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Ovitrap surveillance of the dengue vectors, *Aedes* (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* Skuse in selected areas in Bentong, Pahang, Malaysia

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Abstract. Ovitrap surveillance was conducted in methodically selected areas in Bentong, Pahang, Malaysia from June 2008 till December 2009 in order to identify insular sites with stable *Aedes aegypti* population. Eleven sites were surveyed in Bentong district, Pahang, and one of these locations (N3º33' E101º54') was found to have an ovitrap index of *Ae. aegypti* and *Aedes albopictus* ranging from 8%-47% and 37%-78% respectively, indicating that this site could be a high-risk area for dengue outbreak. *Ae. aegypti* larvae were found in both indoor and outdoor ovitraps ($p>0.05$) while significant difference between the populations of *Ae. albopictus* larvae from indoors and outdoors was observed ($p<0.01$). Data collected in this study could provide important entomological information for designing an effective integrated vector control programme to combat *Aedes* mosquitoes in this area.

INTRODUCTION

Dengue is currently considered an important public health problem all over the world. Its occurrence has been registered in more than 100 countries occurring from the southern USA through most tropical regions, China and into Australia (WHO, 2006). Malaysia is one of the affected countries, with more than 38, 000 notified cases in each of the past six years. From first January 2009 till second January 2010, a total number of 41486 notified cases and 88 deaths were reported by the Disease Control Division, Department of Public Health, Malaysia Ministry of Health (Malaysia Ministry of Health, 2010).

Vector control remains the only option to control dengue and vector surveillance is an important component of vector control programmes. It provides information for developing risk assessment that in turn could be used to qualitatively or quantitatively predict the occurrence of vector-borne disease or pest outbreaks (USAF, 2002; Beech *et al.*, 2009).

Aedes surveillance using ovitraps is one of the cost-effective and most important tools of dengue control. Ovitraps provide a simple and convenient tool for *Aedes* surveillance. Previous studies indicated that ovitrap surveillance could be used for the prediction of dengue outbreak, especially in areas of low *Aedes* infestation, and has been recommended as a surveillance tool in dengue control (Lee, 1992; Tham, 1993; Focks, 2003). This study was conducted to monitor the dengue vector population dynamics in an endemic area.

MATERIALS AND METHODS

Study area

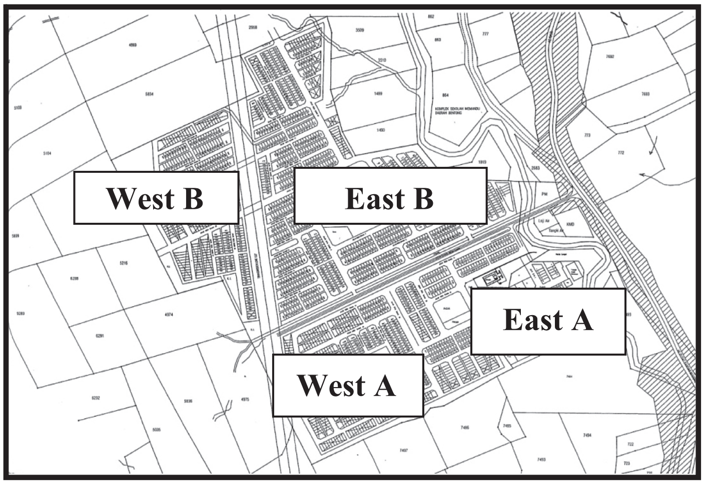
Ovitrap surveillance was conducted in selected areas in Bentong, Pahang, Malaysia in order to identify insular sites with stable *Aedes aegypti* population. The choice of such sites is based on several criteria such as presence of human habitats, dengue cases, population of *Aedes* species, field size, geographical isolation, ecological stability and control site (McKemey *et al.*, 2008). Bentong is a district belonging to the state of Pahang. It comprises an area of 183 119 hectares, with 112 900 inhabitants (Department of Town and Country Planning State of Pahang, 2007).

Preliminary surveys were conducted in 11 sites of Bentong district, and one location, (N03°33' E101°54') was found to be suitable for further study. In 2008, four dengue cases were reported in this area. This site is a geographically isolated suburban residential area covering 29 hectares surrounded by vegetation and greenery. There are approximately 5600 inhabitants living in 1120 houses which consist of single storey terraced houses with a proper concrete storm water drainage system running through the site.

To study the distribution of *Aedes* sp. in the study site, this area was divided into four zones, namely East A, West A, East B and West B (Figure 1). Each zone comprised similar house type; i.e. one storey structure with a kitchen, living room, bathroom and two to three bedrooms.

