

ASSESSING THE TEMPORAL DISTRIBUTION OF DENGUE VECTORS MOSQUITOES AND ITS RELATIONSHIP WITH WEATHER VARIABLES

Masnita Md Yusof^{1,2*}, Nazri Che Dom¹, Rodziah Ismail¹, Ariza Zainuddin²

¹ Faculty of Health Sciences, Universiti Teknologi MARA (UiTM), UiTM Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia

² Pejabat Kesihatan Daerah Jempol, 72120 Bandar Seri Jempol, Negeri Sembilan, Malaysia

*Corresponding author: masnita_yusof@yahoo.com

ABSTRACT

Dengue fever is a vector-borne disease. The infectious disease is often transmitted by mosquito namely *Aedes* species. Understanding of *Aedes* mosquito abundance is essential to plan comprehensive control activities. A cross-sectional study was conducted in Jempol Negeri Sembilan, Malaysia. A total of 960 ovitraps were deployed at Taman Satellite and Felda Raja Alias 4 from April to September 2017 biweekly. These localities were divided to two different setting, which are vegetative and populated environments. This study aims to assess, first, the distribution and abundance of *Aedes* species by measuring two variables which are positive ovitrap index (POI) and mean eggs per ovitraps (MET) in populated and vegetative environments. Thus, ANOVA was used as a statistical analysis in order to determine the difference of POI and MET in difference environment. Second, *Aedes* species was identified to show the index ratio between *Aedes aegypti* and *Aedes albopictus*. And lastly, correlation between POI and MET with weather data in order to determine the relationship between *Aedes* distribution and abundance with temperature, humidity and rainfall. Beside, *Pearson* and *Spearman* coefficient correlation were used to determine the correlation. From the total ovitrap, 945 (98.44%) were inspected with 428 (45.29) were identified positive. A total of 9929 eggs were collected during study session. The highest POI at Felda Raja Alias 4 at vegetative environments was 85%, while populated environment stated the highest POI with 75%. *Aedes* distributions in Raja Alias 4 was significantly higher in vegetative environment compared to populated environment ($p= 0.048$). In Taman Satellite, highest POI in vegetative environment was 50%, while highest POI in populated environment was 45%, therefore significantly, *Aedes* distribution in vegetative environment was higher than populated environment ($p=0.039$). For *Aedes* abundance, the highest MET at Felda Raja Alias 4 at vegetative environment was 50.50 compared to populated environment with 30.13. In Taman Satellite, vegetative environment stated highest MET with 47.38 and populated environment stated highest MET with 42.50. Overall species ratio of *Aedes aegypti* and *A. albopictus* was 1:39. *Aedes albopictus* is a dominant species in this study compared to *A. aegypti*. Positive correlation between POI and temperature was determined ($r= 0.022$, $p\text{-value}= 0.88$). The distribution of *Aedes* mosquito were higher when temperature was arising conversely when humidity and rainfall were decreased. In contrast, POI was negatively correlated with humidity ($r= -0.229$, $p\text{-value}= 0.09$) and rainfall ($r= -0.153$, $p\text{-value}= 0.51$). However, the abundance (MET) of *Aedes* mosquito

shown a negative correlation with weather variables where the correlated with temperature ($r=0.091$, $p\text{-value}=0.41$), humidity ($r=-0.240$, $p\text{-value}=0.87$) and rainfall ($r=-0.209$, $p\text{-value}=0.73$). The abundance of *Aedes* mosquito was higher at lower temperature, humidity and rainfall. As for conclusion, *Aedes* mosquito distribution will arise with high temperature and decrease of humidity and rainfall, thus, *Aedes* mosquito abundance will arise at lower temperature, humidity and rainfall. *Aedes* vectors tend to be vegetative than populated environment, however, populated environment still presence the *Aedes* vectors. This study should be conducted in another geographical condition of localities to create a baseline data and comprehensive dengue management.

Keywords: POI, MET, populated environment, vegetative environment, temperature, relative humidity, rainfall, dengue fever.

