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CLIMATIC INFLUENCES ON *AEDES* MOSQUITO LARVAE POPULATION

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ABSTRACT The impact of climate on *Aedes* larval population was studied. Monitoring of population was done using ovitraps. Ovitrap provides a simple and convenient monitoring method for *Aedes* surveillance as the number of eggs laid in a standard trap within a specific time period give a relative measurement of the number of mosquito in the same area. Ovitraps were set outdoors in selected dengue prone areas in Desa Pandan, Kuala Lumpur for 66 weeks. Weather stations, consisting of a temperature and relative humidity data logger and an automated rain gauge were installed at key locations in the study site. Week-to-week variations of larval densities were correlated against variations in the individual climatic parameters. Results of the study showed that there was a close relationship between the heavy rainfall and the increased mosquito population in the study sites. The study showed that previous week rainfall plays a significant role in increasing the mosquito population.

ABSTRAK Kajian kesan iklim ke atas populasi larva *Aedes* telah dijalankan. Pemantauan populasi telah dilakukan menggunakan ovitrap. Ovitap membekalkan kaedah pemantauan *Aedes* yang ringkas dan mudah, bilangan telur pada perangkap piawai dalam lingkungan jangkamasa yang khusus memberi ukuran relatif bilangan nyamuk di kawasan yang sama. Ovitrap telah diset di luar rumah di kawasan berisiko denggi yang telah dipilih di Desa Pandan, Kuala Lumpur selama 66 minggu.. Stesen cuaca, terdiri daripada data log suhu dan kelembapan relatif, dan alat penyukat jumlah hujan telah dipasang di lapangan. Data mingguan variasi kepadatan larva dan parameter iklim individu telah dikorelasikan. Purata data mingguan jumlah hujan, suhu (minimum dan maksimum) dan kelembapan relatif (minimum dan maksimum) telah digunakan sebagai pembolehubah tidak bersandar. Kajian menunjukkan jumlah hujan pada minggu sebelumnya berperanan secara signifikan dengan populasi nyamuk di mana jumlah hujan yang tinggi akan meningkatkan populasi nyamuk di kawasan kajian.

(**Keywords**: Dengue, *Aedes aegypti*, *Aedes albopictus*, climatic parameter.)

INTRODUCTION

Dengue viral infection is one of the most important public health problems in tropical countries. It was first described by Skae [1] in 1902 following an outbreak in Penang almost exactly a century ago in November-December 1901 [1]. Traders and seafarers who brought in *Aedes aegypti* from Africa, however, could have introduced dengue into Malaysia much earlier. The spread of dengue throughout Malaysia is thought to have followed the pattern of the spread of *Ae. aegypti* that replaced the local *Ae. albopictus* as the main carrier of dengue viruses [2]. By 1960s, dengue had become endemic in Malaysia [3].

Dengue is caused by transmittal of dengue virus to man through mosquito bites. Transmission cycles of dengue virus depend on the interrelationship between the virus and its mosquito vector, which is influenced by environmental conditions [4]. Climate change would directly affect disease transmission by shifting the vector's geographic range and increasing reproductive and biting rates and by shortening the pathogen incubation period [5]. Heat waves in Europe, rises in global mean sea level, summer droughts and wild fires, more intense precipitation, and increasing numbers of large cyclones and hurricanes may be typical example of extreme climate phenomena related to global warming [6].

Temperature directly affects the rate of development of different mosquito life stages, as well as dengue viral replication. Higher ambient temperatures enhance virus replication and shorten the extrinsic incubation period (EIP) in the vectors [7, 8], thereby increasing vectorial efficiency. Mosquito survival is also temperature dependent, which has an influence on the

persistence of free water and relative humidity [9, 10, 11]. For example, dengue outbreaks in the Indian subcontinent frequently occur during the hot, dry season because A. aegypti breeds abundantly in the reservoirs of desert coolers. Aegypti-borne viral disease were widespread in temperature latitude during the Little Ice Age (1600-1700 AD) because water for human consumption was stored in rain barrels, which supported the populations of mosquitoes needed to transmit viruses that were introduced during summer seasons [12]. Temperatures affect the length of the gonotrophic cycle, contributing another factor correlated with seasonality of dengue in tropical Southeast Asia [13].

Warmer temperature can increase the transmission rates of DHF in various ways. First, warmer temperature may allow vectors to survive and reach maturity much faster than at lower temperature [14]. Second, warmer temperature may reduce the size of mosquito larvae resulting in smaller adults that have high metabolism rates, require more frequent blood meal, and need to lay eggs more often [15, 16, 17]. Third, environmental temperature has a marked effect on the length and efficiency of the extrinsic incubation periods (EIPs) of arboviruses in their vectors [14, 17]. This means that mosquitoes exposed to higher temperature after ingestion of virus become infectious more rapidly than mosquitoes of the same species which are exposed to lower temperatures [14]. Therefore the transmission of arbovirus may increase under warmer conditions as more vector mosquitoes become infectious within their life-span. Higher temperature may reduce the length of viral extrinsic incubation periods (EIPs) in mosquitoes [7, 18, 19].

