

## Short Report: High Rates of Inapparent Dengue in Older Adults in Singapore

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**Abstract.** Although the dengue iceberg phenomenon is well known, there is a paucity of data on inapparent dengue. Results from a seroepidemiological study conducted during a dengue epidemic in 2007 in Singapore showed a seroprevalence of 65.9% and an inapparent dengue rate of 78%. Older adults (> 45 years old) had significantly higher rates of inapparent dengue infections ( $P < 0.05$ ).

Since the first report of a dengue epidemic in 1901, Singapore has had outbreaks of dengue fever (DF). Unlike many countries in the region, dengue cases in Singapore are largely reported among the adult population,<sup>1,2</sup> and dengue mortality is mainly observed in adults.<sup>3</sup> Dengue infection is often epitomized by the iceberg effect, in which most cases are clinically inapparent.<sup>4</sup> Although inapparent dengue has been reported in many countries,<sup>5–7</sup> there is little data on its epidemiology or the extent of its severity. During the dengue epidemic in Singapore in 2007, we conducted cross-sectional seroepidemiologic surveys in seven outbreak areas (Figure 1) to estimate the dengue attack rate and the rate of inapparent dengue, and to further our understanding of the disease epidemiology.

All residents at each site were invited to participate in the study, which involved obtaining blood samples and a face-to-face, interviewer-administered questionnaire on demographics, clinical symptoms of dengue, and epidemiologic information. The study was reviewed and approved by the National Environment Agency Bioethics Committee. Signed, informed consent was provided by all study participants.

A total of 3,939 blood samples (participation rates = 3–15%) (Figure 2) were collected, stored on ice during transport, processed, and stored at  $-80^{\circ}\text{C}$  until testing. Serum samples were tested by using a capture dengue IgM enzyme-linked immunosorbent assay (ELISA) and an indirect dengue IgG ELISA (PanBio, Windsor, Queensland, Australia) according to manufacturer's instructions. One study reported the sensitivity and specificity of the indirect dengue IgG ELISA to be 100% and 98%, respectively, and those of the capture dengue IgM ELISA to be 87% and 96%, respectively.<sup>8</sup> The possibility that rheumatoid factors interfering with interpretation of IgM results was eliminated by removal of these factors before samples were assayed. A reverse transcription polymerase chain reaction (RT-PCR) was performed on 400 randomly selected samples by using an in-house assay.<sup>9</sup> Seroprevalence was calculated by adding the number of samples that were positive for IgG and those positive for IgM and dividing this total by the total number of samples.

Historical dengue areas were areas that have consistently reported dengue cases annually over the past 20 years. New dengue areas were those that have reported dengue cases only since 2000. Overall seroprevalence was 65.9% (range = 57.7–81.4%). Historical dengue areas had an average seroprevalence of 76.5%, and new dengue areas had an average seroprevalence of 63.5% ( $P=0.17$ ).

Seroprevalence was also observed to increase with age (Figure 3A). Generally, less than 20% of persons under 15 years of age had previously had dengue. This prevalence increased to 32% in persons 16–25 years of age, and was > 50% for adults 26–35 years of age. Seroprevalence steadily increased until > 90% in persons 66–75 years of age. When analyzed by location, historical dengue areas reported higher seroprevalences in persons  $\leq 45$  years of age compared with persons in new dengue areas ( $P < 0.05$ ) (Figure 3B). This finding indicated that living in historical dengue areas predisposed one to having had dengue at a younger age. There were no gender differences in past exposure to dengue ( $P = 0.39$ ); seroprevalence was 64.8% (range = 63.7–65.9%) in males, and 67.0% (range = 66.0–68.1%) in females.

