# Force of Infection and True Infection Rate of Dengue in Singapore: Implications for Dengue Control and Management

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## WEB APPENDIX 1

### Summary of Seroprevalence Studies in 2004 and 2009

To develop the dengue FOI model of Singapore's dengue transmission, age-specific dengue seroprevalence data independently collected in 2004 and 2009 were integrated with the 2013 and 2017 serological surveys to estimate the historical dengue FOI. Data from the 2004 and 2009 studies were previously reported (1-3).

Briefly, Yew and others (1) determined the presence of dengue IgG in stored blood samples that were collected between September and December 2004 during the National Health Survey (NHS). The study comprised a total of 4152 NHS participants aged 18 years to 74 years who consented to having their residual sera used for further research. The presence of dengue IgG was measured with Panbio dengue IgG Indirect ELISA as per the manufacturer's instruction. Of the study population of adults aged 18–74 years, 59% (2449/4152) tested positive for dengue IgG, with increasing IgG prevalence with age. The lowest age-specific seropositivty of 17.2% was found in the 18–24 year age group and the highest seroprevalence of 88.9% was found in the 55–74 year age group.

To determine the magnitude of dengue infections in the adult population following the 2007 dengue outbreak in Singapore, Low and others (2) reported a dengue serosurvey conducted on residual sera from healthy blood donors in Singapore in 2009. These individuals are not a random sample from the population and as such the serosurvey may not be representative. The presence of dengue IgG was measured with Panbio dengue IgG Indirect ELISA as per the manufacturer's instructions. The weighted

dengue seroprevalence was 50.8% among Singapore residents aged 16–60years, with increasing IgG prevalence with age. Residents in the 16–20 and 21–25 year age groups (16.1% each) presented the lowest IgG prevalence, and residents in 56–60 year age group had the highest IgG prevalence of 86.6%.

The national pediatric seroprevalence survey (NPSS) involved the prospective collection of residual sera in two public acute-care hospitals, KK Women's and Children's Hospital and the National University Hospital from 2008–2010 (which for simplicity we take to be 2009) (3). A total of 1,200 serum samples were collected, comprising 400 in each of the three age groups of 1–6 years (pre-school), 7–12 years (primary school), and 13–17 years (secondary school). The age–ethnic distribution of these subjects by gender was comparable to that of the Singapore resident population aged 1–17 years in 2009. The selection of residual sera was confined to Singapore residents of Chinese, Malay and Indian ethnicity, i.e. the three largest ethnic groups in Singapore, aged 1–17 years attending inpatient services or day surgery at the two hospitals. Sera of patients known to be immunocompromised, on immunosuppressive therapy, or who had been diagnosed with infectious diseases such as dengue were excluded. The sera were tested for IgG antibodies against DENV by ELISA using commercial test kits (EUROIMMUN, Germany) according to the manufacturer's recommended procedure. The overall prevalence of past DENV infection was 10.4% (95%CI: 8.7–12.1%) among children and adolescents aged 1–17 years. The dengue seroprevalence was 11.0% in children aged 1–6 years, 10.0% in those aged 7–12 years, and 10.3% in adolescents aged 13–17 years.

# WEB APPENDIX 2

## Estimation of $R_0$

We used a formula to estimate $R_0$ that makes the following assumptions:

- Dengue in Singapore is a closed system, in the sense that no residents are infected outside the country and none infect anyone else outside the country (more precisely, we assume the numbers of residents infecting foreigners is equal to the number of foreigners infecting residents).
- Each infection is due to a single mosquito. Given the low incidence, the chance of two simultaneous infection events should be vanishingly small.
- Dengue is endemic. There has not been a week since 2000 in which no cases of dengue were notified, so this assumption is clearly met.
- Mixing is homogeneous and thus infections are non-assortative.
- Infection by each serotype is independent conditional on age and the serotypes have approximately the same prevalence.

