ORIGINAL ARTICLE

Ovitrap Surveillance of *Aedes aegypti* and *Aedes albopictus* in Dengue Endemic Areas in Keramat and Shah Alam, Selangor in 2016

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ABSTRACT

Introduction: Entomological surveillance is crucial to determine the abundance of dengue vector and to evaluate breeding areas of *Aedes aegypti* and *Aedes albopictus*. The objective of this study is to determine the distribution and breeding preference for both *Ae. aegypti* and *Ae. albopictus* in dengue endemic areas. **Materials and Methods:** Ovitraps surveillance was conducted in two dengue endemic areas; AU2, Keramat and Seksyen 7, Shah Alam, Selangor. A minimum number of 100 ovitraps were deployed for 5 days in the study sites. Samples collected were brought back to the lab and all larvae recovered were identified to species level. **Results:** The ovitap index (OI) in both localities exceeded the transmission threshold of 10% with the OI recorded ranged from 42.3-79.8% in AU2, Keramat and 16.7-42.9% in Seksyen 7, Shah Alam. *Ae. albopictus* was the dominant species in AU2 Keramat with the highest ratio *Ae. aegypti* to *Ae. albopictus* recorded was 1.00:22.79. Nonetheless, in Seksyen 7, Shah Alam the difference in *Ae. aegypti* to *Ae. albopictus* ratio is not really prominent with 1.00:3.61 for ovitraps deployed outdoor and 3.40:1.00 for ovitraps set indoor. It was determined that single infestation of either *Ae. aegypti* or *Ae. albopictus* is more frequent for ovitraps deployed indoor and/or outdoor, respectively. It was also determined that mixed infestations were found in this study indicating that both species can oviposit in the same container. **Conclusion:** This study indicates that OI is still above transmission threshold in both study sites. While *Ae. aegypti* and *Ae. albopictus* remain as a dominant indoor and outdoor breeder, respectively, mixed breeding of *Aedes* species in a same container was also observed.

KEYWORDS : Ovitraps, surveillance, *Aedes aegypti, Aedes albopictus*, dengue

INTRODUCTION

Dengue fever is an important arthropod-borne viral diseases in the world particularly in tropical regions.[1] In the past, major outbreaks of dengue cases were reported in many countries, causing morbidity and mortality and it is a global public health concern. [2-4] During the past five decades, the incidence rate of dengue has increased up to 30-folds worldwide. It was estimated that 50 to 100 million new infections occur yearly in more than 100 endemic countries.[5] In Malaysia, dengue is endemic and according to the report by the Ministry of Health (MOH), the cumulative numbers of dengue fever cases till the 51 week of 2017 were 82,840 cases and 171 dengue-related deaths were recorded in 2017.[6] Dengue was mainly transmitted by *Aedes aegypti* and *Aedes albopictus*. *Ae. aegypti* prefer clean stagnant water which can be found inside artificial containers and near to human residences while *Ae. albopictus* breed in natural containers and also outdoor man-made containers.[7,8] In several regions of Malaysia, studies reported mixed infestation of two species in the same breeding containers. [9-12]

Vector surveillance through ovitraps is a good and cost effective tool for dengue surveillance. It is reported that ovitrapping is a more efficient tool to

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detect the presence of *Aedes* as compared to larval survey when the infestation rate was low. [13-15] Ovitrap surveillance can provide the information of dengue outbreak forecast particularly in the regions of minor *Aedes* mosquito invasion.[16,17] In this study, a series of ovitrapping activities was conducted to investigate the abundance and distribution of *Ae. aegypti* and *Ae. albopictus* population in the dengue endemic areas.

