International Journal of TROPICAL DISEASE & Health
20(2): 1-10, 2016; Article no.IJTDH.30034
ISSN: 2278–1005, NLM ID: 101632866

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Spatial Circulation of Dengue Serotypes in Eastern Thailand during 2012-2015

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Authors' contributions

This work was carried out in collaboration between both authors. Author SC designed the study and wrote the protocol. Authors SC and YP did the statistical analysis and literature searches while analyses of study was done by author SC. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2016/30034
Editor(s):
(1) Viroj Wiwanitkit, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.
Reviewers:
(1) Pablo A. Méndez-Lázaro, University of Puerto Rico, USA.
(2) Raul H. Morales-Borges, Ashford Institute of Hematology & Oncology, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/17025

Original Research Article

Received 13th October 2016
Accepted 22nd November 2016
Published 25th November 2016

ABSTRACT

Dengue virus infection is an epidemic infectious disease and currently a major public health problem in Thailand. The epidemiology of dengue is characterized by cyclic epidemic activity alternating between years of relatively low and high dengue incidence. The annual change of predominant serotypes was the cause of severity of the disease. This study was to determine the circulating dengue serotype by reverse transcription polymerase chain reaction (RT-PCR) during January 2012 to December 2015. A total of 527 seropositive acute samples were analyzed from dengue fever patients in eight provinces in eastern Thailand. Two hundred and forty five samples were found positive, of which 39.2%, 35.5%, 14.3% and 11.0% were affected with DENV-1, DENV-3, DENV-4 and DENV-2 respectively. From 2012 to 2013, the predominant dengue serotype was DENV-1 whereas DENV-3 and DENV-4 were predominant in 2014. There was an apparent increase in the percentage of DENV-4 from 2014 to 2015 and DENV-4 was predominant in 2015. DENV-2 was the least dengue serotype in this region. The study indicated that all four dengue

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serotypes were circulating in eastern Thailand and the predominant serotypes were dynamic. The identification of dengue viruses infecting the human population provides an important means of early detection of any change in the prevalence of dengue virus serology. Our study has shown the pattern of dengue virus in eight provinces of eastern Thailand from year to year and provided some insight into the dengue epidemic situation in this region. This information should be beneficial to dengue surveillance and prevention and control programs in Thailand.

Keywords: Dengue serotype; epidemiology; eastern Thailand.

1. INTRODUCTION

Dengue is a global mosquito-borne viral disease affecting humans. The primary vector is the *Aedes aegypti* mosquito. Before 1970, dengue was present in the tropical and subtropical regions of the Americas, Africa, Mediterranean, Western Pacific region and Southeast Asia regions [1]. The incidence of dengue has grown dramatically around the world in recent decades. In 2012, imported cases were detected in mainland Portugal and 10 other countries in Europe. Many cases occurred in China, Costa Rica, Honduras and Mexico in 2013. The year 2015 was characterized by large dengue outbreaks worldwide, with the Philippines reporting more than 169,000 cases and Malaysia exceeding 111,000 cases, representing a 59.5% and 16.0% increase in case numbers to the previous year, respectively. Moreover, dengue continues to affect India, the Island of Hawaii of United States of America, Brazil, the Pacific land countries of Fiji, Tonga and French Polynesia [2]. Dengue disease is a public health priority in Southeast Asia, and Thailand contributes substantially to the regional disease burden [3]. The first dengue outbreak occurred in Bangkok in 1958, initially in a pattern with a 2-year cycle, and subsequently in irregular cycles, as the disease spread throughout the country. The largest outbreak was reported in 1987, with an incidence rate of 325 cases/ 100,000 population. In 1999, the Ministry of Public Health, Thailand initiated a dengue control program to reduce the incidence rate to less than 50 cases/ 100,000 population [4]. Over the review period wide yearly variations in incidence occurred, with regular epidemics in 2001, 2002, 2010 and 2013 with dengue disease remaining a highly seasonal disease. The data showed a seasonal peak of dengue disease in the numbers of cases and deaths between May and September annually. The pattern coincides with the rainy season in Thailand. In addition, the age group shifted during the review period from younger towards older, although dengue disease in Thailand remains a childhood disease predominately with higher severity reported in young children [3,5-6]. In recent years, increasingly larger dengue outbreaks have occurred. There were 139,355, 114,800, 116,497, 108,728 cases of dengue reported to the Bureau of Epidemiology in 2001, 2002, 2010 and 2013 respectively [6].

