

Ovitrap Surveillance and Mixed Infestation of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Northern Region and Southern Region of Malaysia

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ABSTRACT: An ovitrap surveillance was conducted to provide recent baseline information on the existence and distribution of *Aedes* larvae in four dengue prone areas located in Penang, Kedah, Johor and Melaka of Peninsula Malaysia. Among these areas, the highest ovitrap index (OI) for both indoors and outdoors were recorded from Sungai Ara, Penang with 62% and 75%, respectively. There was no significant difference for indoor populations of *Ae. aegypti* and outdoor populations of *Ae. albopictus* between all study areas selected ($P>0.05$), whereas the outdoor populations of *Ae. aegypti* and the indoor populations of *Ae. albopictus* in Sungai Ara, Penang were significantly higher than other study areas by 1.91 - 2.61 folds and 1.66 - 3.01 folds, respectively ($P<0.05$). Different population ratios of *Ae. aegypti* to *Ae. albopictus* larvae for both indoors and outdoors were observed in all study areas, respectively. Furthermore, mixed infestation was found in both indoor and outdoor populations in all study areas ranging from 11.36% to 29.03% of the total positive ovitraps. The sensitivity and reliability of ovitrap as a surveillance tool in detecting the presence of more than one mosquito species especially *Aedes* populations were also re-confirmed.

Keywords: Ovitrap, surveillance, Aedes, dengue, mixed infestation

Introduction

Dengue remains as the most important vector-borne disease in many countries including Malaysia. According to WHO (2009), about two fifths of the world's population are now at risk from dengue. As for Malaysia, dengue cases are reported in all states annually. For the year of 2009, 34,975 dengue cases were reported until 7th November 2009 with 75 deaths (Ministry of Health Malaysia, 2009).

Aedes aegypti and *Aedes albopictus* have been incriminated as dengue vectors in the tropical and subtropical regions (Smith, 1956; Hammon, 1966; Rudnick, 1967). *Ae. aegypti* prefers the clean water found in many types of domestic containers inside and near human dwellings, whereas *Ae. albopictus* favors natural containers or outdoor man-made habitats containing a greater amount of organic debris (Rattanaarithikul and Panthusiri, 1994; Chareonviriyaphap et al., 2003). Nevertheless, the

distribution of both species in Peninsula Malaysia overlaps (Yap, 1975).

Ovitrap surveillance is one of the common techniques used in detecting and monitoring the *Aedes* populations. The ovitrap is sensitive, fast, and cost-effective to determine the presence of egg-laying females of *Aedes* mosquitoes (Fay and Eliason, 1966). Hence, the aim of this study was to provide recent baseline information on the presence and distribution of *Aedes* larvae in selected dengue prone areas using ovitraps.

Materials and Methods

Study areas

Ovitrap surveillance was conducted in dengue prone areas of four states in Peninsular Malaysia: Sungai Ara in Penang, Sungai Petani in Kedah, Johor Bahru in Johor and Melaka Tengah in Melaka. The selection of these areas was based on the frequent dengue cases reported annually as provided by the Vector Borne-Diseases Control Programme (VBDCP) of each state.

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Ovitrap surveillance

Standardized ovitraps as described by Lee (1992) were utilized in this study. The ovitrap comprises a 300 ml black plastic container. The opening and the base of the container are both 6.8 cm in diameter and the height of the container is 9.1 cm. An oviposition paddle made from hardboard (10 cm x 2.5 cm x 0.3 cm) consisting of two different types of surfaces was placed diagonally into each ovitrap with the rough surface of the oviposition paddle upwards. Each ovitrap was filled with tap water to a level of 5.5 cm. These ovitraps were used in accordance to the guidelines of Ministry of Health, Malaysia (1997). All ovitraps were placed in proximity to other potential breeding containers with minimum physical and environmental disturbance.

All ovitraps were placed randomly indoors and outdoors which were either partially or totally shaded to avoid from direct sunlight and heavy rain that may cause water spillage. Note that “indoors” refers to the interior of the premise (house, flat), while “outdoors” refers to the outside of the premise but confined to the immediate vicinity of the house.

As this study was originally performed to provide preliminary data of *Aedes* in all study areas to VBDCP of respective states, therefore only one ovitrap surveillance was conducted in each study site selected.

Identification of larvae

All ovitraps were collected after 5 days of deployment and brought back to the laboratory. The contents were poured into individual plastic containers, together with the paddle and topped up with fresh water. A mixture of liver powder, cereals and yeast as well as a small piece of partially-cooked cow liver were added into each container as larval food. The containers were kept covered to avoid other mosquitoes in the vicinity from ovipositing in the containers. All hatched larvae were reared and subsequently counted and identified at fourth instar larvae. The larval numbers were recorded individually for every positive ovitrap.

