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Distribution, seasonal variation & dengue transmission prediction in Sisaket, Thailand

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***Background & objectives*: Environmental factors including weather variables may play a significant role in the transmission of dengue. This study investigated the effect of seasonal variation on the abundance of *Aedes aegypti* and *Ae. albopictus* larvae and explored the impact of weather variability on dengue transmission in Sisaket, Thailand.**

***Methods*: The monthly mosquito larval surveys were carried out in urban and rural areas in Sisaket, Thailand from January to December 2010. Data on monthly-reported cases of dengue fever over the period 2004-2010 were obtained from the Ministry of Public Health. Weather data over the same period were obtained from the Thai Meteorological Department. Chi-square test was used to find the differences relating to seasonal variability, areas of study, and mosquito species factors using entomological survey data. Time series Poisson regression analysis was performed using data on monthly weather variables and dengue cases.**

***Results*: There were more *Ae. aegypti* larvae per household than *Ae. albopictus* larvae in the winter and rainy seasons. More *Aedes* larvae per household were found in the rainy season than in the winter and summer seasons. Relative humidity at a lag of one month and rainy days in the current month were significant predictors of dengue incidence in Sisaket.**

***Interpretation & conclusions*: Increased rain during the current month and less humidity during the previous month might trigger a higher incidence of dengue epidemic in Sisaket. The present findings suggest that the dengue incidence corresponds with the number of *Aedes* larvae. The seasonal patterns of dengue outbreaks coincide with the rainy season.**

Key words *Aedes aegypti* - *Ae. albopictus* - dengue - season - transmission prediction - weather

The incidence and distribution of dengue-related illness have grown dramatically in recent decades[1]. It is estimated that 50 million dengue infections occur annually[1] and about 2.5 billion people live in the regions with potential risk of dengue transmission[1]. *Aedes* mosquitoes transmit dengue virus, causing both classical dengue fever (DF) and potentially fatal dengue haemorrhagic fever (DHF). The first reported epidemic of DHF occurred in Southeast Asia in 1953[2]. Since the first dengue epidemic outbreak in Thailand in 1958[3], there has been an upward trend in the incidence of DHF. In 2010, Sisaket province in Thailand was classified as a dengue high risk area. In that year, the province had the highest number of dengue cases in the northeastern region with 2,618 dengue cases and dengue incidence rate of 180.25 cases per 100,000 population[3].

Environmental factors including weather variables may play a significant role in the transmission of dengue which is a mosquito-borne disease with seasonal distribution[4,5]. Temperature, rainfall, and relative humidity are major parameters influencing the incidence of dengue fever in Thailand[5,6]. The prediction of global climate change and transmission of dengue and its geographic spread has been widely studied[5,7,8]. Since dengue transmission is highly dependent on local environmental factors, it may not be possible to predict dengue incidence outside locations. However, investigations of local weather conditions and dengue incidence in different environmental and regional contexts can improve our understanding of the linkages between weather variables and dengue transmission, and provide strong scientific evidence for predicting future transmission patterns[9].

Monitoring of mosquito larvae, finding mosquito larval indices, and predicting dengue incidence can facilitate early warning and disease control and prevention. In this study, we examined the numbers of *Aedes* larvae and larval indices in both rural and urban areas of Sisaket province over three seasons to assess the impact of weather variables on dengue transmission.

Material & Methods

Study area: Sisaket is one of the northeastern provinces of Thailand. It has a land area of 8,839.976 km^2 and a population of about 1.45 million. The province borders Cambodia to the south. The summer season in Sisaket is from March to May. The rainy season starts in June and ends in October. The winter season follows from November to February. The annual mean temperature is about 26 °C. The annual rainfall is about 1,598 mm. The mean summer and mean winter temperatures are approximately 29 and 24 °C, respectively. The average maximum and minimum temperatures are 35.5 and 18.8 °C, respectively.