In contrast, longitudinal studies in Puerto Rico demonstrated a positive correlation between rainfall and vector abundance, strongest in the dry, south coastal portions of the island. [20]. Precipitation affects adult female mosquito density. An increase in the amount of rainfall leads to an increase in available breeding sites which, in turn leads to an increase in the number of mosquitoes. An increase in the number of adult female mosquitoes increases the odds of a mosquito obtaining a pathogen and transmitting it to a second sensitive host [21]. A distinct seasonal patter in DHF outbreaks is evident in most places. In tropical regions where monsoon weather patterns predominate, DHF hospitalization rates increase during the rainy season and decrease several months after the cessation of the rains [22, 23]. Indoor larval habitats are generally less affected by fluctuations in rainfall compared to outside habitats [24].

Dengue had caused hundreds of epidemics and pandemics thus affecting millions of people throughout the world. Unfortunately, there is still no effective vaccine or specific treatment for dengue. Therefore, dengue surveillance must be carried out in order to control any outbreak by detecting early warning of dengue outbreak. Since dengue is caused by transmittal of dengue virus to man through mosquito bites, an outbreak will be started once the dengue virus is introduced into a human population and circulates within human population [25]. Nakhapakorn and Tripathi [26] reported that, the best way to control an outbreak is to prevent dengue from happening. Ovitraps are simple devices to monitor adult population easily. The development of ovitrap provided a potential new approach for *Aedes* surveillance [27]. Other than that, prevention of dengue is possible if the knowledge about relationship of DF and DHF with climatic is elucidated. This means that, the meteorological data such as rainfall, humidity and temperature are important to predict the outbreak from happening.

MATERIALS AND METHODS

Ovitrap surveillance

Dengue prone area in Desa Pandan, Kuala Lumpur was chosen for this study. The study was conducted from November 2007 until January 2009. A pilot study was conducted for 3 weeks to determine the sample size (number of ovitraps per locality) for the study area. During the pilot study, 20 ovitraps were located randomly outsite occupied house and number of larvae collected was used to estimate the number of ovitrap needed for the study area. Based on this pilot study, a total of 40 ovitraps were set at the study site in Desa Pandan. Ovitraps have been used as a standard tool in studies on mosquitoes [28, 29]. An ovitrap consists of a plastic container of 7 cm in diameter and 9 cm in height, of which the wall of the container is black in colour. An oviposition paddle made from hardboard (10 cm × 3.0 cm × 2.5 cm) was placed into each ovitrap with the rough surface upwards. Each ovitrap was filled with tap water to a level of 5.5 cm [30]. After 7 days, all

ovitraps were collected and replaced with fresh ovitrap and paddle. Ovitraps were set weekly for 66 weeks and lost or damaged ones were recorded and replaced.

Weather stations, consisting of a temperature and relative humidity data logger and an automated rain gauge were installed at key location in the study sites. Meteorological data such as rainfall, maximum and minimum temperature and relative humidity were recorded during the ovitraps collection. Association between the number of larvae and all the climatic parameter were analyzed by correlation coefficient using SPSS program package (SPSS 11.5 Production Facility)

Larvae identification

Ovitraps collected were brought to the laboratory and the contents were poured into a plastic container filled with seasoned water and allowed to further develop in the laboratory. Primary (1º) identification was conducted during which 4th instar larvae were picked up and identified using standard taxonomic keys under compound microscope. Identified mosquito larvae were segregated according to species and date. Paddles were air dried and soaked in the same ovitrap by adding seasoned water after 24 hours. The following 5 days, secondary (2º) identification was done. Water and paddle in each ovitrap was poured again into the same plastic container. Tertiary (3º) identification was conducted another five days. Larvae of *Ae. aegypti* and *Ae. albopictus* were pooled with maximum of 20 larvae per pool and stored in freezer at -70 ºC for dengue virus detection using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

RNA Extraction for RT-PCR

The larvae were homogenized prior to RNA extraction. Samples were added with 300 µl FBS MEM 2% and ground in chilled eppendorf tube. After that, another 500 µl 2% FBS MEM were added before being centrifuged at 3000 rpm for 15 minutes at 4 °C to obtain the supernatant. RNA extraction was performed by using QIAamp Viral RNA Kit. Briefly, 140 µl supernatant of each sample were added to the microcentrifuge tubes containing 560 µl of prepared buffer AVL. After incubation at room temperature for 10 minutes, 560 µl of absolute ethanol were added to the solution. All the solutions were then transferred to a QIAamp Mini spin column and spun at 8000 rpm for 1 minute. The RNA was then washed by 500 µl of Buffer AW1 and spun at 8000 rpm for 1 minute. The same procedure was repeated with Buffer AW2. Finally, the RNA was eluted by adding 60 µl of elution buffer. The eluted RNA was stored in -20 °C till use.