Under these assumptions, each case is infected by exactly one other case in the population (via an intermediate vector), so that if the number of cases is $X$, the number of infection events *to* those cases is also $X$, and the number of infection events *from* those cases is also $X$. Therefore the average number of cases per case, i.e. the effective reproduction number, is 1 averaged over time. (On a week to week basis, the effective reproduction number may fluctuate, but over a time scale of a year, the edge effects at the beginning and end of the time period disappear and we can assume the effective reproduction number in year $y$ to be $R_e(y) = 1$.)

The average case challenges $R_0(y)$ other cases (the basic reproduction number, in the absence of any herd immunity, in year $y$) over its infectious period. Of these, a proportion is lost due to immunity of the recipient of infection. This proportion is related to the age-weighted prevalence of that dengue virus, denoted $P_s(y)$ for year $y$ (suppressing age as an argument for simplicity). Specifically the proportion of challenges lost is $1 - P_s(y)$. To obtain an effective reproduction number of $R_e(y)$ thus requires

$$R_0(y)[1 - P_s(y)] = R_e(y)$$

which by assumption is unity, so

$$R_0(y) = {}^{1}/_{[1 - P_s(y)]}.$$

To obtain the serotype-level prevalence at a given age $a$ (temporarily suppressing time now but reinstating age as an argument) from the overall seroprevalence at the same age, we make two further assumptions, namely that infection status by each serotype is independent conditional on age, so we can write

$$1 - P(a) = \prod_{s=1}^{4} 1 - P_s(a)$$

We further assume that the serotype-specific prevalence is approximately the same for each serotype, so that

$$1 - P(a) \approx \left(1 - P_s(a)\right)^4$$

for any serotype $s$. The serotype specific prevalence is thus approximately

$$P_s(a) \approx 1 - \left(1 - P(a)\right)^{1/4}.$$

The overall serotype specific prevalence at time $y$ can then be obtained by weighting by the number of residents in that age group at that time[1], $N(a, y)$:

$$P_s(y) = \frac{\sum_a P_s(a, y) N(a, y)}{\sum_a N(a, y)}.$$

This is then used to obtain the estimated basic reproduction number for that year.

95% credible intervals are derived for the basic reproduction number by applying the above relationship to each draw from the posterior distribution, thus obtaining a sample from the derived posterior distribution of $P_s(y)$, and taking the 2.5th and 97.5th percentiles, with the point estimate being the posterior median.

It is worth noting the non-exchangeability of the steps above. In particular, the serotype-specific prevalence cannot be obtained after performing the age-weighting of the overall seroprevalence, as these two operations do not commute.

**Sensitivity analysis for the $R_0$ estimates**

We tested the robustness of the $R_0$ estimates with respect to the percentage of annual resident cases being imported from abroad (Source: Ministry of Health, Communicable Diseases Division). Let the numbers of laboratory-confirmed indigenous and imported

[1] Note that demographic data were available to different resolution than the prevalence estimates. In particular age was commonly reported to the level of 5-year age intervals. To accommodate these, we assumed population counts were roughly evenly distributed within each 5-year age interval.

resident cases in year $t$ be denoted as $C_t^{indigenous}$ and $C_t^{imported}$ respectively, and the FOI estimates were adjusted using the equation below:

$$\widehat{\lambda_t^{new}} = \widehat{\lambda_t^{old}} \cdot \frac{C_t^{indigenous}}{C_t^{indigenous} + C_t^{imported}}.$$

Since the percentage of annual laboratory-confirmed resident cases being imported from abroad were only available for 2003~2017, we performed a simulation to assign values to the years before 2003, using a normal distribution $N(1.6\%, 0.5\%^2)$. The mean of the normal distribution, 1.6%, was determined based on the overall percentage of resident cases being imported from abroad during 2003~2017, and we also tested other scenarios where the mean was specified to be 3% and 5% respectively. All the random draws were performed independently, and the resulting adjusted $R_0$ estimates were shown in Web Figure 2.

