

SEROPREVALENCE AND SEROCONVERSION OF DENGUE FEVER IN A HEALTHY POPULATION IN GUANGZHOU, CHINA

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Abstract. Dengue infection may present as a severe febrile illness or may be asymptomatic. In this study, we aim to determine the seroprevalence of the dengue antibodies and the dengue antibody seroconversion rate and the factors significantly associated with conversion in an apparently healthy population in Guangzhou, China between 2015 and 2018 in order to inform dengue virus control efforts in the study area. The study population was Conghua District resident and had not previously been diagnosed with dengue fever. Potential factors associated with seroprevalence and seroconversion were determined by logistic regression model and the Fisher exact test. Thirty-two of the study subjects examined in 2015 were the same subjects as those examined in 2018; the other subjects came from the same study area. An enzyme-linked immunosorbent assay (ELISA) for dengue IgG antibody was examined in all study subjects in 2015 and 2018. A total of 162 subjects were included in the study in 2015 and 80 subjects included in the study in 2018. The mean (\pm standard deviation (SD)) age of subjects in 2015 was 35.2 (\pm 26.6) years; 40.7% males. The mean (\pm standard deviation (SD)) age of subjects in 2018 was 47.9 (\pm 21.7) years; 45.0% males (p -value for differences in mean age and gender between the 2 groups in 2015 and 2018 were 0.000 and 0.665, respectively). A total of 9.3% of subjects in 2015 had dengue IgG antibodies and 11.3% of subjects in 2018 had dengue IgG antibodies ($p=0.626$). Among the 29 study subjects who were in both 2015 and 2018 studies, the seroconversion rate was 13.8%. Logical regression showed that the use of insecticide spray was an important factor associated with seroprevalence ($p=0.037$); the Fisher's exact test showed a significant correlation between residential type (Storied building and Bungalow) and seroconversion ($p<0.001$). No significant difference in dengue antibody-positive rates between 2015 and 2018 was observed. However, such high asymptomatic infection and seroconversion may have an immeasurable effect on the transmission of dengue. More effective control measures are needed to prevent the outbreak of dengue in Guangzhou, China.

Keywords: Dengue fever; Serological survey; Seroprevalence; Seroconversion.

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INTRODUCTION

Dengue fever (DF), a mosquito-borne infection caused by dengue virus (DENV), is increasing an incidence worldwide (Guzman, 2015). DF has been reported from more than 100 countries; there are an estimated 390 million infections per year of which 96 million are symptomatic (Bhatt *et al*, 2013). There has been an increase in dengue fever cases in China in part due to trade with dengue-endemic countries (Chen and Liu, 2015; Li *et al*, 2012; Lai *et al*, 2015).

Humans with dengue infection may have a varying clinical presentation from being asymptomatic to having a severe life-threatening infection, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Gubler, 1998). The proportion of those with an asymptomatic infection may be greater than those with symptoms (Balmaseda *et al*, 2010; Endy *et al*, 2011). Asymptomatic patients are usually not detected or reported but are still a source for the spread of the disease in a population. Asymptomatic cases result in underestimating the incidence of disease in a population, necessitating the need to conduct a serological survey to understand the true epidemiology of a disease in a population (Chao *et al*, 2012; Hladish *et al*, 2016). Socioeconomic (Braga *et al*, 2010) and ecological factors (Hopp and Foley, 2001; Lambrechts *et al*, 2011) can influence the incidence of dengue infections; therefore, it is important to include these factors in studying the epidemiology of dengue infections.

China Guangzhou has always been considered the critical epicenter of DF in Guangdong Province and even in the whole country (Luo *et al*, 2012). The incidence of dengue infection in Guangzhou is rising continuously

(Wang *et al*, 2015). Previous studies have evaluated the epidemiology of dengue infections in urban Guangzhou, but little data is available from underdeveloped and suburban areas (Liu *et al*, 2018). Conghua District, Guangzhou is one of these areas that has been little studied but where in incidence of dengue infections has been increasingly reported in recent years (Luo *et al*, 2008).

