DENGUE VECTOR SURVEILLANCE IN MAJOR TOWNS OF MALAYSIA

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ABSTRACT

Ovitrap surveillance was conducted between February and June 2017 to determine the abundance and distribution of dengue vectors in 13 residential areas located in major towns of different states in Malaysia. Forty ovitraps were placed randomly within the vicinity of buildings which were protected from sunlight and rain. The study revealed that ovitrap index (OI) ranged from 12.50% to 87.50% across all study sites. Both *Aedes aegypti* and *Aedes albopictus* were found breeding in all study sites except for the study sites in Alor Setar, Kedah Gambang, Pahang and Kuala Terengganu, Terengganu, Malaysia in which only *Ae. albopictus* was recorded. The mean number of larvae obtained revealed that *Ae. albopictus* was a more dominant dengue vector as compared to the mean number of *Ae. aegypti* larvae in nine out of thirteen study sites ($P<0.05$). Mixed breeding of both *Aedes* species were found in 4.00% to 28.57% of the total number of recovered ovitraps from all study sites. This study revealed that OIs obtained from all these major towns were >10%, indicating Malaysia is generally at risk of dengue outbreak, and control approaches must be carried out immediately to reduce the vector population to a level below the threshold of transmission.

Keywords: *Aedes aegypti*, *Aedes albopictus*, ovitrap surveillance, towns, Malaysia

ABSTRAK

Kajian taburan ovitrap telah dijalankan di antara Februari dan Jun 2017 bagi menentukan kelimpahan dan sebaran vektor denggi di 13 kawasan-kawasan perumahan yang terletak di

bandar utama di negeri-negeri yang berbeza di Malaysia. Empat puluh ovitrap telah ditempatkan secara rawak di sekitar bangunan-bangunan yang terlindung daripada sinaran matahari dan hujan. Kajian menunjukkan bahawa indeks ovitrap (IO) adalah dalam julat daripada 12.50% hingga 87.50% merentasi semua kawasan kajian. Kedua-dua *Aedes aegypti* dan *Aedes albopictus* telah didapati membiak di semua kawasan kajian kecuali bagi kawasan kajian di Alor Setar, Kedah, Gambang, Pahang, dan Kuala Terengganu, Terengganu, Malaysia yang mana hanya *Ae. albopictus* sahaja direkodkan. Bilangan min jentik-jentik yang diperolehi menunjukkan bahawa *Ae. albopictus* merupakan vektor denggi yang lebih dominan berbanding dengan bilangan min jentik-jentik *Ae. albopictus* di sembilan daripada tiga belas kawasan kajian ($P<0.05$). Pembiakan bercampur melibatkan kedua-dua spesies *Aedes* telah didapati di dalam 4.00% hingga 28.57% daripada jumlah ovitrap yang dikutip semula daripada semua kawasan kajian. Kajian ini menunjukkan bahawa IO yang diperolehi daripada kesemua bandar-bandar utama ini adalah >10%, yang mana membawa maksud bahawa Malaysia secara umumnya berisiko kepada wabak denggi dan kaedah-kaedah kawalan perlu diambil secepat mungkin untuk mengurangkan populasi vektor kepada tahap di bawah paras permulaan penyebaran.

Kata kunci: *Aedes aegypti*, *Aedes albopictus*, kajian taburan ovitrap, bandar-bandar, Malaysia

INTRODUCTION

Aedes aegypti (Linnaeus) and *Aedes albopictus* Skuse are responsible for the transmission of dengue, chikungunya and Zika (Huang et al. 2020). Dengue fever was first reported in Penang, Malaysia in 1901 (Skae 1902). In 2020 until 19th December, 88,845 dengue cases were reported in Malaysia (Ministry of Health Malaysia 2020). In addition, 2,556 chikungunya cases which are likewise transmitted by *Aedes* mosquitoes were also reported within the same period of 2020 (Ministry of Health Malaysia 2020).

