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# Contribution of public places in proliferation of dengue vectors in Penang Island, Malaysia

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ABSTRACT

**Objective:** To determine abundance, distribution and diversity of potential breeding container habitats of the dengue vectors in public places including schools, restaurants, mosques and parks in southwest areas of Penang Island, Malaysia.

**Methods:** Premises at restaurants, schools, parks and mosques were surveyed simultaneously and inspected visually for container habitats and production of immature mosquitoes from March 2015 to March 2016. Abundance (mean ± SE) of breeding containers between sites was compared using One-way ANOVA. Independent sample *t*-test was used to compare total number of *Aedes albopictus* (*Ae. albopictus*) and *Aedes aegypti* (*Ae. aegypti*) surveyed.

**Results:** The surveyed locations yielded a total of 3741 breeding containers and 19537 immature mosquitoes from four areas. Concurrent artificial and natural containers produced 78.4% immature *Ae. albopictus* and 6.3% *Ae. aegypti* mosquitoes in wet season, with 14.2% *Ae. albopictus* and 1.1% *Ae. aegypti* mosquitoes in dry season. Artificial containers accounted for 98.1% of the total containers recorded, with restaurants being the most productive locations (8012) and schools being the least productive (2234).

**Conclusions:** It was concluded that public places are good sources of potential container habitats of *Aedes* mosquitoes in Penang Island, Malaysia and *Ae. albopictus* has exclusively replaced the home-grown *Ae. aegypti* even in urban areas. Therefore, treatment of artificial containers in such locations is critical in *Aedes* mosquito control campaigns during dengue outbreaks.

## 1. Introduction

Dynamism in breeding containers of residential areas is comparably less and numerous studies have been conducted in residential areas, neglecting special units like school, restaurants, mosques and parks despite their potentials in providing good shelter for dengue vectors. Containers that produce excessive numbers of *Aedes aegypti* (*Ae. aegypti*) are termed key containers [1,2]. Traditionally, campaigns for dengue control target artificial water holding containers, *e.g.*, discarded tires, plant pot bases, rainwater tanks and domestic rubbish as well as natural containers [3], and subterranean sites, *e.g.*, wells, mine shafts and service pits [4]. During construction activities in urban areas in Penang, contrasting habitats have been found related to abundance of immature *Aedes* [5]. Eighty percent (24/30) area of the southwest district of Penang Island has been recognized as dengue hotspot. Natural containers or outdoor man-made habitats with great amount of organic debris are more likely prepared by *Aedes albopictus* (*Ae. albopictus*) [6]. *Ae. albopictus* has been found typically inhabiting natural and artificial containers [7].

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Strong positive relationship has been reported between increasing container diameter, container volume, and water surface area with egg numbers over both high and low dengue transmission seasons [8]. *Ae. aegypti* females prefer to oviposit in cups containing cigarette buds over those with water only [7]. In urban areas, *Ae. albopictus* and *Culex pipiens* mainly oviposit and develop in water-holding containers such as bird baths, buckets and trash receptacles [9]. Larvae of *Aedes* mosquitoes required clear but not always clean water to grow and develop [10–13]. *Ae. albopictus* has been found restricting *Ae. aegypti* in breeding containers [14–16]. Out of 65 potential breeding containers identified indoor and outdoor, 86.9% were *Ae. albopictus* and no *Ae. aegypti* was found in either of the containers [17]. Out of 1 873 and 1 807 breeding containers observed, 5.7% and 7.1% were found positive for *Ae. albopictus*, respectively with the highest breeding preference ratio for discarded tires [18]. Therefore, the aim of this study was to determine abundance, distribution and diversity of potential breeding container habitats in schools, restaurants, mosques and parks in southwest areas of Penang Island, Malaysia.

## 2. Materials and methods

### 2.1. Study sites

The study was conducted in southwest district of Penang Island. The climate of the district (5°19′ N, 100°13′ E; population = 196 195; area = 176 $km^2$) is tropical; the wet months (April–December) are hot and humid with characteristic heavy rainfall, while the dry months (January–March) are comparably cooler and moderately humid with reduced or no rain. Non-residential locations (schools, restaurants, mosques and parks) at Batu Maung, Gelugor, Sungai Nibong and Sungai Ara were sampled from March 2015 to March 2016 on a bi-weekly basis. Both localities are the most important urban and rural dengue foci in Pinang Island, Malaysia.

