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OVIPOSITION OF AEDES MOSQUITOES AT A SELECTED RESIDENTIAL AREA IN KUBANG KERIAN, KELANTAN

Nur Nadia Ahmad Fauzi[1] and Siti Marwanis Anua[1*]

[1]*School of Health Sciences, Health Campus Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.*

Corresponding author: Siti Marwanis Anua; E-mail: smarwanis@usm.my ; School of Health Sciences, Health Campus Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan; 097677827

ABSTRACT

Objective: The aim of this study was to determine the preference of the Aedes mosquitoes to oviposit based on the distribution of mosquito eggs, abundance of Aedes species and ovitrap index based on types of water, seasons and breeding sites.

Method: To achieve the aim, 140 ovitraps consisted of seasoned tap water and rain water were placed side by side at 70 different sampling sites in Bandar Baru, Kubang Kerian at a 2-monthly interval between rainy and dry seasons, respectively.

Result: The identified Aedes species in this study were *Aedes Albopictus* and *Aedes Aegypti*. There were significance differences in the distribution of mosquito eggs between types of water (more mosquito eggs were oviposited in rain water) and between seasons (more mosquito eggs were oviposited during wet season). There was a significant association between percentage of mosquito eggs hatched by seasons with more mosquito eggs were hatched during dry season. A significantly different of mosquito eggs oviposition was found at four different locations in studied area with high level of ovitrap index values.

Conclusion: In conclusion, Aedes surveillance using ovitrap is the most sensitive, cost-effective and reliable method to detect the presence of Aedes species.

Keywords: *Ovipostion preferences; Aedes mosquitoes, Ovitrap*

1. Introduction

Aedes aegypti (also known as *Stegomyia aegypti*) and *Aedes albopictus* (also known as *Stegomyia albopictus*) act as the epidemic vector of dengue in the South-East Asia region of WHO countries (WHO, 2011). World widely, dengue has affected 85,111 of people in 106 countries (Leta *et al*., 2018). It was estimated that about two billion of people are at risk of dengue, with over 100 million of infections per year and about 100,000 of death, globally (Harun, 2007). Dengue fever (DF) and dengue haemorrhagic fever (DHF) has become endemic in Malaysia since its first outbreak in 1962 (Lam, 1994). It has been reported previously that number of DF and DHF cases increased dramatically from 43,346 in 2013 to 108,698 in 2014 (Yussof et al., 2017). The highest number of cases ever reported in Malaysia was in 2015 with 120,836 infections. The number of cases has been decreasing since and until recent record, the number of cases dropped to 83,849 with 177 fatalities in 2017 (Ministry of Health Malaysia, 2017). However, it is still two-fold increase compared to 5 years ago. It may be due to the rapid industrial and economic development and urbanisation factors which enhances the creation of man-made environment for the breeding of mosquitoes. Although similar trend is observed in Kelantan, the cases had decreased by 57% from 5937 of dengue cases in 2016 to 2455 cases in 2017 (Yussof et al., 2017; Ministry of Health Malaysia, 2017). However, dengue has remained an endemic, with 171 reported in 2017 and 2 fatalities (Utusan Online, 2017).

Aedes surveillance using ovitrap is one of the dengue vector control which is useful in planning and managing dengue cases (Anis et al., 2016). Oviposition is one of the vector surveillance used in controlling the dengue cases which has become endemic in various regions. Besides, it is a sensitive method in determining the presence of adult female Aedes. The oviposition site selection by females Aedes mosquito is an important element in the reproductive phase of mosquitoes as it help to determine the larval distribution in the field (Navarro et al., 2003). Hence, by knowing about their breeding site preference the potential breeding site can be eliminated and reduced easily. In addition to that, the breeding of Aedes mosquitoes are characterised to be prolific and widespread among residential areas of human population due to also, locally varying rainfall and seasonal temperatures in the tropics (WHO, 2018; Neiderud, 2015; Vanek et al., 2006).

