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3	Authors: Henrik Salje ^{1,2,3,4} , Derek A. T. Cummings ^{4,5,6} , Isabel Rodriguez-Barraquer ⁷ ,	
4	Leah C. Katzelnick ⁵ , Justin Lessler ⁴ , Chonticha Klungthong ⁸ , Butsaya Thaisomboonsuk ⁸ ,	
5	Ananda Nisalak ⁸ , Alden Weg ⁸ , Damon Ellison ⁸ , Louis Macareo ⁸ , In-Kyu Yoon ⁹ , Richard	
6	Jarman ¹⁰ , Stephen Thomas ¹¹ , Alan L. Rothman ¹² , Timothy Endy ^{11,*} , Simon	
7	Cauchemez ^{1,2,3,} *	
8	¹ Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Paris, France	
9	² CNRS UMR2000: Génomique évolutive, modélisation et santé (GEMS), Institut	
10	Pasteur, Paris, France	
11	³ Center of Bioinformatics, Biostatistics and Integrative Biology, Institut Pasteur, Paris	
12	75015, France	
13	⁴ Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health,	
14	Baltimore, USA	
15	⁵ Department of Biology, University of Florida, USA	
16	⁶ Emerging Pathogens Institute, University of Florida, USA	
17	⁷ University of California, San Francisco, San Francisco, California, USA	
18	⁸ Department of Virology, Armed Forces Research Institute of Medical Sciences,	
19	Bangkok, Thailand	
20	⁹ International Vaccine Institute, Seoul, South Korea	

Reconstruction of antibody dynamics and infection histories to evaluate dengue risk

21 ¹⁰ Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, USA

- ¹¹ Department of Medicine, Upstate Medical University of New York, Syracuse, New
- 23 York, USA
- ¹² Institute for Immunology and Informatics, Department of Cell and Molecular Biology,
- 25 University of Rhode Island, Providence, RI USA
- 26 * Joint senior authors
- 27

28	As with many pathogens, most dengue infections are subclinical and therefore
29	unobserved. Coupled with limited understanding of the dynamical behavior of potential
30	serological markers of infection, this observational problem has wide-ranging
31	implications, including hampering our understanding of individual- and population-level
32	correlates of infection and disease risk and how they change over time, assay
33	interpretation and cohort design. We develop a framework that simultaneously
34	characterizes antibody dynamics and identifies subclinical infections via Bayesian
35	augmentation from detailed cohort data (3,451 individuals with blood draws every 91
36	days, 143,548 hemagglutination inhibition assay titer measurements). We identify 1,149
37	infections (95% CI: 1,135-1,163) that were not detected by active surveillance and
38	estimate that 65% of infections are subclinical. Post infection, individuals develop a
39	stable setpoint antibody load after 1y that places them within or outside a risk window.
40	Individuals with pre-existing titers of \leq 1:40 develop hemorrhagic fever 7.4 (95% CI: 2.5-
41	8.2) times as often as naïve individuals compared to 0.0 times for individuals with titers
42	>1:40 (95% CI: 0.0-1.3). PRNT titers \leq 1:100 were similarly associated with severe
43	disease. Across the population, variability in the force of infection results in large-scale
44	temporal changes in infection and disease risk that correlate poorly with age.

46 Despite the large body of literature from observational and cohort studies describing 47 dengue cases, we still have major difficulties in explaining individual- and population-48 level differences in infection and disease risk. These difficulties largely come from a 49 fundamental methodological issue in the research of many pathogens that individual 50 histories of infection are difficult to capture. The four dengue virus serotypes (DENV1-51 4), which are found across tropical and sub-tropical regions with an estimated 390 million 52 infections each year, cause a range of disease manifestations, from asymptomatic infection to death^{1,2}. High levels of subclinical infection mean that even in environments 53 of thorough active surveillance, the majority of infections are missed³. This observational 54 55 problem has wide ranging implications as it hampers our ability to estimate the 56 underlying level of infection in the community, to characterize individual risk factors for 57 infection and severity but also to assess correlates of protection, to dynamically monitor 58 susceptibility at both the population and individual level, to define optimal thresholds for 59 the interpretation of serological assays or to critically assess cohort design.

