

Seroepidemiology of Dengue Virus Infection Among Adults in Singapore

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Abstract

Introduction: To determine the seroepidemiology of dengue virus infection in a representative sample of the adult resident population aged 18 years old to 74 years old in Singapore and to estimate the proportion of asymptomatic dengue infection during the 2004 epidemic. **Materials and Methods:** The study was based on 4152 stored blood samples collected between September and December 2004 from participants aged 18 years old to 74 years old during the 2004 National Health Survey. Sera were tested for IgG and IgM antibodies using a commercial test kit (PanBio Capture/Indirect ELISA). **Results:** Of the study population, 59.0% and 2.6% tested positive for dengue IgG (past infection) and IgM/high-titre IgG (recent infection), respectively. Only 17.2% of young adults aged 18 years old to 24 years old were dengue IgG positive. Multivariate analyses showed that older age, Indian ethnicity and male gender were significantly associated with past infection, whereas only age was significantly associated with recent dengue infection. Based on the dengue cases notified during the period of survey, it was estimated that for every 23 individuals recently infected with dengue, only 1 was reported to the health authority as a clinical case. **Conclusion:** The Singapore population is highly susceptible to dengue epidemics despite its aggressive *Aedes* prevention and control programme. The finding of a high proportion of unreported cases due to asymptomatic and subclinical infection poses a challenge for dengue control.

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Key words: *Aedes* control, Asymptomatic infection, Herd immunity

Introduction

Dengue fever is a mosquito-borne viral disease endemic in Singapore as well as in the surrounding Southeast Asian countries.¹ It is a re-emerging disease of concern.² Although Singapore has a well-established nationwide *Aedes* mosquito control programme that includes source reduction, public health education and law enforcement since the 1970s, there has been an increasing trend in the number of notified dengue cases in the past 15 years with 6-year cyclical epidemics occurring in 1992, 1998 and 2004.³ The *Aedes* premises index (percentage of premises positive for *Aedes* breeding) has been maintained at 1% to 2% since the 1980s. In 2005, Singapore witnessed the largest ever recorded epidemic with a total of 14,209 dengue cases. The epidemiology of dengue has changed. It is no longer a childhood infection, but involved mainly young adults aged between 25 and 34 years of age.⁴ To assess the impact of the cyclical epidemics on the prevalence of dengue virus

infection in the general population, a seroepidemiological study was conducted in 2005.

Materials and Methods

This national dengue seroprevalence study was conducted based on 4152 stored blood samples of the 2004 National Health Survey (NHS 2004)⁵ collected between September and December 2004 when the disease incidence was very high (Fig. 1).

Study Population

The NHS 2004⁵ was a national cross-sectional survey conducted to determine the prevalence of major non-communicable diseases such as diabetes and their associated risk factors among Singapore residents. Six polyclinics equipped with the necessary facilities and geographically well spread out across Singapore were selected. The study population was selected by a combination of disproportionate

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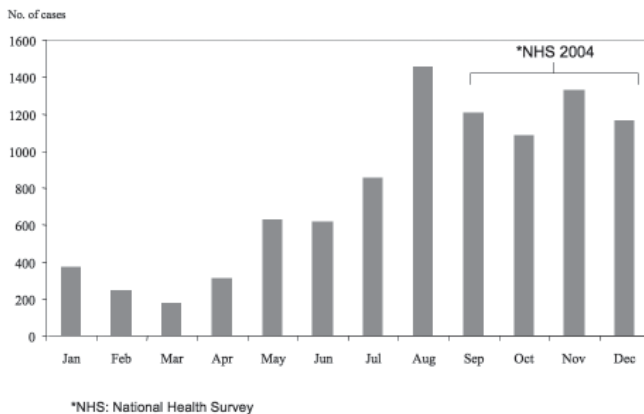


Fig. 1. Monthly number of dengue cases in 2004.

stratified sampling and systematic sampling. Informed consent was obtained after the nature of the study was explained to the participants. The NHS 2004 was approved by the Health Promotion Board (HPB)'s Institutional Review Board (IRB) Ethics Committee.

