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Seroepidemiology of dengue virus infection in the adult population in tropical Singapore

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SUMMARY

To assess the impact of past dengue epidemics in Singapore, we undertook a national seroepidemiological study to determine the prevalence of past dengue virus (DENV) infection in the adult population in 2010 and make comparisons with the seroprevalence in 2004. The study involved residual sera from 3293 adults aged 18–79 years who participated in a national health survey in 2010. The overall prevalence of anti-DENV IgG antibodies was 56·8% (95% confidence interval 55·1–58·5) in 2010. The seroprevalence increased significantly with age. Males had significantly higher seroprevalence than females (61·5% *vs.* 53·2%). Among the three major ethnic groups, Malays had the lowest seroprevalence (50·2%) compared to Chinese (57·0%) and Indians (62·0%). The age-standardized seroprevalence in adults was significantly lower in 2010 (54·4%) compared to 2004 (63·1%). Older age, male gender, Indian ethnicity, permanent residency and being home-bound were independent risk factors significantly associated with seropositivity. About 43% of the Singapore adult resident population remain susceptible to DENV infection as a result of the successful implementation of a comprehensive nationwide *Aedes* surveillance and control programme since the 1970s. Vector suppression and concerted efforts of all stakeholders in the community remain the key strategy in the prevention and control of dengue.

Key words: *Aedes*, dengue infection, epidemics, surveillance.

INTRODUCTION

Dengue, a mosquito-borne arboviral disease, is endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific, with the American, Southeast Asian and the Western Pacific regions most seriously affected [1].

In Singapore, a highly urbanized tropical city-state, the vectors *Aedes aegypti* and *Aedes albopictus* breed throughout the year. The first outbreak of dengue fever (DF) was reported in 1901 [2]. An outbreak of dengue haemorrhagic fever (DHF) involving 70 hospitalized cases was first recorded in 1960 [3, 4]. Since then, dengue has become endemic in Singapore with large epidemics occurring almost annually, and it was a major paediatric problem in the 1960s [5, 6]. Based on surveillance and research data gathered in the field, a comprehensive nationwide *Aedes* prevention and control programme incorporating source reduction, health education and law enforcement was

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launched in 1969 and successfully implemented since 1973, as evidenced by a sharp reduction in *Aedes* premises index (percentage of premises found to be breeding *Aedes* mosquitoes) and low disease incidence [7, 8]. However, since the 1980s, dengue epidemics of increasing magnitude occurred on a 6-year cycle in 1986/1987 [9] (13·0 and 15·7/100 000 population), 1992 [10] (89·1/100 000 population), 1998 [11] (133·9/100 000 population), 2004/2005 [12] (227·0 and 333·1/100 000 population), 2007 [13] (192·3/100 000 population) and 2013 [14] (410·6/100 000 population). During the 10-year period between 2004 and 2013, excluding the epidemic years, the dengue incidence/100 000 population ranged from 87·2 in 2012 to 145·3 in 2008 [15]. All four dengue virus (DENV) serotypes circulate throughout the year; serotype 1 was the predominant serotype in the 2004/2005 epidemic [12], serotype 2 in the 2007 epidemic [13] and serotype 1 in the 2013 epidemic [16].

Several seroprevalence studies were conducted in the Singapore general population aged 6 months to >45 years to monitor the changing immune status and to assess the effectiveness of the national *Aedes* control programmes [17, 18]. These surveys supplemented disease notification in monitoring trends and changing epidemiology of dengue in the community. However, all these seroepidemiological studies undertaken were not representative of the Singapore general population. The first seroprevalence survey representative of the adult population in Singapore was conducted in 2004 [19]. It was based on 4152 residual sera obtained during the National Health Survey (NHS) in 2004. The NHS was a population-based cross-sectional survey conducted by the Ministry of Health (MoH) to determine the prevalence of chronic diseases and lifestyle-related risk factors in Singapore adult residents (Singapore citizens and permanent residents). Collection of blood samples was made between September and December 2004 which coincided with a nationwide dengue epidemic.

To assess the impact of the 2004/2005 and 2007 dengue epidemics, we undertook another national seroepidemiological study in the adult resident population in 2010 to determine the prevalence of past DENV infection in Singapore, and compare this with the findings in 2004.

