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Breeding Characteristics of *Aedes* Mosquitoes in Dengue Risk Area

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Abstract

There are varying ranges of habitats that have different characteristics of water for the breeding of mosquito. A house-to-house cross-sectional entomological survey was carried out at peridomestic area to detect larval breeding sites. *Aedes albopictus* was the predominant species in the container-breeding habitats. Most of the breeding habitats were class into high level of larval density. Turbidity, pH, TOC, magnesium, calcium and sodium is among the characteristics that shows a significant difference with the larval density and species composition respectively. As conclusion, characteristics of the mosquito breeding area can affect the larval density and give impact on the quality of life.

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Keywords: Aedes; mosquito breeding habitat; larval density; physicochemical

1. Introduction

Dengue is one of the urban diseases and keeps increasing annually. There are two mosquito species that responsible for the transmission of dengue in Malaysia which is Aedes aegypti and Aedes albopictus. Both of the species are commonly found nearby human community. Aedes species can be found easily in natural and artificial containers which hold clear and clean water. Some of the preferred breeding sites are containers such as ant traps, earthen jars, flower pots, drums, concrete tanks, coconut shells and discarded tires (Paupy et al., 2009; Nazri et al.,

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2013a). Nazri et al. (2013b) stated that the common method being implemented widely to control larvae is by using a chemical such as abate and it is also crucial for the control of mosquitoes at the larval stage are highly effective. It has been proven that the two Aedes species breed primarily in artificial water containers, and the mosquito's lifecycles are closely associated with human activities. The abundance of mosquitoes has given a significant negative impact on the quality of life to the human surroundings.

In urban surroundings, mosquitos are more prone to utilize water-holding containers as immature developmental stages. Almost all mosquitoes species would prefer to oviposit in a less lighting area or shaded (Dejene et al., 2015) where in that particular area the temperatures are lower compared to the area that exposed with direct sunlight, but there is still many others factor that takes into account for female mosquito oviposition preferences places. One of the critical parameter of the aquatic habitats for mosquitoes is the chemical characteristics which can influence the survival rate of the mosquitoes (Dejene et al., 2015). Some mosquito oviposition cues are water temperature, pH, ammonia, nitrate, sulphate, phosphate and dissolved solids (Oleymi et al., 2010; Bhaskar et al., 2011). Larval mosquitoes develop into four instar stages by consuming detritus and any other related microbes to obtain nutrients. The types of the containers, water quality, and conditions of water containers are necessary for breeding (Dejene et al., 2015). The condition that would lead to mosquito infestation is such as stored water in the container for an extended period, extended rainfall during last rainy season, and ambient relative humidity and temperature (Dejene et al., 2015). Reinfestation of vectors to new geographical areas, warm and humid climate, increased population density, water storage pattern in houses, and storage of trash, for instance, recyclable materials can serve as risk factors for dengue virus infections (Dejene et al., 2015).

The efforts to combat against DF have always met a dead end as there is lack of adequate knowledge, lack of trained workforce, lack of participation from the community and poor waste disposal practices by the resident. The ecological factors such as characteristics of breeding habitat are importance as it can influence the biological characteristics of the mosquito and improve the implementation of the vector management programs (Reji et al., 2013; Thangamathi et al., 2014).

Therefore, in this study, we tried to investigate and identify mosquito species breeding in natural and artificial water storage materials that serve as potential dengue fever (DF) transmission in the central zone of Shah Alam (SA). This study provides baseline information on the types of dengue vectors breeding in natural and artificial water containers in the central zone of SA, providing valuable information to the Ministry of Health and community of the zone. Furthermore, the result of this study enables providing community awareness about the vectors and the protective measures to be taken. This study also provides the foundation for further investigation on DF.