There are various types of factors that contribute in the oviposition site selection which are salinity, acidity and bacteria-related odours (Navarro et al., 2003). Nonetheless, environmental conditions such as rainfall, temperature and relative humidity serve as the factors for the Aedes mosquitoes to fly and lay their eggs (Rozilawati et al., 2007). As there are two seasons in Malaysia which are rainy season and dry season, the distribution and abundance of Aedes mosquitoes may vary across the seasons. Therefore, different vector control approach can be conducted according to their ovipositional behaviour by seasons. In addition, the population of Aedes mosquitoes within the area can be determined by level of ovitrap index (OI). Thus, the results later can help in determine the appropriate control measures which should be applied within the areas.

Kota Bharu district has recorded with the highest case of dengue fever (51.6%) with one fatality and Kubang Kerian is one of the dengue hot spot area in Kota Bharu (Utusan Online, 2017). This study was conducted in one of the dengue hot spot area in Kubang Kerian in which Hasnan et al. (2011) had previously reported six cases of dengue fever at the locality from 15th August to 5^{th} September 2011. The aim of this study was to determine the preference of the Aedes mosquitoes to oviposit based on the distribution of eggs, Aedes species and ovitrap index by types of water, seasons and location of breeding sites at the locality.

2. Materials and Method

This was a cross-sectional study conducted at a residential area located in the middle of Kubang Kerian town, Kelantan. A total of 140 ovitraps were placed randomly during each rainy (November) and dry (February) seasons, two ovitraps each at 70 sampling sites in four divided areas. There were four locations selected namely as location A, location B, location C and location D in order to study about the Aedes distribution and abundance between the areas. Sampling sites of potential breeding spot of Aedes mosquitoes were selected based on the criteria by Jakob & Bevier (1969).

2.1. Preparation of ovitrap and ovipaddle

The ovitraps were made by spraying milk cans in black. The oviposition paddles (ovipaddles) were comprised of two ice cream sticks that were attached together and covered with brown coloured fabric to provide suitable surface for oviposition.

2.2. Preparation of seasoned tap water and collection of rain water

Seasoned tap water was prepared by draining the tap water from pipe directly into plastic containers and was left for 3-5 days to de-chlorinate it (Chen et al., 2007). The rain water was collected from rainfall out in an open space and was stored in plastic containers. The physical measurement of both types of water such as pH and conductivity were determined using YSI multiparameter.

2.3. Mosquito oviposition and larvae sampling

The seasoned tap water and rain water were filled into two different ovitraps at 5.5 cm height and were placed side by side at a distance of 15 cm, each at the identified sampling spots. Ovipaddles with rough surface facing upwards were placed individually into each ovitrap and were left 3 days for the oviposition of adult female mosquitoes. The ovipaddles were collected on the 4th day and water from ovitrap were poured into plastic containers of

13.5×12×6 cm. The collected ovipaddles with mosquito eggs and larvae were transported back to the laboratory.

2.4. Mosquito eggs count and larvae identification

The ovipaddles were left to be dried under room temperature for at least 24 hours before the mosquito eggs oviposited on the ovipaddles were counted under a dissecting microscope. After the counting, the mosquito eggs were placed back and left again into their respective plastic container to hatch. The Aedes larvae that hatched from the eggs were left to grow until the third or fourth instar stage.

The larvae were preserved before identification by submerging in boiling water for 1-2 minutes. Then, it was placed in the universal bottles which contain 70 to 90 percent ethyl or isopropyl alcohol solution (Bloem, 1997). This process kills bacteria in the digestive tract and prevents discolouration. The larvae were mounted on the slide and were identified under the light microscope. In differentiating between the two species of Aedes mosquitoes (Aegypti spp. and Albopictus spp.), several key characteristics for larval Aedes spp. identification were taken into consideration based on Yoshimizu (2013) and Rueda (2004).

The larvae were divided into three main parts which are the head, thorax and the abdomen (Nazri et al., 2013). 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 were observed and identified. Only Aedes mosquito larvae were taken into count and other mosquito larvae were excluded.