Detection of dengue virus using Reverse transcriptase-Polymerase Chain Reaction (RT-PCR)

A master mix was prepared using Titan One Tube RT-PCR kit. The dengue virus consensus primers were TCAATATGCTGAAACGCGCGAGAAACCG and TTGCACCAACAGTCAATGTCTTCAGCTTC-3 [31]. The RT step was carried out in a thermocycler (Eppendorf) at 50 ºC for 30 minutes to produce cDNA which was then amplified by the following steps: 94 ºC for 2 minutes as initial denaturation, 94 ºC for 30 seconds as denaturation step, 55 ºC for 30 seconds as annealing step and 68 ºC for 40 seconds as elongation step. The cycle was repeated 34 times before a final extension at 4 ºC. The RT-PCR products were electrophoresed through 1.5% agarose gel and stained with ethidium bromide. The products were viewed under UV light and the resulting bands were photographed with a digital camera.

RESULTS

Figure 1 shows the number of *Ae. aegypti* and *Ae. albopictus* collected for 66 weeks. *Ae. aegypti* was the dominent species, with 10343 larvae collected compared to 5553 larvae of *Ae. albopictus*. *Ae. aegypti* population was highest at epid week 45th during which was 351 larvae were collected, while the highest number of *Ae. albopictus* was 227 larvae, at epid week 50th. A total of 1143 pools of larvae were collected during this period. From these, 741 pools were *Ae. aegypti* and 402 pools were *Ae. albopictus*.

Weekly data of rainfall, temperature (minimum and maximum) and relative humidity (minimum and maximum) were averaged and used as independent variables. **Figure 2** shows the relationship between total number of *Aedes* larvae and rainfall. The highest amount of rainfall was at epid week 40th during which 9.85 inches of rainfall were recorded with 398 *Aedes*

larvae collected. The graph shows that the number of larvae increases when the rainfall at previous week increases, while decreasing rainfall at current week lowers the number of larvae for the subsequent or following week.

No rainfall was recorded at epid week 6th, during which 285 *Aedes* larvae were collected. The temperature recorded at that week was the highest which is 47.9 °C, with minimum temperature 27 °C (**Figure 3**). A total of 181 *Ae. aegypti* and 104 *Ae. albopictus* larvae were collected. The lowest minimum temperature was recorded at 17th epid week, which is 19.6 °C. The number of larvae collected was 116 larvae for *Ae. aegypti* and 80 larvae for *Ae. albopictus*. The higest total number of *Aedes* larvae collected were recorded at 45th epid week when the temperature ranged from 22.7 °C to 42.1°C.

The highest humidity recorded was 97 %, which were at 2nd, 3rd, 12th, 13th and 14th epid week (**Figure 4).** When the temperature recorded was the highest, the relative humidity recorded was the lowest. At 6th epid week during which the temperature was 47.9 °C, the minimum relative humidity was 12 %. As shown in **Figure 4**, when the relative humidity was high, the following week showed higher number of larvae.

Table 1 shows the coefficients of correlation among rainfall, temperature, relative humidity and *Aedes* densities in Desa Pandan area. *Aedes aegypti* showed positive correlation with minimum and maximum temperature, minimum and maximum relative humidity and rainfall, while *Aedes albopictus* only showed positive correlation with minimum temperature and rainfall.

However, none of the pools showed positive result after RT-PCR was conducted, meaning that, there are no larvae that were infected by dengue virus as showed in **Table 2**.

