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Assessing dengue outbreak areas using vector surveillance in north east district, Penang Island, Malaysia

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ABSTRACT

Objective: To understand the efficacy of ovitrap surveillance and its implementation on monitoring reflection upon case occurrence in relation to climate variables.
Methods: We used routinely setup ovitrap surveillance to monitor the mosquito populations in previous outbreak areas. Ovitraps were installed weekly at three localities that experienced high number of dengue cases (Flat Hamna, Kampung Sungai Gelugor and Kampung Tanjung Tokong) from January 2010 to February 2011. Ovitraps and paddles were brought back to the laboratory and all of the water contents were poured into an enamel pan. Aged tap water was added into the enamel pan and eggs were allowed to hatch. The hatching larvae were counted after 3 days. The hatched larvae were identified at the 3rd instar larval stage. The ovitrap indices and mean number of larvae were analyzed using student *t*-test and One-way ANOVA. Spearmen's rank correlation coefficient was used to determine the relation between meteorology variables and dengue fever cases.
Results: *Aedes albopictus* was found as dominant species followed by *Aedes aegypti* recorded in all three study areas. *Aedes aegypti* preferred to breed outdoor with larvae collection, which was higher than indoor (72.37%). There was a positive correlation between the ovitrap index with the rainfall and humidity except in Kampung Tanjung Tokong. Our result also showed negative correlation between temperature and ovitrap index in all localities.
Conclusions: This study provides useful data to be adapted in dengue vector management. It is very important to understand the fluctuation of vector population according to the seasonal activity, which can help us to improve our control programs. However, other factors might also contribute to the increment of dengue outbreak such as the number of available breeding sites, behavior of the vector against environmental factors and the cleanliness of the environment.

1. Introduction

The first dengue fever (DF) case in the Peninsular Malaysia was reported in 1902 and the first dengue hemorrhagic fever was reported in 1962 in Penang. Between 1962 and 1964, there were 61 cases with five fatalities, which were confirmed by dengue virus isolation and serology[1]. The cases increased with 67 reported cases in 1965[2]. From November 1962 to July 1963, 41 dengue haemorrhagic fever patients were admitted at General Hospital, Penang[3]. Recently, in 2014, 108 698 dengue cases were notified with 215 deaths with an increment of the 151% cases compared to 43 346 cases reported in 2013 in Malaysia. The increasing number of cases was contributed by the peoples' movement, changes in dengue serotype from dengue virus type 2 to dengue virus type 1, climatic factors and lacking in human awareness about dengue[4].

In Asia, *Aedes albopictus* (*Ae. albopictus*) plays a role as an urban vector to spread the dengue viruses[5]. Generally, *Aedes aegypti* (*Ae. aegypti*) distribution is always correlated with the dengue outbreak following rainy season and the spike in *Ae. albopictus* mosquito population matched with the dengue occurrence[6,7]. *Ae. albopictus*

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originated from the tropical forest in Southeast Asia was established in Malaysia[8,9]. The distribution of *Ae. aegypti* and *Ae. albopictus* in Malaysia was found to be overlaps and have adapted to the urban and suburban conditions[9,10]. *Ae. albopictus* is known as a rural mosquito and usually breeds outdoors in the natural habitats such as tree holes, whereas *Ae. aegypti* is abundant in urbanized and densely populated neighbourhoods. However, in 1970, the mosquitoes have successfully adapted to the urban and semi-urban town around the city[11]. In Penang Island, female *Ae. albopictus* has been found indoors or at domestic sites and currently shifted to colonized inside human dwellings, which can increase the opportunities for taking a blood meal[12].

The climatic factor is an important role in the multiplication of *Aedes* mosquito and the transmission of dengue viruses[13,14]. Evidence showed that dengue epidemics have been associated with the rainfall, temperature and relative humidity (RH)[15-20]. Several forecasts modeling also found the relationship between dengue cases and climatic factors[21-24]. Under favourable climatic condition, the probability of vector human interaction and number of breeding sites for mosquitoes are increased[25]. Temperature, RH and rainfall indirectly influence the land-cover and land-used, which can promote or inhibit the growth of the dengue vector populations[26].

