

ORIGINAL ARTICLE

Assessment of Vector Surveillance and Meteorological Factors in Evaluating Dengue Outbreak Areas in South Seberang Perai District, Penang

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ABSTRACT

Introduction: Increasing risk of dengue fever outbreak is associated with inadequate monitoring of distribution and abundance of *Aedes sp.* and the suitable environmental factors for the breeding of mosquitoes. This study aims to evaluate the mosquito (*Aedes sp.*) population in three uprising dengue cases areas located at South Seberang Perai District, Penang, to determine the relationship between dengue cases with the meteorological factors (temperature, relative humidity and rainfall) and to determine spatial pattern of dengue cases. **Methods:** Three localities (Kampung Valdor, Perkampungan Jawi and Jalan Stesen) which were declared as dengue outbreak areas in South Seberang Perai District, Penang from January 2019 to January 2020 were installed with ovitraps weekly. Mosquito larvae were collected from ovitrap weekly and the species were identified. Data analysis were carried out using One-Way ANOVA, Pearson correlation coefficient, Inverse Distance Weighted and Average Nearest Neighbour. **Results:** The dominant vector was *Aedes albopictus* (78.27%) compared to *Aedes aegypti* (21.72%) in all three localities. *Aedes aegypti* preferred breeding inside the home (79%) while *Aedes albopictus* were mostly found in outdoor containers (98.9%). Our result showed no significant correlation between total *Aedes* and dengue cases with all meteorological variable (temperature, relative humidity and rainfall). Dispersed pattern; R value more than 1 ($R=7.57$, $z\text{-score}=21.78$, $p < 0.001$) was shown for dengue cases. **Conclusion:** This study is important in ensuring sustainable dengue management. High population of *Aedes* in the selected localities may potentially lead to a risk factor associated with dengue fever transmission.

Keywords: *Aedes*, Dengue, Ovitrap, Meteorological variables, Public health

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INTRODUCTION

Dengue fever (DF) is prevalent in South-East Asia Region (187,333 dengue cases) and Western Pacific Regions (354,009 cases) which caused 1,075 deaths in the year 2010 (1). The projection of dengue infection incidence was predicted to multiply by nearly four times higher in the year 2020 and almost six times higher in the year 2040 compared to the baseline year in 2010 (2). From December 2019 until July 2020, there were

60,032 dengue cases reported in Malaysia with 96 death cases (3). The primary species responsible for DF transmission in Southeast Asia were identified as *Aedes albopictus* and *Aedes aegypti* (4). *Aedes albopictus* has been identified as a vector of dengue fever and dengue hemorrhagic fever in some countries, including Hawaii, Japan, Indonesia, Singapore and Malaysia (5). Apart from that, other diseases transmitted by *Aedes albopictus* and *Aedes aegypti* are Chikungunya and Zika (6).

Vector surveillance is used to determine vector distribution in the population and identifying outbreak areas (7). One of the monitoring approach for dengue transmission in residential houses is by using ovitrap surveillance and measuring the ovitrap index (OI).

By using the directly collected number of larvae and eggs from the field, OI is calculated as a percentage to monitor the distribution of *Aedes* mosquitoes (8). Vector surveillance and control measures by using ovitrap surveillance act as one prevention and control measures of the dengue diseases (4, 7-8).

The change in meteorological factors such as increasing temperature, rainfall and relative humidity were reported as the prominent factors influencing dengue epidemic and transmission in Malaysia (4, 9). Heavy rainfall can reduce the population of mosquito by flashing out egg, pupae and larvae from the breeding container but at the same time, the remaining water left after the rain can provide a potential breeding area for *Aedes sp.* (10). Temperature at 28°C has been reported to show the highest *Aedes sp.* oviposition rate which in turn may increase the risk of dengue fever (10). In contrast, extreme hot temperature around 36 °C to 40 °C can decrease the risk of dengue transmission by increasing the dengue vector mortality rate (11). The key to control *Aedes* mosquito sp. population is by understanding the environmental factors involved in the infestation of this Culicidae. Some of the conditions which influence the distribution and abundance of mosquito larvae in the breeding container are the oviposition preferences of adult females and the ability of immature stages to proliferate (8).