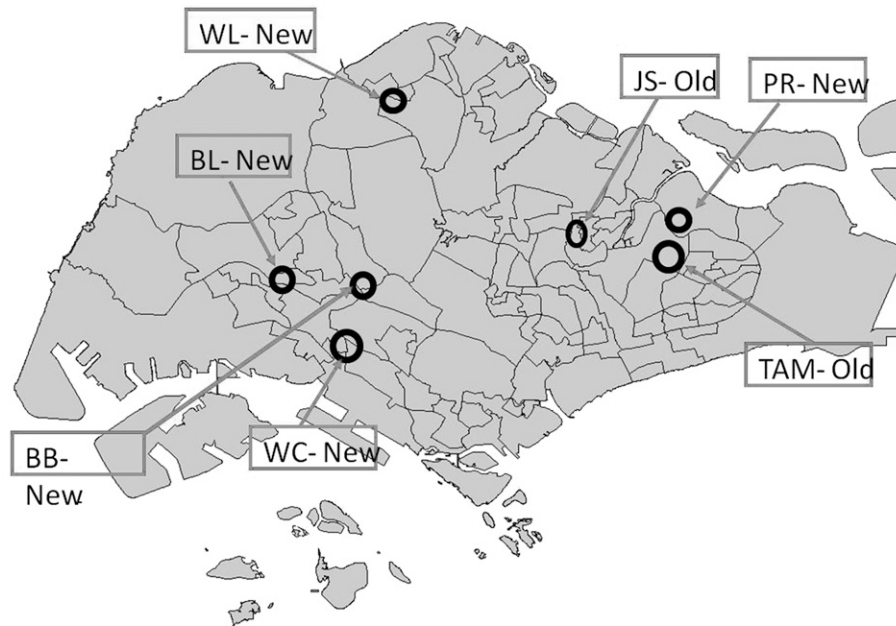
The presence of IgM against dengue indicates a recent infection. The dengue incidence rate was 6.8% (6,803.8 cases/100,000 population) in these outbreak areas during the three months prior to this study. Persons with no recollection of symptoms in the previous three months but who were positive for IgM against dengue were considered as having inapparent dengue. Among persons with recent infections, 78.0% had no recollection of any fever or dengue symptoms in the preceding three months. When stratified into age groups, the rate of inapparent dengue increased with age (Figure 4). Older ( $\geq 45$  years of age) adults had a significantly higher ( $P < 0.005$ ) rate of inapparent dengue (87.9%, range = 85.1–90.6%) than persons < 45 years of age (62.5%, range = 58.2–66.8%).

One person, who did not report any symptoms when a blood sample was obtained, was positive for dengue by RT-PCR (dengue virus serotype 2) and had a viral load of 100 plaque-forming units/mL. As all samples were anonymised before laboratory tests were performed, we could not establish if this person subsequently developed any dengue symptoms. Of persons with recent symptoms of dengue, only five (8.2%) consulted a physician, and only one person was given a diagnosis of dengue.

As our study was conducted in dengue outbreak areas, we report a seroprevalence rate of 65.9%, which was higher than those reported in recent local outbreaks (45–59%). Residents in historical dengue areas, having had more encounters with dengue compared with those in new areas, experienced a higher degree of past exposure. In addition, these persons are more likely to have had dengue at a younger age than persons in new dengue areas.

Our finding of a high rate (78%) of inapparent dengue is consistent with rates previously reported in Singapore,<sup>10,11</sup> and also reaffirmed findings in other countries, in which there are silent transmissions of dengue, resulting in large proportion of dengue infections being subclinical or inapparent.<sup>5–7</sup> The national incidence rate of dengue in Singapore in 2007 was 192.3 cases/100,000 population (Ministry of Health, Singapore).

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<u>Study site</u>	<u>Type of premise</u>	<u>No. of blocks</u>	<u>Approx no. of residents</u>	<u>No. of reported dengue cases</u>	<u>Approx dengue incidence (per 100,000)</u>
<b>Boon Lay (BL)</b>	High-rise public	8	3440	15	436.0
<b>Bukit Batok (BB)</b>	High-rise public	24	15360	89	579.4
<b>Jalan Songket (JS)</b>	Landed private	338	1690	29	1716.0
<b>Pasir Ris (PR)</b>	High-rise public	23	10580	75	708.9
<b>Tampines (TAM)</b>	High-rise public	15	6150	38	617.9
<b>West Coast (WC)</b>	High-rise public	14	5600	25	446.4
<b>Woodlands (WL)</b>	High-rise public	14	6720	61	907.7
<i>National incidence:</i>					<i>192.3</i>

FIGURE 1. Distribution of the seven study sites in Singapore, and estimated dengue incidences in each of the study sites. Because there were no census data available, calculations were based on number of units in each block of high-rise public housing, and the average household size based on data collected during the study.

We report the incidence rate in dengue outbreak areas as 6,803.8 cases/100,000 population, which is 35 times higher than the national average. The higher incidence rate maybe attributed to unreported cases, including those that are inapparent, and the higher incidences in dengue outbreak areas where the study was conducted.

Some studies have reported adults with dengue having more symptoms than children with dengue.<sup>1,12</sup> In contrast, our study demonstrated adults, especially older adults, are less symptomatic. Lye and others also reported no increased morbidity in older adults than in other age groups.<sup>13</sup> The pathogenicity of this phenomenon is not understood. As dengue is known to be an immunogenic disease, high rates of inapparent dengue could be caused by decreased immunologic responses in elderly persons. With advancing age,

cellular and humoral immune responses are modified, and such changes likely affect the general decreased immunoresponsiveness in the elderly.<sup>14</sup> This finding could also be caused by partial cross-protection conferred by previous multiple exposures to other dengue virus serotypes. It is interesting that although older adults are less symptomatic or have inapparent infections, this group is also more vulnerable to dengue mortality.<sup>15</sup> Co-morbidity has been shown to play a significant role in these fatal cases, and more studies are needed for this age group, particularly in Singapore.