The most effective vector control program is environment management, which includes planning, organizing and monitoring activities for the modification of environmental factors with a view to reduce the vector propagation and human vector-pathogen contact. Ovitrap index (OI) is one of the effective monitoring methods for estimation of mosquito populations. If the index is more than 30%, the vector control activity needs to be activated immediately at the particular area including public health education, community participant, active site surveillance, insecticide fogging and clinical surveillance system[27]. Ovitrap has been proven as an effective, simple and easily adaptable device to monitor the distribution of *Aedes* mosquitoes in term of the number of larvae and eggs that was directly collected from the field[28].

The aims of this study were to estimate the *Aedes* mosquito population by using ovitrap as a monitoring tool and its relation between three climatic factors (temperature, humidity and rainfall) to confirm dengue cases. The current strategy of vector control program of the Department of Health Penang is also discussed to facilitate improvement of the preventive vector programme in the future.

2. Materials and methods

2.1. Study sites

Ovitrap surveillance was conducted in three selected localities [Flat Hamna (FH) (5°21' S, 100°17' E), Kampung Sungai Gelugor (KSG) (5°22' S, 100°18' E) and Kampung Tanjung Tokong (KTT) (5°27' S, 100°18' E)] in the north east district of Penang Island, Malaysia. Selection of these three localities was based on registered dengue cases between 2006 and 2010. All of these localities were contributed to the highest number of dengue cases annually for the north east district of Penang Island. Vector surveillance using ovitrap was conducted for a total of 14 months from January 2010 to February 2011.

2.2. Dengue cases data

The dengue cases data from the every weekly DF/dengue hemorrhagic fever cases, 2010–2011 were obtained from Vector Borne Disease Control, Department of Health, Penang, Malaysia. The weekly data were then converted into cumulative case number starting from January 2010 to February 2011.

2.3. Ovitrap surveillance

The purposive survey technique using ovitrap was used as described from a previous study[27,29]. A total of 60 ovitraps were placed in 30 houses. Two ovitraps were located in each house: (1) inside the house (*i.e.* bedroom, living room, kitchen, garage or bathroom) and (2) outside the house (*i.e.* garden, drain or garbage bin). Houses were chosen randomly. Ovitraps and paddles were collected and replaced weekly with a fresh batch. The paddle made of hardwood with a rough side and smooth surfaces on the other side was used as an oviposition substrate. The rough side was the attachment side for the eggs. Any missing paddle was replaced as required. Ovitraps and paddles were brought back to the laboratory and all of the contents were poured into an enamel pan. Aged tap water was added into the enamel pan and eggs were allowed to hatch. The hatching larvae were counted after 3 days and identified at the 3rd instar larval stage using the entomological charts for teaching provided by the Unit of Medical Entomology, Institute Medical Research, Kuala Lumpur.

2.4. Meteorological parameter

Mean temperature, RH and rainfall data were obtained from the Malaysian Meteorological Department of Bayan Lepas station, which is the nearest station to the selected study areas.

2.5. Statistical analysis

The difference in number of mosquitoes found in three localities was analyzed using One-way ANOVA. The OI was calculated as percentage of the positive ovitrap against the number of the ovitrap in each study site. The correlation coefficient among ultra-low volume activity, OI, number of larvae with the meteorological parameters (temperature, RH and rainfall) and number of dengue cases was also analyzed using the Pearson’s correlation coefficient and significant values were determined. All levels of the significance were determined at $P = 0.05$.

3. Results

3.1. Mosquito surveillance and breeding site preference

A total of 15 803 larvae were collected and *Ae. albopictus* was the most abundant mosquito species in north east district of Penang Island (92.4%) and only 7.6% was *Ae. aegypti*. No other species of mosquitoes were recorded in all inspected ovitrap. From the collected larvae, *Ae. albopictus* was found to be the predominant species in all three localities during 14 months of sampling period in

KSG (97.5%), KTT (91.7%) and FH (91.2%), followed by *Ae. aegypti* in FH (8.8%), KTT (8.3%) and KSG (2.5%). *Ae. albopictus* was the most common species in all three localities, while *Ae. aegypti* was more prevalent in FH, which constituted 17.16 ± 2.17 larvae and was significantly different compared to KSG and KTT (F = 43.609, df = 2, P < 0.05) (Table 1).

