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Aedes larval population dynamics and risk for dengue epidemics in Malaysia

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Abstract. Early detection of a dengue outbreak is an important first step towards implementing effective dengue interventions resulting in reduced mortality and morbidity. A dengue mathematical model would be useful for the prediction of an outbreak and evaluation of control measures. However, such a model must be carefully parameterized and validated with epidemiological, ecological and entomological data. A field study was conducted to collect and analyse various parameters to model dengue transmission and outbreak. Dengue-prone areas in Kuala Lumpur, Pahang, Kedah and Johor were chosen for this study. Ovitraps were placed outdoor and used to determine the effects of meteorological parameters on vector breeding. Vector population in each area was monitored weekly for 87 weeks. Weather stations, consisting of a temperature and relative humidity data logger and an automated rain gauge, were installed at key locations in each study site. Correlation and Autoregressive Distributed Lag (ADL) model were used to study the relationship among the variables. Previous week rainfall plays a significant role in increasing the mosquito population, followed by maximum humidity and temperature. The secondary data of rainfall, temperature and humidity provided by the meteorological department showed an insignificant relationship with the mosquito population compared to the primary data recorded by the researchers. A well fit model was obtained for each locality to be used as a predictive model to foretell possible outbreak.

INTRODUCTION

Dengue remains a serious threat for human health in Southeast Asia. As an effective dengue vaccine and anti-viral treatment are not currently available, dengue control relies on controlling the principle vectors of the disease, the mosquitoes *Aedes aegypti* and *Aedes albopictus*. The increase of dengue worldwide is attributed to four major factors, according to the US Centers for Disease Control. They are: uncontrolled urbanisation and concurrent population growth resulting in poor sanitation conducive for increase of *Aedes* population; passive surveillance of cases and reliance on "emergency" control measures; increased air travel and traffic in virus exchange; and poor and ineffective mosquito control approaches.

Malaysia has a good laboratory-based surveillance system for dengue. However, it is basically a passive system and has little predictive capability. Problem may occur if one waits for laboratory confirmation of the case before notification. Delay in notification may lead to delay in control measure, which will further lead to occurrence of outbreak, since dengue needs optimum time management as the transformation of dengue into severe form of dengue will only take a very short period (WHO, 1985).

At present, conventional vector control is usually conducted after the occurrence of dengue cases, not before. Once dengue virus is introduced into a human population through the vectors, it is often too late to kill the infected mosquitoes. Thus fogging after or during the outbreak has little impact on the spread of the disease. This is aggravated by the fact that by the time an outbreak is reported, 7-10 days would have passed before control is instituted.

Furthermore, present control concentrates more on adulticiding, lesser on larviciding. As has been shown in many countries, the larvae are actually the natural reservoir of the dengue virus during inter-epidemic seasons and the occurrence of transovarial transmission of dengue virus by *Ae. aegypti* and *Ae. albopictus* in nature was reported in Malaysia (Rohani *et al.*, 1997, 2007). Therefore, without actively destroying the larvae, the dengue virus can always find a safe haven in them. When these infected larvae multiply in large number under favourable conditions, an outbreak will precipitate.

Studies on the efficacy of source reduction strategy simultaneously at all relevant levels, i.e. vector density, virus transmission dynamics, dengue infection and cost-effectiveness have not been carried out. The efficacy of a source reduction strategy will also depend on various cultural and ecological factors, such as water storage practices, rainfall patterns, temperature, population density and migration. As all these factors are known confounders for the dengue outbreaks, a model employing all four parameters of dengue transmission, namely, vector, human cases, vector infection rate and ecological factor will probably be the most accurate in determining outbreak threshold so that outbreak can be predicted as early as possible.

It is against these backgrounds that new method, novel ideas and concept are urgently needed to revamp the whole dengue control strategies and to check the spread of dengue which apparently at present is unstoppable. Early detection of a dengue outbreak is an important first step towards implementing effective dengue interventions and reducing mortality and morbidity in human populations. A dengue mathematical model would be useful for the evaluation of control measure, to be used in decision-making. However, a mathematical model must be carefully parameterized and validated with epidemiological, ecological and entomological data. A closer interaction is needed between mathematician, epidemiologist and entomologist in order to find better approaches to the measuring and interpretation of the dengue transmissions. Parameter estimation in the field work, localized and comprehensive analysis by using site-specific data will greatly aid the modelling of this disease, making it more realistic and useful.

In this study, a new model is suggested that emphasizes factors contributing to dengue outbreak in Malaysia by concentrating on three major aspects - entomological, epidemiological and environmental. This is the first attempt to model dengue outbreak by including these three major aspects and using time series, econometric automated models and judgments approach. The outcome will lead to a better early warning system for dengue outbreak.