The sample selection was divided into 2 phases. In phase 1, sample selection was based on a modified two-stage stratified design. For the first stage, sampling divisions within close proximity to the 6 selected polyclinics were chosen and dwelling units of each selected sampling division were stratified by house-type and systematically selected in the second stage. A final sample of 12,700 addresses was chosen. This was followed-up by house visits to enumerate all members of the households within the age group of 18 years to 74 years. In phase 2, a random sample of 7,500 persons was selected from all the individuals identified in phase 1. They were first stratified by age and ethnic group, and then systematically selected. A total of 225 persons from the original sample were ineligible to participate in the survey for reasons such as pregnancy, death and overseas sojourn during the survey period. The individual participation rate was 65.9% (4168/7275). Our study comprised a total of 4152 NHS participants aged 18 years old to 74 years old who consented to having their residual sera used for further research.

Sera Samples from NHS 04 Subjects

Sera samples from all NHS 2004 subjects were stored at -80°C at the Department of Pathology, Singapore General Hospital. The stored sera of eligible subjects were sent to the Environmental Health Institute (EHI), National Environment Agency, for analyses of IgG and IgM antibodies against dengue to determine past and recent exposure to the virus using commercial tests, namely, the Panbio Dengue IgM Capture ELISA and Panbio Dengue IgG Capture/Indirect ELISA systems (Panbio, Sinnamon Park, Australia). All

subjects' identifiers such as identification numbers were de-linked before analysis.

Individuals found positive for IgG (PanBio Indirect ELISA), excluding high-titre IgG (PanBio Capture ELISA) were classified as those with "past dengue infection", while individuals found positive for IgM (PanBio Capture ELISA) and/or high-titre IgG (PanBio Capture IgG > 22 Panbio units) were classified as those with "recent dengue infection". The results were sent to the Communicable Diseases Division, Ministry of Health, for statistical analysis. Statistical analysis was performed using the statistical software package, Statistical Package for Social Sciences (SPSS) 14.0. The survey sample data was adjusted to the age, ethnic group and gender distribution of the 2004 Singapore resident population, to ensure that the characteristics of the sample conformed to that of the general population. Comparison between 2 proportions was performed using the Z test. Backward stepwise logistic regression was performed to identify independent predictors of lifetime exposure to dengue. Variables with $P < 0.05$ were retained in the model.

Results

Demographic Profile of Participants

The mean age of the participants was 42.4 years. There was about an equal number of male and female participants in the study. A majority of study participants were ethnic Chinese and lived in public housing apartments and comprised mostly working adults (Table 1).

Characteristics of Individuals with Past Dengue Infection

In all, 2449 (59.0%) of the study population were seropositive for IgG, indicating that they had past dengue infection (95% CI: 57.5%-60.5%).

Table 2 shows the characteristics of individuals with past dengue infection, according to gender, ethnicity, age, main occupational status over the last 12 months and type of residential premises; for multi-storey apartments, the floor level that the individuals resided in. No significant difference in seropositivity between male and female participants was detected. Among the 3 major ethnic groups, Indians had the highest seropositivity (69.3%) as compared to that of Chinese (58.2%) and Malays (57.1%) ($P < 0.0005$). Seropositivity increased with age. The lowest age-specific seropositivity of 17.2% was found in the 18 years to 24 years age group and the highest seroprevalence of 88.9% was found in the 55 years to 74 years age group.

The proportion of individuals with past dengue infection was much lower among students (18.2%) and national servicemen (12.8%) compared to other occupational groups such as the employed (57.5%), the unemployed (70.3%), housewives (76.7%) and retirees (89.5%).

Table 1. Demographic Characteristic of Study Population

Characteristics	Number	Percent
Gender		
Male	2058	49.6
Female	2094	50.4
Ethnic group		
Chinese	3293	79.3
Malay	520	12.5
Indian	339	8.2
Age group (y)		
18-24	512	12.3
25-34	899	21.7
35-44	1046	25.2
45-54	910	21.9
55-74	785	18.9
Main work status over last 12 months*		
Working	2873	69.4
Student	247	6.0
National service	78	1.9
Homemaker/Housewife	673	16.2
Retired	153	3.7
Unemployed	118	2.8
Type of residential premises		
Landed property	264	6.4
HDB ⁺ Flats		
1-3 rooms	843	20.3
4 rooms	1540	37.1
5 rooms	842	20.3
Executive flats and others	326	7.8
HUDC ⁺ and other public flats	38	0.9
Condominium and private flats	299	7.2
Floor level*		
Landed property	264	6.4
HDB, condominium and private flat		
Ground	102	2.4
2-9 floors	2599	62.7
10 floors or higher	1180	28.5

* Numbers do not add up to 4152 due to non-response

⁺ HDB: Housing Development Board

HUDC: Housing and Urban Development Company

Seropositivity was significantly higher among residents of landed properties compared to occupants of public housing apartments (68.2% vs 58.3%, $P < 0.0005$) and private condominiums (68.2% vs 59.2%, $P < 0.0005$).

