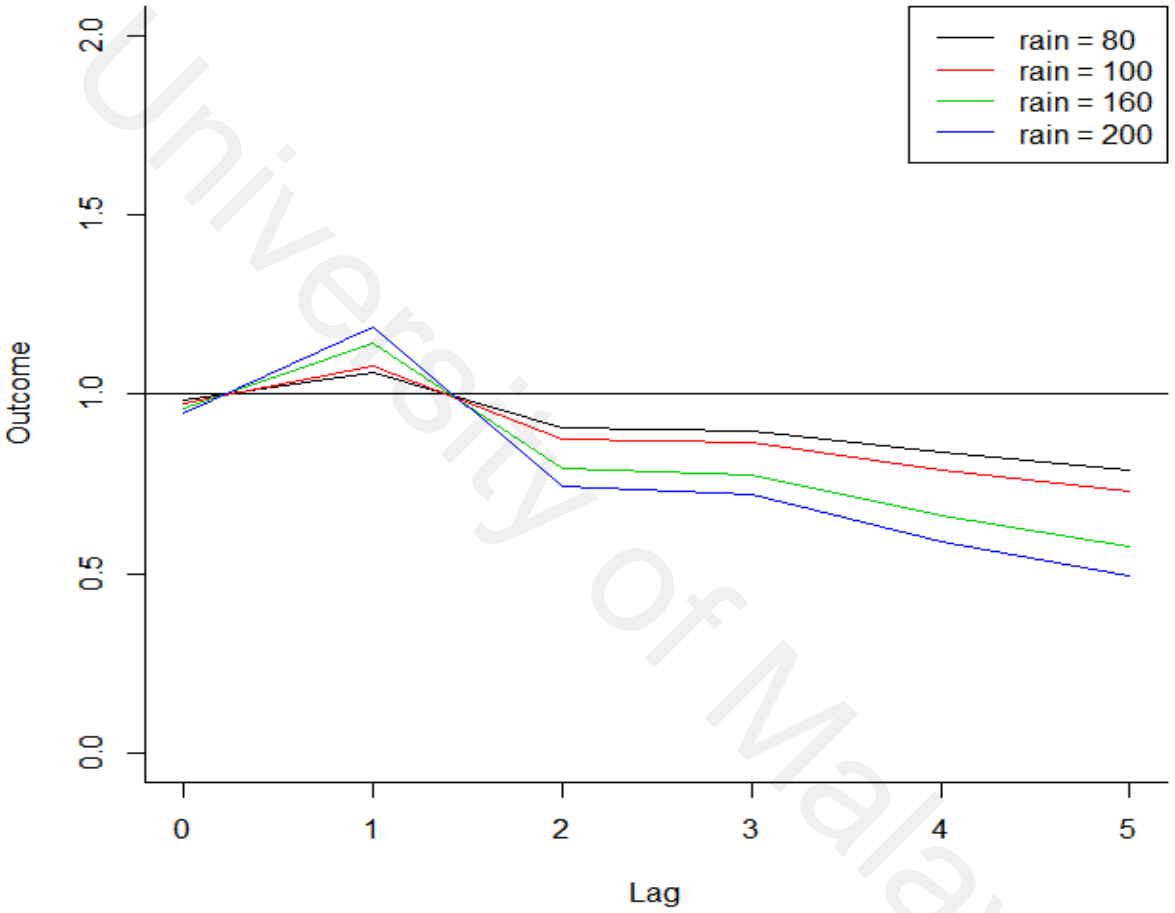

Figure 5.6: Lag-response curves of weekly rainfall on the total numbers of dengue cases, with reference levels at 20 mm rainfall

(c) Humidity

The weekly humidity showed similar trend to that of the number of dengue cases. Correlation analysis between the two variables revealed a significant relationship only for the sixth week, indicating that the number of dengue cases would be higher by a 6-week lag (Figure 5.7).

5.3.3 Total pool of positive-mosquitoes: relationship to climate factors

(a) Temperature

The results of Pearson's correlation test indicated that there was no statistically significant relationship between the temperature and the number of positive mosquito pool. Further correlation analysis on the lag time (2, 3, 4, 5 and 6 weeks) of the total positive mosquito pool and temperature also did not reveal a significant relationship between them ($P > 0.05$). Nevertheless, the plot of lag-response curves (Figure 5.8) for different temperature indicated that the number of positive mosquito pool would be higher at 30^{o}C after 4-week lag.

(b) Rainfall

The results of Pearson's correlation test also indicated that there was no statistically significant relationship between rainfall and the number of positive mosquito pool. Further correlation analysis on the lag time (2, 3, 4, 5 and 6 weeks) of the total positive mosquito pool and rainfall also did not reveal a significant relationship between them ($P > 0.05$). However, the plot of lag-response curves (Figure 5.9) for different