**Estimation of age-weighted prevalence**

To obtain the overall prevalence for a given age $a$ and year $y$ combination from the force of infection $\lambda_t$ for year $t$ (this was used for any given draw from the posterior distribution of the forces of infection), we calculated $P(a, y) = 1 - \exp(-\sum_{t=y-a+1}^{y} \lambda_t)$. This implicitly assumes that all seronegative individuals in the population at time $t$ were exposed to the same risk. To obtain prevalence estimates for older age groups at earlier time periods (for which there was no information on force of infection in the four sero-surveys, as these individuals had mostly died before the studies), we assumed the earliest estimated force of infection applied to these earlier epochs.

To obtain age-weighted overall seroprevalence estimates for a given calendar year, we calculated:

$$P(y) = \frac{\sum_a P(a,y)N(a,y)}{\sum_a N(a,y)}$$

where, as before, $N(a,y)$ is the number of residents aged $a$ in year $y$.

**Goodness-of-fit test**

To evaluate the goodness of fit of the model, and in particular of the assumed smoothness of the seroprevalence curve, we separately assessed it against the four seroprevalence studies (in 2004, 2009, 2013, and 2017). For each seroprevalence study, for each age (in 1-year increments) that was represented in the survey, we performed an independent binomial test using the estimated seroprevalence as the null value of the parameter. Ages which differed significantly (at $\alpha = 0.05$) were tabulated. The expected proportion of 'significant' results if the estimated prevalences truly generated the data is 5% at this level, and the overall proportion was compared qualitatively to that. Because pediatric data were available only for 2009, and there was evidence of a cluster of significant results in the youngest age group for this sample (with more infections than expected), we repeated the calculations excluding those aged less than 5.

**Web Table 1.** Dengue IgG prevalence among residents in 2013 (*n* = 3813)

| Characteristic | No. Positive/ No. Tested | Percentage | Weighted Prevalence (%) | Prevalence Ratio (95% CI) | *P* Value |
|---|---|---|---|---|---|
| Overall | 1874/3813 | 49.1 | 49.8 | - | - |
| Gender | | | | | |
| Female | 839/1808 | 46.4 | 48.6 | 1 (Referent) | NA |
| Male | 1035/2005 | 51.6 | 51.1 | 1.11 (1.04,1.19) | 0.0014* |
| Age group† | | | | | |
| 16 - 20 | 57/373 | 15.3 | 15.3 | 1 (NA) | NA |
| 21 - 25 | 60/338 | 17.8 | 17.6 | 1.16 (0.83,1.62) | 0.3754 |
| 26 - 30 | 77/279 | 27.6 | 27.6 | 1.55 (1.15,2.09) | 0.0037* |
| 31 - 35 | 124/345 | 35.9 | 35.7 | 1.30 (1.03,1.65) | 0.0286* |
| 36 - 40 | 160/421 | 38.0 | 38.2 | 1.06 (0.88,1.27) | 0.5573 |
| 41 - 45 | 218/431 | 50.6 | 50.5 | 1.33 (1.14,1.55) | 0.0003* |
| 46 - 50 | 287/482 | 59.5 | 59.6 | 1.18 (1.05,1.33) | 0.0071* |
| 51 - 55 | 344/481 | 71.5 | 71.5 | 1.20 (1.09,1.32) | 0.0001* |
| 56 - 60 | 332/414 | 80.2 | 80.1 | 1.12 (1.04,1.21) | 0.0024* |
| > 60 | 215/249 | 86.3 | 87.9 | 1.08 (1.01,1.15) | 0.0352* |

CI = confidence interval; NA = not applicable.

* Significant if *P* value is <0.05.

† The preceding age group was used as the referent for calculation of the prevalence ratio.