Ovitrap surveillance

The ovitrap sampling as described by Lee (1992) was used in this surveillance based on the Malaysian Ministry of Health Guidelines (1997). Each ovitrap consisted of a 300 ml black plastic container 6.5 cm in diameter and 9.0 cm in height. Fresh water was added to a level of 5.5 cm and an oviposition paddle (10 cm x 2.5 cm x 0.3



Source: Bentong Municipal Council, 2008

Figure 1. Plan layout of study area

cm) made from hardboard was placed in the water with the rough surface upwards in each ovitrap.

Ovitraps were placed indoor and outdoor in randomly selected houses after obtaining informed consent from the house owner. Ovitraps were collected after seven days and immediately transported to the Institute for Medical Research (IMR) laboratory. The contents of ovitrap were poured individually into labelled and covered plastic containers (15 cm x 7 cm x 8.5 cm) together with the paddle. All larvae were counted and identified under a compound microscope (NIKON ECLIPSE E200, Japan) at third and fourth instar using taxonomy keys prepared by IMR.

Data Analysis

The abundance of *Aedes* sp. larvae in the potential field site were analysed as follows:

- Ovitrap index, the percentage of positive ovitrap against the total number of ovitraps recovered for each site.
- The number *of Aedes* sp. larvae per recovered ovitrap.

The number of *Aedes* larvae collected indoor and outdoor by ovitraps was not normally distributed according to the Kolmogorov-Smirnov normality test (Coakes, 2005). Therefore, Mann-Whitney Test was employed to analyze the mean of differences on larvae per ovitrap recovered between indoor and outdoor at p=0.05.

RESULTS

A total of 1630 ovitraps were placed indoor and outdoor randomly in selected houses from June 2008 till December 2009. During the surveys, 80%–100% of ovitraps were recovered after seven days. In total, 29085 larvae were examined of which 20.00% were *Ae. aegypti*, 76.93% *Aedes albopictus*, 1.21% of other mosquitoes (*Culex* sp. and *Toxorynchites* sp.) and 1.86% of non-mosquito, *Chironomus* sp.

In this study, ovitrap indices of *Ae. aegypti* and *Ae. albopictus* were found to be in the range of 8%-47% and 30%-78% respectively (Figure 1). Overall, the mean number of *Ae. aegypti* larvae per ovitrap was 3.83 ± 2.81 for indoor ovitraps and 4.72 ± 5.28 for outdoor ovitraps. The mean number of *Ae. albopictus* larvae per ovitrap was 10.41 ± 5.93 for ovitraps placed indoors compared to 19.13 ± 9.37 for ovitraps placed outdoors. There was significant difference between the populations of *Ae. albopictus* larvae from indoor and outdoor ovitraps ($p<0.01$), while no such difference was observed for *Ae. aegypti* ($p>0.05$).

This study demonstrated that *Ae. albopictus* populations were dominant in all four zones compared to *Ae. aegypti* populations both indoor and outdoor. The highest percentage of positive ovitrap with *Ae aegypti* and *Ae. albopictus* was 60.96% (indoor-West A) and 99.28% (outdoor-East B), respectively. Mixed breeding was found in both indoor and outdoor populations in all study sites ranging from 7.95% to 29.67% and 5.52% to 44.95%, respectively (Table 1).

Based on Table 2, the distribution of *Ae. aegypti* was more abundant in both East A and West A, while the presence of *Ae. albopictus* population remained stable in all zones. This site also recorded highest *Ae. aegypti/Ae. albopictus* ratio of 1.00 : 39.73 for indoor ovitraps and 1.00 : 36.90 for outdoor ovitraps.

DISCUSSION

In this study, ovitrap data indicated *Ae. albopictus* was the dominant species both indoor and outdoor in the study area. Previous studies had proven that *Ae. albopictus* existed in rural, suburban and urban areas in Peninsular Malaysia (Yap, 1975; Sulaiman & Jeffery, 1986; Lee, 1992; Rozilawati *et al.*, 2007). Braks *et al.* (2003) suggested that habitat affected the abundance of *Ae. aegypti* and *Ae. albopictus* in different ways. *Aedes aegypti* predominated in highly urbanized habitats, *Ae. albopictus* in more rural areas and the

