ADULT *AEDES* MOSQUITO AND DENGUE VIRUS SURVEILLANCE IN RESIDENTIAL AND PUBLIC AREAS OF SELANGOR, MALAYSIA

Lau Sai Ming[1], Tassha Hilda Adnan[2], Ooi Choo Huck[1], Mustafakamal Ibrahim[1], Dahlia Husin[1], Nurul Ainni Abdullah[1], Fatimah Ibrahim[1], Norzaleha Masri[1], Sharifah Malihah Wan Mustapha[1], Ahmad Firdaus Rubani[1], Mohamad Adam Bujang[2] and Tg Mohd Ikhwan Tg Abu Bakar Sidik[2]

[1]State Vector Borne Disease Control Unit, Selangor State Health Department, Shah Alam, Selangor; [2]Biostatistics Unit, National Clinical Research Centre, Level 3, Dermatology Block Kuala Lumpur Hospital, Kuala Lumpur, Malaysia

Abstract. Dengue infection is an important public health problem in Selangor, Malaysia, and its incidence may vary by location. We aimed to determine and compare the density of *Aedes* mosquitoes and dengue virus in public and residential areas in Selangor, Malaysia in order to inform dengue control programs on where to place resources to control this public health problem. This study was conducted in all 9 districts of Selangor during 2014-2016. We used a Selangor Sticky Trap method, described in the paper, to collect mosquitoes in 421 locations, both residential and public. We performed a dengue NS1 antigen test on each of the *Aedes* mosquitoes collected to determine the presence of dengue virus. A total of 85.5% of sampled locations in the residential areas had *Aedes* mosquitoes, of which 15.7% had a positive dengue NS1 antigen test. A total of 84.4% of sampled locations in public areas had *Aedes* mosquitoes, of which 15.0% had a positive dengue NS1 antigen test. There were no significant differences in percentages of locations with *Aedes* mosquitoes or mosquitoes positive for the NS1 antigen between residential and public areas surveyed ($p>0.05$). Among public areas, *Aedes* mosquitoes were most often detected in commercial areas, railway stations and recreational areas. The above findings are concerning and suggest that control measures must be evenly divided between public and residential areas. The relatively high percentage of dengue-infected mosquitoes in public areas is concerning, since the likelihood of spread is greater in these areas, resulting in spread to previously uninfected residential areas.

Keywords: *Aedes*, dengue, surveillance, Selangor, Malaysia

Correspondence: Mohamad Adam Bujang, Clinical Research Centre, Sarawak General Hospital, JalanTun Ahmad Zaidi Adruce, 93586 Kuching, Sarawak, Malaysia.
Tel: +6082 276820; Fax: +6082 276823
E-mail: adam@crc.gov.my

INTRODUCTION

Dengue infection is a mosquito-borne viral infection and is a global public health problem, endemic in more than 100 countries including Southeast Asian countries (WHO, 2016). Malaysia has experienced an increase in dengue cases since 2013, with a current range of 145-396 cases per 100,000 population (Rose, 2015; Kementerian Kesihatan Malaysia, 2016). There were 86,428 cases of dengue infection in Malaysia by week 41 of 2016, with 51% of the cases identified in Selangor State (Ministry of Health, 2016b). The incidence of dengue infection is generally expected to increase four times by the year 2020 compared to 2010, and to increase nearly six times by the year 2040, compared to 2010 (Bujang *et al*, 2017). Therefore, dengue is a major public health problem in Malaysia associated with morbidity and mortality (Rose *et al*, 2018).

The Malaysian government requires all dengue infection cases be reported to the Malaysian government within 24 hours (Ministry of Health Malaysia, 2002; Malaysia MoHMAoM, 2015; Ministry of Health Malaysia, 2016c). Cases of dengue infection are investigated by assistant environmental health officers, who record the particulars and travel histories of dengue-infected patients in order to determine where they contracted the infection and to perform control measures, such as insecticide spraying and destruction of breeding sites. Inadequate information regarding breeding sites and underreporting of subclinical cases makes control of dengue infection difficult. Selangor State, Malaysia has a high population density and limited resources. Most dengue control measures in Malaysia target the areas in which patients with dengue reside, rather than the location where a patient might have contracted the infection.