Analysis of data

Data obtained in this study were analyzed as:

- (i) Ovitrap Index (OI): the percentage of positive ovitraps to the total number of recovered ovitraps for each study site.
- (ii) Mean number of *Ae. aegypti* and / or *Ae. albopictus* larvae per recovered ovitrap.

One-way ANOVA analysis was performed using computer- aided statistical programme. All levels of statistical significance were determined at $P = 0.05$.

Results and Discussion

TABLE 1 showed the ovitrap index (OI), the mean number of larvae per recovered ovitrap and the ratio of *Ae. aegypti* to *Ae. albopictus* collected in every study site. More positive ovitraps were found outdoors rather than indoors in all study areas which were similar to the findings by Dibo et al. (2005) in Mirassol, the state of Sao Paulo, Brazil. Sungai Ara, Penang had the highest OI for both indoors and outdoors with 62.00% and 75.00%, respectively. Moreover, the highest mean number of *Ae. aegypti* and *Ae. albopictus* larvae per recovered ovitrap for both indoors and outdoors was also obtained from Sungai Ara, Penang. There was no significant difference for indoor populations of *Ae. aegypti* and outdoor populations of *Ae. albopictus* between all study areas selected ($P > 0.05$), whereas the outdoor populations of *Ae. aegypti* and the indoor populations of *Ae. albopictus* in Sungai Ara, Penang were significantly higher than other study areas by 1.91- to 2.61-folds and 1.66- to 3.01-folds, respectively ($P < 0.05$). The population ratios of *Ae. aegypti* to *Ae. albopictus* larvae in all study areas were generally different from one another for both indoors and outdoors, respectively.

TABLE 2 described the distribution of *Aedes* larvae in positive ovitraps collected from all study areas. Both *Ae. aegypti* and *Ae. albopictus* remained as the predominant indoor and outdoor mosquitoes in all study areas, respectively, except for indoor populations in Sungai Ara, Penang and Taman Kenanga, Melaka where positive ovitraps with only *Ae. albopictus* larvae were found to be higher than positive ovitraps with *Ae. aegypti* larvae alone. In general, these results indicated that *Ae. aegypti* and *Ae. albopictus* in all study areas have similar role in transmitting dengue virus. However, for Sungai Ara, Penang and Taman Kenanga, Melaka, the *Ae. albopictus* populations in both study areas were found to be more dominant compared to the *Ae. aegypti* populations which could suppress the indoor populations of *Ae. aegypti* by ovipositing inside the premises as well. In fact, many recent studies had suggested that *Ae. albopictus* is now invading many residential habitats especially in urban zones (Chareonviriyaphap et al., 2003). Furthermore, according to Cheah et al. (2006), although *Ae. albopictus* is an outdoor breeder, it will still migrate indoors in the absence of *Ae. aegypti*.

Both *Ae. aegypti* and *Ae. albopictus* share nearly the same habitat, especially in urban areas (Nazni et al., 2009). In line with this, mixed infestation was found

in both indoor and outdoor populations in all study areas which was from 11.36% to 29.03% of the total positive ovitraps, respectively. These results were relatively similar to the findings by Chen et al. (2006) who reported 10% to 32% of mixed infestation observed indoors and outdoors of four localities with different environments in Selangor, Malaysia. However, the results were much higher than the mixed infestation rates of *Aedes aegypti* and *Aedes albopictus* recorded from four suburban communities in Selangor, Malaysia which were only 3.11% to 8.21% and 5.11% to 9.76% of positive indoor and

outdoor ovitraps, respectively (Lee, 1992). Furthermore, only 6.30% and 15.40% of positive ovitraps collected from Kg Pasir Gebu and Taman Permai Indah in Penang, respectively, were found with mixed infestation of *Ae. albopictus*, *Ae. aegypti* and *Culex quinquefasciatus* (Rozilawati et al., 2007). In fact, surveys conducted in Singapore also found merely 7.1% of mixed infestation in natural habitats (Chan et al., 1971). These findings indicated that more than one mosquito species could oviposit in a single ovitrap (Chen et al., 2006).

