Original Article

Seasonal Monitoring of Dengue Infection in *Aedes aegypti* and Serological Feature of Patients with Suspected Dengue in 4 Central Provinces of Thailand

Jakkrawarn Chompoosri[1] Usavadee Thavara[1] Apiwat Tawatsin[1] Surapee Anantapreecha[1] Padet Siriyasatien[2]*

Abstract

This study aimed to determine seasonal dengue infection rates in *Ae. aegypti* mosquitoes and dengue infection in suspected patients in 4 central provinces of Thailand. *Ae. aegypti* mosquitoes collected during three seasons and blood specimens taken from patients with suspected dengue were detected and serotyped for dengue viruses using RT-PCR. The biting behavior of *Ae. aegypti* females was studied by 24-hour mosquitoes collection once a month using human bait for determination of seasonal biting rate. Dengue infection rates in *Ae. aegypti* females obtained from all the 4 provinces were highest in hot season and varied from place to place ranging from 64.4% to 77.5%, whereas morbidity rates of DHF appeared to be highest in rainy season. The occurrence of transovarial transmission was found in local *Ae. aegypti* larvae and males in all provinces investigated ranging from 18.3 % to 46.9 % and from 12.0% to 46.3%, respectively. Serotyping of dengue viruses in *Ae. aegypti* showed that DENV 3 and DENV 1 were the two most predominant serotypes, followed by DENV 2 and DENV 4. Similarly, DENV 1 and DENV 3 were the two most prevalent serotypes detected in the serum of suspected patients, followed by DENV 2 and DENV 4. The highest biting activity of *Ae. aegypti* females took place in hot season with the biting rate of 30 mosquitoes/person/hour. Data obtained from this study could be used as powerful tools for virological surveillance in *Ae. aegypti* populations before the occurrence of dengue outbreaks in endemic and newly dengue-introduced areas.

Keywords: *Aedes aegypti*, biting behavior, dengue infection, dengue viruses, patients with suspected dengue

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บทคัดย่อ

การตรวจติดตามการติดเชื้อไวรัสเดงกีของยุงลายบ้านในแต่ละฤดูกาลและการตรวจน้ำเหลืองของผู้ป่วยที่สงสัยว่าติดเชื้อไวรัสเดงกีใน 4 จังหวัดภาคกลางของประเทศไทย

จักรวาล ชมภูศรี[1] อุษาวดี ถาวระ[1] อภิวัฏ ธวัชสิน[1] สุรภี อนันตปรีชา[1] เผด็จ สิริยะเสถียร[2]*

งานวิจัยนี้ได้ตรวจติดตามการติดเชื้อไวรัสเดงกีของยุงลายบ้านในแต่ละฤดูกาลและตรวจน้ำเหลืองของผู้ป่วยที่สงสัยว่าติดเชื้อไวรัสเดงกีใน 4 จังหวัดภาคกลางของประเทศไทย โดยการตรวจและจำแนกเชื้อไวรัสเดงกีแต่ละซีโรทัยป์ในตัวอย่างลูกน้ำ ยุงลายบ้าน และเลือดของผู้ป่วยโดยวิธี RT-PCR นอกจากนี้ ยังได้มีการศึกษาพฤติกรรมการกัดของยุงลายบ้านโดยการให้อาสาสมัครนั่งจับยุงลายบ้านตลอด 24 ชั่วโมง เดือนละ1 ครั้ง เพื่อหาอัตราการกัดของยุงลายบ้านในแต่ละฤดูกาล จากการศึกษาในครั้งนี้พบว่า ยุงลายบ้านของทุกจังหวัดมีอัตราการติดเชื้อไวรัสเดงกีสูงสุดในฤดูร้อนระหว่างร้อยละ 64.4-77.5 และผู้ป่วยไข้เลือดออกมีอัตราป่วยสูงสุดในฤดูฝน นอกจากนี้ยังตรวจพบการถ่ายทอดเชื้อไวรัสเดงกีจากแม่สู่ลูกในลูกน้ำและยุงลายบ้านเพศผู้ของทุกจังหวัดในอัตราที่แตกต่างกันไปในแต่ละจังหวัด โดยมีอัตราการตรวจพบอยู่ระหว่างร้อยละ 18.3-46.9 ในลูกน้ำและร้อยละ 12.0-46.3 ในยุงลายบ้านเพศผู้ ในการจำแนกซีโรทัยป์ของไวรัสเดงกีในยุงลายบ้านพบว่า ซีโรทัยป์ 3 และ 1 เป็นสองซีโรทัยป์ที่พบมากที่สุด รองลงมา คือ ซีโรทัยป์ 2 และ 4 ซึ่งพบว่ามีความคล้ายคลึงกับซีโรทัยป์ที่ตรวจพบในผู้ป่วยไข้เลือดออก โดยพบว่า ซีโรทัยป์ 1 และ 3 เป็นสองซีโรทัยป์ที่พบมากที่สุด รองลงมา คือ ซีโรทัยป์ 2 และ 4 จากการศึกษาพฤติกรรมการกัดของยุงลายบ้านในแต่ละฤดูกาลพบว่า ยุงลายมีอัตราการกัดสูงสุดในฤดูร้อน คือ 30 ตัว/คน/ชั่วโมง ซึ่งข้อมูลที่ได้จากงานวิจัยนี้เป็นประโยชน์ในการเฝ้าระวังโรคไข้เลือดออกโดยสามารถใช้ข้อมูลอัตราการติดเชื้อไวรัสเดงกีในยุงลายไปประกอบการพิจารณาหามาตรการที่เหมาะสมในการตัดวงจรการระบาดของโรคในพื้นที่ที่มีไข้เลือดออกเรื้อรังและในพื้นที่ที่พบเชื้อไวรัสเดงกีสายพันธุ์ใหม่เข้ามาได้อย่างทันเวลา