Aedes aegypti is a domestic mosquito which dwells near human habitat and prefers indoor environment; and it breeds in man-made containers such as earthen jars, concrete tanks and flowerpots, whereas, *Ae. albopictus* is commonly found feeding and breeding outdoors especially near vegetations (Masnita et al. 2018; Wan Fatma & Aminoddin 2019; Yap et al. 1995). It was reported that the dengue virus can be transmitted to the offspring through transovarial transmission in *Aedes* (Nor Aliza et al. 2019; Lee 2000).

To date, there are no drugs and effective vaccines for the treatment of dengue. The prevention of dengue infection relies heavily on vector control to reduce the populations, which involves the usage of insecticides and implementation of source reduction (Salazar et al. 2019). To control the transmission of dengue, the first step to be considered is to determine the distribution and abundance of the dengue vector, since the increase of dengue cases are highly associated with the abundance of *Aedes* (Tham 2000). Therefore, vector surveillance forms an integral part of the dengue control programme in monitoring vector population.

Ovitrap surveillance is the most effective used method to monitor the distribution and abundance of mosquitoes in Malaysia (Yap et al. 1995). There were number of surveillances conducted in specific districts or several regions of Peninsular Malaysia to study *Aedes* populations (Ahmad et al. 2015; Chen et al. 2005; Lee 1991; Hamid et al. 2020; Hasnan et al. 2017; Rozilawati et al. 2015; Wan-Norafikah et al. 2011; Zhaki et al. 2019). In other words, there is no comprehensive nationwide dengue vector surveillance conducted that covered major

towns of different states in Malaysia, especially in the East Coast of Peninsular Malaysia and East Malaysia (located on Borneo Island). Therefore, the aim of this study was to determine the abundance and distribution of *Aedes* in residential areas in major towns across different states in Malaysia.

MATERIALS AND METHODS

Study Sites

Aedes aegypti and *Ae. albopictus* were collected from 13 residential areas in major towns of different states in Malaysia by using ovitrap surveillance. This study was carried from February 2017 until June 2017 which was during the dry season in Malaysia. The geographical description of all study sites is shown in Table 1.

Table 1. Mosquito collection sites in major towns within Malaysia

Malaysia	Region	State	District	Study Site	Geographical Coordinate
Peninsular	Northern	Kedah	Alor Setar	Kampong Tanjung Bendahara (KTB)	N 06° 06’ 47.378” E 100° 22’ 20.902”
		Penang	Gelugor	Taman Pekaka (TP)	N 05° 21’ 8.639” E 100° 17’ 42.394”
		Perak	Menglembu	Taman Bukit Merah (TBM)	N 04° 33’ 26.086” E 101° 02’ 52.139”
	Central	Kuala Lumpur	Kuala Lumpur	Taman Sri Endah (TSE)	N 03° 03’ 51.298” E 101° 41’ 49.405”
		Selangor	Ampang	Taman Muda (TM)	N 03° 06’ 55.362” E 101° 45’ 31.406”
	Southern	Johor	Batu Pahat	Taman Setia Jaya (TSJ)	N 01° 52’ 3.377” E 102° 56’ 45.444”
		Melaka	Bukit Baru	Taman Bahagia (TB)	N 02° 13’ 8.615” E 102° 16’ 12.759”
		Negeri Sembilan	Titi	Taman Desa (TD)	N 02° 59’ 32.894” E 102° 04’ 31.992”
	East Coast	Kelantan	Tanah Merah	Kampung Banggol Maka (KBM)	N 05° 50” 24.253” E 102° 07’ 31.065”
		Pahang	Gambang	Kampung Sungai Belat (KSB)	N 03° 42’ 28.725” E 103° 06’ 47.675”
		Terengganu	Kuala Terengganu	Taman Padang Nanas (TPN)	N 05° 24’ 50.131” E 103° 03’ 51.894”
East Malaysia	East	Sabah	Kota Kinabalu	Taman Selesa (TS)	N 05° 57’ 56.099” E 116° 05’ 30.807”
	West	Sarawak	Kuching	Tabuan Desa (TAB)	N 01° 31’ 35.909” E 110° 23’ 1.032”