### 2.2. Sampling methods

Initial school, restaurant, mosque and park were selected randomly; subsequent locations within 500–1 000 m at each area were logically frequent and sampled if permitted. The premises in the locations were surveyed simultaneously and inspected visually for water-holding containers. Confirmation of breeding container and collection of immature mosquitoes were done by dipping, using pipette or dipper [19], depending on location and container size [20,21]. Each mosquito breeding container was characterized, thoroughly sampled and recorded. Representative samples of larvae and pupae were collected and returned to laboratory in a plastic bag (205 mm × 133 mm) for further identification; and the contents were poured into enamel pans (850 cm diameter) filled with 500 mL of deionized water and fed with dried yeast powder and larval food (1:1 ratio). The water was replaced daily. Only 3rd and 4th instar larvae were checked under microscope (Meiji EMZ, Meiji Techno Co. Ltd, Tokyo, Japan) using keys provided by Kumar *et al.* [22] and immature larvae were counted to assess yield. Samples were linked to their location of origin by labelling all plastic bags according to name and description of location. Pupae were raised to adults and identified relatively to estimate number of each species. The density of *Aedes* mosquitoes per location per area was determined by combining the results of individual location between areas surveyed.

### 2.3. Statistical analysis

Total number and type of all surveyed water-holding containers were analyzed using descriptive statistics such as percentages and mean ± SE. Descriptive statistics were used to obtain means for categories of containers within locations. Abundance (mean ± SE) of breeding containers between sites was compared using One-way ANOVA. Percent of total breeding sites and positive breeding containers was compared using *Chi*-square test. Independent sample *t*-test was used to compare total number of *Ae. albopictus* and *Ae. aegypti* surveyed. All statistical significance was expressed by taking $P < 0.05$ as a value in all analysis using IBM SPSS statistics version 21.

## 3. Results

### 3.1. Abundance of breeding container

A total of 3 741 water-holding breeding containers were found during the wet and dry season in all locations surveyed. All locations have been found to house a number of categories of containers. Restaurants, parks, mosques and schools comprised 38.17%, 23.31%, 21.57% and 16.95% of the total containers, respectively (Table 1). Restaurants contributed to higher number of breeding containers compared to other three locations. A *Chi*-square test indicated that number of container differed significantly at the four locations and study areas during the period of study ($\chi^2 = 25.000$, $df = 2$, $P < 0.05$). Comparison of mean abundance of breeding containers per location per area indicated that a total of 28.68 ± 5.95 containers were found at Batu Maung, followed by Gelugor (23.15 ± 5.09), Sungai Nibong (11.73 ± 3.06) and Sungai Ara (7.03 ± 2.04).

### 3.2. Container type

The containers found in all locations surveyed were classified into plastic, metal, cement/clay, natural, rubber, glass and paper (Table 2). Out of 3 741 containers identified, plastic types were the most abundant (53.9%), with glass type being the least (0.6%). The *t*-test indicated that number of container differed significantly by type in all locations at four sites during surveys (mean ± SE = 1.50 ± 0.44, $df = 27$, $P < 0.05$).

### 3.3. Species composition

An estimated 19 537 immature mosquitoes were recorded during the wet and dry season's surveys, with 84.7% collected during the wet season and 15.3% in the dry season. *Ae. albopictus* and *Ae. aegypti* comprised 92.6% and 7.4% of the total immature population, respectively (Table 3). *Ae. albopictus* mosquitoes were found dominant in all locations and during both wet (mean ± SE = 3 520.500 ± 891.51, $df = 3$, $P < 0.05$) and dry (mean ± SE = 636.250 ± 187.43, $df = 3$, $P < 0.05$) seasons. The total number of immature *Aedes* mosquitoes collected from the four locations was 8 012 (restaurants), 5 709 (parks), 3 582 (mosques) and 2 234 (schools). *Ae. albopictus* and *Ae. aegypti* were dissimilarly widespread in both wet (78.4%

**Table 1**
Abundance of breeding containers recorded at schools, restaurants, mosques and parks in four study areas [*n* (mean ± SE)].