MATERIALS AND METHODS

Entomological data collection

This study was conducted by simulating the field conditions. Dengue prone areas (five consecutive years of high dengue cases) in Kuala Lumpur, Pahang, Kedah, and Johor were chosen for this study. A total of 50 (Taman Sejahtera, Kulim, Kedah); 40 (Desa Pandan, Kuala Lumpur); 30 (Indera Mahkota 2, Pahang) and 26 (Taman Perumahan Uda, Johor) ovitraps were set at the study sites. Mosquito population was estimated based on number of larvae collected using ovitrap which was located outside occupied houses. Mosquito larvae collected from the ovitraps were identified using standard taxonomic keys. Identified mosquito larvae were segregated according to species, site, and date. Vector population in each area was monitored weekly for 87 weeks.

A pilot study was conducted for three weeks to determine the sample size (number of ovitraps per locality) for each study area. During the pilot study, 20 ovitraps were located randomly outside occupied houses and number of larvae collected was used to estimate the number of ovitrap needed for the study for each locality. A training regarding methodology of the project such as setting up ovitraps, mosquito collection, identification and preservation, collecting rainfall, temperature and humidity data and relevant explanation about the project were also conducted. The actual data collection started in October 2007 until June 2009.

Meteorological data collection

Weather stations, consisting of a temperature and relative humidity data logger and an automated rain gauge (Onset Computer Corporation, MA, USA) were installed at key locations in each study site to collect primary meteorological data. Secondary data (rainfall, temperature and humidity) were provided by the meteorological department based on the nearest weather stations to the study areas.

Epidemiological data collection

The number of notified dengue cases reported in each locality during the study period was obtained from the local health authorities. Case definitions were used according to the World Health Organization (WHO) classification system and were identical for all the study areas. A database was prepared for data-entry purposes and co-ordinate storage of data. Data collection was anonymous and data confidentiality was guaranteed. Fogging activities carried out during outbreak in each study area were also recorded.

Predictive model for dengue outbreak

The aim of this part of the research is to study factors contributing to dengue outbreak in Malaysia by concentrating on three major aspects - entomological, epidemiological and environmental. We assessed the usefulness of larval indices for identifying high-risk areas for dengue virus transmission. Climatic sensitivity of the model was determined by correlating week-to-week variations in larvae densities against variations in the individual climatic parameters. Statistical correlations between climate parameters, vector and virus transmission dynamics were analyzed. The formula used is as follows;

Pearson correlation coefficient, $r = \frac{S_{xy}}{\sqrt{S_{xx}S_{yy}}}$ where,

$$S_{xx} = \sum x_i^2 - \frac{\left(\sum x_i\right)^2}{n}$$

$$S_{yy} = \sum y_i^2 - \frac{\left(\sum y_i\right)^2}{n}$$

$$S_{xy} = \sum x_i y_i - \frac{\left(\sum x\right)\left(\sum y\right)}{n}$$

Modelling Technique

Eighty seven (87) weeks of data were collected and partition into 2 parts. First part was for model estimation which used the first 83 data points and second part was for model evaluation which used the last four data points. The Autoregressive Distributed Lag (ADL) Model was used to obtain the predictive model, where the general unrestricted Autoregressive Distributed Lag (ADL) model can be written in the form of,

$$y_{it} = \alpha_{i0} + \sum_{j=1}^{J} \alpha_{ij} y_{i(t-j)} + \sum_{k=1}^{K} \sum_{j=0}^{J_k} \phi_{ikj} x_{ik(t-j)} + \varepsilon_{it}$$

where j is the lag length, i = 1,2,…,N, t = 1,2,…,T (time periods) and y_{it} is the dependent variable which is the total larvae. x_{ikt} is the independent variables which are rainfall, minimum- maximum temperature and maximum humidity, ε_{it} are identically independently distributed random errors with mean zero and variance $\sigma^2_{\varepsilon_{it}}$, α and $\emptyset$ are unknown parameter to be estimated using Ordinary Least Squares (OLS). Lag one for each of the variables was also included in the initial model. The general-to-specific strategy was used to simplify the model with the help of PcGets software (Hendry & Krolzig, 2001). The models were evaluated using Root Mean Square Errors and is written as,

$$RMSE = \sqrt{\frac{\sum_{t}^{n} e_t^2}{n}}$$

where $e_t = y_t - \hat{y}_t$, y_t is the actual observation at t, and $\hat{y}_t$ is the forecast of y_t generated from the models using week 1-83 observations. Thus, with the estimation period being week 1-83, week 84-87 data were used to evaluate the model.

