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Relationships between dengue virus infection in mosquito vector, *(Aedes aegypti)*, dengue cases and weather conditions in Samut Sakhon Province, Thailand

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Background : *Dengue is commonly found in the tropical and subtropical regions. The disease is still affecting the population of the world especially in Southeast Asia. The current increase in both morbidity and mortality rates was associated with the potentiality of the viral transmission. Surveillance focusing on the virus infection in principal dengue vector, weather conditions and number of dengue cases should be evaluated to develop an effective control approach, therefore reduce the emergence of dengue disease within the endemic and/or new areas.*

Objectives : *To characterize the transmission pattern of dengue virus in the mosquito vector (Aedes aegypti) according to the seasons and to determine the relationship between dengue virus infections in the mosquito, monthly dengue case reports and weather conditions in a highly endemic area of Thailand.*

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Research design : *Descriptive study.*

Setting : *Ban Phaeo District, Samut Sakhon Province, Thailand*

Methods : *Ae. aegypti mosquitoes were collected from the study site during the rainy season of 2012, winter, dry and rainy seasons of 2013. Dengue infection in the mosquitoes was determined by nested reverse-transcription polymerase chain reaction. The seasonal prevalence pattern of dengue in the mosquitoes was compared to the dengue cases, and also with to local-weather condition within the same periods.*

Results : *Four dengue serotypes were detected in the individual mosquito samples. The highest rate of infection was shown in the rainy season of 2012 (August - November). The infection rate in mosquitoes declined in the winter and dry season of 2013. However, the infection rate in the mosquitoes was increasing in the rainy season of 2013. The trend of the dengue cycle in mosquito vector likely associated with that from the cycle of dengue cases or the morbidity rate in the study area. Interestingly, those were also associated with the changes of local weather conditions, i.e. temperature and relative humidity.*

Conclusions : *The result showed significant association between the pattern of dengue case, morbidity and the dengue infection in the mosquito vectors. Incidence trends of the disease were also accompanied with the consecutive data of both humidity and temperature. Therefore, the data could improve the surveillance and contribute to better prediction of the magnitude for the dengue outbreak.*

Keywords : *Aedes aegypti mosquito, dengue virus, dengue transmission cycle, weather conditions, nested reverse-transcription polymerase chain reaction (nested RT-PCR).*

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วีรยุทธ กิตติชัย, ประกายแก้ว มนตริวัต, จักรวาล ชมภูศรี, พายุ ภักดีนวน, ธีรกมล เพ็งสกุล, อภิวัฏ ธวัชสิน, อุษาวดี ถาวระ, เผด็จ สิริยะเสถียร. ความสัมพันธ์ระหว่างการติเชื้อไวรัสเดงกีในยุงลายบ้านพาหะ *Aedes aegypti* จำนวนผู้ป่วยและข้อมูลสภาพอากาศ ณ จังหวัดสมุทรสาคร. จุฬาลงกรณ์เวชสาร 2558 ก.ค – ส.ค.; 59(4): 347 – 63

ความรู้พื้นฐาน : *ไข้เลือดออกเป็นโรคที่พบบ่อยในเขตร้อนและร้อนชื้น ซึ่งยังคงมีผลกระทบต่อประชากรของโลกโดยเฉพาะในประเทศแถบเอเชียตะวันออกเฉียงใต้ อุบัติการณ์ที่เพิ่มขึ้นของโรคในปัจจุบันรวมทั้งอัตราป่วยและอัตราการตายนั้นมีพบว่าความ สัมพันธ์กับประสิทธิภาพการติดต่อของเชื้อไวรัส การสำรวจเพื่อเฝ้าระวังโรคโดยเน้นศึกษาการติดเชื้อไวรัสเดงกีในยุงลายพาหะรวมถึงพิจารณาข้อมูลสภาพภูมิอากาศและจำนวนผู้ป่วยควรจะถูกใช้ในการประเมินเพื่อเป็นข้อมูลในการพัฒนาวิธีการควบคุมโรคที่มีประสิทธิภาพและลดอุบัติการณ์การเกิดโรคในพื้นที่ระบาดและ/หรือ พื้นที่แห่งใหม่ได้*

วัตถุประสงค์ : *เพื่อระบุรูปแบบการระบาดของเชื้อไวรัสเดงกีของแต่ละฤดูกาลในยุงลายบ้าน (Aedes aegypti) และศึกษาความสัมพันธ์ระหว่างอัตราการติดเชื้อในยุงกับจำนวนผู้ป่วยไข้เลือดออกและข้อมูลสภาพภูมิอากาศจากพื้นที่ระบาดแห่งหนึ่งของประเทศไทย*