2. Methodology

2.1. Study area

The epidemiology data on daily DF cases from 2012 to 2014 were obtained from Majlis Bandaraya Shah Alam (MBSA) and further analyzed. From the spatial analysis of DF cases, several characteristics of DF epidemic may be described by comparing the zones for the incidence of DF. The central zones of SA were recorded higher DF cases as compared to other zones in SA (north and south). The DF cases in the central zone are cluster and have high population density. Therefore, based on this information, the central zones will be selected as study focused area. In this study, dengue risk area in the central zone of SA area is chosen as the main research site which has a significant public health implication in relation to the control and prevention of dengue. SA is divided into 3 zones which are Northern Zone, Central Zone and Southern Zone.

The main landscape characteristic of this area is the urban area with rapidly developed. The major land area in this study area is the residential, commercial and industrial area. Besides that the forested areas and construction area also have shown the rapid changes trend, and this might influence the pattern of DVs distribution.

2.2. Larval surveys

A house-to-house cross-sectional entomological survey was carried out at the peridomestic area to detect larval breeding. The level of infestation of Aedes mosquito can be influenced by the quality of the surrounding environment. Natural and artificial water containers were visually inspected with a minimum of 100 houses per locality for the presence of water and mosquito larvae and pupae (Rozilawati et al., 2015). Peridomestic area was inspected thoroughly and the houses inspected were chosen randomly in each locality. Each container was recorded for container type, sun exposure, lid status, and water type.

The immatures collection will be collected by using dipping method (Kumar et al., 2016). Dipper is the most commonly used tools for collecting mosquito larvae from a wide variety of habitat. Water samples will be collected by using a dipper and were collected accordingly with the volume (mL) needed for each parameter tested in this study. For the water breeding habitat that contains little amount of water, composite sampling method will be used. Composite sampling method will be done by mixing water from some breeding sites until the needed volume of samples is enough. Three dips will be taken from each mosquito breeding habitat found from the surveillance, and the mean number of larvae per dip will be calculated to determine the larval density (Mogi & Wada, 1973; Mogi & Sota, 2012). Table 1 shows the indicator used to determine the density level of the larvae collected.

Table 1. Indicator for larval density level (Mogi & Wada, 1973; Mogi & Sota, 2012)

Density level	Mean number of larvae per dip
Low	< 0.23
Moderate	0.23 – 5.09
High	> 5.09

2.3. Larval identification

Larvae collected from the field were identified to determine the species. The equipment that was used for identification is the compound microscope. The identification was aided by the Pictorial Key for Identification of Mosquito by Leopoldo M. Rueda (Rueda, 2004; Nazri et al., 2013b). The larvae are divided into three main parts which are the head, thorax, and the abdomen. All three parts were observed to identify the setae, the segment VIII, the siphon, and the anal segment or the segment X, which resembles the parts and segments of an Aedes mosquito larvae. All larvae collected were identified, and only Aedes mosquito larvae were being taken into count. Other mosquito larvae will be excluded.

2.4. Breeding characteristics

Physical properties of water which are pH, turbidity (NTU), dissolve oxygen (DO), and temperature (°C) were tested on-site at the time of collection while hardness and total organic carbon (TOC) will be tested in the laboratory using TOC Analyzer. Certain laboratory equipment was brought together to the field to measure that parameter's reading. The pH reading and temperature of the water were taken by using pH meter meanwhile turbidity was measured using turbidity meter. Hardness was measured using DR5000 spectrometer and DO were measured using DO meter. The dissolved micronutrients such as magnesium, calcium, sodium and potassium will be analyzed by using FAAS (Flame Atomic Absorption Spectrometer).

2.5. Data analysis

The mean characteristics of breeding water parameter in relation with larval density and species composition were compared using one-way ANOVA and Kruskal-Wallis Test. The statistical analysis was carried out using IBM SPSS 20 statistical analysis.

3. Results

3.1. Laval density and species identification

Ten dengue risk area in the central zone of SA was selected based on the analysis of DF data retrieved from MBSA. Table 2 shows the number of positive container that has been classified according to the level of larval density. Based on the result of larval survey, most of the localities have a high larval density. The highest number of positive container collected was at Seksyen 17 with 15 containers and 60% of the positive container being class into high larval density followed by Seksyen 13 with 12 containers collected with 58.3 % of the containers were identified as the high level of larval density.