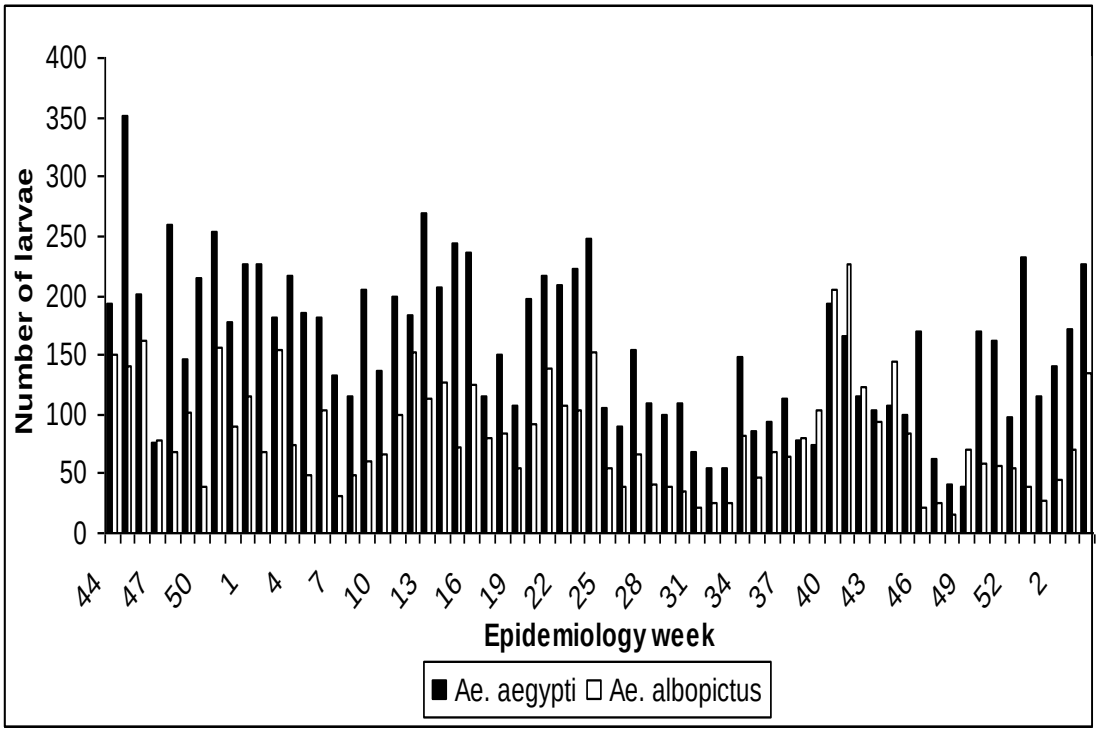

Figure 1. Total number of *Aedes aegypti* and *Aedes albopictus larvae* collected from Desa Pandan using 40 ovitraps

