CLIMATIC INFLUENCES ON *Aedes* MOSQUITO LARVAE POPULATION

Malinda Madi¹, Rohani Ahmad¹, Noor Azleen Mohd Kulaimi¹, Wan Najdah Wan Mohamad Ali¹, Suzilah Ismail² and Lee Han Lim¹

¹Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur
²Department of Statistics, Faculty of Quantitative Science, Universiti Utara Malaysia, 06010 Sintok, Kedah

**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.

**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 the 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
A. aegypti breeds several months after the rainy season because A. aegypti breeds abundantly in the reservoirs of desert coolers. Aeegypti-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 pattern 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]. Naklapakorn 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**

**Ovitraps 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 outside 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 °C 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 TCAATATGCTGAAACGCGAGAAACCG and TTGCACCAACAGTCAATGTCTTCAGCTTC-3 [31]. The RT step was carried out in a thermocycler (Eppendorf) at 50 °C for 30 minutes to produce cDNA which was then amplified by the following steps: 94 °C for 2 minutes as initial denaturation, 94 °C for 30 seconds as denaturation step, 55 °C for 30 seconds as annealing step and 68 °C for 40 seconds as elongation step. The cycle was repeated 34 times before a final extension at 4 °C. 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 dominant 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 highest 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](image-url)
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.

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Temperature</th>
<th>Relative Humidity</th>
<th>Previous week rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td>Min.</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>-0.339*</td>
<td>0.371*</td>
<td>-0.340*</td>
</tr>
<tr>
<td>Significant level</td>
<td>(0.005)</td>
<td>0.002</td>
<td>(0.005)</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>-0.261*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Significant level</td>
<td>(0.035)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* significant at 5%

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Total larvae pools</th>
<th>Positive dengue</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
<td>741</td>
<td>0</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>402</td>
<td>0</td>
</tr>
</tbody>
</table>
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 previously 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 °C favored the transmission of dengue since temperature ranging from 21 °C to 33 °C favored the breeding of mosquitoes. This study was also supported by a study by Nakhapakorn and Tripathi [26] who stated that higher than 20 °C 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.
ACKNOWLEDGMENTS

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REFERENCES