Table 2. Number of container according to level of larval density in all ten (10) localities

Localities	Number of container according to level of larval density, n (%)			**TOTAL**
	Low	Medium	High	
Seksyen 2	0 (0)	1 (12.5)	7 (87.5)	**8**
Seksyen 4	5 (50)	4 (40)	1 (10)	**10**
Seksyen 6	3 (50)	3 (50)	0 (0)	**6**
Seksyen 7	0 (0)	4 (80)	5 (20)	**9**
Seksyen 8	1 (9)	5 (45.5)	5 (45.5)	**11**
Seksyen 13	2 (16.7)	3 (25)	7 (58.3)	**12**
Seksyen 17	2 (13.3)	4 (26.7)	9 (60)	**15**
Seksyen 18	3 (30)	4 (40)	3 (30)	**10**
Seksyen 20	1 (20)	0 (0)	4 (80)	**5**
Seksyen 24	0 (0)	2 (20)	8 (80)	**10**
TOTAL	**17 (17.7)**	**30 (31.3)**	**49 (51)**	**96**

The larvae species identified in each locality are varied between Ae. albopictus, Ae. aegypti and mixed breeding (Figure 1). Aedes aegypti are the least number of species being recorded with highest percentage record with only 3% in Seksyen 7. From the ten localities, the highest percentage of Aedes species are Ae. albopictus and mixed breeding with both of the categories are highest in five localities each. But, although the percentages of mixed breeding are high, Ae. albopictus are considered as the main Aedes vector at the central zone of SA. The highest percentage of Ae. albopictus are found in Seksyen 13 with 100% while the highest percentage of mixed breeding was recorded at Seksyen 7 with 94%.

