Tropical Biomedicine 24(1): 83–94 (2007)

Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang, Malaysia

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Received on 29 December 2006; received in revised form 5 April 2007; accepted 8 April 2007

Abstract. Ovitrap surveillance was conducted in a selected urban area and suburban area, ie. Taman Permai Indah(TPI) and Kampung Pasir Gebu (KPG) in Penang for 14 months. It was found that *Aedes albopictus* was the most abundant *Aedes* species in both study areas, even though a small percentage of *Aedes aegypti* and *Culex quinquefasciatus* were found to breed simultaneously in the same ovitrap. This study indicated that the main dengue vector was *Ae. albopictus*. A strong correlation was found between rainfall and egg population in both of the study sites (r= 0.982 and r=0.918).

INTRODUCTION

Mosquito-borne diseases such as dengue fever (DF) and dengue hemorrhagic fever (DHF) are the most important arthropod-borne viral diseases of public health significance. Their geographical spread is increasing: only 5 countries documented dengue in the 1950's but to date there are more than 100 countries reporting the incidence of DF and DHF (Guha –Sapi & Schimmer, 2005). Several important factors have influenced the epidemiology of dengue.

Aedes (Stegomyia) albopictus (Skuse), known as the Asian tiger mosquito and *Aedes (Stegomyia) aegypti* (Linnaeus), mosquitoes are the principal dengue vectors and to date have become the main vectors in the transmission of dengue and dengue haemorrhagic fever in the tropical and subtropical regions (Smith, 1956; Rudnick *et al*. 1965; Hammond, 1966; Knudsen, 1995). The distribution of *Ae. aegypti* and *Ae. albopictus* in peninsular Malaysia has been well established (Lee, 1990). In Malaysia, the first reported DHF cases was in Penang in 1962 (Rudnick *et al*., 1965) while classical dengue fever was first reported in 1901-1902 in Penang by Skae (1902). Major outbreaks were reported in 1974, 1978, 1982, 1990 and 1995 (Lam, 1993; Poovaneswari, 1993; Farizah *et al*., 2003). Since then, the disease has become endemic throughout the country (Singh, 2000). Up until November 2005, there were 3098 cases reported in Penang, with 7 deaths (MOH, 2005). In the last decade, cases of dengue have become more severe (Farizah *et al*., 2003). The infection is predominant in urban areas where 61.8% of the total population lives and the rapid industrial and economic development created many man made opportunities for *Aedes* mosquito breeding (Teng & Singh, 2001).

Aedes albopictus is indigenous in tropical Asia but presently the distribution is world wide. The abundance of dengue is closely associated with the abundance of the vectors. It was also reported that the abundance of vectors was associated with the environmental factors such as the rainfall, temperature and relative humidity (Okogun *et al*., 2003), while the wet seasons are associated with the higher prevalence of mosquito borne diseases. This study was conducted in two dengue prone areas in Penang. The main objective of this study was to determine the density, distribution and other physical parameters relating to the fluctuations of *Ae. albopictus*.

MATERIALS AND METHODS

Study sites

A general survey of Penang state was carried out before the study was conducted. Two areas comprising a suburban and an urban site were selected for this study. The suburban site was Kampung Pasir Gebu and the urban site was Taman Permai Indah. The choice of the sites were based on the relative abundance of mosquito species, the location, (close to the laboratory of VCRU, USM) and the record of high incidence of dengue cases in the year of 2002-2003.

Ovitraps

Continuous ovitrap surveillance was conducted biweekly between March 2003 until March 2004 for a period of 56 weeks in both study sites. The ovitrap was a simple device consisting of a black painted milk can, filled with 250ml tap water and a hole each side of tin, to avoid overflow of water during heavy rain (Yap & Thiruvengadam, 1979). An oviposition paddle with two different substrates made from hardboard (2cm x 12.5cm x 0.3czm) was suspended vertically in the ovitrap to provide a suitable surface for oviposition.

One hundred ovitraps were placed randomly outdoors confined to the immediate vicinity of the houses (Lee, 1992). All the ovitraps were collected after 5 days and replaced with fresh ovitraps and paddles on the following week. The paddles placed in a clear plastic bag were brought back to the laboratory. All collected ovitraps from the sites were brought back to the laboratory and their contents were poured into plastic trays. Tap water was added into the trays and all larvae collected were allowed to grow to adults in the laboratory. The paddles were left to dry under room temperature for at least 24 hours before the eggs on the paddles were counted under a dissecting microscope as described by Hornby *et al.* (1994).