คำสำคัญ: ยุงลายบ้าน พฤติกรรมการกัด การติดเชื้อไวรัสเดงกี ไวรัสเดงกี ผู้ป่วยที่สงสัยว่าติดเชื้อไวรัสเดงกี

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Introduction

Dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) are diseases transmitted by the bite of *Aedes* mosquitoes, principally *Aedes aegypti*, infected with one of the four dengue viruses, DENV 1-4 (WHO, 2011). DF/DHF currently occurs in over 100 countries worldwide and more than 2.5 billion people are at risk of infection. Around 975 million people live in urban areas in tropical and sub-tropical countries in Southeast Asia, the Pacific and the Americas (WHO, 2007). In other countries, Africa and Eastern Mediterranean including rural communities, are extensively being affected with dengue transmission as well. There are up to 50 million cases reported annually with 500,000 cases of DHF and 22,000 deaths mainly among children (WHO, 2011).

DHF in Thailand was first reported in Bangkok in 1949 (Wangroongsarb, 1997), while the first epidemic appeared in 1958 (Ungchusak and Kunasol, 1988). The highest mortality rate of DHF (1.88 per 100,000 populations) was documented in 1987 (Nisalak et al., 2003). After the first DHF outbreak occurred, the disease has spread across the country and become a major public health problem. At present, there is no specific medication for treatment of dengue infection available. The prevention and control measures of dengue disease have emphasized vector mosquito management. Attempts have been made to focus on surveillance for planning to reduce disease burden, changing behaviors to improve vector control and responding to disease epidemic (WHO, 2000).

In this study, *Ae. aegypti* larvae and adults were collected during three seasons from 4 central provinces of Thailand for detection and serotyping of dengue viruses. Data on DHF patients reported in those provinces and the suspected patients confirmed as DHF could be taken into consideration with data on dengue infection in mosquitoes for planning in response to imminent dengue occurrence. Seasonal biting activity of *Ae. aegypti* females was studied and used as supporting factor on explanation of dengue incidence. The data obtained herein might be employed in constituting an early warning monitoring system for dengue outbreak in all those endemic areas.

Materials and Methods

Study areas: Four provinces in the central region of Thailand: Nakhon Pathom, Nonthaburi, Ratchaburi and Samut Sakhon were chosen as the study areas. In each province, three districts (2 sub-districts per district, 2 villages per sub-district, and 40 dwellings per village) were conducted for collections of *Ae. aegypti* larvae and adults. The selection of those areas was based on three reasons: 1) the provinces at least once ranked on top fifteen DHF incidence (morbidity rate of DHF per 100,000 populations) reported in Thailand between 2002 and 2006, 2) the abundance of *Ae. aegypti* mosquitoes, and 3) the travel convenience for mosquito collection.

