อุบัติการณ์ของการติดเชื้อไวรัสเด็งกี่ในตัวอย่างสิ่งส่งตรวจที่สงสัยการติดเชื้อไวรัสเด็งกี่ของผู้ป่วยโรคไข้เลือดออกโรงพยาบาลศิริราช ระหว่างปี พ.ศ. 2555-2559

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บทคัดย่อ

โรคไข้เลือดออกเป็นโรคติดเชื้อไวรัสที่มียุงลาย Aedes aegypti เป็นพาหะ พบในเขตร้อนและกึ่งเขตร้อน ซึ่งประเทศไทยเป็นหนึ่งในพื้นที่ที่มีการระบาดของไข้เลือดออกตามฤดูกาล การวินิจฉัยเชื้อไวรัสเด็งกี่สามารถทำได้โดยการตรวจหา NS1 แอนติเจนในซีรั่มของผู้ป่วยในช่วงเริ่มต้น (1-7 วันหลังติดเชื้อ) หรือการตรวจหาแอนติบอดี ชนิด IgM ซึ่งพบได้ระหว่าง 3-5 วันหลังจากเริ่มมีไข้ ส่วนแอนติบอดี ชนิด IgG จะตรวจพบในช่วงท้ายของการติดเชื้อและคงอยู่นาน การตรวจ IgG ได้ผลบวก จึงบ่งบอกว่าผู้ป่วยเคยได้รับเชื้อมาก่อน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาอุบัติการณ์ของการติดเชื้อไวรัสเด็งกี่ในผู้ป่วยที่สงสัยว่าติดเชื้อไวรัสเด็งกี่ที่มารับการตรวจรักษาที่โรงพยาบาลศิริราช ช่วงระหว่างเดือนมกราคม พ.ศ. 2555 - ธันวาคม พ.ศ. 2559 จากการวิเคราะห์ผลการตรวจหาแอนติเจน NS1 ของเชื้อไวรัสเด็งกี่ และ/หรือ แอนติบอดี ชนิด IgM ด้วยวิธี immunochromatographic assay ในซีรั่มผู้ป่วยจำนวน 5,196 ราย พบว่าผู้ป่วยจำนวน 1,813 ราย ให้ผลบวกต่อการทดสอบแอนติเจน NS1 ของเชื้อไวรัสเด็งกี่ และ/หรือ แอนติบอดี ชนิด IgM ผลการศึกษาพบว่าอัตราการติดเชื้อไวรัสเด็งกี่อยู่ที่ 36.38% (ความเชื่อมั่นร้อยละ 95 = 32.91, 39.99), 40.75% (ความเชื่อมั่นร้อยละ 95 = 37.71, 43.86), 34.64% (ความเชื่อมั่นร้อยละ 95 = 30.82, 38.67), 43.33% (ความเชื่อมั่นร้อยละ 95 = 40.93, 45.76) และ 17.78% (ความเชื่อมั่นร้อยละ 95 = 15.69, 20.07) [ในปี พ.ศ. 2555, 2556, 2557, 2558 และ 2559] ตามลำดับ ค่าเฉลี่ยอุบัติการณ์ของเชื้อไวรัสเด็งกี่ 5 ปีเท่ากับ 34.89% (33.60%, 36.21%) พบการติดเชื้อไวรัสเด็งกี่สูงสุดในปี พ.ศ. 2558 (43.33%) ในขณะที่อัตราต่ำสุดในปี พ.ศ. 2559 (17.78%) โดยพบการติดเชื้อสูงในช่วงเดือนพฤศจิกายนถึงธันวาคม ทั้งนี้การติดเชื้อไวรัสเด็งกี่พบมากที่สุดในผู้ป่วยที่มีอายุระหว่าง 15-24 ปี และอัตราการติดเชื้อไวรัสเด็งกี่ระหว่างเพศหญิงและเพศชายไม่มีความแตกต่างกันอย่างมีนัยสำคัญ (หญิง:ชาย = 1.2:1; p = 0.29)

คำสำคัญ: เด็งกี่ ไข้เลือดออก อุบัติการณ์

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Incidence of dengue virus infection in suspected dengue virus-infected patients' samples at Siriraj Hospital during 2012-2016