TABLE 1- Ovitrap index (OI), mean number larvae per recovered ovitrap and ratio of *Aedes aegypti* to *Aedes albopictus* for indoors and outdoors in four study areas

Study site	Ovitrap placement	Ovitrap Index (OI)	Mean number larvae per recovered ovitrap		<i>Ae. aegypti</i> : <i>Ae. albopictus</i>
			<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	
Sungai Ara, Penang	Indoors	62.00 %	4.17 ± 0.68 ^e	5.62 ± 0.78 ^g	1.00 : 1.35
	Outdoors	75.00 %	4.85 ± 0.92 ^f	9.60 ± 1.25 ^h	1.00 : 1.98
Taman Keladi, Kedah	Indoors	51.76 %	3.47 ± 0.69 ^e	2.80 ± 0.67 ^g	1.24 : 1.00
	Outdoors	65.22 %	2.54 ± 0.51 ^f	8.40 ± 0.96 ^h	1.00 : 3.31
Taman Skudai Kanan, Johor	Indoors	28.00 %	2.31 ± 0.55 ^e	1.87 ± 0.45 ^g	1.23 : 1.00
	Outdoors	60.00 %	1.86 ± 0.70 ^f	7.12 ± 1.26 ^h	1.00 : 3.83
Taman Kenanga, Melaka	Indoors	43.00 %	2.65 ± 0.81 ^e	3.38 ± 0.88 ^g	1.00 : 1.28
	Outdoors	51.00 %	1.88 ± 0.56 ^f	5.64 ± 0.95 ^h	1.00 : 3.00

^e (F = 1.61, P>0.05, df = 3); ^f (F = 4.14, P<0.05, df = 3); ^g (F = 5.81, P<0.05, df = 3); ^h (F = 2.55, P>0.05, df = 3)

TABLE 2- Distribution of *Aedes* populations in the ovitraps deployed in four study area

Study site	Ovitrap placement	No. of re-covered ovitrap	No. of positive ovitrap	No. of positive ovitrap with each <i>Aedes</i> sp.		Percentage of positive ovitrap with each <i>Aedes</i> sp.		No. of positive ovitrap with mixed infestation	Percentage of positive ovitrap with mixed infestation	Ratio of <i>Ae. Aegypti</i> : <i>Ae. albopictus</i> in mixed infestation
				<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>			
Sungai Ara, Penang	Indoors	100	62	18	26	29.03 %	41.94 %	18	29.03 %	1.00 : 1.30
	Outdoors	100	75	14	42	18.67 %	56.00 %	19	25.33 %	1.71 : 1.00
Taman Keladi, Kedah	Indoors	85	44	24	15	54.55 %	34.09 %	5	11.36 %	1.18 : 1.00
	Outdoors	115	75	18	44	24.00 %	58.67 %	13	17.33 %	1.00 : 2.20
Taman Skudai Kanan, Johor	Indoors	150	42	20	15	47.62 %	35.71 %	7	16.67 %	1.70 : 1.00
	Outdoors	50	30	5	17	16.67 %	56.67 %	8	26.67 %	1.00 : 2.71
Taman Kenanga, Melaka	Indoors	100	43	13	25	30.23 %	58.14 %	5	11.63 %	1.00 : 1.04
	Outdoors	100	51	10	35	19.61 %	68.63 %	6	11.76 %	1.00 : 1.38

Taman Keladi, Kedah; Taman Skudai Kanan, Johor and Taman Kenanga, Melaka are suburban residential areas consisting of terraced houses and flats. On the other hands, Sungai Ara, Penang comprises structured-villages with brick and wooden houses. However, all study areas shared almost similar environment where many ornamental plants and vegetations were observed around the

compounds of the premises. These surroundings are ideal as breeding habitats especially for *Ae. albopictus*. Recent studies by Honorio et al. (2009) found that *Ae. albopictus* was more abundant in vegetated areas compared to *Ae. aegypti*. Nevertheless, few containers of water storage could be found in all study areas as these areas are supplied with piped water, but unattended and unused

artificial containers such as flower pot plates, plastic containers, buckets and bottles could still be found inside and outside the compounds of some premises. All study areas are also constructed with concrete drainage systems but certain portions along the drains were not well-managed and were found to be clogged resulting in the formation of stagnant water. The clear stagnant water in the drains was found to support the breeding of all stages of *Aedes* mosquitoes (Chen et al. 2006).

Conclusion

Both *Ae. aegypti* and *Ae. albopictus* were the potential dengue vectors in all study areas. However, the suppression of *Ae. albopictus* populations in Sungai Ara, Penang and Taman Kenanga, Melaka onto the indoor populations of *Ae. aegypti* is underway. Besides, this study also confirmed the sensitivity and reliability of ovitrap as a tool in detecting the presence of more than one mosquito species especially *Aedes* populations as well as their abilities to lay eggs within the same ovitrap.

Although many types of adulticides and larvicides are available in the market, the implementation of the source reduction remains as the best solution in preventing the existence of the unnecessary mosquito breeding habitats indoors and outdoors. Hence, the awareness of the community members towards the seriousness of dengue and the importance of the source reduction activities should be enhanced, for instance, through the health education. This is because the transmission of dengue could not be minimized or totally interrupted without the participation and cooperation of the whole communities.

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