รูปแบบการวิจัย : *การศึกษาเชิงพรรณนา*

สถานที่ทำการศึกษา : *อำเภอบ้านแพ้ว จังหวัดสมุทรสาคร*

วิธีการศึกษา : *เก็บตัวอย่างยุงลายบ้าน (Ae. aegypti) จากพื้นที่ในระหว่างฤดูฝนปี 2555 และฤดูหนาว ฤดูร้อน และฤดูฝนของปี 2556 การติดเชื้อไวรัสเดงกีอาศัยวิธี Nested reverse-transcription polymerase chain reaction แล้วเปรียบเทียบรูปแบบของความชุกของเชื้อไวรัสเดงกีกับจำนวนผู้ป่วยและข้อมูลสภาพอากาศในแต่ละฤดู*

ผลการศึกษา : *พบการติดเชื้อไวรัสเดงกีทั้งสี่ซีโรทัยป์จากตัวอย่างยุงลายบ้าน โดยในฤดูฝนปี 2555 มีอัตราการติดเชื้อสูงสุด อัตราการติดเชื้อลดลงในฤดูหนาวและฤดูร้อนของปี 2556 อย่างไรก็ตาม ยังพบอัตราการติดเชื้อเพิ่มขึ้นในฤดูฝนของปี 2556 จากการผลศึกษาดังกล่าว แนวโน้มของวงจรการติดเชื้อในยุงลายพาหะมีลักษณะที่สัมพันธ์กับจำนวนผู้ป่วยหรืออัตราป่วยของประชากรในพื้นที่ ที่น่าสนใจวงจรของการติดเชื้อดังกล่าวยังมีความสัมพันธ์กับข้อมูลการเปลี่ยนแปลงของสภาพภูมิอากาศด้วย*

สรุป : *ผลการศึกษานี้ แสดงความสัมพันธ์ระหว่างรูปแบบของจำนวนผู้ป่วย อัตราป่วยและอัตราการติดเชื้อในยุงพาหะอย่างมีนัยสำคัญ ซึ่งแนวโน้มของข้อมูลข้างต้นยังมีความสัมพันธ์กับทั้งอุณหภูมิและความชื้นด้วย ดังนั้นข้อมูลที่ได้จากการศึกษาสามารถนำมาปรับปรุงการเฝ้าระวังการเกิดโรค อันจะนำมาซึ่งการพยากรณ์ขนาดการระบาดของโรคได้*

คำสำคัญ : *ยุงลายบ้าน, เชื้อไวรัสเดงกี, สภาพภูมิอาศ, ปฏิกิริยาลูกโซ่โพลีเมอร์ เรสแบบ nested revers-transcription.*

Dengue viruses (DENV) are members of the *Flavivirus* genus of the *Flaviviridae* family and are classified into four serological serotypes (DENV1 - 4). The disease is transmitted to susceptible human host by a bite of infected female mosquito especially *Ae. aegypti*.[1] The virus is mainly dominant in endemic zones with different distribution patterns. Dengue virus can cause dengue diseases: classical dengue fevers (DF) and its severe form, namely, dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS).[2] Annual dengue cases of 50 - 100 million (10 - 15%) out of 390 million infected patients were symptomatic, which led to 500,000 hospitalizations and eventually developed into severe form.[3] This is especially true for the population who inhabit the tropical and subtropical regions [2], where annual hospitalization and death rates of patients by the severe form is highest; most countries of Southeast Asia, southern and central America, and the Caribbean and south Pacific but with a lower rate of infection in Africa were reported possibly due to poor surveillance.[4]

Co-circulating DENV viruses have been intensifying in various scales where they had been referred as hyperendemicity within the relevant countries.[5] Although numerous efforts have been taken for the disease control but the outbreaks of the disease still occur. The weather-sensitivity of the dengue virus results in the globally-geographic distribution. Interestingly, the duration of the viral replication in the mosquito depends on the temperature and relative humidity.[9, 10] Control efforts currently focus on the biology of the vector with limited resources and limited success in many endemic areas.[6 - 8] However, the fundamental knowledge of the relationship between the dynamic of the disease and the environment could effectively guide an assessment of the magnitude of risk and then indicate possible use of the resources.

Thailand experiences hyper-endemic dengue spread with seasonal phases of disease that unpredictably diverge in degree across the provinces (each year-round).[11] All four dengue viruses (DENV-1 to 4) have been circulating in the area attributed to various risk factors,[12] contributed the introduction of DENV viruses to the population of the area. Dengue transmission was associated with the population density of both mosquitoes and human hosts.[13] In 2012, the incidence rate of DHF at Samut Sakhon Province has been reported as 252.26/100,000 people,[14] and was classified as a severe epidemic year of dengue disease with 90th percentile.[15] The highest rates of DHF were found in students, contract workers, agriculture workers, housekeepers, and traders.[16] An understanding of the seasonal transmission of dengue serotype virus which takes into account the geographical is essential. We characterized the transmission cycles of the dengue virus in the mosquito vector and also in the monthly case reports from a highly endemic area of Thailand, located in the central region that annually experienced the disease outbreaks. Additionally, the trend of consecutive weather data and human movement were concurrently studied with the dengue transmission cycle, the key factor that associates the dengue spread. Therefore, using the potential surveillance by narrowing into each small scale of the endemic zones should enable recognition of the interaction between weather-based and non-weather-based regulation of transmission.