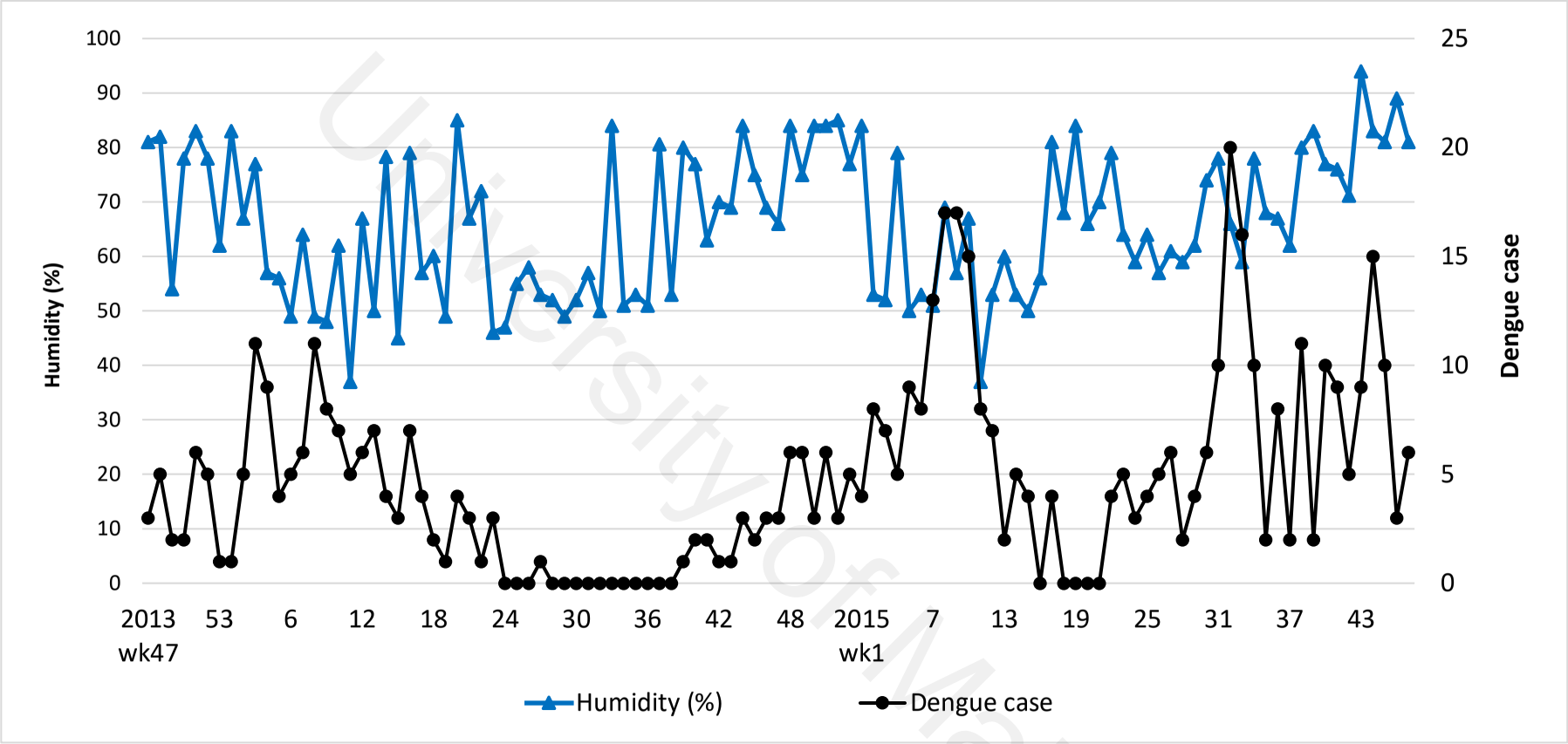

Figure 5.7: Comparison between humidity (%) and the total number dengue cases with lag time analysis (Lag time 0, 1, 2, 3, 4, 5, 6 weeks) using Pearson's product-moment correlation as Lag time 0 week: r=0.0710 (*P* > 0.05), Lag time 1 week: r=0.1088 (*P* > 0.05), Lag time 2 week: r=0.0882 (*P* > 0.05), Lag time 3 week, r=0.0341 (*P* > 0.05), Lag time 4 week, r=0.1400 (*P* > 0.05), Lag time 5 week, r=0.1668 (*P* > 0.05) and Lag time 6 week, r=0.1985 (*P* < 0.05)

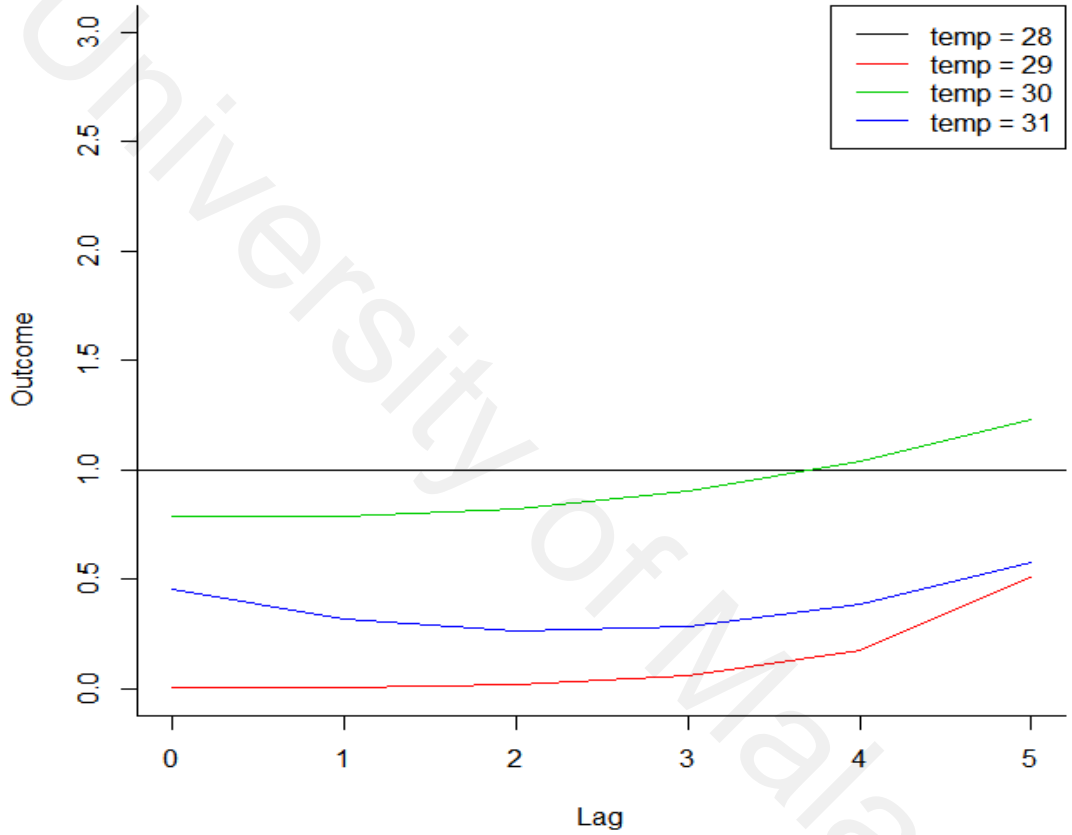

Figure 5.8: Lag-response curves of temperature on a weekly total NS1 pool mosquito positive, with reference levels at 28°C

