SEROPREVALENCE OF NEUTRALIZING ANTIBODY AGAINST DENGUE VIRUS IN HEALTHCARE WORKERS IN BANGKOK, THAILAND

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Abstract. Recent epidemiological studies from Southeast Asia demonstrated an increased incidence of dengue disease and a predominantly adult age distribution. Population density/urbanization is an important risk factor for an increased burden of dengue infection. However, little is known regarding dengue serostatus among Thai adults in an urban setting. The study determined dengue seroprevalence and factors affecting dengue serostatus among healthcare workers (HCWs) in Bangkok, Thailand. A cross-sectional survey was conducted among 400 HCWs (with relatively equal distribution among four age groups: 21-30, 31-40, 41-50, and 51-60 years) during a routine annual health check-up. Antibodies against the four dengue virus serotypes using a plaque reduction neutralization test (PRNT) revealed 95% of participants had positive dengue serology, 20% of whom reported having previously been diagnosed with dengue and 89% ($n = 355$) with antibodies against all four serotypes. PRNT titer (geometric mean ± SD) for serotype 1, 2, 3, and 4 was 678 ± 677, 450 ± 430, 432 ± 474, and 102 ± 93, respectively. Logistic regression analysis indicated the youngest age group (21-30 years) was associated with negative dengue serostatus for serotype 2, 3, and 4 with adjusted odds ratios (95% confidence interval) of 2.69 (1.18-6.14), 2.28 (1.03-5.05), and 2.41 (1.20-4.83), respectively. Dengue seroprevalence in Thai HCWs in urban Bangkok was very high and the majority of the individuals had been exposed to all four dengue virus serotypes.

Keywords: dengue, health care workers, neutralizing antibody, seroepidemiology, seroprevalence, Thailand

INTRODUCTION

Despite the recent advances in medical care and available public health intervention programs, dengue infection has become one of the world most important mosquito-borne viral illness (WHO, 2016). Dengue has spread from only nine countries during 1970 and is currently endemic in more than 100 countries especially in tropical and subtropical countries (Tan, 2015). The World Health Organization (WHO) ranks dengue as

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the fastest spreading vector-borne disease in 2013 with a 30-fold increase in global incidence during the prior half century (WHO, 2013).

Dengue has been known traditionally as childhood illness, but recent epidemiological studies from Southeast Asia demonstrated a shift toward an older age group and apparent increased incidence of the disease in the adult population (Cummings *et al*, 2009). The virus is transmitted to humans by bites of infected *Aedes* mosquitoes, in particular *Ae. aegypti* and/or *Ae. albopictus*. These vectors are urban day-biting mosquitoes rendering the use of insecticide-treated bed nets ineffective. As a result, these mosquitoes have successfully adapted to a transmission cycle in densely-populated urban areas (WHO, 2013).

Although population density/urbanization is known as important risk factor for the increased burden of dengue infection (Gubler, 2011), limited data are available on dengue serostatus among adults in an urban setting. In general, national surveillance data do not represent the true epidemiological situation due to a large proportion of underreported and undifferentiated infections.

A seroprevalence survey in Singapore during 2009-2010 of 4,000 adult blood donors indicated dengue seroprevalence increased with age, with lowest prevalence (16.1%) in 16-20 years of age group and highest (86.6%) in 56-60 years of age (Low *et al*, 2015). Plaque reduction neutralization test (PRNT) on samples of young adults (16-30 years of age) revealed lower dengue seroprevalence of each dengue virus serotype ranging from 5.4% to 20.3% compared to those of the older age groups. In addition, the predominant monotypic PRNT pattern in most young adults was suggestive of single infection with increasing proportion of multitypic PRNT pattern with increasing age; adults >26 years of age exhibit prevalence to three or more serotypes (23-40%). A dengue seroprevalence survey among adult blood donors in the French West Indies in 2011 demonstrated a seroprevalence ranging from 90.7% in Martinique to 96.2% in Guadeloupe with 80% seroprevalence against all four dengue virus serotypes (L'Azou *et al*, 2015). A study from Sri Lanka showed dengue seroprevalence has increased 2.5 folds from 2003 to 2014, although 68.2% of children and adult population attending primary health facility were seropositive, only 11.5% were hospitalized due to dengue (Jeewandara *et al*, 2015). In Santo Domingo, Dominican Republic, dengue seroprevalence among adult blood donors is 98% (Yamashiro *et al*, 2004). In the Solomon Islands 39% dengue seroprevalence was reported, with 28% monotypic-positive by PRNT for serotype 2 (Darcy *et al*, 2001).