Ae. aegypti collection: The collections of *Ae. aegypti* larvae from clean water-containing containers indoor and adults using human bait (WHO, 1997) in the study areas were carried out during three seasons between March 2007 and February 2008: the winter, from November to February, the summer, from March to May, and the rainy season, from June to October. The dwellings for larval and adult mosquito collections were selected from the villages which had experienced recent DHF cases. The mosquito collection time started from 09.00 am to 05.00 pm. Six volunteers positioned themselves in dark areas of the room where most biting activity occurs. All bared their legs between knees and ankles, collected all landing and biting mosquitoes individually with plastic vial and capped it. The collectors caught mosquitoes indoors for 20 min per dwelling. The collected larvae and mosquitoes were visually identified for *Ae. aegypti* species. The live adult mosquitoes were inactivated in a refrigerator, separated by localities and sexes. All *Ae. aegypti* larvae and adults were pooled and then kept in cryogenic vials (5 larvae or mosquitoes/pool/vial) to store in liquid nitrogen for subsequent dengue viral detection. The temperature and relative humidity were recorded at the study areas.

Viral detection in Ae. aegypti: The method for detection of dengue viruses in *Ae. aegypti* larvae and adults was modified from that described by Tuksinvaracharn et al. (2004). Mosquito wings and legs were removed and the remaining bodies were processed for RNA extraction using Invisorb® Spin Virus RNA Mini Kit (Invitex Gmbh, Germany). Five oligonucleotide primers used in this study were designed by Lanciotti et al. (1992), D1 and 4 type-specific primers (TS1, TS2, TS3 and TS4). Multiplex RT-PCR was performed using BluePrint™ One Step RT-PCR Kit. Target RNA was amplified in 25-µl volume containing 2x One Step BluePrint™ Buffer, 25 pmol of each primer, One Step BluePrint™ RT Enzyme Mix, and RNase-free water. The thermal cycler was programmed to incubate at 50^0C for 30 min, 94^0C for 2 min and then to proceed with 40 cycles of 94^0C for 30 sec, 50^0C for 30 sec and 72^0C for 30 sec, the last 1 cycle of 72^0C for 7 min and final holding at 4^0C. A 6-µl product was electrophoresed through a 2% agarose gel at 100 volts, stained with ethidium bromide, visualized on a UV transilluminator and confirmed the positive bands by nucleotide sequencing analysis. Mosquitoes intrathoracically inoculated with dengue viruses were used as positive controls. These viruses were DENV-1 strain Hawaii, DENV-2 strain TR 1751, DENV-3 strain H87, and DENV-4 strain H241. Uninfected laboratory-reared *Ae. aegypti* mosquitoes were used as negative controls. The larval or adult pools positive for dengue viruses were determined for dengue infection rate which was calculated from the number of positive pools divided by the total number of tested pools x 100.

Collection of blood specimens: Blood specimens were taken from DHF patients admitted to hospitals in the study areas in 2007. The blood samples were drawn into tubes with EDTA anticoagulant, and centrifuged to obtain plasma. The plasma specimens were kept in liquid nitrogen tanks and then transported to the Arbovirus Laboratory, National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand for determination of dengue serotypes.

Viral determination in blood samples: RT-PCR was performed to determine dengue serotypes (Yenchitsomanus et al., 1996; Chanama et al., 2004). A 100 µl-patient plasma was extracted for viral RNA using QIAamp viral RNA mini kit (Qiagen Gmbh, Hilden, Germany). One-step RT-PCR kit (Qiagen) was used together with type-specific primers in RT-PCR reactions. Positive and negative controls were added in each run as well. PCR products were electrophoresed through agarose gel, stained with ethidium bromide and visualized on a UV transilluminator.

