

AEDES SURVEILLANCE AND OVIPOSITION PREFERENCES: A CASE STUDY WITHIN UNIVERSITY CAMPUS AND HOSPITAL AREA

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Abstract

Investigations on oviposition preferences of dengue vectors are critical for effective vector breeding control. The aim of this case study was to determine the prevalence and preference of the Aedes mosquitoes to oviposit based on the container index, types of container and types of water. To achieve the aim, 58 ovitraps consisted two types, modified can and plastic material Aedes Larva Ovitrap (ALOT), each were placed side by side at selected sampling sites within a university campus and teaching hospital area. Five different types of water were used (rain water, tap water, reverse osmosis water, pond water and well water). Overall, higher prevalence of positive mosquito eggs were found in modified can (66 %, n=38) compared to ALOT (31 %, n=18). The identified Aedes species in this study were higher for *Aedes Albopictus* compared to *Aedes Aegypti*, with more mosquito eggs were oviposited in modified can (n=26). There was no significant association between types of water and presence of mosquito egg/larva for modified can ($p>0.05$) but, significant association was found for ALOT ($p=0.026$). In conclusion, Aedes surveillance using ovitrap is the most sensitive, cost-effective and reliable method to detect the presence of Aedes species.

Keywords: Oviposition preferences; Aedes mosquitoes; Ovitrap

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1.0 INTRODUCTION

Dengue fever is a common infectious disease in Malaysia due to its occurrence or outbreaks. Malaysia has recorded 13,374 cases with Selangor having the highest prevalence [1]. Worldwidely, a total of 785,736 cases had been reported [2]. Dengue is caused by an arbovirus which is vector-borne, transmitted by the vector of Aedes species mosquitoes. There are many traits of Aedes species but among all, the two mosquitoes that carry the virus of dengue are *Aedes aegypti* and *Aedes albopictus*.

Ae. aegypti which is known as yellow fever mosquito acts as the main vector of dengue virus worldwide. The average distance that *A. aegypti* could travel is about 400 metres [3]. If taken into consideration the weather factor, *Ae. aegypti* could fly more than the average distance by the presence of the wind. In comparison, the virus has been spread faster through human spread between and inside communities and places. The ability of mosquito females to distinguish among potential oviposition sites that will or will not support the growth, development and survival of their offspring is critical for the maintenance of the mosquito population [4]. Moreover, in Malaysia, rapid urbanization has resulted in the creation of suitable habitats for Aedes mosquito.

Ae. albopictus is recognized as Asian tiger mosquito that is likely to be zoophilic due to its habitat originated in the forests of Southeast Asia. The spread of *Ae. albopictus* is an effect of globalization however has less contribution to major health problem. It is believed to have a potential to control *Ae. aegypti* because of the competition between both species [5]. *Ae. albopictus* is abundant in Kuala Lumpur, Singapore and Tokyo that generally can occupy rural and suburban areas of Asia where plants persist, according to Hawley [6] as cited in [5]. The district of Kota Bharu has recorded with the highest case of dengue fever and Kubang Kerian is one of the dengue hot spot area [7]. A recent news reported an increase of 250.7 % dengue fever cases in Kelantan, with 830 cases in 2023 compared to 236 in 2022 [8].

Thus, by determining the prevalence of potentially dengue vector such as *Ae. aegypti* and *Ae. albopictus* at the selected area, the further step of prevention occurrence of dengue fever could be taken immediately and effectively. In order to prevent the spread of virus through both species, a vector control is an important approach. The vector control is designed and targeted to prevent mosquito development at an early stage of either from egg or larvae stage. The Aedes mosquito attracted to black thing to lay their eggs after feeding blood. This study aims to determine the prevalence of mosquitos' species between *Ae. albopictus* and *Ae. aegypti* at potential breeding sites of selected area of study, to determine the types of preferred container between modified can or ovitrap plastic material (ALOT), and types of water preference for mosquitoes breeding sites by determining its association with the presence of mosquitos' eggs and larvae. Eventually, these data would help the respected responsible party to do fogging at the right places. In addition to that, the local community should practice healthy environment such as ensuring no stagnant water of preference for mosquito's breeding to avoid the outbreak of dengue fever happened around the campus and hospital as well as nearby residential area.

2.0 METHODOLOGY

2.1 Study location and period

A total 58 self-modified can and Aedes Larvae Ovitrap (ALOT) ovitraps, each, were placed side by side at selected locations within a university campus and teaching hospital area, that have the potential of Aedes breeding sites. This study was conducted starting 18th of July until 4th of August, 2016.

2.2 Data collection

2.2.1 Preparation of ovitrap

The modified can ovitraps were made following our previous study [9] by spraying milk cans in black. Two ice cream sticks were attached together and covered with brown coloured fabric to provide suitable surface for oviposition (ovipaddles).