Ovitrap Surveillance

Ovitraps were prepared according to Lee (1992a). The ovitrap consists of a 300ml plastic cup, with the outer wall painted with black paint, 9.0cm in height, with base diameter of 6.5cm, and opening diameter of 7.8cm. An oviposition paddle that is made of hardboard measuring 2.5cm (width) x 10.0cm (length) x 0.3cm (thickness) was placed diagonally into the cup. The ovitraps

were filled with chlorine free tap water up to the height of 5.5cm. As recommended by Chen et al. (2006a) on the deployment of ovitraps with not less than 10% from the total number of houses at each study site, a total of 40 ovitraps were placed randomly at each study site which were within the vicinity of the buildings both indoor and outdoor (Chen et al. 2005). These ovitraps were protected from direct sunlight and rainfalls such as in the garage, under the car porch as well as shaded walkways, near stairways, underneath of shoe racks and under extended roofs of buildings. The ovitraps were collected after five days and transported back to the entomology laboratory at the Institute for Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. All larval samples obtained from these ovitraps were maintained at 28±2ºC and 75±5% relative humidity with 12:12 lighting hours in the laboratory.

Identification of Mosquitoes

The contents together with the oviposition paddles of each ovitrap were transferred into individual plastic cup. Fresh water was added into every plastic cup and these cups were kept covered. The first instar (L1) and second instar (L3) larvae were fed with a quarter of a teaspoon of beef liver powder once daily while the third instar (L3) and fourth instar (L4) larvae were provided with small pieces of half-cooked beef liver. The species of larvae were identified at L3 or L4 larval stage and recorded separately for each ovitrap.

Data Analysis

Results obtained from the ovitrap surveillance were analysed as below:

(i) ***Ovitrap Index (OI)***

Ovitrap Index is the percentage of positive ovitraps to the number of recovered ovitraps, which indicates the infestation rate (%) of the *Ae. aegypti* and *Ae. albopictus* in the study sites (Lee 1992b).

The formula of OI is as follows:

$$\text{Ovitrap Index (\%)} = \frac{\text{number of positive ovitrap}}{\text{number of recovered ovitrap}} \text{ x } 100\%$$

(ii) ***Percentage of ovitraps with breeding of each Aedes species***

The percentage of ovitraps with breeding of each *Aedes* species per study site was calculated as below:

$$\text{Number of ovitrap with breeding of one } \textit{Aedes} \text{ species per study site} = \frac{\text{Number of ovitrap with breeding of one } \textit{Aedes} \text{ species}}{\text{Total number of positive ovitraps}} \text{ x } 100$$

(iii) ***Percentage of ovitraps with breeding of both Aedes species***

The percentage of ovitraps with breeding of both *Ae. aegypti* and *Ae. albopictus* per study site was calculated as below:

$$\text{Number of ovitrap with breeding of both } \textit{Ae. aegypti} \text{ and } \textit{Ae. albopictus} \text{ per study site} = \frac{\text{Number of ovitrap with breeding of both } \textit{Aedes} \text{ species}}{\text{Total number of positive ovitraps}} \text{ x } 100$$

(iv) ***Density of larvae per ovitrap***

The density of *Aedes* mosquitoes in the study site was determined by calculating the mean number of larvae per ovitrap.

$$\text{Mean number of larvae} = \frac{\text{Total number of larvae (ovitrap 1+ovitrap 2+…+ovitrap 40)}}{\text{Number of recovered ovitraps}}$$

(v) ***Comparison on larval density between Ae. aegypti and Ae. albopictus larvae per study site***

Any significant difference between the mean number of *Ae. aegypti* larvae and *Ae. albopictus* larvae for each study site at 95% confidence limit was determined by performing the independent samples t-test by using the IBM SPSS 23.0 statistical software.