Multiple studies existed on spatial distribution and abundance of *Aedes sp.* in urban regions (8,12) and relationship between *Aedes* with socioeconomic and environmental factors (4,7,9,13). In line with this, our study is another effort integrating the distribution and abundance of *Aedes sp.* using ovitrap index, meteorological aspects (temperature, relative humidity and rainfall) and preliminary spatial pattern analysis to determine the baseline data in South Seberang Perai District that can help authorities improve their vector control management.

This study aimed to assess the *Aedes* mosquito population in the selected dengue outbreak localities, to determine the relationship of dengue cases with the meteorological factors (temperature, relative humidity and rainfall) and to determine preliminary spatial pattern of dengue cases. This study can provide the baseline data for local authorities such as the Ministry of Health to improve their strategy in vector control management, especially when no cases are reported in the localities. This study is also in line with the United Nation’s Sustainable Development Goals number 3, good health and well-being and goal number 11, sustainable cities and communities. Marselle et al., (14) have highlighted the relationship between Vector Borne diseases (VBDs) like dengue fever with the SDG goal number 3. VBDs are responsible for more than 17% of the worldwide burden of infectious diseases. In SDG goal number 11, ending VBDs will contribute towards achieving sustainable

urban development.

METHODS AND MATERIALS

Study areas

This research was conducted in three frequently outbreak areas of dengue fever in South Seberang Perai District, located in the mainland of Penang. South Seberang Perai District has an area around 242 km² with an estimated 120,000 population. The selected dengue outbreak areas for this study were Kampung Valdor (KV) (5°14'25.88" N, 100°29'32.25" E), Perkampungan Jawi (PJ) (5°12'41.04"N, 100°29'41.74"E) and Jalan Stesen (JS) (5°13'23.31"N, 100°29'31.67"E). These three localities were selected based previous number of reported dengue cases around the year 2017 to 2019 and the number of dengue outbreaks episode that was declared at these areas. These areas are mostly covered with residential areas as shown in Figure 1 (15). Continuous vector surveillance has been conducted by Vector Borne Disease Control Unit, South Seberang Perai Health District Office, Penang since the outbreak. For this study, we have used vector surveillance data for about 12 months from January 2019 to January 2020 using the ovitrap method.

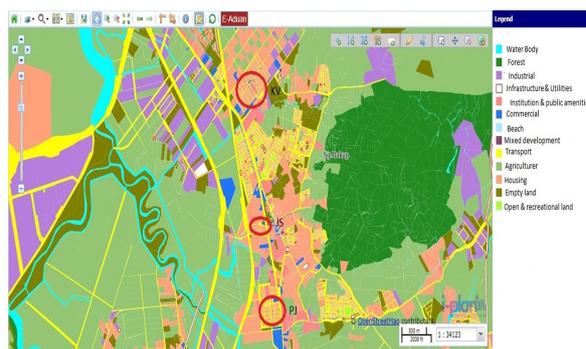


Fig. 1 Three selected localities as the study area in Perkampungan Jawi (PJ), Jalan Stesen (JS) and Kampung Valdor (KV), Seberang Perai, Penang marked in red circles. (Iplan, Malaysia,2019).

Ovitrap surveillance

The ovitrap consists of a base diameter of 6.8 cm and 9.1 cm in height and is made of a 300 mL plastic container coated in black both inside and outside. The ovitrap also consisted of an oviposition paddle made from hardboard with a measurement of 10 cm x 2.5 cm x 0.3 cm. In each ovitrap, two different types of surfaces was positioned diagonally with the rough surface of the oviposition paddle positioned upwards. The *Aedes* mosquito prefers to oviposit at the rough surface (16). Each ovitrap was filled with clean tap water that has been stored for 24-48 hours. These ovitraps were used by following the guidelines on the use of ovitrap for *Aedes Surveillance* by the Ministry of Health, Malaysia (17). The use of ovitraps has provided important information on the temporal distributions of *Aedes aegypti* that inhabit containers (18,19). A total of 90 ovitraps were used in this study with 30 ovitraps placed in each selected localities. For