RESULTS

Figure 1 shows the number of *Ae. aegypti* and *Ae. albopictus* larvae in ovitraps collected for 87 weeks for each study site. The results indicated that Kuala Lumpur had the highest percentage of *Ae. aegypti* which was 66.68%, followed by Kedah (55.21%). Johor Bharu showed the lowest number of *Ae. aegypti* collected, 3.04%.

Figure 2 shows total number of larvae and amount of rainfall recorded every week for 87 weeks study period in Taman Sejahtera, Kulim, Kedah. From the plot and correlation analysis, there was significant but weak relationship (r=0.236) between the total number of larvae and amount of the same week rainfall, but moderate (r=0.525) relationship was observed between total number of larvae and rainfall data of the previous week, indicating that current week and previous week rain helped to increase the number of larvae (mosquito population). Based on Table 1, the study site at Indera Mahkota 2, Pahang, showed that rainfall of the previous week contributed to a higher number of larvae but the amount of rain of the same week showed negative weak correlation (r=-0.188). Taman Perumahan Uda, Johor showed similar results as Kedah. As for Desa Pandan, Kuala Lumpur, only rainfall data of the previous week showed significant relationship with the number of larvae. Based on the data obtained, a small amount of rainfall (approximately 0.1 inches and above) was enough to encourage the number of larvae to increase but if there is no rain for 2 to 3 consecutive weeks, the number of larvae would be reduced.

We performed a statistical analysis to examine the effect of maximum and minimum temperatures on the number of mosquitoes larvae found in ovitraps (Figure 3 and Table 2). Results showed that the number of larvae collected from the ovitraps was negatively associated with minimum temperature. At higher minimum (26ºC and above) and lower minimum temperatures (21-22ºC), the number of larvae decreased.

On the other hand, positive correlation was found between the maximum temperature and number of larvae. The rise of maximum temperature influenced the increment of larvae. The obvious temperature was at 34ºC and 35ºC where it contributed to high number of larvae, but at higher maximum temperature (36ºC and above), a reverse association was observed. However, the temperature must be supported with the amount of rain to influence the multiplication of larvae.

Maximum humidity was positively associated with the mosquito larvae collected for all the study sites but a reverse association was found with minimum humidity (Figure 4 and Table 3). The obvious reading of humidity that influenced the number of larvae was 90% and above. The humidity also must be supported with the amount of rain to influence the multiplication of larvae.

The secondary data of rainfall, temperature and humidity provided by the meteorological department based on the nearest weather station to the study areas were not showing similar reading with the primary meteorological data. Thus, no significant relationship was obtained between number of larvae and the secondary meteorological data. This indicates that the secondary meteorological data were not suitable to be used in the modeling.

The study models were obtained based on mosquito population (number of larvae) as the dependent variables and environmental factors (rainfall, minimum and

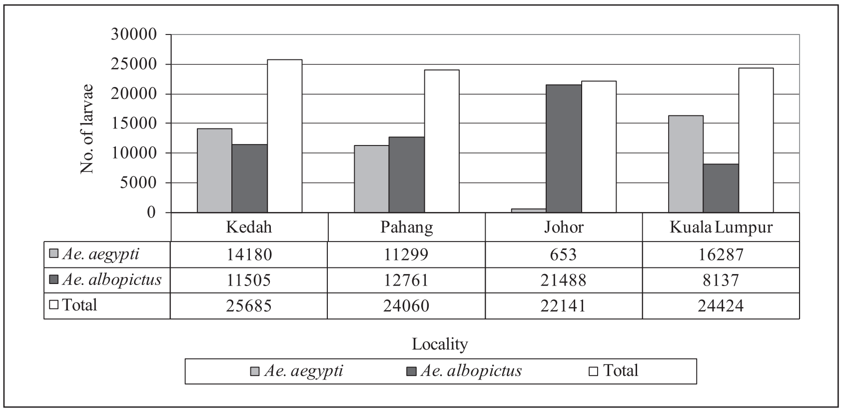
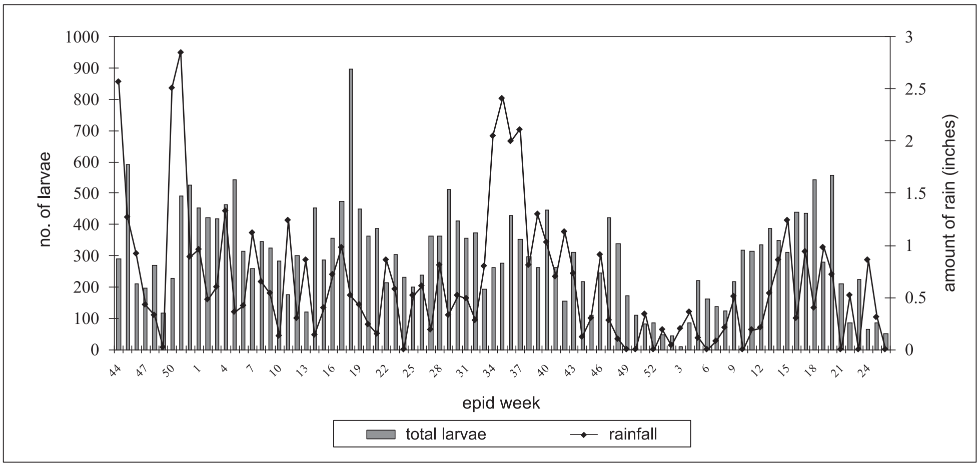