Biting activity test: The study of seasonal biting activity pattern of *Ae. aegypti* mosquitoes was simulated and conducted once a month between March 2007 and February 2008 at the research station in Bang Bua Thong district, Nonthaburi. This station, where a married couple live, contains basic facilities like the general dwellings. Twenty earthen jars (200 l in capacity) were fully filled with city hydrant water. Five hundred large *Ae. aegypti* pupae which mostly become female mosquitoes, were picked out for the experiment. Twenty five pupae were added to each jar for adult emergence. After 5-day pupal addition, the biting activity test of *Ae. aegypti* was performed through 24-hour period from 06.00 am to 06.00 am of the following day. Three volunteers sat down on small plastic chairs provided in a row. Five meter distance was set up for each volunteer. All bared their legs between knee and ankle, collected all the landing mosquitoes individually with plastic vial and capped it. The collectors caught mosquitoes indoors by the first 20-minute period of each hour. The collected mosquitoes were visually identified for *Ae. aegypti* females and pooled together with those captured in the same hour of other months by the same season. The seasonal biting rate was calculated and expressed as an average number of all mosquitoes collected in each season /person/hour. The temperature and relative humidity were recorded at the study area.

Incidence of DHF in the study areas: DHF data reported from the study areas in 2007 was obtained from the Bureau of Epidemiology, Department of

Disease Control, Ministry of Public Health, Thailand. The data was expressed as the morbidity rate of DHF per 100,000 populations.

Data analysis: Comparison of dengue infection rates determined in each seasons of all the 4 provinces was conducted using One-Way ANOVA at a significant level of 0.05.

Results

RT-PCR analysis: RNA extracted from *Ae. aegypti* larval and adult pools was processed for dengue detection by multiplex RT-PCR using dengue serotype-specific primers. The PCR products generated were analyzed by agarose gel electrophoresis to visualize the characteristic band sizes of 482 bp (DENV 1), 119 bp (DENV 2), 290 bp (DENV 3), 392 bp (DENV 4) and co-existence of more serotypes against positive controls as shown in Fig 1A and Fig 1B, respectively.

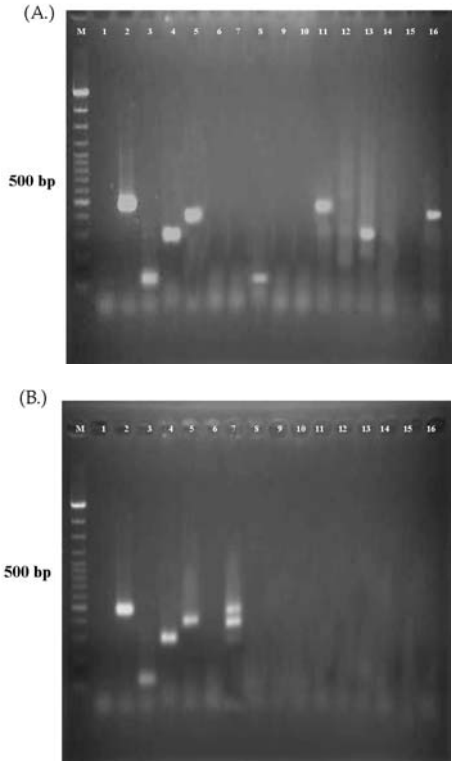

Figure 1 Agarose gel analysis of the PCR products generated by multiplex RT-PCR assay. (A.) Amplification of DNA amplicons reverse-transcribed from dengue RNA extracted from *Ae. aegypti* pools, M: DNA marker, Lane 1: negative control (uninfected *Ae. aegypti* RNA), Lane 2: positive control (DENV 1: 482 pb), Lane 3: positive control (DENV 2: 119 bp), Lane 4: positive control (DENV 3: 290 bp), Lane 5: positive control (DENV 4: 392 bp), Lane 8: sample positive for DENV 2, Lane 11: sample positive for DENV 1, Lane 13: sample positive for DENV 3, Lane 16: sample positive for DENV 4, Lanes 6,7,9,10,12,14 and 15: samples negative for dengue viruses. (B.) Co-existence of two dengue serotypes found in individual pools, M: DNA marker, Lane 1: negative control, Lane 2: positive control (DENV 1), Lane 3: positive control (DENV 2), Lane 4: positive control (DENV 3), Lane 5: positive control (DENV 4), Lane 7: sample positive for DENV 1 and DENV 4, Lanes 6 and 8-16: samples negative for dengue viruses.