each localities, 30 houses were selected randomly to place the ovitrap; 15 ovitraps were placed indoors while the other 15 ovitraps were placed outdoors. The basis for selection was due to the coverage of the area. Each ovitrap was placed within 200 m from one another, which was sufficient to cover the area of study. The distance between the selected house to the others must be within 200 meter radius as specified by the guideline (17). Usually, indoor ovitraps were placed at the living hall, kitchen or bedroom while outdoor ovitraps were placed at the shaded area, far from direct sunlight and rainfall outside the house. Ovitrap were collected and replaced weekly by a new one. The ovitrap must not be left more than a week to ensure safety issues. This is the common routine monitoring practiced by the Vector Borne Disease Control Unit, South Seberang Perai Health District Office, Penang.

Data collection

Firstly, after collection, the ovitraps were brought back to the lab. Record was taken in terms of the number of ovitrap and if there were any broken ones (normally there are, involving those that were placed outdoors). The number of larvae were recorded and the larvae species were identified. The ovitraps were not left more than a week to ensure optimum safety measures were practised. Ovitrap is part of a routine monitoring and evaluation process and no safety issues have been raised if the ovitrap is installed and left for a week

After one week, not all ovitraps contained larvae. From here, the ovitrap index calculation were made and the localities with more presence of adult mosquitoes were determined. Ovitrap is a universal and effective method used to identify the distribution and abundance of mosquito species in study areas. The use of ovitrap is crucial in integrated vector management (IVM) so that control measures can be carried out earlier. It is also effective for evidence-based decision making which is one of the elements of IVM in Malaysia).

The paddle is one of the components found in the ovitrap which is installed together with the ovitrap container. When collection was made, the paddle in the ovitrap container were brought to the lab and left for 3 days. The reason for the 3 day observation was to make sure if there are any eggs on the paddle or in the ovitrap container that has not yet hatched. They were observed until hatching were completed. After 3 days, re-identification of the larvae were carried out and proliferation were recorded.

The ovitraps and larvae were brought back to the lab for identification process. The mosquito larvae in the ovitrap were sucked using a pipette and put into a container filled with warm water to kill the larvae before the larvae were placed on the microscope slide and identification was carried out. *Aedes albopictus* and *Aedes aegypti* were identified using the compound microscope based

on the observation of 3rd instar or 4th instar stage of the mosquito larvae. The larvae comb teeth morphology was observed based on the entomological chart by Medical Entomology Unit, Institute of Medical Research, Kuala Lumpur. After all processes were completed in the lab, the specimens were discarded since all larvae were dead.

Mean temperature, relative humidity and rainfall data from January 2019 to January 2020 were procured from Malaysian Meteorological Department Malaysia for Butterworth station (the nearest station to the selected study area). The weekly dengue cases and incidents of dengue fever outbreaks data in the three selected study area in the year 2019 were obtained from the Vector Borne Disease Control Unit, South Seberang Perai District Health Office, Penang, Malaysia.

Data analysis

The ovitrap index (OI) that represents the *Aedes sp* distribution (equation 1) and the total number of *Aedes sp* larvae per recovered ovitrap were calculated. Normality tests were conducted and data were found to be in normal distribution using the Skewness, Kurtosis ($p > 0.05$), Kolmogorov-Smirnov test ($p > 0.05$) and Shapiro-Wilk test ($p > 0.05$). One-way ANOVA test was carried out to find the significant difference in numbers of larvae found at the three different localities. The Pearson's correlation coefficient analysis was conducted among the number of larvae and OI with the meteorological parameters (temperature, rainfall and relative humidity) and the number of dengue cases reported. P-value, <0.05 was considered statistically significant at all levels.

$$\text{Ovitrap index} = \frac{\text{number of positive ovitrap}}{\text{number of ovitrap}} \times 100 : (\text{equation 1})$$