MATERIALS AND METHODS

As in the last seroepidemiological study [19] on DENV infection conducted in 2004 in Singapore adult residents, we made use of residual sera obtained during the NHS in 2010 [20]. Selection of the survey population was by a combination of disproportionate stratified sampling and systematic sampling. Five government primary-care clinics and one community club geographically well spread out across Singapore were selected as the sites for NHS 2010. The household units in the vicinity of these survey sites provided a good combination of the different types of dwelling and the three major ethnic groups in Singapore. The fieldwork for the NHS was carried out over a 3-month period from 17 March to 13 June 2010. There was no increase in dengue incidence during this period. Ethical approval was given by the Ethics Committee of the Health Promotion Board, Singapore (reference number 006-2010). Only sera from survey participants who had consented to having their residual sera used for further research were included in this study. Personal identifiers of survey participants were permanently removed and new study numbers tagged to the residual sera, so as to ensure strict anonymity of the participants via this delinking process.

A total of 4337 persons out of an eventual sample of 7512 eligible Singapore residents aged 18–79 years participated in NHS 2010, giving a response rate of 57·7%. Residual sera from 3293 participants aged 18–79 years from NHS 2010 (75·9%) were included in the dengue seroprevalence study.

The stored residual sera from NHS 2010 were screened for anti-DENV IgG antibodies by ELISA using a commercial test kit (EUROIMMUN, Germany) according to the manufacturer's recommended procedure. Levels $\geqslant 20$ relative units/ml were considered to be reactive. For the earlier study based on residual sera from NHS 2004, a commercial test, the Panbio Dengue IgG Capture/Indirect ELISA system (Panbio, Australia), was used to determine past exposure to DENV [19]. Individuals that tested positive for IgG (PanBio Indirect ELISA), excluding high-titre IgG (PanBio Capture ELISA), were classified as having past DENV infection in 2004.

To ensure that the characteristics of the sample for NHS 2010 conformed to that of the general population, post-stratification weights were computed based on the age, gender, ethnic group and dwelling type attributes of the Singapore resident population. The overall sample weight was the product of weights for unequal probability of selection and non-response from the household enumeration exercise and survey fieldwork, respectively, and post-stratification weight.

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Table 1. *Socio-demographic profile (%) of 3293 study subjects and resident population aged 18–79 years in Singapore, 2010*

Demographic characteristics	Study sample (n = 3293)	Resident population (Census 2010)
Age group (years)		
18–29	20·2	21·6
30–39	20·7	21·4
40–49	22·2	21·9
50–59	20·8	19·1
60–69	10·3	10·5
70–79	5·8	5·5
Gender		
Male	43·1	49·2
Female	56·9	50·8
Ethnic group		
Chinese	73·8	75·6
Malay	12·8	12·3
Indian	9·6	8·8
Other	3·8	3·2
Type of dwelling		
HDB flats*		
1–3 rooms	21·1	21·0
4 rooms	34·2	34·2
5-room, executive flats and other public flats	29·2	27·6
Condominium and private flats	10·0	9·4
Landed property†	4·9	6·7
Others‡	0·6	1·1

* HDB, Housing Development Board, refers to public apartments.
† Refers to bungalow/detached house, semi-detached house and terrace house.
‡ Refers to temporary residences and dormitories.

The weighted respondent sample of 4337 participants of NHS 2010 was similar to the Singapore resident population in socio-demographic profile [20]. The socio-demographic profile of 3293 survey subjects included in our dengue seroprevalence study and the Singapore resident population aged 18–79 years was also found to be similar (Table 1).

The χ^2 test or Fisher's exact test, where appropriate, was used to test for group differences. Proportions between two groups were compared using two-sample independent z tests, with standard error estimated using pooled value of the two proportions. The Mantel–Haenszel linear-by-linear association χ^2 test was used to evaluate whether there was linear trend in seroprevalence in the age groups.

Age-standardization of dengue seroprevalence was calculated by the direct method, using the 2010 census Singapore resident population as the standard. Differences in the age-standardized rates between genders and ethnic groups were computed and tested for statistical significance using the z test [21]. The 95% confidence intervals (CI) were reported.

