LARVAL SURVEILLANCE AND HABITAT CHARACTERIZATION OF DENGUE VECTORS IN TANGKAK, JOHOR, MALAYSIA

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ABSTRACT

Entomological surveillance based on larval surveys, provides vital information for dengue management. In addition, the knowledge on breeding habitat of *Aedes* species is crucial to provide better understanding of their population densities and distribution. The larval surveillance method was conducted in ten outbreak areas in Tangkak District, Johore, Malaysia. The larval surveillance indices; House Index (HI), Container Index (CI), and Breteau Index (BI) were evaluated to measure the density of *Aedes* mosquito population. Then, habitat characterization was analyzed by calculating the prevalence, productivity and efficiency of the collected container. Approximately 1163 houses were inspected in Tangkak District and 86 (7.4%) houses were classified as positive premise. The highest HI were found in Taman Sialang (HI: 25 %) meanwhile the highest CI was recorded at Taman Jalan Ong Siong (CI: 71.4%). Eight out of 10 localities were found exceed the BI threshold value of 5%. The highest BI was logged in Taman Sialang (BI: 70%). *Ae. albopictus* represent 58% of the collected larvae meanwhile another 42% was *Ae. aegypti*. Some of the most effective container that contribute to the *Aedes* breeding in the study area were CID2: Flower pot, CID3: Plastic scrap, and CID4: Pail, with the productivity of 30%, 27%, and 19.8%, respectively. However, only CID2 and CID3 indicate higher prevalence of *Aedes* larvae with container prevalence of 31.1% and

26.9% respectively. In overall, CID2 was found to be the most preferred container for *Aedes* breeding since this container record the highest container efficiency (CE) value of 9.6%. The findings of this study could assist the health authority in the early prevention and control of dengue outbreak in Tangkak, Johor.

Keywords: Dengue, larval surveillance, habitats characterization, Tangkak

ABSTRAK

Pengawasan entomologi berdasarkan kajian larva menyumbang kepada maklumat utama pengurusan penyakit denggi. Selain itu, ilmu pengetahuan mengenai habitat berkaitan spesies *Aedes* adalah sangat penting untuk memahami kaitannya dengan kepadatan populasi dan taburan nyamuk**.** Kajian pengawasan larva ini dijalankan di10 kawasan wabak di Tangkak, Johor, Malaysia. Kajian pengawasan larva; Indeks Rumah (HI), Indeks Bekas (CI), dan Indeks Breteau (BI) dinilai bagi mengukur kepadatan populasi nyamuk *Aedes*. Kemudian, pencirian habitat dianalisis dengan mengira kadar prevalens, produktiviti dan keberkesanan bekas yang dikutip. Sebanyak 1163 buah rumah telah diselidik di daerah Tangkak dan 86 (7.4%) rumah diklasifikasikan sebagai positif. HI yang paling tinggi dicatatkan di Taman Sialang (HI: 25 %) manakala CI yang paling tinggi rekodkan di Taman Jalan Ong Siong (CI: 71.4%). Lapan daripada 10 lokaliti berada pada Kadar BI melebihi 5%. BI yang paling tinggi dicatatkan di Taman Sialang (BI: 70%). *Ae. albopictus* mewakili 58% daripada jumlah larva yang dikutip manakala 42% lagi adalah *Ae. aegypti***.** Antara bekas yang paling efektif dalam pembiakan nyamuk *Aedes* adalah CID2: Pasu bunga, CID3: Bekas plastik dan CID4: Baldi yang menunjukkan produktiviti bekas sebanyak 30%, 27%, and 19.8%. Walau bagaimanapun, hanya CID2 dan CID3 menunjukkan prevalens larva nyamuk yang lebih tinggi dengan peratusan prevalens bekas sebanyak 31.1% dan 26.9%. Secara keseluruhannya, CID2 merupakan bekas yang menjadi pilihan nyamuk *Aedes* untuk bertelur kerana mencatatkan keberkesanan bekas tertinggi (9.6%). Penemuan kajian ini boleh digunakan untuk membantu pihak berkuasa kesihatan di dalam pengawalan dan pencegahan awal wabak di Tangkak, Johor.