In Thailand, recent surveys indicated a significant increase in mean age of dengue seropositivity in 2010 compared with 1980s (8-10 years) and 2002-2008 (18 years old) (Kongsomboon *et al*, 2004; Cummings *et al*, 2009; Rodriguez-Barraquer *et al*, 2013). Based on a 1980 survey, 96% of the Thai population were exposed to dengue by the age of 11 (Kongsomboon *et al*, 2004), but by 2010 only 65% were children 11 years of age and 18% of the group 18 years of age still remained susceptible to dengue virus infection (Rodriguez-Barraquer *et al*, 2013).

In December 2015, the first dengue vaccine, Dengvaxia® (CYD-TDV) developed by Sanofi Pasteur, was licensed in Mexico (WHO, 2016) for individuals 9-45 years old. CYD-TDV has since been licensed in several endemic countries including the Philippines in 2015 (Shim,

2016). Dengvaxia® demonstrated higher vaccine efficacy (78.2%) in subjects with pre-existing dengue seropositivity compared to those seronegative (38.1%) (Hadinegoro *et al*, 2015). As the vaccine has become available in Thailand in late 2016, the study determined dengue seroprevalence and factors affecting dengue seropositivity among adults aged 21-60 years living in Bangkok. Information obtained should be useful in predicting at-risk population for dengue infection and dengue vaccine effectiveness in adults.

MATERIALS AND METHODS

Study group

A descriptive cross-sectional study among healthcare workers (HCWs) (n = 400) undergoing annual routine health check-up at Queen Sirikit National Institute of Child Health in 2015. Participating HCWs were composed of approximately equal numbers among four age groups: 21-30, 31-40, 41-50, and 51-60 years (Table 1). Serum was prepared from 3 ml aliquot of blood sample from each participant, transported at 4°C within 3-5 hours to the Institute of Molecular Biosciences, Mahidol University (WHO-approved Reference Laboratory for serology and virology of dengue and Japanese encephalitis viruses) and stored at -20°C until used. Demographic data were obtained from a self-administered questionnaire.

The study received approval from Ethics Committee, Queen Sirikit National Institute of Child Health (58-083 REC.027/2559) and prior written consent was obtained from each participant.

PRNT

PRNT was performed using rhesus monkey LLC-MK2 kidney cells (American Type Culture Collection, Manassas, VA) in a single overlay plaque technique as previously described (Russell *et al*, 1967). In brief, serum was heat-inactivated at

Table 1
Demographic characteristics of participating health care workers enlisted at Queen Sirikit National Institute of Child Health, Bangkok, Thailand in 2015.

Characteristic	n (%) (n = 400)
Male	53 (13)
Age group (years)	
21-30	100 (25)
31-40	102 (25.5)
41-50	98 (24.5)
51-60	100 (25)
Prior clinical diagnosis of dengue	80 (20)
Age (years) diagnosed symptomatic dengue (n = 78)	
0-10	32 (41)
11-20	21 (27)
21-30	14 (18)
31-40	6 (8)
41-50	4 (5)
>50	1 (1)

56°C for 30 minutes and 10-fold serial dilution using phosphate-buffered saline containing 20% (v/v) fetal bovine serum. An equal volume of each diluted Dengue 1 (strain 16007), Dengue 2 (strain 16681), Dengue 3 (strain 16562), and Dengue 4 (strain 1036) (all strains obtained from University of Hawaii, Honolulu, HI) (40-60 plaque forming unit/0.2 ml) was added to each serum solution, incubated at 37 °C for 60 minutes, then 0.2 ml aliquot was removed from each tube and inoculated in duplicate onto a well of a 6-well flat-bottom plate containing confluent LLC-MK2 cells. Each plate was incubated at 37°C for 90 minutes and then each well monolayer was overlaid with 4 ml aliquot of 3.0% carboxymethyl cellulose/MEM (Sigma-Aldich, St Louis, MO). Plates were incubated for 7 days at 37 °C under a humidified atmosphere containing 5% CO_2, followed by plaque counting and titer at 50% reduction was recorded.

RESULTS

HCWs (n = 400) were enrolled with mean age (SD) of 40 (11) years (range, 21-60 years) and 87% being females (Table 1). Dengue seroprevalence tended to increase with age for all serotypes but PRNT levels were different across the four serotypes with serotypes 1 and 2 being among the highest and serotype 4 the lowest (Table 2). Some 95% of the subjects experienced at least one serotype of dengue infection, with 45 (11%) subjects having at least one negative serological test for dengue antibody (PRNT titer <10) and 21 (5%) no evidence of any prior dengue infection (Table 3). Eighty-nine percent (n = 355) of the participants expressed antibodies against all four serotypes. One fifth of the subjects had been diagnosed with clinically significant dengue infection and among those with seropositivity for at least one dengue virus serotype, 20% reported having been diagnosed with clinical dengue.