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Abstract

Dengue fever is an Aedes aegypti mosquito-borne viral infectious disease found in tropical and subtropical areas. Thailand is one of the endemic areas in which seasonal dengue outbreaks usually occur. Laboratory diagnosis of dengue infection can be done by NS1 antigen detection in the patient's serum at the early stage of disease (1-7 days after infection). Another method is to detect IgM anti-dengue antibodies, which usually rise 3-5 days after the onset of fever. The IgG anti-dengue antibodies are detected at the later stage of infection and usually stay positive for a long time, thereby indicating the previous dengue infection. This study aims to investigate the incidence of dengue virus infection in suspected dengue virus-infected patients, who visited Siriraj Hospital during January 2012 - December 2016. Here, we analyzed the test results of 5,196 patients' serum samples that were processed for dengue NS1 antigen and/or IgM antibody detection using immunochromatographic assay and found that 1,813 serum samples were positive for either dengue NS1 antigen or dengue specific IgM antibody or both. The result demonstrated that dengue virus infection rate was 36.38% (95% CI = 32.91, 39.99), 40.75% (95% CI = 37.71, 43.86), 34.64% (95% CI = 30.82, 38.67), 43.33% (95% CI = 40.93, 45.76) and 17.78% (95% CI = 15.69, 20.07) in 2012, 2013, 2014, 2015, and 2016, respectively. The five-year average incidence of dengue infection was 34.89% (33.60%, 36.21%). The highest numbers of dengue-infected cases were in 2015 (43.33%), while the lowest case numbers were in 2016 (17.78%). High rate of dengue virus infection was observed during November to December. Interestingly, dengue virus infection is the mostly detected in patients aged between 15-24 years. Infection rates between females and males were not significantly different (Female : Male = 1.2:1; p = 0.29).

Keywords: dengue virus, dengue fever, incidence

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Introduction

Dengue is a mosquito-borne viral infection caused by four closely related serotypes of dengue virus (DENV1, DENV2, DENV3, and DENV4). Dengue viruses are transmitted by infected *Aedes aegypti* mosquitoes. Dengue is present in the tropical and subtropical region[1]. Dengue infection can be asymptomatic or a broad spectrum of clinical illness ranging from undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[2]. Not only that almost half of the global population is living in the countries where dengue is endemic[3,4], but dengue incidences worldwide also have been dramatically increased over the past five decades[5]. The disease severity is often associated with subsequent infection by a different serotype, which involves the antibody-dependent enhancement (ADE) mechanism[6].

Dengue specific immunoglobulin M (IgM) and protective IgG antibodies are generated in individuals infected by any of the DENV serotypes. The IgM antibodies are the first immunoglobulin isotype to rise upon infection, and therefore are commonly used for clinical diagnosis of DENV-infected cases. IgM can be detected between 3-5 days after the onset of fever, peak around two weeks after the onset of symptoms and generally decline over a period of 2-3 months. The specific IgG antibodies are typically found in serum at the end of the convalescent period (9-10 days) in a primary infection. It may also be detected earlier in the case of a secondary infection and remains at high titers up to 30-40 days after infection. The specific IgG antibodies may remain detectable for decades, allowing the identification of individuals who have been in contact with the virus, and therefore are generally used for epidemiological study. Apart from detecting dengue specific antibodies, the laboratory diagnosis of dengue infection can also be achieved by detecting viral components. The detection of viral non-structural protein 1 (NS1) in sera of patients at early DENV infection (day 1-7 of illness) is highly recommended for clinical diagnosis of DENV-infected cases[7].