Dengue infections are caused by four closely related viruses named DENV-1, DENV-2, DENV-3, and DENV-4. These four viruses are called serotypes because each has different interactions with the antibodies in human blood serum. The four dengue viruses are similar — they share approximately 65% of their genomes — but even within a single serotype, there is some genetic variation. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms [7]. Dengue virus causes varying clinical symptoms ranging from dengue fever (DF) and dengue haemorrhagic fever (DHF) to dengue shock syndrome (DSS) according to the World Health Organization criteria [8]. Recovery from infection by one serotype provides lifelong immunity against that particular serotype [7]. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing dengue haemorrhagic fever [9-10]. Gubler et al. [11] and Lam et al. [12] reported that virological surveillance, which involves monitoring of dengue virus infection in humans, has been used as an early warning system to predict outbreaks. Such surveillance, based on the isolation and identification of dengue viruses infecting the human population, provides an important means of early detection of any change in the prevalence of dengue virus serotypes. Nisalak et al. [13] reported that the predominant dengue serotype in the outbreaks in Bangkok during 1997–1998 was DENV-3. Anantapreecha et al. [14] detected the predominant serotypes DENV-1 and DENV2 in six provinces across Thailand during 2001– 2002. Veeraseatakul et al. [15] reported the predominant serotypes DENV-2 in four provinces of northern Thailand during

2002-2006. In 2010, Bureau of Epidemiology, Department of disease control, Thailand reported that the most common dengue serotype was DENV-2, representing over half of all those isolated [5]. However, dengue serotypes in eight provinces of eastern Thailand have not yet been well elucidated. Thus, the present study is aimed at clarifying the pattern of circulating dengue serotypes in this region with a view to better understand the epidemiological complexities of the epidemics of dengue infection.

2. MATERIALS AND METHODS

2.1 Patients

A total of 527 seropositive acute samples were subjected to dengue serotype examination by RT-PCR at the Regional Medical Sciences Centre, Chonburi, Ministry of Public Health, Thailand. These samples were collected from DF/DHF patients according to WHO criteria in eight eastern provinces that included Chonburi, Rayong, Chanthaburi, Samutprakan, Chachoengsao, Prachinburi, Sakaeo and Trat during 2012-2015 (Fig. 1) and were confirmed for dengue infection by IgM/IgG ELISA [16]. There were 114, 89, 86, 61, 54, 61, 35 and 27 respectively (Table 1).

Table 1. Summary of seropositive dengue cases in eight provinces of Eastern Thailand, 2012 – 2015

Province	Year				Total
	2012	2013	2014	2015	
Chonburi	35	43	24	12	114
Rayong	25	28	12	24	89
Chanthaburi	12	5	63	6	86
Samutprakan	21	16	15	9	61
Chachoengsao	20	21	5	8	54
Prachinburi	20	28	5	8	61
Sakaeo	15	5	9	6	35
Trat	10	5	6	6	27
Total	158	151	139	79	527