Table 1
Comparison of the *Ae. aegypti* and *Ae. albopictus* larvae collected from three localities.

Species	Localities	Mean ± SE
Ae. aegypti	FH	17.16 ± 2.17^{a}
	KSG	1.29 ± 0.33^{b}
	KTT	2.91 ± 0.64^{b}
Ae. albopictus	FH	177.84 ± 24.50^{c}
	KSG	50.75 ± 7.96^{d}
	KTT	32.80 ± 2.88^{d}

Same letter indicated no significant difference; Different letter indicated significant difference at P < 0.05.

All of the three localities showed the distribution pattern of the OI with the range between 8%–77% for 14 months from January 2010 to February 2011 of sampling (Figure 1). Classification of OI was determined: normal routine activities was taken at OI < 10%, house inspection and enforcement of destruction disease bearing insect act (DDBIA), and search and destroy activities were taken at 10% ⩽ OI ⩽ 30% as well full coverage of house inspection and enforcement of DDBIA, while search and destroy activities, preventive space spraying and health promotion education actions were taken at OI ⩾ 30%. All actions were taken by health authority. The OI showed statistically significant difference between three localities (FH, KSG and KTT) as determined by One-way ANOVA [F (2.39) = 47.872, P = 0.000]. However, there was no significant difference between KSG and KTT against the OI (P = 0.192). At FH, increasing pattern of OI was observed for the first 8 months and attained a peak in August 2010. Only slight changes of OI were recorded at KTT during the sampling period. *Ae. albopictus* was found to be the principal mosquito species in outdoor areas for all localities [KSG (73.01% ± 2.43%), KTT (64.23% ± 1.97%) and FH (56.71% ± 1.34%)]. *Ae. aegypti* was also found to breed more in outdoor area compared to indoor area. From the independent sample t-test, it was found that there was significant difference (P < 0.05) between number of mosquito species collected in both breeding sites (outdoor and indoor) for all three localities (Table 2).

Table 2
Comparison of percentage of *Ae. aegypti* and *Ae. albopictus* breeding site in FH, KSG and KTT. %.

Ovitrap placement	Locations	*Ae. aegypti*	*Ae. albopictus*
Indoor	FH	6.38 ± 0.71^{a}	28.59 ± 1.10^{b}
	KSG	0.15 ± 0.15^{a}	19.94 ± 1.59^{b}
	KTT	0.32 ± 0.22^{e}	24.10 ± 1.80^{b}
Outdoor	FH	8.32 ± 0.84^{a}	56.71 ± 1.34^{b}
	KSG	5.11 ± 0.91^{a}	73.01 ± 2.43^{b}
	KTT	11.36 ± 1.19^{a}	64.23 ± 1.97^{b}

Data were expressed as mean ± SE. Means within a column followed by the same letter were not significantly different (P > 0.05).

3.2. The relationship between meteorological data and mosquito population

The average air temperature was approximately between 26 and 29 °C during the period of January 2010 to February 2011. OI in FH (r = -0.653) and KSG (r = -0.670) showed significant negative correlations with the temperature (Table 3). Whereas, only RH showed significant positive correlation with OI in FH. The highest OI was recorded in FH (77.92%) which contributed to the high population of *Aedes* mosquitoes and was found to be correlated with temperature and RH.

Table 3
Pearson correlation analysis result between temperature, RH, rainfall and OI in three selected areas in Penang Island, Malaysia.

Locations	Temperature	Rainfall	RH
FH	-0.653^{*}	0.446	0.726^{**}
KSG	-0.670^{**}	0.141	0.495
KTT	-0.285	-0.384	-0.317

*: Significant at 0.05; **: Significant at 0.01.