Materials and methods

Study sites

The study was conducted in two residential areas which were in AU2, Keramat and in Seksyen 7, Shah Alam in Selangor (Fig. 1). Localities were chosen since the locations were identified as dengue hotspot areas as suggested by the Ministry of Health (MOH) Malaysia. Both sites were identified as the sub-urban and urban residential areas during study period. The areas had appropriate drainage system and high dense vegetation surrounding the areas. (Table 1)

Figure 1 Sampling site AU2, Keramat and in Seksyen 7, Shah Alam

Table 1 The study localities in AU2, Keramat and Seksyen 7, Shah Alam

Locality	GPS Coordinate	Category	Houses/ Residential Type	Ecological condition
AU2, Keramat	3°10'24.0"N 101°44'57.0"E	Suburban	Planned terraces	Surrounded with various vegetation such as trees and shrubs.
				Well-managed and generally clean area.
Seksyen 7, Shah Alam	3°04'00.5"N 101°28'59.6"E	Urban	Planned terraces	Have some area with vegetation, trees and shrubs.
				Well-managed and clean area.

Ovitrap surveillance

Ovitrapping was done to obtain baseline data of local wild mosquito population and it was conducted following the guidelines of MOH. [18] Black plastic containers of 300 mL volume was used as the ovitrap. Hard-board measuring 10 cm x 2.5 cm x 0.3 cm was used as an oviposition paddle and was placed in the ovitrap container to allow mosquito to lay eggs on its surface. Clean tap water was added to a level of 5.5 cm.[19] In this study, indoor was referred to as the interior of the house and outdoor as outside the house. The ovitraps were recovered after 5 days of placing in designated areas and brought back to the Medical Entomology Unit laboratory, Institute for Medical Research (IMR) for the larvae species identification.

In AU2, Keramat; the sampling was completed after five independent trips (Table 2). For each trip, a total of 100 ovitraps were placed. 50 ovitraps were placed indoors and outdoors respectively for placement on 8th August 2016 in randomly selected houses. Meanwhile, 100 ovitraps were placed outdoors for the remaining 4 placements, respectively. The surveillance at Seksyen 7, Shah Alam was also completed after four independent trips (Table 2). For each trip, a total of 100 ovitraps were randomly placed indoors and outdoors in selected houses.

Table 2 Ovitrap Index (OI),percentage of *Ae. aegyptiand Ae. albopictus* for indoor and outdoor ovitrap and percentage of mixed breeding

			AU2, Keramat					
			Indoor			Outdoor		
Ovitrap placement trip	Date trap collected	Ovitrap Index (%)	*Ae. aegypti* (%)	*Ae. albopictus* (%)	Mixed Breeding (%)	*Ae. aegypti* (%)	*Ae. albopictus* (%)	Mixed Breeding (%)
1st Trip	8-Aug-16	42.3	53.8	23.8	30.8	0	73.8	26.2
2nd Trip	26-Sep-16	77.4	n/a	n/a	n/a	2.8	79.1	18.1
3rd Trip	10-Oct-16	62.7	n/a	n/a	n/a	9.2	73.9	16.9
4th Trip	24-Oct-16	67.0	n/a	n/a	n/a	2.9	79.7	17.4
5th Trip	27-Feb-17	79.8	n/a	n/a	n/a	0	78.5	21.5
			Seksyen 7, Shah Alam					
1st Trip	29-Aug-16	29.8	50.0	0	50.0	38.5	42.3	19.2
2nd Trip	17-Oct-16	16.7	80.0	20.0	0	50.0	50.0	0
3rd Trip	31-Oct-16	31.8	100.0	0	0	31.6	63.1	5.3
4th Trip	14-Nov-16	42.9	66.7	16.6	16.7	29.6	48.2	22.2

Identification of larvae

The contents of ovitrap were transferred into plastic containers, and the paddle was then placed into the container to allow eggs on it to hatch. The larvae were routinely fed on liver powder (BD Difco™, USA) and bovine liver chunk diet. Larvae identification was conducted when the larvae reached the L3 stage. All larvae were identified to species according to specific criteria [20] and the number of larvae in each ovitrap were also recorded.