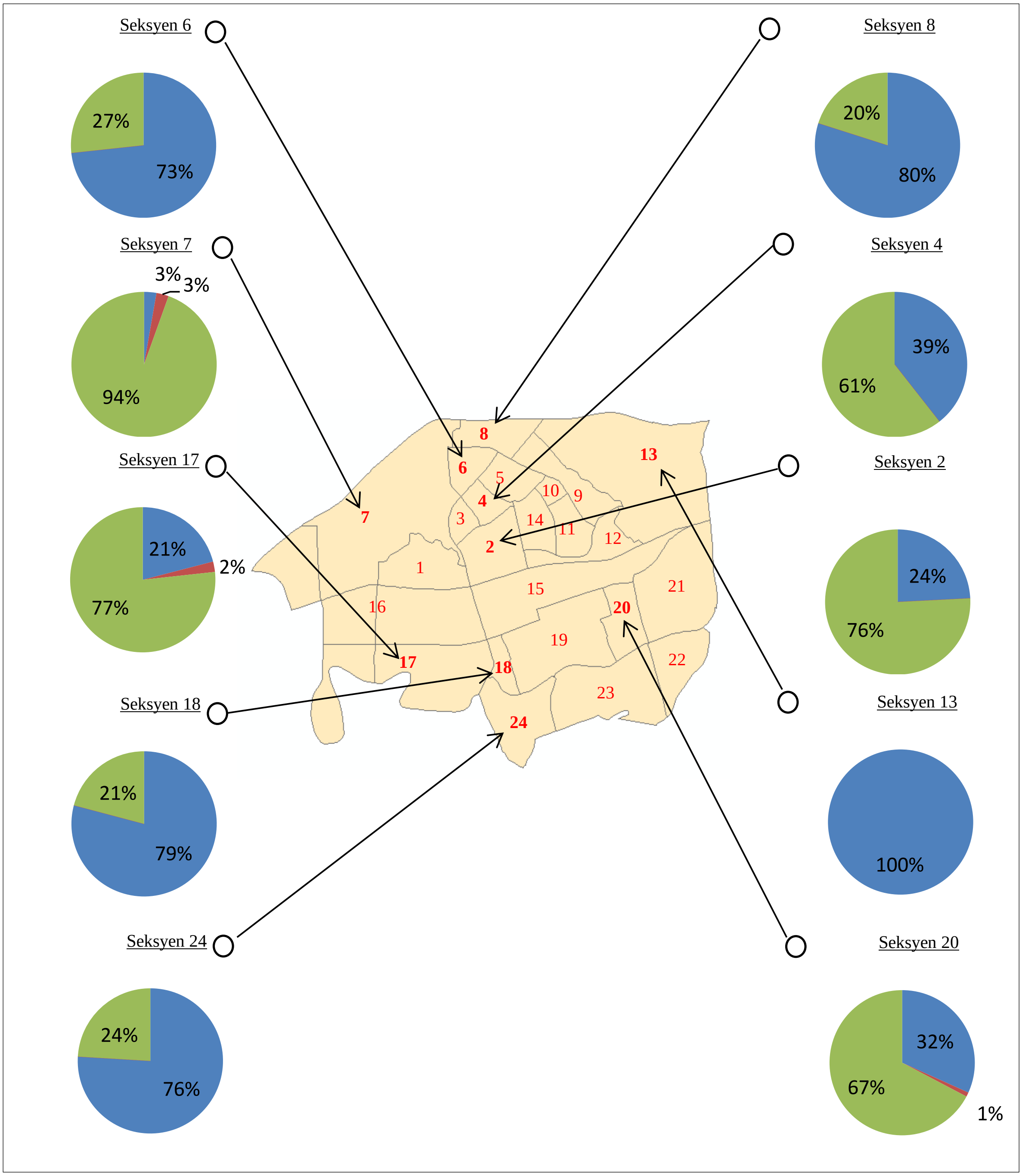

Fig. 1. Spatial distribution of *Aedes* species at ten (10) dengue risk area in central zone SA, Selangor; Noted: Blue color indicates *Ae. albopictus*, red color indicates *Ae. aegypti* and green color indicates mixed breeding.

3.2. Characteristics of aquatic breeding habitat for Aedes mosquito

A total of 1258 houses were being surveyed for *Aedes* larvae and 96 containers were classified as positive container (PC). PC is defined as the presence of *Aedes* larvae in the container. The characteristics of the aqueous larval habitat were also being measured and further analyze in order to understand the influenced of larval density on the characteristics of breeding habitat. Table 3 shows the relationship of the habitat characteristics with its different level of larval density. From the results, three parameters have showed a significant different on the level of larval density which is turbidity (*p*-value: 0.003), TOC (*p*-value: 0.008) and magnesium (*p*-value: 0.008). Other parameters were concluded as do not possess any influence to the level of larval density presence in the water.

Table 3. Mean, median and p-value to show the differences between characteristics of aqueous larval habitat and level of larval density

Characteristics of the aqueous larval habitat		Larval density	Mean ± SE	Median (IQR)	*p*-value
Physical characteristics	Temperature (NTU)	Low	28.53 ± 0.25		
		Medium	29.18 ± 0.21		[a]0.114
		High	29.28 ± 0.20		
	pH	Low	6.52 ±0.12		
		Medium	7.06 ± 6.78		[a]0.037*
		High	6.86 ± 0.99		
	DO (mg/l)	Low	90.29 ± 0.92		
		Medium	89.45 ± 1.09		[a]0.692
		High	90.56 ± 0.84		
	Turbidity (NTU)	Low		6.43 (12.22)	
		Medium		6.43 (12.22)	[b]0.003*
		High		6.43 (12.22)	
Chemical characteristics	Hardness (mg/l)	Low		0.20 (6.75)	
		Medium		0.20 (6.75)	[b]0.976
		High		0.20 (6.75)	
	TOC (mg/l)	Low		20.84 (43.10)	
		Medium		20.84 (43.10)	[b]0.008*
		High		20.84 (43.10)	
	Calcium (mg/l)	Low		13.72 (21.02)	
		Medium		13.72 (21.02)	[b]0.296
		High		13.72 (21.02)	
	Magnesium (mg/l)	Low		1.18 (2.05)	
		Medium		1.18 (2.05)	[b]0.008*
		High		1.18 (2.05)	
	Potassium (mg/l)	Low		0.00 (0.00)	
		Medium		0.00 (0.00)	[b]0.124
		High		0.00 (0.00)	
	Sodium (mg/l)	Low		0.00 (0.00)	
		Medium		0.00 (0.00)	[b]0.098
		High		0.00 (0.00)	