Mosquito surveillance is important for mosquito-borne disease control programs. In Malaysia, *Aedes* larval surveys and ovitraps are the most commonly used tools for *Aedes* surveillance (Tham, 2000). Larval surveys are conducted to inspect for *Aedes* breeding sites (Ministry of Health Malaysia, 2016c). However, larval surveillance is labor intensive and plagued by difficulties in accessing houses, especially in urban areas (Sivagnaname and Gunasekaran, 2012). Larval surveillance has been reported to be inappropriate for predicting occurrences of dengue infection cases (de Melo *et al*, 2012). The ovitrap method was first used in a study for estimating the abundance and distribution of *Aedes* species on Penang Island, Malaysia (Yap, 1975). The ovitrap encourages mosquitoes to lay eggs in the trap. It has been used to survey *Aedes* populations, especially in areas with low infestation rates (Ministry of Health Malaysia, 1997). Although the ovitrap method is more sensitive than other mosquito-traps, such as the sticky trap method (Resende *et al*, 2013) and the Breteau index (Chau *et al*, 2005), it does not detect risk for dengue transmission (Focks, 2004; Dibo *et al*, 2008). The adult *Aedes* survey is a preferable method since it counts the number of adult female *Ae. aegypti* mosquitoes. The adult *Aedes* survey method is more accurate than the other methods that detect the immature mosquito stages, which correlate poorly with the number of adult mosquitoes (Focks, 2004; Lourenço-de-Oliverira *et al*, 2008).

The sticky trap method is cheaper, requires less skilled labor, is easy to maintain (de Santos *et al*, 2012), and collects more *Aedes* mosquitoes than backpack aspirators (Facchinelli *et al*, 2008); however, it is less sensitive than the Biogents Sentinel method (Kroeckel *et al*, 2006; Ritchie *et al*, 2014). There are different types of sticky traps used to collect *Aedes* mosquitoes, such as the Gravid *Aedes* Trap

(GAT) (Ritchie *et al*, 2014), Gravitraps (Lee *et al*, 2013), MosquiTRAP (Steffler *et al*, 2011) and the double sticky trap (DST) (Chadee and Ritchie, 2010). The sticky trap can trap mosquitoes with dengue virus, and the virus can then be detected using PCR (Ritchie *et al*, 2014) or the Dengue NS1 Antigen test kit (Lee *et al*, 2013; Lau *et al*, 2015). Combined use of the Gravid Mosquito Ovipositing in the Sticky Trap (GOS) as a sticky trap and the Dengue NS1 antigen test kit has been assessed for dengue virus surveillance in dengue endemic areas of Selangor (Lau *et al*, 2015; *ibid*, 2017).

Dengue has been found in many urban and suburban areas in tropical and subtropical climates worldwide. Dengue vector distribution is affected by climate and environmental factors associated with human activity (Higa, 2011). A study from Rio de Janeiro, Brazil found the highest seroprevalence of dengue to be in commercial areas with a large concentration of humans, but large concentrations of mosquitoes did not correlate with the areas with the highest prevalence of dengue (Honoria *et al*, 2009). Barbosa *et al* (2014) reported similar findings. In Malaysia, the seroprevalence of dengue is higher in urban than in rural areas (Chew *et al*, 2016). Selangor, which is the most developed state in Malaysia with many urban areas, has the largest seroprevalence of dengue in Malaysia (Hassan *et al*, 2012).

In Selangor, dengue has been difficult to control owing to unplanned urban development and the large number of construction sites. Dengue control programs only target areas around the homes of dengue victims, rather than identifying and targeting sites where dengue victims may have contracted the infection. Thus, this means control efforts are reactive rather than proactive. The aim of this study was to determine whether there are any significant differences between residential and public areas in terms of the presence of mosquito vectors and dengue infected mosquitoes, so as to inform dengue control measures.