Inverse Distance Weighted was used as a preliminary spatial interpolation of temperature, rainfall and relative humidity data to visualize the geographical pattern of the meteorological parameters. Additionally, average nearest neighbour (ANN) analysis was conducted in ArcMap 10.5 software by observing the shape of the ANN curve as a function of neighbour for the dengue outbreak cases and based on the OI to determine whether the distribution is clustered, random or dispersed (20). The ANN ratio is calculated as the observed average distance divided by the expected average distance. If the ratio is less than 1, the data exhibits a clustered pattern, whereas a value greater than 1 indicates a dispersed pattern in our data (8).

RESULTS

Aedes mosquito population using Ovitrap surveillance and breeding site assessments

Out of the total 7866 collected mosquito larvae, 3039

larvae (38.63%) from KV, 1667 larvae (21.19%) from PJ and 3160 larvae (40.17%) from JS (Fig. 2). The total *Aedes* larvae collected from these three localities were significantly different (One way ANOVA, $F=9.624$, $df = 2$, $p < 0.05$). Total *aedes* collected in KV showed a significant difference compared to PJ (Tukey test, $p < 0.05$) but was not significantly different with JS (Tukey test, $p > 0.05$).

Aedes albopictus was the most abundant mosquito species collected from the three localities (78.27%) while only 21.73% were *Aedes aegypti* species. Referring to *Aedes aegypti* alone, this species was more prevalent in JS (68.46 ± 6.98). In the three localities, *Aedes albopictus* were more likely to be found breeding in outdoor ovitrap (98.9%) compared to the indoor ovitraps. *Aedes albopictus* was highly detected in the outdoor areas in all three localities, KV (161.54 ± 18.64), PJ (105.77 ± 10.93) and JS (172.62 ± 15.95). *Aedes aegypti* were found to breed more in the indoor areas with the highest data recorded in JS (66.54 ± 7.41).

Based on Fig. 3, OI was found to be within the range of 6.67% to 37.8% from January 2019 to January 2020. The OI was highest in JS in January 2019 (37.8%) while the lowest OI was recorded in PJ in September 2019 (6.67%). OI showed a statistically significant difference between KV, PJ and JS (One Way ANOVA, $F= 24.277$, $df = 2$, $p < 0.05$). However, KV and JS was not significantly different between one another (Tukey test, $p = 0.987$).

The relationship between meteorological data, dengue cases and mosquito population and Preliminary Spatial Pattern of Dengue Cases

Total *aedes* in KV was negatively correlated with the dengue cases ($r = -.056$) as shown in Table I. No

significant correlation was found between the total *aedes* with the meteorological data (temperature, rainfall and RH). Nevertheless, an increasing number of total *Aedes* was found from June 2019 to August 2019 (Fig. 4). Using ANN analysis, dengue cases was observed to show dispersed pattern ($R \text{ value} > 1$, $R=7.57$, $z\text{-score} = 21.78$, $p < 0.01$) in Fig. 5. IDW showed the visual patterns of the higher rainfall distribution in the upper part of mainland Penang than the lower ones (Fig. 6).

DISCUSSION

Higher total larvae in JS and KV compared to PJ (Fig. 2) could be related to the type of houses in the area and the

Table I: Pearson correlation analysis between dengue cases, temperature, relative humidity (RH), rainfall and total *aedes* in the three selected areas.

Localities	Temperature	Rainfall	RH	Dengue cases
KV	0.148	0.166	0.275	-.056*
PJ	0.500	-.154	-3.53	0.500
JS	0.476	-.305	-0.10	-.194

*Correlation significant at 0.05 level.