Kata kunci: Denggi, kajian pengawasan larva, pencirian habitat, Tangkak

INTRODUCTION

Mosquitoes can be found all over the world and commonly known to pose a significant threat to public health. There are approximately 3500 mosquito species in the world, of which 200 species can spread diseases to humans (Rufalco et al. 2016). The common fear of mosquitoes is their role as vectors that can spread diseases such as dengue, malaria, filariasis, yellow fever etc. In Malaysia, the outbreak of dengue cases is one of the major problems caused by mosquitoes which is a global issue as well. Dengue is transmitted by *Aedes* mosquitoes, specifically *Ae. aegypti* and *Ae. albopictus* (Elia-Amira et al. 2019). Despite the close monitoring and continuous effort from the Ministry of Health, Malaysia and other local authorities, the number of dengue cases is still on the rise.

One of the important elements in dengue prevention program is vector surveillance. This method employs several entomological indicators that have been developed to assess the risk of outbreaks occurring. The indicators include House Index (HI), Breteau Index (BI) and Container Index (CI) (Dom et al. 2016). The surveillance approach is important to determine the factors related to dengue transmission in order to prioritize areas and seasons for vector control (WHO 2020). In addition to entomological vector surveillance, the knowledge on where mosquitoes breed also crucial for mosquito control strategies (Dejenie et al. 2011). The profiling of the mosquito's breeding habitat is critical in providing baseline data for the improvement in the implementation of the programs for vector control by the local authorities.

Most of the surveillance studies were conducted at the urban and congested area such as Kuala Lumpur, Penang and Selangor (Lau et al. 2013; Saifur et al. 2012). The surveillance studies conducted at suburban areas were rarely reported although the increasing number of dengue cases have been recorded (Yusof et al. 2017). In this study, the suburban area of Tangkak, Johore was selected as the study area due to the high occurrence of dengue outbreak cases in certain localities. The dengue cases in this area increased from 21 in 2013 into 114 cases in 2018 (MOH 2018). The objectives of this study are to perform the *Aedes* larvae surveillance and investigate the breeding container characteristics (types of container) at the study area. The characterization of dengue vector habitat is important to determine their influence on the distribution and densities of mosquitoes thus explaining the variations observed in dengue transmission intensity.

MATERIALS AND METHODS

Study Areas

Tangkak Johor is divided into six counties (*mukim*) which are Kundang, Bukit Serampang, Serum, Gersik, Tangkak and Kesang, Johore, Malaysia. The district has a tropical climate with a significant rainfall at 2,698 mm yearly with a moderate annual temperature around 27°C. Ten dengue outbreak locations in Tangkak district have been identified from E-Notifikasi and E-Wabak system, Ministry of Health, Malaysia (MOH 2018), and adopted as study areas for this research. A dengue outbreak area can be defined as the area when two or more dengue cases occur within a 200-meter radius of the index case within 14 days of the index case notification date (iDengue 2020).

Sample Collection and Statistical Analysis

A cross-sectional ecological comparative study was carried out to examine the distribution of *Aedes* mosquito and its breeding site characteristics. The larval surveillance was conducted by a group of three between 9 am to 12 pm from October 2018 to April 2019. A minimum of 100 houses were inspected at every location for detection of larval breeding of *Aedes* mosquito. All the containers from the study sites were inspected but only positive containers were collected for larval identification. The House Index (HI), Container Index (CI) and Breteau Index (BI) were calculated according to equation 1, 2 and 3 (Dom et al. 2016).

$$\text{House index (HI)} = \frac{No.\,of\ Positive\ house}{Total\ inspected\ house} \times 100 \qquad (1)$$

$$\text{Container index (CI)} = \frac{No.\,of\ positive\ container}{Total\ \ no.\,of\ inspected\ container} \times 100 \qquad (2)$$

$$\text{Breteau index (BI)} = \frac{No.\,of\ positive\ container}{Total\ inspected\ house} \times 100 \qquad (3)$$