MATERIALS AND METHODS

Study sites

This study was conducted during 2014-2016 in all nine districts of Selangor State. Data regarding the total number of mosquitoes caught, mosquito species and virus detection in caught mosquitoes were collected from residential and public areas. The collection sites were based on dengue epidemiology at the time; thus, there were differences in the number of study sites for each district. In our study, residential areas were those areas used mainly for houses and apartments. Public areas were those places visited by the public and used mainly for common purposes, such as commercial areas, railway stations and parks.

Selangor is located in the center and on the west coast of Peninsular Malaysia and consists of nine districts; Petaling, Hulu Langat, Gombak, Klang, Hulu Selangor, Sepang, Kuala Selangor, Kuala Langat and Sabak Bernam. The population densities of the study areas are shown in Table 1. Four districts, Petaling, Hulu Langat, Gombak and Klang are more urbanized with higher population densities. A total of 224 localitions in residential areas and 197 localities in public areas were sampled in the nine districts of Selangor (Table 1). A total of 30 to 100 sticky traps were set both indoors and outdoors in an area with a radius of 200 m. The number of traps varied by location based on ac-

Table 1
Population densities total samples sites per study area.

District	Population per km²	Total sample sites		
		Residential areas	Public areas	Total
Petaling	3,742.64	30	40	70
Hulu Langat	1,394.42	38	31	69
Gombak	1,049.45	45	49	94
Klang	1,373.99	63	27	90
Hulu Selangor	113.84	18	15	33
Sepang	352.47	6	7	13
Kuala Langat	261.83	2	10	12
Kuala Selangor	175.46	22	13	35
SabakBernam	106.08	0	5	5
Total	684.45	224	197	421

Source of the data on total population per km^2 (Wikipedia, 2018).

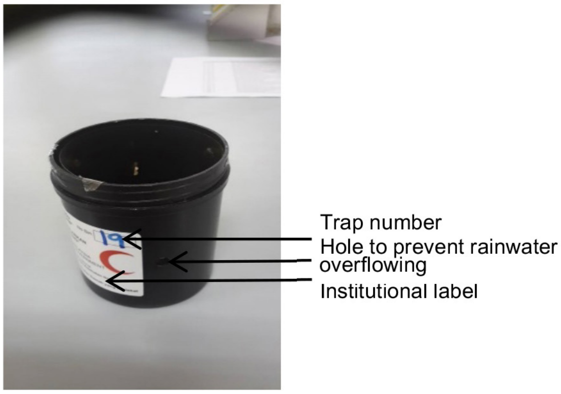

Fig 1–Selangor Sticky Trap.

cessibility. Indoor areas were defined as those with a roof and outdoor areas as those without a roof.

Selangor Sticky Trap (SST)

The Selangor Sticky Trap (SST) used in this study was modified from the conventional ovitrap. The conventional ovitrap is a 260 ml black plastic container. It is factory-made and costs USD0.50 each. The inner wall of the ovitrap is lined with a 5.5 cm x 24 cm transparent plastic sheet (Fig 1). The inner plastic sheet is covered with liquid insect glue (RA-PS100). The plastic sheet is attached to the container using adhesive tape on both sides of the top of the container. A hole is drilled about 3cm above the bottom to avoid flooding of the trap with rain water. Each container had an institutional label and a trap series number. Each SST was placed in the field for one week and then collected and brought back to the laboratory for further processing.

Detection of dengue virus in mosquitoes

The species of each mosquito caught by the SST was identified using a stereomicroscope. Female *Aedes* mosquitoes were tested for dengue virus antigen using the SD Bioline® NS1 Antigen Kit (Standard Diagnostics, Seoul, Korea) following a previously described procedure (Lau *et al*, 2015). Five collected mosquitoes were added to a 1.5 µl centrifuge tube along with 50 µl of phosphate buffer solution. The contents of the tube were then homogenized using either a pestle manually or a handheld homogenizer (Kontes Thompson Scientific, Minneapolis, MN). The tube was then centrifuged for 1 minute using a mini centrifuge (MLX-210 Thermo-Line, China) at 10,000 rpm. Three drops of the contents from each tube were then pipetted into the well of a dengue NS1 antigen test kit and after 10 - 15 minutes the result was read as either positive or negative.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 18.0 (IBM, Chicago, IL).