3.3. Vector surveillance and registered DF cases

In 2010, FH was recorded the highest registered DF cases with 48 cases compared to other two localities. However, the number of dengue cases decreased in the year 2011 with 79.17% reduction in FH and more than 50% reduction of the registered DF cases in KSG (Figure 2). In FH, the first DF was reported in early February

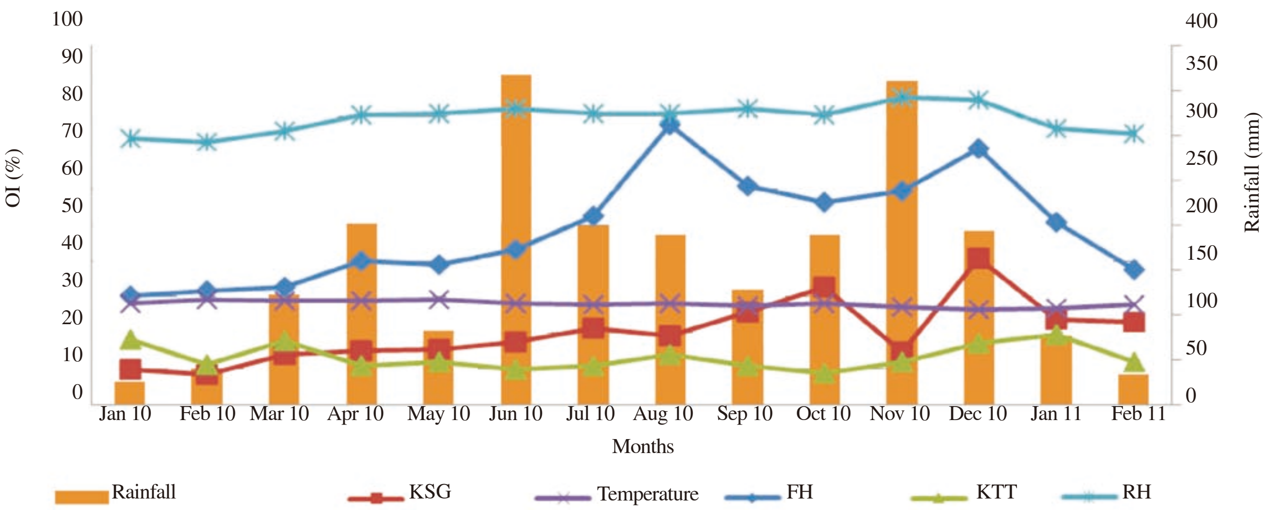

Figure 1. Correlation between OI and physical parameter (rainfall, temperature and RH) in three localities from January 2010 to February 2011.

2010 and the peak of DF cases was reported in August and September 2010 (Figure 3A). This is due to the higher number of *Aedes* mosquitoes found during these two months period. In KSG, only one case was reported in January 2010 and no DF cases were reported afterward until June. However, the DF cases were lingering in July, September and December (Figure 3B). In KTT, only three cases were reported in July throughout the year 2010 (Figure 3C). The correlation between the number of DF cases and OI was found highly significant in FH (r = 0.640) and KSG (r = 0.624). Surprisingly, negative correlation was found in KTT (r = 0.200). Results showed that the DF cases were paralleled with the high OI.

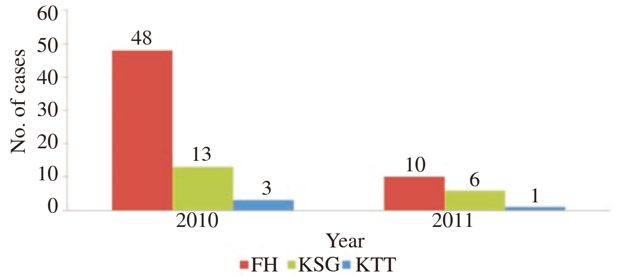

Figure 2. Total number of registered DF cases from three selected localities in Penang Island from 2010 to 2011.

4. Discussion

In the present study, *Aedes* mosquitoes density was monitored using ovitrap surveillance. FH, KSG and KTT were selected as the sampling areas due to high number the *Aedes* larval density and OI was positively correlated with the confirmed dengue cases. Ovitrap is a rapid, non-expensive and sensitive tool for monitoring dengue vector and widely been used in mosquito surveillance in mosquito control program. It can also be used to enhance the information of the vector in a smaller area and has a good predictive power[30]. More mosquitoes can be collected by using ovitrap technique compared to the conventional larval survey[31].