Note: KV-Kampung Valdor, PJ-Perkampungan Jawi, JS-Jalan Stesen

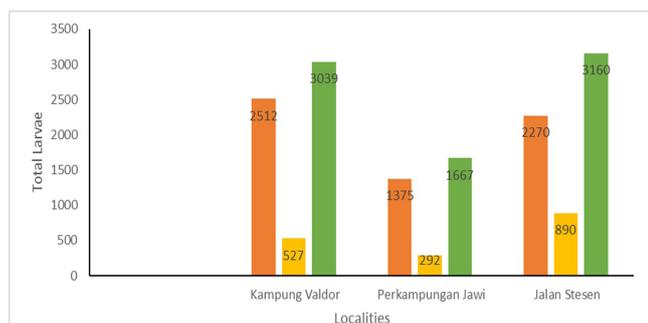


Fig. 2 Total number of *Aedes* larvae by species collected in the three localities from South Seberang Perai District, Penang

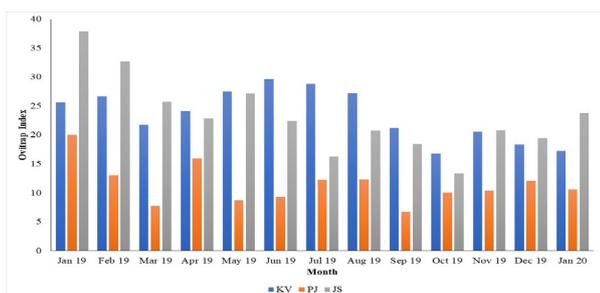


Fig. 3 Ovitrap index (OI) from the three localities, Kampung Valdor (KV), Perkampungan Jawi (PJ) and Jalan Stesen (JS) in South Seberang Perai District, Penang

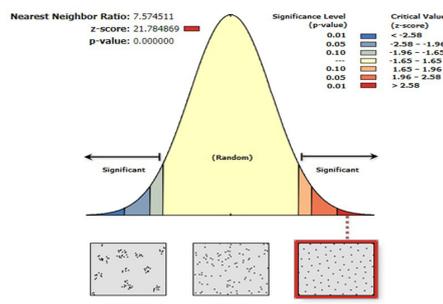


Fig. 4 The relationship between total *Aedes* with the meteorological factor (temperature, rainfall, relative humidity) in three localities, Kampung Valdor (KV), Perkampungan Jawi (PJ) and Jalan Stesen (JS) from January 2019 to January 2020.

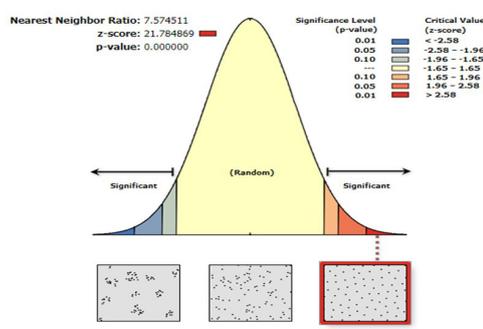


Fig. 5 Average Nearest Neighbour Analysis (ANN) spatial pattern analysis on dengue cases

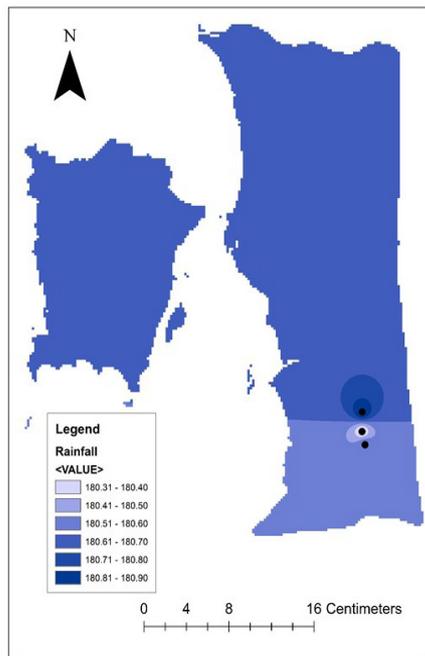


Fig. 6 Inverse Distance Weighted (IDW) for rainfall distribution in South Seberang Perai district.

cleanliness of the surroundings. Based on observation, JS is a traditional village with scattered house arrangement while KV and PJ are organised in lot houses of residential land area with a standardised arrangement of houses in rows. Similar findings were also observed in Central Shah Alam which stated that mixed houses including squatter were found to have higher number of dengue incidence rate than interconnected houses like terraces (21).