Materials and Methods

Demographic and epidemiological data of study site

Ban Phaeo District, Samut Sakhon Province was selected as the study site due to its high incidence of DHF as previously described. The province locates in the central part of Thailand (latitude 13.48 - 13.69° and longitude 100.02-100.27°) around 29 km from the capital city, Bangkok is (Department of Provincial Administration, Ministry of Interior, Statistical Forecasting Bureau, National Statistical Office, Thailand). Among the districts of Samut Sakorn Province, Ban Phaeo District is a relatively populous area with 94,278 residents, and is also containing 23,530 housing structures; all are located in an area of 245.031 km^2 (derived from the Department of Provincial Administration, Ministry of Interior, Statistical Forecasting Bureau, National Statistical Office, Thailand).

Mosquito collection

Mosquitoes were randomized collected inside and around the resident's houses using a method described by Kovats *et al*., 2001. [18] Seven villages of the districts were the targets of the collection and selected for collections of larva, pupa, male and female of *Ae. aegypti* mosquitoes. The field captured mosquito was done after obtaining written permission from the residents. Human baiting was performed by officers with highly experience from National Institute of Health, Thailand. [17] The study was undertaken in all Thai seasons comprising at twice in rainy seasons (September and October) in 2012 and at once in the winter (February), dry (April) and rainy-seasons (July) in 2013. The living individual samples were collected, vial and collection occurred between 8.00 a.m. and 11.00 a.m. (based on the suitable period of host-blood seeking) once or twice each season. The mosquito samples were collected from approximately 10% (150 samples) of the study site per season. In the same day, all insect stages were transferred to the laboratory of Molecular Entomology, Department of Parasitology, Faculty of Medicine, Chulalongkorn University.

Viral RNA extraction

The viral RNA was extracted and purified from the mosquito samples (larva, pupa, and adult) following the instruction Invisorb® Spin Virus RNA Mini kit (Invitek Gmbh, Germany). RNA concentration and purity are quantified by Nano Drop 2000c spectrophotometer (Thermo scientific, USA).

Dengue detection, typing and sequencing based on the E protein gene

The purified RNA samples from the mosquito were amplified for dengue infection by nested RT-PCR following the instruction of QIAGEN® OneStep RT-PCR Kit (QIAGEN GmbH, Germany) and using the protocols provided with BIOTAQ™ PCR Kit (Qiagen, Germany), respectively. cDNA synthesis and nested PCR were done by using the gene-specific primers targeted the E gene. [19] The template RNA was amplified by using universal primer (DEUL; 5'- TGGCTGGTGCACAGACAATGGTT and DEUR; 5'-GCTGTGTCACCCAGAATGGCCAT) by all serotypes producing 641 bp in lenghts, as described previously. The dengue-specific primer sets including D1L: 5'-GGGGCTTCAACATCCCAAGAG and D1R: 5'- GCTTAGTTTCAAAGCTTTTTCAC producing 504 bp for DENV 1; D2L: 5'-ATCCAGATGTCATCAGGA

AAC and D2R: 5'- CCGGCTCTACTCCTATGATG producing 346 bp for DENV 2; D3L: 5'-CAATGTGC TTGAATACCTTTGT and D3R: 5'-GGACAGGCTCC TCCTTCTTG producing 198 bp for DENV 3, and D4L: 5'-GGACAACAGTGGTGAAAGTCA and D4R: 5'- GGTTACACTGTTGGTATTCTCA producing 143 bp amplicons for DENV 4, respectively. The following positive controls and template concentrations were used: Hawaii strain (DENV-1, 5×10^5 PFU/mL; NGC strain (DENV-2), 4.75×10^6 PFU/mL; H87 strain (DENV-3), 2.75×10^5 PFU/mL; and 814609 strain (DENV-4), 2.5×10^5 PFU/mL. The optimized condition was obtained within a thermal cycler (Eppendorf, USA). The PCR product was run on 2% agarose gel (w/v), stained by ethidium bromide and a specific band was visualized by gel based UV-visualization. Viral sequences (submitted to 1st base company, (Malaysia) were aligned using ClustalW (Bioedit v2.0.2). Consensus sequences excluded the primer sequence were tested with their homology sequence in Genbank nucleotide database using the basic alignment program of the National Center for Biotechnology Information (NCBI) GenBank database webserver (http://blast.ncbi.nlm.nih.gov/Blast). The nucleotide sequences were deposited in GenBank with the following accession numbers: KM003944-KM003962 (DENV-1), KM003971-KM003982 (DENV-2) and KM003983-KM003984 (DENV-4). DENV3 sequences were not submitted to Gen Bank since these lengths are less than 200 bp.