For comparison with the dengue seroepidemiological study based on 4152 residual sera from NHS 2004 [19], the study sample in 2010 was confined to 3091 Singapore residents aged 18–74 years who were ethnic Chinese, Malay and Indian; subjects aged 75–79 years and other ethnic groups were omitted. Tests for statistical significance between the two studies were based on age-standardized seroprevalence.

Bivariate analysis using logistic regressions was performed to compute crude odds ratios of demographic characteristics for DENV seroprevalence. Multivariable logistic regression analysis was used to determine independent risk factors of past DENV infection, using forward stepwise selection based on maximum partial likelihood estimates. All P values reported were two-sided and statistical significance was taken at $P < 0·05$. Statistical analysis was performed using SPSS Statistics software, v. 19·0 (IBM, USA).

RESULTS

The overall prevalence of anti-DENV IgG antibodies in the adult population aged 18–79 years was 56·8% (95% CI 55·1–58·5) in 2010. The seroprevalence increased significantly from 17·8% in young adults aged 18–29 years to 77·8% in those aged 50–59 years (test for trend, $P < 0·0005$) (Table 2). It was maintained at 92·4% or higher in older age groups of 60–69 years and 70–79 years. Males (61·5%) had a significantly higher seroprevalence than females (53·2%) ($P < 0·0005$). Of the three major ethnic groups, the seroprevalence in Chinese (57·0%) and Indians (62·0%) ($P = 0·09$) were similar, but Malays had a significantly lower seroprevalence (50·2%) than Chinese ($P = 0·01$) and Indians ($P = 0·001$). No significant difference was detected by residency status (Singapore citizens *vs.* permanent residents, $P = 0·10$).

We observed no significant difference in the seroprevalence by the three main types of dwelling ($P = 0·34$). Of those living in public and private housing apartments, there was also no discernible trend in seroprevalence by floor level ($P = 0·25$).

In the multivariable logistic regression analysis, older age, male gender, Indian ethnicity, residency status

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Table 2. *Seroprevalence (%) and odds ratios of DENV infection in logistic regression analysis by demographic characteristics in 3293 adults aged 18–79 years in Singapore, 2010*

		Bivariable analysis			Multivariable analysis		
Demographic characteristics	Seroprevalence (%)	Crude OR	95% CI	*P* value	Adjusted OR	95% CI	*P* value
Age group (years)				<0·0005			<0·0005
18–29	17·8	1·00	Referent		1·00	Referent	
30–39	42·1	3·36	(2·62–4·32)	<0·0005	3·06	(2·29–4·10)	<0·0005
40–49	60·4	7·08	(5·52–9·07)	<0·0005	7·09	(5·31–9·47)	<0·0005
50–59	77·8	16·21	(12·39–21·21)	<0·0005	16·36	(11·98–22·34)	<0·0005
60–69	92·4	56·52	(36·13–88·42)	<0·0005	49·75	(30·28–81·74)	<0·0005
70–79	92·7	58·12	(32·69–103·35)	<0·0005	45·45	(23·84–86·66)	<0·0005
Gender				<0·0005			<0·0005
Male	61·5	1·00	Referent		1·00	Referent	
Female	53·2	0·71	(0·62–0·82)	<0·0005	0·69	(0·58–0·83)	<0·0005
Residency				0·109			0·018
Singapore citizen	57·4	1·00	Referent		1·00	Referent	
Permanent resident	53·4	0·85	(0·70–1·04)	0·109	1·34	(1·05–1·70)	0·018
Ethnic group				0·007			<0·0005
Chinese	57·0	1·00	Referent		1·00	Referent	
Malay	50·2	0·76	(0·62–0·94)	0·010	0·91	(0·71–1·17)	0·478
Indian	62·0	1·23	(0·97–1·57)	0·088	1·73	(1·31–2·30)	<0·0005
Other	61·6	1·21	(0·84–1·75)	0·307	1·75	(1·15–2·68)	0·010
Main work status over last 12 months				<0·0005			0·029
Working	53·5	1·00	Referent		1·00	Referent	
Student/national serviceman	18·9	0·20	(0·14–0·29)	<0·0005	1·05	(0·69–1·59)	0·817
Homemaker	70·9	2·11	(1·72–2·59)	<0·0005	1·33	(1·03–1·71)	0·028
Retired	93·5	12·17	(7·21–20·56)	<0·0005	1·85	(1·02–3·35)	0·043
Unemployed/ unknown	56·3	1·12	(0·74–1·70)	0·586	0·71	(0·44–1·13)	0·148
Type of dwelling				0·506			
Landed residential property*	60·2	1·00	Referent				
Public housing apartment	56·7	0·86	(0·62–1·18)	0·348			
Private flat and condominium	53·5	0·75	(0·51–1·10)	0·141			
Others†	100·0	0·00	–	0·998			
Floor level of residential premises				0·055			
Landed residential property*	64·4	1·51	(1·08–2·10)	0·015			
Public and private apartment							
Ground	51·5	0·86	(0·57–1·29)	0·458			
2nd–9th floor	57·3	1·10	(0·95–1·29)	0·215			
10th floor or higher	54·7	1·00	Referent				