Data analysis

All the data were analyzed as follow:

a. Ovitrap index (OI) = (Number of positive traps / Number of recovered traps) X 100%
b. Mean larvae per ovitrap = Total number of larvae / Number of recovered ovitraps.

One-way ANOVA analysis was performed using SPSS (Version 19.0; IBM, Armonk, NY). The levels of statistical significance were resolute at P=0.05.

Results

Ovitrap surveillance

Table 2 shows the ovitrap index (OI), percentage of *Ae. aegypti* and *Ae. albopictus* for indoor and outdoor ovitraps and percentage of mixed breeding. The OI in both localities exceeds the transmission threshold of 10% [21] and is summarized in Table 2. In general, the OI for AU2, Keramat were higher than Seksyen 7, Shah Alam (Fig. 2). The OI in AU2, Keramat and Seksyen 7, Shah Alam ranged from 42.3 -79.8% and 16.7-42.9% respectively. Both sites recorded a higher percentage of single infestation of *Ae. aegypti* as compared to *Ae. albopictus* and/or mixed infestation for ovitraps deployed indoor. On the other hand, ovitraps that were set outdoor recorded a higher single infestation of *Ae. albopictus* than single infestation of *Ae. aegypti* and/or mixed infestation except for the 2nd placement trip at Seksyen 7, Shah Alam which recorded the same single infestation percentage for each species. Mixed infestation was consistently observed in ovitraps deployed outdoors but at a low percentage (5.3% to 26.2%). A non-consistent but a higher percentage (16.7% to 50.0%) mixed breeding was recorded indoors.

The total number of *Aedes sp.* larvae collected using ovitraps, mean number of larvae per recovered ovitrap and ratio of *Ae. aegypti* to *Ae. albopictus* collected at AU2, Keramat and Seksyen 7, Shah Alam is shown in Fig. 3 and Table 3. In this study, a very high *Ae. albopictus* population was found in AU2, Keramat. A total of 12,707 *Aedes* larvae were collected using ovitraps, comprising of 11,625 *Ae. albopictus* and 1,082 *Ae. aegypti* which indicated the former population is about ten times higher than the latter. In each placement trip, more *Ae.*

albopictus than *Ae. aegypti* were collected with the highest mean larvae per trap recorded was 33.57 ± 3.64 with no significant difference observed among the placement trips ($p>0.05$). Based on the ratio of *Ae. aegypti* to *Ae. albopictus* infestation outdoors, the latter was found to be a dominant outdoor breeder with the highest ratio of 1.00:22.79. Nonetheless, due to a limited data for indoor population, the comparison for infestation ratio cannot be made for indoor population for AU2, Keramat. Meanwhile, in Seksyen 7, Shah Alam, a total of 2,482 larvae comprising 1,119 *Ae. albopictus* and 1,363 *Ae. aegypti* were collected which indicated the population for both species in this locality was comparable. Similar trend was recorded in Shah Alam with *Ae. albopictus* dominating the outdoor traps with mean larvae per trap was 5.83 ± 1.66 with the highest ratio *Ae. aegypti* to *Ae. albopictus* breeding of 1.00:3.61. *Ae. aegypti* was the dominant indoor breeder with mean larvae per trap recorded of 19.74 ± 7.43 and the highest infestation ratio of *Ae. aegypti* to *Ae. albopictus* recorded indoor was 3.40:1.00.