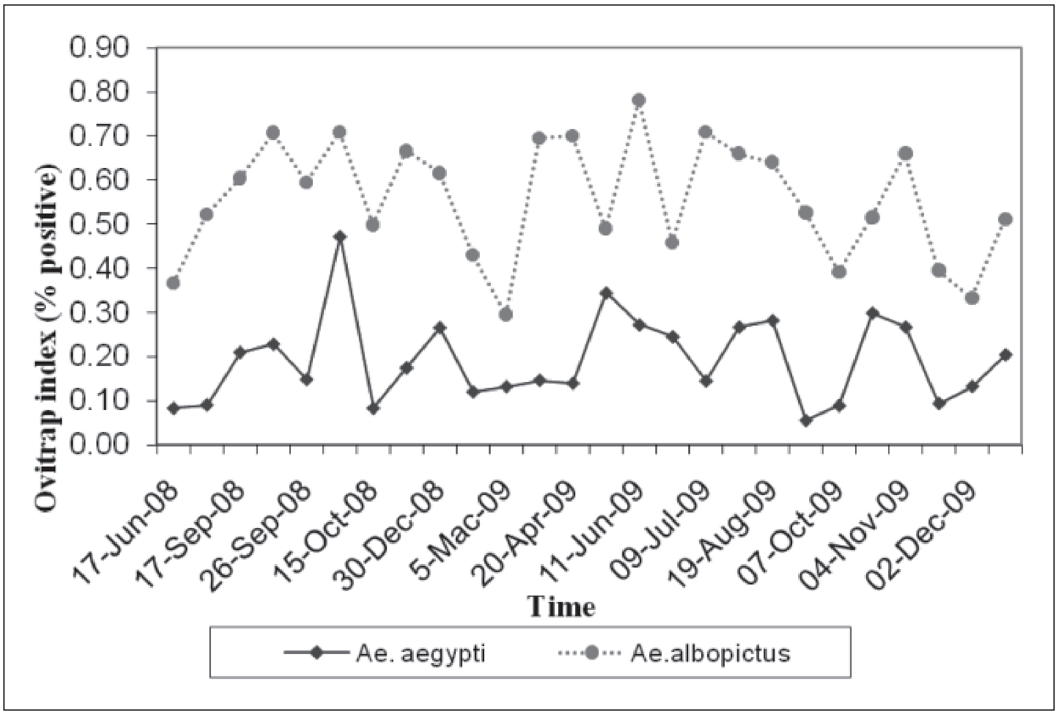


Figure 2. Ovitrap index of *Ae. aegypti* and *Ae. albopictus* over time in study area

Table 1. Distribution of *Aedes* populations in the four zones of study area based on the ovitrap placement

Indoor

Study area	No. of recovered ovitrap	No. of positive ovitrap	No. of positive ovitrap with		Percentage of positive ovitrap with		No. of positive ovitrap with mixed breeding	Percentage of positive ovitrap with mixed breeding
			Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*		
East A	210	108	57	82	52.78%	75.93%	27	25.00%
West A	131	82	50	58	60.96%	70.73%	24	29.67%
East B	201	98	17	89	19.10%	90.82%	8	8.16%
West B	188	88	10	84	12.04%	95.45%	7	7.95%

Outdoor

Study area	No. of recovered ovitrap	No. of positive ovitrap	No. of positive ovitrap with		Percentage of positive ovitrap with		No. of positive ovitrap with mixed breeding	Percentage of positive ovitrap with mixed breeding
			Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*		
East A	221	156	72	133	46.15%	85.26%	40	25.64%
West A	144	109	60	98	55.06%	89.91%	49	44.95%
East B	218	139	11	138	7.91%	99.28%	8	5.76%
West B	200	145	9	143	6.38%	98.62%	8	5.52%

Table 2. Distribution of the ovitrap index, mean larvae per recovered ovitrap and ratio of *Ae. aegypti* and *Ae. albopictus* (indoor and outdoor) in four zones of the study area

Study area	Ovitrap placement	Ovitrap index		Mean larvae per recovered ovitrap		*Ae. aegypti*: *Ae. albopictus*
		Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	
East A	Indoor	0.26 ± 0.15	0.41 ± 0.22	6.45 ± 5.98	9.81 ± 9.40	1.00 : 1.30
	Outdoor	0.33 ± 0.17	0.64 ± 0.24	7.60 ± 7.76	17.46 ± 9.83	1.00 : 2.10
West A	Indoor	0.36 ± 0.27	0.54 ± 0.28	8.82 ± 9.59	9.18 ± 9.40	1.00 : 1.00
	Outdoor	0.38 ± 0.26	0.75 ± 0.25	6.05 ± 9.35	20.43 ± 20.31	1.00 : 2.16
East B	Indoor	0.08 ± 0.12	0.45 ± 0.26	0.69 ± 1.27	10.71 ± 7.67	1.00 : 13.03
	Outdoor	0.05 ± 0.09	0.68 ± 0.21	0.59 ± 1.21	18.93 ± 14.71	1.00 : 33.57
West B	Indoor	0.04 ± 0.07	0.43 ± 0.25	0.15 ± 0.33	11.43 ± 12.48	1.00 : 39.73
	Outdoor	0.03 ± 0.09	0.75 ± 0.22	0.47 ± 1.60	19.79 ± 13.48	1.00 : 36.90