The type of inspected container from the study site were recorded and will be given a container identification identity (CID) based on the WHO operational guidelines for assessing the productivity of *Aedes* breeding sites (WHO 2011). In this study, all the positive containers were classified into 8 CID which are CID1: Toilet flush, CID2: Flowerpot, CID3: Plastic scrap, CID 4: Pail, CID5: Spray can, CID6: Natural containers, CID7: Water storage and CID8: Plastic chair. CID1, CID4 and CID7 were further classified as indoor setting, meanwhile CID2, CID3, CID5, CID6 and CID8 were classified as outdoor setting. The prevalence, productivity and efficiency of each type of containers were calculated according to equation 4, 5 and 6 (Madzlan et al. 2018).

$$\text{Container Prevalence} = \frac{No. of\ container\ in\ each\ CID}{Total\ number\ of\ all\ CID} \times 100 \quad (4)$$

$$\text{Container Productivity} = \frac{No. of\ larvae\ in\ each\ CID}{Total\ collected\ larvae} \times 100 \quad (5)$$

$$\text{Container Efficiency} = \frac{Container\ Productivity}{Container\ Prevalence} \quad (6)$$

RESULTS AND DISCUSSION

Larval Surveillance

There are various types of dengue vector surveillance indicator available such as larval, pupae and adult surveys. The selection of suitable indicators depends on the research objectives, time consideration, resources and infestation level of the selected area. The most commonly used vector surveillance indicator to evaluate the population densities of the mosquito in developing countries is larval survey which include determination of House Index (HI), Container Index (CI) and Breteau Index (BI) (Dom et al. 2016). Destruction of *Aedes* breeding habitats through larvaciding and communal work are required if the AI, CI and BI threshold value were more than 1%, 10% and 5% respectively (MOH 2012).

Figure 1 shows the result of larval surveillance (HI, CI, and BI) for both *Ae. aegypti* and *Ae. albopictus* from the selected ten outbreak areas in Tangkak.. Approximately 1163 houses were inspected in Tangkak District and 86 (7.4%) houses were classified as positive premise. All the localities reported the HI above the threshold limit of 1%. The two highest HI index were found in Taman Sialang (HI: 25 %) followed by Taman Tangkak Jaya (HI: 13.3%). Only three locations were found below than 5% which were Taman Serom Utama (HI: 3.3%), Taman Mulia (HI: 3.6%) and Kampung Baru (HI: 2.7%) respectively. Generally, HI represent the percentage of positive houses for *Aedes* larvae. A HI greater than 5% for any locality is always used as indicator for dengue sensitive area (Basker et al. 2013). HI has been used to determine risk areas for control measures. In addition, it also important to indicate a potential spread of virus through an area once an infected case is established.