ABSTRAK

Demam denggi merupakan penyakit bawaan vektor. Penyakit berjangkit ini ditularkan oleh nyamuk *Aedes* spesies. Kefahaman mengenai kepadatan *Aedes* sangat penting bagi merancang aktiviti kawalan secara komprehensif. Kajian *cross-sectional* telah dijalankan di Jempol, Negeri Sembilan, Malaysia di mana sebanyak 960 ovitrap telah dipasang dua kali sebulan iaitu setiap dua minggu sekali di Taman Satelite dan Felda Raja Alias 4 dari bulan April sehingga bulan September 2017. Lokaliti-lokaliti ini telah pecahkan kepada dua persekitaran yang berbeza, iaitu persekitaran vegetatif dan persekitaran berpenduduk. Fokus kajian ini adalah, pertama, untuk mengakses penyebaran dan kepadatan *Aedes* spesis menggunakan dua variable iaitu index ovitrap positif (POI) dan min telur per ovitrap (MET) di dua persekitaran tersebut. Ujian ANOVA telah digunakan sebagai analisa statistik untuk mengenalpasti perbezaan POI dan MET di dua persekitaran yang berbeza. Kedua, untuk memaparkan nisbah indeks antara kehadiran *Aedes aegypti* dan *Aedes albopictus*. Dan terakhir, untuk menghubungkan antara POI dan MET dengan faktor cuaca untuk mengenalpasti perhubungan antara penyebaran dan kepadatan *Aedes* dengan suhu, kelembapan dan taburan hujan. Korelasi koefisien *Pearson* dan *Spearman* telah digunapakai untuk mengenalpasti hubungkait tersebut. Dari jumlah keseluruhan ovitrap, sebanyak 945 (98.44%) ovitrap telah diperiksa dan sebanyak 428 (45.29) dikenalpasti positif. Sejumlah 9929 telur telah dikutip sepanjang kajian ini. POI tertinggi dikenalpasti di Felda Raja Alias 4 mencatatkan 85% di persekitaran vegetatif, manakala persekitaran berpenduduk mencatatkan POI tertinggi sebanyak 75%. Penyebaran *Aedes* di Raja Alias 4 secara signifikan lebih tinggi di persekitaran vegetatif berbanding dengan persekitaran berpenduduk dengan $p\text{-value}=0.048$. Di Taman Satelite pula, POI tertinggi mencatatkan 50% di persekitaran vegetatif, berbanding 45% di persekitaran berpenduduk, dan secara signifikan didapati persekitaran vegetatif lebih tinggi berbanding dengan persekitaran berpenduduk dengan $p\text{-value}=0.039$. Bagi kepadatan *Aedes* di Felda Raja Alias 4, MET mencatatkan bacaan tertinggi iaitu 50.50 di persekitaran vegetatif jika dibandingkan persekitaran berpenduduk dengan bacaan 30.13. Di Taman Satelite pula, persekitaran vegetatif mencatatkan MET tertinggi sebanyak 47.38 manakala persekitaran berpenduduk tertinggi mencatatkan 42.50. Secara keseluruhan, nisbah indeks *Aedes aegypti* dan *A. albopictus* mencatatkan 1:39. *Aedes albopictus* merupakan spesis dominan dalam kajian ini berbanding *A. aegypti*. Korelasi positif dikenalpasti antara POI dan suhu dengan bacaan ($r=0.022$, $p\text{-value}=0.88$). Penyebaran nyamuk *Aedes* didapati meningkat apabila suhu meningkat, berbeza dengan kelembapan dan taburan hujan, di mana kedua-dua faktor ini menurun apabila POI meningkat. POI berkorelasi negative terhadap kelembapan ($r=-0.229$, $p\text{-value}=0.09$) dan taburan hujan ($r=-0.153$, $p\text{-value}=0.51$). Bagaimanapun, kepadatan (MET) nyamuk *Aedes* menunjukkan korelasi negatif dengan faktor cuaca iaitu suhu ($r=-0.091$, $p\text{-value}=0.41$), kelembapan ($r=-$

0.240, p -value= 0.87) dan taburan hujan (r = -0.209, p -value= 0.73). Kepadatan nyamuk *Aedes* lebih tinggi pada suhu, kelembapan dan taburan hujan yang rendah. Kesimpulannya, penyebaran nyamuk *Aedes* akan meningkat apabila suhu, kelembapan dan taburan hujan menurun. Nyamuk *Aedes* lebih cenderung di persekitaran vegetatif, namun begitu, masih lagi terdapat kehadiran nyamuk *Aedes* di persekitaran berpenduduk. Kajian ini perlu dijalankan di lokaliti-lokaliti yang mempunyai keadaan geografi yang berbeza dari kajian ini untuk mewujudkan data asas dan pengurusan denggi yang komprehensif.

Kata kunci: POI; MET; persekitaran berpenduduk; persekitaran vegetatif; suhu, kelembapan, taburan hujan, demam denggi.

INTRODUCTION

This study aims to investigate the distribution and abundance of dengue vectors (DV's) in relation to the population density with weather variables at the highest prevalence rate of dengue outbreak localities. The selected localities are based on secondary data of dengue cases from 2011 to 2015. It is hope to have a clear understanding of the interaction of DV's on weather in terms of their development and survival. Regarding to Ministry of Health (2016), dengue cases in Jempol shows the increased figure from 2011 to 2014 and the figure decreased in 2015. Jempol district is classified as sub-urban area with high population. The high population areas are usually experiencing dengue fever cases, which is a leading cause of illness and death in the area (World Health Organization 2009). Therefore, populated environment may indicate abundance of dengue vectors as the larval habitat is increasing rapidly in those areas (Dom et al. 2013). Ovitrap surveillance is one of the observational studies that can be used to monitor, as well as giving more understanding regarding the breeding population of mosquitoes and help to formulate control strategies in controlling the increasing of *Aedes* population in our country. It is usually preferred and has been installed to control at eggs stage of dengue vector. Ovitrap appear to be the ideal location for *Aedes aegypti* to lay their eggs especially on the paddle (WHO 2016). Ovitrap surveillance in public areas especially sub-urban area would aid as an alternative method for entomological surveillance of dengue vector (Jacob and Bevier 1969; Tanner 1969; Mogi et al. 1990). A previous study by Ritchie (1984) stated ovitraps have provided useful information on the temporal distributions of *A. aegypti* that inhabit by containers. Ai Leen et al. (2000) stated in their study that ovitrap is a sensitive and efficient technique to monitor the density of dengue vectors. This fact also supported with a study conducted by Lee (1991), stated a sensitive method using the ovitrap has been adopted to supplement larval survey.

This study was conducted to assess the temporal distribution and abundance of dengue vector and its relationship with weather variables in sub-urban area. Most of the demographic studies in Malaysia only focused on entomological in urban area. Other than that, most of the studies concentrated on the relationship between entomological with climate in the high population areas. It will appear the unfair data to give a real picture for dengue situation in Malaysia. Consequently, a study in sub-urban or rural areas should be conducted to show the density of dengue vectors in other area. Thus, this study is crucial to help other researchers in exploring the dengue vector in a multiplicity habitat. Therefore, this study may improve the previous study in the term of the association between the density and weather variables. Rather than that, this study will determine the relationship between the weather variables in the locality and the density of dengue vector populations. Among the environmental factors, rainfall, temperature and relative humidity are the key factors that may determine the density of the mosquito population (Wongkoon et al. 2013). Most of the research only focused on ovitrap

surveillance and density of dengue vector in their study but ignore the weather variables where the larvae and eggs are found. Therefore, this study may improve the previous study in terms of the association between the density and weather variables.