**Web Table 2.** Dengue IgG prevalence among residents in 2017 (*n* = 4002)

| Characteristic | No. Positive/ No. Tested | Percentage | Weighted Prevalence (%) | Prevalence Ratio (95% CI) | *P* Value |
|---|---|---|---|---|---|
| Overall | 1828/4002 | 45.7 | 48.6 | - | - |
| Gender | | | | | |
| Female | 835/1964 | 42.5 | | 1 (Referent) | NA |
| Male | 993/2038 | 48.7 | 50.6 | 1.29 (1.13, 1.46) | <0.001 |
| Age group[†] | | | | | |
| 16 - 20 | 46/334 | 13.8 | 13.8 | 1 (NA) | NA |
| 21 - 25 | 63/365 | 17.3 | 17.3 | 1.31 (0.85, 2.48) | 0.212 |
| 26 - 30 | 97/389 | 24.9 | 24.9 | 1.59 (1.10, 2.31) | 0.012* |
| 31 - 35 | 109/396 | 27.5 | 27.5 | 1.14 (0.82, 1.59) | 0.418 |
| 36 - 40 | 150/421 | 35.6 | 35.6 | 1.46 (1.07, 1.98) | 0.013* |
| 41 - 45 | 187/436 | 42.9 | 42.9 | 1.36 (1.02, 1.81) | 0.030* |
| 46 - 50 | 232/418 | 55.5 | 55.5 | 1.66 (1.26, 2.20) | <0.001* |
| 51 - 55 | 287/439 | 65.4 | 65.4 | 1.51 (1.14, 2.01) | 0.003* |
| 56 - 60 | 324/417 | 77.7 | 77.7 | 1.85 (1.35, 2.53) | <0.001* |
| > 60 | 333/387 | 86.0 | 85.0 | 1.77 (1.21, 2.61) | 0.002* |

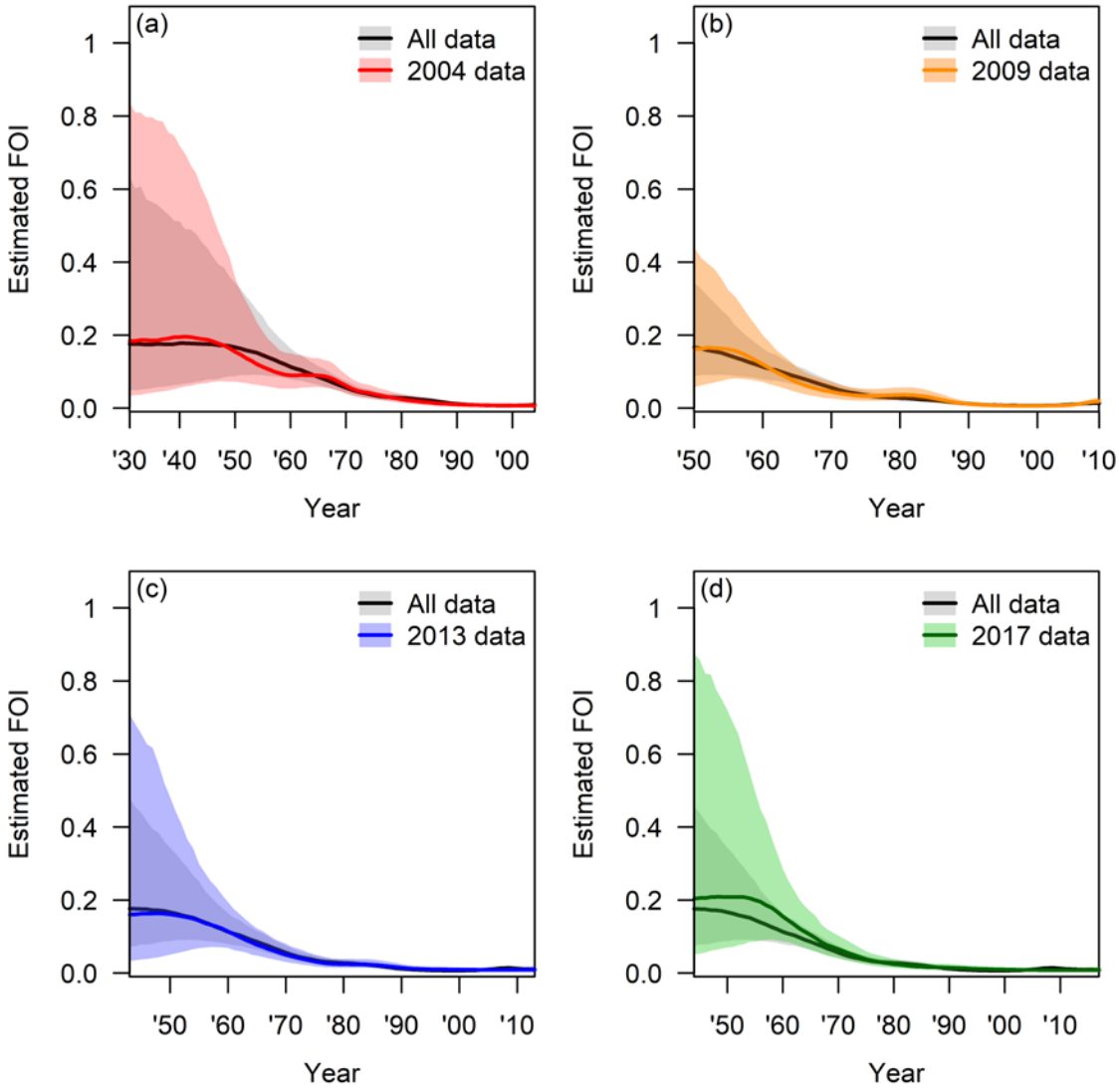
CI = confidence interval; NA = not applicable.