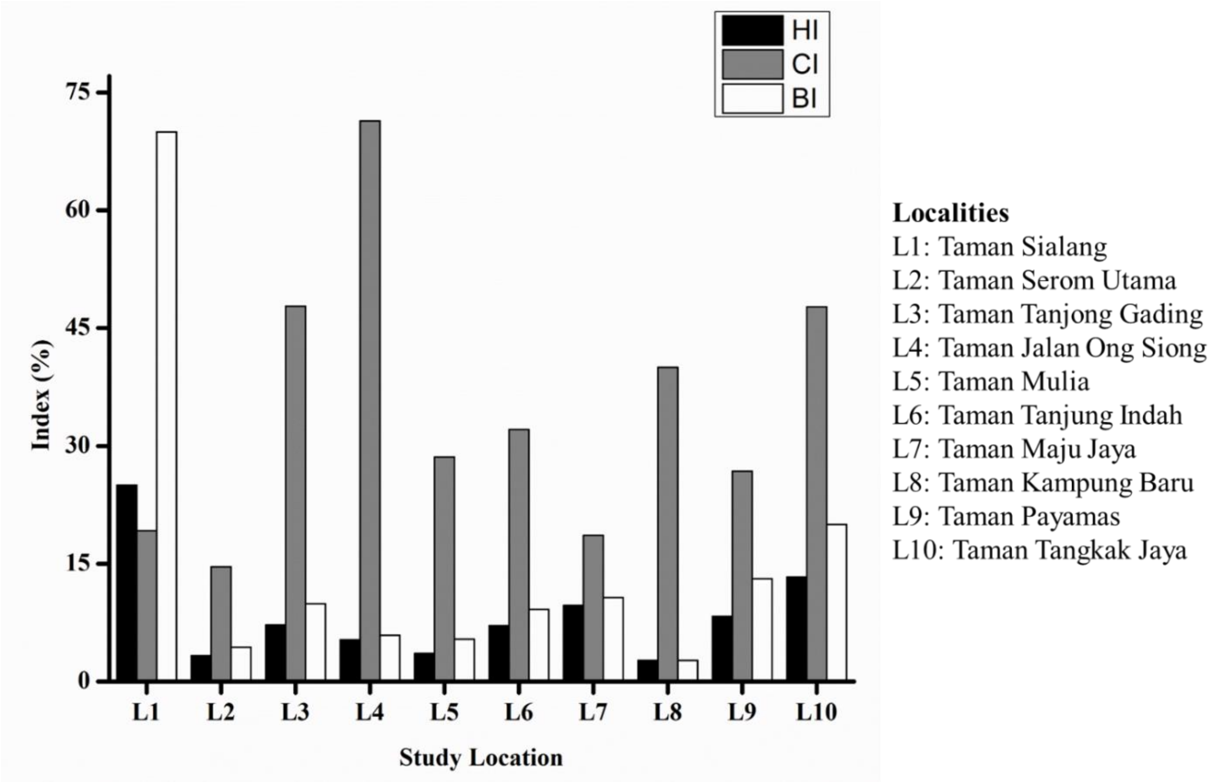

Figure 1. *Aedes* Larval Surveillance from ten outbreak locations in Tangkak, Johor from October 2018 – April 2019

House Index (HI) only shows the number of positives houses without considering the number of positive containers available. Thus, additional information of Container Index (CI) was required to provide information on the proportion of positive container against the total number of containers in the study area. A total of 283 out of 798 containers are found positive with *Aedes* breeding across the ten localities. All the localities recorded the CI above the threshold limit of 10%. The highest CI was recorded at Taman Jalan Ong Siong (CI: 71.4%) followed by Taman Tanjung Gading (CI: 47.8%) and Taman Tangkak Jaya (CI: 47.4%). Based on the field observation, most of the houses in Taman Jalan Ong Siong were vacant and abandoned. According to Seng & Jute (1994), abandoned house and vacant plots provide a suitable habitat for *Aedes* breeding. The lowest CI was found in Taman Serom Utama which indicates 14.6%. Higher CI value portray the presence of large potential breeding containers with high density *Aedes* mosquito infestation. Regular activity for destruction of potential breeding sites are required to reduce the density of mosquito population.

The Breteau Index (BI) establishes a relationship between positive containers and houses and is considered to be the most informative index. Based on the surveillance activity, eight out of 10 localities were found above the threshold value of 5%. Two of the localities scored more than 20% which require immediate implementation of comprehensive vector control program (MOH 2012). The highest BI score was detected in Taman Sialang Utama (BI: 70%) followed by Taman Tangkak Jaya (BI: 20%). Field observation identified the sanitation at Taman Sialang and Taman Tangkak Jaya as relatively poor. In addition, regular occurrence of water shortage require many inhabitants to store their rainwater in their house that contribute to the higher breeding site at both locations. The lowest BI was observed in Kampung Baru (BI: 2.7%). The dominant demographic profiles of the community in Kampung Baru are pensioners and senior citizens. This community always support the health campaign organized by health ministry and other non-government organizations (NGOs). Good environmental management in this area can minimize vector propagation and human contact with the vector pathogen.