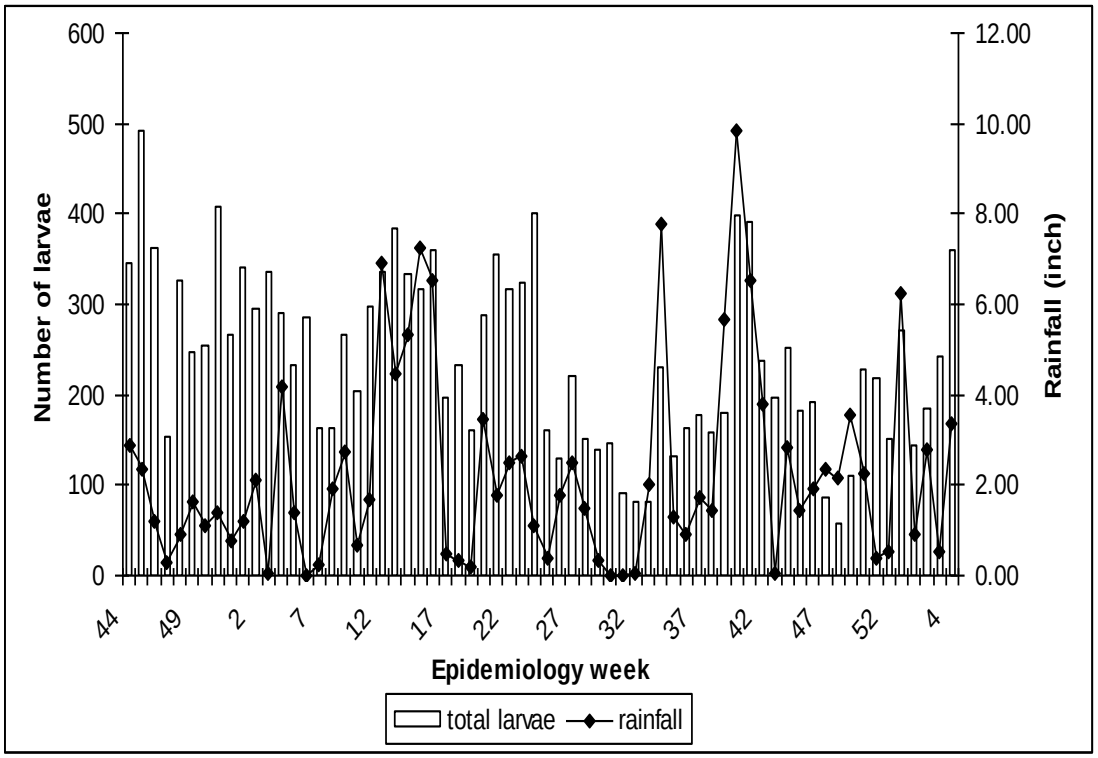

Figure 2. Relationship of total number of *Aedes* larvae and rainfall

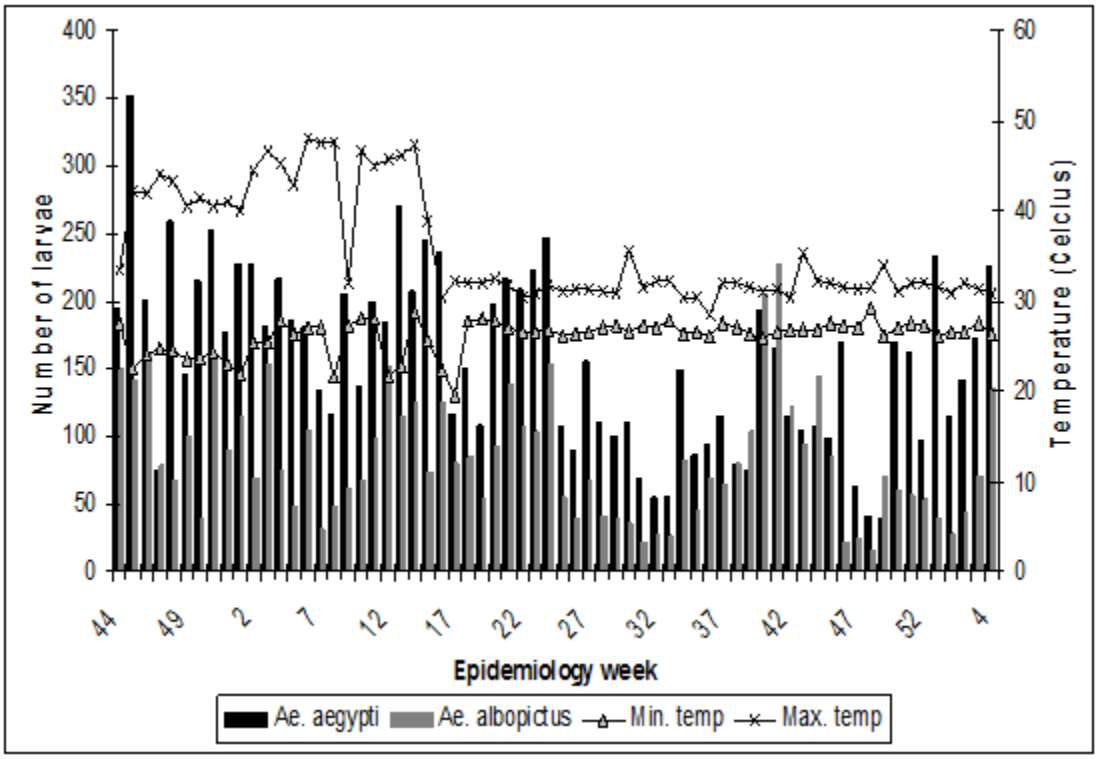

Figure 3. Relationship of total number of *Aedes* larvae and temperature

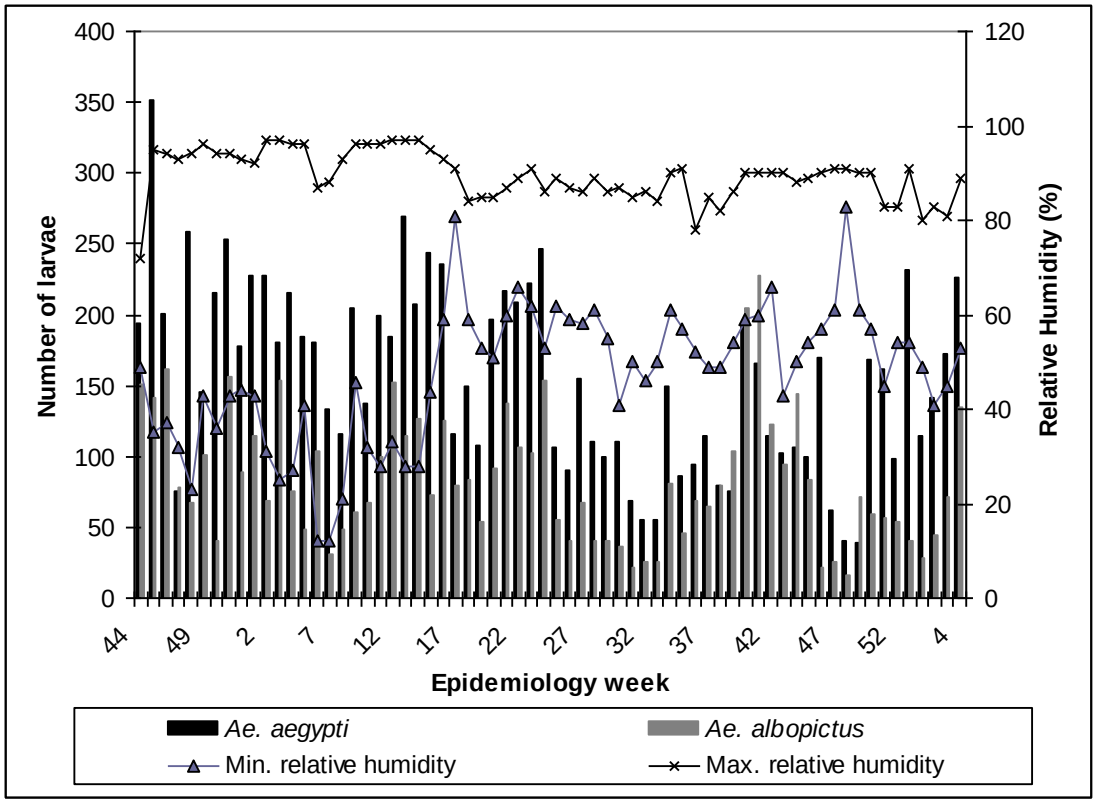

Figure 4. Relationship of total number of *Aedes* larvae and relative humidity

Table 1. Coefficient of correlation among rainfall, temperature, relative humidity and *Aedes* densities in Desa Pandan areas.