MATERIALS AND METHOD

Study Location

Jempol is one of seven districts in Negeri Sembilan. It is classified as a sub-urban area. Ovitrap surveillance was focused at Felda Raja Alias 4 and Taman Satellite based on the highest prevalence rate of dengue outbreak from 2011 to 2015. The selection of localities were based on a guideline on the use of ovitrap for *Aedes* surveillance. From the guidelines, priority areas for dengue control concentrated to identify the right localities for this study. According to Ministry of Health, Malaysia (1997), P1 refers to localities where an outbreak of dengue fever or dengue hemorrhagic fever or case of dengue has been recorded in the past.

Data Collection

The study location was characterized to two sections which are Zone A and Zone B. Zone A represents the high populated environment meanwhile high vegetative environment represents Zone B. Zone A consists of terrace buildings that have small distance between each other due to its highly-populated characteristics. The vegetative environment in Zone B consists of small plantation and bush areas. The summary of the area description of the study location is shown in Table 1 below:

Table 1 Ecological description of the study location

Sampling area	Description
Zone A	Less vegetation highly populated with terrace buildings and both old and new buildings are common. The distance between each house is very small.
Zone B	Abundance natural vegetation like trees and shrubs, also there is small plantations.

Ovitrap surveillance

Tham (2000) stated that ovitrap can be used to access *Aedes* population fluctuation in epidemiological studies. The ovitrap setup is standardized as described by Lee (1992) in this study. The ovitrap contains 300 ml black plastic container. The specification of the ovitrap is 6.8cm in diameter, 6.8cm for the base and 9.1cm in height. An oviposition paddle made from hardboard with 10cm x 2.5cm x 0.3cm size. The paddle consists of two different types of surface. The rough surface of the oviposition paddle must be upwards. The fresh paddles were put in the ovitraps and water level were adjusted so that they would remain moist. The ovitrap must be filled with 5.5cm of tap water. The ovitrap was set up based on the guidelines of Ministry of Health, Malaysia (1997). All ovitraps should be set up in nearness to other potential breeding containers with minimum environmental and physical disturbance.

A total of nine hundred and sixty ($n=960$) black ovitraps will be randomly placed at populated and vegetative environment where they should be either partially or fully shaded to avoid direct sunlight and heavy rain as to avoid the water spillage. Two traps were positioned in each site at a distance of 150 to 200 m from each other. Knowledge about *Aedes* vectors

flight range is crucial for control the disease (Lee 2000). A total of 480 ovitraps were placed at Taman Satelite while another 480 ovitraps have been placed at Felda Raja Alias 4. Zone A consists of highly populated environment which can be defined by area that are packed with inhabitants or humans meanwhile Zone B which is a highly vegetative environment that can be defined as an area consisting of plantation and trees. The ovitrap surveillance was conducted temporally in six-months, twice every one month with twelve intervals to determine the distribution of *Aedes* mosquito by weekly. The eggs in each ovitraps were collected four days after ovitraps deployment for egg enumeration process.

Ovitrap collection and identification of eggs.

After four days, the ovitraps were collected in the study locations. For transportation process, the paddles were placed in clear plastic zipper bags while the ovitrap containers were collected and bring back to the laboratory for the next process. In the laboratory, the paddles were left to dry under room temperature for at least 24 hours before the eggs on the paddles being counted under a microscope as described by Hornby et al. (1994). At the same time, water remaining in the ovitrap container was filtered using a filter paper to proceed the eggs hatching process. The process was conducted carefully to avoid the eggs from being damaged. Subsequently, the eggs enumeration process is done, the paddles were put into container with fresh water to hatch the eggs. The hatched eggs are monitored and recorded daily for five days. The eggs are taken out from the water and dried for two days. Then, the eggs are left again in the water for five days. The procedure was followed for three times and ended after five days' period. This procedure is based on a study conducted by Delatte et al. (2009) to avoid the immature stages influencing the results. The larvae were put into a glass bottle for counted process before species identification. Subsequently, the species were identified by microscope.

Data Analysis

To measure the population abundance of *Aedes* mosquito eggs, the entomological indices recommended by the Ministry of Health was calculated such as positive ovitrap index (POI) and mean eggs per trap (MET). The POI value characterizes the mosquitoes' distribution while MET value indicates the vector's population abundance. The descriptive analysis was performed in order to understand the distribution and abundance of *Aedes* mosquitoes in both dengue risk areas. In order to fine the POI and MET, the number of positive ovitrap and the number of ovitrap collected have to be identified. Ovitrap Data Analysis (Rozilawati et al. 2015). Table 2 shows the ovitrap index of each locality that has been classified by the sector of Vector-borne Disease, Disease Control Division, Ministry of Health, Malaysia (2005).

Table 2 The classification of Ovitrap Index Level

Classification of Risk Level	Ovitrap Index (OI)	Required Action
Level 1	$O.I < 10\%$	<ul style="list-style-type: none"> ▪ Routine action should be continue
Level 2	$10\% \leq O.I \leq 3.0$	<ul style="list-style-type: none"> ▪ Premises inspection and enforcement of DDBIA ▪ Destroy the breeding source activities including larvaciding
Level 2	$O.I \geq 30\%$	<ul style="list-style-type: none"> • Overall premises inspection activities and enforcement of DDBIA • Destroy the breeding source activities including larvaciding ▪ Preventive Thermal fogging ▪ Intensive health education

The effect of weather to the breeding site

Ministry of Health has stated that the disease is widespread with local variations in risk influenced by rainfall, temperature, humidity and unplanned rapid urbanization. Temperature is the main factor that influences the breeding of mosquito population. Since dengue transmission and the density of the mosquito population is highly dependent on the weather variables, investigations on the temperature, rainfall and humidity factors can be conducted to increase understanding on the relationship between those factors and density of mosquito population. Studies by Stewart et al. (2013) indicates that warmer air and warmer temperature can increase the rates of larvae development and adult biting rates. A study was conducted by Rodrigues et al. (2015) stated that weather variables affected mosquito populations.