All larvae present in the ovitraps were identified and counted. *Aedes* larvae that hatched from the eggs were identified at the third or fourth instar stage. Any larvae that could not be identified during the larval stages were allowed to grow to adults and the adults were identified.

The following guidelines served as the basis for the selection of locations for ovitraps (Jacob & Bevier, 1969; Evans & Bevier, 1969):

i. The traps are to be located near other potential breeding containers except tyres as the tyres are black and often contain water, therefore ovitraps do not compete well,

ii. The traps are to be located in partial or total shade, not under direct sunlight and also at suitable resting or breeding areas,

iii. The traps are to be placed at ground level, avoiding disturbance by children, and

iv. The traps are to be located at the rear or sides of premises than in front of the yard near the street.

Using the standard rearing methods the mosquitoes were reared under laboratory conditions at 25 ± °C and 65±20% RH. During the study period, all the data on total rainfall, mean temperature and mean relative humidity were obtained from the Malaysian Meteorological Services for Bayan Lepas and Butterworth which covered the study sites.

Data analysis

To evaluate the distribution and the abundance of the species in the study area, several parameters were considered:

i) The ovitrap index (OI) for both locations:

$$\text{Ovitrap Index (OI)} = \frac{\text{Number of ovitraps positive}}{\text{Total number of ovitraps examined}} \times 100\%$$

ii) The mean number of eggs in the ovitraps:

$$\text{Mean number of eggs} = \frac{\text{Total number of eggs in ovitraps}}{\text{Total number ovitraps examined}}$$

iii) The correlation between OI and mean number of eggs

iv) The correlation between OI and mean number of eggs with the meteorological parameters (mean temperature, rainfall and relative humidity)

v) The percentage and ratio of adults produced from the eggs, and

vi) The sex ratio of adults emerged.

To evaluate the relationship between the eggs collected and the factors investigated (mean temperature, rainfall and relative humidity), the correlation among experimental variables was evaluated using the Pearson's correlation coefficient (r) and its significance was determined. All statistical tests were considered significant at the a=0.05 probability level. Data was analysed using SPSS version 11.5.

RESULTS

Both locations showed fluctuation patterns of ovitrap indices with a range of 66-99% (Figure 1). This implicated that both areas have a high *Ae. albopictus* population. Figure 1 describes the distribution of *Ae. albopictus* and other mosquitoes present in the ovitraps. Only 6% to 15% of the ovitraps contained other species which were *Cx. quinquefasciatus* and *Ae. aegypti*. *Ae. albopictus* was found in 84.6% and 93.7% of positive ovitraps in Taman Permai Indah and Kg Pasir Gebu, respectively. The mix population of *Ae.aegypti* and *Cx. quinquefasciatus* in Taman Permai Indah and Kg Pasir Gebu was 15.4% and 6.3%, respectively (Figure 2 A, B).

Egg- abundance

Figure 3 described the mean number of eggs collected for both study area for 14 months of ovitraps surveillance.

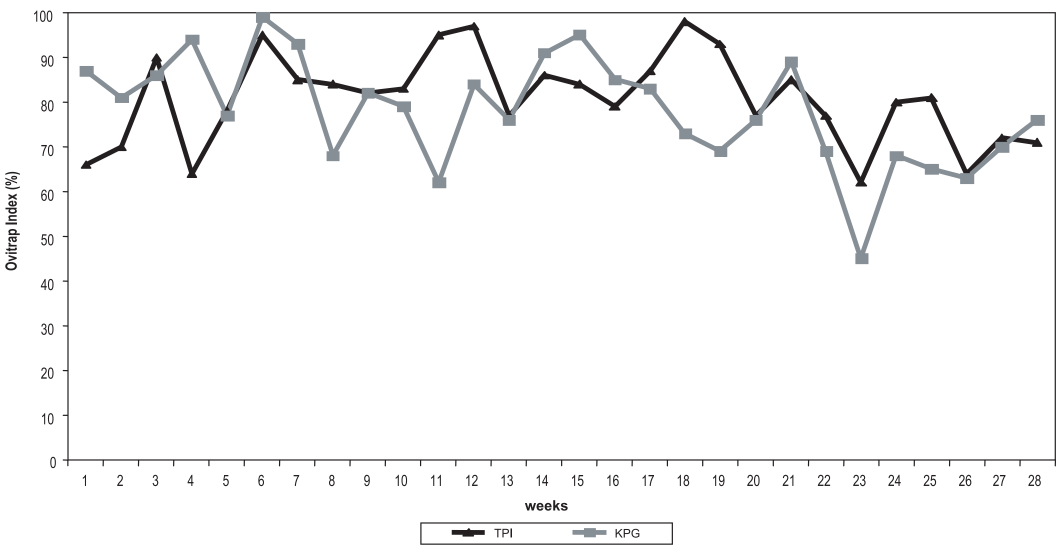

Figure 1. Ovitrap indices in Taman Permai Indah and Kg Pasir Gebu for 14 months of sampling.