Although the first case of DENV infection was recognized in Thailand in 1949 and several dengue outbreaks were reported in Thailand[8,9], the study of epidemiology of DENV infection in Thailand was limited[10]. This is the results of an inadequate human and vector surveillance, non-reporting of illness syndromes. Moreover, poor diagnostic capacity limits the detection of dengue virus infection in rural area, sometimes resulting in delays in outbreak recognition. In the absence of laboratory confirmation, diagnosis of DENV infection based on merely clinical presentation could be inaccurate. Accordingly, serological studies to unveil the incidence of DENV infection has a potential

to yield information of the burden of DENV infection and also urge health personnel and the general population at risk to adopt preventive measures for the disease. In this study, we analyzed the laboratory result data and calculated the positive rate of detection of dengue NS1 antigen and/or dengue specific IgM from DENV suspected patients' serum samples sent to Serology laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital during January 2012 to December 2016. The characteristics of serological confirmed DENV infected-cases, such as age groups and gender, and the seasonality of DENV infection were also analyzed and discussed.

Materials and Methods

Ethical Statement

This study was approved by the Institutional Review Board of The Faculty of Medicine Siriraj Hospital, Mahidol University (COA: Si 438/2017).

Serum samples

A total number of 5,196 serum samples from DENV suspected cases at Siriraj Hospital during January 2012 to Decenber 2016 were sent to Serology laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital to investigate for either dengue NS1 antigen or dengue specific IgM antibody or both.

Serology test

Serum samples of suspected DENV-infected cases were previously examined for dengue NS1 antigen and/or dengue specific IgM antibodies depending on the physicians request using immunochromatographic-Dengue Early Rapid, NS1 antigen kit (sensitivity 31.89%, specificity 98.39%) and Dengue Duo Cassette, antibody IgG & IgM kits (sensitivity 85.1%, specificity 91.6%), respectively (Panbio, Republic of Korea). All kits were performed according to manufacturer's instruction.

Data and statistical analysis

The confirmed case of DENV infection was defined as having positive dengue NS1 antigen and/or positive dengue specific IgM antibody. The incidence of DENV infection was calculated from ratio of confirmed cases of DENV infection to suspected cases of DENV infection, whose serum samples were sent to examine for dengue NS1 antigen and/or dengue specific IgM antibody.

Descriptive statistics, such as numbers, ratio, and percentage were used to present positive rate of DENV-infected case detection. Confidence interval (95% CI) for a proportion was calculated using an online tool: Correlation between positive test result for DENV infection and patients' clinical data, which are categorical variables, was analyzed using Chi-square test.

Results

Laboratory confirmed dengue cases

During 2012-2016, a total of 5,196 serum samples of suspected DENV-infected cases were sent to serology laboratory for detection of dengue NS1 antigen or dengue-specific IgM/IgG antibodies. Of this number, 1,950 serum samples were requested for dengue NS1 antigen detection only; 879 serum samples were requested for only dengue specific IgM antibody detection only, and 2,367 serum samples were requested for both dengue NS1 antigen and specific IgM antibody detection. Confirmed dengue cases, which were identified from positive NS1 antigen and/or dengue-specific IgM antibody, were detected in 1,813 out of 5,196 (34.89%; 95%CI (33.60%, 36.21%)) serum samples. Dengue positive rate was 36.38% (95% CI = 32.91, 39.99), 40.75% (95% CI = 37.71, 43.86), 34.64% (95% CI = 30.82, 38.67), 43.33% (95% CI = 40.93, 45.76) and 17.78% (95% CI = 15.69, 20.07) in years 2012, 2013, 2014, 2015 and 2016, respectively (Table 1). Highest rate of DENV-infected cases (43.33%) was shown in 2015 whereas the lowest rate of DENV-infected cases (17.78%) was shown in 2016.

The result demonstrated that 866 out of 1,950 serum samples (44.41%) requested for NS1 antigen detection only; 532 out of 879 serum samples (60.52%) requested for dengue specific IgM detection only and 415 out of 2,367 serum samples (17.53%) requested for both NS1 antigen and IgM detection were laboratory confirmed as dengue positive cases (Table 2).

Dengue infection in different age groups

The highest incidence of DENV infection (44.22%; (95%CI = 41.32, 47.16)) was found in serum samples from patients aged group 15-24 years old, followed by aged group 5-14 (39.70%; (95%CI = 36.41, 43.08)) whereas the dengue positive rate was the lowest (21.30%; (95%CI = 17.59, 25.53)) in older aged group (≥65 years old). Our result showed an evidence of shifting DENV-infected cases from children towards adolescents and young adults (Table 1).