RESULTS

A total of 960 ovitraps were deployed, and 9929 eggs were collected. Off these, a total of 6166 were *Aedes albopictus* and 157 were *A. aegypti* were hatched. The number of *Aedes* species specimens varied according to the environment background throughout the study. *Aedes albopictus* and *A. aegypti* were more abundance in vegetative environment as shown in Table 3. Dengue vectors abundance preferring in high plantation and bushes area. The result shows no relationship between weather and the abundance of DV’s. Sample description was analyzed using SPSS version 22. Table 3 was described sample characteristics.

Positive Ovitrap Index (POI) and Mean Eggs per Ovitrap (MET)

The hatching process is carried out to identify the mosquito species. As much as 9929 eggs are collected from the ovitrap surveillance that has been conducted within 6 months’ period (12 laps). The pattern of abundance and distribution of *Aedes* species showed the highest of POI in Week 3, August with 85%. However, the highest MET shows in Week 4, April with 50.5. Of these eggs, 7276 eggs were hatched into larvae. A total of 6166 larvae are identified as *A. albopictus* while 157 larvae are identified as *A. aegypti* as shown in Table3. The highest number of eggs collected is at Week 4 in April 2017 whereas the lowest is at Week 3 in June 2017. The highest of *A. aegypti* has been identified in April 2017 with the total of 73 larvae.

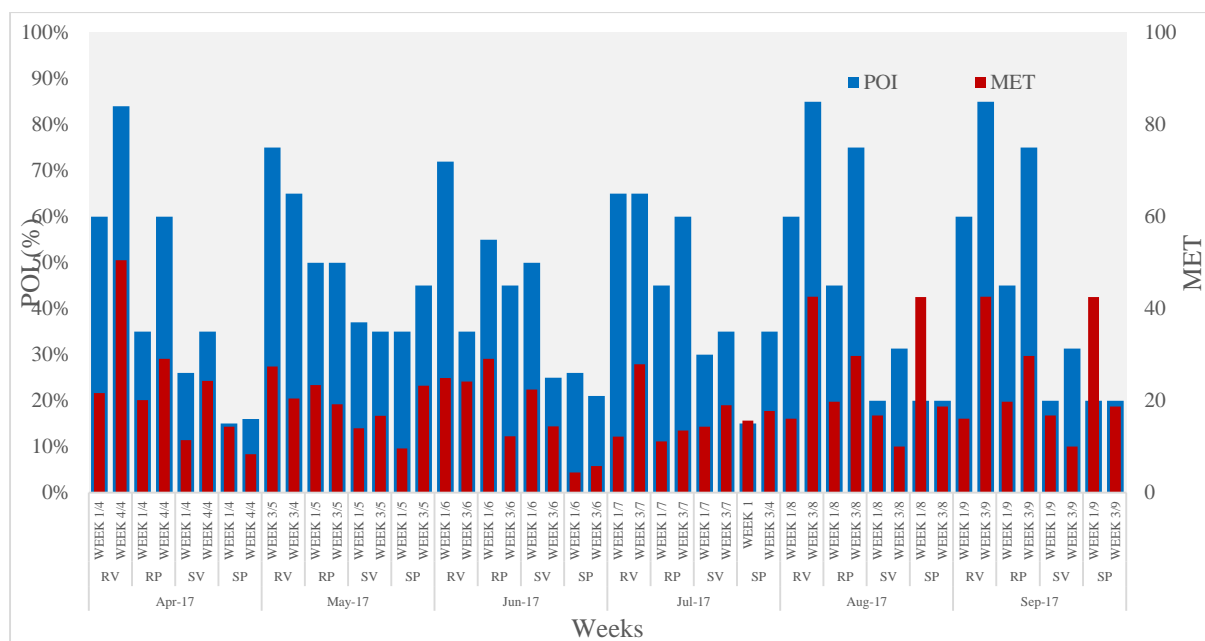


Figure 1 Dengue vectors temporal distribution and abundance.

Positive ovitrap index was analysed using SPSS with p -value < 0.05 , while mean eggs per trap stated p -value > 0.05 . Table 3 shows the comparison of POI in both environments using one-way ANOVA statistical analysis.

Table 3 Comparison of dengue vector mosquito distribution between vegetative and populated environment.

	Environment	Mean	F	df
POI	Vegetative	50.08	3.130	1
	Populated	40.33		46

The mean of POI at vegetative environment was higher with 50.08 than populated environment with 40.33. Both of the environment shows the consistency of variant with p -value > 0.05 . Sum of square (SS) between both of environment with value $F = 3.130$ ($p > 0.005$), and it proved both of the environment mean score was not significant different. The ANOVA analysis also prove that no significant different between both environments with value of $F(1, 46) = 3.130$, $p < 0.005$. The result retains the null hypothesis. A Kruskal Wallis test was generated hypotheses to determine the different of MET in two different environments. It was retained the null hypotheses, which is no different of MET between vegetative and populated environment.

The abundance of *Aedes* species in different environments

Aedes species found to be more concentrated in the vegetative environment rather than the populated environment as shown in Table 4. After being identified under the microscope, 2.48% are *A. aegypti* species. This species is discovered to be as much as 53.5% in the populated environment compared to 46.5% in the vegetative environment, and the presence of *A. aegypti* in outdoors and bushes area was determined. The finding shows that the number of *Aedes* eggs was greatly obtained at vegetative environment with 5879 eggs (59.5%) than populated environment with 40.5%. The highest mean eggs per trap (MET) were captured at Felda Raja Alias 4 which was vegetative environment with 50.5 in Week 4, April 2017. The highest POI was captured in Week 3, August 2017 with 85% from the same locality. The populated environment showed the lowest of POI and MET.

Table 4 Mean number of eggs/ larvae \pm STDEV in study sites based on type of environments.