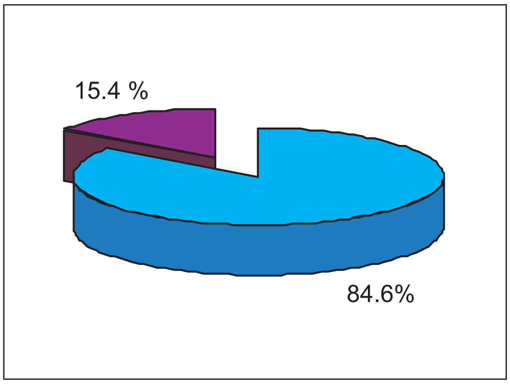

Figure 2(A). The distribution of *Aedes albopictus* and other mosquitoes present in the ovitraps in Taman Permai Indah.

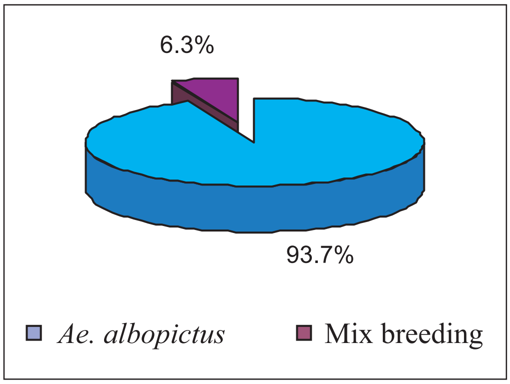

Figure 2(B). The distribution of *Aedes albopictus* and other mosquitoes present in the ovitraps in Kampung Pasir Gebu.

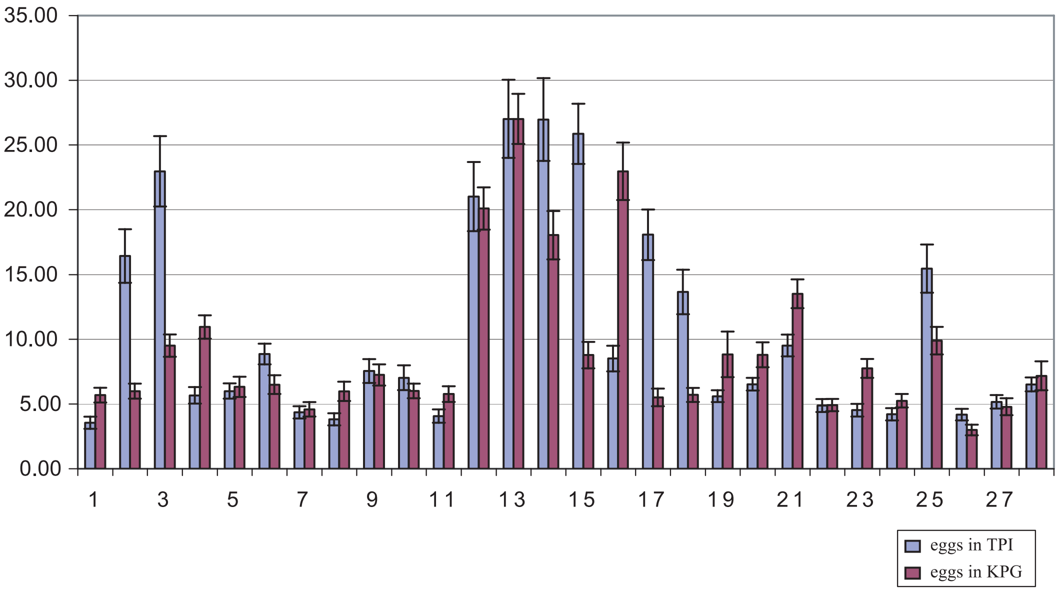

Figure 3. Mean number of the total eggs collected in both study areas from 14 months of sampling.