Dengue IgG antibodies usually appear in the disease recovery phase, so they can be used as a marker of previous recent infection (Anderson *et al*, 2014; Nascimento *et al*, 2018). To better understand the prevalence of previous dengue infections in the Conghua District and the incidence of new cases, we performed two cross-sectional studies of dengue IgG antibodies in 2015 and 2018 in order to understand the epidemiology of asymptomatic dengue infection in the study area to inform dengue infection control programs in this area.

MATERIALS AND METHODS

Study area

The Conghua District, Guangzhou City, has a total area of 2,009 km² and a population of 538,200 (Guangzhou Statistics Bureau at URL: <http://tjj.gz.gov.cn/>). The climate is rainy and subtropical with an average temperature of 19.5-21.4°C and has an average annual rainfall of 1,800-2,200 mm. The village selected for the study were Dingkeng Village and Xincun Village which have industrial and agricultural production. *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes are present year-round and are the dominant mosquito species (Jiang and Yan, 2018).

Study design

In this study, there were two parts. The first part consisted of an initial cross-

sectional dengue IgG seroprevalence survey of subjects conducted in July 2015 and a follow up cross-sectional survey or dengue IgG antibodies to determine the incidence of dengue infection conducted in July 2018. Not all the same subjects were examined in both surveys; those who participated in both surveys and had a negative result of the first survey and a positive result on the second survey were used to determine the incidence of dengue infection during the intervening 3-year period between the two surveys. Inclusion criteria for study subjects were as follows: (1) residing in Conghua District; (2) have not been previously diagnosed with dengue fever. Those with incomplete personal data and information were excluded from the study.

A blood sample was obtained from each subject from each survey and tested for dengue IgG antibodies using the Australian Panbio enzyme-linked immunosorbent assay (Standard Diagnostics Inc, Brisbane, Australia). The results were reported following the manufacturer's instructions, *ie* >11 units as positive results, 9-11 units as equivocal, and <9 units as negative results.

Data analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc, Chicago, IL). Age-and gender-specific antibody prevalences, were calculated. Univariate analysis of potential demographic and behavioral factors associated with dengue infection was performed. Logistic regression analysis with odds ratio (OR) and 95% CI were calculated. The Fisher's exact test was used to assess the association between dengue seroconversion and associated factors. A *p*-value <0.05 was considered statistically significant.

Ethics approval and consent to participate

This study was approved by the Ethics Committee Guangzhou Center for Disease Control and Prevention. Study subjects and their parents or guardians for children, gave informed consent prior to participation in the study.

RESULTS

Study population

In 2015, a total of 162 subjects were included in the study. The mean (range) age of subjects was 35.2 (0.8-83) years, 40.7% male. Forty-nine point four percent of subjects were from Dingkeng Village and 50.6% from Xincun Village. Thirty-seven point seven percent of subjects were aged <19 years, 14.8% were aged 19-40 years, 30.9% were aged 41-65 years and 16.7% were aged >65 years. Greater than 50% of subjects were professionals and business people.

In 2018, a total of 80 subjects were included in the study, of whom 40.0% had participated in the 2015 survey. The mean (range) age of subjects was 47.9 (5-86) years, 45.0% male. All the subjects in the 2018 survey were from Dingkeng Village. Fifteen percent of subjects were aged <19 years, 16.3% were aged 19-40 years, 42.5% were aged 41-65 years and 26.3% were aged >65 years. Seventy-eight point eight percent of subjects were professionals and business people.

The prevalence of dengue infection

In 2015, 15 of the 162 subjects had dengue IgG antibodies giving an overall seroprevalence of 9.3%; females: 9.4%; males: 9.1%. The seroprevalences of dengue IgG antibodies among the 2015 subjects aged <19, 19-40, 41-65 and >65 years were 8.2%, 4.2%, 12.0% and 11.1%, respectively. There was no significant

association between gender ($p=0.951$) or age group ($p=0.183$) and the presence of dengue IgG antibodies.

In 2018, 9 of the 80 subjects had dengue IgG antibodies giving an overall seroprevalence of 11.3%; males: 11.1%; females: 11.4%. The seroprevalences of dengue IgG antibodies among the 2018 subjects aged <19, 19-40, 41-65 and >65 years were 8.3%, 15.4%, 8.8%, and 14.3%, respectively. According to statistics, there was no significant difference between gender ($p=0.972$) or age group ($p=0.304$) and the presence of dengue IgG antibodies (Table 2).