2.5. Data analysis

The collected data were analysed using SPSS version 24 at level of statistical significance of $p < 0.05$. The comparison between preference of water by Aedes mosquitoes with number of eggs to oviposit and comparison on distribution of eggs by seasons were analysed using Mann Whitney test. Whereas the distribution of mosquito eggs by location was tested using Kruskal Wallis. The association of percentage of eggs hatched between seasons was determined using Pearson's Chi Square. The ovitrap index (OI) was calculated based on the formula given (Rozilawati et al., 2007):

$$OI = \frac{no.of\ ovitraps\ positive\ for\ oviposition}{total\ no.\ of\ ovitraps} \times 100$$

3. Results

3.1 Physical measures for seasoned tap water and rain water

The pH value of rain water was 6.57 whereas seasoned tap water was only 7.87 indicated that rain water was more acidic compared to seasoned tap water. The flow of electrical current by ions in rain water was lower than seasoned tap water as the conductivity of the rain water was lower compared to seasoned tap water which only 0.04 ms/cm compared to 0.08 ms/cm.

3.2 Distribution of mosquito eggs in different types of water, season and location

A total of 4,089 mosquitoes eggs were collected in this study. Out of the total, 38.3 % (n = 1565) of the eggs were oviposited in seasoned tap water whereas 61.7 % (n = 2524) of the eggs were oviposited in rain water. There was a significant difference between the number of mosquito eggs oviposited between the two types of water ($p = 0.001$). Whereas higher percentage of mosquito eggs were collected during rainy season (52.3 %, n = 2132) compared to dry season (47.7 %, n = 1957). A significantly higher median number of mosquito eggs found during rainy season compared to dry season ($p=0.006$). Therefore, it may indicate that Aedes mosquitoes preferred to oviposit in rain water rather than in seasoned tap water and during rainy season. Majority of mosquito eggs were collected from location A (45.1 %, n = 1843) and B (29.9 %, n = 1221) (Table 1).

3.3 Association between percentages of hatched mosquito eggs by different types of water, season and location

Table 2 shows that percentage of eggs hatched to larvae stage in seasoned tap water was 44.2 % (n = 19) whereas in rain water was 55.8 % (n = 24). There was no significant association found between the percentage of hatched mosquito eggs in different types of water, $p = 0.407$. Hence, this indicates that neither seasoned tap water nor rain water may have contributed in the mosquito eggs hatching process to larvae stage. There was higher percentage of mosquito eggs hatched during dry season (69.8 %, n =

30) compared to rainy season (30.2 %, n = 13) and the association was significant (p = 0.005). Whereas for location, the highest frequency of mosquito eggs hatched were collected from location A (39.5 %, n = 17) compared to other locations.

Table 1: The comparison of mosquito eggs oviposition by types of water, season and location

Variables	Mosquito eggs oviposition		
	Frequency (%)	Median (IQR)	p-value
Types of water			**[a]0.001***
Tap Water	1565 (38.3)	6 (3 - 16)	
Rain Water	2524 (61.7)	11 (5 - 25)	
Types of season			
Rainy	2132 (52.3)	11 (5 - 21)	**[a]0.006***
Dry	1957 (47.7)	6 (3 - 17)	
Location			
A	1843 (45.1)	18 (6 - 29)	**[b]0.001***
B	1221 (29.9)	10 (3 - 23)	
C	660 (16.1)	6 (4 - 11)	
D	370 (9)	5 (3 - 10)	

Statistical test - [a]Mann Whitney, [b]Kruskal Wallis, *p < 0.05

Table 2: The association of hatched mosquito eggs by different types of water, season and location

Variables	Frequency (%)		p-value
	Hatched	Not Hatched	
Types of water			
Tap Water	19 (44.2)	121 (51.1)	0.407
Rain Water	24 (55.8)	116 (48.9)	
Types of season			
Rainy	13 (30.2)	127 (53.6)	**0.005***
Dry	30 (69.8)	110 (46.4)	
Location			
A	17 (39.5)	63 (26.6)	0.130
B	11 (25.6)	69 (29.1)	
C	13 (30.2)	67 (28.3)	
D	2 (4.7)	38 (16.0)	

Statistical test - Chi square test, *p<0.05

3.4 Aedes species in different types of water, season and by location

There was no significant association found between the types of Aedes species in different types of water (p = 0.235) nor between seasons (p = 0.491). It may indicate that there were no specific Aedes species of either *Ae. Albopictus* or *Ae. Aegypti* significantly prefer to oviposit based on types of water nor season (Table 3).