Ethical considerations

This study was approved by the National Institutes of Health Malaysia and registered under the National Medical Research Registry (NMRR ID: NMRR-16-1841-32956).

RESULTS

A total of 421 locations were surveyed; 85.3% of locations in residential areas and 84.8% of locations in public areas had *Aedes* mosquitoes (Table 2). No significant difference in the percentage of areas with *Aedes* mosquitoes was seen between residential and public areas (p= 0.560) and no significant differences were seen by district (p=0.111). Greater than 90% of locations sampled in urban districts had *Aedes* mosquitoes, except in Hulu Langat such as Petaling, Gombak and Klang.

In residential areas, 15.7% of

Table 2
Study sites where *Aedes* mosquitoes were found.

Study sites	Residential area			Public area		
	Total number of sites	Total number of sites positive for *Aedes* mosquitoes	% of sites positive for *Aedes* mosquitoes	Total number of sites	Total number of sites positive for *Aedes* mosquitoes	% of sites positive for *Aedes* mosquitoes
Petaling	30	30	100.0	40	39	97.5
Hulu Langat	38	30	78.9	31	15	48.4
Gombak	45	44	97.8	49	49	100.0
Klang	63	61	96.8	27	23	85.2
Hulu Selangor	18	16	88.9	15	13	86.7
Sepang	6	6	100.0	7	7	100.0
Kuala Langat	2	2	100.0	10	9	90.0
Kuala Selangor	22	2	9.1	13	8	61.5
SabakBernam	0	0	NA	5	4	80.0
Total	224	191	85.3	197	167	84.8

NA: not available

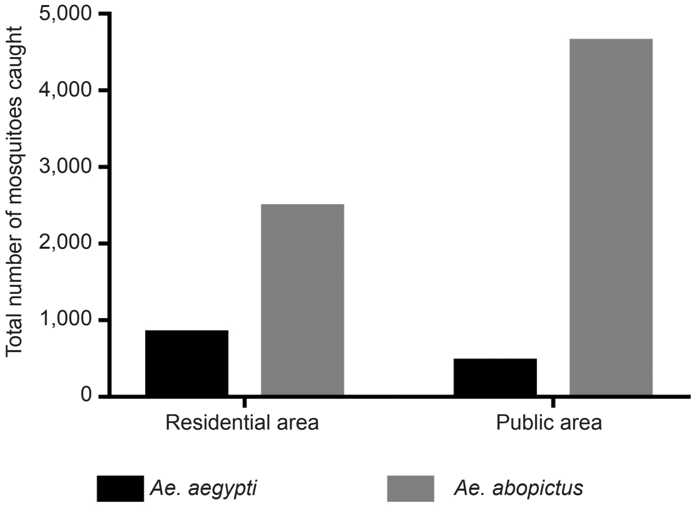

Fig 2–Total number of vector species caught by type of area.

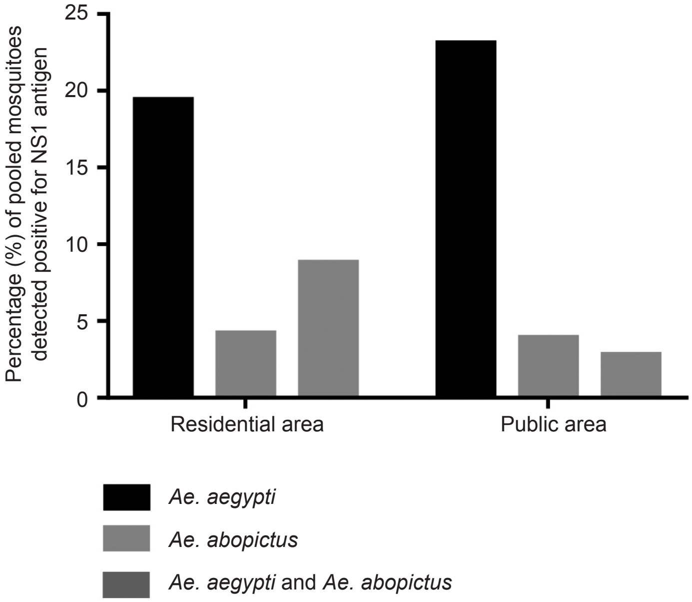

Fig 3–Percentages of pooled mosquitoes with positive NS1 test results.