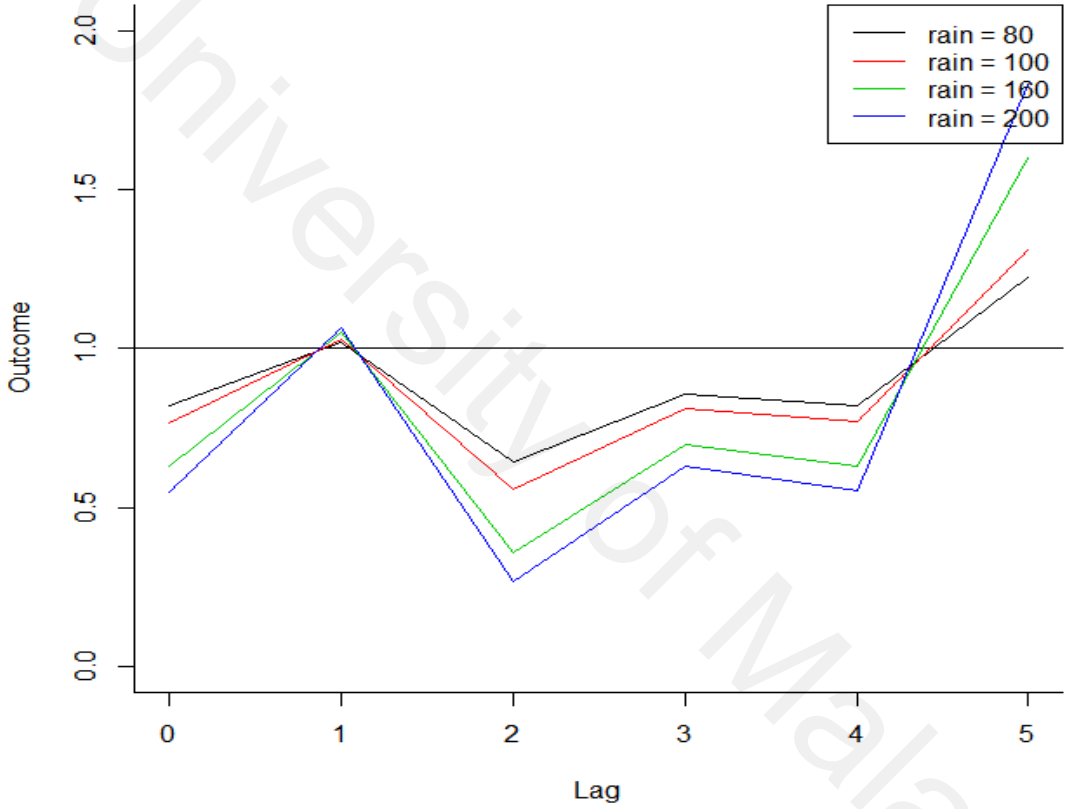

Figure 5.9: Lag-response curves of weekly rainfall on a weekly total NS1 pool mosquito positive, with reference levels at 20 mm rainfall

rainfall indicated that the number of positive mosquito pool would be higher at the different level of rainfall after 4 week lag.

(c) Humidity

The weekly humidity had an almost similar trend to that of the number positive mosquito pool. Correlation analysis between the two variables revealed a significant relationship only at the seventh week, indicating that the number of positive mosquito pools would be higher by a 7-week lag (Figure 5.10).

5.3.4 Total number of mosquito eggs: relationship to climate factors

(a) Temperature

The results of Pearson's correlation test indicated that there was no statistically significant relationship between the temperature and the number of mosquito eggs. Further correlation analysis on the lag time (2, 3, 4, 5 and 6 weeks) of the total mosquito eggs and temperature also did not reveal a significant relationship between them ($P > 0.05$). However, the plot of lag-response curves (Figure 5.11) indicated that temperature of 29^{o}C had a positive effect on higher number of mosquito eggs production compared to a higher temperature (30-31^{o}C). However, a higher temperature such as 30-31^{o}C showed the increasing mosquito eggs only after 2 weeks lag.