Figure 1. Total number of *Ae. aegypti* and *Ae. albopictus* collected from Kedah, Pahang, Johor and Kuala Lumpur



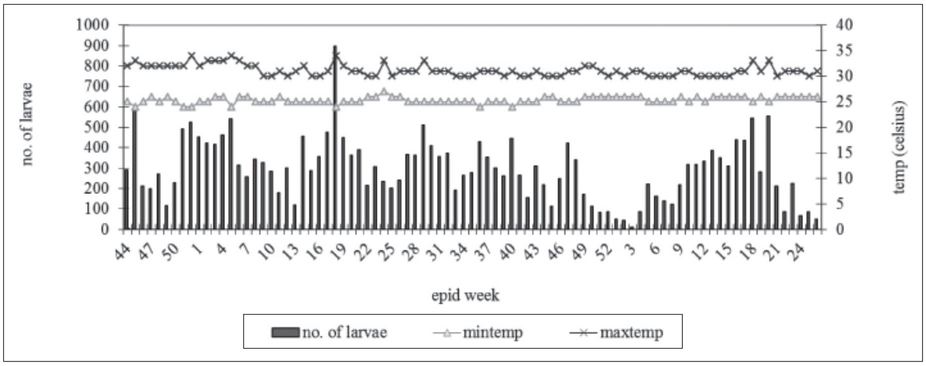
Correlation		Rainfall	Last week rainfall
Larvae	Pearson correlation Sig.	0.236 (0.029)	0.525 (0.000)

Figure 2. Number of mosquito larvae and rainfall by week in Taman Sejahtera, Kulim, Kedah

Table 1. Correlation between numbers of mosquito larvae with rainfall and previous week rainfall respectively according to locality

Locality	Correlation: Larvae and Rainfall	Correlation: Larvae and Previous Week Rainfall
Kedah	0.236**	0.525***
Pahang	-0.188*	0.419***
Johor	0.241**	0.536***
Kuala Lumpur	0.157	0.565***

*** Sig. at 1%, ** Sig. at 5%, * Sig. at 10%



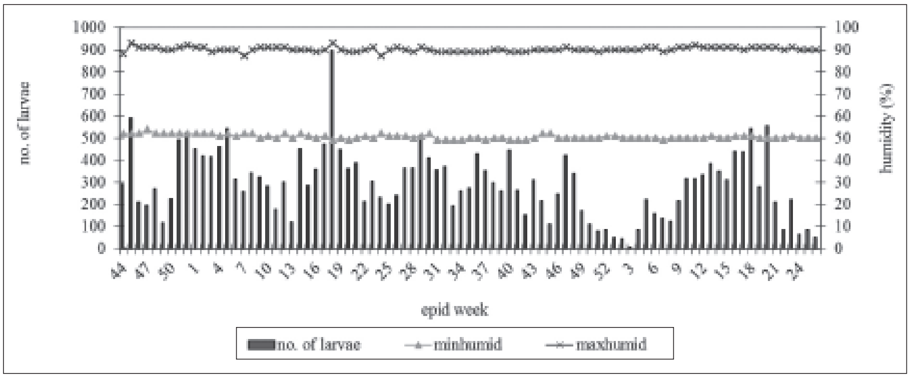
Correlation		Min temperature	Max temperature
Larvae	Pearson correlation Sig.	-0.591*** (0.000)	0.537*** (0.000)

Figure 3. Number of mosquito larvae, maximum and minimum temperatures by week in Taman Sejahtera, Kulim, Kedah

Table 2. Correlation between numbers of mosquito larvae with maximum and minimum temperature respectively according to locality

Locality	Correlation: Larvae and Minimum Temperature	Correlation: Larvae and Maximum Temperature
Kedah	-0.591***	0.537***
Pahang	-0.809***	0.363***
Johor	-0.727***	0.228**
Kuala Lumpur	-0.406***	0.237**