Another interesting finding was the one person who was dengue positive by RT-PCR and had a viral load of 100 plaque-forming units/mL. We do not know if dengue transmission is sustainable with such a low viremia. Likewise, we do not know if persons with inapparent infections were capable of

Age (years)	% of participants in each study population						
	JS	BL	WC	TAM	PR	WL	BB
7-15	8.29	10.00	6.88	0.15	15.81	11.70	9.28
16-25	17.68	10.26	7.81	5.84	10.33	10.57	12.80
26-35	9.39	14.36	17.02	15.12	8.29	10.75	15.21
36-45	19.89	21.28	14.34	20.21	26.76	23.40	20.22
46-55	27.07	18.21	19.11	27.69	20.50	19.43	22.63
56-65	30.94	14.36	21.79	16.02	7.51	11.89	13.73
66-75	20.44	8.97	9.91	9.58	6.26	8.49	4.27
76-85	4.97	1.79	3.50	3.29	1.88	2.64	1.11
<b>Gender</b>							
Male	42.54	43.08	46.15	52.25	40.53	57.55	49.17
Female	57.46	54.36	53.61	49.10	59.31	42.45	50.83

FIGURE 2. Age and gender distribution of participants per study site, Singapore. Values are percentages. No significant difference in age and gender was observed between populations.

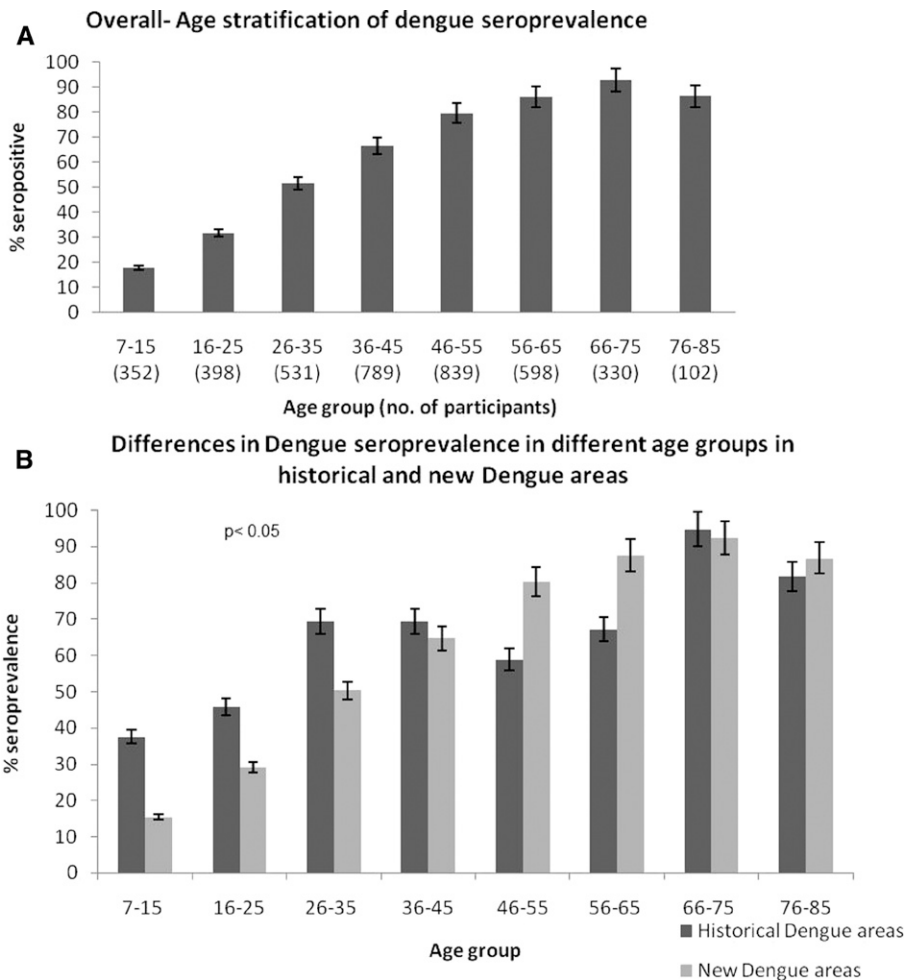


FIGURE 3. **A**, Overall dengue seroprevalence and age stratification in outbreak areas, Singapore. Past exposure to dengue increased with age. **B**, Differences in dengue seroprevalence in different age groups in historical and new dengue areas. Younger persons (< 45 years old) who lived in historical dengue areas had a significantly higher chance of being exposed compared with their counterparts in new dengue areas ( $P < 0.05$ ). Error bars indicate mean  $\pm$  SD.