test (Table 3); there were no significant differences by area (p=0.490) or by district (p=0.586). Mosquitoes positive for dengue virus were found in residential areas in most districts, whereas for public areas, mosquitoes positive for dengue virus were mostly detected in Petaling, Hulu Langat, Gombak and Klang.

A total of 1,293 *Ae. aegypti* and 7,115 *Ae. albopictus* mosquitoes were collected from 421 locations in this study. *Ae. aegypti* was found more often in residential (25.11%) than public (9.06%) areas (Fig 2). The total number of *Ae. albopictus* collected in residential areas in this study was 2.9 times more than the number of *Ae. aegypti*, but the total number collected in public areas was 10 times higher.

Table 4 shows that only *Ae. albopictus* species were found in most of the locations (38.7% of residential area and 61.0% of public areas). NS1 antigen testing was positive in a total of 32 collections of pooled mosquitoes (3.69%) in residential areas and in 32 pooled collections (6.85%) in public areas. Fig 3 shows that localities that had only *Ae. aegypti* species tended to have the highest percentage of pools in which dengue virus testing was positive, in both residential and public ar-

collected mosquitoes sampled had a positive dengue NS1 antigen test and in public areas 15.0% of collected mosquitoes sampled had a positive dengue NS1 antigen

Table 3
Study sites with mosquitoes having a positive NS1 antigen test.

Study sites	Residential areas			Public areas		
	Total number of sites	Total number of sites positive for NS1 antigen	% of sites positive for NS1 antigen	Total number of sites	Total number of sites positive for NS1 antigen	% of sites positive for NS1 antigen
Petaling	30	6	20.00%	39	14	35.90%
Hulu Langat	30	1	3.30%	15	1	6.70%
Gombak	44	5	11.40%	49	2	4.10%
Klang	61	15	24.60%	23	7	30.40%
Hulu Selangor	16	0	0.00%	13	0	0.00%
Sepang	6	1	16.70%	7	0	0.00%
Kuala Langat	2	2	100.00%	9	0	0.00%
Kuala Selangor	2	0	0.00%	8	0	0.00%
SabakBernam	0	0	NA	4	1	25.00%
Total	191	30	15.70%	167	25	15.00%

NA, not available.

eas (19.4% and 23.1%, respectively). In terms of localities where both vector species were present, residential areas had a higher percentage of pooled collections in which NS1 antigen testing was positive (8.8%) than public areas (2.8%). Table 4 also shows that localities that had only *Ae. aegypti* species tended to have the highest percentage of detected dengue virus in mosquitoes. In Petaling District, the district in Malaysia with the greatest dengue problem, localities in public areas had higher percentages of positive dengue virus detection (Table 4).

A total of 21,266 SSTs were set; 45% of the traps were set indoors, while the rest were set outdoors. Little differences were observed in the percentages of SSTs positive for mosquitoes between indoors and outdoors, (21.25% and 27.11%, respectively). The mean (SD) number of *Aedes* per trap in residential areas was 0.36 (0.362) and that in public areas was 0.526 (0.798). The highest number of *Aedes* per trap recorded in this study was 9.43, with a total of 282 *Aedes* mosquitoes caught in a recreational park in Klang. Analysis of variation (ANOVA) indicated that there was a significant difference between these two types of areas (p=0.006). The mean number of *Aedes* mosquitoes per trap ranged from 0.092 to 0.825 per district in Selangor. There were statistically significant differences between districts determined by the one-way ANOVA (p=0.001). A

Table 4
Study sites where *Aedes* mosquitoes were tested by NS1 antigen.