Distribution of Mosquito Species

All the larvae from 283 positive breeding sites were collected to determine the distribution of the mosquito's species in the study area. The larvae collected from CID1, CID4 and CID7 were categorize as indoor environment meanwhile CID2, CID3, CID5, CID6 and CID8 were classified as outdoor setting. A total of 1199 mosquito larvae were collected with 67.4% of the larvae were found in the outdoor setting, meanwhile another 32.6% were found indoors. The species composition consist of 42% *Ae. aegypti* and 58% *Ae. albopictus* (Figure 2). Generally, *Ae. albopictus* is the dominant species found across the study site. *Ae. albopictus* is the prominent species found in urban and suburban area (Rozilawati et al. 2015). It has been known as an outdoor species that breeds in a broad range of natural environment, as well as artificial water holding containers. In contrast, *Ae. eegypti* prefer indoor environment such as under furniture, inside closets and on any dark and moist surface (Dunbar et al. 2019).

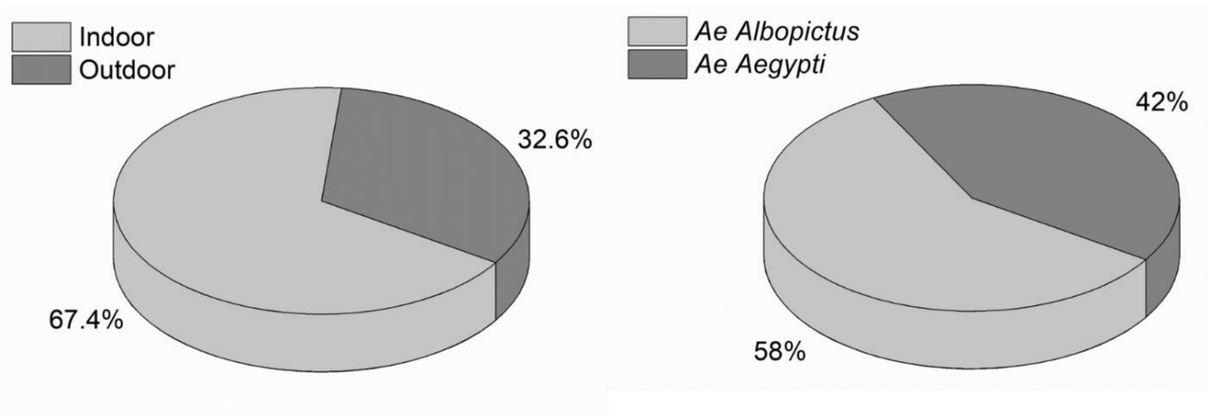

Figure 2: Distribution of *Aedes* distribution and species

Mosquito Breeding Container Characteristics

Container prevalence, productivity, and efficiency

The productivity, prevalence and efficiency of each category of containers in selected study area were evaluated and shown in Table 1. The container productivity (CPt) was calculated to determine *Aedes* breeding preference on various types of CID. Meanwhile container prevalence (CPv) was used to determine the abundance of *Aedes* larvae in each type of CID. Across the 10 localities, CID2: Flower pot, CID3: Plastic scrap and CID4: Pail were found to be the most productive container with the CP value of 30%, 27.1% and 19.8% respectively. These containers hold the most number of larvae compared to other CID. Meanwhile, water storage and plastic chair were the least preferable site with the CP value of 2.1% and 1.6%. CID2 and CID3 also represent the highest container prevalence with the CPv value of 31.1% and 26.9% respectively. However, CID4 only indicate 6% of CPv despite of high value of CPt.

Table 1. Container prevalence, productivity and efficiency

CID	**No of larvae**	**No of container**	**[a]Container prevalence (CPv)**	**[b]Container productivity (CPt)**	**[c]Container efficiency (CE)**
1:Toilet flush	128	15	5.3	10.7	2.0
2: Flower pot	359	88	31.1	30.0	9.6
3: Plastic scrap	325	76	26.9	27.1	1.0
4: Pail	238	17	6.0	19.8	3.3
5: Spray can	45	12	4.2	3.8	0.9
6: Natural containers	60	8	2.8	5.0	1.8
7: Water storage	25	45	15.9	2.1	0.1
8: Plastic chair	19	22	7.8	1.6	0.2

Note:
[a]Number of container in each CID/Total containers*100
[b]Number of larvae in each CID/all collected larvae*100
[c]Container productivity/Container prevalence.
Total container: 283 and Total Larvae: 1199.