Data of Dengue-case report, Weather and Migration

The monthly data of dengue cases during 2012-2013 (both DF & DHF/DSS) derived from Samut Sakhon Provincial Department of Disease Control, Ministry of Public Health of Thailand. The number of DHF cases was indicated as the morbidity rate of dengue cases per 100,000 populations. Local-weather data of max/mean/minimum-values of temperature and relative humidity during 2012-2013 were derived from Asian Start Regional Center. Temperature was shown as degree Celsius, and relative humidity as percentage.

Dengue transmission cycle

Seasonal transmission cycle of dengue viruses was evaluated from the infective mosquitoes, and infection rates were determined by samples of each serotype. The rate was calculated and analyzed from the clusters of positive samples. Then the data was used to construct the line-graphs accordance with Thai season during 2012 - 2013. Relationships between the dengue cycles (in mosquito as above) and from the number of case report during the study periods were analyzed. Additionally, the cycle was compared with the local weather conditions including maximum/mean/minimum values of temperature and relative humidity. Trend of dengue incidence was assessed with the data of in- and out-migration.

Data analysis

Associations of seasonal rates of the dengue virus and serotype infections during 2012-2013 were determined using Minitab 16 statistical software based on Chi-square test at the significant level of 0.05.

Results

Dengue detection and typing

Dengue infection rate in mosquitoes was determined by total samples and serotypes within the season. The significantly highest rate was shown in the rainy season (collected during August - November, 2012) by 63.1% (81/129) when compared to the rate of other seasons (df = 3, $p < .05$), 4.24% (5/119) in February 2013 of the winter in 2013, 4.35% (5/116) in April 2013 of the dry season and 8.7% (5/58) in July 2013 of the rainy season (Fig 1A).

In addition, the dengue typing from the individual samples was also achievably discriminated by nested RT-PCR mentioned as above. According to the result of dengue typing in the rainy season of 2012, we found 29.41% for DENV-1, 27.06% for DENV-2, 48.24% for DENV-3 and 20% for DENV-4, respectively (Fig 1B). Four dengue serotypes circulating in the rainy season of 2012 showed significant difference, of which, DENV-3 was more statistically significant prevalence than that of DENV-1, DENV-2 and DENV-4 ($p < .05$). The consecutive winter of 2013, we found 1.69%, 3.39%, 4.24% and 3.39% of DENV1-4 respectively. Only DENV-4 was found in the dry season of 2013: moreover, 2.17% of DENV-1 and 4.35% of DENV-3 were found in the rainy season of 2013.

Transmission cycles of dengue incidence and weather conditions

Seasonal transmission cycles during 2012 - 2013 were evaluated by the rate of the mosquito infection. The highest rate of the mosquito infection was found in the rainy season of 2012 and it declined in the winter; the lowest rate in dry season of 2013. Again, the rate increased in the rainy season of 2013 (Fig 1). The trend of the cycle is likely associated with that from the number of monthly dengue cases and the morbidity rate (Fig 2A). Of which, there were 867 cases in the rainy season during May to November of 2012, and this represented the highest rate of morbidity in November as 40.08/ 100,000 people (204 cases). In the winter from December 2012 to February 2013, there were 398 cases with highest rate in December (32.06/ 100,000). The lowest rate was found during the dry season as 12.50/ 100,000 and 7.74/ 100,000 in March and April of 2013. By contrast, 498 cases were reported in the rainy season of 2013, the rate has increased with the highest (21.23/ 100,000) in August. In the rainy season during the study period, the seasonal dynamics of both temperature and relative humidity are shown (Table 1). Declined values of the humidity and increased degree of temperature were observed in the winter and dry season.

Discussion

Effective drugs and vaccine against dengue infection are not yet available for treatment and protection of the disease. Control of the mosquito vectors before the disease outbreak is essential, therefore the improvement of dengue surveillance system is highly crucial. Barbazan, Yoksan and Gonzalez, (2002) suggested that the seasonal transmission of DENV serotypes in an endemic area was related to the prevalence and virulent strains, associated to the high pathogenesis. [24] Previous studies suggested that using information of dengue detection from an active school-based dengue case could be used to reduce the longitudinal risk of the viral transmission in the rural areas. [25] Assessment of the dengue outbreak though the viral prevalence in the field from caught *Ae. aegypti* mosquitoes has been proposed and it seems to be more practical and effective tool for planning dengue control.[15, 26, 27]