Uni- and multivariate analyses revealed the youngest age group (21-30 years) has a significantly higher probability of being seronegative against all dengue serotypes except serotype 1 (Table 4). This finding corroborates existing literature reporting dengue virus serotype 1 is the most prevalent serotype in Thailand (Limkittikul *et al*, 2014) and thus exposure to this serotype may have occurred early in life. Of note, HCWs living in the inner Bangkok area were associated with negative seroprevalence against serotype 3 dengue virus.

DISCUSSION

Seroprevalence surveys are essential for understanding the true epidemiological disease burden in a population and for evaluation of the impact of disease control programs. Serum samples collected from a group of health care workers in Bangkok, 21-60 years of age, showed nearly all subjects had prior dengue infection of at least one serotype. As expected, the older the age, the higher the dengue seroprevalence and PRNT50 titer, but the latter varied against each dengue serotype. Geometric mean titer for dengue neutralizing antibodies were comparable across age except for dengue virus serotype 3, antibody levels against which increased with age.

The ratio of clinical recognizable dengue infection among those with evidence or prior infection by serology was 1:5, but this ratio was likely to be a substantial underestimation due to the retrospective nature of data collection and reliance on participant's memory rather than medical documentation. A school-based prospective surveillance in a Thai province in

Table 2
Dengue seropositivity according to age group and dengue serotype of health care workers enlisted at Queen Sirikit National Institute of Child Health, Bangkok, Thailand in 2015.

Dengue virus serotype/age group (years)	PRNT50 geometric mean titer (SD)	*p*-value*	PRNT50 titer ≥10 *n* (%)	*p*-value**
Serotype 1[a]	678 (677)	0.218	374 (93)	0.054
21-30[b]	625 (720)	-	89 (89)	-
31-40[c]	603 (651)	-	94 (92)	-
41-50[d]	703 (665)	-	93 (95)	-
51-60[e]	784 (666)	-	98 (98)	-
Serotype 2[a]	450 (430)	0.821	374 (93)	0.005
21-30[b]	459 (459.1)	-	87 (87)	-
31-40[c]	417.8 (472)	-	95 (93)	-
41-50[d]	450 (424)	-	93 (95)	-
51-60[e]	474 (359)	-	99 (99)	-
Serotype 3[a]	432 (474)	< 0.001	371 (93)	0.025
21-30[b]	337 (415)	-	87 (87)	-
31-40[c]	344 (35)	-	95 (93)	-
41-50[d]	453 (460)	-	91 (93)	-
51-60[e]	596 (595)	-	98 (98)	-
Serotype 4[a]	102 (93)	0.181	360 (90)	0.002
21-30[b]	94 (95)	-	83 (83)	-
31-40[c]	93 (97)	-	91 (89)	-
41-50[d]	103 (87)	-	88 (90)	-
51-60[e]	119 (92)	-	98 (98)	-

[a]$n = 400$. [b]$n = 100$. [c]$n = 102$. [d]$n = 98$. [e]$n = 100$. *Between each age group within each dengue serotypes using analysis of variance. **Between each age group within each dengue serotype using chi-square for contingency table. PRNT50, plaque reduction neutralization test (50% reduction).

Table 3
Seropositivity status against dengue virus serotypes of health care workers enlisted at Queen Sirikit National Institute of Child Health, Bangkok, Thailand in 2015.

Number of serotypes	Dengue seropositivity ($n = 400$)	
	PRNT50 < 10, *n* (%)	PRNT50 ≥ 10, *n* (%)
At least one	21 (5)	379 (95)
At least two	23 (6)	377 (94)
At least three	32 (8)	368 (92)
All four	45 (11)	355 (89)

PRNT50, plaque reduction neutralization test (50% reduction).

Table 4
Factors associated with the lack of dengue seropositivity among healthcare workers enlisted at Queen Sirikit National Institute of Child Health, Bangkok, Thailand in 2015.