Taman Permai Indah (TPI)

A total of 29 805 eggs were collected from 14 months of ovitrap-sampling. The highest amount of eggs recorded was on the 13th week yielding 2702 eggs, whereas the lowest was on the 1st week yielding 355 eggs. The amount of eggs seemed to be moderate during the high and low rainfall. However, from the independent sample t-test, it was found that there is a significant difference ($p<0.05$) between the mean number of eggs collected during the high and low rainfall seasons. For the ovitrap index, no significant difference was found both during high and low rainfall seasons ($p>0.05$).

Kampung Pasir Gebu, Penaga

A total of 25 665 *Ae. albopictus* eggs were collected from this study site. On the 13th week, the maximum number of eggs (2702) recorded, whereas in the 26th week, a minimum of 300 eggs were recorded. From the first week to the tenth week, there was fluctuation with an irregular increase from 11th week to 15th week. An irregular decrease and increase were recorded in the following weeks. The ovitrap index and mean number of eggs collected were found to be significantly different (independent sample t test, $p<0.05$).

Correlations

(a) Taman Permai Indah

The ovitrap index (OI) showed a significant correlation with the mean number of eggs (r=0.374), lag 1 and lag 2 rainfall (r=0.377, r=0.506). Figure 4 described the correlation between ovitrap index and mean number of eggs collected in Taman Permai Indah. The mean number of eggs produced have also been correlated with the amount of rain at study area, temperature and percentage of environmental relative humidity using the Pearson Correlation test at 0.05 significance level. Figure 5 described the correlation between ovitrap index and rainfall in Taman Permai Indah. The correlation between mean number of eggs was highly significant with the lag 1 rainfall and lag 2 rainfall, which are r= 0.982 and r=0.771, respectively. (Figure 6: Correlation between mean number of eggs and rainfall in Taman Permai Indah). Meanwhile, a significant negative correlation between the mean number of eggs and the mean temperature was also recorded (r=-0.374). The mean number of eggs and relative humidity were also found to be significantly correlated (r= 0.477).

(b) Kampung Pasir Gebu, Penaga

The correlation between the mean number of eggs collected from the field and rainfall at the site was found to be highly significant (r=0.918) (Fig. 7). However, no significant correlation was found between mean temperature or relative humidity and mean number of eggs collected. Besides, Kampung Pasir Gebu also showed a significant correlation between the ovitrap and the rainfall at the study area (r=0.387) (Fig. 8).

Total larvae hatched

A total of 28 714 mosquito larvae were produced from 29 805 (96.34%) eggs collected in Taman Permai Indah, whereas from the total of 25 665 eggs collected in Pasir Gebu, 22 301 (86.89%) hatched to larvae stage. This indicated that high

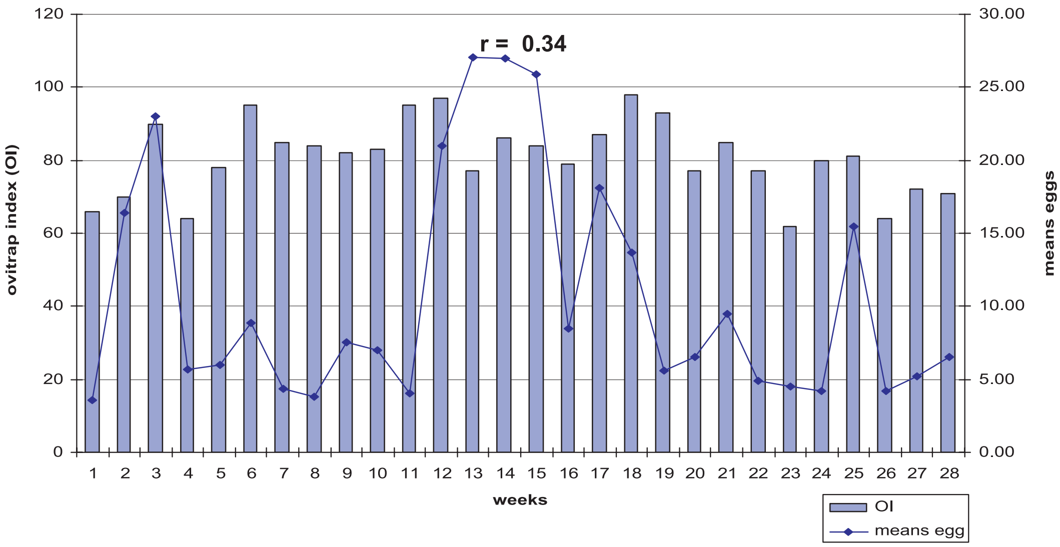

Figure 4. Correlation between ovitrap index and mean number of eggs collected in Taman Permai Indah.