Study district	*Ae. aegypti*			*Ae. albopictus*			Mixed *Ae. aegypti* and *Ae. albopictus*		
	Total sites tested	Total sites positive for NS1 antigen	% of sites positive for NS1 antigen	Total sites tested	Total sites positive for NS1 antigen	% of sites positive for NS1 antigen	Total sites tested	Total sites positive for NS1 antigen	% of sites positive for NS1 antigen
Residential areas									
Petaling	1	1	100.0	20	4	20.0	9	1	11.1
Hulu Langat	9	0	0.0	7	0	0.0	14	1	7.1
Gombak	0	0	NA	13	0	0.0	31	5	16.1
Klang	10	4	40.0	21	4	19.0	30	7	23.3
Hulu Selangor	4	0	0.0	9	0	0.0	3	0	0.0
Sepang	4	1	25.0	2	0	0.0	0	0	NA
Kuala Langat	0	0	NA	0	0	NA	2	2	100.0
Kuala Selangor	0	0	NA	2	0	0.0	0	0	NA
SabakBernam	0	0	NA	0	0	NA	0	0	NA
Total	28	6	21.4	74	8	10.8	89	16	18.0
Public areas									
Petaling	5	2	40.0	23	7	30.4	11	5	45.5
Hulu Langat	2	0	0.0	5	0	0.0	8	1	12.5
Gombak	0	0	NA	30	1	3.3	19	1	5.3
Klang	0	0	NA	14	6	42.9	9	1	11.1
Hulu Selangor	0	0	NA	12	0	0.0	1	0	0.0
Sepang	1	0	0.0	2	0	0.0	4	0	0.0
Kuala Langat	0	0	NA	6	0	0.0	3	0	0.0
Kuala Selangor	0	0	NA	7	0	0.0	1	0	0.0
SabakBernam	1	1	NA	3	0	NA	0	0	NA
Total	9	3	33.3	102	14	13.7	56	8	14.3

NA, not available.

Table 5
Total number of *Aedes* per trap in each district and the comparisons between districts.

District	*n*	Mean	Std. deviation	95% CI	*p*-value
					<0.001[a]
Gombak	124	0.825	0.470	(0.742 - 0.909)	reference
Petaling	69	0.426	0.445	(0.319 - 0.533)	<0.001[b]
Hulu Langat	80	0.184	0.213	(0.137 - 0.231)	<0.001[b]
Klang	99	0.457	1.045	(0.249 - 0.665)	<0.001[b]
Hulu Selangor	37	0.160	0.175	(0.101 - 0.218)	<0.001[b]
Sepang	13	0.151	0.118	(0.080 - 0.222)	0.003[b]
Kuala Langat	12	0.154	0.106	(0.087 - 0.222)	0.005[b]
Kuala Selangor	15	0.150	0.189	(0.050 - 0.255)	0.001[b]
Sabak Bernam	5	0.092	0.156	(0.102 - 0.286)	0.137[b]

[a]*p*-value was derived from One-way ANOVA test.
[b]*p*-values were derived from post-hoc comparisons using Tukey HSD test between all districts and Gombak. All other comparisons were not significant.

significant difference was found between Gombak District and other districts except Sabak Bernam (Table 5).

DISCUSSION

In this study, *Aedes* mosquitoes positive for dengue virus were found not only in the locations where dengue patients were residing but also in public areas. There were no statistically significant differences between the two types of areas in either percentage of localities found to have vector present or infected *Aedes* mosquitoes. Although dengue spatial distribution analysis has been performed in many previous studies, information was lacking regarding the cases of dengue infection either in humans or mosquitoes in public areas. However, a seroprevalence study conducted by Honório *et al* (2009) found an acommercial activity area located at the entrance of two communities was a dengue hotspot. Those findings suggest large-scale human movement may be responsible for the spread of the virus to smaller neighborhoods. Our study found mosquitoes positive for dengue virus in public areas were more concentrated around bus stations, recreational areas, railway stations and parks in Selangor. Public areas are places challenging to target for dengue control, since it is difficult to track patient movement and determine the source of dengue infection during case investigations. However, this study revealed the percentage of localities with mosquitoes positive for dengue virus in public areas was not significantly different from residential areas. Therefore, dengue cases would be difficult to control if there were no control measures in public areas.