*** Sig. at 1%, ** Sig. at 5%, * Sig. at 10%



Correlation		Minimum humidity	Maximum humidity
Larvae	Pearson correlation Sig.	0.113 (0.301) – not sig.	0.320*** (0.003)

*** Sig. at 1%, ** Sig. at 5%, * Sig. at 10%

Figure 4. Number of mosquito larvae, maximum and minimum humidity by week in Taman Sejahtera, Kulim, Kedah

Table 3. Correlation between numbers of mosquito larvae with maximum and minimum humidity respectively according to locality

Locality	Correlation: Larvae and Minimum Humidity	Correlation: Larvae and Maximum Humidity
Kedah	0.113	0.320***
Pahang	0.102	0.851***
Johor	0.152	0.760***
Kuala Lumpur	-0.096	0.698***

*** Sig. at 1%, ** Sig. at 5%, * Sig. at 10%

Table 4. Estimated Autoregressive Distributed Lag (ADL) Models

Variable	Kedah	Pahang	Johor	Kuala Lumpur
Constant	-1892.09*	-575.49	-26.11	-1013.53***
Total larvae_1 (X_1)	0.54***	–	0.28***	0.31***
Rainfall_1 (X_2)	44.80**	9.30**	27.95***	17.72***
Min. temp. (X_3)	-90.40***	-42.14***	-51.52***	–
Min. temp_1 (X_4)	58.94***	-16.23***	–	–
Max. temp (X_5)	52.71***	12.69***	–	–
Max. temp_1 (X_6)	-35.16***	–	–	–
Max. humidity (X_7)	25.01**	20.01***	15.54***	12.71***
Adj R^2	0.66	0.85	0.76	0.61

*** Sig. at 1%, ** Sig. at 5%, * Sig. at 10%
- ~ these variables are not significant even at 10% significant level. Therefore it is not included in the equation.
E.g. of writing the equation: Kedah:

$$\hat{Y} = -1892.09 + 0.54X_1 + 44.80X_2 - 90.40X_3 + 58.94X_4 + 52.71X_5 - 35.16X_6 + 25.01X_7$$

$\hat{Y}$ ~ Dependent variable ~ Total Larvae
Independent Variables:
X_1 ~ Total larvae_1 ~ previous week number of larvae or lag 1 of larvae
X_2 ~ Rainfall_1 ~ previous week amount of rainfall in inches
X_3 ~ Min. temp ~ Minimum temperature in degree Celsius
X_4 ~ Min. temp_1 ~ previous week minimum temperature
X_5 ~ Max. temp ~ Maximum temperature in degree Celsius
X_6 ~ Max. temp_1 ~ previous week maximum temperature
X_7 ~ Max. humidity ~ Maximum humidity

To obtain fitted values, just substitutes the X_1 to X_7 with the data (real values) obtained from the study.

maximum temperature, maximum humidity and lag one for each of the variables was also included in the initial model) as the independent variables.

The first 83 weeks were used to fit the initial models, while the remaining 4 weeks, were used for model evaluation. After appropriate simplification, the estimated ADL models for each locality are shown in Table 4. Previous week number of larvae (Total Larvae _1) was included in 3 of the models (Kedah, Johor and Kuala Lumpur). In other word if previous week larvae increase, current week larvae will also increase. Previous week rainfall (Rainfall_1) was included in all of the models (Kedah, Pahang, Johor and Kuala Lumpur). Maximum humidity was also included in all models. This indicates that previous week rainfall (Rainfall_1) and maximum humidity is very important factors in influencing mosquito population for all localities. Minimum temperature was included in 3 models (Kedah, Pahang and Johor) but maximum temperature was significant in 2 models (Kedah and Pahang only). All models pass the assumptions and the values of adjusted R^2 ranging from 0.61 to 0.85 which is acceptable to be used for forecasting.

Figure 5 to 8 shows the actual and fitted number of larvae for each locality in 87 weeks. The fitted values were produced by substituting the independent variables with their actual values in the models (as in Table