In ovitrap surveillance, we successfully collected 15 803 mosquito larvae comprising of 92.4% *Ae. albopictus* and 7.6% *Ae. aegypti* from indoor and outdoor breeding sites. No other mosquito species was found in this study. This is similar with previous study in Penang Island using ovitrap and other entomological survey which showed high number of *Ae. albopictus* collections[12,31,32]. This species typically displayed outdoor breeding behaviour. However, we found the high frequency of *Ae. albopictus* breeding in indoor containers compared with *Ae. aegypti*. This suggested that *Ae.*

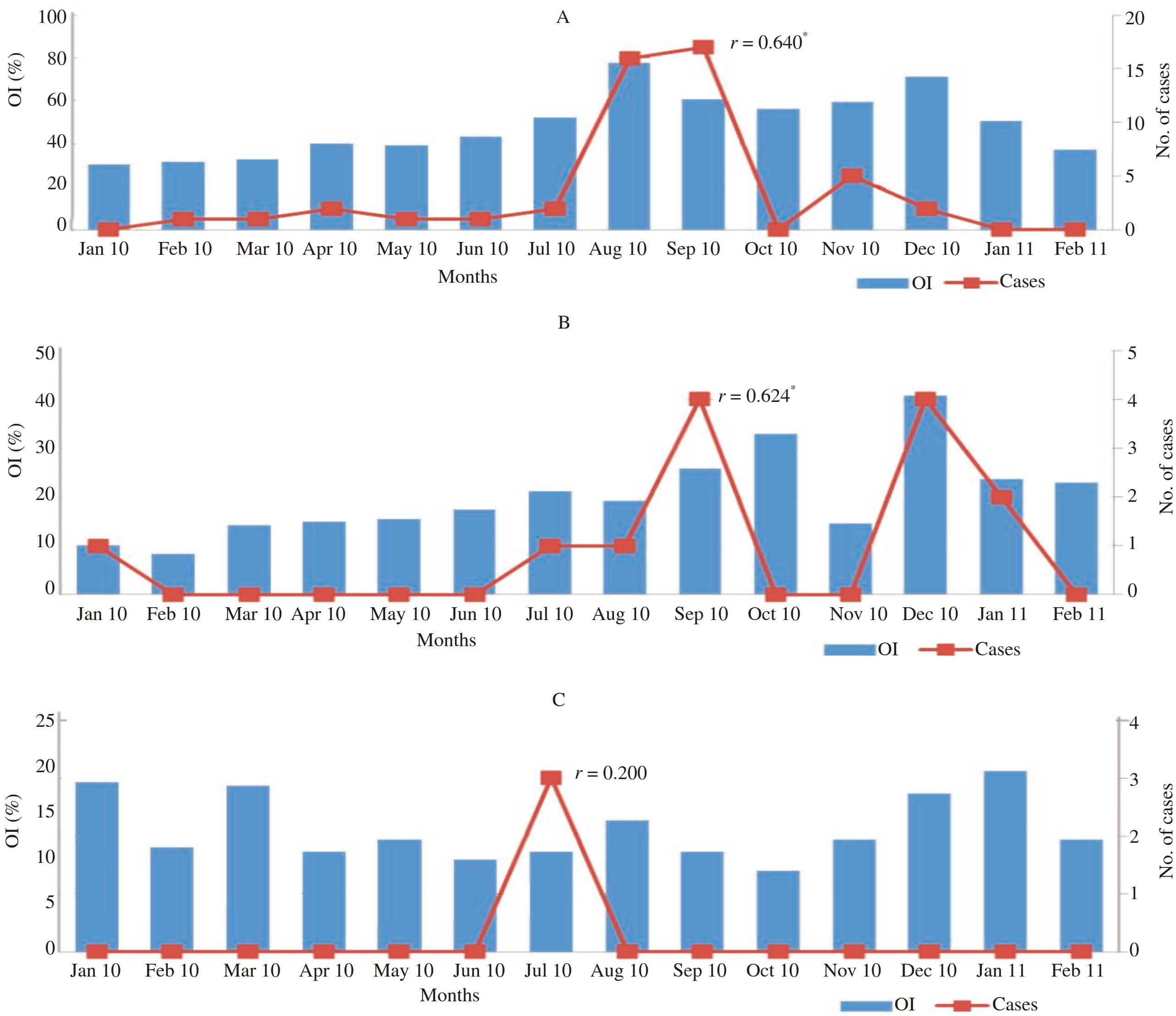

Figure 3. The number of registered DF in relation with the OI.
A: FH; B: KSG; C: KTT.

albopictus species has become an urban species, adapting to new indoor environments, well distributed in urban and forest habitats and producing niche overlap between these two species[33,34]. *Ae. albopictus* showed a high biting activity with a shorter feeding time compared to *Ae. aegypti*. This species also has a larger size, large diapausing eggs production and presents as one of the superior species in term of survival rate[35].