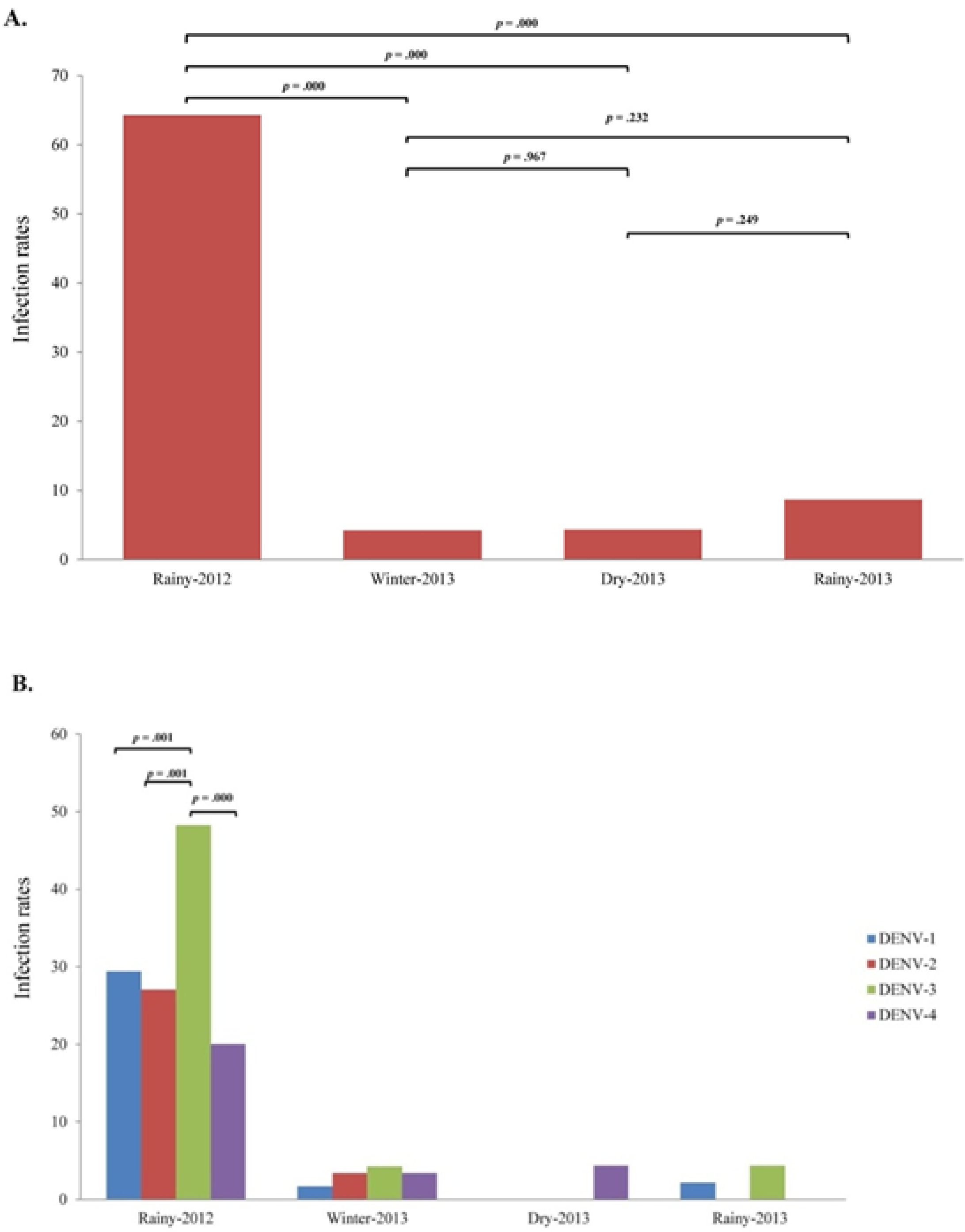

Figure 1. Seasonal prevalence of in the mosquito vector during the rainy season of 2012 and winter, dry and rainy seasons of 2013: (A) dengue prevalence in total samples for each season: (B) prevalence of dengue serotypes in mosquitoes by season. Chi-square statistic-based *p*-values represented the probability of significant different rates of dengue infection by seasons if the value was less than 0.05.

Table 1. Local weather conditions in 2012 - 2013 according to the three seasons in Thailand, which was represented by mean values of temperature and percent of relative humidity (ranges by season) with their minimum and maximum values. The data were concluded from Figure 2B and 2C (the raw data of weather conditions retrieved from Asian Start Regional Center).