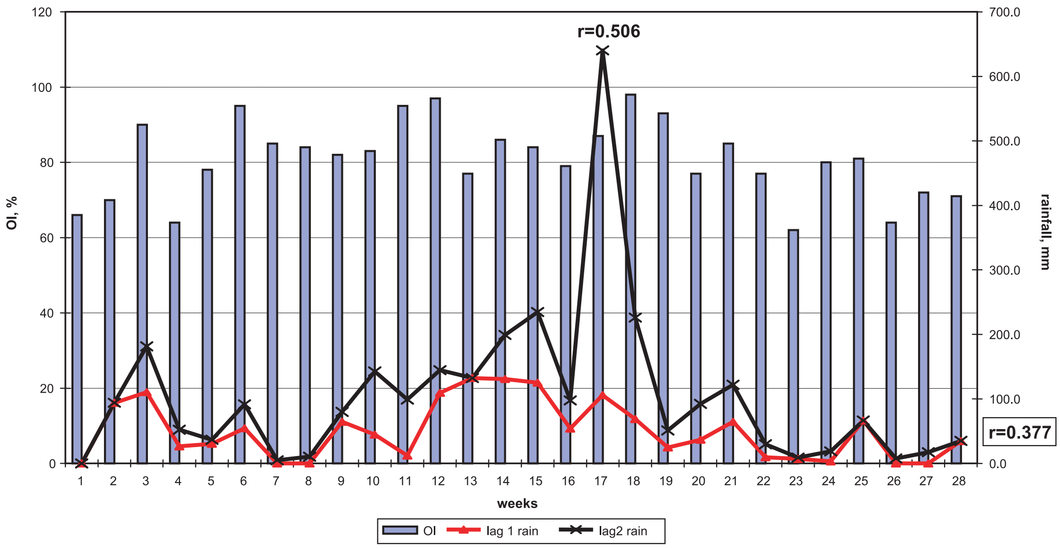

Figure 5. Correlation between ovitrap index and rainfall in Taman Permai Indah.

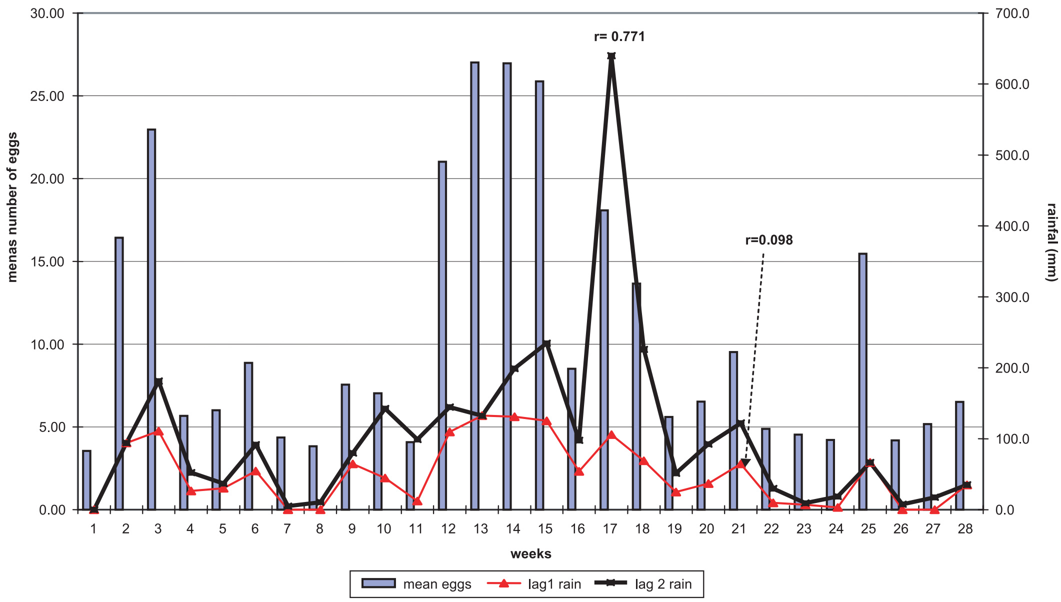

Figure 6. Correlation between mean number of eggs and rainfall in Taman Permai Indah.