In the multivariate logistic regression model, ethnic Indian and older aged individuals remained as independent risk factors significantly associated with subjects with past dengue infection (Table 2). Male gender was now found to be significantly associated with the risk of past dengue infection, after controlling for the other variables.

Characteristics of Individuals with Recent Dengue Infection

The proportion of study participants with recently acquired dengue infection was 2.6% (95% CI: 2.1%-3.1%). Table 3 shows the characteristics. There were no significant differences in the prevalence of recent dengue infection by gender and ethnic groups ($P > 0.1$). The proportion of individuals with recent dengue infection also increased with age. It was lowest in the 18 years to 24 years age group (1.2%) and highest in the 45 years to 54 years age group (3.2%).

Students and national servicemen had the lowest prevalence of recent infection (1.2% and 1.3%, respectively). Among the other occupational groups, retirees had the highest prevalence of recent dengue infection (3.3%), followed by housewives (2.8%), the employed (2.7%) and the unemployed (2.6%).

Among occupants of high-rise apartments, the prevalence was highest for ground floor residents (3.9%) compared to residents living on the second floor and above (2.5%).

In the multivariate logistic regression model, only age group remained significantly associated with the risk of recent dengue infection.

Discussion

In Singapore, seroepidemiological surveys are conducted periodically to monitor the changing immune status of the population against dengue virus infection and to evaluate the efficacy of the *Aedes* control programme. It supplements disease notification in monitoring trends and changing patterns of dengue virus infection in the population. Seroprevalence data have also been used in mathematical modelling on historical changes in the force of infection (the per capita rate at which susceptible individuals acquire infection) for dengue.⁶ Serological surveys were carried out between 1982 to 1984,⁷ in 1988,⁸ 1990 to 1991,⁹ 1993,¹⁰ 1998,¹¹ 2001¹² and 2004.¹³

Comparison with Past Studies

In this study, the seroprevalence of adults above 18 years of age with past dengue infection (59.0%) had not

Table 2. Analysis of Past Dengue Infection Among the General Population by Selected Characteristics

Characteristics	No. positive/ No. tested	Bivariate analysis			Multivariate analysis*			
		(%)	Odds ratio	95% CI	P value	Adjusted OR	95% CI	P value
Gender					0.097			0.006
Male	1238/2058	(60.2)	1.00	Referent	-	1.00	Referent	-
Female	1211/2094	(57.8)	0.90	(0.79, 1.02)	0.097	0.80	(0.68, 0.94)	0.006
Ethnic group					<0.0005			<0.0005
Chinese	1917/3293	(58.2)	1.00	Referent	-	1.00	Referent	-
Malay	297/520	(57.1)	0.95	(0.78, 1.14)	0.564	1.10	(0.88, 1.38)	0.404
Indian	235/339	(69.3)	1.68	(1.31, 2.16)	<0.0005	2.25	(1.69, 3.01)	<0.0005
Age group (y)					<0.0005			<0.0005
18-24	88/512	(17.2)	1.00	Referent	-	1.00	Referent	-
25-34	307/899	(34.1)	2.57	(1.97, 3.37)	<0.0005	2.19	(1.53, 3.14)	<0.0005
35-44	645/1046	(61.7)	8.30	(6.38, 10.80)	<0.0005	7.03	(4.90, 10.09)	<0.0005
45-54	711/910	(78.1)	19.91	(14.98, 26.47)	<0.0005	16.80	(11.52, 24.50)	<0.0005
55-74	698/785	(88.9)	52.12	(36.94, 73.55)	<0.0005	41.37	(26.44, 64.72)	<0.0005
Main work status over last 12 months					<0.0005			0.032
Student	45/247	(18.2)	1.00	Referent	-	1.00	Referent	-
National service	10/78	(12.8)	0.64	(0.31, 1.35)	0.242	0.62	(0.29, 1.31)	0.207
Working	1652/2873	(57.5)	6.26	(4.50, 8.70)	<0.0005	1.18	(0.75, 1.84)	0.475
Unemployed	83/118	(70.3)	10.99	(6.55, 18.46)	<0.0005	1.90	(1.00, 3.59)	0.050
Homemaker/ Housewife	516/673	(76.7)	16.11	(11.11, 23.37)	<0.0005	1.60	(0.96, 2.67)	0.072
Retired	137/153	(89.5)	56.58	(27.97, 114.44)	<0.0005	1.56	(0.69, 3.54)	0.289
Type of residential premises					0.012			
Landed property	180/264	(68.2)	1.00	Referent	-			
HDB flat	2092/3589	(58.3)	0.66	(0.51, 0.87)	0.003			
Condominium and private flat	177/299	(59.2)	0.68	(0.48, 0.97)	0.035			
Floor level					0.005			
Landed property	180/264	(68.2)	1.48	(1.11, 1.97)	0.009			
HDB, condominium and private flat								
Ground	68/102	(66.7)	1.47	(0.94, 2.30)	0.132			
2-9 floors	1500/2599	(57.7)	0.96	(0.83, 1.10)	0.398			
10 floors or higher	699/1180	(59.2)	1.00	Referent	-			