OR, Odds ratio; CI, confidence interval.
* Refers to bungalow/detached house, semi-detached house and terrace house.
† Refers to temporary residences and dormitories.

(permanent resident) and occupation (homemaker and retiree) were independent risk factors significantly associated with past DENV infection (Table 2).

Compared to 2004, the seroprevalence in 2010 was significantly lower in all age groups except in the youngest (18–24 years) and oldest (55–74 years) age

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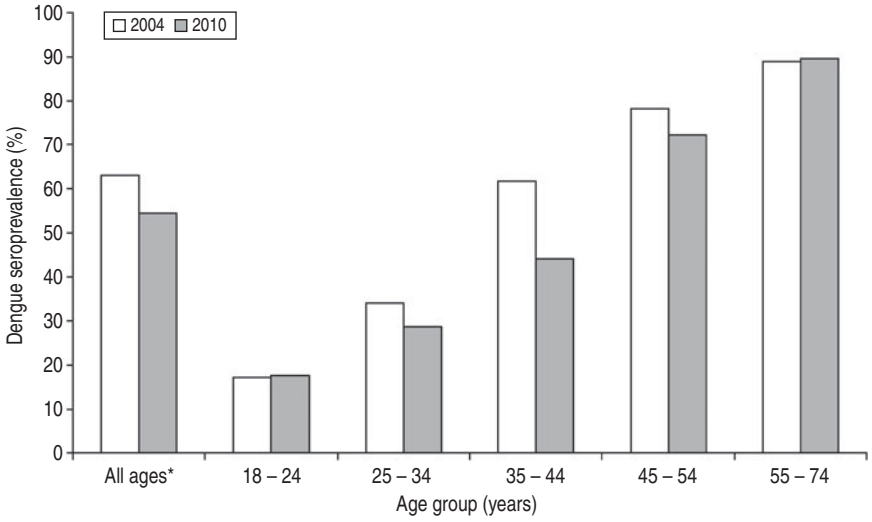

Fig. 1. Age-specific DENV seroprevalence in adults aged 18–74 years in Singapore, 2004 and 2010. *Age-adjusted using the 2010 census population as the standard.

groups (Fig. 1). The decrease in seroprevalence was most marked in the 35–44 years age group; 61·7% in 2004 *vs.* 44·2% in 2010 ($P < 0{\cdot}0005$).

The age-standardized seroprevalence in 2010 (54·4%, 95% CI 52·7–56·2) was significantly lower than in 2004 (63·1%, 95% CI 61·6–64·6) ($P < 0{\cdot}005$). There were significant decreases in the age-standardized seroprevalence for both genders and in the three major ethnic groups between 2004 and 2010 (all $P < 0{\cdot}01$).

While there was a significant difference in seroprevalence by gender in 2010, no significant difference was observed in 2004 ($P = 0{\cdot}13$). In 2004, Indians had a significantly higher seroprevalence (69·3%) compared to Chinese (58·2%) and Malays (57·1%) ($P < 0{\cdot}0005$). There were no significant differences in seroprevalence for each type of dwelling (landed residential property, public housing apartment, private flat, condominium) between 2004 and 2010 (all $P \geq 0{\cdot}10$).