The information from CPt and CPv can be used to estimate the container's efficiency (CE). The most efficient container is CID2: Flower pot (CE: 9.63). CID2 is one of the most suitable places for mosquitoes to lay their eggs. Trees, shrubs and flowers planted near the resident's housing provide harborage sites for mosquitoes (Sunahara et al. 2002). Getachew et al. (2015) reported that female mosquitoes tend to lay their eggs in household containers such as flowerpot, tires, cement tanks and sink pipe. Frequent contact with water for example rainwater, will allow the mosquito's eggs to hatch. Mosquito breeding will increase if the residents did not regularly manage their compound and ensure unused containers are discarded properly (Saleeza et al. 2013). Pail (CE: 3.3) was found to be the second highest efficient container. The residents in Tangkak usually use pails as a routine activity to wash their cars, doing laundry, and watering plants. It is usually left outside of the house without being covered. The remaining water from these activities or rainwater can accumulate in the pails leaving a clean and stagnant water exposed to the mosquitoes. This condition makes it suitable for mosquito breeding (Sahay 2018).

The lowest efficient container is CID7: Water storage (CE: 0.1). Although the water storage provided a clean and stagnant water that is suitable for mosquito breeding, the regular prevention control by Tangkak Health District by distribution of free larvacide to the residents reduces the infestation of mosquitoes. Treating standing water with larvacide such as the Abate is one of the easiest and most effective way to control mosquitoes and other insects in a community. One of the functions is to combat the juvenile *Aedes* before they reach maturity and it can last for three months (Boissière et al. 2012).

CONCLUSION

This study has provide information on larval surveillance of *Aedes mosquitoes* and the characteristics of its breeding container. This surveillance activity allows for the early detection of changes in abundance and species variety, which provides valuable information to health authorities so that control measures can be implemented to decrease the impact of vector populations on public health. In addition, the present study also observed that poor waste management that probably contributed to the potential source of *Aedes* breeding. It is recommended that environmental management be implemented in order to eradicate these species with the participation of the local community.

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REFERENCES

Basker, P., Kannan, P., Porkaipandian, R.T., Saravanan, S., Sridharan, S. & Kadhiresan, M. 2013. Study on entomological surveillance and its significance during a dengue outbreak in the District of Tirunelveli in Tamil Nadu, India. *Osong Public Health and Research Perspectives* 4(3): 152-158.

Boissière, A., Tchioffo, M.T., Bachar, D., Abate, L., Marie, A., Nsango, S.E., Shahbazkia, H.R., Awono-Ambene, P.H., Levashina, E.A., Christen, R. & Morlais, I. 2012. Midgut microbiota of the malaria mosquito vector Anopheles gambiae and interactions with Plasmodium falciparum infection. *PLoS Pathogens* 8(5): e1002742.

Dejenie, T., Yohannes, M. & Assmelash, T. 2011. Characterization of mosquito breeding sites in and in the vicinity of tigray microdams. *Ethiopian Journal of Health Sciences* 21(1): 57-66.

Dom, N.C., Madzlan, M.F., Nur, S., Hasnan, A. & Misran, N. 2016. Water quality characteristics of dengue vectors breeding containers. *International Journal of Mosquito Research* 3(1): 25-9.

Dunbar, M.W., Correa-Morales, F., Dzul-Manzanilla, F., Medina-Barreiro, A., Bibiano-Marín, W., Morales-Ríos, E., Vadillo-Sánchez, J., López-Monroy, B., Ritchie, S.A., Lenhart, A. & Manrique-Saide, P. 2019. Efficacy of novel indoor residual spraying methods targeting pyrethroid-resistant Aedes aegypti within experimental houses. *PLoS Neglected Tropical Diseases* 13(2): e0007203.