Mosquito larval survey: The monthly mosquito larval surveys were carried out by examining all containers in indoor and outdoor water containers in both urban and rural areas from January to December 2010. The urban area was in Kanthararom district with population density of 141 inhabitants/km^2. The rural areas covered 15 sub-districts (Epad, Tam, Buanoi, Muang-Noi, La-Tay, Hnong Wang, Hnong Kaw, Hnong Bua, Doon, Non Sang, Du, Kam Niam, Pak Paw, Yang and Hnong Hua Chang) with average population density of nine inhabitants/km^2. Mosquito larvae were collected from both indoor and outdoor containers using fine-meshed fishnets. The outdoor larval surveys were conducted within 15 m of houses[10,11]. All containers holding water were sampled for *Aedes* immatures (III/IV instars). Water in very small containers was emptied into the fishnet. Larger water containers were sampled by dipping the net in the water, starting at the top of the container and continuing to the bottom in a swirling motion that sampled all edges of the container[11]. Immediately after collection, mosquito larvae were put on plastic bags with water-filled from the water container until processed later that same day. In the laboratory with a stereoscopic microscope, all live mosquito larvae were identified to species level using Rattanarithikul and Panthusiri's keys[12]. In this study, the first, second instars and pupae were discarded because immature mosquitoes at these stages could not be identified and found in such a low number (less than five percentage of total mosquito larvae collected). Immature mosquitoes at these stages were never found in water containers without the third and fourth instars. Discarding immature mosquitoes at these stages should not affect mosquito larval indices [*i.e.* Container Index (CI), House Index (HI) and Breteau Index (BI)][13,14].

There were a total of 21 container categories in this study. Indoor containers were all containers inside the house that held some water such as small water jars, large water jars, cement tanks, plastic tanks, ant guards, flower vases and refrigerator drainages. Outdoor containers were all containers within 15 m of the house, such as small water jars, large water jars, cement tanks, plastic tanks, areca husks, coconut shells, used cans, used tires, plastic bottles, discarded objects, animal pans, pot saucers, plant pots and bamboo clumps. For the water jar, we classified water jars into two categories: small water jars (<500 L) and large water jars (≥500 L).

Data collection for predictive model: The computerized data sets were obtained on monthly-reported dengue cases in Kanthararom district for the period of January 2004-December 2010 from the Kanthararom Health Office, Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health. Weather data over the same period were obtained from the Thai Meteorological Department (TMD) consisting of monthly rainfall, the number of rainy days, daily maximum rainfall, relative humidity, min/mean/max temperatures, sunshine and evaporation.

Statistical analysis: Chi-square test was used to find the differences between seasonal, area and mosquito species factors of the number of *Ae. aegypti* and *Ae. albopictus* larvae. Mosquito larval indices - *i.e.* CI, HI and BI were calculated. *Ae. aegypti* and *Ae. albopictus* larval indices were developed as per standard WHO guidelines[1]. All tests were two-tailed.

Spearman's correlation analysis was conducted to examine the relationship between monthly dengue incidence and weather variables with a lag of zero to three months. Poisson regression adjusted for auto-correlation, secular trend and lag effects, was used to quantify the relationship between weather variables and dengue incidence. In order to control for a potential long-term trend in the number of cases over the study period, a year variable was included in the regression model.

Results

Aedes larvae, breeding habitats, season and area: A total of 2,326 *Ae. aegypti* larvae and 300 *Ae. albopictus* larvae were found during the one year sampling period. There were more *Ae. aegypti* larvae per household than *Ae. albopictus* larvae per household in the winter ($P<0.05$) and rainy ($P<0.001$) seasons but the number of *Ae. aegypti* and *Ae. albopictus* larvae did not differ in the summer season (Fig. 1). There were more *Aedes* larvae per household in the rainy season than in the winter ($P<0.05$) and summer seasons ($P<0.001$) in both urban and rural areas. The number of *Aedes* larvae per household found in the urban area did not differ from that for the rural area.