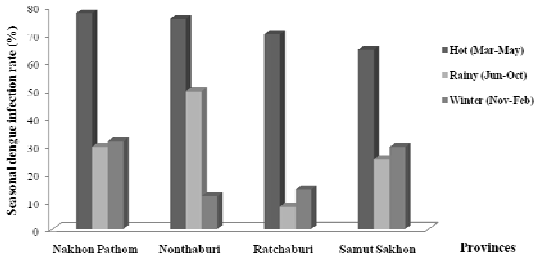

Figure 2 The dengue infection in *Ae. aegypti* females collected in each season in 4 studied provinces. All female mosquito pools separated by season and province were assayed for dengue infection rate.

Seasonal dengue infection in Ae. aegypti females: *Ae. aegypti* female pools grouped by season and province were assayed for dengue infection. In Fig 2, data showed that the dengue infection rates in female mosquitoes collected in summer from all the 4 provinces were higher significantly than those collected in winter (p= 0.000) and rainy season (p= 0.001). However, there were no statistical differences of infection rates in *Ae. aegypti* females collected between winter and rainy season (p= 0.482) The infection rates determined in summer in Nakhon Pathom, Nonthaburi, Ratchaburi and Samut Sakhon were 77.5% (31/40 pools), 75.5% (77/102 pools), 70% (7/10 pools), and 64.4% (29/45 pools), respectively. In winter collections in Nakhon Pathom, Samut Sakhon and Ratchaburi, the infection rates were 31.6% (30/95 pools), 29.4% (15/51 pools), 14.3% (6/42 pools) ,while those in rainy season were 29.4% (82/279 pools), 25% (31/124 pools) and 8% (9/113 pools), respectively. Only in Nonthaburi, the infection rate in rainy season with 49.5% (53/107 pools) was higher than that in winter with 11.8% (10/85 pools). The minimum and maximum temperature (^{0}C)/relative humidity (%RH) were recorded at the study areas in summer, rainy season and winter as follows: Nakhon Pathom: 22.3-39.1^{0}C/70-79%RH, 22.1-35.5^{0}C/77-79%RH, and 13.3-35.0^{0}C/72-79%RH, Nonthaburi: 23.8-38.0^{0}C/70-76%RH, 22.3-37.2^{0}C/72-76%RH, and 17.9-36.3^{0}C/61-69%RH, Ratchaburi: 21.0-39.5^{0}C/73-83%RH, 22.5-36.5 ^{0}C/79-85%RH, and 15.5-35.6^{0}C/70-78%RH, Samut Sakhon: 23.0-38.0^{0}C 70-76%RH, 23.1-37.0^{0}C/74-76%RH, and 17.0-36.1^{0}C/63-69%RH.

Transovarial dengue transmission in Ae. aegypti larvae and males: All *Ae. aegypti* larvae or males collected from each province were assayed for transovarial dengue infection; where infected female mosquitoes transmitted dengue viruses to their offspring via the eggs. It revealed that the occurrence of transovarial dengue transmission was found indeed in both local *Ae. aegypti* larvae and males of all the 4 provinces investigated (Fig 3). The transovarial dengue infection rates in the mosquito larvae obtained from Nakhon Pathom, Nonthaburi, Samut Sakhon, and Ratchaburi were 46.9% (99/211 pools), 45.0% (143/318 pools), 31.8% (49/154 pools), and 18.3% (11/60 pools), respectively. In adult males, the dengue infection rates found in Nonthaburi, Samut Sakhon, Nakhon Pathom and Ratchaburi were 46.3% (57/123 pools), 39.4% (71/180 pools), 37.7% (85/225 pools), and 12.0% (14/117 pools), respectively.

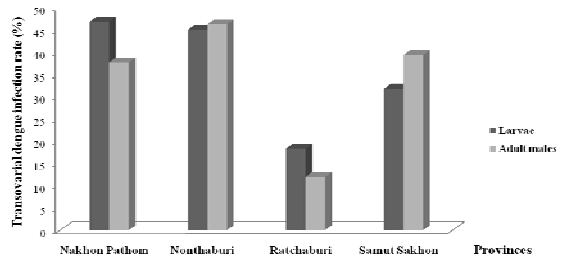

Figure 3 Transovarial dengue transmissions in *Ae. aegypti* larvae and adult males collected from the 4 studied provinces. All larval and adult male pools positive for dengue viruses from each province were tested for the transovarial dengue infection rate.