Study sites	Mean number of eggs/ larvae per recovered ovitrap \pm STDEV							
	Felda Raja Alias 4				Taman Satelite			
Types of env.	Vegetative environment		Populated environment		Vegetative environment		Populated environment	
Eggs collected	352.75	± 207.97	252.75	± 125.83	140.67	± 98.05	82.25	± 59.88
Eggs hatches	260.33	± 131.97	183.42	± 80.39	108.92	± 94.88	53.67	± 38.44
<i>Aedes albopictus</i>	195.50	± 67.93	167.17	± 75.52	100.83	± 83.47	50.33	± 33.83
<i>Aedes aegypti</i>	5.42	± 16.96	3.67	± 6.53	0.67	± 2.02	3.33	± 7.45

Out of the total number of eggs collected by the ovitrap surveillance method, 73.3% of them were hatched to identify the DV's species. *Aedes albopictus* identified as the most dominant species in all study environments. However, 2.5% of *A. aegypti* was recognized. Despite the small percentage, it they are still able to transmit hemorrhagic dengue fever if not in control.

Table 5 Positive ovitrap with larvae identification

Months	Number of ovitrap			%	Number of eggs		
	Installed	Collected	Positive		Hatched	<i>Aedes albopictus</i> (%)	<i>Aedes aegypti</i> (%)
April 1	80	79	27	34.2	516	96.17	3.83
April 4	80	78	38	48.7	1352	88.45	11.55
May 1	80	79	39	49.4	810	100.00	0.00
May 3	80	80	39	39.0	784	97.41	2.59
June 1	80	75	38	50.7	868	97.63	2.37
June 3	80	79	25	31.6	374	100.00	0.00
July 1	80	80	31	38.8	391	100.00	0.00
July 3	80	80	39	48.8	782	97.79	5.21
August 1	80	80	29	36.3	608	100.00	0.00
August 3	80	76	41	54.0	1295	100.00	0.00
Sep. 1	80	80	42	52.5	964	99.71	0.29
Sept. 3	80	79	37	46.8	1185	97.04	2.96
Total	960	945	425		9929	97.52	2.48

Relationship between number of dengue cases with weather variables.

There are some studies that stated there are significance between dengue cases and weather variables and some of researcher objected. The weather pattern data has affected the dengue occurrence as shown in Figure 2. The graph shows the annual rainfall amount that is obtained from the Malaysian Meteorological Department. From the year 2011 to 2016, the number of annual dengue cases can be attributed with the rain factor and relative humidity. The number of annual cases of dengue fever increases as the annual rainfall increases, whereas the number of annual rainfall decreases, the number of annual dengue cases decreases as shown in Figure 2. This can be attributed to the accumulated amount of humidity, in which, the higher the amount of rainfall, the higher the amount of humidity as shown in same figure. Typically, as the amount of rainfall increases, it subsequently will produce many reservoirs that is the breeding ground of dengue's vector. Besides that, *A. aegypti* is positively associated with the high relative humidity and high vegetation (Stewart et al. 2013). In terms of rainfall, it can increase the mosquito density by increasing the availability of potential breeding sites. However, heavy rainfall may also decrease the density by flushing away all the larvae and eggs from the containers.