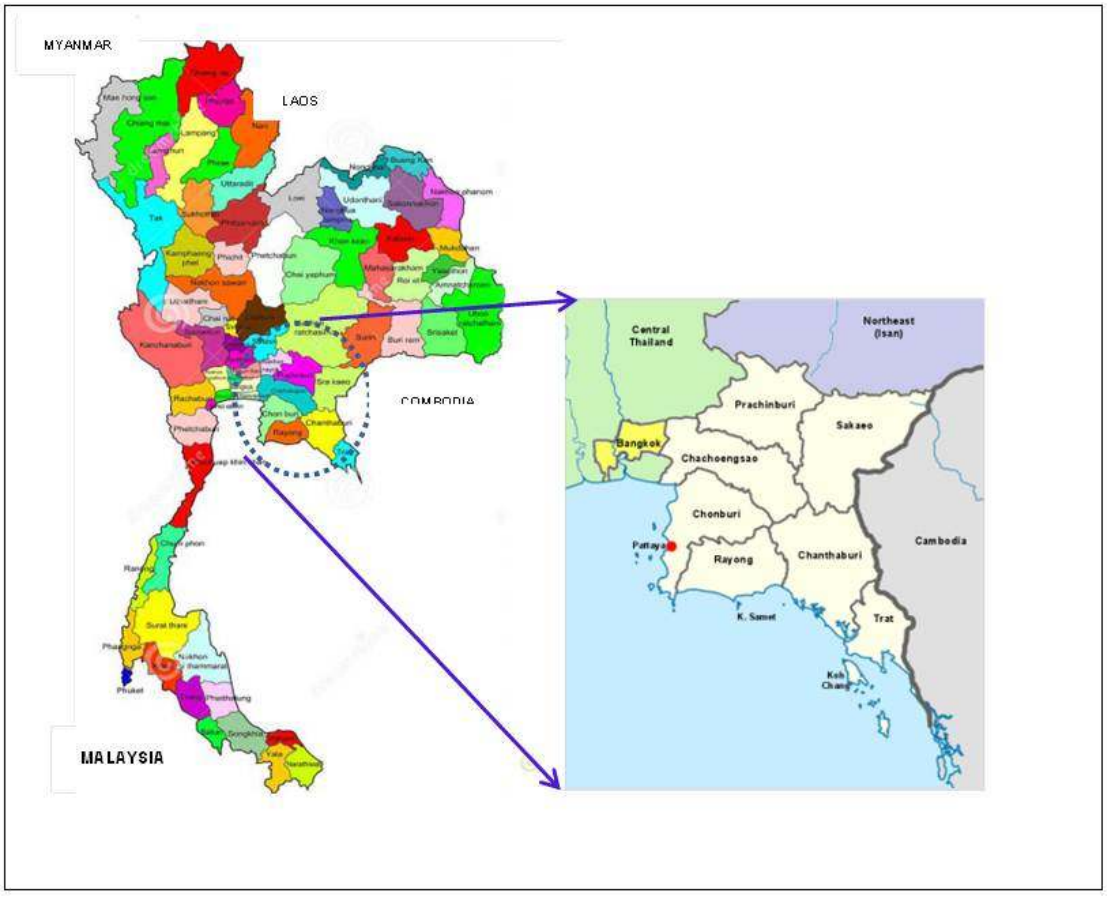

Fig. 1. Map of eight provinces of Eastern Thailand
Source: www.dreamstime.com

2.2 RNA Extraction

Viral RNA was isolated by using QIAamp viral RNA Mini Kit (QIAGEN, GmbH, Hilden, Germany). Briefly, the serum (100 µl) was added and mixed with 400 µl of AVL/RNA carrier solution (lysis buffer). After incubation at room temperature for 10 min, 400 µl of absolute ethanol was added to the solution. All the solutions were then transferred to a spin column and were spun at 8,000 rpm for 1 min. The RNA was then washed by adding 500 µl of AW1 (washing buffer 1) and spun at 8,000 rpm for 1 min and following the same procedure with AW2 (washing buffer 2). Finally, the RNA was eluted by adding 60 µl of elution buffer and spun at 8,000 rpm for 1 min. The eluted RNA was kept in -70℃ until use.