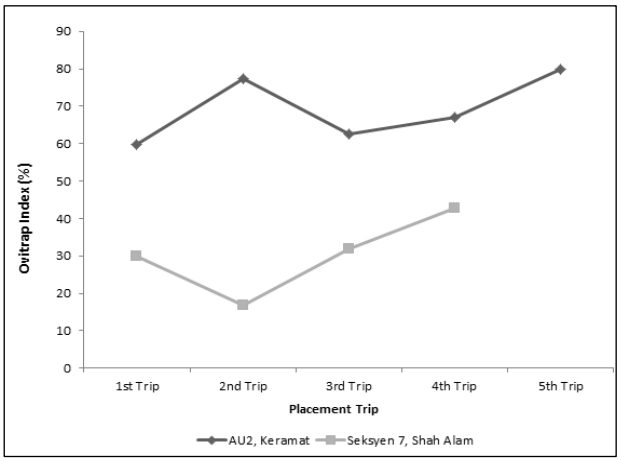

Figure 2 Ovitrap index in AU2, Keramat and Seksyen 7, Shah Alam

Discussion and Conclusion

This study utilized ovitrapping technique for *Aedes* surveillance purpose and has been reported as an economical and effective tool as compared to larval survey especially when the *Aedes* infestation rate is low. The cryptic breeding sites of dengue vectors makes the conventional larval survey technique less sensitive and less efficient.[13-15] Based on the total number of *Aedes* species collected during this study, it was found that *Ae. albopictus* population is highly abundant and dominated the area in AU2, Keramat. This species is significantly dominating the ovitraps deployed outdoors in line with many ovitrap surveillance studies which reported *Ae. albopictus* as an outdoor breeder.[10,22,23] The high population of *Ae. albopictus* in this site may be due to the suburban environment with dense vegetation. Besides, during the study duration; there was an active construction site located adjacent to the sampling area, which may have contributed to formation of water puddles and eventually providing natural breeding sites for *Ae. albopictus* to oviposit. This study however, with its limited data for indoor population for AU2, Keramat showed that the ratio for *Ae. albopictus* is ten times higher than *Ae. aegypti* in indoor setting. Nonetheless, it may be possible that the presence of a very low number of

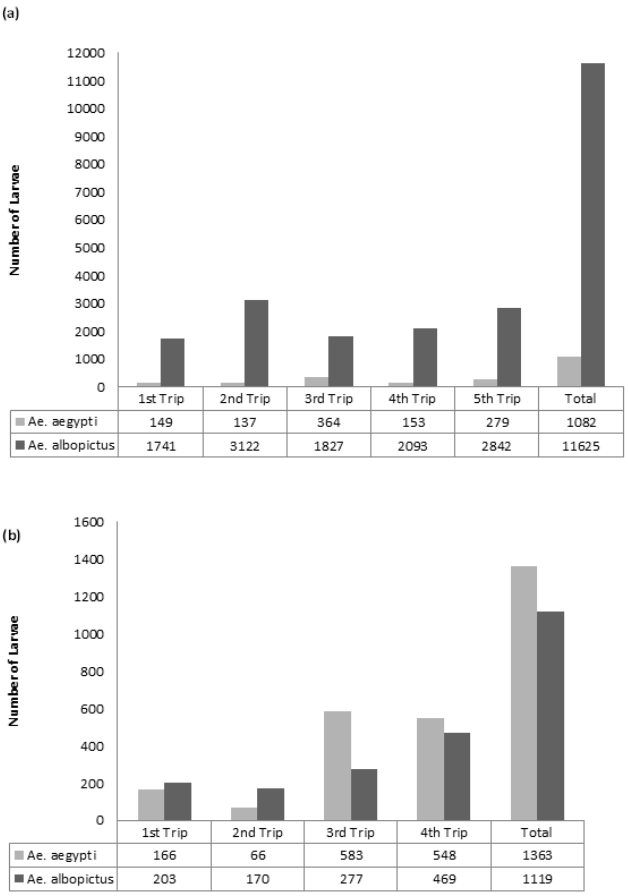

Figure 3 : Total number of *Aedes* sp. Larvae collected using ovitraps in (a) AU2, Keramat and (b) Seksyen 7, Shah Alam

Ae. aegypti may contribute to this figure. Moreover, in studies where the absence of *Ae. aegypti* were reported, *Ae. albopictus* was also found to breed in ovitraps deployed indoors .[19,22]

In Seksyen 7, Shah Alam; the abundance of both species *Ae. aegypti* and *Ae. albopictus* is comparable and the OI showed that these two species as an