Mosquito Species / Level of significant	**Temperature**		**Relative Humidity**		**Previous week rainfall**
	Min.	**Max.**	**Min.**	**Max.**	
Aedes aegypti	-0.339*	0.371*	-0.340*	0.409*	0.293*
Significant level	(0.005)	0.002	(0.005)	(0.001)	(0.017)
Aedes albopictus	-0.261*	-	-	-	0.435*
Significant level	(0.035)	-	-	-	(0.000)

* significant at 5%

Table 2. Result in detection of dengue virus in *Aedes* sp. larvae.

Species	**Total larvae pools**	**Positive dengue**
Aedes aegypti	741	0
Aedes albopictus	402	0

DISCUSSION

In this study, both *Ae. aegypti* and *Ae. albopictus* were identified. The number of *Ae. aegypti* larvae collected was higher than *Ae. albopictus.* This outcome supported previous study of Chen et al. [32] who concluded that, *Ae. aegypti* had become dominant mosquito when they replaced the previously common mosquito, *Ae. albopictus.*

The relationship of rainfall and *Aedes* population is important to determine dengue outbreak. According to Viroj Wiwanitkit [33], the prevalence of dengue infection in central region of Thailand may depend on rainfall. Our study also showed positive linear regression between total rainfall and *Aedes* population. The study showed that previous week of high amount of rainfall played a significant role in mosquito population. Indeed, high rainfall is reported to exhibit strong correlation with the breeding of the vector mosquitoes [34]. Larvae will colonize at bamboo, leaf axils, flower pots, tires and others since water will be collected and retained for a prolong period of time. Therefore, adults *Aedes* are able to lay eggs and increase their population size. Mouchet et al. [35] reported that rainfall can promote transmission by creating breeding sites, but heavy rains can have flushing effect, cleansing such sites of the mosquitoes.

According to this study, the higher the temperature, the higher the total number of *Aedes* population recorded. Ratho et al. [36] reported that, temperature higher than 20 ºC favored the transmission of dengue since temperature ranging from 21 ºC to 33 ºC favored the breeding of mosquitoes. This study was also supported by a study by Nakhapakorn and Tripathi [26] who stated that higher than 20 ºC is the favourable temperature for *Ae. aegypti* mosquito. Ratho et al. [36] also reported that, high temperature favoured the breeding of mosquito and induced mosquitoes to bite more frequently. Daily maximum and minimum temperatures affect the pathogen's rate of multiplication within the insect, which in turn affects the rate of salivary gland infection and hence the likelihood of successful transmission to another host. If the development time of the pathogen exceeds the life span of the insect, transmission cannot occur; vector longevity is thus very important which can be shortened by elevated temperature [37, 38]. In addition, the development of mosquito larvae is faster in warm climates than cold ones, and thus with global warming, the mosquito will become a transmitting adult earlier in the season. The implication is that with warmer temperatures, not only would there be a wider distribution of *Ae. aegypti* and faster mosquito metamorphosis, but also the viruses of dengue and yellow fever would have a shorter extrinsic incubation period and thus would cycle more rapidly in the mosquito [39]. In the laboratory, the rate of dengue virus replication in *Ae. aegypti* mosquitoes increases directly with temperature [40]. Therefore, when there were areas with high temperature, residents over there should increase their awareness in order to prevent the dengue from happening.

Besides that, this study also supported previous studies that when the relative humidity was high, higher number of the larvae was found [33, 36 and 41]. The relationship of relative humidity and *Aedes* population is important in order to control an outbreak of dengue. Goncalves Neto and Rebelo [42] reported a positive correlation of larval population with the amount of rainfall and relative humidity. Similar results were also reported by other study groups [43, 44]. Relative humidity is always high if rainfall is high. High relative humidity always increases *Aedes* population. High relative humidity may favour the breeding of mosquito. Ratho et al. [36] found that, dengue cases were increasing during October and November due to the high relative humidity. Study also found that, survival and growth of mosquito is facilitated when the relative humidity is high [26].

In this study, RT-PCR technique was used to detect dengue virus in *Aedes* mosquitoes collected. As reported by Lee and Rohani [25], this technique was more effective and efficient compared to cell culture and PAP staining technique. However, none of the pools showed positive result after RT-PCR was conducted, showing that, the dengue outbreak in Desa Pandan, Kuala Lumpur was well controlled. Perhaps, residents at the study site there are aware of the increase of dengue cases. Larval density was reduced to very low levels after the breeding sites were removed by resident. As reported by Nakhapakorn and Tripathi [26], cleaning, emptying and removing the containers and sites where mosquitoes oviposit was able to reduce the transmission of dengue.