Table 1 Incidence of confirmed cases of acute DENV infection in Siriraj hospital during years 2012-2016 categorized by age group

Age (year)	Number of laboratory confirmed cases/number of suspected cases (%)					Total (95%CI)
	Year: 2012	2013	2014	2015	2016	
4	16/60 (26.67)	34/107 (31.78)	13/63 (20.63)	33/139 (23.74)	17/142 (11.97)	113/511 (22.11) (18.64, 26.01)
5-14	59/135 (43.70)	89/190 (46.84)	41/94 (43.62)	115/239 (48.12)	35/196 (17.86)	339/854 (39.70) (36.41, 43.08)
15-24	89/181 (49.17)	103/211 (48.82)	45/110 (40.91)	213/417 (51.08)	55/223 (24.66)	505/1142 (44.22) (41.32, 47.16)
25-34	40/123 (32.52)	70/156 (44.87)	40/104 (38.46)	131/261 (50.19)	31/187 (16.58)	312/831 (37.55) (34.26, 40.95)
35-44	27/86 (31.40)	47/115 (40.87)	25/65 (38.46)	74/175 (42.29)	20/126 (15.87)	193/567 (34.04) (30.17, 38.13)
45-54	17/59 (28.81)	25/75 (33.33)	15/57 (26.32)	73/172 (42.44)	22/120 (18.33)	152/483 (31.47) (27.39, 35.85)
55-64	15/50 (30.00)	26/76 (34.21)	11/38 (28.95)	38/106 (35.85)	17/106 (16.04)	107/376 (28.46) (24.01, 33.36)
≥65	4/40 (10.00)	18/81 (22.22)	13/55 (23.64)	38/141 (26.95)	19/115 (16.52)	92/432 (21.30) (17.59, 25.53)
Total (95%CI)	267/734 (36.38) (32.91, 39.99)	412/1011 (40.75) (37.71, 43.86)	203/586 (34.64) (30.82, 38.67)	715/1650 (43.33) (40.93, 45.76)	216/1215 (17.78) (15.69, 20.07)	1813/5196 (34.89) (33.60, 36.21)

Laboratory confirmed of acute DENV-infected cases classified in eight age groups are demonstrated yearly and accumulatively. Data represent raw number of laboratory confirmed cases (positive serum samples)/number of suspected cases (serum samples being investigated for DENV infection). The percentage (%) of positive cases and 95%CI are shown in parenthesis.

Table 2 Laboratory confirmed of acute DENV-infected cases categorized by type of serological tests

Number of serum samples having positive results/number of serum samples being investigated for each serological test (%)				
Year	Test for NS1 Ag only	Test for IgM only	Test for both NS1 Ag and IgM	Total (95%CI)
2012	131/182 (71.98)	99/177 (55.93)	37*/375 (9.87)	267/734 (36.38) (32.91, 39.99)
2013	195/311 (62.70)	154/189 (81.48)	63*/511 (12.33)	412/1011 (40.75) (37.71, 43.86)
2014	84/218 (38.53)	84/122 (68.85)	35*/246 (14.23)	203/586 (34.64) (30.82, 38.67)
2015	365/719 (50.76)	101/222 (45.50)	249*/709 (35.12)	715/1650 (43.33) (40.93, 45.76)
2016	91/520 (17.50)	94/169 (55.62)	31*/526 (5.89)	216/1215 (17.78) (15.69, 20.07)
Total (95%CI)	**866/1950 (44.41) (42.19, 46.65)**	**532/879 (60.52) (57.19, 63.75)**	**415*/2367 (17.53) (16.03, 19.14)**	**1813/5196 (34.89) (33.60, 36.21)**

Laboratory confirmed of acute DENV-infected cases categorized by type of serological tests: 1) NS1 Ag only, 2) Dengue-specific IgM, only 3) Both NS1 Ag and dengue-specific IgM in Siriraj Hospital during years 2012-2016 are demonstrated yearly and accumulatively. Data represent raw number of serum samples having positive results/number of serum samples being investigated for each serological test. The percentage (%) of positive cases and 95%CI are shown in parenthesis. Accumulatively (year 2012-2016), 1,950 serum samples were requested for NS1 Ag detection only and yielded 866 positive samples (44.41%); 879 serum samples were requested for dengue-specific IgM antibody detection only and yielded 532 positive samples (60.52%); 2367 serum samples were requested for both NS1 Ag and dengue-specific IgM antibody detection and yielded 415 positive samples* (17.53%).