Table 3: The association of Aedes species by different types of water and season

Variables	Frequency (%)		p-value
	Ae. Albopictus	***Ae. Aegypti***	
Types of water			
Tap Water	11 (37.9)	8 (57.1)	0.235
Rain Water	18 (62.1)	6 (42.9)	
Types of season			
Rainy	10 (34.5)	3 (21.4)	0.491
Dry	19 (65.5)	11 (78.6)	

Statistical test - Chi square test

The ovitrap index at location A and location C were the same 90 % (n = 36) respectively. The ovitrap index at location B were 80 % (n = 32) and for location C was 80 % (n = 16).

Table 4: Ovitrap Index and Aedes species by location

Location	Ovitrap Index	Frequency (%) of Aedes species	
		Ae. Albopictus	***Ae. Aegypti***
A	90	13 (44.8)	4 (28.6)
B	80	8 (27.6)	3 (21.4)
C	90	7 (24.1)	6 (42.9)
B	80	1 (3.4)	1 (7.1)

4. Discussion

The oviposition site selection by female Aedes mosquitoes is an important element in the reproductive phase of mosquitoes as it helps to determine the larval distribution in the field (Navarro et al., 2003). In this study, two types of water were used which were seasoned tap water and rain water. Based on the physical measurement, both of the water served as a suitable place for oviposition as the pH value falls in the range of 6-8 which is conducive for the growth of the larvae.

The median of mosquito eggs collected in rain water was significantly higher compared to seasoned tap water which indicates that Aedes mosquitoes preferred to oviposit in rain water rather than in seasoned tap water. This may be due to the chlorine content in the water (Navarro et al., 2003) even though the water had undergo dechlorination as suggested in the study by Chen et al. (2007). However there was no association between the percentages of the hatched mosquito eggs with the types of water. This shows that the process of mosquito eggs hatching occur at the same rate in both types of water. Any standing water represents a potential Aedes mosquito breeding site for mosquito larvae to mature and complete their growth cycle. Similarly, there was no significance association in distributions of Aedes species between the two types of water. Although *Ae. Albopictus* was found to be the more dominant species compared to *Ae. Aegypti*. It has been long known that both Aedes species are container breeders with *Ae. albopictus* prefers to breed outdoors while *Ae. aegypti* generally prefers indoor (Nazri et al., 2013).

The distribution of mosquito eggs between the rainy seasons and dry seasons showed a significance difference. This correlated with the study by Rozilawati et al. (2007) where total number of mosquito eggs were most abundant during rainy season and remain low during dry season. Rainfall has become an important factor in regulating the abundance of outdoor breeding mosquito populations. Okogun et al. (2003) too stated that rainy seasons are associated with higher prevalence levels of mosquito diseases. This might be because mosquitoes are known to breed more during rainy seasons (Yam, 2013). Nazri et al. (2013) cited that the development cycle of mosquito in rainy season was the quickest hence the number of adults emerging at the end of the developmental cycle was higher in the cold season than the hot season. Aedes mosquito eggs have a special characteristics which they need an adequate retention of moisture for successful embryonation (Hill et al. 2006). However, heavy rainfall might as well give a negative impact on the number of larvae or eggs due to excess water from ovitraps (Foo et al., 1985). Thus, it may result in death of immature stages of mosquitoes (Rohani et al., 2001) and loss of egg.

During dry season, the percentage of mosquito eggs hatched was higher compared to rainy season. This was supported by Rueda et al. (2004) where high rise in water temperature cause shorter time for the larvae to mature. But it was contradicted with Roberts (2001) that stated the mosquito eggs that are already present in the habitat can be stimulated to hatch by the raindrops as the vibration of water can cause agitation. Based on the results in this study, during dry season the percentage of mosquito eggs hatched were higher compared to rainy season. There was a research stated that high rise in water temperature cause shorter time for the larvae to mature (Rueda et al., 2004) which may serve as the reason for the mosquito eggs to hatch more in dry season compared to rainy season. However, in comparison for association between percentages of hatched mosquito eggs in different seasons by types of water, there was no significance difference. There was no significant association between the distributions of Aedes species in rainy season and dry season ($p>0.05$). Hence, this may indicate that the distribution of *Ae. Albopictus* and *Ae. Aegypti* does not related to either types of season.