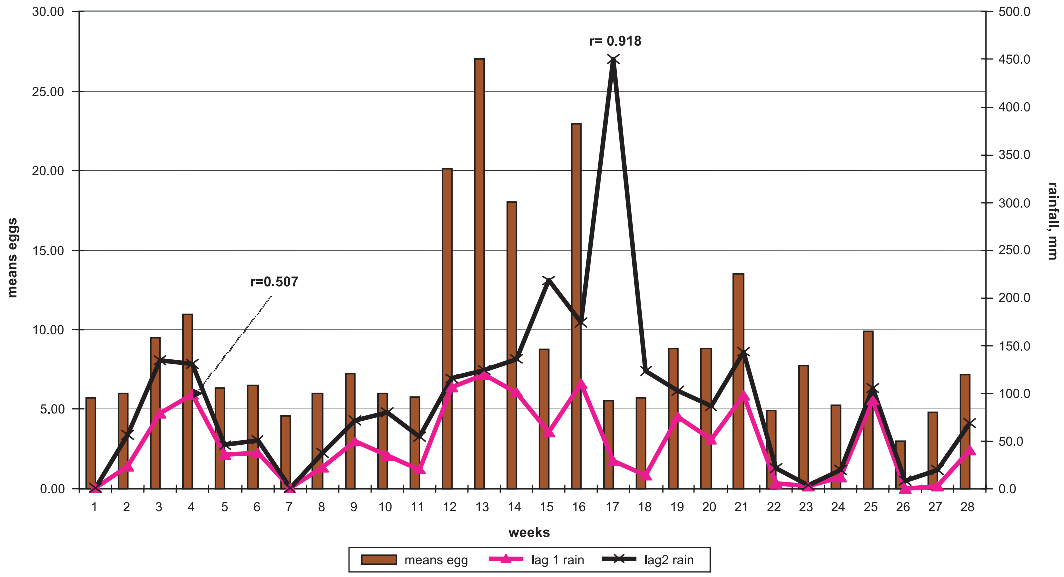

Figure 7. Correlation between mean number of eggs and rainfall in Kampung Pasir Gebu.

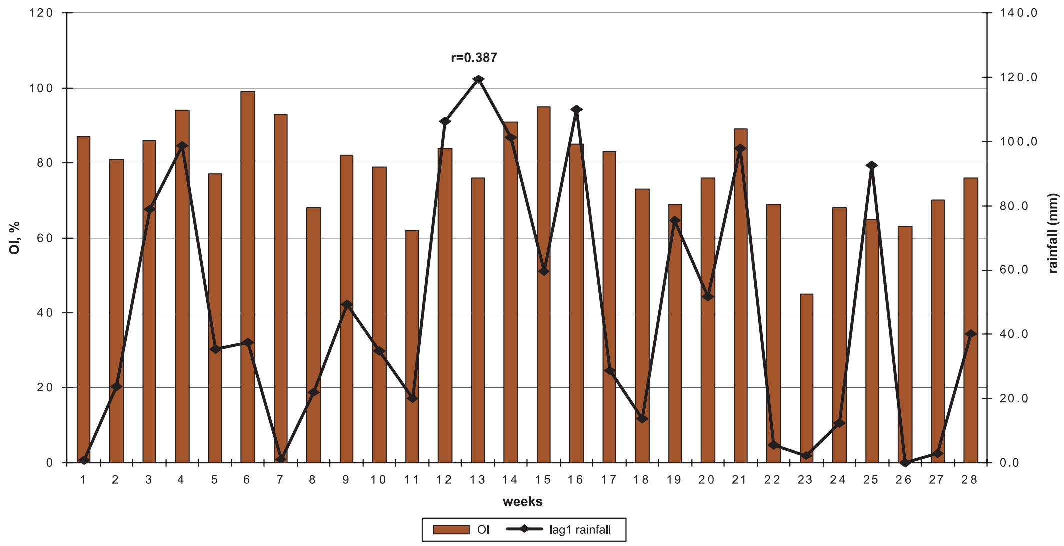

Figure 8. Correlation between ovitrap index and rainfall in Kampung Pasir Gebu.

number of eggs can be hatched and reared to the larval stages. All the larvae hatched in the laboratory were *Ae. albopictus*. From this total, the sex ratio (male: female) of adults that emerged from the larvae was close to one is to one (Table 1). These findings indicated that *Ae. albopictus* could survive well in both study areas.