In the winter season, *Ae. aegypti* larvae were found mostly in outdoor plastic tanks in the urban area whereas *Ae. aegypti* larvae were found mostly in used tires, pot saucers and outdoor plastic tanks in the rural area. *Ae. albopictus* larvae were found mostly in outdoor concrete tanks both in the urban and rural areas. In the summer season, *Ae. aegypti* larvae were found mostly in plastic bottles in the urban area whereas, *Ae. aegypti* larvae were found mostly in bamboo clumps and animal pans in the rural area. No *Ae. albopictus* larvae were found in the urban area. *Ae. albopictus* larvae were found mostly in indoor large water jars. In the rainy season, *Ae. aegypti* larvae were found mostly in indoor cement tanks both in the urban and rural areas. *Ae. albopictus* larvae were found mostly in bamboo clumps and used tires in the rural area.

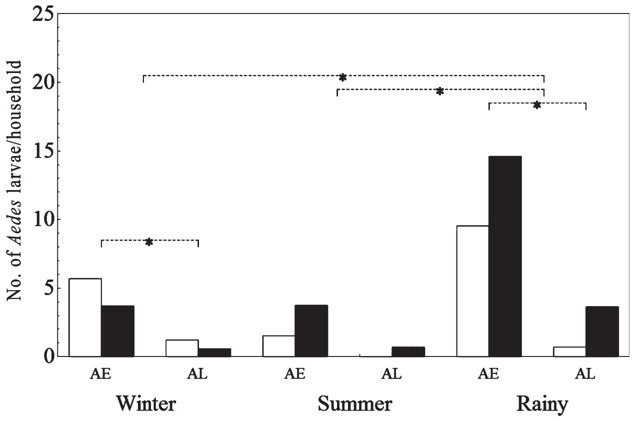

Fig. 1. Number of *Ae. aegypti* (AE) and *Ae. albopictus* (AL) larvae per household in urban (□) and rural (■) areas in winter, summer and rainy seasons at Sisaket, Thailand from January to December 2010. Horizontal lines indicate the statistical differences between the means.

Larval indices, season and area: CI for *Ae. aegypti* was higher than CI for *Ae. albopictus* in all seasons. CI in the rainy season was higher than CI in the winter and summer seasons. HI for *Ae. aegypti* was higher than HI for *Ae. albopictus* in all seasons. The HI in the summer season was lower than HI in the winter and rainy seasons. BI for *Ae. aegypti* was higher than that for *Ae. albopictus* in all seasons. BI in the summer season was lower than BI in the winter and rainy seasons (Table I).

Impact of weather variability on dengue transmission: Monthly rainfall, the number of rainy days, daily maximum rainfall, relative humidity, and min/mean temperature, at a lag between zero to three months, were positively associated with dengue incidence in Sisaket over the study period (Table II). Maximum temperature, sunshine and evaporation were negatively correlated with dengue incidence at a lag of zero to three months (Table II).

The incidence rate was first-order autoregressive indicating that the dengue incidence rate in the current month was related to the incidence rate occurring in the previous month ($\beta = 0.431$, $P<0.001$, Table III). Relative humidity at a lag of one month had a negative effect on dengue incidence ($\beta = -0.045$, $P<0.001$, Table III). The number of rainy days had a positive effect on dengue

Table I. Container, House and Breteau indices (CI, HI, BI) of *Ae. aegypti* and *Ae. albopictus* larvae in different seasons

Larval indices	*Ae. aegypti*						*Ae. albopictus*					
	Winter		Summer		Rainy		Winter		Summer		Rainy	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
CI	25.28	25.73	19.51	28.33	34.88	30.57	5.58	4.97	0.00	6.44	4.07	7.10
HI	72.55	62.60	39.47	42.86	74.47	71.49	21.57	15.45	0.00	13.19	8.51	20.82
BI	133.33	92.68	42.11	72.53	127.66	114.93	29.41	17.89	0.00	16.48	14.89	26.70

Table II. Spearman correlation coefficients between dengue incidence rate and weather factors at Sisaket, Thailand

Weather factors	Spearman correlation coefficient	Time Lag (months)
Monthly rainfall (mm)	0.596^{**}	0
Rainy days (days)	0.630^{**}	0
Daily maximum rainfall (mm)	0.561^{**}	0
Relative humidity (%)	0.617^{**}	1
Minimum temperature (° C)	0.630^{**}	0
Mean temperature (° C)	0.449^{**}	0
Maximum temperature (° C)	-0.618^{**}	3
Sunshine (h)	-0.410^{*}	1
Evaporation (mm)	-0.346^{*}	3

P *<0.05, **<0.001

incidence of the same month (β = 0.060, P<0.001, Table III). The year of occurrence was included in the model as an independent variable (β = 0.094, P<0.05, Table III) indicating that there was a long-term decline and increase in the number of dengue cases notified over the study period (Fig. 2).