Table 3 Mean number of Ae. aegypti and Ae. albopictus and the species ratio collected indoor and outdoor

		AU2, Keramat					
		Indoor			Outdoor		
Ovitrap placement trip	Date trap collected	*Ae. aegypti* (Mean±SE)	*Ae. albopictus* (Mean±SE)	*Ae. aegypti:* *Ae. albopictus*	*Ae. aegypti* (Mean±SE)	*Ae. albopictus* (Mean±SE)	*Ae. aegypti:* *Ae. albopictus*
1st Trip	8-Aug-16	0.79 ± 0.40	7.98 ± 3.70	1.00:10.00	2.35 ± 1.08	28.53 ± 4.78	1.00:12.16
2nd Trip	26-Sep-16	n/a	n/a	n/a	1.49 ± 0.51	33.57 ± 3.64	1.00:22.79
3rd Trip	10-Oct-16	n/a	n/a	n/a	3.57 ± 1.24	17.91 ± 2.43	1.00:5.02
4th Trip	24-Oct-16	n/a	n/a	n/a	1.44 ± 0.52	20.32 ± 2.59	1.00:13.68
5th Trip	27-Feb-17	n/a	n/a	n/a	2.82 ± 0.92	28.71 ± 2.89	1.00:10.19
		Seksyen 7, Shah Alam					
1st Trip	29-Aug-16	1.21± 1.14	0.36 ± 0.36	3.40:1.00	2.00 ± 0.71	2.92 ± 0.90	1.00:1.46
2nd Trip	17-Oct-16	0.96 ± 0.70	0.85 ± 0.85	1.14:1.00	0.64 ± 0.30	2.31 ± 1.28	1.00:3.61
3rd Trip	31-Oct-16	19.74 ± 7.43	0	n/a	1.98 ± 1.38	4.25 ± 1.32	1.00:2.14
4th Trip	14-Nov-16	7.33 ± 4.35	3.56 ± 2.01	2.06 : 1.00	5.47 ± 2.17	5.83 ± 1.66	1.00:1.07

indoor and outdoor breeder respectively. Extensive ovitrap and larval surveys in many urban and suburban areas in peninsular Malaysia[24,25] reported that although these two species can be found both indoors and outdoors, *Ae. aegypti* was predominantly found to be dominant indoors and *Ae. albopictus* to be dominant outdoors.

Although single infestation of either *Ae. aegypti* or *Ae. albopictus* is more frequent for ovitraps deployed indoor and/or outdoor, a consistent trend of mixed breeding of the two species was also observed especially for the ovitraps deployed outdoors. Several reports have indicated the mixed infestations of both species in ovitraps deployed in Selangor, Kuala Lumpur and Penang. [9,13,26] The frequency of either species was influenced by placement of the traps, with *Ae. aegypti* recorded a higher frequency than *Ae. albopictus* for ovitraps set indoor and vice versa. Mixed infestations were found in both our study areas which was from 5.3% to 50%. Other studies reported that mixed infestation in different localities in Kuala Lumpur and Selangor was from 3.03% to 32.0%. [9,13]

In conclusion, this study indicates that *Ae. aegypti* and *Ae. albopictus* still remain as dominant indoor and outdoor breeders accordingly. Also, ovitrap is a sensitive tool to attract gravid females of more than one *Aedes* species to oviposit.

ACKNOWLEDGEMENTS

The authors thank the Director-General of Health, Malaysia and the Director, Institute for Medical Research (IMR), for permission to publish this study. This study was supported by study grant from the Ministry of Health, Malaysia. We acknowledge the Wolbachia team members from the Medical Entomology Unit, IMR particularly Faiz A, Muhammad Irfan Norman FR, Muhammad Kamarul Ridhuan G, Thohir H, Siti Nor Syazwani A, Nur Syamimi HS, Nur Ruqqayah MM and Nur Zatil Aqmar MZ for their technical assistance in collecting and identifying the mosquitoes.

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