Asterisk (*) Either only dengue NS1 Ag or dengue IgM antibody was positive or both dengue NS1 Ag and dengue IgM antibody were positive

Dengue infection in female and male patients

Incidence of DENV infection was similar between female (35.54%) and male (34.15%). Highest incidence was detected in 2015 for both female (43.26%) and male patients (43.43%) whereas the lowest incidence was detected in 2016 for both gender (18.76% for female and 16.72% for male). There was no significant difference in dengue incidence between genders ($p > 0.05$ in all studied years) (Table 3).

Table 3 Laboratory confirmed of dengue virus suspected infection in female and male cases in Siriraj Hospital during years 2012-2016

Year	Laboratory confirmed/Suspected cases (%)		*p* (Chi square test)
	Female	Male	
2012	141/382 (36.91)	126/352 (35.80)	0.75
2013	212/533 (39.77)	200/478 (41.84)	0.50
2014	116/309 (37.54)	87/277 (31.41)	0.12
2015	401/927 (43.26)	314/723 (43.43)	0.94
2016	118/629 (18.76)	98/586 (16.72)	0.35
Total (95%CI)	**988/2780 (35.54) (33.76, 37.36)**	**825/2416 (34.15) (32.27, 36.09)**	**0.29**

Laboratory confirmed of acute DENV-infected cases categorized by gender are demonstrated yearly and accumulatively. Data represent raw number of laboratory confirmed cases (positive serum samples)/number of suspected cases (serum samples being investigated for DENV infection). The percentage (%) of positive cases and 95%CI are shown in parenthesis. *P*-value calculated by Chi square test was included.

Seasonality of dengue virus infection

The percentages of laboratory confirmed dengue virus infection in male and female during 2012-2016 were demonstrated as month by month of each year. Notably, the major peaks of new DENV-infected cases were observed yearly during November to December (Fig. 1).