| Location | Batumaung | Gelugor | Sungai Nibong | Sungai Ara | Total |
|---|---|---|---|---|---|
| School | 231 (4.36 ± 0.90)[a] | 236 (4.45 ± 0.87)[a] | 105 (1.98 ± 0.55)[b] | 62 (1.17 ± 0.38)[b] | 634 (12.00 ± 2.70) |
| Restaurant | 589 (11.11 ± 2.03)[b] | 443 (8.36 ± 1.76)[b] | 243 (4.58 ± 1.28)[a] | 153 (2.89 ± 0.81)[a] | 1428 (26.90 ± 5.88) |
| Mosque | 334 (6.30 ± 1.26)[a] | 264 (4.98 ± 1.02)[a] | 134 (2.53 ± 0.62)[b] | 75 (1.42 ± 0.39)[b] | 807 (15.23 ± 3.29) |
| Park | 366 (6.91 ± 1.76)[a] | 284 (5.36 ± 1.44)[a] | 140 (2.64 ± 0.61)[b] | 82 (1.55 ± 0.46)[b] | 872 (16.36 ± 4.27) |
| Total | 1520 (28.68 ± 5.95)[a] | 1227 (23.15 ± 5.09)[a] | 622 (11.73 ± 3.06)[b] | 372 (7.03 ± 2.04)[b] | 3741 (70.49 ± 16.14) |

Means within a column and between row (for total) followed by different letters are significantly different ($P < 0.05$).

**Table 2**
Types and number of container recorded at schools, restaurants, mosques and parks at the four study sites.

| Container types | Location | | | | Total | Percent |
|---|---|---|---|---|---|---|
| | School | Restaurant | Mosque | Park | | |
| Plastic | 310 | 866 | 439 | 402 | 2017 | 53.9 |
| Metal | 85 | 305 | 118 | 195 | 703 | 18.8 |
| Cement/clay | 101 | 106 | 99 | 48 | 354 | 9.5 |
| Natural | 29 | 12 | 16 | 13 | 70 | 1.9 |
| Rubber | 64 | 16 | 71 | 167 | 318 | 8.5 |
| Glass | 0 | 8 | 11 | 5 | 24 | 0.6 |
| Paper | 45 | 115 | 53 | 42 | 255 | 6.8 |
| Total | 634 | 1428 | 807 | 872 | 3741 | 100.0 |

being a place where people with different life style and socio-economic backgrounds patronize. Traditional source reduction operations for dengue vectors often exclude restaurants, schools, mosques and parks. Failure to treat these special institutions would compromise the success of dengue control operations.

The high number of *Ae. albopictus* (92.6%) as against *Ae. aegypti* (7.4%) in this study corroborated with findings by Chen *et al.* [11] where 86.9% of 65 potential breeding containers identified indoor and outdoor were *Ae. albopictus* and no *Ae. aegypti* was found in either of the containers. Comparably, artificial containers were found highest (98.1%) in abundance compared to natural containers (1.9%) in all locations and sites. Of the total artificial containers found in this study, plastic types were the most abundant (53.9%), followed by metal (18.8%), cement/clay (9.5%), rubber (8.5%), paper (6.8%) and glass (0.6%). Artificial containers had been observed to comprise 95% of total container positive for immature *Aedes* in Pulau Pinang with only 4.2% natural containers [16]. Buckets have been found the most abundant and important larval breeding habitats [23–25]. Roof gutters were among largest containers (capacity approximately 10 L and above) recorded in this study and have been found to be productive source of *Ae. aegypti* in both wet and dry seasons and its treatment is critical in *Ae. aegypti* control campaigns [26]. Presence of *Ae. albopictus* at various developmental stages in containers kept inside a cage holding birds in Penang was reported [27], and similarly, birds drink containers were found positive for immature *Aedes* in this study. Toilet flush tanks were among the containers comprising high percentage (29.78%) of total containers recorded by size. Similarly, toilet flush tank has been reported to have contained *Aedes* eggs in a house in urban area of Penang Island [27,28].