Years	Seasons	Temperature ^{o}C (ranges)	Min & max values of temperature	% RH (ranges)	Min and Max values of RH
	Dry	$31.67^{o}C$ ($31.12^{o}C$-$32.23^{o}C$)	$26.88^{o}C$-$39.02^{o}C$	63.90% (59.20-68.60^{o}C)	42.35%-87.53%
2012	Rainy	$30.32^{o}C$ ($29.52^{o}C$-$31.39^{o}C$)	$25.81^{o}C$-$37.67^{o}C$	71.45% (53.47%-78.75%)	43.99%-90.18%
2013	Winter	$29.18^{o}C$ ($27.04^{o}C$-$30.42^{o}C$)	$21.62^{o}C$-$36.87^{o}C$	52.94% (48.50%-56.85%)	38.15%-79.53%
	Dry	$32.21^{o}C$ ($31.88^{o}C$-$32.54^{o}C$)	$26.51^{o}C$-$38.69^{o}C$	58.95% (55.59%-62.30%)	44.82%-85.18%
	Rainy	$30.06^{o}C$ ($28.84^{o}C$-$33.71^{o}C$)	$24.28^{o}C$-$41.04^{o}C$	72.59% (54.22%-81.58%)	45.13%-93.12%

DENV1-4 viruses were found circulated in the mosquitoes collected from the study area, which could be represented as a hyperendemicity. (11, 21, 28) All serotypes were detected in the rainy season of 2012 with the highest rate of infection when compared to other seasons; especially, DENV-3 was the most prevalence. The result differs from a previous report studied by Chompoosri *et al*. (2012) who suggested that high-dengue prevalence was found in the dry season. (26) The different results might depend on the period of sample collection, the sensitivity of detection method and environmental factors within the study period. In this study, the result was done using the high sensitivity technique, nested RT-PCR with limited-detection ranges of 0.1-1 pfu/ml. (19) Memman *et al*. (2008) suggested that although there were lower number of mosquito population circulating in the endemic area, only the infective vector was significant for transmitting the dengue virus to infect the susceptible human host. (25) Lifespan of the infected mosquito is another significant factor that shapes the transmission rate. (8)

Interestingly, the similar incidence of four-dengue spreads remained in the winter of 2013. Infection rates by total samples and by serotypes were significantly less than that in the previous rainy season and only DENV-4 was found only in the dry season. Additionally, DENV-1 and DENV3 were found in the rainy season of 2013, shown by a report of Nisalak *et al*. (2003). (12) The trend of the seasonal prevalence of dengue detected from the mosquito was likely correspond with that of the morbidity pattern reported within the same period (Fig 1 and Fig 2A). Although the significant association of DHF cases and dengue prevalence in the mosquito in the country mentioned as above has been suggested by Scott *et al.* (2000), [6] no study has proposed the compatibility of seasonal patterns between these two related-parameters all-year-round. Moreover, spatial and temporal dynamics of dengue vector and dengue cases in the urban areas of a Brazilian oceanic island, Fernando de Noronha, showed the strong relation between each other. [29] The vector density could be associated with the effect of weather conditions on the distribution of mosquito vectors, and also have increased the relative risk to spread the dengue disease later.

The cycle of case reports and infection rates in the mosquito not only showed more associations, but the trend of them is also associated within each season based on the different degrees of the local weather conditions, temperature and relative humidity. By contrast, it showed the decline with lowest magnitudes in the winter and in dry season. An increase of the number of dengue cases was found again in the rainy season of 2013. Watts *et al*. (1987) suggested that the important factor that possibly regulate the disease spread throughout the season, i.e. the weather conditions. [20]

In this study, we show the patterns of both the humidity and temperature, each of them was associated with the trend of dengue prevalence and dengue cases within the study period (Figure 2). It is already known that temperature is conversely correlated with the relative humidity and both factors affected on the growing of the vector population density. [34] From our studied mosquito infection rate in rainy season 2012 was 63.1 % while in rainy season 2013 mosquito infection rate was only 8.7 %, the mosquito infection rate depends on several factors such as weather conditions and number of infected persons. Previous studies suggested that temperature not only shorten the length period of viral incubation time in the mosquito, [10] but it also affects on the size of each development stage (larva, pupa and adult) and frequency of females blood feeding. [24, 27] Dengue case report in 2012 was higher than 2013 in the study area this also showed association between number dengue case and infection rate in mosquito.

Speculation of the naturally transmission dynamics of the virus in small scales (regions, provinces, in and out-municipal or district) could be focused. This is because the small area may contribute an origin of the disease spread. Generally, Thailand is the one of dengue endemic nations located between neighboring countries which has been exposed to all four serotypes but with different dominant strains such as in Loa PDR as DENV-1 & 3 outbreak in 2007-2012, and Singapore as DENV-2 outbreak in 2008-2010. [22, 23] Travelling or human movements were important factors that play a role on the geographical spreading of the disease. [30, 31] In the study site, the massive movement of the labors to non-municipal area occurred in 2008 - 2009 (Fig 3). In 2012 migration to the municipality (37.75%) was higher than that of non-municipal (29.16%) which was from abroad; Myanmar (84%) and Macau (16%). According to data of disease incidence in 2012 (CDC, Thailand 2012), the number of dengue cases was the most found in non-municipal areas as 64.11%, considered as highest morbidity by regions which showed that there are central (150.87/ 100,000 people) > southern (149.54/ 100,000 people) > northern (109.62/ 100,000 people) > northeastern region (96.17/ 100,000 people).