[a] p-value by *Oneway ANOVA* Test; [b] p-value by *Kruskal Wallis* Test

Both *Aedes* species are found in the container in all study area. There are only one species in a single container plus there are also mixed breeding which both species in a single container. The preferences of *Aedes* mosquito to oviposit in term of water characteristics were being analyzed as shown in Table 4. The results indicate that calcium and sodium have a significant difference with the *p*-value of 0.014 and 0.025 respectively. This indicates that certain species prefer a specific characteristic of aqueous habitat or calcium and sodium play a significant role in larval survival rate.

Table 4. Mean and p-value to show the differences between characteristics of aqueous larval habitat and the larvae species

Characteristics of the aqueous larval habitat		Species	Mean ± SE	Median (IQR)	*p*-value
Physical	Temperature (NTU)	*Ae.albopictus*	29.19 ± 0.17		
		Ae.aegypti	29.44 ± 0.61		[a]0.614
		Mixed breeding	28.96 ± 0.23		
	pH	*Ae.albopictus*	6.75 ± 0.09		
		Ae.aegypti	7.06 ± 0.48		[a]0.132
		Mixed breeding	7.03 ± 0.10		
	DO (mg/l)	*Ae.albopictus*	90.47 ± 0.76		
		Ae.aegypti	85.88 ± 1.79		[a]0.207
		Mixed breeding	90.28 ± 0.90		
	Turbidity (NTU)	*Ae.albopictus*		6.43 (12.22)	
		Ae.aegypti		6.43 (12.22)	[b]0.726
		Mixed breeding		6.43 (12.22)	
Chemical	Hardness (mg/l)	*Ae.albopictus*		0.20 (6.75)	
		Ae.aegypti		0.20 (6.75)	[b]0.188
		Mixed breeding		0.20 (6.75)	
	TOC (mg/l)	*Ae.albopictus*		18.73 (47.55)	
		Ae.aegypti		18.73 (47.55)	[b]0.530
		Mixed breeding		18.73 (47.55)	
	Calcium (mg/l)	*Ae.albopictus*		0.34 (0.53)	
		Ae.aegypti		0.34 (0.53)	[b]0.014*
		Mixed breeding		0.34 (0.53)	
	Magnesium (mg/l)	*Ae.albopictus*		0.30 (0.05)	
		Ae.aegypti		0.30 (0.05)	[b]0.284
		Mixed breeding		0.30 (0.05)	
	Potassium (mg/l)	*Ae.albopictus*		0.00 (0.00)	
		Ae.aegypti		0.00 (0.00)	[b]0.270
		Mixed breeding		0.00 (0.00)	
	Sodium (mg/l)	*Ae.albopictus*		0.00 (0.01)	
		Ae.aegypti		0.00 (0.01)	[b]0.025*
		Mixed breeding		0.00 (0.01)	

[a]p-value by *Oneway ANOVA* Test; [b]p-value by *Kruskal Wallis*Test

4. Discussions

Dengue is one of the acute arboviral diseases in tropical and subtropical countries (Nazri *et al*., 2013b). Many factors contribute in influencing the current pandemic directly or indirectly. Rapid urbanization, high population growth rate and globalization are the factors that contributed in creating more larval breading habitat and thus increase the mosquito density. The quality of living also can be affected by the number of DF cases that keep arising which indicate that health status of the particular areas is low. All ages group are susceptible, but the most distinct age group would be teenagers and adults which their daily routine have force them to be indirectly exposed to the dengue vectors (DV) which are *Aedes* mosquito.