* The model retained significant predictors ($P < 0.05$) using backward (likelihood ratio) stepwise method

Table 3. Analysis of Recent Dengue Infection Among the General Population by Selected Characteristics

Characteristics	No. positive/ No. tested	(%)	Odds ratio	95% CI	P value
Gender					0.451
Male	57/2058	(2.8)	1.00	Referent	-
Female	53/2094	(2.5)	0.86	(0.59, 1.27)	0.451
Ethnic group					0.272
Chinese	89/3293	(2.7)	1.00	Referent	-
Malay	11/520	(2.1)	0.78	(0.41, 1.47)	0.445
Indian	10/339	(2.9)	1.59	(0.80, 3.14)	0.183
Age group (y)					<0.0005
18-24	6/512	(1.2)	1.00	Referent	-
25-34	23/899	(2.6)	2.59	(1.07, 6.28)	0.035
35-44	29/1046	(2.8)	5.02	(2.11, 11.93)	<0.0005
45-54	29/910	(3.2)	11.17	(4.67, 26.70)	<0.0005
55-74	23/785	(2.9)	23.02	(9.23, 57.40)	<0.0005
Main work status over last 12 months					<0.0005
Student	3/247	(1.2)	1.00	Referent	-
National service	1/78	(1.3)	1.31	(0.14, 12.41)	0.816
Working	79/2873	(2.7)	5.44	(1.54, 19.29)	0.009
Unemployed	3/118	(2.6)	8.53	(1.61, 45.07)	0.012
Homemaker/Housewife	19/673	(2.8)	11.08	(2.93, 41.99)	<0.0005
Retired	5/153	(3.3)	36.72	(7.10, 189.91)	<0.0005
Type of residential premises					1.000
Landed property	5/264	(1.9)	1.00	Referent	-
HDB flat	97/3589	(2.7)	0.99	(0.41, 2.42)	0.988
Condominium and private flat	8/299	(2.7)	0.99	(0.32, 3.05)	0.983
Floor level					0.588
Landed property	5/264	(1.9)	1.2	(0.46, 3.10)	0.707
HDB, condominium and private flat					
Ground	4/102	(3.9)	2.07	(0.65, 6.61)	0.219
2-9 floors	75/2599	(2.8)	1.26	(0.79, 1.98)	0.331
10 floors or higher	26/1180	(2.2)	1.00	Referent	-

increased despite cyclical epidemics in 1992, 1998 and 2004. In fact, it was slightly lower than the weighted prevalence for the corresponding age group reported in 1993 (71.4%)¹⁰ and 1998 (64.0%).¹¹ However, in making this comparison, it should be noted that in previous studies, a smaller population group comprising mainly of healthy volunteers and facility-based healthy children and adults was examined. In comparison, the participants of this study were more representative of the Singapore general population. Furthermore, the haemagglutination-inhibition

(HI) method was used in the 1993 and 1998 studies. In contrast, the Panbio Dengue Indirect ELISA method was used for IgG assay in this study. The sensitivity and specificity of the test may be different from that of the HI method.¹⁴