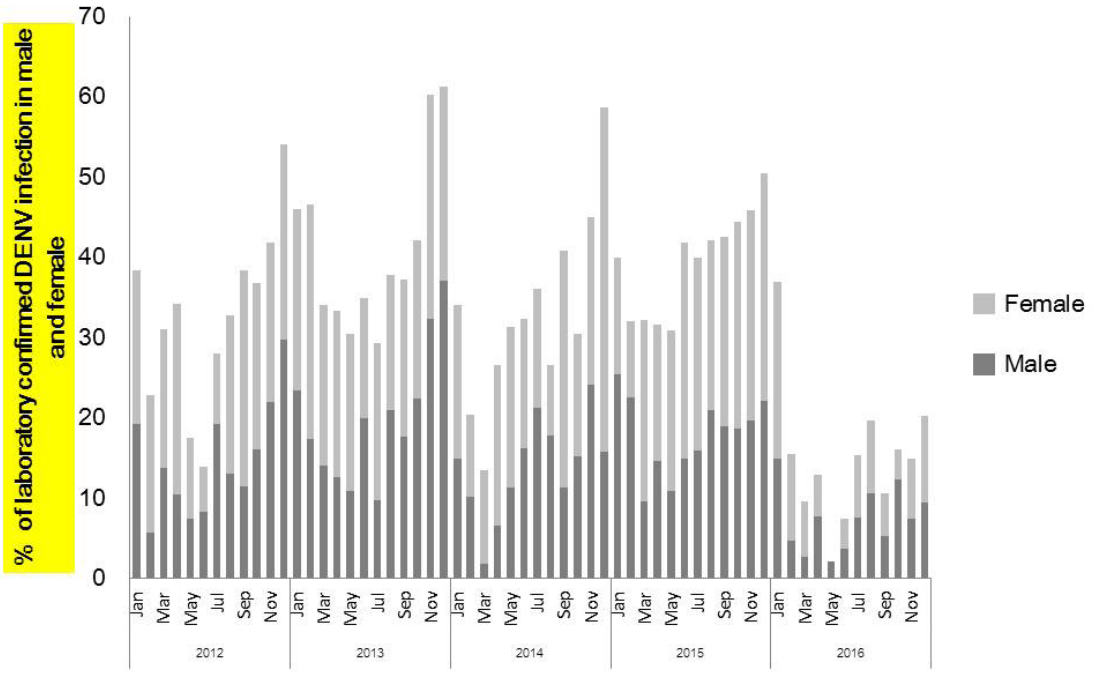

Fig. 1 Laboratory confirmed dengue cases of both genders demonstrated by months during 2012-2016

Laboratory confirmed of acute DENV-infected cases of both male and female are demonstrated monthly in bar graph. The X-axis shows months of the year from 2012-2016. The Y-axis shows percentage of laboratory confirmed DENV infection in male (dark bar) and female (light bar).

Discussion

The study of incidence of DENV infection is important for understanding the burden of disease in population, especially in endemic area like Thailand. In this study, we analyzed the serological test results of DENV suspected cases, who visited Siriraj Hospital, a tertiary hospital setting in Bangkok, Thailand, during January 2012 to December 2016.

Serum samples from suspected DENV infection were sent to laboratory to confirm diagnosis of acute DENV infection by using serological assays, namely NS1 antigen and/or specific IgM detection. During 2012-2016, the highest percentage of DENV positive cases in our study was shown in 2015: 43.33% (40.93%, 45.76%) of suspected DENV-infected cases' serum samples were reported positive by either NS1 antigen detection and/or dengue specific IgM antibody detection. Compare to our data, a report from World Health Organization (WHO) on dengue incidence in Southeast

Asia showed that the highest incidence of dengue cases in Singapore was observed in 2013 (200-900 cases per week) whereas the highest number of dengue cases in Laos was shown in 2014 (100-600 cases per week). Apart from other Asian countries, Australia reported the highest incidence of dengue cases in 2016 (50-300 cases per week) [11]. The discrepancy of numbers of dengue cases in each country might result from difference geographic regions and different dengue surveillance system in each country.

Even though dengue fever is considered a childhood disease, typically affecting children aged 5-9 years old, there are evidences of increased dengue incidence in older age groups as have been reported from many countries, including Thailand [12-14]. Our data also indicated that the DENV-affected group has shifted towards older aged as the highest incidence (44.22%) was found in young adults aged 15-24 years old.

The higher ratio of males to females in dengue infection cases was reported in Southeast Asia including Singapore and Malaysia[15]. However, this was not the case in our study, which male-to-female ratio showed no significant difference. In agreement with our study, other studies in Thailand also showed no association between gender and dengue infected cases (1:1)[16-20]. These differences in finding between Thailand and other countries in South East Asia might be caused by environmental factors and geographic regions that drive disease pattern in a community.

The limitation of this study was that the study process was to analyze the test results of serum samples previously sent to serology laboratory during 2012-2016, in which the type of serological assays were requested by the physicians. Therefore, not all serum samples were investigated for both dengue NS1 antigen and dengue-specific IgM antibody. It would be more informative if all serum samples were investigated for both tests and were correlated with onset of diseases. Interestingly, our results showed that the majority of serum samples (2,367 samples) were sent for both dengue NS1 antigen and dengue-specific IgM antibody but yielded positive results only 17.53%. In contrast, the serum samples that were sent for NS1 antigen only (1,950 samples) and dengue specific IgM (879 samples) yielded higher positive rate of detection, which were 44.41%, and 60.52%, respectively (Table 2). It is also possible that the negative serum samples for DENV were positive for other infectious pathogens.

In summary, our data reported the incidence of DENV infection in a tertiary hospital setting in Bangkok, Thailand during 2012-2016. The information on acute DENV-infected cases over the 5-year period, which shows high incidence of DENV infection in particular age group (15-24 years old) and specific months of the year (November-December), would serve as fundamental data for disease prognostic prediction and preventive intervention of dengue infection, especially for population at risk in Thailand.