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REFERENCES

1. Skae, F.M.T., (1902). Dengue fever in Penang. *British Medical Journal* **2**: 1581-1582.

2. Smith, C.E.G., (1956). The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti. Journal of Tropical Medicine and Hygiene* **59**: 243-251.

3. Abu Baker, S. and Shafee, N., (2002). Outlook of dengue in Malaysia: a century later. *Malaysian Journal of Pathology* **24**(1): 23-27.

4. Thongrungkiat, S., Jirakanjanakit, N., Apiwatnasorn, C., Prummongkol, S. and Samung, Y., (2003). Comparative of susceptibility to oralinfection of dengue viruses among local strains of *Aedes aegypti* (Diptera: Culicidae) collected at different seasons of the year. *Journal of Vector Ecology* **28**:166-170.

5. Patz, J.A., Epstein, P.R., Burke, T.A. and Balbus, J.M., (1996). Global climate change and emerging infectious diseases. Journal of the American Medical Association **275**(3):217-223.

6. Mutsuo, K., Osamu, K. and Naoko, N., (2008). Global Warming and vector –borne Infectious Disease. *Journal of Disaster Research* **3**(2): 105-112.

7. Watts, D.M., Burke, D.S., Harrison, B.A.. Whitmire , R.E. and Nisalak, A., (1987). Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. *American Journal of Tropical Medicine and Hygiene* **36**:143-152.

8. Rieter, P., (1988). Weather, vector biology and arboviral recrudescence. In: Monath TP, ed. *The Arboviruses: Epidemiology and ecology*. Boca Raton, FL: CRC Press. **1**:245-255.

9. Rueda, L.M., Patel, K.J., Axtell, R.C. and Stinner, R.E., (1990). Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* **27**:892-898.

10. Tun-Lin, W., Burkot, W. and Kay, B.H., (2000). Effects of temperature and larval diet on development rate and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Medical and Veterinary Entomology* **14**:31-37.

11. Hopp, M.J. and Foley, J.A., (2001). Global scale relationships between climate and the dengue fever vector, *Aedes aegypti*. Climate Change **48**:441-463.

12. Halstead, S.B., (2008). Dengue virus-Mosquito Interactions. *Annual Review of Entomology* **53**:273-91.

13. Pant, C.P., Yasuno, M., (1973). Field studies on the gonotrophic cycle of *Aedes aegypti* in Bangkok, Thailand. *Journal of Medical Entomology* **10**:219-23.

14. Lindsay, M. and Mackenzie, J., (1997). Vector-borne viral disease and climate change in the Australian region: major concerns and the public heath response. In: Climate changes ad human health in the Asia Pacific region Curson, P., Guest, C., Jackson, E., Eds., *Australian Medical Association and Greenpeace International*, *Canberra*, pp. 47-62.

15. Jetten, T.H. and Focks, D., (1997). Potential changes in the distribution of dengue transmission under climate warming. *American Journal of Tropical Medicine and Hygiene* **57**(3): 285-297.

16. Barbazan, P., Yoksan, S. and Gonzalez, J.P., (2002). Dengue hemorrhagic fever epidemiology in Thailand: description and forecasting of epidemics. *Microbes and Infection* **4**(7): 699-705.

17. McMichael, A.J., Haines, A., Slooff, R. and Kovats, S., (1996). Climate changes and human health. World Health Organization, Geneva.

18. Harrington, L.C., Buonaccorsi, J.P., Edman, J.D., Costero, A., Kittayapong, P., Clark, G.G. and Scott, T.W., (2001). Analysis of survival of young and old *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico and Thailand. *Journal of Medical Entomology* **38**(4):537-547.

19. Keating, J., (2001). An investigation into the cyclical incidence of dengue fever. *Social Science and Medicine* **53**(12): 1587-1597.

20. Moore, C.G., Cline, B.L. and Tiben, E.R., (1978). Aedes agypti in Puerto Rico environmental determinants larval abundance and relation to dengue virus transmission. *American Journal of Tropical Medicine and Hygiene* **27**:1225-31.

21. Kuno, G., (1997). Factors influencing the transmission of dengue viruses. In: *Dengue and dengue hemorrhagic fever*, Gubler, D.J. and Kuno, G. Eds., London: CAB International Ltd, pp. 61-87.

22. Eamchan, P., Nisalak, A., Foy, H.M. and Charoensook, O.A., (1989). Epidemiology and control of dengue virus infections in Thai villages in 1987. *American Journal of Tropical Medicine and Hygiene* **41**(1):95-101.

23. Gratz, N.G., (1993). Lessons of Aedes aegypti control in Thailand. *Medical and Veterinary Entomology* **7**(1):1-10.