DISCUSSION

Periodic seroprevalence surveys conducted in Singapore since 1982 show a declining level of immunity against dengue infection in the population aged 6 months to >45 years. The overall proportion who possessed haemagglutination-inhibition (HI) antibody to DENV serotype 2 decreased from 45·6% in 1982–1984 to 39·6% in 1993 and 29·4% in 1998 [17, 18]. Dengue is no longer a childhood infection, as only 4·3% of children and young adults aged <20 years possessed HI antibody to DENV serotype 2 in 1998. The low seroprevalence in children aged 6–15 years was also observed in a survey conducted between 1996 and 1997 when less than 7% tested positive for anti-DENV IgG antibodies [22]. In a national paediatric seroprevalence survey (NPSS) involving residual diagnostic sera collected prospectively from 1200 Singapore residents aged 1–17 years at two public acute-care hospitals from August 2008 to July 2010, the low prevalence in children and adolescents was again confirmed with 10·4% having evidence of past DENV infection. The median age of reported dengue cases has gradually increased from 14 years in 1973 [23] to 27 years in 1996 [6], 31 years in 2005 [12] and 37 years in 2007 [13], with the highest incidence rate of indigenous cases in adults aged 35–44 years in 2010 (132·8/100 000 population) [24]. The increasing age of dengue cases poses new challenges, including increased risk of severe disease [14].

The first serological survey representative of the adult population aged 18–74 years who participated in NHS 2004 showed an overall DENV seroprevalence of 59·0% (95% CI 57·5–60·5) [19]. This was within the range from 44·6% in a survey of 298 asymptomatic volunteers aged 18–45 years comprising staff and visitors to a large public tertiary hospital in 2002 [25] to 65·9% in another study conducted during the 2007 epidemic involving 3939 blood samples from residents in seven outbreak areas [26]. In the second study representative of the adult population which was conducted during the non-epidemic period in 2010, the overall prevalence of anti-DENV IgG antibodies was 56·8% (95% CI 55·1–58·5). When we combined the results from NPSS 2008–2010 and our study

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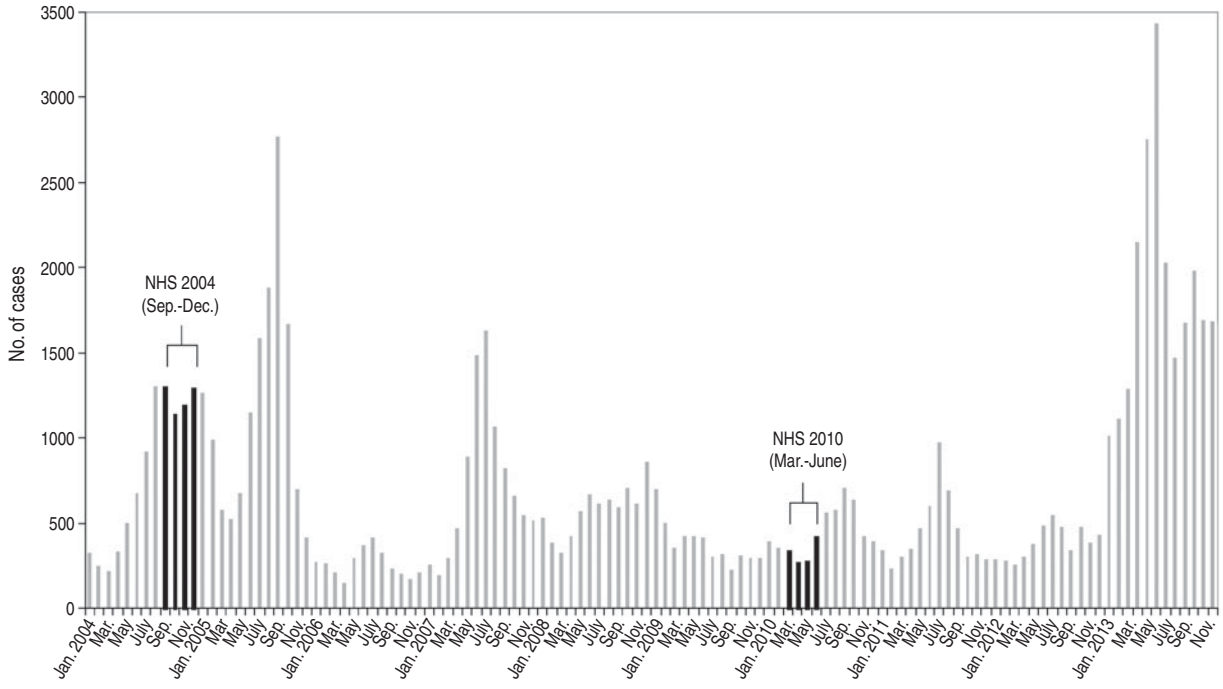