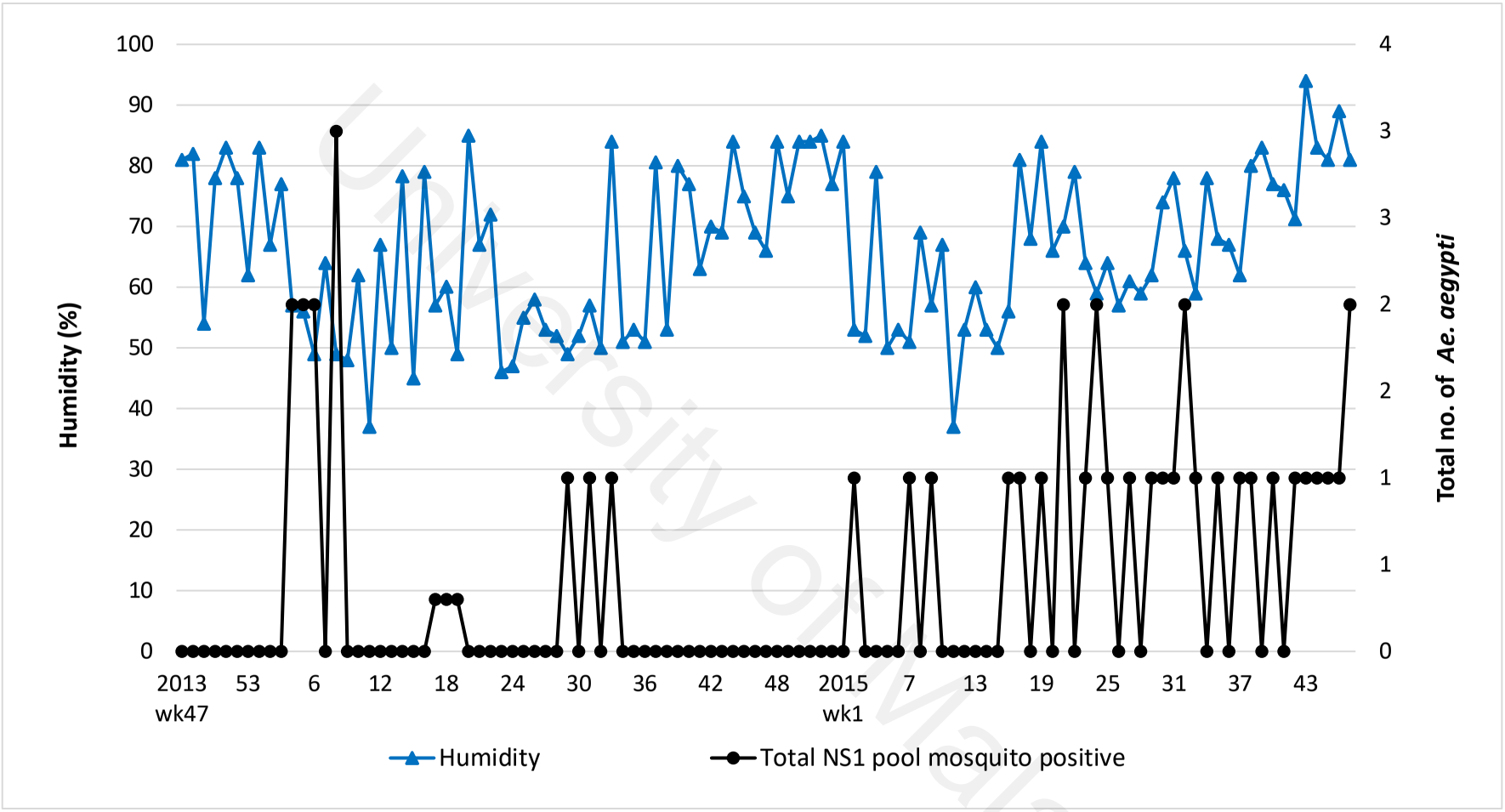

Figure 5.10: Comparison between humidity (%) and the total NS1 pool mosquito positive with lag time analysis (Lag time 0, 1, 2, 3, 4, 5, 6 weeks) using Pearson's product-moment correlation as Lag time 0 week: r= -0.0270 (*P* > 0.05), Lag time 1 week: r= 0.0876 (*P* > 0.05), Lag time 2 week: r= 0.0811 (*P* > 0.05), Lag time 3 week, r= 0.1267 (*P* > 0.05), Lag time 4 week, r= 0.0875 (*P* > 0.05), Lag time 5 week, r= 0.1143 (*P* > 0.05) and Lag time 6 week, r= 0.079 (*P* > 0.05)

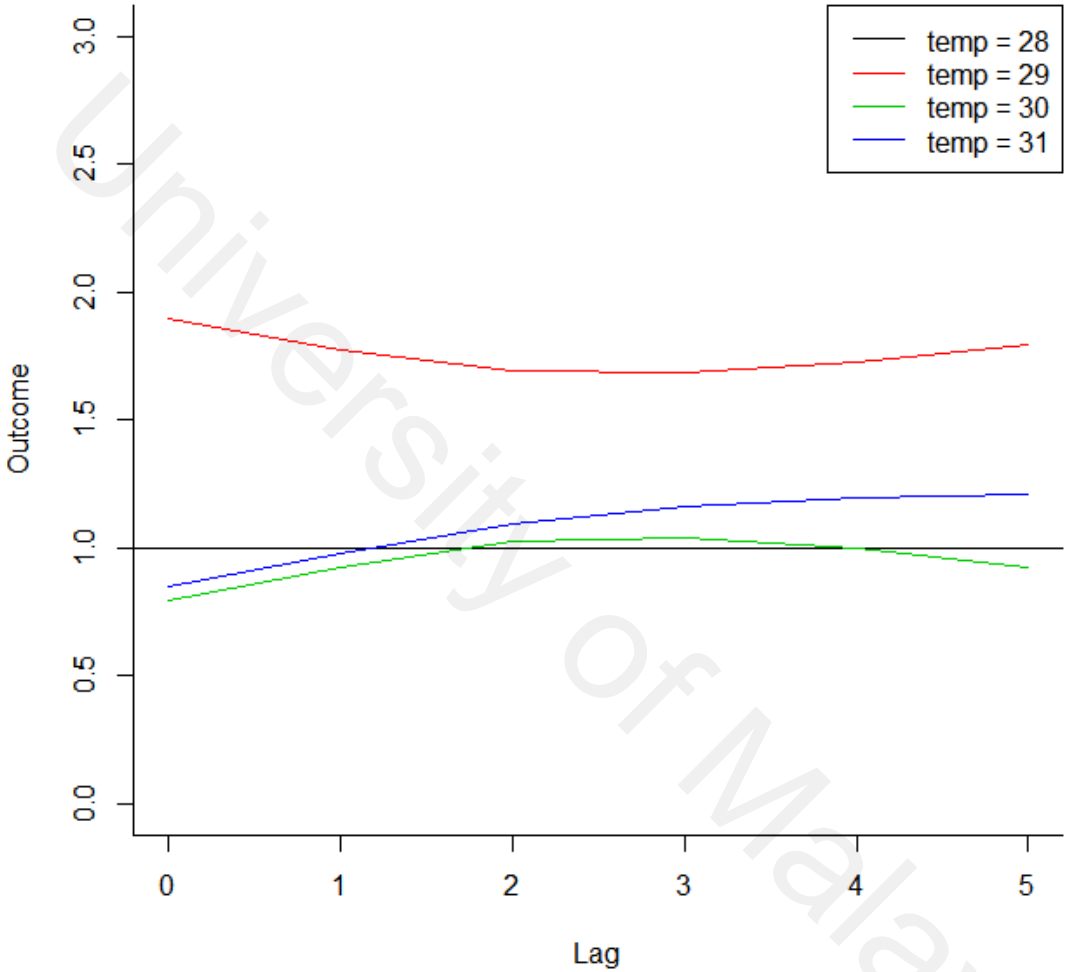

Figure 5.11: Lag-response curves of temperature on the total number of mosquito eggs, with reference levels at 28°C

(b) Rainfall

The results of Pearson's correlation test also indicated that there was no statistically significant relationship between rainfall and the number of mosquito eggs. Further correlation analysis on the lag time (2, 3, 4, 5 and 6 weeks) of the total mosquito eggs and rainfall also did not reveal a significant relationship between them ($P > 0.05$). However, the plot of lag-response curves (Figure 3.12) revealed that rainfall had a positive effect on the increasing egg productivity after 2-3 weeks.

(c) Humidity

The results of Pearson's correlation test also indicated that there was no statistically significant relationship between humidity and the number of mosquito eggs. Further correlation analysis on the lag time (2, 3, 4, 5 and 6 weeks) of the total mosquito eggs and humidity also did not reveal a significant relationship between them ($P > 0.05$) (Figure 5.13).