2.3 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR method was performed as previously described by Yenchitsomanus et al. [17]. Briefly, 5 µl of RNA solution was mixed with reagents of one step RT-PCR kit (QIAGEN) and specific oligonucleotide primers of dengue-envelope (E) gene; DUL and DUR. The amplification was performed in a thermal cycler. (MJ research PTC-100, USA) The amplification reaction comprised 40 cycles of denaturation at 94℃ for 1 min, annealing at 45℃ for 1 min and extension at 72℃ for 1 min. One µl of the primary PCR product was used as the template for the second PCR with four serotype-specific primer pairs; D1L, D1R, D2L, D2R, D3L, D3R, D4L and D4R (Table 2). The PCR step was the same as above with the annealing temperature set at 62℃. Negative and positive dengue controls were used. The secondary PCR products were analyzed in 2% agarose gel electrophoresis and then visualized by ethidium bromide staining.

Table 2. Primer sequences of dengue virus and serotyping by RT-PCR

Primer	Sequence 5'- 3'
DUL	GCTGTGTCACCCAGAGTGGCCAT
DUR	TGGCTGGTGCACAGACAATGGTT
D1L	GGGGCTTCAACATCCCAAGAG
D1R	GCTTAGTTTCAAAGCTTTTTCAC
D2L	ATCCAGATGTCATCAGGAAAC
D2R	CCGGCTCTACTCCTATGATG
D3L	CAATGTGCTTGAATACCTTTGT
D3R	GGACAGGCTCCTCCTTCTTG
D4L	GGACAACAGTGGTGAAAGTCA
D4R	GGTTACACTGTTGGTATTCTCA

2.4 Statistical Methods

The statistical analysis for significance was done using Chi-square. $P<0.05$ was considered significant. The data were analysed using EPI-inflo 7 computer package.

3. RESULTS

The number of seropositive acute samples and dengue serotypes in the eight provinces during 2012-2015 are shown in Table 3. Two hundred and forty-five dengue viral samples were detected with an average positivity rate of 46.5% by RT -PCR. All the four dengue serotypes were detected during this study. The total numbers of positive dengue samples were analyzed. DENV-1 was the most predominant serotype as 39.2%, followed by DENV-3, DENV-4 and DENV-2 as 35.5%, 14.3% and 11.0% respectively. The pattern of dengue serotypes in eastern Thailand by year during 2012-2015 is shown in Fig. 2. From 2012 to 2013, a total of 158 and 151 samples showed the proportion of predominant serotype DENV-1 as 42.3% and 47.3%, followed by DENV-3 as 36.5% in 2012 and 33.6%in 2013. In 2014, a total of 139 samples showed the proportion of predominant serotype DENV-3 as 46.2% and DENV-4 as 46.2%. In 2015, a total of 79 samples showed the proportion of predominant serotype DENV-4 as 80%. DENV-1 was not found from any study samples during 2014-2015. DENV-2 was found to be the least circulating serotype during 2012 to 2015 but remained in circulation in eastern region throughout this 4-year period.

The distribution of the predominant dengue serotypes by provinces was analyzed. In Chonburi province, the predominant serotypes were DENV-1 (45.5% and 40.7%) in 2012 and 2013. In Rayong province, the predominant serotypes were DENV-3 (42.9%, 50.0% and 100.0%) from 2012 to 2014 respectively and DENV-4 (100.0%) in 2015. In Chanthaburi province, the predominant serotypes were DENV-3 (42.9% and 50.0%) in 2012 and 2013, and DENV-4 (50.0%, 64.7% and 100.0%) from 2013 to 2015 respectively. In Samutprakan province, the predominant serotypes were DENV-1 (57.1% and 66.7%) in 2012 and 2013 and DENV-2 (40.0%) in 2014. In Chachoengsao province, the predominant serotype was DENV-1 (46.2% and 52.6%) in 2012 and 2013 and DENV-3 (46.2%) in 2012. In Prachinburi province, the predominant serotype was DENV-1 (75.0% and 73.7%) in 2012 and 2013. In

Sakaeo province, the predominant serotype was DENV-3 (60.0%, 33.3% and 100%) from 2012 to 2014 respectively and DENV-2 (100%) in 2015. In Trat province, the predominant serotype was DENV-3 (80.0%, 100.0 % and 100%) from 2012 to 2014 respectively and DENV-4 (100%) in 2015 (Table 3).