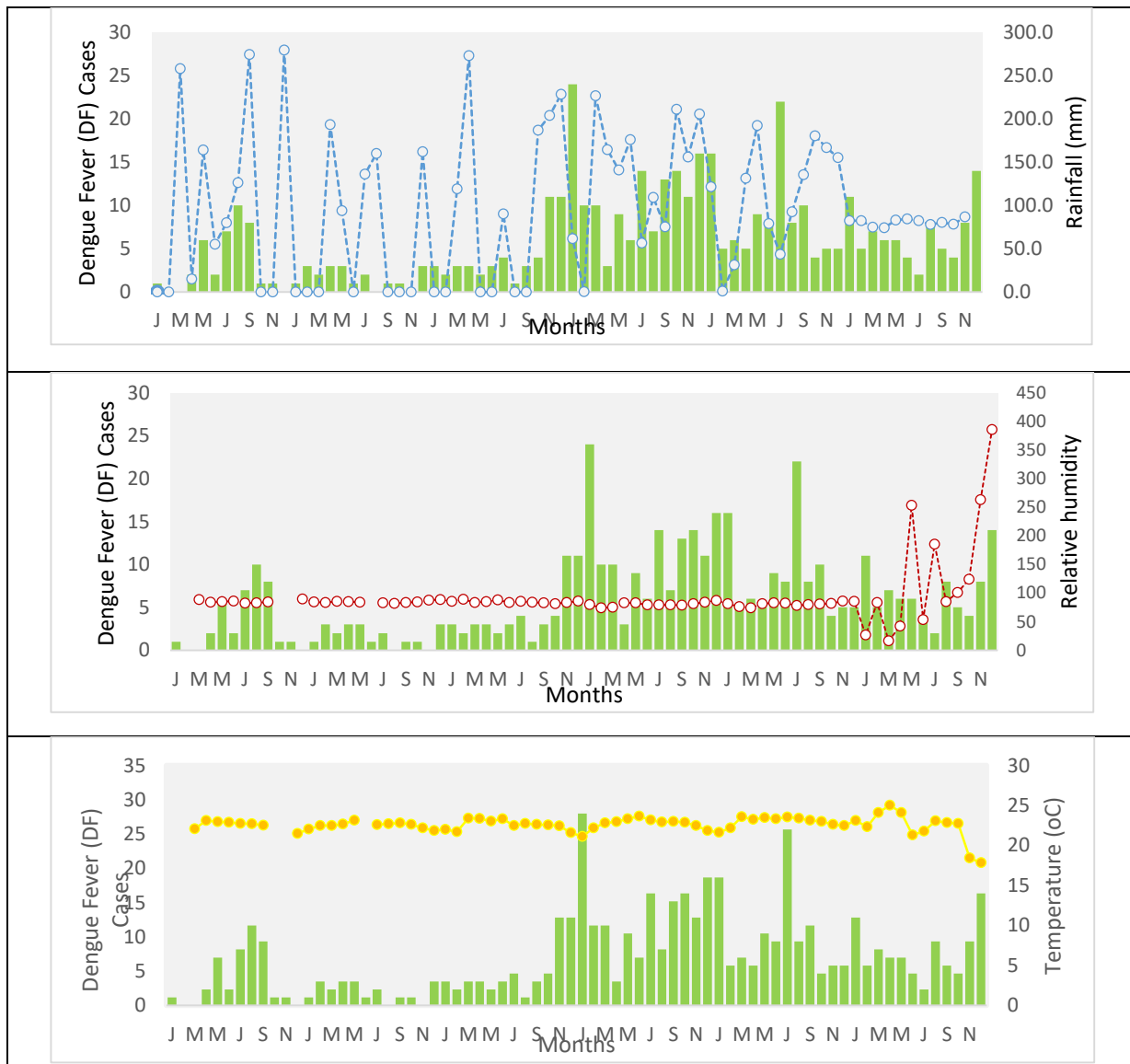


Figure 2 The Relationship of (A): Rainfall, (B): Humidity and (C): Temperature Variables with Dengue Cases from 2011 to 2015 in Jempol District (Meteorology Department 2017)

Relationship between POI and MET with weather variables.

Positive Ovitrap Index (POI) refers to the mosquito abundance while Mean Eggs per Ovitrap (MET) refers to the dengue distribution. Using the ovitrap methods, these two parameters were calculated and it shown the dengue situation in the study location. The association of dengue vectors with weather variables shows in Figure 3. A positive correlation was initiate between the temperature with Positive Ovitrap Index (POI), and between humidity with Mean Eggs per Trap (MET). Positive Ovitrap Index (POI) refers to the mosquito abundance while Mean Eggs per Ovitrap (MET) refers to the dengue distribution. Using the ovitrap methods, these two parameters were calculated and it shown the dengue situation in the study location. The association of dengue vectors with weather variables shows in Figure 3. A positive correlation was initiated between the temperature and the number of eggs.

From the correlation analysis, positive ovitrap index was increase with the increasing of temperature. However, when the humidity and rainfall stated the decrease number, the positive

ovitrap index was raised. Different with mean eggs per trap, where it was increased with arising number of humidity, and when the temperature and rainfall decreased. The association of dengue vectors with weather variables shows in Table 7. A positive correlation was initiate between the humidity and the number of eggs.

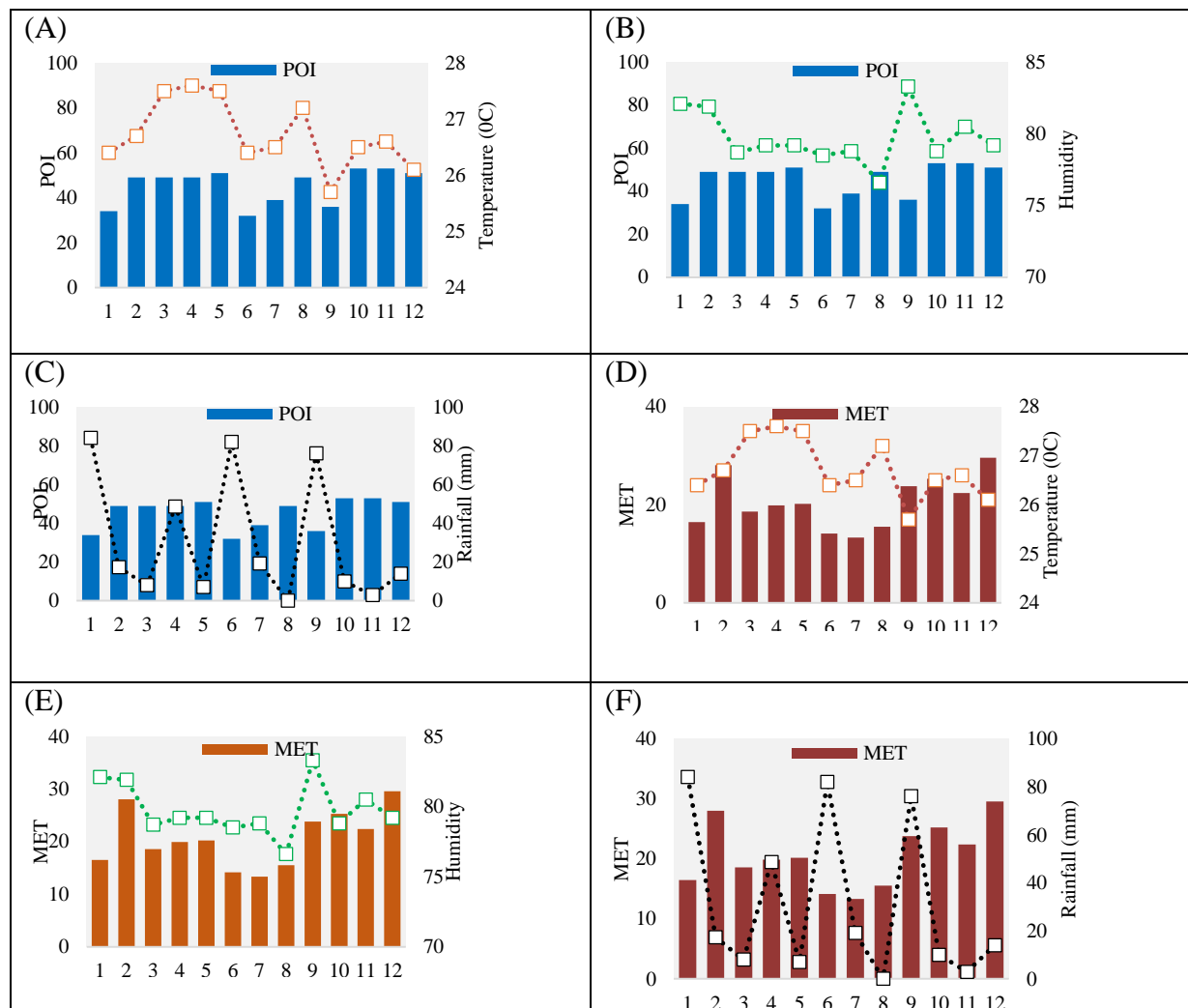


Figure 3 Weekly distribution of positive ovitrap index (POI) (Blue histogram) and mean eggs per trap (red histogram) with climatic variables; temperature (red dotted line), relative humidity (%) (Green dotted line) and rainfall (mm) (black dotted line).