Ae. albopictus is the most distributed outdoor species probably due to the structure of the house. The housing areas consist of an apartment with five levels of floors, built up with brick and cement. The ground floor structure is not well maintained, as it always collects water during rainy season and becomes a major potential breeding site in these areas. This is similarly with other finding in Selangor, which showed that in a clean residential environment, the areas were still infested with a high number of *Ae. aegypti* and *Ae. albopictus* species. The possible breeding habitats are concrete drainage and the structure of the house, other man-made structures such as drain, sand trap and other artificial containers such as empty paint cans, plastic sheet, plastic containers, trays and tin pots[36,37]. This showed that *Aedes* mosquitoes had adapted to some new breeding sites (not only in clear water, but also possibly breeding in turbid water).

Generally, *Aedes* mosquito larvae require clean water, but not clean water is necessary for their development. For example, in septic tank, after the settling of organic matter, a clean water zone is formed on the top layer where large number of *Aedes* larvae can be found[38]. In Central Africa, *Ae. albopictus* was found to breed outdoor despites rich organic matter in the water. This showed the ability of this species to adapt in a new environment due to their behavior and genetic structure, in which this species has been shaped by additional abiotic and biotic factors[39]. In some highly urbanized areas in Thailand, an intensive *Aedes* control campaign has been done to eliminate artificial and natural breeding sites for *Ae. albopictus*[40]. This study also showed that the outdoor containers were more suitable and conducive as breeding sites compared to indoor containers. The *Aedes* preferred to deposit their eggs in an open container rather than covered. In urbanized areas, the changes in the environmental factors became more suitable for the growth and development of *Ae. Albopictus*. The condensed human populations produced more containers that served as larval habitat[41]. Both species can also breed indoors and outdoors which was similar with findings from Mexico and India[42-44].

The absence of *Ae. aegypti* indoor was probably due to more enforcement activity. Also community starts to increase their awareness and takes more time to check and eliminate indoor containers rather than outdoor containers. To survive, *Ae. aegypti* will move from indoor to outdoor containers. Study in Putrajaya found similar result that *Ae. aegypti* was found to breed outdoors along with *Ae. albopictus*[45]. Although, water temperature in outdoor containers was higher than indoor containers, *Ae. aegypti* still preferred to breed in outdoor containers. However, a major decline in *Ae. aegypti* population was reported in association with the high prevalence number of *Ae. albopictus*[46]. Laboratory and field studies found that adult *Ae. albopictus* had higher survival rate than *Ae. aegypti*[47]. This might due to *Ae. albopictus* is more competent in surviving and replacing the primary vector[48].

The transmission of *Aedes* mosquitoes is climate sensitive as the mosquitoes need water to breed. Ambient temperature and RH are critical factors for larval development and affecting feeding behaviors[49,50]. Temperature was found to be correlated with the larval development, egg viability, adult longevity and dispersal[51]. At low RH, adult *Aedes* mosquito collection was high and usually associated with a lower temperature[52]. The temperature affected the mortality and hatching rate of *Aedes* mosquitoes[53,54]. Study in Texas showed that low temperature (15–27 °C) and high RH (55%–75%) caused the increase of egg hatching percentages, but at 32 and 35 °C, the hatchability rate were decreased[55]. However, the RH alone did not effect *Aedes* mosquitoes activity[56].

In Malaysia, the abundance of *Ae. albopictus* has always been correlated with rainfall[57-59]. Rainfall is one of the most important factors contributing to the density of *Aedes* mosquito populations and dengue transmission[51,60-63]. The wet season is usually associated with high number of *Aedes* mosquitoes. Rain fills artificial containers and creates natural breeding sites, especially in housing areas (*e.g.* vases and tires)[64]. Commonly during the wet season, the number of dengue cases is high compared to the dry season due to the number of breeding sites increased and influenced the increasing number of DF incidence. However, during heavy rains, the excessive rain will flush larvae out from the containers and decreased DF is often observed[65]. The effect of rainfall on dengue prevalence is a very important study to forecast variation in incidence and risk related to the impact of changes in climatic variations[66].