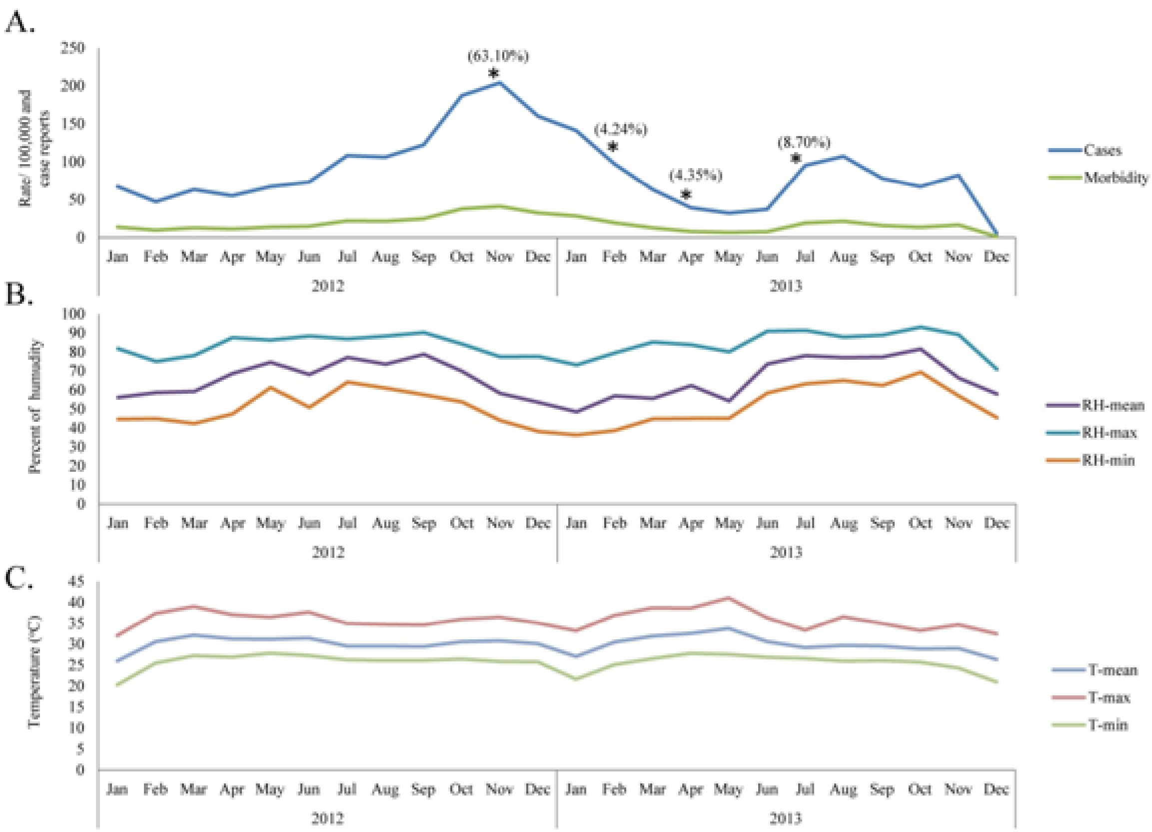

Figure 2. Relationships of the dengue cycles by case report and morbidity and the cycle of weather conditions (temperature and relative humidity) during 2012-2013, (Raw data were obtained from the number of dengue cases derived from Samut Sakhon Provincial Department of Disease Control, Ministry of Public Health of Thailand and the raw data of weather conditions derived from Asian Start Regional Center). Asterisk (*) represented the seasonal collection time-periods of the field-caught mosquito vector and the percentage of seasonal dengue infection was concurrently shown in figure 2A.

Immigration within the province and from the other provinces located in the Central region and Northeastern region and Bangkok were the most commonly found (Fig 3). Migration within the province was the highest rate as 27.59% in 2008 and 33.73% in 2009; however, in 2011 the most migration by crossing from the province in the Central region was as high as 36.92% and the highest rate of 33.08% were from Bangkok in 2012. Considering the occupational-movements, we suggested that they worked in manufacturing (80.60%) > wholesale and retail trades (8.70%) > construction (7.80%) > education (2.98%) > entertainment and arts (0.53%) (Fig. 4).

Behaviors and measurable characteristics of human population could explain the predictions of dengue spread by different people bitten by the mosquitoes and contributions to pathogen transmission. [13, 32] Although the geographical pattern of dengue epidemiology in Thailand is hardly different from time to time or year to year [33], better surveillance could also be considered, as well as the effect of the human migration for planning of the disease control. [32] This may significantly shape and potentially develop the dengue dynamics in the countries of the region.