Direct observation at the studied site, especially at KV showed that many residents neglect the cleanliness of the house and were storing some containers that can be potential breeding sites for *Aedes* mosquitoes. Favourable breeding conditions for *Aedes* were mostly found in plastic containers, flower vases and lids of buckets outside the house which were exposed to rainwater accumulation (13,21). Neglected backyards which were not regularly checked also contributed as one of the factors that increase the breeding area for *Aedes* (22).

More *Aedes albopictus* larvae (78.27%) were collected relative to the *Aedes aegypti* larvae (21.73%). The finding of this study was similar to the other previous studies in Malaysia reporting a higher abundance of *Aedes albopictus* compared to *Aedes aegypti* (4, 23). *Aedes albopictus* and *Aedes aegypti* are anthropophilic mosquitoes that live in close relationship with human territories, particularly in residential areas where human hosts are available easily (7, 24).

The location of the breeding ground is an important clue for the Ministry of Health to formulate effective integrated

vector management (25). More *Aedes albopictus* was found to breed outdoors compared to indoors. In order to control *Aedes albopictus*, control measures should be more focused on the breeding search and destroy, thermal fogging and ultra-low volume (ULV) and biological control measure through larviciding activities using biological agent, *Bacillus thuringiensis israelensis* (Bti) (26). Meanwhile, more *Aedes aegypti* was found indoors, suggesting that more control measures should be strengthened on home breeding inspection and the use of aerosol insecticide.

In terms of their breeding site, our study has reported that 5719 (93%) of *Aedes albopictus* larvae were found at the outdoor ovitraps. In comparison, only 438 (7%) *Aedes albopictus* larvae were found breeding indoors. In contrast, the breeding behaviour of *Aedes aegypti* species was found mostly in indoor ovitrap (1648 larvae) compared to outdoor ovitrap (61 larvae). This finding is because *Aedes aegypti* is an endophilic mosquito that takes shelter inside the house and portrays an endophagic behaviour which highly likely feed inside the house. Meanwhile, *Aedes albopictus* is considered as exophilic and exhibited an exophilic mosquito behaviour which probably stays outside the house and bites its prey outside the house (27). This finding is also consistent with the study by Hii et al., (28), reporting similar findings in terms of the breeding preference of *Aedes albopictus* outdoors while *Aedes aegypti*, preferred to breed in the houses.

Müller et al., (14) stated that the attempt to reduce the incidence of VBDs such as DF are unsuccessful due to the inadequacy of community awareness and knowledge regarding the relationships between disease, vectors and viruses. A more pro-active and consistent collaboration between community and the responsible agencies may lead to a better control of dengue fever (29). Malaysia has begun community approach called Communication for Behavioural Impact (COMBI) to focus on community behavioural change and mobilisation to prevent together and control communicable diseases such as dengue fever (30). Localities that implemented COMBI program had found to reduce the dengue cases by 80% (31). Frequent proliferation of dengue fever were found in man-made settings, therefore, human behavioural control is without a doubt, the most significant way forward in managing the issues associated with the dengue vectors (8).

The OI value characterises the mosquitoes' distribution in the studied area (23). Referring to Fig. 3, only JS recorded the OI of more than 30%. According to Vector-borne Disease, Disease Control Division, Ministry of Health, Malaysia (31), the OI is classified into three risk level; level 1 for $OI < 10\%$, level 2 for $10\% \leq OI < 30\%$ and level 3 $OI \geq 30\%$. Level of OI implied the types of action which must be taken by the authority. No preventive action is done when OI is reported as level

1, but when OI achieves level 2, the mosquito breeding inspection and larviciding activities will be initiated. Once the ovitrap index achieves or exceeds 30%, *Aedes* mosquito survey, search and destroy, thermal fogging and ULV will be mobilised. Unfortunately, the control measures based on OI as outlined by the Ministry of Health only comes into practice when there are dengue cases reported.