The comparison of the predominant dengue serotypes among the eight provinces by year was analyzed. In 2012, DENV-1 was predominant in four province (Chonburi, Samutprakan, Chachoengsao and Prachinburi) and DENV-3 was predominant in five provinces (Rayong, Chanthaburi, Chachoengsao, Sakaeo and Trat. In 2013. DENV-1 was predominant in five provinces (Chonburi, Samutprakan, Chachoengsao and Prachinburi and Sakaeo). DENV-3 was predominant in four provinces (Rayong, Chanthaburi, Sakaeo and Trat). In 2014, DENV-3 was predominant in four provinces (Rayong, Samutprakan, Sakaeo and Trat) and DENV-4 was predominant in one province (Chanthaburi). In 2015, DENV-2 was predominant in one province (Sakaeo) and DENV-4 was predominant in three provinces (Rayong, Chanthaburi and Trat). In addition, we detected predominant serotypes with equal percentages in 2012 (DENV-1 and DENV-3) and in 2013 (DENV-1, DENV-2 and DENV-3). This result has shown that the spread of predominate DENV-1 was increasing from four provinces to five provinces during 2012–2013; after that it was not found in any province whereas predominate DENV-3 was decreasing from five provinces to four provinces during 2012-2014; after that it was also not found in any provinces. In contrast, the spread of predominate DENV-4 was increasing from one province to three provinces during 2014-2015 (Table 3).

4. DISCUSSION

From the 527 seropositive acute samples collected from confirmed DF/DHF patients with positive anti-dengue IgM antibody by ELISA for this study, only 245 (46.5%) samples could be found positive for dengue virus. It is possible that some patients visited the hospital in the late period of viremia, as it has been reported that the samples from patients which were collected on fever day 1 were 50% positive for dengue virus [17]. Moreover, other factors could be influenced by the outcome of laboratory determination, such as sample collection, handling and storage from hospital to the Regional Medical Sciences Centre, Chonburi.

Our study found that all the four dengue serotypes were circulating continuously in eight provinces of eastern Thailand, with one serotype emerging as the cause of a periodic dengue infection. These results were broadly similar to the other regions [6,15,18-21].