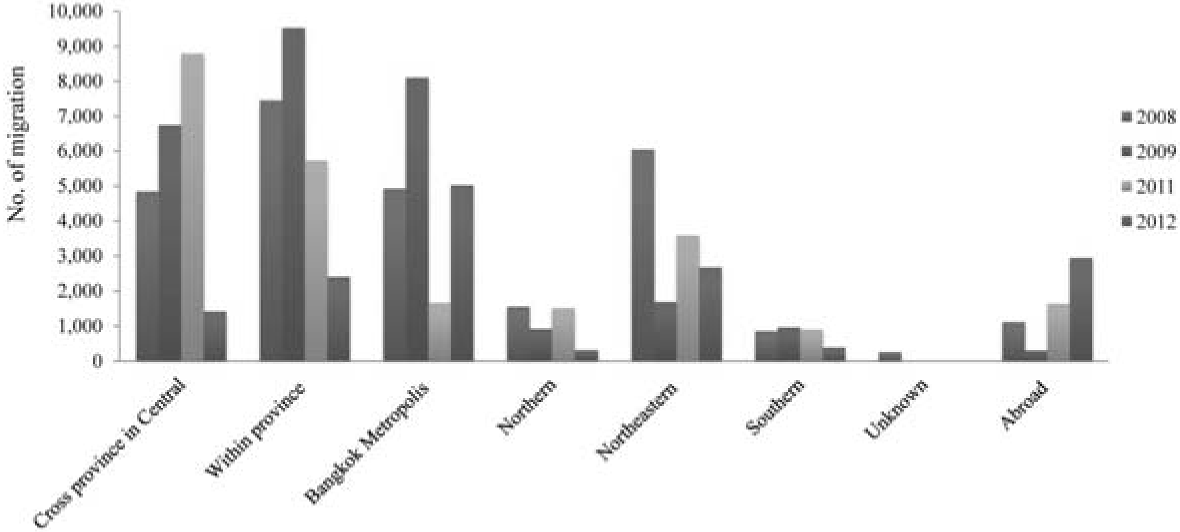

Figure 3. Immigration Samut Sakhon Province during 2008-2009 and in 2011 - 2012, was separated according to region of origin

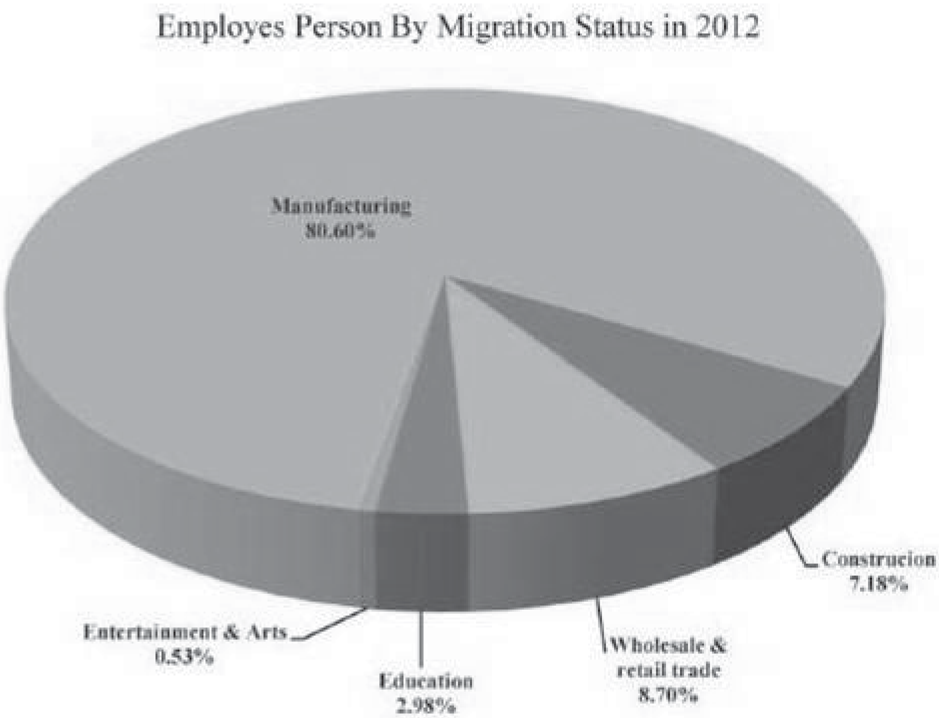

Figure 4. Immigration study site in 2012, which was separated by occupational status.

Conclusions

We demonstrate the relationships between DHF cases, weather-based and seasonal transmission cycles of the DENV in *Ae. aegypti* mosquitoes at Ban Phaeo District, Samut Sakhon Province, Thailand. The possible associations between the dengue cases/ morbidity and the dengue cycle in the vectors, the trend of which accompanied the consecutive data of both the humidity and temperature are shown. Human movements would be considered another factor driving the dengue outbreak in certain geographical areas. Hence, year-round surveillance of the dengue infection rate in mosquito vectors should be performed, as it would be beneficial for planning the effective dengue control.

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