Fig. 2. Monthly incidence of reported dengue cases in Singapore, 2004–2013.

in adults aged 18–79 years, we estimated an overall seroprevalence of 46·9% in the Singapore general population aged 1–79 years in 2010. This was not much different from the finding that 45·6% had HI antibody to DENV serotype 2 in 1982–1984 [17].

There were two dengue epidemics which occurred in 2005 and 2007 before the seroprevalence study was conducted in 2010. In fact, the 2005 epidemic was the largest epidemic recorded prior to the survey until it was overtaken by an even larger epidemic in 2013 (Fig. 2). The dengue incidence rate in the Singapore resident population was highest in the 15–24 years age group in the 2004/2005 and 2013 epidemics, while it was the highest in the elderly aged $\geqslant$55 years in the 2007 epidemic. Singapore adolescents and young adults had less cumulative exposure to DENV as discerned from the age-specific incidence rate of dengue cases, which could have accounted for the low dengue seroprevalence in those aged 25–54 years over the 6-year period. Those who had been infected during large dengue epidemics in the 1960s would have reached the age of $\geqslant$55 years in 2004. This age group was the most affected in the 2007 epidemic and as the older cohorts died and were replaced by new cohorts, it was not unexpected that no significant difference in seroprevalence in those aged 55–74 years was detected between 2004 and 2010. Similarly, the relatively unchanged seroprevalence in those aged 18–24 years over the 6-year period could also be partly attributed to replacement of new cohorts in this age group. In periodic studies on blood donors conducted by the Environmental Health Institute (EHI) under the National Environment Agency (NEA), decreases in the prevalence of anti-DENV IgG antibodies were observed in the 20–40 years age group and older adults aged >40 years, while the seroprevalence remained stable in those aged <20 years between 2004 and 2009 [27]. The overall declining trend of dengue seroprevalence observed in about 4000 blood donors tested each year between 2004 and 2013 corroborated with our finding.

The overall prevalence of anti-DENV IgG antibodies in adults has remained relatively stable despite the two large epidemics in 2005 and 2007. This could also be partly attributed to the unprecedented national efforts to curb the first largest ever recorded epidemic in 2005 with the formation of an Inter-Ministerial Committee, an Interagency Dengue Coordination Committee to ensure that various policy initiatives were well coordinated, a Dengue Watch Committee to coordinate outreach to the community, an Expert Panel comprising local and international experts to advise the government on prevention and control measures, and an Interagency Dengue Task Force to

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enhance communication and coordination on dengue control efforts among various government agencies and private organizations. Manpower was increased by almost 400%, indoor and outdoor inspections for *Aedes* breeding increased by 50% compared to the previous year and a 2-month 'carpet combing' exercise was carried out for all the electoral constituencies [12]. Similar enhanced *Aedes* prevention and control measures were implemented in the 2007 epidemic [13].