Comparison with other Dengue Endemic Countries

Our prevalence of 17.2% in young adults, between 18 years and 24 years of age, is lower than that of other endemic countries. It was 97% in those less than 20 years

old in the Dominican Republic,¹⁵ 53% in children aged 7 years to 9 years of age in Bangkok,¹⁶ 86% in children of 13 years of age in Southern Vietnam¹⁷ and 79% in those aged 15 years to 24 years of age in Mexico¹⁸ (Table 4). The comprehensive nationwide *Aedes* control programme and the aggressive vector control measures implemented in suppressing dengue transmission during both epidemic and inter-epidemic periods have resulted in a lowering of the herd immunity of Singapore children and young adults despite more frequent outbreaks with greater intensity.⁴ In fact, mathematical modelling showed that there has been a marked reduction in the force of dengue infection due to *Aedes* control, which resulted not only in an overall reduction in herd immunity, but also an increase in the incidence of clinical disease by exposing a higher proportion of older age groups.⁶

Demographic Determinants of those who had Past Dengue Infection

Older age groups were found to have higher seropositivity as compared to younger age groups in this study. The odds ratio of past infection with dengue increased by more than 2 times for every 10 year increase in age. The increased seroprevalence with age has also been observed in other studies. This is expected and is consistent with the fact that levels of IgG antibodies remain detectable once a person is infected.²¹

Male gender was found to be significantly associated with subjects who had past dengue infection. While it has been suggested that this could be attributed to the higher proportion of working males compared to working females,²² the finding that housewives, retirees and the unemployed had higher seropositivity than those employed do not support the hypothesis. Moreover, our serological finding reveals that recent dengue infections displayed no gender difference. Thus factors such as movement history that contribute to the higher seroprevalence among males should be investigated further.

Environmental Determinants of Subjects who had Past Dengue Infection

This study also demonstrated that residents of landed property had a higher seroprevalence compared with public housing apartment dwellers. However, the type of residence did not remain as a significant risk factor after controlling for other risk factors. Previous studies⁸⁻¹¹ have demonstrated that those living in landed properties have a higher risk of dengue infection, as there are more potential breeding habitats on landed property. The proportion of landed property found breeding *Aedes* mosquitoes was significantly higher than that of public housing apartments.^{23,24}

Demographic Risk Factors of Recent Dengue Infection

In this study, 2.6% of the subjects had serological evidence of recent dengue infection. Based on univariate analysis, age group and occupational status in the past 12 months were significantly associated with recent dengue infection. However, only age group remained as an independent risk factor for recent dengue infection after controlling for other risk factors. There is however no gender difference for recent dengue infection. The Northern Thailand study²¹ also reported that persons aged 30 years or older had more than 5 times higher risk of acquiring recent dengue infection compared with persons younger than 30 years old.

Comparison with Profile of Notified Dengue Cases during the Period

Based on the finding that 2.6% of the subjects had serological evidence of recent dengue infection, it was estimated that about 69,000 residents could have been infected when this seroprevalence estimate was projected to the resident population (Chinese, Malay and Indians) aged 15 years old and above during the study period. Singapore residents invited to participate in the NHS 2004 most likely would have consented to the survey if they were healthy at that time. Only a total of 3104 individuals developed clinical symptoms and were notified to the Ministry of Health as laboratory-confirmed dengue during the period between September and December 2004.³ Therefore, it is estimated that 1 out of 23 cases are diagnosed and notified. This is consistent with the findings of previous serological surveys as well as findings in other countries^{16,25-27} that a majority of dengue virus infection is either asymptomatic or subclinical, and notified cases only represent a fraction of the actual extent of infection in the population. This is especially so among children with silent transmission occurring in the majority.^{16,27-29} However, there could be an overestimate of the ratio of reported to unreported cases in our study. As shown in a longitudinal cohort study³⁰ of dengue infection in adults in Indonesia, the incidence of symptomatic dengue disease was 18 cases per 1000 person-years compared to an estimated 56 asymptomatic cases per 1000 person-years. In another study in West Jakarta, among adults, the ratio of symptomatic to asymptomatic infection was 3:5.²⁵ As viraemia has been demonstrated in asymptomatic cases, these unreported cases potentially form an important virus reservoir for further transmission of infection, especially in areas where the vector population is high.