Factor	PRNT50 (n = 400)															
	Dengue virus serotype 1				Dengue virus serotype 2				Dengue virus serotype 3				Dengue virus serotype 4			
	Univariate analysis			Multivariate analysis aOR (95% CI)	Univariate analysis			Multivariate analysis aOR (95% CI)	Univariate analysis			Multivariate analysis aOR (95% CI)	Univariate analysis			Multivariate analysis aOR (95% CI)
	<10 n=26 n (%)	≥10 n=374 n (%)	cOR (95% CI)		<10 n=26 n (%)	≥10 n=374 n (%)	cOR (95% CI)		<10 n=29 n (%)	≥ 10 n=371 n (%)	cOR (95% CI)		<10 n=40 n (%)	≥ 10 n=360 n (%)	cOR (95% CI)	
Female (n = 347)	22 (6.3)	325 (93.7)	1.206 (0.399-3.648)	0.854 (0.279-2.617)	23 (6.6)	324 (93.4)	0.845 (0.245-2.919)	1.210 (0.343-4.271)	25 (7.2)	322/371 (92.8)	1.051 (0.351-3.151)	1.009 (0.331-3.076)	36/40 (10.4)	311/360 (89.6)	0.705 (0.240-2.068)	1.487 (0.501-4.413)
Young age group (21-30 years) (n = 100)	11 (11)	89 (89)	2.348 (1.041-5.298)	2.010 (0.871-4.641)	13 (13)	87 (87)	3.299 (1.474-7.381)	2.686 (1.175-6.144)	13 (13)	87/371 (87)	2.652 (1.228-5.730)	2.284 (1.033-5.051)	17/40 (17)	83/360 (83)	2.467 (1.258-4.836)	2.409 (1.201-4.833)
Living in inner Bangkok area (n = 248)	20 (8.1)	228 (91.9)	2.135 (0.837-5.441)	1.905 (0.740-4.907)	20 (8.1)	228 (91.9)	2.135 (0.837-5.441)	1.845 (0.712-4.777)	24 (9.7)	224/371 (90.3)	3.150 (1.175-8.441)	2.831 (1.047-7.656)	29/40 (11.7)	219/360 (88.3)	1.697 (0.822-3.507)	1.569 (0.751-3.279)
Living at least one month outside Bangkok (n = 188)	16 (8.5)	172 (91.5)	1.860 (0.823-4.207)	0.634 (0.274-1.465)	18 (9.6)	170 (90.4)	2.674 (0.134-6.302)	0.466 (0.193-1.124)	17 (9.0)	171/369 (91.0)	1.640 (0.762-3.531)	0.751 (0.340-1.659)	20/40 (10.6)	168/358 (89.4)	1.131 (0.588-2.174)	1.089 (0.552-2.147)

aOR, adjusted odds ratio; CI, confidence interval; cOR, crude odds ratio; PRNT50, plaque reduction neutralization test (50% reduction).

1980 indicated 87% of dengue infection in children were either asymptomatic or minimally symptomatic, and those with preexisting dengue immunity have 6.5 times higher risk of symptomatic dengue hemorrhagic fever (Burke *et al*, 1988). An active case surveillance of school children in a rural setting in northern Thailand in 2000 reported an overall prevalence of dengue infection of 2.2% with a ratio of symptomatic to asymptomatic infection of 1:1.75 (Endy *et al*, 2002). Subsequent surveillance surveys conducted during 2004 to 2008 demonstrated an overall symptomatic to asymptomatic infection ratio of 1:3 (Endy *et al*, 2010; *ibid*, 2011). During a large dengue outbreak in Nakhon Pathom Province (central Thailand) in 2001, a population-based survey indicated 8.8% of asymptomatic individuals living in the dengue outbreak area have had a recent infection (determined by IgM positivity) indicative of silent dengue transmission (Poblap *et al*, 2006).

In the majority of surveillance programs, the starting point for reporting dengue infection is a visit to a health center or hospital (WHO, 2016).According to national surveillance data in Thailand and several other countries, dengue disease burden are, therefore, likely to be under-reported. A recent prospective cohort analysis indicated an average under-recognition of total dengue cases is 8.7 and 9.1 folds in Thailand and Cambodia, respectively (Vicente *et al*, 2016). The high dengue seroprevalence in the present indicates Thailand contributes substantially to the regional dengue disease burden. However, the high level of exposure to dengue among young adults may contribute to a strong herd immunity and low vulnerability to dengue outbreak. On the other hand, those without complete protection against all four dengue virus serotypes may remain susceptible to severe, secondary dengue infection (Soo *et al*, 2016).

The study has a number of limitations: firstly, the relatively small number of subjects compared to the overall Bangkok population, and secondly, the possible higher dengue seroprevalence among HCWs compared to the whole adult population in Bangkok owing to potential nosocomial transmission, such as needle prick injury or mucocutaneous contact with infected blood (de Wazières *et al*, 1998; Chen and Wilson, 2004; Nemes *et al*, 2004; Wagner *et al*, 2004; Chen and Wilson, 2005; Gupta *et al*, 2008; Morgan *et al*, 2015).

In conclusion, this study contributes to a better understanding of dengue seroepidemiology among a small cohort of adult health care workers in Bangkok, revealing the vast majority of participants were seropositive for dengue neutralizing antibodies and, as expected, dengue seropositivity with age. Future studies should not only verify these findings using a larger cohort of subjects and having different occupations and areas of residence, but also focus on questions such as whether presence of neutralizing antibodies is indicative of protective immunity against symptomatic dengue infection.

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CONFLICTS OF INTEREST

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