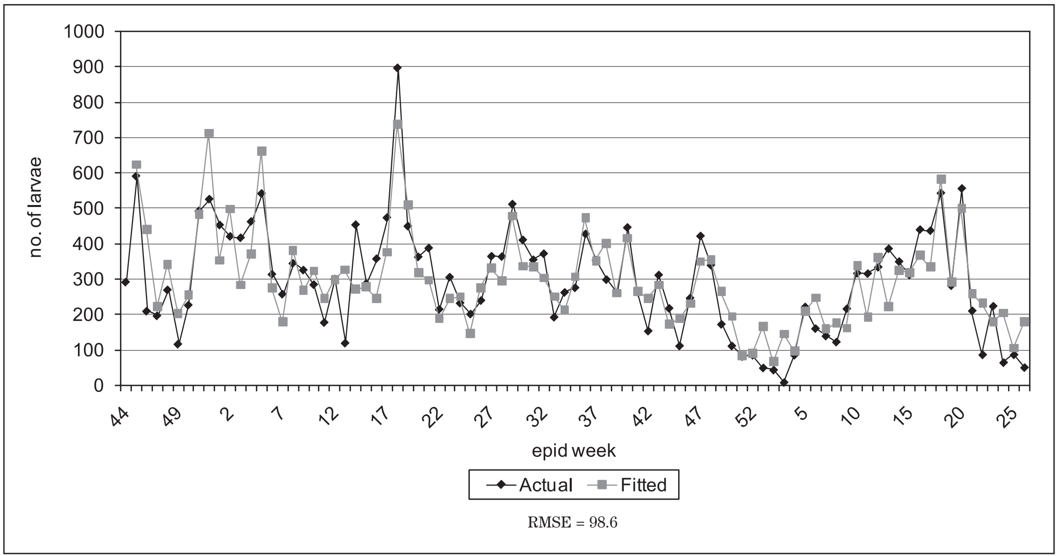


Figure 5. Actual number of larvae against modeled number of larvae in Taman Sejahtera, Kulim, Kedah

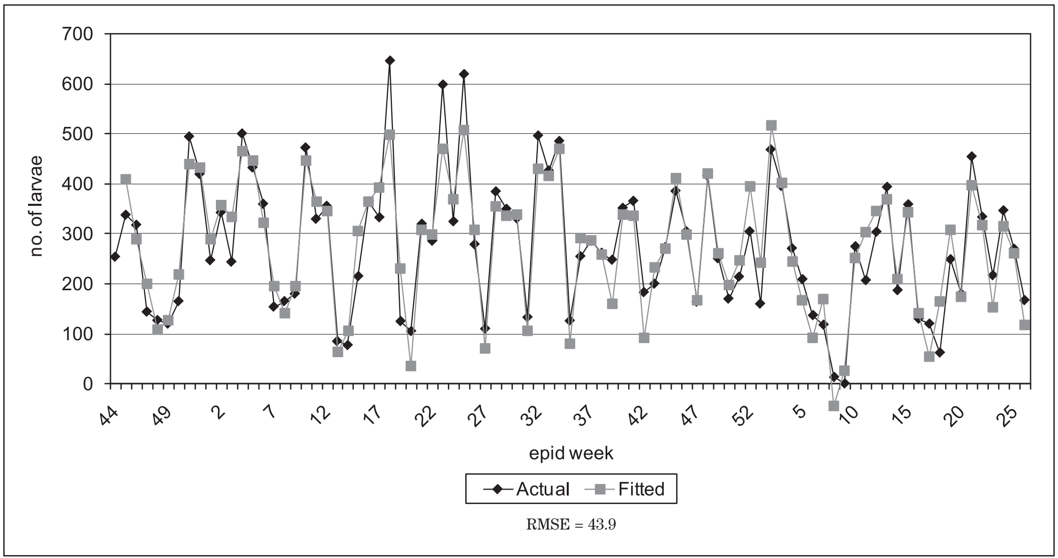


Figure 6. Actual number of larvae against modeled number of larvae in Indera Mahkota 2, Kuantan, Pahang