Table 1. Total no. eggs collected and the total no.of larvae produced including the sex ratio of adults produced from the eggs collected at both study sites

Locality	No. of Eggs	No.of Adults emerged	Male: Female
* TPI	29 805	28 714 (96.34%)	0.93 ± 0.33
* KPG	25 665	22 301 (86.89%)	0.97 ± 0.42

* TPI = Taman Permai Indah
* KPG= Kampung Pasir Gebu

DISCUSSIONS

The ovitraps were likely to be mostly colonized by *Ae. albopictus* since this species is mainly a container breeder. This finding is similar to the study by Rawlins *et al.* (1998) where, *Ae. albopictus* was found associated with numerous container-inhabiting mosquitoes such as *Aedes*, *Culex*, *Toxorhynchites* and *Armigeres*. However, in general it is the most abundant species present in shared habitats, it often occurs alone. In fact, *Culex quinquefasciatus* is also found to be attracted to oviposit in ovitraps. *Aedes aegypti* is strictly domilicary, preferring less vegetation, biting indoors and primarily found indoors, while *Ae. albopictus* is found commonly outdoors and breeds in all types of natural containers (Sucharit *et al.*, 1978: Foo *et al.*, 1985;). One reason why we obtained more *Ae. albopictus* than *Ae. aegypti* may be due to the fact that the ovitrap surveillance was done only outdoors.

Dengue is a disease associated with areas where breeding of *Aedes* mosquitoes is most prevalent such as in slum areas (Chan & Counsilman, 1985). However, in this study *Aedes* was not only associated with residential areas, but also associated with numerous natural and artificial containers which provided good habitats for breeding. Even in the urban area, which generally has a cleaner environment with piped water supply, the residents store water in containers outdoor for the purpose of watering their plants and other activities. Furthermore, the concrete drainage (in Taman Permai Indah) with clear stagnant water can also become a potential habitat for the breeding of *Aedes*. This finding is supported by the study by Lee in 1990, which showed that *Aedes* requires clear but not necessarily clean water. According to Chen *et al.* (2005) in their study of dengue vector surveillance in urban and settlement areas in Selangor, even in clean residential environment with no water storage, the area still had high *Ae. aegypti* and *Ae. albopictus* populations and the only possible breeding habitat was the concrete drainage system outside the houses. The drains had clear stagnant water with fallen tree leaves and other debris. Therefore, the drains served as a good artificial breeding habitats for *Ae. aegypti* and *Ae. albopictus* (Chen *et al.* 2005).

The study on biology and ecology of the eggs gave much valuable information. Egg survey is particularly useful with species which remain in the egg state for many months. Potential larval habitats can be recognized and enumerated without waiting for the larvae to appear. Ovitraps which trapped eggs to reflect adult density are very useful tools for the surveillance of *Aedes* vector. Although the use of ovitraps is a method that is operationally viable, if an ovitrap remains in the field for more than a maximum of seven days, it becomes a potential breeding site by itself (Santos *et al.*, 2003). Therefore, any ovitrap monitoring should be done within an interval of less than seven days in the field.

Rainfall is an important factor which regulates the abundance of outdoor breeding mosquito populations. The wet seasons are associated with higher prevalence levels of mosquito diseases

(Okogun *et al.* 2003). The onset of rainfall supports the development of additional mosquito breeding sites, hatching of eggs following oviposition, high relative humidity (Igbinosa, 1989), and growth of vegetation cover and cool shaded environment for the development of the aquatic stages and the recruitment of young adults and their survivors (Evan, 1938). In this study, total number of eggs were most abundant in the wet season (high rainfall). High peak of egg population was observed during 1 week after the rainfall peaks. Rain supplied the water for *Aedes* breeding purpose in the artificial or natural containers. Rainfall was either correlated or uncorrelated to insects. As a dengue vector, *Ae. albopictus* population has always been related with rainfall in Asia including Malaysia (Lo & Narimah, 1984). However, heavy rainfall might give a negative impact on the number of larvae or eggs due to excess water from ovitraps (Foo *et al.*, 1985) that flushed out the immature stages thus preventing any oviposition. Heavy rain accompanied by strong winds might disturb the flight activity of *Ae. albopictus* females, resulting in difficulties to find hosts and suitable breeding sites. According to Hornby *et al.* (1994), in a study in the United State, the egg population kept increasing at the beginning but decreased at the end of the wet seasons due to the occurrence of heavy rain. However, the run-off hole at the sides of the ovitraps lessen the effect of heavy flushing, therefore the decrease of eggs in our sample was not too obvious in this study.