We observed differences in the demographic profile of the study group with recent dengue infection and those notified to the Ministry for dengue.³ Although males consistently had a significantly higher reported incidence rate compared to females (374.7 per 100,000 and 282.8

Table 4: Seroprevalence of Dengue Virus infection in Some Endemic Countries

		Study Descriptions					
Study	Singapore NHS	Santo Domingo, Dominican Republic	Bangkok Thailand ¹⁶	Southern Vietnam ¹⁷	Texas-Mexico Border ¹⁸	Complex Urban Area, Salvador, large city in north east Brazil, 1998 ¹⁹	Brazil ²⁰
Population	participants	adults and children ¹⁵					
Sampling method	Combination of disproportionate stratified and systematic sampling	Healthy adult volunteers at blood bank and children visiting a hospital	Students 4-16 yrs at a Bangkok school	Two primary schools participated, non-randomised	Cross-sectional serosurvey at household level	Prospective cohort study with sampling from spatial sample of 30 neighbourhood	3-stage sampling approach
Sample size	4152	1008 (adults) 201 (children <10 yrs)	1757	961	600 households	2149	1585
						Participation rate: 70%	
Laboratory method	PanBio IgG Capture ELISA, IgG Indirect ELISA, IgM Capture ELISA	ELISA DEN virus specific IgG	Blood samples collected before and after dengue season	Indirect IgG ELISA	DUO IgM /IgG capture ELISA (PanBio)- Indirect IgG ELISA for past dengue infection (PanBio)	Titres by HI of 1:20 or higher	DEN IgM/Ig G antibodies by ELISA
Seroprevalence	Adults >18 yrs: 59% +ve 18-24 yrs: 18.4% +ve	Adults: 98% +ve <10 yrs: 56% <20 yrs: 97% +ve	7-9 yrs: 53% +ve	7 yrs: 53% +ve 13 yrs: 86% +ve	15-24 yrs: 79% +ve	0-4 yrs: 39.0% +ve <15 yrs: 57.4% +ve >15 yrs: 76.1% +ve 30-39 yrs: 76.4% +ve	5-14 yrs: 22.2% +ve >15 yrs: 32.2% +ve

per 100,000, respectively in 2005), serological evidence showed no gender difference in recent infection.

Among the 3 major ethnic groups, the Chinese had the highest incidence rate followed by Malays and Indians. The incidence rate of residents of compound houses was 3 times that of HDB residents. On the other hand, age remained the only significant determinant for recent dengue infection. Different health seeking behaviour and differential severity of dengue infection may account for these observations. Working adults who are symptomatic would seek medical attention from a registered medical practitioner as they require medical certification to be excused from work. They will then be notified to the Ministry if dengue is suspected. Those identified with recent dengue infection in the seroprevalence study were most likely to be asymptomatic or had non-specific or subclinical illness and did not see a need to seek medical treatment or preferred to self-medicate. This is especially so if they are not working, for example, the retired, homemaker/housewife and unemployed.

Strengths and Limitations

This study was based on testing on residual sera that was collected from NHS 2004.⁵ The laboratory method used to indicate seroprevalence of past dengue infection was PanBio Indirect ELISA where the sensitivity and specificity are reported to be 99.2% and 96.2%, respectively when compared to the hemagglutination-inhibition method.^{14,31} PanBio Capture ELISA was used to detect cases of recent dengue infection with positive IgM or high-titre IgG. The combined use of IgM and IgG in PanBio Capture ELISA demonstrated high sensitivity and specificity (99% and 96%, respectively) when compared to the hemagglutination-inhibition method.^{32,33} It also has high sensitivity and specificity when compared to both RT-PCR and virus neutralisation tests.³⁴ The PanBio ELISA tests also detect cross-reactive antibodies to Japanese encephalitis (JE) and yellow fever (YF) viruses. However, false positives due to cross-reactivity are minimal because JE incidence is very low in Singapore, and YF has never been detected in this region. The number of participants included in this study was representative of Singapore's general population as compared to previous serological surveys.⁷⁻¹¹ Therefore the seroprevalence of dengue infection would be closer to the actual situation in Singapore. This allows for better planning and implementation of specific dengue prevention and control programmes. However, there were limitations to this study that need to be considered. Risk factors examined in our study were limited to demographics, housing types as well as main work status. Further information such as any recent travel history and length of stay in a particular location were not examined. A cohort study could be conducted in the future to enable risk factors for the acquisition of dengue

infection such as specific environmental determinants to be determined.

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