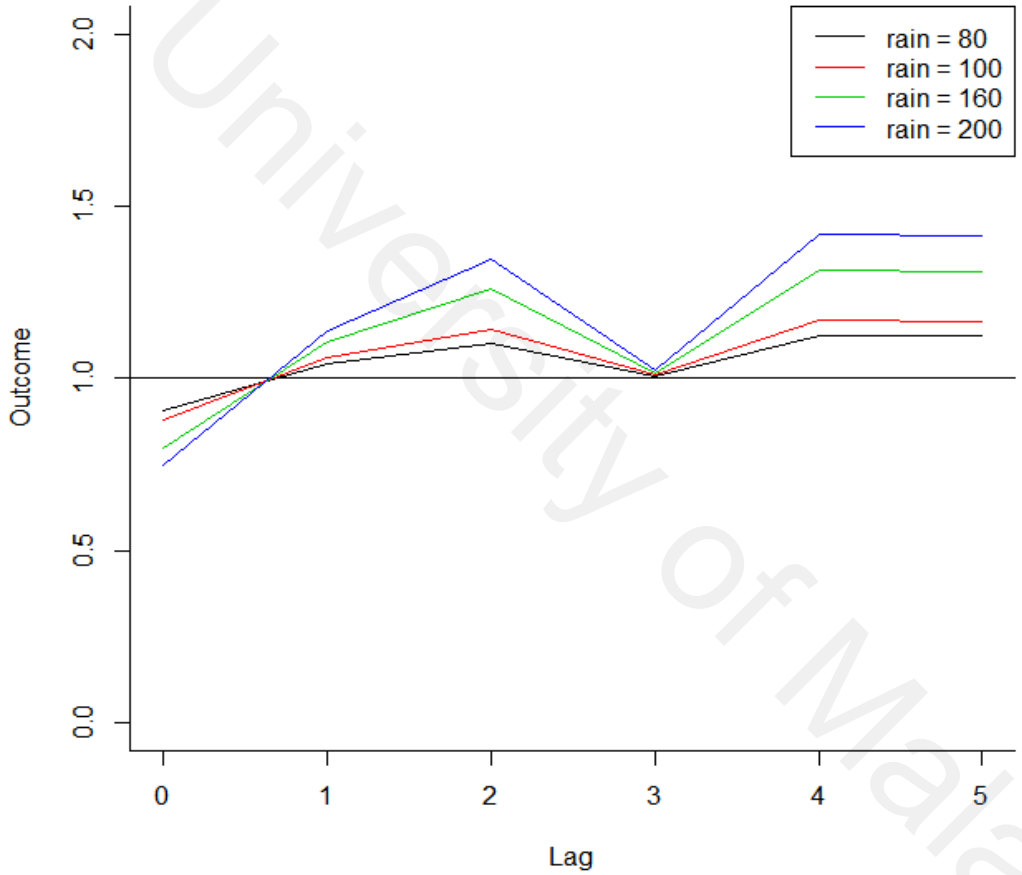

Figure 5.12: Lag-response curves of total number of mosquito eggs, with reference levels at 20 mm rainfall

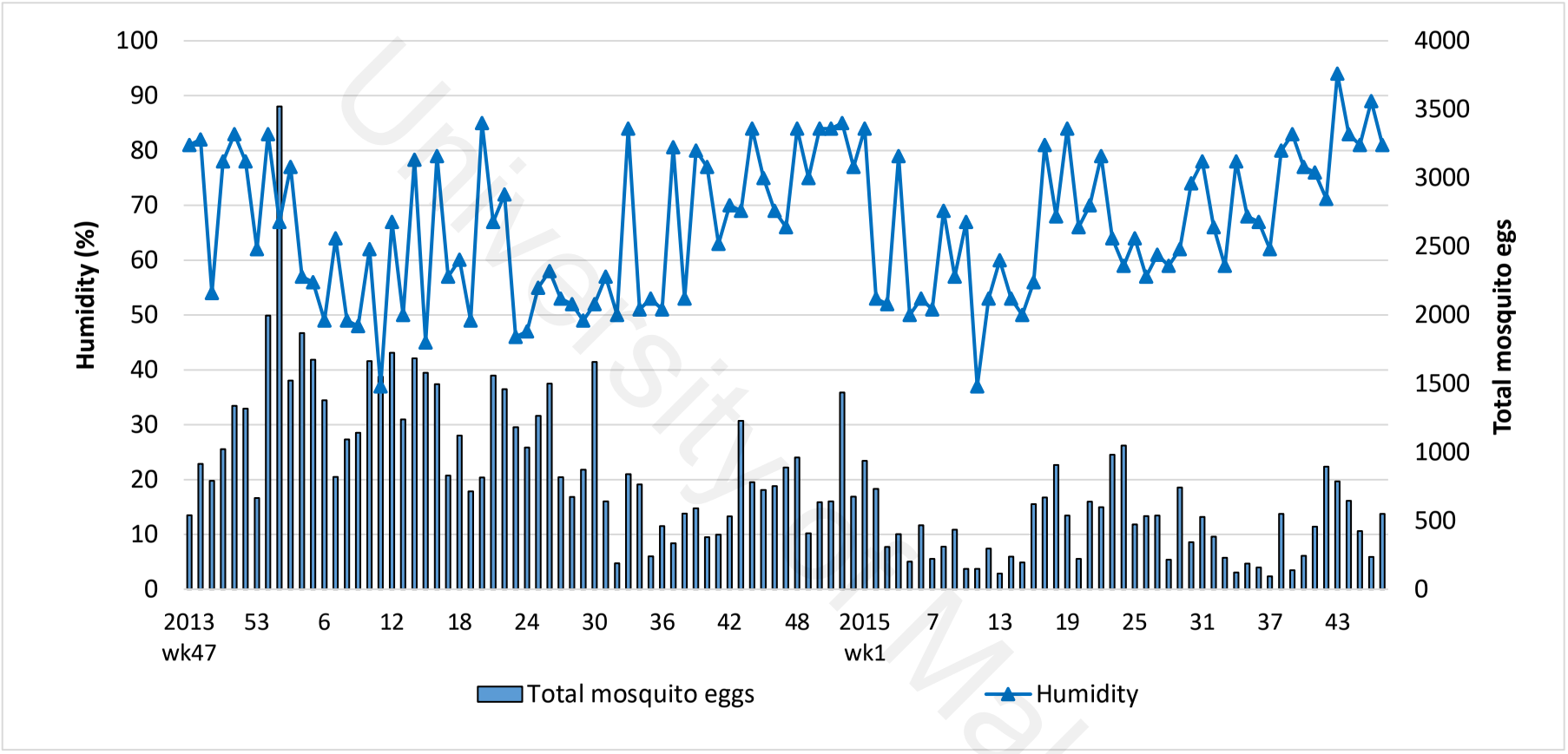

Figure 5.13: Comparison between humidity (%) and total mosquito eggs with lag time analysis (Lag time 0, 1, 2, 3, 4, 5, 6 weeks) using Pearson's product-moment correlation as Lag time 0 week: r= -0.02208221 (*P* > 0.05), Lag time 1 week: r= -0.01635824 (*P* > 0.05), Lag time 2 week: r= -0.03691872 (*P* > 0.05), Lag time 3 week, r= -0.01173688 (*P* > 0.05), Lag time 4 week, r= 0.1126809 (*P* > 0.05), Lag time 5 week, r= -0.0001750885 (*P* > 0.05), Lag time 6 week, r= -0.05816665 (*P* > 0.05) and Lag time 7 week, r= 0.2197033 (*P* < 0.05)