Serotyping of dengue viruses in Ae. aegypti and DHF patients: All larval and mosquito pools collected from all the 4 studied provinces and found positive for dengue viruses were grouped into 5 categories: 4 dengue serotypes and coexistence of more serotypes. The result showed that all four serotypes of dengue viruses were detected in both *Ae. aegypti* larvae and adults in all provinces examined. Of 2,798 pools tested, 1,047 pools (37.4%) were positive for dengue viruses. Among the 4 serotypes presented, DENV3 and DENV 1 were the two most prevalent serotypes with the frequencies of 17.0% (178 pools) and 16.9% (177 pools), followed by DENV 2 and DENV 4 with the frequencies of 14.7% (154 pools) and 8.2% (86 pools) as shown in Fig 4. In separated positive pools, there were 43.2% (452 pools) of co-existence of more serotypes found in individual pools. It revealed that the multiple dengue serotypes were circulating in a dwelling. Of 908 blood specimens taken from suspected patients admitted in all those provinces confirmed for dengue serotypes, only 415 suspected patients (45.7%) were positive for dengue serotypes. The proportion of each serotype showed that DENV1 and DENV 3 were the two most predominant serotypes with the frequencies of 51.3% (253 pools) and 28.8% (142 pools), followed by DENV 2 and DENV 4 with the frequencies of 14.4% (71 pools) and 5.5% (27 pools). There was no double or multiple dengue infection reported among all those serotyped DHF patients (Fig 5).

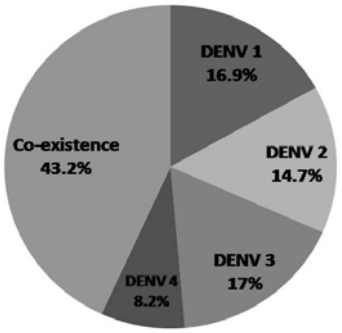

Figure 4 Serotyping of dengue viruses in *Ae. aegypti* collected from the 4 studied provinces.

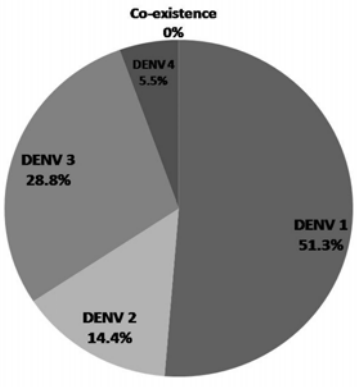

Figure 5 Serotyping of dengue viruses in suspected patients admitted to hospitals in the 4 studied provinces.

Seasonal biting activity of Ae. aegypti females: The data obtained from 3 seasons revealed that the highest biting activity occurred in summer with the biting rate of 30 mosquito/person/hour, followed by in rainy season and in winter with the biting rates of 19.8 and 15.8 mosquito/person/hour (Fig 6). The peak of biting activity in summer was presented in the afternoon at 14.00-15.00 hr, while that in rainy season and winter happened in the morning at 08.00-09.00 hr and 09.00-10.00 hr, respectively. In all three seasons, the biting activity decreased after sunset at 18.00-19.00 in winter and delayed an hour at 19.00-20.00 hr in summer and rainy season. An increase in biting activity started up again after sunrise at 06.00-07.00 hr in summer and rainy seasons and deferred an hour at 07.00-08.00 hr in winter. The minimum and maximum temperature (^{0}C)/relative humidity (%RH) were recorded at the study area in summer, rainy season and winter as follows: 23.8-38.6^{0}C /70-76%RH, 22.3-37.2^{0}C/72-76%RH, and 17.9-36.3^{0}C/ 61-69%RH.

Incidence of DHF in the study areas of the 4 provinces: DHF cases reported in various districts of the study areas in 2007 were expressed as morbidity rate per 100,000 populations. As shown in Fig 7, the morbidity rates of DHF in all the 4 studied provinces were highest in rainy season with 129.3 (Nonthaburi), 123.8 (Nakhon Pathom), 115 (Ratchaburi) and 107.3 (Samut Sakhon). Following that, the morbidity rate of DHF among those provinces appeared to be low in winter and summer with less than 50 per 100,000 populations.