The time series Poisson regression model was constructed with the data for the period January 2004-December 2010 (Fig. 3). The model demonstrated goodness-of-fit with a correlation between observed and predicted number of dengue incidence rate of 72.74 per cent (Table III, Fig. 3). The goodness-of-fit analyses revealed that the model fitted the data reasonably well.

Discussion

This study demonstrated that the number of *Aedes* larvae was higher in the rainy season than in the winter and summer seasons. Many studies have reported similar findings in many other parts of Thailand[10,11,15,16] and Côte d'Ivoire[17]. The seasonality of *Aedes* larvae in Sisaket showed a similar pattern to that observed by Mogi *et al*[15] in Chiang Mai, Thailand, that *Aedes* larvae remained low in summer and winter seasons, but increased in the rainy season. However, *Aedes* larvae in Bangkok showed a non-seasonal fluctuation pattern due to water-filled containers being present year round[18]. Scarcity of larval habitats seems to be a limiting factor.

Our results support previous findings that the number of *Ae. aegypti* larvae was higher than that of *Ae. albopictus* larvae in the winter and rainy seasons[10,11]. Although *Ae. aegypti* was introduced to Thailand a long time ago, possibly via rubber tires, and *Ae. albopictus* is native to Thailand[12], *Ae. aegypti* is now the primary dengue vector and has greater prevalence than *Ae. albopictus*. This could be due to four possible reasons. Firstly, *Ae. aegypti* larvae are competitively superior

Table III. Poisson regression coefficients of weather data on dengue cases in Sisaket, Thailand

Variables	β	SE	z-Statistic	AIC
Case (lag1)	0.431	0.124	3.466^{**}	Log likelihood:
Relative humidity (lag 1)	-0.045	0.013	-3.448^{**}	-53.9865
Rainy days (lag 0)	0.060	0.015	4.064^{**}	R^2=0.7274
Year	0.094	0.045	2.088^{*}	
Constant	-185.593	90.328	-2.055^{*}	

AIC, Akaike's information criterion; β, coefficients; SE, standard error
P *<0.05, **<0.001