5.3.5 Correlation of dengue case in relation to climatic factors and infected mosquitoes

The impact of the climate such as temperature and rainfall on *Aedes* density and dengue risk using distributed lag non-linear models (DLNM) and the generalized linear models (glm), while effect for humidity was analyzed using Pearson correlation on lag time effect are summarized in Table 5.. However, the same analysis (DLNM and glm) which has been described in Chapter 4 demonstrated that the number of NS1-positive mosquito pools have 2-3 weeks lag effect for dengue cases. This outcome of the analysis showed that occurrence of the dengue case can be better predicted by infected mosquitoes, rather than climatic factors at microhabitat.

Table 5.1: Relationship between climate (temperature, rainfall and humidity) and the total number of adult mosquito, mosquito eggs, pool of positive-mosquito and dengue cases

Total	Temperature	Rainfall	Humidity
Adult mosquitoes	peak after 2-3 weeks lag	positive effect by 3 weeks lag	significant relationship after 5-6 weeks lag
Mosquito eggs	positive relationship at temperature 29^{o}C, however higher temperature such as 30-31^{o}C showed positive effect only after 2 weeks	positive effect after 2-3 week	No relationship
Pool of positive mosquito	positive relationship after 4 weeks lag at 30^{o}C.	positive relationship after 4 weeks.	significant relationship after 7 weeks lag
Dengue cases	No relationship	Positive relationship only by 1 weeks lag	significant relationship by 6 weeks lag

5.4 Discussion

This study analyzed the association of weather and *Ae. aegypti* abundance at the micro-level to determine its suitability as a surveillance tool for dengue control. The results showed that if the temperature increased from 28 to 31°C the abundance of *Ae. aegypti* would increase with a lag of 2 weeks, while after rainfall the increment would be with a lag of three weeks and the effect lag for humidity was 5 weeks. The lag time is needed perhaps for the development of the mosquitoes due to favourable environment. Many dengue forecasting studies focus on the weather factors such as temperature, total rainfall and humidity (Cheong et al., 2013, Descloux et al., 2012, Karim et al., 2012, Morin et al., 2013), however additional new factors correlated with the disease such as female mosquito infection rates are important needed to enhance the prediction accuracy of the predictive model (Siriyasatien et al., 2016).

The positive effect of climate was demonstrated in San Juan City, Puerto Rico by Barrera et al. (2011). There were significant changes in the density of adult mosquitoes in correlation with rainfall and temperature (Barrera et al., 2011). Mogi et al. (1988) reported that the rainy season was associated with marked seasonal changes in *Ae. aegypti* oviposition, with maximum numbers occurring at a one-month lag. In Ekiti, Western Nigeria, temperature and rainfall were highly correlated with the abundance of mosquito vectors, the temperature between 26^{o}C and 32^{o}C with an average humidity of 55% facilitated the higher mosquito abundance (Simon-Okie & Olofintoye, 2015). Moreover, a relative humidity of at least 50 – 55% prolonged mosquito survival (Simon-Okie & Olofintoye, 2015). However, there were also studies which had no effect of the climate on *Aedes* mosquito density. In Australia there was no significant effects of rainfall on *Ae. aegypti* dynamics using BG traps at any time lags, but significant effects of relative humidity were observed lag of two weeks and mean daytime temperature at lag 0 (Azil

et al., 2010). While in Puerto Rico, it was confirmed that the areas where rainfall was uniformly distributed there were no correlation between rainfall and *Aedes* dynamics (Scott et al., 2000) but in areas where rainfall was more seasonal there was a strong correlation with *Aedes* density and dengue cases (Reiter, 2007).

Besides, the effect of the climate on dengue cases has been widely studied. Most studies showed that the transmission of dengue was highly sensitive to climatic conditions, especially temperature, rainfall and relative humidity (Naish et al., 2014). In Singapore, analysis of the effects of weather (absolute humidity, temperature, rainfall, relative humidity, wind speed) on dengue cases from 2001 to 2009 showed that an absolute humidity was the best predictor and indicator for dengue incidence (Xu et al., 2014). In Malaysia climatic factors such as temperature, rainfall, and humidity have been associated with dengue, however these relationships were not consistent (Hii et al., 2016). Cheong et al. (2013) demonstrated that (in Selangor, Kuala Lumpur, and Putrajaya) between 2008 - 2010 the incidence of dengue cases was positively associated with increased minimum temperature (from 25.4°C to 26.5°C) with a lag of 51 days for the highest effect. Increasing bi-weekly accumulated rainfall (215 mm to 302 mm) had a strong positive effect on the incidence of dengue cases, with a lag of 26 – 28 days for the highest effect (Cheong et al., 2013). High temperature constrains the development of infection in mosquitoes (Peña-García et al., 2016) and decreases mosquito life expectancy and subsequently infective life expectancy, thus reducing the incidence of dengue cases (Goindin et al., 2015).