Elia-Amira, N.M.R., Chen, C.D., Lau, K.W., Low, V.L., Lee, H.L., Cyril-Tham, Y.S. & Sofian-Azirun, M. 2019. Dengue vector surveillance in West Coast and Kudat division, Sabah, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* 50(1): 55-63.

Getachew, D., Tekie, H., Gebre-Michael, T., Balkew, M. & Mesfin, A. 2015. Breeding sites of *Aedes aegypti*: potential dengue vectors in Dire Dawa, East Ethiopia. *Interdisciplinary Perspectives on Infectious Diseases.* 2015:706276. doi: 10.1155/2015/706276.

Lau, K.W., Chen, C.D., Lee, H.L., Izzul, A.A., Asri-Isa, M., Zulfadli, M. & Sofian-Azirun, M. 2013. Vertical distribution of Aedes mosquitoes in multiple storey buildings in Selangor and Kuala Lumpur, Malaysia. *Tropical Biomedicine* 30(1): 36-45.

iDengue. 2020. Statistik Kes Denggi Malaysia. https://idengue.mysa.gov.my/pdf/statistik.pdf#page=3 [20 December 2020].

Madzlan, F., Dom, N.C., Zakaria, N., Hasnan, S.N.A., Tiong, C.S. & Camalxaman, S.N. 2018. Profiling of dengue vectors breeding habitat at urban residential areas in Shah Alam, Malaysia. *Serangga* 22(2): 185-216.

MOH (Ministry of Health Malaysia). 2012. Garis panduan pencegahan dan kawalan penyakit. https://www.moh.gov.my/moh [20 December 2020].

MOH (Ministry of Health Malaysia). 2018. Laporan Denggi Tahunan Pejabat Kesihatan Daerah Tangkak, Johor.

https://jknjohor.moh.gov.my/bmv/ [20 December 2020].

Rozilawati, H., Tanaselvi, K., Nazni, W.A., Masri, S.M., Zairi, J., Adanan, C.R. & Lee, H.L. 2015. Surveillance of *Aedes albopictus* Skuse breeding preference in selected dengue outbreak localities, Peninsular Malaysia. *Trop Biomed.* 32(1): 49-64.

Rufalco, M.P., Schweigmann, N., Bergamaschi, D.P. & Sallum, M.A.M. 2016. Larval habitats of Anopheles species in a rural settlement on the malaria frontier of southwest Amazon, Brazil. *Acta Tropica* 164: 243-258.

Sahay, S. 2018. Climatic variability and dengue risk in urban environment of Delhi (India). *Urban Climate* 24: 863-874.

Saifur, R.G., Hassan, A.A., Dieng, H., Ahmad, H., Salmah, M.R.C., Satho, T., Saad, A.R. & Vargas, R.E.M. 2012. Update on temporal and spatial abundance of dengue vectors in Penang, Malaysia. *Journal of the American Mosquito Control Association* 28(2): 84-92.

Saleeza, S.N.R., Norma-Rashid, Y. & Sofian-Azirun, M. 2013. Mosquito species and outdoor breeding places in residential areas in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* 44(6): 963-969.

Seng, C.M. & Jute, N. 1994. Breeding of Aedes aegypti (L.) and *Aedes albopictus* (Skuse) in urban housing of Sibu town, Sarawak. *Southeast Asian Journal of Tropical Medicine and Public Health* 25: 543-543.

Sunahara, T., Ishizaka, K. & Mogi, M. 2002. Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. *Journal of Vector Ecology* 27: 8-20.

WHO (World Health Organization). 2020. Dengue control; vector surveillance. https://www.who.int/denguecontrol/monitoring/vector_surveillance/en/ [20 December 2020].

WHO (World Health Organization) 2011. Operational guide for assessing the productivity of *Aedes aegypti* breeding site. https://www.who.int/tdr/publications/tdr-research-publications/sop-pupal-surveys/en/ [20 December 2020].

Yusof, M.M., Dom, N.C. & Zainuddin, A. 2017. Spatial pattern distribution of dengue fever in sub-urban area using GIS tools. *Serangga* 21(2): 127-148.