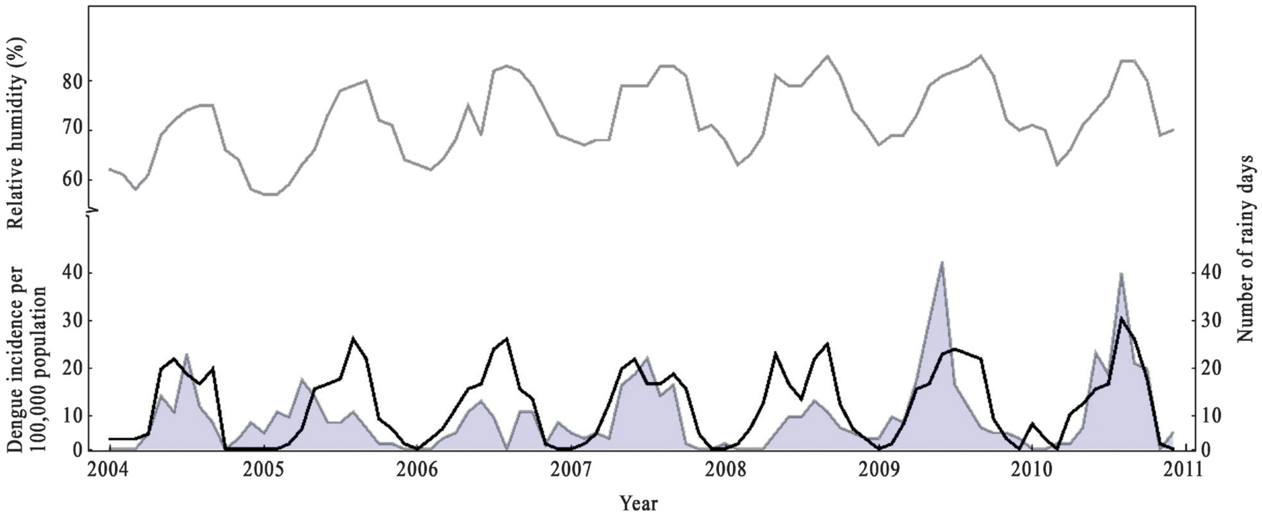

Fig. 2. Relative humidity (%) (gray line), the number of rainy days (black line) and dengue incidence per 100,000 population (shaded area) in Sisaket, Thailand from January 2004 to December 2010.
Weather Data Source: Thai Meteorological Department.

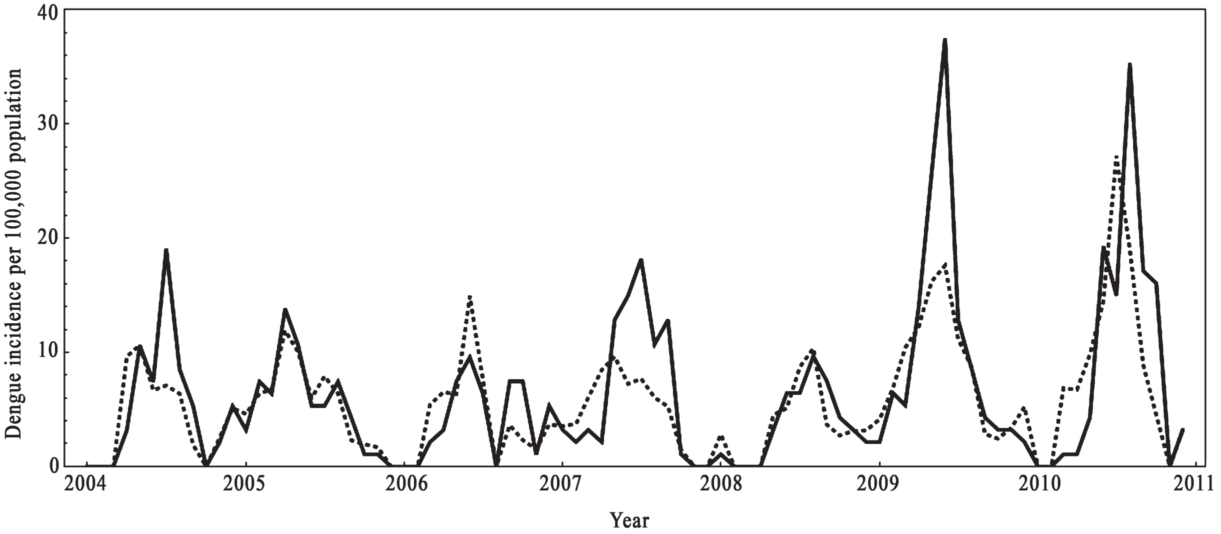

Fig. 3. The actual dengue cases (continuous line) and the predicted dengue cases (dotted line) from January 2004 to December 2010 by the Poisson regression model in Sisaket, Thailand.
Source: Kanthararom Health Office, Bureau of Epidemiology, Department of Disease Control, Kanthararom.

to *Ae. albopictus* larvae[19]. Secondly, *Ae. aegypti* has a higher net reproductive rate than *Ae. albopictus*[15]. Thirdly, eggs of *Ae. aegypti* are more desiccation-resistant than those of other *Aedes* species[17]. Fourthly, the females of these two species select different ovipositing habitats. *Ae. aegypti* females prefer to oviposite in all types of artificial and natural indoor water containers, whereas *Ae. albopictus* females prefer to oviposite in outdoor habitats, especially trash containers[10,11]. This could be due to the fact that *Ae. albopictus* larvae are more tolerant of food shortage than *Ae. aegypti* larvae[15].