In the present study, rainfall was found to have positive effect for the increasing number of dengue cases by 1-week lag and humidity was 6 weeks lag effect, whereas there was no association with dengue incidence for temperature. Nevertheless, other studies showed the different time lag effect of the climate to the dengue cases (Halstead,

2008b, Hii et al., 2009, Fairos et al., 2010, Rohani et al., 2011). In Bangkok, the incidence of dengue cases increased 2 months after heavy rainfall (Halstead, 2008b). Study in Singapore also demonstrated that dengue incidence increased linearly at time lag of 5 – 16 and 5 – 20 weeks succeeding elevated temperature and precipitation (Hii et al., 2009). Fairos et al. (2010) revealed that the daily temperature and wind speed significantly influenced the incidence of dengue fever after a 2 – 3 weeks lag, while the effect of humidity appeared to be significant only after 2 weeks. Rohani et al. (2011) reported that rainfall, temperature, and humidity were associated with dengue cases at a lag of up to 1 week. A study in Cambodia showed that the association between dengue incidence and weather factors apparently varies by locality, with temperature having a 3-month lag effect and rainfall have 0 – 3 months lag (Choi et al., 2016). Time lag for the effect on the climatic variables on dengue incidence could be explained by climatic factors which do not directly influence of dengue cases, which need to go through their effect on the life-cycle dynamics of both vector and virus. For the vector, it need to go through mosquito hatching, larval, pupal development and adult emergence. While, the virus need to go through virus amplification in vectors, incubation in humans culminating in a dengue outbreak (McMichael et al., 1996).

However, climate has an effect on the mosquito infection rate for certain reasons. Peña-García et al. (2016) reported that the relative humidity positively affected mosquito infection rate with a lag time of a month or more, and temperature negatively affected mosquito infection rate with a lag time of 2-6 weeks, but association between precipitation and mosquito infection rate varies with locality as local habits of water storage results in the availability of breeding places without the requirement for rain. Another study also reported that the increase in density of *Ae. aegypti* was not directly related to climate change, but rather to human activities related to domestic water storage (Beebe et al., 2009; Padmanabha et al., 2010; Stewart et al., 2013). Therefore, rainfall can

have non-linear contrasting effects on dengue risk (Githeko, 2012; Hii et al., 2009). Heavy rainfall may flush away eggs, larvae and pupae from containers but the residual water can create breeding habitats in the long-term effect (Sarfraz et al., 2012), however dry climate can lead to human behaviour to save water which may cause increase of breeding sites for *Aedes* mosquito (Dieng et al., 2012). Study by Lambrechts et al. (2011) demonstrated that larger diurnal temperature range (DTR) will reduce virus infection in mosquitoes, but not the duration of the virus extrinsic incubation period (EIP). This also could explain why average temperature which does not vary seasonally could lead it to higher seasonally DENV transmission at locations, in which mosquito abundance is not associated with dengue incidence. The same study also reported that the highest risk of dengue cases occurred within a small temperature range (Cheong et al., 2013). Increased temperature could increase dengue risk by increasing the rate of mosquito development and reducing the virus incubation time (Focks et al., 1995; Kuno, 1995; Patz et al., 1996). Conversely, extreme hot temperature can increase the rate of mosquito mortality (Hii et al., 2009). Thus, climatic conditions have the influence on virus, the vectors and human behaviour both directly and indirectly (Gubler, 2000). This study indicated that temperature and rainfall have 4 weeks lag effect for the total pool of positive-mosquitoes, while humidity effect was by 7 weeks lag.

This present study demonstrated that the temperature at 29^{o}C has positive effect on the number of mosquito eggs. However, a higher temperature such as 30-31^{o}C would have an effect only after 2 weeks lag. Whereas, rainfall showed 2- 3 weeks effect on the mosquito eggs density, but not with the humidity. Most studies showed that the temperature has positive effect for mosquito eggs count but not for rainfall. Serpa et al. (2013) reported that temperature has an effect on the oviposition activities of *Ae. aegypti* in the peridomiciliary environment in term of positive ovitrap indices (POI) and mean egg counts per trap (MET), but no correlation with rainfall. The statistically significant

association between the temperature and trap positivity as well as the mean egg count was also reported by Dibo et al. (2005). A study by Resende et al. (2013) also demonstrated that the temperature has a positive relationship was with adult capture measurements and egg collections, whereas precipitation and frequency of rainy days exhibited a negative relationship. While, temperature and humidity were significantly associated with ovitrap index in early post-rainy and late post-rainy seasons (Ejaz Mahmood et al., 2017), but the association with rainfall was significant for all seasons (Ejaz Mahmood et al., 2017; Mogi et al., 1988).

Chapter 4 has described that the infected mosquitoes played a better role to predict dengue cases instead of the density of mosquitoes. Whereas, this chapter showed that the climate has the lag effect on the density of mosquitoes but not so clear for dengue cases. However, climatic variations alone do not explain the *Ae. aegypti* and dengue distribution, many other factors should be considered in the design of explanatory epidemiological models of dengue occurrence such as the abundance of the breeding sites, domestic behavior of the vector that protects it against fluctuations in temperature and humidity and the degree of immunity of the population against the dengue virus serotypes as proposed by Dibo et al. (2008).

This perhaps explains why epidemics of dengue have not decreased in Malaysia despite warning issued by the Ministry of Health every time when heavy rain occurs. Thus, it seems that climatic variables are not very good proactive measures that can be used as surveillance tool to prevent dengue epidemics.

CHAPTER 6: GENERAL CONCLUSIONS

6.1 General Conclusions

In Malaysia, dengue is taking a toll on the public health resources. To add to the existing challenges, its mosquito vectors, *Ae. aegypti* followed by *Ae. albopictus* are also vectors for Chikungunya (serious outbreaks in 2008-2009) and Zika (has spread very rapidly in the Americas in 2016-2017 and has emerged in Singapore). Given the commonality of their vector, the successful control of dengue via its mosquito vector control will automatically control the other two diseases as well. At present, surveillance is dependent on household *Aedes* larval surveys and notifications of lab-confirmed human infections (Mudin, 2015). Unfortunately, both of these strategies have major shortcomings, there is no correlation between larval indices and cases of dengue, and of the proportion of people that seek medical care following infection (Dom et al., 2013, Chang et al., 2011). It is known that some asymptomatic people are infectious to mosquitoes (Duong et al., 2015). Therefore, the existing reactive programme lacks sensitivity and is delayed, and has proven insufficient to stave off epidemics. It needs to be replaced with a proactive strategy.