* Significant if *P* value is <0.05.

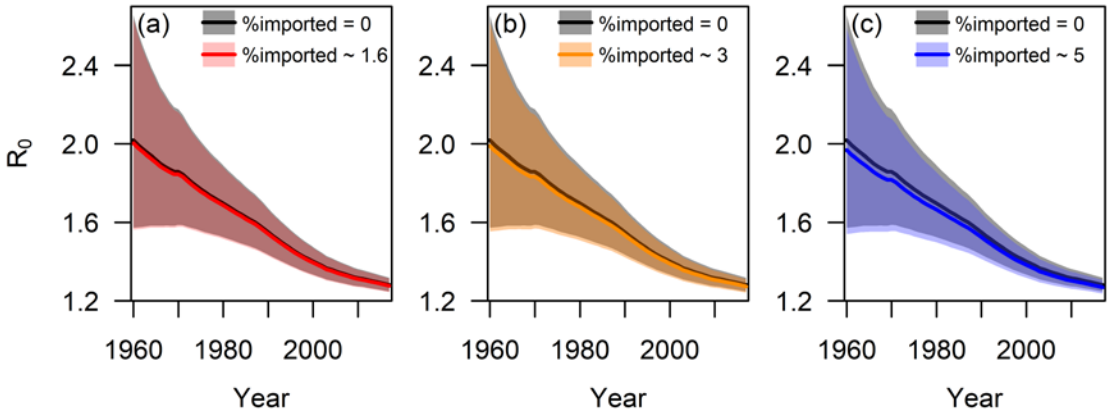
[†] The preceding age group was used as the referent for calculation of the prevalence ratio.