Ae. aegypti is a highly domesticated mosquito and is widely found in urban and residential areas [15]. *Aedes* mosquito has shown some adaptation toward urbanization. For example, *Aedes* eggs in urban areas, where there is limited vegetation and low humidity, are more resistant to desiccation than conspecific strains in rural areas[20]. As water supply is readily available in the urban area, residents have no need to store water inside and around the house. The possible larval habitat for *Aedes* mosquitoes in the urban area is the concrete drainage systems. *Aedes* larvae require clear, but not necessarily clean water[21]. Our results contradict the previous findings that the number of *Aedes* larvae in the urban area did not differ from that in the rural area. This could be due to the fact that degree of urbanization in Sisaket province is not yet significant enough to cause the difference. In some highly urbanized areas of Southeast Asia, such as Bangkok and Manila, *Ae. aegypti* has replaced *Ae. albopictus*[22]. The growing urban population and the increasing population mobility with rapid development of transportation are the risk factors that may contribute to further geographic expansion and density of *Aedes*[23].

In this study, all three *Aedes* larval indices (CI, HI, BI) for *Ae. aegypti* were higher than that for *Ae. albopictus* in all seasons, and significantly higher than those accepted by WHO[1]. Larval indices are important to vector control efforts because these provide the means to prioritize locations or categories of larval habitats and evaluate the entomologic effectiveness of control measures.

It has been proposed that understanding weather variables can increase the predictive power of dengue models[24]. The relationship between weather and dengue has been assessed in multiple settings using different statistical methods[5-8]. However, mosquito population dynamics vary in different geographic regions where dengue is transmitted, suggesting that the influence of weather on dengue may be site-specific[25]. Our results indicated that an increase in the number of rainy days and a decrease in relative humidity were associated with an increase of dengue incidence in Sisaket. Rainfall, daily maximum rainfall, minimum/maximum/mean temperatures, sunshine and evaporation were associated with the dengue incidence, while these failed to enter the best fitting predictive model.

Production of mosquitoes is determined by the availability of suitable and sufficient habitat for the larval stages and this is dependent on rainfall[26]. Rainfall has been found to correlate with dengue in many countries such as Thailand[5,6] and Brazil[8]. This study found that rainfall, rainy days and daily maximum rainfall were positively associated with the dengue incidence. Increased rain may increase larval habitat and vector population size by creating a new habitat or increase adult survival[27]. However, in tropical areas in particular, extensive and continuous rainfall can delay the build-up of some mosquito species until late in the season and thus delay transmission[26].

Relative humidity influences longevity, mating, dispersal, feeding behaviour, egg production, oviposition of mosquitoes and dengue virus transmission[28,29]. Adult survival and hatching rate are affected by the rise in temperature and lower humidity[30]. In the predictive model, relative humidity at a lag of one month was negatively associated with dengue incidence. This suggests that less humidity of the previous month might trigger a higher incidence of dengue epidemics. This is because relatively lower humidity in the surrounding environment could assist mosquitoes in seeking target hosts and facilitate disease transmission[7].

The regression model indicates that the number of dengue cases in a current month can be estimated by observing the number of dengue cases occurring in the previous month. This may provide a warning indicator to the local communities and health authorities. The dengue incidence corresponds with the number of *Aedes* larvae in the study site; the number of *Ae. aegypti* and *Ae. albopictus* larvae was highest in the rainy season when dengue incidence outbreak was observed in the area. Previous studies conducted in Thailand[10,11] suggest that the seasonal patterns of dengue outbreaks coincide with the rainy season. Two peaks were observed in 2009 and 2010 with 37.51 and 35.32 cases respectively per 100,000 population and might be affected by the weather conditions.

In conclusion, the number of *Ae. aegypti* larvae and larval indices for *Ae. aegypti* were higher than that for *Ae. albopictus* in Sisaket. An increase in the number of rainy days and a decrease in relative humidity were associated with increase in dengue incidence.

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