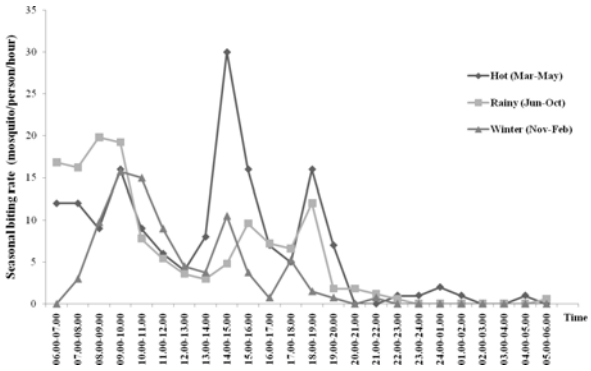

Figure 6 Seasonal biting activity of *Ae. aegypti* females. The experiment was conducted once a month throughout 12 months during March 2007 and February 2008.

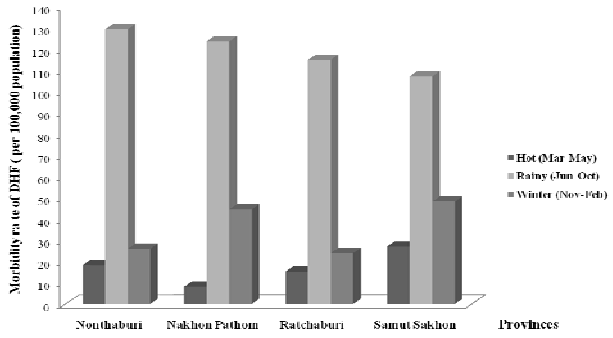

Figure 7 Incidence of DHF in the study areas of the 4 provinces**.** DHF cases reported in 2007 in the study areas of various provinces were expressed as morbidity rate per 100,000 populations.

Discussion

Virological presence of dengue viruses in field-caught *Ae. aegypti* populations has been suggested for prediction of the dengue outbreaks (Urdaneta et al., 2005; Chen et al., 2010). However, during the appearance of the disease epidemics, the environmental factors such as temperature, rainfall and humidity affecting dengue transmission should be considered as well (Ooi and Gubler, 2009). As shown in our study, the highest dengue infection rates in *Ae. aegypti* females collected from all the 4 provinces were obtained in summer. It can be explained that a moderately high temperature in the environment may influence vector efficiency and dengue virus transmission. Scott et al. (2000) reported that temperature involved in transition rate from larvae to pupae and pupae to young adults because it accelerated the development rate of *Ae. aegypti*. A warmer temperature may allow mosquito vectors to adapt themselves, survive in unsuitable conditions and reach maturity more rapidly. Consequently, mosquito biology is presumably changed by size reduction of mosquito larvae that develop smaller adults with high metabolism rates, more frequent blood feeding, and more often egg-laying (Jetten and Focks, 1997; Barbazan et al., 2002; McMichael et al., 2007). Besides, the environmental temperatures affect the extrinsic incubation period (EIP) of arboviruses in their vectors (McMichael et al., 1996; Lindsay and Mackenzie, 1997). The increase in temperature can reduce the length of viral extrinsic incubation periods in mosquito vectors (Harrington et al., 2001; Keating, 2001). As reported by Watts et al. (1987), only mosquitoes infected with high dose of DENV 2 and incubated at 30^0C with 12-day EIP, and at 32-35^0C with 7-day EIP can transmit the virus to monkeys, but no viral transmission by mosquitoes maintained at 26^0C. In addition, a 5-day decrease in EIP resulted in a three-fold higher transmission rate of dengue (Koopman et al., 1991).

In theory, it is mentioned that high temperatures should accelerate biting and egg laying activities of mosquitoes (Reiter, 2001). Our result indicated that it was possible due to the highest biting rate of *Ae. aegypti* females obtained in summer. In fact, the frequency in mosquito biting until complete feed is unknown and could be 2 or more from interrupted feeding attempts with resumption on the same or different host (Focks et al., 1995). As stated by Focks and Barrera (2006), the warmer temperature with 2 or 3^0C could induce doubling of the expected number of replete feeds which is equal to a doubling of *Ae. aegypti* density. Therefore, the mosquito vectors feeding on infected human multiply or the infected mosquitoes biting human more frequently can enhance the natural cycle of dengue disease involving human-mosquito-human transmission. However, a morbidity rate of DHF reported patients in our study areas was highest in rainy season in which populations of the mosquito females peaked each year during May-June in Thailand (Scott et al., 2000). The peak for DHF cases occurred about 2 months after the peak of *Ae. aegypti* populations (Halstead, 1966). Additionally, our results supported the former observations on the seasonal feeding pattern of *Ae. aegypti* suggesting that annual DHF epidemics was more likely the result of increased frequency of feeding on humans during the hot-dry and rainy seasons (Yasuno and Pant, 1970; Pant and Yasuno, 1973).