The incidence of dengue infection

A total of 32 subjects participated in both two surveys. Nine point four

percent of subjects (3/32) had dengue IgG antibodies in 2015 and 15.6% (5/32) did in 2018 ($p=0.626$). Two of the 3 subjects with a positive test in 2015 were aged <19 years. Of the 3 subjects with a positive test for dengue IgG antibodies in 2015, 2 tested negative in 2018, only 1 tested positive. Four of the 5 subjects who tested positive in 2018 had tested negative in 2015, all of whom were aged >65 years. Given that the seroconversion in this article refers to the sero-negative-to-positive, therefore, the seroconversion rate of dengue infection among the subjects between 2015 and 2018 was 4/29 (13.8%).

Demographic and behavioral risk factors

On univariate analysis, using insecticide spray when going out of

Table 1
Demographic and socioeconomic characteristics of study subject by year.

Variables	2015		2018	
	Number	%	Number	%
Age in years				
<19	61	37.7	12	15.0
19-40	24	14.8	13	16.3
41-65	50	30.9	34	42.5
>65	27	16.7	21	26.3
Gender				
Male	66	40.7	36	45.0
Female	96	59.3	44	55.0
Occupation				
Services (professional, business, etc.)	91	56.2	63	78.8
Student	58	35.8	15	18.8
Housewife	13	8.0	2	2.5
Education				
No Education	57	35.2	6	7.5
Primary	50	30.9	37	46.3
Secondary or above	55	34.0	37	46.3

Table 2
Dengue IgG antibodies among study subjects in 2015 and 2018.

Variables	2015			2018		
	Number positive	Total number	Percent positive	Number positive	Total number	Percent positive
Age in years						
<19	5	61	8.2	1	12	8.3
19-40	1	24	4.2	2	13	15.4
41-65	6	50	12.0	3	34	8.8
>65	3	27	11.1	3	21	14.3
Gender						
Male	6	66	9.1	4	36	11.1
Female	9	96	9.4	5	44	11.4

the house was significantly associated with seroprevalence (OR=0.14; 95%CI: 0.02-0.89, $p = 0.037$) (Table 3). However, there was no significant association in prevalence by age, gender, occupation, education or village.

The results of the univariate analysis described in Table 4 showed that seroconversion was significantly associated with residential type ($p < 0.001$), while there was no statistical difference in gender, age, number of screen windows inside household, number of air conditioners inside household, number of bed nets inside household, using mosquito coils, attendance in public gatherings.

DISCUSSION

In our study, we determined the prevalence of dengue IgG antibodies among study subjects and factor associated with a positive result. We also examined a small subset of these subjects 3 years later to determine the seroconversion rate to a positive test. The overall prevalence

of dengue IgG antibodies in 2015 and 2018 were 9.3% and 11.3% respectively. These were lower than those reported from Brownsville, Mexico (59%) and Matamoros, Mexico (67.2%) (Brunkard *et al*, 2007) but lower than that reported from Brazil (84.4%) (Braga *et al*, 2010).

We found that the seroprevalence of healthy populations in 2015 (9.3%) was similar to that reported in Conghua District in the same year (9.4%) (Jing *et al*, 2019). Although the seroprevalence rate was lower than those reported in other central urban areas of Guangzhou (Liu *et al*, 2018), it was still higher than the 5.1% seroprevalence rate reported in Zengcheng (Jing *et al*, 2019).

In our study, the seroconversion rate was 13.8% (4 subjects), all asymptomatic. A previous prospective cohort study in Nicaragua found that the incidence of asymptomatic dengue cases far exceeded the rate of symptomatic infections (Balmaseda *et al*, 2010), which may explain why no cases of symptomatic infections were reported during our observation

Table 3

Univariate analysis of factors associated with dengue IgG antibodies among study subjects in 2015.