two species co-occurred in the suburban areas where mosquitoes were found. *Aedes albopictus* is commonly found outside houses, prefers vegetated areas (Hawley, 1988) and breeds in artificial and a variety of natural water containers.

Although *Ae. albopictus* population was higher than *Ae. aegypti*, *Ae. aegypti* population was shown to be stable with ovitrap indices ranging from 8% to 47%. An ovitrap index above 10% for *Aedes* species in an area may indicate a possible risk of dengue outbreak (Lee, 1992; Malaysia Ministry of Health, 1997). *Aedes aegypti* in Malaysia was mostly found exclusively inside houses, breeds in artificial water containers and feed mostly indoor (Rudnick, 1986). It also may be found in housing areas lacking gardens and vegetation, such as heavily congested urban cities in Kuala Lumpur (Chen *et al.*, 2005, 2006). In contrast, study performed in West Java, Indonesia concluded that *Ae. aegypti* was an outdoor rather than indoor mosquito since the number of larvae caught in the outdoor ovitraps was 25.18% higher than indoor (Syarifah *et al.*, 2008). Nevertheless, in this study *Ae. aegypti* did oviposit in both indoor and outdoor ovitraps, underlining the plasticity of its behaviour.

In this study, *Ae. albopictus* seems to have a homogenous distribution throughout the area but it is not the case for *Ae. aegypti*. The ovitrap index and number of larvae per ovitrap of *Ae. aegypti* were lower in both East B and West B compared to East A and West A. This outcome was probably a result of mosquito control activity namely Communication for Behavioural Impact (COMBI) that was organized in East B and West B since 2007.

COMBI participation in East B and West B involved approximately 40 volunteers from the neighbourhood. The COMBI activities included visit from house to house on foot by three to four volunteers in a team in order to deliver the anti dengue message, to inspect and destroy the *Aedes* breeding sites indoor and outdoor mainly in the weekends. The COMBI approach in Hulu Langat successfully demonstrated that correct problem identification synergized with community engagement can potentially reduce *Aedes* proliferation and dengue morbidity (Rozhan *et al.*, 2006).

Interestingly, our results demonstrated higher number of *Ae. aegypti* in both East A and West A. This finding was most probably due to the presence of shop houses exclusively in this area. This building was a double storied, built in bricks and cements where a portion of this premise was used for business while the rest for residence. Studies conducted in Puerto Rico and Thailand indicated that in rural habitats with abundance of human hosts and oviposition sites, *Ae. aegypti* did not disperse far from their development site in and around homes and tended to be spatially clustered at the household level (Harrington *et al.*, 2005).

In addition, results of larval survey by the health authorities in this study area indicated that *Ae. aegypti* was mostly found in the shop houses, mainly in pails and toilet tank (Malaysia Ministry of Health, 2008). In contrast, Sulaiman *et al.* (1991) reported *Ae. albopictus* apparently was dominant to *Ae. aegypti* breeding inside premises of houses, shop houses and factories. However, in this study, no significant difference was found between the presences of *Ae. aegypti* larvae found inside houses and shop houses (p>0.05).

In a study of *Ae. aegypti* in Trinidad, Rawlins *et al.* (1998) compared the use of visual larval surveillance system with ovitrapping to determine the presence of eggs. It was found that ovitrapping was more sensitive than the visual inspection of larvae. Lee (1992) also reported that ovitrapping was more sensitive than visual larval surveys. On the other hand, Chadee (2004) suggested that visual larval surveillance could be made more efficient by evaluating key containers and premises. Our results provide an example of how the *Aedes* population can be monitored continuously throughout the entire study period by using ovitraps. Moreover, this study could serve as a early warning system and assist decision makers in initiating vector control for both dengue and chikungunya prevention

In conclusion, this study indicated that Bentong district has a high risk for outbreaks of *Aedes* mosquito-borne diseases such as dengue and chikungunya. Data collected in this study are likely to aid in the selection of test and control sites and at the same time could provide important entomological information for the design of an effective integrated vector control programme to combat *Aedes* mosquitoes in this area.

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