(vi) ***Ratio of mixed breeding***

The ratio of mixed breeding between *Ae. aegypti* and *Ae. albopictus* for each study site that was positive with mixed breeding was also manually calculated.

RESULTS

Table 2 describes the ovitrap index (OI) obtained from thirteen study sites. The OI ranged from 12.50 to 87.50% across all study sites. The highest OI were obtained from the study sites in Menglembu, Perak (87.50%), followed by Kuala Lumpur (74.36%) and Gelugor, Penang (70.00%). *Aedes aegypti* was found breeding in 8.00–58.82% of ovitraps, whereas *Ae. albopictus* was found breeding in 23.53–100.00% of ovitraps. Interestingly, only *Ae. albopictus* was found breeding in three study sites which were located in Alor Setar, Kedah; Gambang, Pahang; and Kuala Terengganu; Terengganu.

The mean numbers of *Ae. aegypti* and *Ae. albopictus* larvae obtained from all study sites and the horizontal analysis on the statistical difference between the mean number of *Ae. aegypti* larvae and *Ae. albopictus* larvae per ovitrap for each study site are presented in Table 3. The mean number of *Ae. albopictus* larvae per ovitrap recorded for nine study sites which ranged from 2.98±1.10 to 32.95±4.80, were significantly higher as compared to the mean number of *Ae. aegypti* larvae per ovitrap in these study sites, respectively. These results indicate that *Ae. albopictus* was a dominant dengue vector in these residential areas rather than *Ae. aegypti*. Although the mean number of *Ae. aegypti* larvae per ovitrap was higher than the mean number of *Ae. albopictus* larvae per ovitrap obtained in the study sites at Ampang, Selangor and Titi, Negeri Sembilan, these results were not significantly different ($P>0.05$).

In addition, mixed breeding of *Ae. aegypti* and *Ae. albopictus* was detected in 4.00% to 28.57% of ovitraps obtained from nine out of thirteen study sites (Table 4). *Aedes aegypti* was found to be dominant in mixed breeding ovitraps in the study sites in Gelugor, Penang; Ampang, Selangor; Kuala Lumpur; and Batu Pahat, Johor by 1.67 to 5.00 folds in comparison to *Ae. albopictus* larval populations. On the contrarily, the number of *Ae. albopictus* larvae recorded in mixed breeding ovitraps obtained from the study sites in Menglembu, Perak; Titi, Negeri Sembilan; Bukit Baru, Melaka; Tanah Merah, Kelantan; and Kuching, Sarawak were higher by 1.26 to 27.00 folds as compared to the number of *Ae. aegypti* larvae.

Table 2. Ovitrap Index (OI) and distribution of *Aedes* population obtained from 13 study sites in Malaysia

Study site	**No. of recovered ovitrap**	**No. of positive ovitrap**	**Ovitrap index (%)**	**Number of positive ovitrap according to species of *Aedes***			**Percentage of positive ovitrap according to species of *Aedes* (%)**		
				Ae. aegypti	***Ae. albopictus***	***Ae. aegypti* + *Ae. albopictus***	***Ae. aegypti***	***Ae. albopictus***	***Ae. aegypti* + *Ae. albopictus***
KTB, Alor Setar, Kedah	40	6	15.00	0	6	0	0.00	100.00	0.00
TP, Gelugor, Penang	40	28	70.00	0	26	2	0.00	89.29	10.71
TBM, Menglembu, Perak	40	35	87.50	0	25	10	0.00	71.43	28.57
TM, Ampang, Selangor	27	14	51.85	4	8	2	28.57	57.14	14.29
TSE, Kuala Lumpur	39	29	74.36	4	22	3	13.79	75.86	10.34
TD, Titi, Negeri Sembilan	32	17	53.13	10	4	3	58.82	23.53	17.65
TB, Bukit Baru, Melaka	35	23	65.71	9	9	5	39.13	39.13	21.74
TSJ, Batu Pahat, Johor	40	24	60.00	2	20	2	8.33	83.33	8.33
KBM, Tanah Merah, Kelantan	40	25	62.50	2	22	1	8.00	88.00	4.00
TPN, Kuala Terengganu, Terengganu	40	10	25.00	0	10	0	0.00	100.00	0.00
KSB, Gambang, Pahang	40	15	37.50	0	15	0	0.00	100.00	0.00
TS, Kota Kinabalu, Sabah	40	5	12.50	2	3	0	40.00	60.00	0.00
TAB, Kuching, Sarawak	33	22	66.67	0	21	1	0.00	95.45	4.55
TOTAL	**486**	**253**	**-**	**33**	**191**	**29**	**13.04**	**75.49**	**11.46**