Variables	Seropositive <i>n</i> (%)	Seronegative <i>n</i> (%)	OR (95% CI)	<i>p</i> -value
Age in years				0.717
< 19 years (children)	4 (7.02)	53 (92.98)	1	
19–84 years (adults)	11 (10.48)	94 (89.52)	0.81 (0.26,2.50)	
Sex				0.871
Male	6 (8.82)	62 (91.18)	1	
Female	9 (9.57)	85 (90.43)	1.09 (0.37,3.23)	
Occupation				0.816
Services (professional, business, etc.)	8 (8.79)	83 (91.21)	1	
Non-wage earning (student, housewife, etc.)	7 (9.86)	64 (90.14)	1.14 (0.39,3.29)	
Education				0.975
No Education	5 (8.77)	52 (91.23)	1	
Primary	5 (10.00)	45 (90.00)	0.96 (0.26,3.52)	
Secondary and above	5 (9.09)	50 (90.91)	1.11 (0.30,4.09)	
Village				0.786
DingKeng	7 (8.75)	73 (91.25)	1	
XinCun	8 (9.76)	74 (90.24)	1.16 (0.40,3.36)	
Whether to use sprays when going out.				0.037*
No	2 (40.00)	3 (60.00)	1	
Yes	13 (8.28)	144 (91.72)	0.14 (0.02,0.89)	

*Significant at $p \leq 0.05$.

period. Of course, this is also related to the study sample size and observation time. Large samples and longer follow-up observations are needed in the future to observe the occurrence of symptomatic infections.

Besides, we found that two of the three asymptomatic infections in 2015 turned negative in 2018, indicating that the asymptomatic infections may have lower antibody titers. Previous studies have found that asymptomatic infections are more likely to cause antibody-negative conversions than those with symptomatic

dengue infections with lower antibody concentrations (Luo *et al*, 2018).

The most common mosquito control measures practiced in China are the uses of bed nets, mosquito coils, and insecticide sprays. We found that the seroprevalence of DF in participants who used the insecticide spray was lower than that of the non-user. This is consistent with previous studies demonstrating the use of insecticide spray can reduce opportunistic infections of dengue (Lenhart *et al*, 2008).

In our study, seroconversion was significantly associated with the type

Table 4

Univariate association between dengue seroconversion (IgG) of dengue and selected explanatory variables during follow-up survey (2018).

Variables	Seropositive <i>n</i> (%)	Seronegative <i>n</i> (%)	<i>p</i> -value
Age in years			0.464
< 19 years (children)	0 (00.00)	3 (100.00)	
19–84 years (adults)	4 (15.38)	22 (84.62)	
Sex			0.779
Male	1 (11.11)	8 (88.89)	
Female	3 (15.00)	17 (85.00)	
Residential type			<0.001*
Storied building	2 (7.41)	25 (92.59)	
Bungalow	2 (100.00)	0 (00.00)	
Number of screen windows inside the household			0.224
None	0 (0.00)	7 (100.00)	
≥1	4 (18.18)	18 (81.82)	
Number of air conditioning inside the household			0.052
None	4 (25.00)	12 (75.00)	
≥1	0 (0.00)	13 (100.00)	
Number of bed nets inside the household			0.300
None	3 (11.54)	23 (88.46)	
≥1	1 (33.33)	2 (66.67)	
Use mosquito coils to control mosquitoes			0.965
No	3 (13.64)	19 (86.36)	
Yes	1 (14.29)	6 (85.71)	
Attendance in public/mass gatherings(Garden/Park)			0.138
No	1 (100.00)	0 (0.00)	
Yes	3 (10.71)	25 (89.29)	

*Significant at $p \leq 0.05$.

of resident. We found that people who live in bungalows have higher dengue seroconversion rates than those living in houses. This may be due to the poor basic public facilities in the bungalows, which may cause mosquito breeding and increase the risk of dengue infection.

Our study had some limitations. First, only 32 subjects participated in both

surveys while many subjects being lost to follow up. Second, we only used the ELISA to detect dengue IgG antibodies. ELISA can lead to false-positive results due to the cross-reaction of flaviviruses.

In conclusion, we found asymptomatic dengue infections were presented and not uncommon. However, the small number of subjects prevents from making further

conclusions. Further studies are needed with larger numbers to clarify the factors associated with infection so as to inform dengue infection control programs.

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