60

61 Here, we develop an analytical framework that can address this challenge, leading to new 62 insights on a broad range of questions. We use it to jointly characterize antibody changes 63 following infection and identify infection events missed by surveillance from the analysis 64 of longitudinal data from cohort studies. We apply it to data from a school-based cohort 65 study in Thailand (N=3,451, mean age at recruitment of 9y, interquartile range 8-11) 66 where blood was taken on average every 91 days for up to five years and when illnesses were detected through active surveillance⁴. Active fever and school absence surveillance 67 68 was conducted during June to mid-November when DENV circulation is concentrated⁴.

Hemagglutination inhibition (HI) tests were used to measure antibody titers to each
serotype in each sample (143,548 HI measurements in all). PRNT titers were also
measured on a subset of 1,771 samples. HI titers correlate closely with PRNTs (Pearson
correlation of 0.91) and with inhibition ELISAs, although titer values differ by laboratory
and assay⁵⁻⁸.

74

75 To track the evolution of an individual's measured antibody titers (Figure 1A), we place 76 titers on an adjusted \log_2 scale (titers of 1:10 are given a value of 1, 1:20 of value of 2 77 etc.). There were 274 detected symptomatic DENV infections (Figure 1B); 62 were 78 hospitalized (23%), 36 with dengue hemorrhagic fever (DHF) (13%). For those where the 79 infecting serotype is known (79% of cases through PCR, Table S1), we observe a sharp 80 rise and subsequent decay in log₂-titers following symptom onset (Figure 1C-D). The 81 mean log₂-titer to the infecting serotype was 0.79 (95% CI: 0.74-0.84) times the log₂-titer 82 to the non-infecting serotype in the three months prior to symptom onset compared to 83 0.94 (95% CI: 0.93-0.96) times in the six months after symptom onset (Figure 1E). As 84 86% of symptomatic infections had detectable titers to at least one serotype prior to 85 infection, the higher antibody titer to non-infecting serotypes likely captures responses to prior infections⁹. 86

87

We reconstruct individuals' antibody trajectories by assuming that infection leads to a
rise in titers that subsequently decays exponentially¹⁰. We also explore biphasic responses
(Figure S1). We allow for variability in antibody kinetics across individuals and
infections, and for differential rises for the infecting versus the non-infecting serotypes

92 for primary infections but undifferentiated responses for subsequent infections. We use 93 data augmentation techniques to impute undetected infections (subclinical infections 94 during active surveillance or unknown symptom status outside the surveillance windows) and to identify the serotype for undetected primary infections¹¹. Instead of relying on 95 96 fixed cutoffs to identify infections, data augmentation allows us to incorporate 97 uncertainty in the existence, timing and serotype of unobserved infection events and 98 therefore probabilistically assess whether differences in measured titers are due to 99 infections or assay variability.

100

101 We find that following post-primary infection there is a mean 5.8 (95% CI: 5.6-5.9) rise 102 in log₂-titers across serotypes, which declines by 76% after one year. For primary 103 infections (i.e., individuals without detectable titers prior to infection) the mean log₂-titer 104 rise is 7.6 (95% CI: 7.4-7.8) for the infecting serotype and 6.6 for the non-infecting 105 serotypes (95% CI: 6.4-6.7). The similarity of titers of infecting and non-infecting 106 serotypes coupled with assay variability suggests that in a clinical setting individual HI 107 measurements cannot reliably determine the infecting serotype. We find that titers largely 108 stabilize one year after infection to a set-point (the 'set-point antibody load') (Figure 1D). 109 There is significant variability between infections: the interquartile range of the \log_2 -titer 110 rise one year after infection is 0.7-2.2 across all infections (Figure S2A). We find that 111 even after accounting for historic infection status, measured DENV-2 titers are 112 systematically lower than other serotypes (0.85 lower than DENV1) (Figure S2B, Table 113 S2), which could point to technical considerations of the DENV2 assay or inherent 114 differences in immune responses to DENV2. We estimate the measurement error in the

HI assay (i.e., the standard deviation in any reading) as 0.49 (95% CI: 0.49-0.50), which
is similar with that empirically estimated using repeated testing on the same serum and
2.6 times error estimates for the plaque reduction neutralization test (PRNT) (Figure
S2C)¹². Despite the variability in individual readings, as we use many readings from four
serotypes for each participant and titers appear to behave in a stable and predictable
manner, we can nevertheless make robust inferences when considering the ensemble of
the measurements.