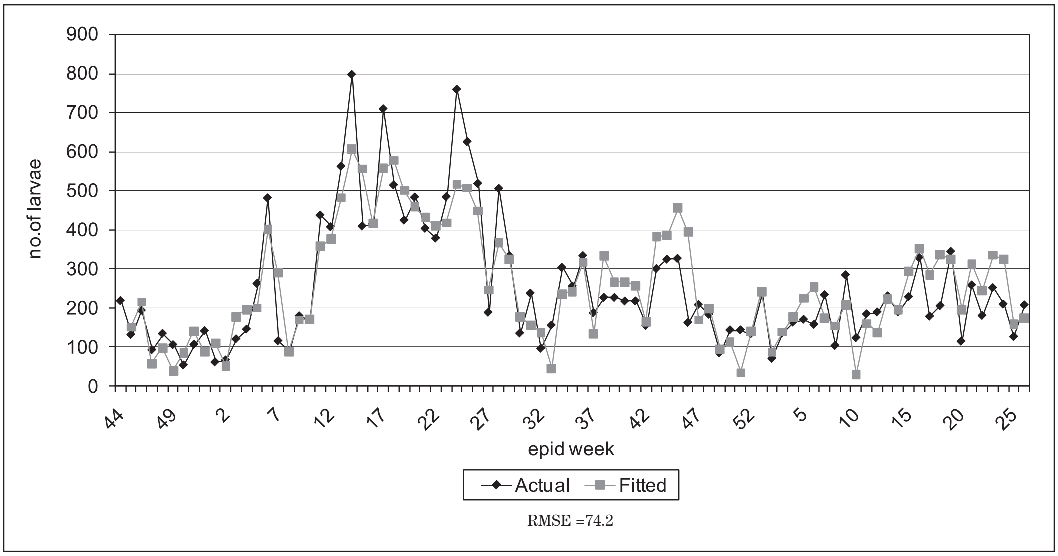


Figure 7. Actual number of larvae against modeled number of larvae in in Taman Perumahan Uda, Johor

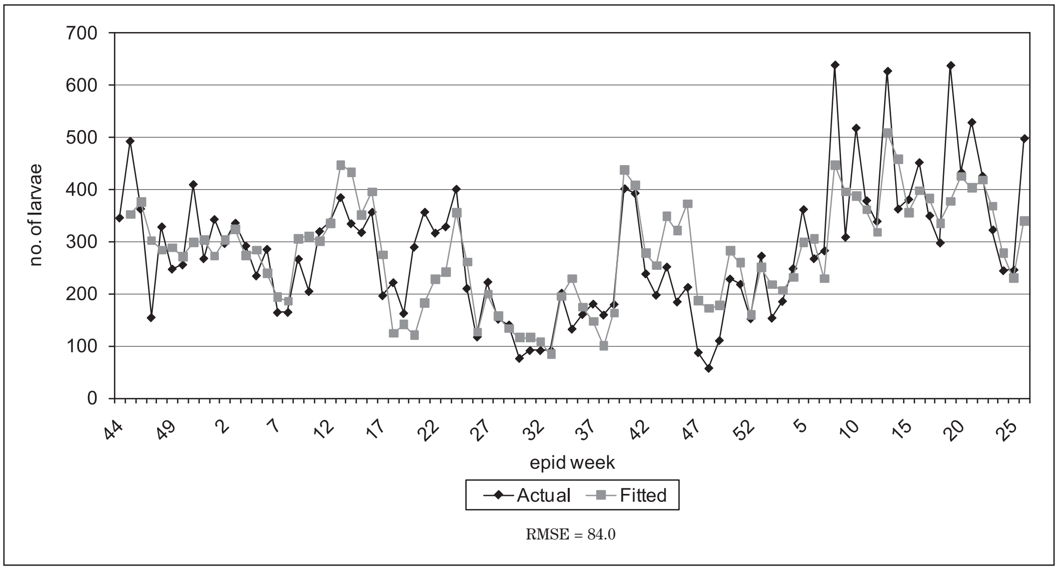


Figure 8. Actual number of larvae against modeled number of larvae in Desa Pandan, Kuala Lumpur

3) where these models are mathematical equations. The actual and fitted values are very close to each other and the error (RMSE) is very small ranging from 43.9 to 98.6. This indicates that the estimated models are good fit models and can be used as forecasting models.

DISCUSSION

Using mosquito egg traps for vector surveillance seems to be a current trend in dengue endemic countries, since this method allows better assessment of infestation densities than the conventionally used methods based on the search for larvae (Morato *et al*., 2005). We used ovitraps, set out weekly, to monitor *Ae. aegypti* and *Ae. albopictus* populations. Week-to-week variations in simulated larva densities were correlated against variations in the individual climatic parameters.

With the build-up of entomological, climatologically and epidemiological databases, the next logical step would be to develop models to test hypotheses concerning vector and disease relationships and the nature and processes of disease transmission. Mathematical models remain a powerful tool for epidemiological analyses and are likely to play a prominent role in the study of epidemic dengue. However, multiple intrinsic factors, both human and vector, as well as extrinsic environmental factors such as temperature, rainfall and humidity affect epidemic dengue virus transmission (Ooi & Gubler, 2009). Correlation and Auto regressive Distributed Lag (ADL) model were used to study the relationship among these variables. In this investigation, the strongest relationship occurs between variations in mosquito larvae densities and both previous week rainfall and maximum humidity. Wu *et al*. (2007), using autoregressive integrated moving average models, found an association of dengue incidence in Taiwan with temperature and relative humidity and Focks *et al*. (1995) using simulation approach models described the daily dynamics of dengue virus transmission in the urban environment in San Pedro Sula, Honduras.