**Web Table 3.** Comparison of FOI and $R_0$ estimates with previous studies in Singapore

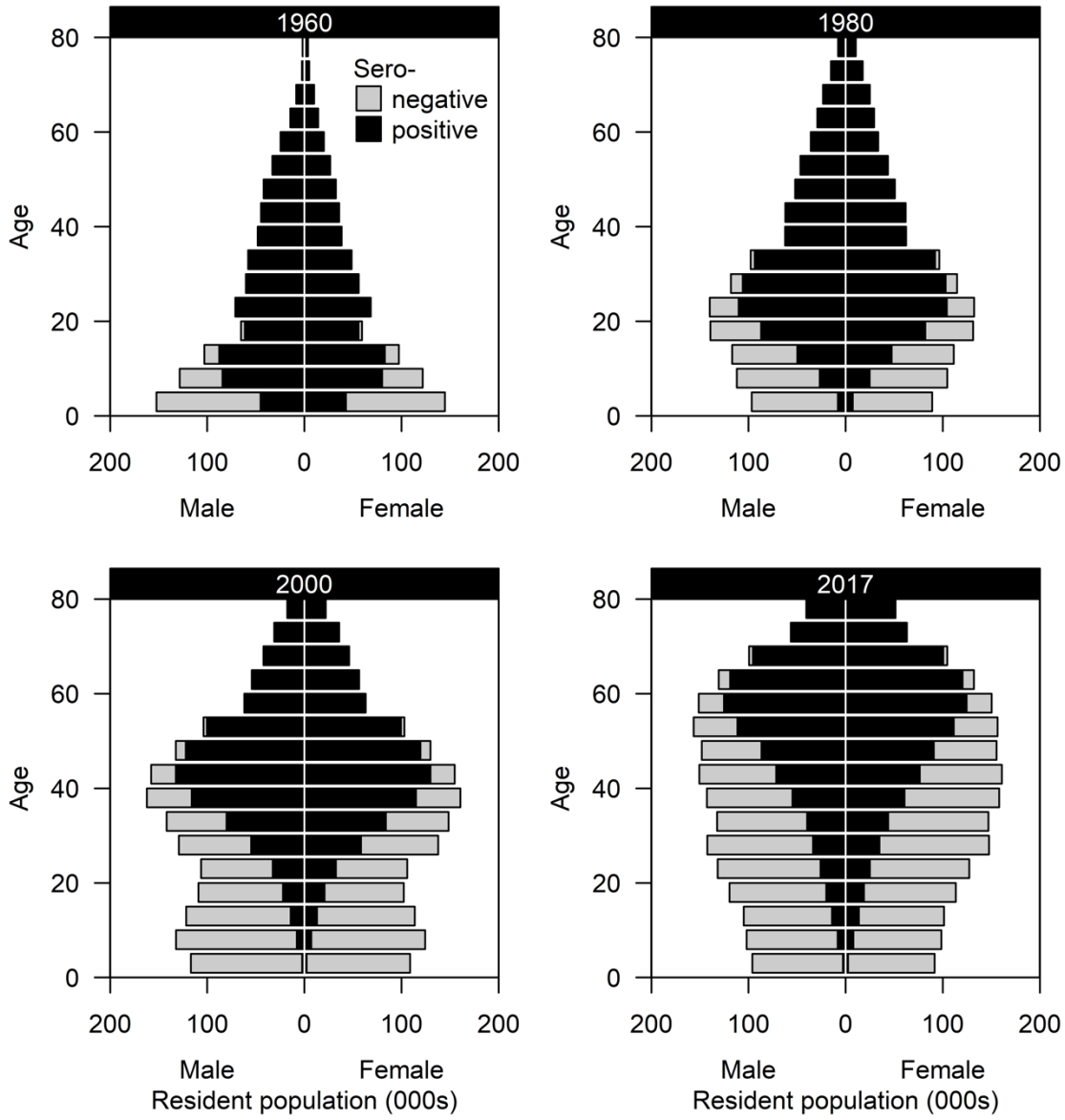
| Author | Data Set | FOI and $R_0$ Estimates | FOI and $R_0$ Estimates From Our Study | Reasons for Discrepancies in Analysis |
|---|---|---|---|---|
| Egger et al. (4) | Five serosurveys conducted between 1984 and 2002 | Similar temporal trend but peak of FOI of about 0.1 to >0.2 | <u>FOI estimates</u><br>0.023 in 1984<br>0.009 in 2004<br>0.013 in 2007<br><br><u>$R_0$ estimates</u><br>1.36 in 2004 | • Different data set was used.<br>• Different methodology in FOI estimation |
| Imai et al. (5) | Serosurveys conducted in 1984 (6), 2004 (1) and 2007 (7) | <u>FOI estimates</u><br>2 to 3-folds higher than data from our study<br><br>0.055 to 0.089 in 1984<br>0.023 to 0.040 in 2004<br>0.027 to 0.051 in 2007<br><br><u>$R_0$ estimates</u><br>Comparable to our study<br><br>1.21 to 1.33 in 2004 | | • Data from these serosurveys were excluded for the following reasons.<br><br>1984 – Different detection assay was used.<br><br>2007 – Conducted in active dengue clusters that may not represent national situation.<br><br>• Age-specific prevalence data from the 2004 serosurvey was used in our study whereas Imai *et al* used the same prevalence across age bands. |