The controls of DF have become a major problem in many countries. With the absence of effective vaccines, control of DV has become the common method which being implemented in most of the countries in combatting this disease. Breeding habitat selections for *Aedes* vector are important as it can be related to the control and reduction of dengue transmission. It also can act as a factor in the survival rates and population dynamics of dengue vectors. Knowledge and understanding of the ecosystem are crucial especially in relating the influence of dengue vectors distribution and abundance. One of the key components of dengue vectors breeding habitat is the quality of the water which can influence the oviposition process and larval development stages (Oyewole *et al*., 2009; Nazri *et al*., 2013b).

From the results, *Aedes albopictus* are more abundance than *Aedes aegypti*. This can be explained as the survey was conducted at the peridomestic area of the residential area. Most of the containers were found in the backyard area. From visual analysis, most of the backyard area was not properly managed by the owner and management. The characteristics of the backyard would be full with abandoned vegetation area. Lots of crops not being handle properly. Similar to the study by Nazri *et al*. (2013b) stated that female *Aedes albopictus* prefer to oviposit in a natural and outdoor manmade container that contain a high amount of organic debris while *Aedes aegypti* prefer to oviposit in the clear water nearby human community and indoor domestic container. The larval density also high as this reflects the abundance of mosquito in the area. From the results, the most susceptible localities would be Seksyen 13 and Seksyen 17. Both of the localities recorded the highest number of positive container collected from the survey and also the larval density is among the highest with more than half of the positive container collected class into high level of larval density. Seksyen 13 shows a clear dominant of species composition with 100% are *Aedes albopictus* while Seksyen 17 shows 77% of species composition are mixed breeding. There is previous study conducted by Thangamathi *et al*. (2014) states that both of the *Aedes* species would prefer the same breeding sites regard to turbidity, pH, alkalinity, chloride and phosphate levels. But, they do not favor the water conditions or mosquito breeding habitat characteristics. The abundance of mosquito can lead to higher virus transmission and decrease the quality of life in the particular area.

The characteristics of aqueous larval habitat also play an important role as it can provide nutrients and suitable environment that can increase the survival rate of the larvae. From the results, turbidity, pH, TOC and concentration of magnesium shows a significant difference when being compare with the larval density. This has been achieved by other study (Nazri *et al*., 2013b) that also agrees that turbidity affects the larval density. Both of the *Aedes* species have their own preference of turbidity level. *Aedes aegypti* would prefer less turbid water while *Aedes albopictus* prefer more turbid water. The TOC also showed that had effects the larval density, and this can be support with the study by Thangamathi *et al*. (2014) indicate that the sulphate content in *Aedes* species larvae breeding habitat are high compared to other species. Moreover, *Aedes* species are also has a fast adaptation to the environment and surroundings condition. According to Reji et al. (2013), the survival of *Aedes aegypti* larvae was found to be in the maximum in the pH range of 6.5-8. pH can be used as weapons to control the DF by spraying of biopesticides such as neem oil. Magnesium also plays a role in determining larval density, and there is study by Thangamathi *et al*. (2014) expressed that calcium, potassium, sodium, sulphur and magnesium can give an ideal breeding ground for *Aedes aegypti*. The characteristics of aqueous larvae habitat also being analyze and relate to the species of larvae presence. From the result, calcium and sodium give a significantly different result from the analysis which can support the previous statement which calcium and sodium can provide a good breeding ground for *Aedes* species.

5. Conclusion

In the study area, the community set up a small garden at the backyards but does not manage the area well. There is also some of the resident store water in different containers for an extended period for a purpose to watery all the crops and domestic use. There are varied types of discarded containers that hold rainwater for a long period. As the results of the study, most of the containers were infested with *Aedes* larvae which could serve as the vector of dengue disease. From the investigation, there are no doubts that the area is a dengue risk area and have a potential of dengue viral infection spreading. However, to determine whether the mosquito is transmitting DV or not; further investigation is required to looking for the virus in the mosquito. In conclusion, characteristics of larvae breeding habitat can affect the larval density thus the abundance of mosquito will increase. The quality of life is profoundly affected by DF especially people that lives in high population density area where the disease will transmit faster. Data obtain from this study will serve as a baseline for the control of the dengue vector mosquito.

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