The seasonal changes in oviposition also are a consequence of seasonal changing in weather conditions, and the availability of sites for laying eggs. The increase in *Ae. albopictus* might be the result of greater female activity, due to an increase in the temperature and lower relative humidity during this month. High survival rate of immature stages at the beginning of the dry season led to a rise in the number of emergent adults.

A higher ovitrap index containing *Aedes* in the low rainfall season than in high rainfall season might be a consequence of the relative attraction to the ovitrap due to the scarcity of other suitable artificial containers near the ovitrap. However the egg population remained low in the dry seasons, increasing at the beginning and decreasing at the end of the raining seasons. It was also reported that females of *Ae. aegypti* lay a few eggs in several containers, a behaviour described as 'skip oviposition' (Chadee et al.,1993, Corbet & Chadee, 1993; Reiter *et al.*, 1995; Colton *et al.*, 2003). The possible reason for the lower egg values recorded may be that the females lay a smaller number of eggs but in more containers like ovitraps that provide a good habitat for them to lay their eggs. When the water temperature rises, the larvae take shorter time to mature (Rueda *et al.*, 1990) and consequently there is a greater capacity to produce more offspring during this season and adult females mosquitoes digest blood faster and feed more frequently in warmer climates, thus increasing the oviposition activity (Gillies, 1953). However without any rain, there is no source for breeding. When rain is abundant, mosquitoes can lay eggs continuously and with ideal temperature a cycle can be completed within one week.

Many researchers have reported in their studies, especially in Southeast Asian countries including Myanmar (Khai Ming *et al.*, 1974), Thailand (Watts *et al.*, 1987; Thongrungkiat *et al.*, 2003) and Malaysia (Sulaiman *et al.*, 1991), that the seasonal patterns of outbreaks of dengue, coincides with the rainy season. In the study by Foo *et al.* (1985) in Selangor, it was found that the monthly incidence of dengue for the period 1973-1982, was associated with the monthly rainfall during the first wet season, and concluded that the increase of the cases was correlated to the increase of vector abundance. Since Malaysia is a tropical country, the abundance of any species depends more upon the availability of breeding places than the season. The ovitraps set in the study areas are the breeding habitats for the mosquitoes. Therefore, there is not much variability in the number of eggs collected since the breeding source has existed throughout the sampling period.

Eggs survival may also depend on a combination of density dependent and density independent selective pressures (Estrada-Franco & Craig, 1995). Factors that may promote egg loss include desiccation, predation, and freezing (Estrada-Franco & Craig, 1995). The number of *Ae. albopictus* eggs that survive low humidity appears to depend upon the development stage of the embryos before they are exposed to dry conditions (Estrada – Franco & Craig, 1995). In a study by Gubler (1970), it was found that *Ae. albopictus* eggs were highly resistant to dry conditions if they were kept under humid conditions for four days before being exposed to drought. Therefore in this study, the eggs were allowed to dry at high relative humidity (± 80% RH) to avoid embryo development being disturbed. On the filter paper, the eggs were also been kept in a moist condition. As a result, the hatching rate of the eggs was very high.

With efficient ovitrap surveillance, *Ae. albopictus* distribution and abundance can be studied but it is more difficult to control because of the wide range of breeding habitats. The seasonal fluctuation of *Ae. albopictus* abundance described in this study may allow the implementation of control measures. During the wet or dry seasons (high or low rainfall), elimination of containers would help to reduce vector population (mainly eggs and immature stages) (Reiter *et al.*, 1995; Edman *et al.*, 1998). This would help to save time and money in control programme. However, continuous monitoring should be done by local authorities in order to prevent the increase of *Ae. albopictus* population since the abundance of vector is associated with outbreak. The same concept can be applied in order to control other mosquitoes than *Aedes*. With public participation, breeding sites can be minimized by eliminations of containers, bush clearing around houses, tree holes filling and drainage clearing. By understanding the mosquito fauna available at the area, one may understand more about the diseases associated with vector mosquitoes which may be helpful for a successful control program. More than that, early precaution or warning can be provided, thus making the public more aware of the control measures that can be applied not only by the local authorities but also by themselves.

Acknowledgements. The authors would like to thank the Director of the Institute for Medical Research, Kuala Lumpur for giving permission to publish this paper, the VCRU staff for being so helpful throughout the study and the Coordinator of Vector Control School of Biological Sciences, USM.

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