Both species of *Ae. albopictus* and *Ae. aegypti* have been observed occupying various containers of different types in all locations and sites, and this is consistent with findings which

**Table 3**
Immature *Ae. albopictus* and *Ae. aegypti* surveyed in restaurants, mosques, parks and schools during wet and dry period in four study sites.

| Period | Survey location | Positive containers | No. of immature *Ae. albopictus* | No. of immature *Ae. aegypti* |
|---|---|---|---|---|
| Wet season (April–December 2015) | Restaurants | 531 | 6172 | 497 |
| | Parks | 341 | 4560 | 367 |
| | Mosques | 308 | 2806 | 226 |
| | Schools | 259 | 1777 | 143 |
| | Total | 1439 | 15315 | 1233 |
| Dry season (January–March 2016) | Restaurants | 164 | 1243 | 100 |
| | Parks | 107 | 724 | 58 |
| | Mosques | 78 | 509 | 41 |
| | Schools | 67 | 291 | 23 |
| | Total | 416 | 2767 | 222 |

and 6.3%) and dry (14.2% and 1.1%) season surveys, respectively. Immature mosquitoes are either *Ae. albopictus* or *Ae. aegypti* in all locations and *Ae. albopictus* was more abundant during both seasons (92.6%) as against *Ae. aegypti* (7.4%). However, all locations were productive sites.

## 4. Discussion

Knowledge of breeding ecology of these vectors is crucial when it comes to management of the mosquito. Abundance of *Aedes* mosquito breeding container habitats and immature populations in public places of restaurants, parks, mosques and schools was quantified for the first time in Pinang Island, Malaysia. However, because only a sample of the entire schools, restaurants, mosques and parks was surveyed, estimates of containers may be in error. Nonetheless, the number of containers ($n = 3741$) collected from only a sample of locations indicates that these places are indeed the sources of key containers for *Aedes* mosquitoes in Penang Island. The disproportionate production of mosquitoes breeding sites by schools, restaurants, mosques and parks is probably due to the characteristic attributes of the sites,

described *Ae. aegypti* and *Ae. albopictus* as sympatric species occupying similar ecological niches [20,29]. *Ae. aegypti* possess the characteristic ability to breed in habitats close to humans and can be found in urbanized areas [30–32]. This characteristic is shared with *Ae. albopictus* [33]. A number of studies showed *Ae. albopictus* and *Ae. aegypti* coexisted [34–36]. In this study, *Ae. albopictus* were 18 107 (92.6%) of the 19 537 total immature populations collected in all sites surveyed, while *Ae. aegypti* were only 1 430 (7.4%) in abundance. Likewise, a total of 79 451 immature *Aedes* mosquitoes have been recorded in houses from four areas of Pantai Jerjak, Bayan Lepas, Batu Maung and Balik Pulau in Penang Island, and comprised 92.3% *Ae. albopictus* and 7.7% *Ae. aegypti* [16]. Study on distribution of *Ae. albopictus* appeared to be occurring at the expense of *Ae. aegypti* which are found restricted to forest and disturbed habitats [37], while *Ae. aegypti* as domestic species are found in houses and depend on human blood [38,39]. In contrast, it is established in this study that *Ae. albopictus* have exclusively replaced the home-grown *Ae. aegypti* even in urban areas in Penang Island. Another dissimilar findings reported *Ae. aegypti* to have wholly substituted the indigenous *Ae. albopictus* in municipal areas [40–42]. In numerous studies, extensive sharing of ovitrap by *Ae. aegypti* and *Ae. albopictus* has been reported [43–45]. Correspondingly, species of *Aedes* mosquitoes have been found to have shown equal preference for varying container habitats in several suburban communities in Malaysia and Bangladesh [27,46].

In conclusion, this study has established that public places like schools, restaurants, mosques and parks are good sources of potential container habitats of *Aedes* mosquitoes in Penang Island. Artificial containers are the most abundant and more prepared by *Aedes* mosquitoes. Comparably, plastic containers were found more abundant, more productive and most prepared. Distribution of *Ae. aegypti* was restricted by *Ae. albopictus* in all locations surveyed. Since these public places are always being patronized by people with varying cultural and socioeconomic background, and in large number at a time, this might have contributed to the characteristic abundance and diversity of container habitats at the locations. Probably, the rules and regulations enforced in schools could have been a reason for the less number of breeding containers observed in the schools than in other locations surveyed. The researchers recommend the continuousness and extension of the survey to places like university campuses, hospitals, prison yards and home of the aged in Penang Island.

## Conflict of interest statement

We declare that we have no conflict of interest.

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