Table 3. Mean number (±S.E.) of larvae per ovitrap obtained from 13 study sites in Malaysia

Study Site	**No. of recovered ovitrap**	**Total number of larvae**	***Ae. aegypti***			***Ae. albopictus***			**Significance level for the difference between the mean number of *Ae. aegypti* and *Ae. albopictus* larvae per study site**
			n	**%**	**Mean number (±S.E.) of larvae per ovitrap**	**n**	**%**	**Mean number (±S.E.) of larvae per ovitrap**	
KTB, Alor Setar, Kedah	40	73	0	0.00	0.00±0.00	73	100.00	1.83±0.98	P<0.05
TP, Gelugor, Penang	40	700	66	9.43	1.65±1.20	634	90.57	15.85±2.64	P<0.05
TBM, Menglembu, Perak	40	1423	105	7.38	2.63±0.98	1318	92.69	32.95±4.80	P<0.05
TM, Ampang, Selangor	27	397	274	69.02	10.15±4.15	123	30.98	4.56±1.42	P>0.05
TSE, Kuala Lumpur	39	758	94	12.40	2.41±1.02	664	87.60	17.03±3.67	P<0.05
TD, Titi, Negeri Sembilan	32	404	278	68.81	8.69 ± 2.78	126	31.19	3.93±1.57	P>0.05
TB, Bukit Baru, Melaka	35	615	213	34.63	6.09±2.32	402	65.37	11.49±3.00	P>0.05
TSJ, Batu Pahat, Johor	40	533	33	6.19	0.83±0.54	500	93.81	12.50±3.57	P<0.05
KBM, Tanah Merah, Kelantan	40	527	14	2.66	0.35±0.28	513	97.34	12.83±3.38	P<0.05
TPN, Kuala Terengganu, Terengganu	40	152	0	0.00	0.00±0.00	152	100.00	3.80±1.52	P<0.05
KSB, Gambang, Pahang	40	119	0	0.00	0.00±0.00	119	100.00	2.98±1.10	P<0.05
TS, Kota Kinabalu, Sabah	40	17	2	11.76	0.05±0.03	15	88.24	0.38±0.26	P>0.05
TAB, Kuching, Sarawak	33	309	1	0.32	0.03±0.03	308	99.68	9.33±2.28	P<0.05
TOTAL	**486**	**6027**	**1080**	**17.92**	**-**	**4947**	**82.08**	**-**	**-**

Table 4. Mixed breeding of *Aedes aegypti* and *Aedes albopictus* in nine study sites in Malaysia

Study site	No. of positive ovitrap	Mix breeding ovitraps		Ratio *of* ***Ae. aegypti* : *Ae. albopictus***
		n	%	
TP, Gelugor, Penang	28	2	7.14	2.38 : 1.00
TBM, Menglembu, Perak	35	10	28.57	1.00 : 10.61
TM, Ampang, Selangor	14	2	14.29	5.00 : 1.00
TSE, Kuala Lumpur	29	3	10.34	2.86 : 1.00
TD, Titi, Negeri Sembilan	17	3	17.65	1.00 : 1.26
TB, Bukit Baru, Melaka	23	5	21.74	1.00 : 16.56
TSJ, Batu Pahat, Johor	24	2	8.33	1.67 : 1.00
KBM, Tanah Merah, Kelantan	25	1	4.00	1.00 : 6.00
TAB, Kuching, Sarawak	22	1	4.55	1.00 : 27.00