The presence and abundance of *Ae. aegypti* and *Ae. albopictus* are vital to the transmission of dengue. Various researches have investigated the relationship between dengue transmission and *Aedes* population, expressed as larval indices (Pontes *et al*., 2002; Teixeira *et al*., 2002). Scot & Morrison (2004) showed that traditional larval indices in Peru were correlated with the prevalence of human dengue incidence. Regis *et al*. (2008) used the egg average to identify areas with high concentration of mosquitoes. They considered this strategy a good one to detect and prevent *Ae. aegypti* population outbreaks and consequently, a good measure of dengue risk.

This study found direct relationships between the mean numbers of larvae collected by oviposition traps with temperature. Temperature affects the potential spread of dengue virus through each stage in the life cycle of the mosquito. Lower temperatures adversely effected the survival of adult and immature *Aedes* mosquitoes (Lu *et al*., 2009) while higher minimum temperatures might assist larvae survival. Donalisio & Glasser (2002) reported that the minimal temperature was the most important factor in determining the levels of vector infestation. Our results indicated that temperature deviation was the most significant predictor for the adult population to increase. Rising temperatures may accelerate the mosquito's rates of development and consequently, one might expect increases in mosquito abundance.

In this study it was found that maximum humidity had a positive association with the *Aedes* larvae densities but not minimum humidity. Relative humidity influenced longevity, mating, dispersal, feeding behavior and oviposition of mosquitoes and rapid replication of the virus (Hales *et al*., 2002). At high humidity, mosquitoes generally live longer and disperse further. Relative humidity also directly affects the evaporation rates of vector breeding sites.

The study also indicated that humidity is high only when rainfall and temperatures are high and these are conditions that are conducive to breeding and survival of vector populations and rapid replication of virus. Wu *et al*. (2007) reported that temperature and relative humidity are the major determinants in the fluctuation of dengue fever incidence in Kaohsiung City, Taiwan. Vezzani *et al*. (2004) reported that higher population of *Ae. aegypti* larvae were found during periods of higher temperature and greater rainfall. Pontes *et al*. (2000) reported that the seasonal fluctuation of rainfall, *Aedes* larval indices and dengue incidence showed a strong relation in the patterns of the three series.

Precipitation is an important factor in the transmission of DHF. All mosquitoes have aquatic larval and pupal stages and therefore require water for breeding (Lindsay & Mackenzie, 1997). The study showed that timing of rainfall is as important as the amount and frequency of rain. The pattern of rainfall may also play apart. Extremely heavy rainfall may flush mosquito larvae away from breeding sites or kill them outright (Promprou *et al.*, 2005). More frequent, lighter rains may replenish existing breeding sites and maintain higher levels of humidity that assist in dispersal and survival of adult mosquitoes.

The study also showed that rainfall data of the previous week showed positive relationship with mosquito population in all study areas. When the amount of rain increases, larva population also increases. These could be partially explained by looking at entomologic indices since *Aedes* needs 6-10 days to develop from egg to larval stage (Lee, 1992). Our findings with respect to rainfall are in general agreement with the findings of Loh & Song (2001) who reported similar results for *Ae. aegypti* and *Ae. albopictus* in Singapore. Based on this study, it may be useful in assessing and more importantly, responding to the risk of a dengue fever outbreak in a localized area based on current entomological surveillance data and rainfall data of the previous week. Such study can confirm the possible influence of rainfall on the prevalence of dengue. Therefore, intensification of surveillance and control of mosquitoes during the period of high temperatures and frequent rainfall is recommended.

However, rainfall is geographically stochastic, meaning that data collected as close to the area is most desirable (Williams *et al.*, 2008). Our study of rainfall here revealed some local variation in rainfall totals but strong correlation with the data that were recorded at weather stations installed at key locations in each study site. Secondary data provided by meteorological department are not showing significant relationship. The modeling data of this study showed that the actual and fitted are closed to each other because we collected our own weather data at each locality for 87 weeks and not using secondary data provided by meteorological department. This indicates that the estimated models are good fit models and can be used as forecasting models to predict dengue outbreak. The model will be more accurate if the respective institutions set their own weather station at dengue prone areas.

A mosquito forecast for higher-than-normal densities in a particular region, could motivate health officials to alert the public to the increased risk of acquiring dengue and increase mosquito control efforts. A mosquito density forecast for a particular area, together with information on which dengue virus is circulating, as well as knowledge of current mosquito control efforts, can be a component of an early warning system for dengue. However, prediction model of small scale by using localized parameters, including weather parameters, host condition, vector density and other environmental variables was the one that could accurately predict the actual risk of human cases. Such a finding could be applied to assist in establishing an early warning system based on weather forecasts and making decision on public health prevention programme such as vector control, other environmental intervention and personal protection promotion. In conclusion, this study indicated that *Aedes* larval population showed strong relation with maximum temperature and rainfall data of the previous week.

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