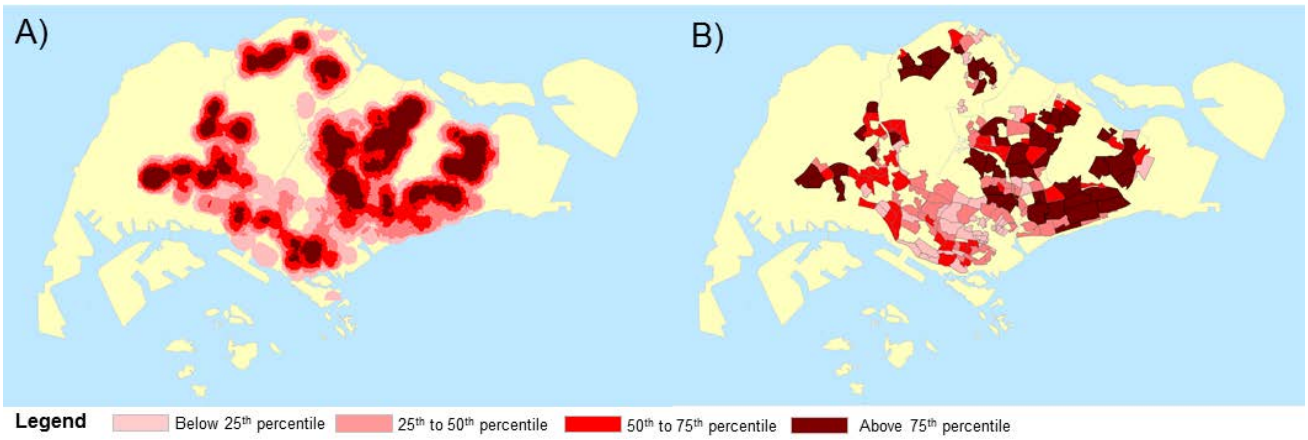

**Web Figure 1. FOI estimates derived using individual and overall data sets, with 95% BCI.** BCI = Bayesian Credible Interval. Similar trends in FOI estimates were observed in models generated using individual data sets of 2004, 2009, 2013 and 2017.




**Web Figure 2. Sensitivity analysis for the $R_0$ estimates.** For each year between 2003 and 2017, the percentage of resident cases being imported from abroad (hereinafter referred to as % imported) were computed using laboratory-confirmed case data from Singapore Ministry of Health. For each year earlier than 2003, %imported was unavailable, and drawn from a normal distribution with mean 1.6% (based on 2003~2017 average) and standard deviation 0.5%. We also tested two more scenarios, where the mean was increased to 3% and 5% respectively. The resulting $R_0$ estimates under each scenario were compared with the original estimates (%imported = 0).




**Web Figure 3. Demographic structure and dengue seroprevalence in the Singapore resident population in 1960, 1980, 2000 and 2017.** Graphical representation of the population structure and modelled seroprevalence by age groups.




**Web Figure 4. Density maps to represent the geographical distribution of (A) 2013 serosurvey samples and (B) overall residents in 2013.** Overall, the spatial distribution of 2017 serosurvey samples did not differ greatly from 2013. Twenty-two out of 26 postal districts showed no significant difference (Adjusted *P* value: > 0.001923).

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