The current study unfolds a proactive and innovative paradigm shift in vector surveillance. The creation of an in-house user-friendly technique to detect dengue virus in mosquitoes for early detection of dengue cases is an important and timely study which has been completed with promising results. Important finding of this study showed that cheaper methods such as GOS traps (less than US$1) were able to capture *Aedes* mosquitoes and NS1 antigen test kit can be used to detect the dengue virus antigen in mosquitoes. In this study, *Ae. aegypti* was the predominant *Aedes* mosquitoes (95.6%)

caught in GPS traps and 23% (43/187 pools of mosquitoes each) were found to be positive for dengue using NS1 antigen kit. This method also can easily be used by public health workers for the surveillance of dengue vectors. Currently epidemics of dengue are not being controlled in our country due to limitation of resources such as manpower to cover all houses for the control measures such as larval surveys and chemical control, besides most of houses are locked during the activities carried out and also many cryptic breeding sites are not found during larval surveys. Besides, this novel strategy also can help to detect infectious mosquitoes, thus immediate subsequent control measures can be carried out before the next epidemics occurs. While fogging is only carried out when cases are reported and the control can be missed for asymptomatic cases which is more infectious to mosquitoes (Duong et al., 2015). GOS trap unlike other *Aedes* mosquitoes collecting traps such as BG-Sentinel trap and backpack aspirators which are costlier, labour intensive, intrusive and also depend on the skill and diligence of the personnel to operate it. In addition, the NS1 antigen test kit which is used for detecting dengue virus antigen in patients also was confirmed can be used for mosquitoes. It is easier and cheaper than other techniques such as RT-PCR, and thus, can be used as a new paradigm for dengue surveillance.

This study also showed that climate has the lag effect on the density of mosquitoes but not so clear for dengue cases. However, numerous studies showed correlation between climate and dengue case (Cheong et al., 2013; Hii et al., 2016; Naish et al., 2014; Xu et al., 2014). In this study, infected mosquitoes demonstrated better role to predict dengue cases instead of density of mosquitoes. Confirmed cases of dengue were observed with a lag of one week after positive *Ae. aegypti* were detected. *Ae. aegypti* density as analyzed by distributed lag non-linear models, will increase lag of 2-3 weeks for temperature increase from 28 to 30°C, and lag of three weeks for increased rainfall. Thus, effect of the climate was localized and thus it is very difficult to use these factors in general for a

particular district, state or country to predict dengue case and density of mosquitoes. Methods to improve sensitivity and reduce delays in dengue detection are desperately needed.

6.2 Recommendation

This study has revealed that GOS trap is a cheap and effective way to collect *Aedes* mosquitoes. Whereas, the NS1 antigen kit is a simple tool that can be used by public health staff to demonstrate the presence of an infected mosquitoes thus preventive action can be taken before an epidemic occurs. Therefore, this study has shown the use of GOS traps and NS1 kit represents one possible way forward to forewarn and reduce dengue outbreaks which are increasing yearly and projecting a global disease burden. For a start the strategy provides early warning system where swift action can be taken by public health workers to reduce dengue outbreaks. High dengue transmission rates across Southeast Asian countries with extensive diversity in population density, climate, and geology may be explained by the infectiousness of asymptomatic cases to *Ae. aegypti* (Duong et al., 2015). The situation is exacerbated due to a long or delayed response time for fogging and ULV space spraying after a case has been reported. The response may be more efficient when timely vector control measures are implemented after the immediate detection of an infected mosquito from the GOS trap.

Novel techniques such as the release of genetically modified mosquitoes (RIDL) and the use of the bacteria *Wolbachia* to control the population of the *Ae. aegypti* are still under trial (Harris et al., 2011, Harris et al., 2012, Hoffman et al., 2011, Frentiu et al., 2014). However, urgent effective strategies for control are required ahead of the evidence from these trials, which would also require a lengthy process to access the environmental

and ecological impact of these intervention. Public and community support will also be needed.

This innovative usage of GOS trap coupled with NS1 detection in mosquito provides a comprehensive early warning and surveillance system that has the predictive capability for epidemic dengue. However, it is crucial to test the application of this innovative paradigm shift strategy in a randomized cluster design with the inclusion of intervention and control groups. Thus, the future study should address: 1) diagnosis and case management. 2) proactive integrated vector control measures to pre-empt an outbreak (GOS Trap and NS1 kit). 3) sustainable vector control measures. And 4) health education and community participation.

6.3 Study Limitation

a) This study was unable to incorporate part of Geographic Information System (GIS) or GIS modelling application to calculate the dengue risk as has been planned at the beginning of this study due to only one site being involved and the sampling was not expanded to other sites in order to develop the spatial database.

b) During the study, about 0.84% GOS trap were spoilt or lost, this could be due to the disturbance from the public and animals such as cats. Besides, it is hard to set the mosquito traps inside the houses since most of the houses were locked and mostly people were not in the house (away at work). Therefore, most of the GOS trap were set along the corridor of the unit house.

c) Due to lack of funding, it was not possible to test all negative mosquitoes by RT-PCR.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

a. Publication

(ISI Journal)

Lau SM, Vythilingam, I., Jonathan ID., Shamala, DS, Chua, TH, Wan Yusof WS, Karuthan C., Yvonne Lim AL & Venugopalan B. (2015). Surveillance of adult *Aedes* mosquitoes in Selangor, Malaysia. *Tropical Medicine and International Health*, doi:10.1111/tmi.12555 (Appendix D).

Lau, S. M., Chua, T. H., Sulaiman, W.-Y., Joanne, S., Lim, Y. A.-L., Sekaran, S. D., . . . Vythilingam, I. (2017). A new paradigm for *Aedes* spp. surveillance using gravid ovipositing sticky trap and NS1 antigen test kit. *Parasites & vectors*, 10(1), 151. doi:10.1186/s13071-017-2091-y (Appendix E)..

b. Presentation

Lau, SM., Vythilingam, I., Venugopalan, B., Wan Yusoff, W.S., Karuthan, C., Yvonne Lim, A.L. & Ahmad Safri, M. (2014). Gravitraps for surveillance and control of dengue in Selangor. *6th ASEAN Congress of Tropical Medicine and Parasitology, 4 – 6 March 2014*, Kuala Lumpur, Malaysia: International Hotel.

Lau SM, Vythilingam, I., Jonathan ID., Shamala, DS, Chua, TH, Wan Yusof WS, Karuthan C., Yvonne LAL & Venugopalan B. (2015). New tools for the surveillance of adult *Aedes aegypti* and detection of dengue virus in adult *Aedes aegypti* (L.). *An International Symposium "New challenges & Winning strategies", WAR against mosquitoes-borne disease, 19 – 20th August 2015*, Kota Kinabalu, Sabah: Shangri-la's Tanjung Aru Resort & SPA.