The nationwide vector surveillance and control programme implemented since the early 1970s has reduced the overall *Aedes* premises index from more than 25% in the 1960s to 1–2% since 1985 [7, 28]. With less exposure to infected female *Aedes* mosquitoes, the herd immunity of the human population has declined over the decades as evidenced by the findings of periodic seroprevalence surveys. This has resulted in a paradoxical situation in that epidemics tend to occur more frequently and with greater intensity since the 1980s. Mathematical modelling shows that dengue transmission by the *Aedes* mosquito becomes more efficient with declining herd immunity of the human population [29, 30]. In another mathematical model based on local seroprevalence data, the rise in dengue incidence in Singapore was attributed to a declining trend in the force of infection (defined as *per capita* rate at which susceptible individuals in the community acquire infection), partly due to a vector-control-driven reduction in herd immunity and an increase in the average age of first infection since the comprehensive *Aedes* control programme was implemented nationwide [31]. Susceptibility in developing clinical dengue is higher in older than younger individuals [32].

In both the 2004 and 2010 studies of the adult population, older age, male gender and Indian ethnicity were identified as independent risk factors significantly associated with past DENV infection. In addition, homemakers or retirees were also found to be at higher odds of past DENV infection in 2010 after adjusting for age in the regression model. The increase in seroprevalence with age was expected, due to long-term or lifelong persistence of anti-DENV IgG antibodies following infection [33, 34]. The higher seroprevalence in retirees was probably age-related. The higher seroprevalence in those aged $\geqslant 50$ years in 2010 was also anticipated, as large epidemics had occurred almost annually in the 1960s. The higher seroprevalence in men corresponded to the pattern of gender-specific dengue incidence rates/100 000 population. This gender differential in the risk of DENV infection could be attributed to specific behaviour such as less tendency towards individual protection (i.e. use of insect repellents) that resulted in higher exposure to *Aedes* mosquito bites in men [35].

In the multivariable logistic regression analysis of the 2010 study, residency status was found to be an independent risk factor; compared to Singapore citizens, permanent residents were at significantly higher odds of past DENV infection (adjusted odds ratio 1·34, 95% CI 1·05–1·70). This could be due to the influx of foreign immigrants over the decades from other regions with higher level of dengue endemicity such as Malaysia where the majority of the adults (91·6% of a Malaysian cohort in 2008) have been found to be seropositive [36].

Dengue seroprevalence was highest in residents of landed residential properties, although it was not significantly different from that of other types of dwelling, whereas significant difference had been detected in past serological studies [18, 37]. The annual incidence rates of indigenous dengue have consistently been the highest in residents of landed residential properties where more potential breeding habitats are detected [38, 39].

The main limitation in our study in comparing the dengue seroprevalence between 2004 and 2010 is that the laboratory methods employed in the two studies were different. However, based on the manufacturer's document (EUROIMMUN, Germany), there was a 100% agreement between the results obtained using the EUROIMMUN Anti-Dengue Virus IgG ELISA and the Panbio Dengue Capture IgG ELISA, excluding borderline results from either test. Moreover, the significant decline in overall prevalence of anti-DENV IgG antibodies in adults between 2004 and 2010 was consistent with the trend observed in EHI's periodic studies on blood donors, who are screened to exclude potential donors with dengue infection without symptoms [40].

Another limitation is the comparison of findings with other serological surveys which were not representative of the general population; these should be interpreted with caution. Serotype-specific seroprevalence has not been determined and we only measured total IgG antibodies in the two nationally representative studies in 2004 and 2010, as there is no serological test that can accurately detect the serotype associated with past DENV infections due to cross-reactivity of anti-DENV antibodies [41]. The most widely used and recommended method to determine

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serotype-specific antibodies against DENV is the plaque reduction neutralization test (PRNT) [33, 41, 42].

In conclusion, despite epidemics occurring between the surveys in 2004 and 2010, the herd immunity of the adult population in Singapore is low, as about 43% remain susceptible to DENV infection. The city-state continues to be receptive to dengue despite sustained vector suppression measures implemented during both epidemic and non-epidemic periods to maintain a consistently low *Aedes* population. It is also vulnerable to the introduction of different DENV strains because of the large influx of travellers and visitors from other endemic regions. The only definitive long-term solution to the control of dengue in Singapore is vaccination of the population when a safe and effective dengue vaccine against all the four dengue serotypes becomes commercially available. In the meantime, more aggressive vector suppression through concerted efforts of all stakeholders in the community remains the main strategy in the prevention and control of dengue in Singapore.

DECLARATION OF INTEREST

None.

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