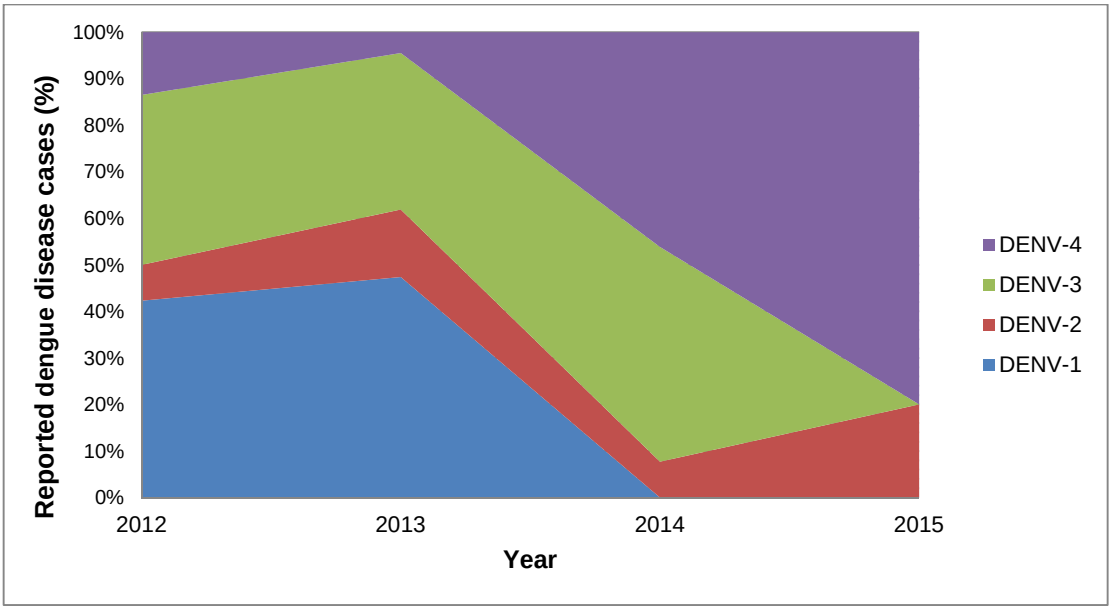

Fig. 2. Pattern of circulating dengue virus serotypes in eastern region by year, 2012-2015

Table 3. Summary of seropositive dengue cases and dengue serotypes in eight provinces of Eastern Thailand, 2012 – 2015

Year	Province	Seropositve acute sample	Positive dengue by RT-PCR	Dengue serotype							
				DENV-1	(%)	DENV-2	(%)	DENV-3	(%)	DENV-4	(%)
2012	Chonburi	35	22	10	(45.5)	2	(9.1)	6	(27.3)	4	(18.2)
	Rayong	25	21	8	(38.1)	1	(4.8)	9	(42.9)	3	(14.3)
	Chanthaburi	12	7	1	(14.3)	1	(14.3)	3	(42.9)	2	(28.6)
	Samut prakan	21	14	8	(57.1)	1	(7.1)	3	(21.4)	2	(14.3)
	Chachoengsao	20	13	6	(46.2)	1	(7.7)	6	(46.2)	0	(0.0)
	Prachinburi	20	12	9	(75.0)	1	(8.3)	1	(8.3)	1	(8.3)
	Sa Kaeo	15	10	1	(10.0)	1	(10.0)	6	(60.0)	2	(20.0)
	Trat	10	5	1	(20.0)	0	(0.0)	4	(80.0)	0	(0.0)
	Total	158	104	44	(42.3)	8	(7.7)	38	(36.5)	14	(13.5)
2013	Chonburi	43	27	11	(40.7)	7	(25.9)	8	(29.6)	1	(3.7)
	Rayong	28	20	6	(30.0)	3	(15.0)	10	(50.0)	1	(5.0)
	Chanthaburi	5	4	0	(0.0)	0	(0.0)	2	(50.0)	2	(50.0)
	Samut prakan	16	15	10	(66.7)	1	(6.7)	4	(26.7)	0	(0.0)
	Chachoengsao	21	19	10	(52.6)	2	(10.5)	7	(36.8)	0	(0.0)
	Prachinburi	28	19	14	(73.7)	2	(10.5)	2	(10.5)	1	(5.3)
	Sa Kaeo	5	3	1	(33.3)	1	(33.3)	1	(33.3)	0	(0.0)
	Trat	5	3	0	(0.0)	0	(0.0)	3	(100.0)	0	(0.0)
	Total	151	110	52	(47.3)	16	(14.5)	37	(33.6)	5	(4.5)
2014	Chonburi	24	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Rayong	12	2	0	(0.0)	0	(0.0)	2	(100.0)	0	(0.0)
	Chanthaburi	63	17	0	(0.0)	0	(0.0)	6	(35.30)	11	(64.7)
	Samut prakan	15	5	0	(0.0)	2	(40.0)	2	(40.0)	1	(20.0)
	Chachoengsao	5	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Prachinburi	5	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Sa Kaeo	9	1	0	(0.0)	0	(0.0)	1	(100.0)	0	(0.0)
	Trat	6	1	0	(0.0)	0	(0.0)	1	(100.0)	0	(0.0)
	Total	139	26	0	(0.0)	2	(7.7)	12	(46.2)	12	(46.2)