DISCUSSION

The ovitrap surveillance method was first developed by Jakob & Bevier (1969) in the United States during *Ae. aegypti* eradication programme. Ovitrap is a sensitive tool for mosquito sampling even when the population number of mosquitoes is low (Lee 1992a). The sensitivity of ovitraps compared to larval traps have also been proven in other studies outside of Malaysia (Furlow & Young 1970; Marques et al. 1993; Rawlins et al. 1998). According to Lee (1992b), there is a risk of dengue infection in a site if its ovitrap index (OI) is more than 10%. Ovitrap indexes (OIs) of all major towns selected in this study were more than 10%, proving that Malaysia, in general, is at risk of *Aedes* borne diseases outbreaks.

In this study, *Ae. albopictus* was found dominating in most urban and sub-urban residential areas, as documented in other local studies (Ahmad et al. 2015; Ho et al. 2014; Lim et al. 2011; Norzahira et al. 2011; Saleeza et al. 2011; Rozilawati et al. 2015). It was revealed in this study that the study sites in Alor Setar, Kedah; Gambang, Pahang; and Kuala Terengganu, Terengganu recorded 100% of species composition of *Ae. albopictus* larvae. *Aedes albopictus* is commonly found outdoor and breeds in natural containers such as tree hole, leaf axils and bamboo stumps as well as artificial containers that contain high amount of organic matters (Chareonviriyaphap et al. 2003; Yap et al. 1995). As observed in these three study sites, the existence of vegetations provided ideal breeding sites for *Ae. albopictus*. Moreover, artificial containers especially flowerpots, plastic containers, buckets and water storage tanks could also be found around these residential areas which could function as potential breeding habitats of this mosquito species. However, it is also reported that *Ae. albopictus* moves indoors in the absence of *Ae. aegypti* (Cheah et al. 2006). According to Isaacs (2006), *Aedes* breeds in any habitat that retains water and associated with human dwellings. In addition, clogged concrete drainage system which creates stagnant water near residential areas could also provide mosquitoes a possible breeding site (Chen et al. 2005).

The present study revealed the co-occurrence of *Ae. aegypti* and *Ae. albopictus* in nine study sites, in which this phenomenon was also reported in other local studies (Chen et al. 2006b; Wan-Norafikah et al. 2011; Wan-Norafikah et al. 2012; Wan-Norafikah et al. 2020). Studies conducted in Singapore by Chan et al. (1971) and in Thailand by Chareonviriyaphap et al. (2003) also reported that mixed infestation occurred for both *Aedes* species by 7.1% and 20%, respectively. This phenomenon could be due to the changes in environmental conditions and rapid urbanization which caused by the decreased number of natural breeding sites in urban

areas (Ahmad et al. 2015; Jirakanjanakit et al. 2007). Mixed breeding is associated with urbanized areas, where *Ae. aegypti* is found to spread with highly urbanized and populated regions due to its endophilic and endophagic nature (Jansen & Beebe 2010; Saifur et al. 2012). However, the abundance of *Ae. albopictus* (based on mean number, percentage and ratio obtained) in Malaysia displayed the species' invasiveness and aggressiveness due to its ability to invade and compete in urban regions with other species in various ways (Chareonviriyaphap et al. 2003; Gratz 2004).

CONCLUSION

In conclusion, many major towns in Malaysia are at risk of *Aedes*-borne diseases outbreaks. Thus, continuous control measures must be carried out. This study provides information for local authorities to plan and carry out effective measures to suppress *Aedes* populations. Integrated Vector Management (IVM) which involves vector surveillance, biological control, chemical control, personal protection, source reduction as well as public awareness and education should be implemented simultaneously to reduce the risk of dengue infection in Malaysia.

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