Table 6 Associated factor of meteorological factors in entomological indices, n=12

	POI	MET	Temp	Humid	Rainfall
POI	1	0.556 ^b	0.483 ^a	-0.338 ^a	-0.768 ^b
MET	0.556 ^b	1	-0.165 ^b	0.519 ^b	-0.182 ^b
Temp	0.483 ^a	-0.165 ^b	1	-0.319 ^b	-0.510 ^b
Humid	-0.338 ^a	0.519 ^b	0.370 ^b	1	0.370 ^b
Rainfall	-0.768 ^{**b}	-0.182 ^b	0.370 ^b	0.370 ^b	1

Correlation is significant at level 0.05 (2-tailed). ^{**} Significant at p value < 0.05, *Pearson*^a, *Spearman*^b

DISCUSSION

A total of 960 ovitraps have been installed at two localities in 12 laps within 6 months from April 2017 to September 2017. The ovitraps were left for four days after being installed in the DV's breeding ground. It aims to give time for the mosquitoes to lay eggs in the ovitrap container. Ovitrap should be collected according to the time that has been set based on the mosquitoes' life cycle. Egg counting is conducted in the laboratory for POI and MET every time the ovitrap was collected. The results showed that the number of eggs collected in Felda Raja Alias 4 localities is higher than Taman Satellite. This is due to the geographical conditions of the Felda area surrounded by rubber estates and palm oil of plantations.

Additionally, the Felda area is not under the custody of Jempol District Council. In terms of solid waste management, it needs to be managed by the local community themselves. The results of the monitoring revealed that solid waste disposal around the housing area with surrounding bushes contributed to the high DV density. This is evidenced by the calculation of POI and MET as shown in Table 3. According to the Sector of Vector-borne disease, Disease Control Division, Ministry of Health Malaysia (2005), locality with OI 10% to 30% is categorized as level 2, and OI of 30% or more as level 3. From the result, there are three study sites were categorized as level 3 besides one in level 2. At level 3, ground inspection, enforcement of DDBIA implementation, abolishment of breeding receptacles, thermal fogging and health education to arising the awareness among residents are compulsory to be conducted. Different with level 2, the activities should be conducted including ground inspection, enforcement of DDBIA implementation and abolishment of breeding receptacles.

In the present study, *A. albopictus* was more frequently encountered than *A. aegypti*. The dominant species might be a crucial role in the transmission of *Aedes*-borne disease in these areas. Based on Forattini (2002), the suburban areas favour *A. albopictus* and urban environment favors *A. aegypti*. The factor of environmental effect might be related biology, ecology the species behavior. *Aedes* surveillance by ovitrap also conducted by Rozilawati et al. (2007) in two different areas which urban and suburban area within Penang which specified *A. albopictus* as the most prevalent species.

After the calculation process, the eggs are separated according to localities category that are vegetative environment and populated environment. The calculation revealed high density of eggs in vegetative environment. The findings show that these environments are characterized by greenery, with many plants and bushes. In contrast to the populated environment, the ovitraps were placed in the area protected by roof of the house. It shows a condition in which the DV's are more likely to lay their eggs in vegetative environment nearby human population and DV's flying distance of 200m is capable of carrying this vector to a human-occupied area.

The eggs then proceed to the hatching process according to the procedure described in the methodology section to identify the DV's species in the study area. A total of 73.3% of the total number of eggs collected undergoes hatched whereas 84.7% of a total hatched larvae go through identification larvae. From this study, it is found that *A. albopictus* was found in the populated and vegetative environment. It also shows that *A. aegypti* was more commonly found in the vegetative environment than the populated environment. This study proves that there was a presence of *A. aegypti* in the outdoors. Mean eggs per trap can be determined using a positive trap in ovitrap study. It is crucial to monitor the abundance of dengue vectors. It is also supported by the weather data to determine the relationship between the oviposition and rainfall

factors. During the study, positive traps were recorded at the highest number when rainfall was the least. In Week 1, April 2017 stated the highest rainfall, however, based on Figure 3 it was stated 34.2% of positive traps. The highest number of rainfall may cause the eggs to flush out from the container. In term of this finding, the number of positive traps are based on the rainfall factor.

The present study proved the effect of temperature on oviposition activities because a positive association was found between temperature and the number of traps for dengue vectors. Temperature indicates a positive correlation with the oviposition of dengue vectors in two different environments. Temperature consequently affecting the vector behavior that need supplementary studies. The association between the oviposition activity and the temperature supported by the reduction in POI and MET when the temperature decreases. A study conducted by Dibo et al. (2005), stated that statistically significant association found among temperature and trap positivity and the mean eggs per trap. There is limitation needed during ovitrap installation process. The ovitrap was damaged and there was also a missing ovitrap. In the first week, one ovitrap in the vegetative environment was solved. There are likely to be people who are worried about the presence of this unknown container. There was also an ovitrap which was not found during the collection. However, the number of problematic ovitraps were not included in the calculation to avoid biased data. Selected localities recorded high dengue fever cases every year and dengue outbreaks occur almost every year. However, throughout this ovitrap installation, no dengue cases were reported from the locality. From the observational study, it is proven that the installation of the ovitrap can control dengue fever cases. This is likely due to increased awareness among the population to keep their environment clean. This is due to the monitoring carried out bi-weekly during the six-month period as well as making a huge impact in preventing the chain of dengue fever disease from occurring. However, further studies have to be carried out to prove this statement. This follows the findings beyond the scope of the study which has been conducted and it requires another study for the purpose of proving the statement.