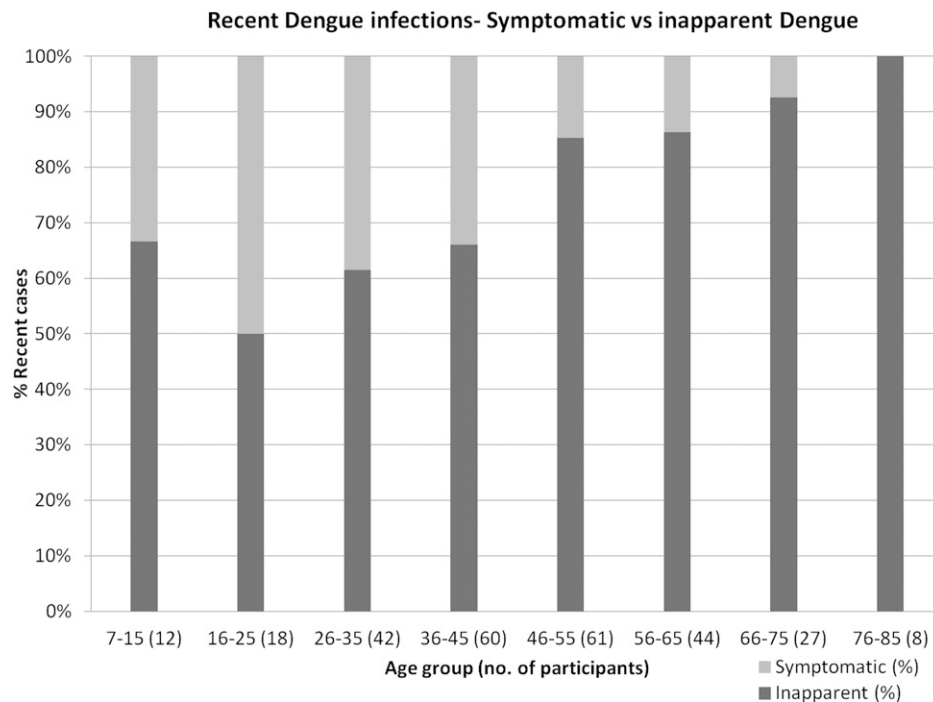


FIGURE 4. Dengue symptomatic and inapparent rates in different age groups, Singapore. Inapparent dengue rates increased with age.

maintaining dengue transmission. To explain the epidemiology of inapparent dengue infections, future studies are planned to ascertain the minimal viral load necessary for uptake of dengue virus by mosquitoes.

The health-seeking behavior and low dengue diagnostic rate demonstrated in this study, despite the presence of a convenient and accessible primary health care system in Singapore, serve to further corroborate the iceberg phenomena that most dengue infections are either asymptomatic or mild (undifferentiated fever).

There are three noteworthy limitations to this study. First, because this study was a cross-sectional study, selection bias cannot be ruled out. The participants in this study might have had a different serologic profile for dengue than persons who did not participate. The low response rate is also a concern and can bias the findings. Nevertheless, the generalizability of these research findings is limited. Second, there is a potential of recall bias influencing the research findings. As data were collected by using a questionnaire, some degree of subjectivity is inevitable. However, it is also worth noting that this is not a study to estimate the association between a risk factor and a health problem, in which case any selection and information bias may lead to erroneous conclusions. Third, because the duration of IgM against dengue can range from three to nine months, our prevalence of inapparent infections may be an overestimate because our questionnaire asked about symptoms restricted only to the preceding three months. Nevertheless, because we conducted our study in areas that were having dengue outbreaks and, by inference, because there were no dengue cases in these areas before the outbreaks, IgM against dengue infections more than three months ago should not be present.

Received March 7, 2012. Accepted for publication August 22, 2012.

Published online April 22, 2013.

Note: Supplemental questionnaire appears at [www.ajtmh.org](http://www.ajtmh.org).

Acknowledgments: We thank Lim Hwee Hua, Amy Khor, Ngeow Pack Hua, Arthur Fong, Ong Kian Min, and Teo Chee Hean for assistance during the study; the staff and students of the Environmental Health Institute for providing field and laboratory assistance; Professor Chia Kee Seng for providing technical advice; and the volunteers for participating in the study.

Financial support: This study was supported by the National Environment Agency, Singapore.

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