2.2.2 Ovitrap placement and mosquito eggs/larvae sampling

All the labeled ovitraps (modified can and ALOT) were placed at the potential breeding sites of Aedes in order to identify the Aedes population in the university campus. All the ovitraps were filled with water until reaching $\frac{3}{4}$ levels. Five different types of water were used: rainwater, tap water, reverse osmosis water, pond water and well water. The aim of using different types of water was to identify the preference type of water for Aedes breeding. The placement of ovitraps was conducted within 8.30 a.m. until 12.30 p.m. in different days. The ovitraps were left for four days in the specific places and then were collected.

2.2.3 Sample preparation

All the ovitraps were observed for the presence of eggs or larvae. Then, the presence of eggs and/or larvae in the ovitraps were recorded. All the eggs and/or larvae were collected by using a dropper and then transferred into labeled glass bottles. All the glass bottles were covered with parafilm that had been punctured in order to provide oxygen to the eggs and/or larvae. The remaining water in the ovitraps was removed into the soil instead of into the sink or drain to prevent from water accumulation because the water accumulation can become another Aedes breeding site.

2.2.4 Sample identification

One of the larvae which presence in the glass bottle was taken and then put on the glass slide. Next, the cover slide was put on it and then observed under light microscope in order to identify the genus of mosquito. The magnification used in this study was 40X. If Aedes, it will be further identified into specific species either *Ae. aegypti* or *Ae. albopictus* based on different characteristics. Several key characteristics for larval Aedes spp. identification were taken into consideration based on Yoshimizu [10] and Rueda [11]. Then, the picture of each identified species was captured and recorded. The mosquito eggs that were present in the labeled glass bottle were left for 2 to 7 days and waited for the transformation into larvae. The samples in the labeled glass bottles were checked everyday. Then, the same steps as above were repeated for new larva identification.

2.3 Statistical analysis

The data obtained was tested using Statistical Package for Social Science (SPSS) Version 24 software and Microsoft office Excel. In this study, Chi-square test and Fisher exact test were used to summarize the relationship between two categorical variables which are different types of water and presences of mosquito larvae or eggs. The probability of the

differences in the data was described using p-value at the significance level set which is 0.05.

3.0 RESULTS AND DISCUSSION

3.1 Types of container preference

The main goal of this project is to determine the prevalence of mosquitoes in potential breeding sites of selected area. The findings showed that 66 % (n=38) positive mosquito breeding sites were found in modified can and 31 % (n=18) were found in plastic ovitrap ALOT. The two types of container used is investigated whether it is a factor of Aedes prevalence and preferences in this area. The data obtained were recorded and interpreted using Container Index (CI) (Figure 1).

The CI Prevalence of Mosquito for Modified Can is: $38/58 \times 100 = 66 \%$
 The CI Prevalence of Mosquito for ALOT is: $18/58 \times 100 = 31 \%$

Figure 1. Container Index (CI)

The vector surveillance for larva is divided into three types which are House Index (HI), Container Index (CI) and Breteau Index (BI) (Table 1). HI is the percentage of houses infested with larvae and/or pupae; CI is the percentage of water-holding containers infested with larvae or pupae; and BI is the number of positive containers per 100 houses inspected. Although a previous study [12] mentioned that BI is the top priority among the three indices because it takes into account for both container and house index. However, in this study, CI was used due to time constraint and convenience.

Table 1. The density of Aedes larva using Larva Index (LI)

Density Figure (DF)	House Index (HI)	Container Index (CI)	Breteau Index (BI)
1	1-2	1-2	1-4
2	4-7	3-5	5-9
3	8-17	6-9	10-19
4	18-29	10-14	20-34
5	20-37	15-20	35-49
6	38-49	21-27	50-74
7	50-59	28-31	75-99
8	60-76	32-40	100-199
9	≥77	≥41	≥200

The CI in this study for modified can was higher, thus it portrays that mosquitoes might prefer modified can compared to plastic ovitrap ALOT. There are many factors affecting container preference such as the type of material, size, volume, the purpose of the container and color [12, 13].

3.2 Prevalence of Aedes species

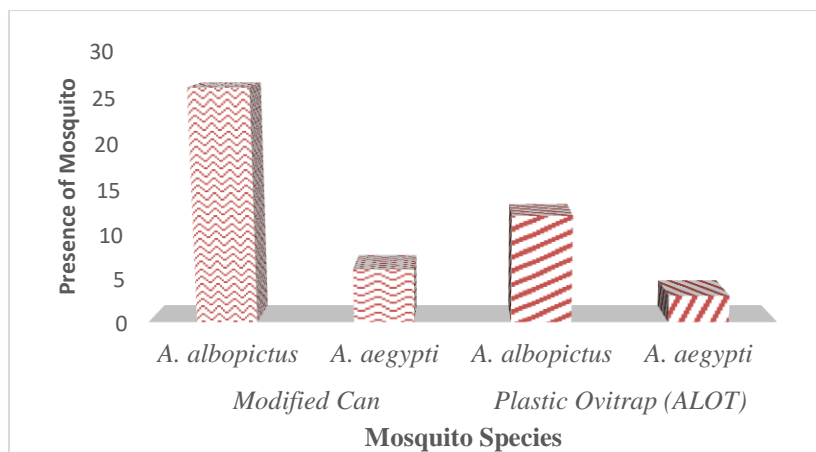


Figure 2. Presence of mosquito species between modified can and ALOT

Recorded observation under the microscope that was made to distinguish between *Ae. albopictus* and *Ae. aegypti* is presented in Figure 2. Based on the figure, *Ae. albopictus* has the higher prevalence for both types of container with presence of eggs/larva in 26 modified cans and 12 ALOTs. On the other hand, *Ae. aegypti* were only present in 6 modified cans and 3 ALOTs.