Year	Province	Seropositve acute sample	Positive dengue by RT-PCR	Dengue serotype							
				DENV-1	(%)	DENV-2	(%)	DENV-3	(%)	DENV-4	(%)
2015	Chonburi	12	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Rayong	24	2	0	(0.0)	0	(0.0)	0	(0.0)	2	(100.0)
	Chanthaburi	6	1	0	(0.0)	0	(0.0)	0	(0.0)	1	(100.0)
	Samut prakan	9	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Chachoengsao	8	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Prachinburi	8	0	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
	Sa Kaeo	6	1	0	(0.0)	1	(100.0)	0	(0.0)	0	(0.0)
	Trat	6	1	0	(0.0)	0	(0.0)	0	(0.0)	1	(100.0)
	Total	79	5	0	(0.0)	1	(20.0)	0	(0.0)	4	(80.0)
Total		527	245(46.5%)	96	(39.2)	27	(11.0)	87	(35.5)	35	(14.3)

The pattern of dengue serotypes in geographical locations in Thailand is dynamic and there may have been many factors associated with these dynamic changes such as human and vector population in terms of the number and their movement, environment, social factors and public health infrastructure [10]. This is also seen the dynamic changes of dengue serotype in Puerto Rico and The Caribbean. In Peurto Rico, DENV-3 emerged in 1998 after 21-year absence. The rapidly expansion of DEN-3 on the island correlated with the withdrawal of the other serotypes for 7 years. The DENV-3 declined in 2008 and remain undetected whereas all four serotypes circulated in the Caribbean area, but predominantly DEN-1 and DENV-2 during the period 2001-2007 [22,23]. At the time of this study, the serotype data show a reduction in the proportion of DENV-1 and a slight increase in the proportion of DENV-4. DENV-1 peaked during 2012-2013 and then was not found during 2014-2015 whereas DENV-4 was found throughout the 4-year period and peaked in 2015. During 2014-2015, however, the percentage of DENV-4 considerably increased up to 46.2(n=12) and 80.0 (n=4) ($p<0.05$), which also seemed to be predominant during this year.

Broadly, there was a reduction in the proportion of DENV-1 and increase in the proportion of DENV-4 in this region. The predominant serotypes were dynamic and changed between DENV-1 and DENV-4. This study is in agreement with another study demonstrating that DENV-1 was replaced by DENV-4 [24]. The study has shown that serotype predominance can shift from one to another. This finding provides an important starting point of change to predominance from one serotype to another in the eastern region and might be a predictive indicator of the continuous pattern of dengue serotype predominance in the next year. However, associations were proposed between both the burden and severity of diseases and the specific DENV serotypes circulating in the population, the sequence of dengue serotypes causing primary and secondary infection, which indicate the multifactorial processes that influence dengue diseases severity. Each dengue serotype has characteristics that affect the nature of dengue epidemic and disease severity. DENV-1 has been linked with high morbidity and low mortality whereas DENV-4 was linked to lower levels of virulence [25-27]. Dengue shock syndrome has associated with secondary infection attributable to DENV-2 [28]. Our study found that DENV-2 was the least proportion but remained in circulation throughout this 4-year period. Finding such as these have prompted suggestions that changes in predominant serotypes are associated with changes in disease severity [7,9-10,15]. Therefore, this study would be beneficial to the description and understanding of dengue epidemiology and to development surveillance systems of dengue infection control.

5. CONCLUSION

This study has shown the pattern of dengue virus serotypes in eight provinces of eastern Thailand from year to year and provided some insight into the dengue epidemic situation in this region. Further studies on heterogeneous temporal circulation for at least 10 year would allow observation overtime and studies through several epidemics would also accurately reflect recent changes in dengue epidemiology. This information should be beneficial in long-term dengue surveillance and future work can focus on using this pattern of dengue serotype circulation to develop a predictive model of dengue disease in this region.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by institution ethics committee. Regional Medical Sciences Center. Department of Medical Sciences. Ministry of Public Health, Thailand.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17025