Climatic factors may affect the dengue ecology and incidence like mosquito population, virus replication, and dynamic interactions between mosquito and human (32). However, in this study, relative humidity was found not correlated with total *Aedes* population in the study area (Table I). This factor may point out that other causality based on observations of the study area such as the existence of numerous natural and artificial containers as well as the concrete drainage system which may trigger breeding in the study area. This situation is in line with the study by Arcari et al., (33) that found the impact of relative humidity was not significant in dengue cases in Indonesia and Malaysia. The local relative humidity in these areas were within the range of 70–80% throughout the year.

Despite finding no significant correlation between temperature and total *Aedes*, temperature is one of the most vital climatic factors for dengue transmission. Evidence has proven that rising temperature within the range of 25 °C to 30°C may accelerate viral replication rate, promote *Aedes* mosquitoes' oviposition rate, larvae development and survival (34), and increase female mosquitoes' blood feeding behaviours (32). Our study showed maximum *Aedes* mosquitoes population collected at the average temperature of 28.1 °C at KV, 29.5 °C at JS and 28.8 °C at PJ (Fig. 4).

Fan et al., (35) have stated that between 22°C and 29 °C was the peak positive temperature-dengue association. This range of temperature was also closed to the study by Shen et al., (36) at Guangzhou, China with a temperature-dengue associations between 21.6°C – 32.9 °C. The temperature range between 22 °C to 27 °C were optimum for dengue fever transmission in the tropical climate zone and subtropical climate zone in Australia (37).

Although no significant relationship of rainfall and the dengue cases and total *Aedes* population was recorded in this study (Table I), rainfall can be an important part in dengue transmission by creating a favourable breeding site to *Aedes* mosquito. The population of *Aedes* mosquitoes were higher starting from June 2019 until August 2019 (Fig. 4) when the moderate total rainfall was recorded with the reading around 116.8 mm to 123.2 mm. After any rainy events, the number of dengue cases increases in the first few months due to plausible tendency of rainwater accumulation (38). This pattern is shown in a study in Dhaka which showed that rainfall

increases dengue incidence (39). Rainfall also has been reported to cause increasing dengue incidence by increasing *Aedes* mosquito density in Puerto Rico (40). However, higher intensity of rainfall in short time effect can clear out breeding site, eggs and larvae, causing negative impact on mosquito populations but in the long haul, intense rainfall may create abundant breeding sources (32). The influence of rainfall on dengue fever is related to multiple factors such as rainfall frequency, duration, intensity and magnitude as well as human-induced factors such as cleanliness, storage patterns of household and management of drainage systems (41).

ANN in other studies such as Hasim et al. (42) also showed dispersed distance, influenced by high complexity of the ecology. Dispersed spatial pattern is more difficult to provide control measures than clustered pattern (42-43). The dispersed distribution shown on dengue cases could imply that implementing effective control, prevention and surveillance programme would not be as direct as clustered pattern which is driven by a factor causing dengue epidemiology in an area (20). Dispersed pattern in dengue cases indicates multiple factors may cause dengue epidemiology ranging from socioeconomic, environmental, land use changes, breeding areas, which require further spatial and temporal analysis in this study area.

CONCLUSION

In brief, the prevalence and transmission of dengue fever is a complex and multi-factor process. Although the mosquito population is highest in JS, the DF cannot happen without the presence of dengue virus itself and humans as the host. *Aedes albopictus* was more dominant outdoor while *Aedes aegypti* was more prevalent indoors. Thus, VBDs management should be more focused based on their breeding site. From this study, no significant relations between total *Aedes*, dengue cases and meteorological factors may be masked by other factors. Meteorological climate variability may be one of the plausible factors causing dengue fever transmission, especially associated with the growth of dengue fever vectors, *Aedes* mosquitoes. The higher presence of *Aedes* population in the area can be regarded as a higher potential for transmission of dengue fever. This study provides beneficial data for the health authorities in improving their integrated vector management and assists them in establishing the best method to be used for control and prevention of DF by focusing on their breeding site in South Seberang Perai district, Penang.

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