With regards to the Aedes distribution and abundance between the four selected areas, location A is an area of flat houses which does not have a proper solid waste management system. Thus, it can be observed that there were no proper storage of household waste such as artificial containers likes tin cans and bottles which can lead to a wide range of potential habitats for Aedes mosquitoes. This container may serve as a suitable breeding site for Aedes mosquitoes as they can retain water for a long period of time.

Based on this study, the distribution of mosquito eggs in these four locations have shown significant differences ($p<0.05$). Location A had the highest median value of mosquito eggs oviposition whereas location C had the lowest median value. The reason for this may because of the artificial containers found around location A as *Ae. Albopictus* and *Ae. Aegypti* were known and well establish as the species which breed in the artificial container (Madzlan et al., 2016). These Aedes species which known as container dwelling mosquitoes are able to exploit the containers and make it as larval habitats. Mahfodz et al. (2017) stated that plastic container ranked as the highest, followed by cans and tyres, natural container, vase and old furniture, bottles and finally water dispenser as preferred breeding sites of mosquitoes.

As for the location B and C, the area is more clean compared to location A with less artificial container that can retain the water. However, natural container likes stumps and ground pools can be found within these area. Apart from that, there was also concrete drainage system outside of the houses. Most of the drains contained clear stagnant water with fallen tree leaves. Based on Chen et al. (2005), concrete drainage system can become a good place for mosquito breeding sites although the residential area is clean. Whereas, most of the houses in location C were double-storey houses which is the most clean compared to other locations with no water storage and artificial containers that can retain the water.

There was no significant association between the percentages of mosquito eggs hatched in these four different locations. As for the association between types of species by different locations, there was also no significance different between them. However, between the two types of Aedes species, *Ae. Albopictus* more likely to be found in these four locations. This may be because *Ae. Albopictus* are outdoor breeders and the ovitrap surveillance in this study were done outdoors. This finding was correlated with the study by Rozilawati et al., (2007).

The ovitrap index (OI) obtained from these four locations were between 80% and 90% where location A showed the highest OI value whereas location D showed the lowest OI value. There were four categories in ovitrap index classification where different actions would be taken according to the levels (Table 4). The population of Aedes in four different locations showed the highest level of OI value (level 4). This may due to the wide range of potential breeding sites within the area.

Table 4: Classification of Ovitrap Index (OI)

Classification	**Ovitrap Index level (%)**
Level 1	OI <5
Level 2	5≤ OI <20
Level 3	20≤ OI <40
Level 4	OI ≥ 40

Source: Yam (2013)

Preventive measures such as 'gotong-royong' programme and frequent surveillance need to be done to control the Aedes population. According to the suggestion by Cheung and Fok (2009), the best controlled for OI levels 1 and 2 is through source reduction by eliminating the potential breeding sites such as plastic containers and tin cans. When the OI level is 3 the vector control department should carried out a special control operations in collaboration with the community. Finally when the OI level reach 4, an insecticide spraying should be carried out to control and contain the mosquito population. For the fact that, such insecticide fogging has been frequently conducted as necessary at the respective area however unfortunately the effectiveness of such control measure was not investigated in this study.

5. Conclusion

The identified Aedes species in this study were *Ae. Albopictus* and *Ae. Aegypti*, in which, the Aedes mosquitoes in this study preferred to oviposit in the rain water compared to seasoned tap water as the number of mosquito eggs collected in the rain water was higher compared to seasoned tap water. Neither seasoned tap water nor rain water contributes in the mosquito eggs hatched process. During rainy seasons, the mosquito eggs collected was significantly higher compared to dry season. The percentage of mosquito eggs hatched was higher during dry season compared to rainy seasons. Location A showed highest number of collected mosquito eggs and ovitrap index was highest in Location A and C. Based on the ovitrap index level, all of the locations showed the highest level of OI value. This may be due to the wide range of potential breeding site presence within the area. In conclusion, Aedes surveillance using ovitrap is the most sensitive, cost-effective and reliable method to detect the presence of Aedes species.

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