The density of mosquitoes in our study was high in both residential and public areas as well as urban areas of Selangor, where abundant *Aedes* mosquitoes were found in more than 80% of localities. A survey conducted in 1980 in Selangor, also found no significant difference in the percentage of *Ae. aegypti* and *Ae. albopictus* present in urban and rural settings (Ho and Vythilingam, 1980). In a survey conducted in Putrajaya and Kuala Selangor, *Ae. albopictus* was found to be the dominant mosquito species breeding outdoors; other species were *Ae. aegypti* and *Culex quinquefasciatus* (Saleeza *et al*, 2011). That study also reported localities with *Ae. aegypti* present, being higher risk for transmission of dengue. *Ae. albopictus* testing positive for dengue virus was found in both areas in that study.

In our study public areas had a higher mean number of mosquitoes caught per trap than residential areas. Gombak District had a significantly higher mean number of mosquitoes caught per trap (0.82) compared to other districts, followed by Petaling District (0.42). The previous record for the highest number of mosquitoes caught per trap was 0.50 in the Mentari Court Apartments, which is a dengue hotspot in Selangor (Lau *et al*, 2017). The median number (0.39) with a capture rate in a dengue-endemic area was reported from Brazil (Maciel-de-Freitas *et al*, 2008). Alhough <1 *Aedes* mosquitoes per trap were caught by the MosquiTRAP at the study sites in Brazil (Codeço *et al*, 2015), the highest entomological index was reported to be 0.83, for three municipalities in Brazil (Eiras and Resende, 2009). In Australia, a sticky ovitrap index above one mosquito/trap is the threshold for risk assessment for dengue transmission (Duncombe *et al*, 2013), while in Brazil, the alert threshold is 0.2 mosquitoes/MosquitoTRAP and more than 0.4 mosquitoes/MosquitoTRAP indicates a critical situation (Eiras and Resende, 2009).

In Selangor, 15.7% of localitions were positive for infected mosquitoes in residential areas and 15.0% in public areas, with the percentage of positive pooled mosquitoes being about 3.69% in residential areas and 6.85% in public areas. However, in another study conducted in 11 localitions in Singapore, 54.5% were reported to be positive for dengue virus in 2010 and the positivity rates ranged from 2.8% to 13.6% per location (Lee *et al*, 2013). A previous study conducted in the Mentari Court Apartment in Petaling District, Selangor, using NS1 antigen detection kits, found 8 pooled groups of *Ae. aegypti* were positive for dengue virus giving a minimum infection rate of 38.02 (per 1,000 mosquitoes) (Lau *et al*, 2015). A study conducted two years later found 43 pooled groups of *Aedes* mosquitoes (22.9%) were positive (Lau *et al*, 2017). However, in samples collected from 12 states in Malaysia between 1993 and 1995, about 0.48% (1 out of 207 *Ae. Albopictus* mosquitoes) were positive for dengue virus using the peroxidase anti-peroxidase (PAP) staining technique (Ahmad *et al*, 1997).

Another study conducted in Venezuela during 2000- 2001 found 5.2% of mosquito pooled groups were positive for dengue virus in dengue-infected houses, whereas in the neighboring houses the rate was 12.7% (Urdaneta *et al*, 2005). A percentage of localitions in Selangor with infected mosquitoes whether residential or public areas, may act as continuous sources of dengue transmission to other localitions. Therefore, a solution to allow detection of dengue at an early stage is to initiate an intensive vector control program to identify sites that cannot be traced by case investigation but have numerous infected mosquitoes prior to the occurrence of dengue epidemics. A limitation of this study was the data were collected randomly at one time only from selected localitions but may have missed other areas.

In conclusion, *Aedes* mosquitoes from both residential and public areas in Selangor were found to have the dengue virus. There was no significant difference between residential and public areas in the percentages of localitions with dengue virus infected mosquitoes, although the public areas had a higher number of *Aedes mosquitoes* per trap than residential areas. Resources to control dengue virus need to focus equally on both residential and public areas.

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