3.3 Types of water preference

Out of 29 modified can ovitraps with well water, 13 (44.8 %) cans had no presence of mosquito egg/larva while 16 (55.2 %) cans had presence of mosquito egg/larva. From 29 cans with rain water, 12 (41.4 %) cans had no presence of mosquito egg/larva while 17 (58.6 %) cans had presence of mosquito/larva (Table 2). There was no significant association between types of water and presence of mosquito egg/larva for modified can. This was similar to our previous study [9].

Table 2. Association between presence of mosquito and types of water for modified can

Presence of Mosquito Egg	Types of Water		Chi-square statistic (df)	p-value
	Well Water	Rain Water		
No	13 (44.8%)	12 (41.4%)	0.070 (1)	0.791
Yes	16 (55.2%)	17 (58.6%)		

Statistical test – Chi Square

Out of 13 plastic ovitraps (ALOT) with well water, 6 (46.2 %) had no presence of mosquito egg/larva while 7 (53.8 %) had presence of mosquito egg/larva. From 25 plastic ovitraps with rainwater, 14 (56.0 %) had no presence of mosquito egg/larva while 11 (44.0 %) had presence of mosquito egg/larva. Among 17 plastic ovitraps with pipe water, 16 (94.1 %) had no presence of mosquito egg/larva while only 1 (5.9 %) had presence of mosquito egg/larva. Out of 2 plastic ovitraps with R.O water and one with pond water, none of them had presence of mosquito egg/larva (Table 3).

Table 3. Association between presence of mosquito and types of water for ALOT

Presence of Mosquito Egg	Types of Water					p-value
	Well Water	Rain Water	Pipe Water	Reverse Osmosis Water	Pond Water	
No	6 (46.2%)	14 (56.0%)	16 (94.1%)	2 (100.0%)	1 (100.0%)	0.026*
Yes	7 (53.8%)	11 (44.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	

Statistical test – Fisher Exact Test, *p<0.05

The study revealed that the more dominant species in the area was *Ae. albopictus* (n=38) in both types of containers, while *Ae. aegypti* found in nine places. This was similar to previous reported findings [9, 13]. Most sampling site of study location were outdoors, therefore there was bias in location selection. *Ae. albopictus* can be found outdoors, inhabits rural and suburban areas, day feeder and it is able to breed in a wide range of containers from natural to artificial [5]. On the contrary, *Ae. aegypti* usually stays indoors, inhabits urban areas, nocturnal feeder and usually breeds in artificial containers where there is lacking of vegetation [15].

3.4 Study limitation

One of the factors investigated in this study was water preference. The containers were filled with five different types of water; well water (n= 29), rain water (n=29), pipe water (n=17), reverse osmosis (R.O.) water (n=2) and pond water (n=1). Supposedly, for future study, two different containers in a same place were filled with the same type of water to avoid bias. For instance, a gravid *Aedes* mosquito might prefer the modified can, but, found that the water was unfavorable for laying eggs. Thus, it had to choose plastic container with water of preference instead. Some of the modified can failed to record any *Aedes* breeding as it toppled due to the heavy rain. Some modified cans also had no holes, therefore the eggs/larva might have been washed away due to overflow of water due to rain. Apart from that, some ovitraps were not positioned in the shady areas and thus exposed to direct sunlight which could cause overheating resulting in egg/larva to destroy.

There might be error in certain observation due to error in preparation of slide. The larval saddle might have overlapped with the siphon which could cause confusion in identification. Besides, some container only consist of 1st instar larva that had not fully developed their comb scales which made it harder for identification process. Some photographs of larva might not be clear as it was taken from a phone camera and the photographs were taken few days after the slides were prepared. The samples were not fixed with alcohol and few samples became dried and destroyed. Hence for future study, these limitations should be considered for improvement.

4.0 CONCLUSION

In conclusion, this study indicated that the selected university campus and hospital area has a potentially high risk for outbreaks of *Aedes* mosquito-borne diseases such as dengue and chikungunya. The two distinct components may be involved which are the female selection for oviposition. *Aedes* population has been observed higher in the modified can compared to plastic ovitrap ALOT. This may be due to availability of natural potential breeding sites such as bamboo tree, tree holes and broken containers in outdoor environment and because of the strong smells of the plastic ovitrap itself.

Thus, the *Ae. albopictus* was more significant than *Ae. aegypti* and this may be due to the bias throughout the survey. Lastly, the modified can was the most frequently infested and it appears as the key breeding site.

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