As well-known, *Ae. aegypti* females feeding on viremic humans in acute phase of infection become infected with dengue viruses. Subsequently, the mosquitoes maintain the viruses for life and transmit them to susceptible individuals (WHO, 1998). More efficiently, the infected female mosquitoes are able to pass on the viruses to their offspring through the eggs as demonstrated in *Ae. aegypti* larvae and adult males both infected experimentally and collected from the field (Joshi et al., 2002; Usavadee et al., 2006; Thongrungkiat et al., 2011). It is known that transovarially infected mosquitoes are capable of transmitting the virus through the bite (Mourya et al., 2001). Therefore, we demonstrated this phenomenon and found the occurrence of transovarial transmission of dengue viruses in local *Ae. aegypti* collected from all the 4 studied provinces. It is noted that there had been transovarial maintenance of dengue viruses in those endemic areas. This vertical transmission may be one of the factors that provides a mechanism for viruses to survive in dry season and winter with low mosquito populations (Rosen et al., 1983).

Determination of dengue serotypes in *Ae. aegypti* mosquitoes could be used as an important surveillance indicator to investigate the epidemiological situation of DHF (Kow et al., 2001; Mendez et al., 2006; Chen et al., 2010). Our study showed that all four dengue serotypes were found in the *Ae. aegypti* larvae and adults in which DENV 3 and DENV 1 were the two most prevalent serotypes. Serotyping of dengue viruses in blood specimens of suspected patients showed similar results as obtained in mosquitoes in that DENV 1 and DENV 3 were the two most predominant. Additionally, it was found that there is co-existence of more serotypes of dengue viruses circulating in the same household of the study areas. Previous work reported that the occurrence of multiple infections with different DENV serotypes was possible in regions of hyperendemicity

(Mackenzie et al., 2004). As reported by Gubler et al. (1985), there was a case of natural concurrent infection with DENV 1 and DENV 4 but with only mild symptoms during a 1982 outbreak in Puerto Rico. However, more severe symptoms of dengue disease, DHF/DSS, are caused by secondary infection with a different dengue serotype (Halstead et al., 1970; Vaughn et al., 2000; Nisalak et al., 2003) due to antibody-dependent enhancement of disease (Porterfield, 1986). The perception of the presence of multiple serotypes of dengue viruses co-circulating in those endemic areas can ignite public consciousness those who have never experienced dengue infection or were previously infected with serotypes different from those circulating in their dwellings.

Since no specific treatment or effective vaccine against all four serotypes of dengue viruses are currently available, preventive measures have emphasized vector control and personal protection measures. However, public health practitioners still lack adequate knowledge on mosquito biology and ecology which is necessary to operate control of mosquito vectors efficiently. For personal and household protection, commercial products have been less used in response to any public health concerns, but employed to mitigate the nuisance of biting mosquitoes. Furthermore, insufficiency of data on dengue infection and biting behavior of mosquito vector as well as dengue infection history of the patients are important reasons that cause failure of dengue control efforts. This study provided advantageous data on vector and dengue controls and revealed the highest dengue infection rate and highest biting rate of *Ae. aegypti* females obtained in summer including the phenomenon of transovarial transmission found in all provinces investigated. In addition, the data on dengue serotypes detected in mosquito vectors and DHF patients were reported in our study as well. Knowledge of all these factors are beneficial to public health practitioners in planning and implementing dengue management programs. Implementation of integrated vector management would diminish dengue outbreak usually occurring during the rainy season. Information gathered in our study could be used as an early warning monitoring system for dengue outbreaks and for the detection of newly introduced virus serotypes in endemic areas.

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