122

123 We probabilistically identify 1,149 undetected infections (95% range across model 124 iterations: 1,135-1,163), of which 507 (494-520) occurred during active surveillance 125 periods and were therefore subclinical (Figure 1B). Overall, we estimate 35% of 126 infections are symptomatic (95% CI: 34-36). The temporal distribution of subclinical 127 infections was correlated with that of symptomatic infections (Pearson correlation 0.78, 128 95% CI: 0.70-0.84). Using augmented primary infections where we could confidently 129 assign the infecting serotype (same serotype implicated by >50% of iterations), we find 130 that 34% of undetected primary infections (and 39% of subclinicial primary infections) 131 were due to DENV-4, compared to only 3% of all symptomatic infections (none of which 132 were primary infections) (Figures S3-S4). We find consistent results using a more 133 stringent cutoff to assign the infecting serotype (Figure S5). These findings are consistent 134 with a reduced risk of disease from DENV-4 compared to other serotypes resulting in a 135 largely silent DENV-4 epidemic. This is supported by a phylogenetic analysis that found DENV-4 was widespread in Thailand throughout this period (Figure S4 in *Salje et al.*,¹³). 136 137 This suggests the serotype distributions from hospital-based or community-based

surveillance may not be representative of infections in the population and supports previous evidence that the transmissibility of a serotype can be delinked from the propensity to cause symptomatic and/or severe disease^{14,15}. Further they imply that factors that contribute to transmission potential (e.g., viral replication, peak titers or infection length) are not predictive of adverse outcomes¹⁶.

143

144 We find that the underlying probability of infection and the probability of developing 145 disease are strongly linked to the mean antibody titer at the time of exposure. Overall, an 146 individual's annual risk of infection was 17%, varying from 21% for individuals with 147 mean measured \log_2 -titers <2, to 16% for those with \log_2 -titers of 2-3 and 11% for those 148 with \log_2 -titers of >3 (Figure 2A). Using logistic regression, we find that for \log_2 -titers 149 >2, each unit increase in \log_2 -titers is associated with a 0.71 times relative risk of 150 infection (95% CI: 0.67-0.76). The annual probability of having a symptomatic infection 151 varies from 6.4% (95% CI: 4.9-8.4) for primary infections to 8.4% (95% CI: 7.8-9.1) for 152 individuals with pre-existing \log_2 -titers $\leq 3 (\leq 1:40 \text{ on a linear scale})$ and 4.0% (95% CI: 153 3.0-5.0) for those with \log_2 -titers >3 (Figure 2B). The annual probability of being 154 hospitalized during a primary infection was 1.2% (95% CI: 0.5-2.1), compared to 2.4% 155 (95% CI: 2.1-2.7) during a subsequent infection for those with pre-existing \log_2 -titers ≤ 3 156 and 0.3% for those with \log_2 -titers >3 (95% CI: 0.09-0.6) (Figure 2C). Even more stark 157 was the risk for developing DHF, which ranged from 0.2% (95% CI: 0.0-0.6) for primary 158 infections compared to 1.5% (95% CI: 1.3-1.7) for subsequent infections in those with 159 \log_2 -titers ≤ 3 and 0.0% for \log_2 -titers > 3 (95% CI: 0.0-0.4) (Figure 2D). Within this study 160 population, an average of 54% of the population had detectable \log_2 -titers of ≤ 3 at any

161 time. Time-varying cox proportional hazards models that specifically account for the

162 dependence of titer observations within individuals gave similar results (Figure S6)¹⁷.

163 Using log2-titers to probabilistically identify the cohort participants with detectable titers

164 that will develop DHF has an AUC of 0.66 (Figure S7).

165

166 Considering only infected individuals, we observe no difference in the probability of 167 subclinical infection by titer; however, the probability of hospitalization and DHF 168 remains greatest in those with pre-existing \log_2 -titers of ≤ 3 (Figure S8). Only one 169 individual with pre-infection log₂-titers >3 developed DHF during surveillance compared 170 to 146 who did not but had titers at infection within the same range. This suggests that in 171 the event that infection takes place, antibodies are not protective of developing symptoms 172 per se but, conversely, are associated with the development of severe disease. We observe 173 no difference in the risk of disease given infection across years (Table S3) or age (Table 174 S4). Other studies are needed to see if younger age groups than those included here 175 nevertheless have increased risk. PRNTs form the basis of current discussions on immune 176 correlates. Among those infected, individuals with detectable PRNT \log_2 -titers of ≤ 4.5 177 (equivalent to approximately $\leq 1:100$) have 7.5 times (95%CI: 2.4-11.6) increased risk of 178 DHF compared to previously naïve individuals, compared to 0.0 times for those with 179 higher titers (Figure S9). Cross-reactive titers that result from exposure to non-DENV 180 flaviviruses such as Japanese encephalitis and Zika may be included in these risk 181 estimates.