24. Chan, K.L. (1985). Methods and indices used in the surveillance of dengue vectors. *Mosquito borne diseases bulletin 1985a*. **1**:79-88.

25. Lee, H.L. and Rohani, A., (2005). Transovarial transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus* in relation to dengue outbreak in an urban area in Malaysia. *Dengue Bulletin* **29**:1-6.

26. Nakhapakorn, K. and Tripathi, N.K., (2005). An information value based analysis of physical and climatic factors affecting dengue fever and dengue haemorrhagic fever incidence. *International Journal of Health Geographic* **4**(13):1-11.

27. Fay, R.W. and Perry, A,S., (1965). Laboratory studies of ovipositional preferences of *Aedes aegypti. Mosquito News* **25**: 276-281.

28. Arunachalam, N., Samuel, P.P., Hiriyan, J. and Gajarana, A., (1999). A comparative study on sampling techniques for *Aedes aegypti* (Diptera: Culicidae) surveillance in Madurai, South India. *Tropical Biomedicine* **10**: 25-29.

29. Richards, S.L., Apperson, C.S., Ghosh, S.K., Cheshine, H.M. and Zeichner, B.C., (2006). Spatial analysis of *Aedes albopictus* (Diptera: Culicidae) oviposition in neighborhoods of a piedmont community in North Carolina. *Journal of Medical Entomology* **43**(5): 976-989.

30. Lee, H.L., (1992). *Aedes* ovitrap and larval survey in several suburban community in Selangor, Malaysia. *Mosquito Borne Disease Bulletin* **9**(1): 9-15.

31. Lanciotti, R.S., Calisher, C.H., Gubler, D.J., Chang, G,J. and Vorndam, A.V., (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *Journal of Clinical Microbiology*. **30**: 545-551.

32. Chen, C.D., Benjamin, S., Mahamad, M.S., Chiang, Y.F., Lee, H.L., Nazni, W.A. and Mohamad, S.A., (2005). Dengue vector surveillance in urban residential and settlement areas in Selangor, Malaysia. *Tropical Biomedicine* **22**(1): 39-43.

33. Viroj Wiwanitkit, M.D., (2005). Strong correlation between rainfall and prevalence of dengue in central region of Thailand in 2004. *Journal of Rural and Tropical Public Health* **4**: 41-42.

34. Indaratna, K., Hutubessy, R., Chuprapawan, S., Sukapurana, C., Tao, J., Chunsuttiwat, S., Thimasarn, K. and Crissman, L., (1998). Application of geoghrapical information system to co-analysis of disease and economic resources: dengue and malaria in

Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **29**: 669-684.

35. Mouchet, J., Faye, O., Juivez. J. and Manguin, S., (1996). Drought and malaria retreat in the Sahel, West Africa. *Lancet* **348**: 1735-1736.

36. Ratho, R.K., Mishra, B., Kaur, J., Kakkar, N. and Sharma, K., (2005). An outbreak of dengue fever in peri urban slums of Chandigarh, India with social reference to entomological and climatic factors. *Indian Journal of Medical Sciences* **59**(12): 519-527.

37. Gubler, D.J., Reiter, P., Ebi, K.L., Wendy, Y., Nasci, R. and Patz, J.A., (2001). Climate Variability and Change in the United States: Potential Impacts on Vector and Rodent-Borne Diseases. *Evironmental Health Perspectives* **109**(2): 223-233.

38. Watts, D.M., Burke, D.S., Harrison, B.A., Whitmire, R.E. and Nisalak, A., (1987). Effect on temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. *American Journal of Tropical Medicine and Hygiene* **36**:143-152.

39. Shope, R., (1991). Global Climate Change and Infectious Disease. *Environmental Health Perspectives* **96**: 171-174 pp.

40. Rohani, A., Wong, Y.C., Zamree, I., Lee, H.L. and Zurainee, M.N., (2009). The effect of extrinsic incubation temperature on development of dengue serotype 2 and 4 viruses in *Aedes Aegypti* (L.). *Southeast Asian Journal of Tropical Medicine and Public Health* **40**(5): 942-950.

41. Zeicler, J.D., Acosta, P.O.A., Barreto, P.P. and Cordeiro, J.D.S., (2008). Dengue virus in *Aedes aegypti* larvae and infestation dynamics in Roraima, Brazil. *Revista de Saude Publica* **42**(6):9-14.

42. Goncalves Neto, V.S. and Rebelo, J.M., (2004). Epidemiological characteristics of dengue in the Municipality of Sao Luis, Maranhao, Brazil, 1997-2002. *Cad Saude Publica* **20**: 1424-1431.

43. Guzman, M.G. and Kouri, G., (2003). Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *Journal of Clinical Virology* **27**: 1-13.

44. Chakravarti, A. and Kumaria, R., (2005). Eco-epidemiological analysis of dengue infection during an outbreak of dengue fever, India. *Journal of Virology* **2**:32; 1-7.