This study can confirm the possible influence on rainfall which indicated that the peak prevalence happened during heavy rainfall. The analysis of the seasonal pattern of OI in the study areas indicated that high density occurred during August 2010 to September 2010. This could be contributed by the fasting month. During this period, all of the localities were occupied with the night market (Bazaar Ramadhan) nearby which sold food and drinks. In these localities, the usage of plastic bags and polystyrene containers by the public increased during both months. Irresponsible publics undiscriminating discarded polystyrene container, plastic containers and other containers after use and created breeding sites for *Aedes* mosquitoes when fill in with water from rain[43,67]. The massive amount of plastics used globally created innumerable non-biodegradable ideal habitats for mosquitoes to breed[68,69]. This can be used as an alternative indicator for the density of vector, serving extra supplement to access the risk of dengue in the particular areas[70].

The statistical analysis was performed between mosquito density (OI) and DF. The mosquito density showed a positive correlation with dengue cases reported from FH and KSG. Various of studies have investigated the relationship between dengue transmission and *Aedes* population[64,71]. Recently, study in Sisaket, Thailand showed that the high larval indices were correlated with the prevalence of human dengue infections which was associated with the rainy season[72]. This may produce a good warning system to the health authorities and local communities. However, there is no clear indication of consistent association between dengue cases and vector indices. Most of the study showed weak study design, lack of basic knowledge of epidemiology and virus transmission. It is suggested to quantify the relationship between vector population, dengue transmission and clinical database which carefully match temporally and spatially[62].

Based on the guidelines by Ministry Health of Malaysia, the OI is classified into three categories with different strategies and actions will be applied for each level of OI. For *Aedes* mosquito survey, search and destruction, thermal fogging and ultra-low volume will be mobilized, once the index reaches or exceeds 30%. The epidemiologist and entomologist will review and decide on whether the OI = 30% is reasonable for control activities according to the current situation. However, in some problematic areas with history of high DF cases, OI of 15% is considered the threshold of control activities. In our cases, FH is one of the localities which needs more attention for *Aedes* control effort base on previous high reported DF cases and high number of *Aedes* population found in that area.

During the 2010 dengue outbreak in Penang, integrated vector strategies were applied in all of outbreak localities in north east district. The implementation of control strategies is by using simple strategies such as source reduction, combination use of *Bacillus thuringiensis* and fogging, ovitrap monitoring, health education, communication for behavioral impact activities and the enforcement of DDBIA. Inspection in all public centers, school, shopping complex, recreational theme park and markets was regularly conducted. A cleanup campaign organized by community leader together with the residence, public health workers, children and local administration authorities of Penang was held regularly to maintain the cleanliness around the communities. Garbage collection, drainage and canal maintenance by the local authority was also implemented.

Although the effectiveness of the current strategies in dengue vector control is still unclear, the number of dengue cases reported in 2011 decreased 77% compared to 2010. It is very difficult to evaluate the effectiveness of vector control activities since we have started the integrated vector management. Surveillance, source reduction, biological control agents, insecticide application, public education and awareness, and enforcement have been implemented in all of the outbreak areas in north east district till date. Source reduction activities conducting by health worker, community and other agencies are the most important activity to reduce mosquito habitats in the targeted areas[73,74]. Integrated vector management is still the most effective method to control dengue[75].

This study provided useful data for dengue control management. The relationship between dengue vector density and the meteorological data is responsible for the decision support in deciding the best method to be used for dengue vector management in Northeast Penang, Malaysia. It is also important for us to evaluate and understand the vector populations according to the seasonal activity, which can help to improve the effectiveness of our control program. However, other factors that might also contribute to the increment of dengue outbreak such as the number of available breeding sites, behavior of the vector against environmental factors and the cleanliness of the environment need to be considered.

Conflict of interest statement

We declare that we have no conflict of interest.

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