Conclusion

The information on number of suspected cases and incidence of DENV infection could be useful for the estimation of the burden of disease on population, especially in an endemic area like Thailand. Importantly, this information potentially provides basis guidance for serology laboratories in hospital settings to plan for diagnostic test kit supplies and laboratory staff workload in case of dengue seasonal variation and epidemics in Thailand.

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References

1. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature 2013;496(7446):504-7.
2. Murrell S, Wu S-C, Butler M. Review of dengue virus and the development of vaccine. Biotechnol Adv 2011;29(2):239-47.
3. Simmons CP, Farrar JJ, Nguyen VVC, et al. Dengue. N Engl J Med 2012;366(15): 1423-32.
4. Rather IA, Parray HA, Lone JB, et al. Prevention and control strategies to counter dengue virus infection. Front Cell Infect Microbiol 2017;7:336.
5. Murray NEA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol 2013;5:299-309.
6. Halstead SB. Dengue antibody-dependent enhancement: Knowns and unknowns. Microbiol Spectr 2014;2(6).
7. Peeling RW, Artsob H, Pelegrino JL, et al. Evaluation of diagnostic tests: dengue. Nat Rev Microbiol 2010;8(12 Suppl):S30-8.
8. Halstead SB. Dengue haemorrhagic fever-a public health problem and a field for research. Bull World Health Organ 1980;58(1):1-21.
9. Hammon WM. Dengue hemorrhagic fever--do we know its cause? Am J Trop Med Hyg 1973;22(1):82-91.
10. Limkittikul K, Brett J, L'Azou M. Epidemiological trends of dengue disease in Thailand (2000-2011): a systematic literature review. PLoS Negl Trop Dis 2014;8(11):e3241.
11. World Health Organization. Update on the dengue situation in the Western Pacific region [Internet]. 2017 [cited 2017 February 24]. Available from: http://www.wpro.who.int/emerging_diseases/dengue_biweekly_20170131.pdf.
12. Egger JR, Coleman PG. Age and clinical dengue illness. Emerging Infect Dis 2007: 13(6):924-5.

13. Thai KTD, Nishiura H, Hoang PL, et al. Age-specificity of clinical dengue during primary and secondary infections. PLoS Negl Trop Dis 2011;5(6):e1180.
14. Cavalcanti LP, Vilar D, Souza-Santos R, et al. Change in age pattern of persons with dengue, northeastern Brazil. Emerging Infect Dis 2011;17(1):132-4.
15. Bhatia R, Dash AP, Sunyoto T. Changing epidemiology of dengue in South-East Asia. WHO South East Asia J Public Health 2013;2(1):23-7.
16. Bureau of epidemiology in Thailand. Reported cases and deaths by sex of dengue haemorrhagic fever, Thailand 2012 [Internet]. 2012 [cited 2018 February 13]. Available from: http://www.boe.moph.go.th/Annual/AESR2012/main/AESR55_Part2/table15.pdf.
17. Bureau of epidemiology in Thailand. Reported cases and deaths by sex of dengue haemorrhagic fever, Thailand 2013 [Internet]. 2013 [cited 2018 February 13]. Available from: http://www.boe.moph.go.th/Annual/AESR2013/table/table15.pdf.
18. Bureau of epidemiology in Thailand. Reported cases and deaths by sex of dengue haemorrhagic fever, Thailand 2014 [Internet]. 2014 [cited 2018 February 13]. Available from: http://www.boe.moph.go.th/Annual/AESR2014/table.php.
19. Bureau of epidemiology in Thailand. Reported cases and deaths by sex of dengue haemorrhagic fever, Thailand 2015 [Internet]. 2015 [cited 2018 February 13]. Available from: http://www.boe.moph.go.th/Annual/AESR2015/aesr2558/Part%202/table12.pdf.
20. Bureau of epidemiology in Thailand. Reported cases and deaths by sex of Dengue haemorrhagic fever, Thailand 2016 [Internet]. 2016 [cited 2018 February 13]. Available from: http://203.157.15.110/annual/AESR2016/static/documents/table/Table13.pdf.