CONCLUSION

Mosquito surveillance should be a prime phase in vector control approaches. Therefore, the abundance of mosquito species in each area and their oviposition behavior are crucial to be identified. Using this method, the health department and local authorities may decide the most suitable action to control the vector. It also may be a long-term efficiency of mosquito control plan with involvement of public to eliminate all the potential breeding sites. So that, this study aims to access the temporal population density of dengue vector in sub urban area. Using ovitrap methods, it may lead to create the baseline data that we need for the dengue high-risk area. The input of this study can be used as a potential baseline reference for sustainable dengue management.

ACKNOWLEDGEMENT

The authors sincerely thanked Dr. Zainuddin bin Ali, the Director of Public Health JKNNS, for the permission to conduct this study. Also thanks to Mr. Nur Amri b Nazaruddin, Mrs Bibi Radhiah binti Abdull Rahman, Mr S. Zulain bin Sh. Roslen and Mr Mohamad Suhaimi bin Taib for their help during the sampling.

REFERENCES

- Ai-Leen, Tan, G. & Song, R. 2000. "The use of GIS in ovitrap monitoring for dengue control in Singapore". *Dengue Bulletin* 24(65): 110–116.
- Cawangan Penyakit Bawaan Vektor, Bahagian Kawalan Penyakit, Kementerian Kesihatan Malaysia. 2005. Protokol pengawasan dan pemantauan vektor melalui ovitrap. MMP/Pasukan Ovitrap: 17022004. Malaysia.
- Delatte, H., Elissa, N., Quilici, S. & Fontenille, D. 2009. *Aedes* (Diptera: Culicidae) vectors of arboviruses in Mayotte (Indian Ocean): distribution area and larval habitats. *Journal of medical entomology* 46 (2): 198-207.194.
- Dom, N. C., Ahmad, A. H. & Ismail, R. 2013. Habitat characterization of *Aedes* sp. breeding in urban hotspot area. *Procedia-Social and Behavioural Sciences* 85: 100-109.
- Forattini, O. P. 2002. *Identificacao, Biologia e Epidemiologia. Culicideologia Medica*. vol. 2 Brazil: Sao Paulo.
- Vector Control Unit, Vector-Borne Disease Section, Ministry of Health. 1997. Guidelines on the use of ovitrap for *Aedes* surveillance. Malaysia.
- Hornby, J.A., Moore, D.E. & Miller, T.W.Jr. 1994. *Aedes albopictus* distribution, abundance and colonization in Lee County, Florida and its effect on *Aedes aegypti*. *Journal of American Mosquito Control Association* 397-402.
- Lee, H.L. 2000. *Aedes*: Mosquitoes that spread dengue fever. In Ng, F.S.P. & Yong, H.S. (eds.). *Mosquitoes and The Mosquito-Borne Disease*, pp. 45-61. Kuala Lumpur: Akademik Sains Malaysia.
- Lee, H.L. 1992. Sequential sampling: its application in ovitrap surveillance of *Aedes* (Diptera: Culicidae) in Selangor, Malaysia. *Tropical Biomedicine* 9: 29 – 34.
- Lee, H. L. 1991. Analysis of limiting factors affecting breeding of *Aedes* vectors in urban towns of Peninsular Malaysia- nationwide resurvey. *Tropical Biomedicine*. 8: 185-189.
- Ministry of Health Malaysia. 1997. Guidelines on the use of ovitrap for *Aedes* surveillance. Malaysia: Putrajaya.
- Organization, W.H. 2009. *Dengue Guidelines for Diagnosis, Treatment, Prevention and Control. New Edition*. Malaysia: WHO.
- Ritchie, S.A. 1984. The production of *Aedes aegypti* by a weekly ovitrap survey. *Mosquito. News* 44: 77-79.
- Rodrigues, M.M., Marques, G.R.A.M., Serpa, L.L.N., Arduino, M.B., Voltolini, J.C., Barbosa, G., Andrade, V.R. & Lima, V.L.C. 2015. Density of *Aedes aegypti* and *Aedes albopictus* and its association with number of residents and meteorological variables in the home environment of dengue endemic area, Sao Paulo, Brazil. *Parasite & Vectors* 8:115.

- Rozilawati, H., Zairi, J. & Adenan, C. R. 2007. "Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang, Malaysia." *Tropical Biomedicine* 24(1): 83-94.
- Rozilawati, H., Tanaselvi, K., Nazni, W.A., Mohd, Masri. S., Zairi, J., Adanan, C.R., & Lee, H.L. 2015. Surveillance of *Aedes albopictus* Skuse breeding preference in selected dengue outbreak localities, Peninsular Malaysia. *Tropical Biomedicine* 32: 49-64.
- Stewart Ibarra, A.M., Ryan, S.J., Beltra'n, E., Meji'a, R. & Silva, M. 2013. Dengue Vector Dynamics (*Aedes aegypti*) Influenced by Climate and Social Factors in Ecuador: *Implications for Targeted Control*. *PLoS ONE* 8(11): e78263.
- Wongkoonn, S., Jaroensutasinee, M. & Jaroensutasinee, K. 2013. Distribution, seasonal variation & dengue transmission prediction in Sisaket, Thailand. *Indian Journal of Medical Research*. 138: 347-353.
- World Health Organization and the Special Programme for Research and Training in Tropical Diseases (TDR). 2009. *Dengue Guidelines for Diagnosis, Treatment, Prevention and Control*. New edition. Switzerland: Neglected Tropical Disease (NTD).