182

183	Our findings suggest that post-infection set-point antibody loads appear important to
184	determining individual infection and disease risk. Post infection, we estimate the daily
185	probability of a subsequent infection and the development of DHF disease as a function
186	of titer dynamics. We demonstrate that the probability of both infection and disease
187	stabilizes after 1y (Figure 3). Based on our observation in Figure 2 that individuals with
188	detectable titers of \leq 3 had increased risk of infection and disease, we explored the
189	temporal evolution of risk following infection for those with setpoint antibody loads (i.e.,
190	the titer at 1y following infection) above and below this threshold. At 1 year, we observe
191	a 2.1 times increased risk of infection (irrespective of disease outcome) for those with
192	setpoint antibody loads of ≤ 3 compared to those with greater antibody loads and an 8.9
193	times increased risk of infection that leads to DHF. Overall, we find that three years
194	following infection 34% of individuals with setpoint antibody loads of \leq 3 suffer a
195	subsequent infection, irrespective of severity (95% CI: 33%-35%) compared to 23% for
196	those with greater loads (95%CI: 20%-26%). After this delay 3.5% of individuals with
197	setpoint loads of \leq 3 develop DHF disease (2.4%-4.4%) compared to none in those with
198	higher loads. The apparent stability of setpoint antibody loads points to an ability to
199	assess an individual's long-term risk.

Our findings are consistent with low titers generated by some candidate vaccines in
previously naïve individuals 'priming' individuals for severe disease upon their first
exposure¹⁸. A hypothesis supported by previous evidence that primary infections in
infants with maternal antibodies and secondary infections in older individuals are
associated with severe disease^{19,20}. Further, a Nicaraguan study found elevated risk of

severe disease for those with low iELISA titers at prior annual blood draws⁸. Previously 206 207 naïve individuals given the Dengvaxia vaccine had mean PRNT titers within our risk window (Figure 4D)²¹. Further work is required to understand whether immunity 208 209 acquired from vaccination and natural infection are qualitatively similar and whether the 210 risk window described here is relevant for vaccine recipients. T-cell immunity, which is 211 not captured by these assays, might compensate for antibody titers in this window. 212 Vaccine studies should carefully assess the criteria used to define seroconversion, and 213 how titers correlate with disease risk over time. Our work suggests that previously used 214 criteria (PRNT titer >1:10) do not adequately correlate with reduction in disease risk and 215 suggest that HI titers >1:40 or PRNT titers of >1:100 may provide a starting point for any 216 vaccine in identifying a targeted neutralizing antibody response. Placebo arm data from the Dengvaxia vaccine trials also suggests higher PRNT titers are linked to protection ²². 217 218 The targeted vaccination of individuals that have pre-existing antibody titers within our 219 zone may be a viable approach to minimize the public health burden from dengue by 220 moving individuals away from the risk window (Figure 4D). Even in an endemic setting 221 such as our cohort, there is considerable temporal variability in the serological status of 222 9y individuals (Figure S10) suggesting that the current WHO guidance surrounding 223 Dengvaxia or similar guidance based on serostatus at vaccination will have to carefully 224 consider this variation or specifically screen individuals.

225

226 Our approach allows us to consider wider problems concerning drivers of dengue

227 epidemiology. The assumption that population-wide immunity varies in time and dictates

228 multi-annual dynamics of dengue pervades the literature and dominants current

hypotheses about what drives large outbreaks of dengue in particular settings^{18,23-26}. More 229 230 generally, the idea that temporally varying population immunity drives temporal dynamics of pathogens pervades infectious disease epidemiology²⁷⁻²⁹. However, 231 232 quantitative evidence that any population varies in dengue immune status over time is 233 largely lacking, as is a link between the immune status of a population and the risk of 234 epidemics in empirical data. Here, though we have only a short time series, we show that 235 underlying heterogeneity in the size of annual epidemics mean the risk of having titers 236 within-the risk zone for different birth-cohorts are more correlated by epidemic time-237 point (Figure 4A, mean correlation of 0.70) than by age (Figure 4B, mean correlation of 238 (0.23). While both the probabilities of being naïve and having \log_2 -titers above the risk-239 zone are correlated with age, there also exist strong birth-cohort effects (Figure S10-S11). 240 For example, among 9 year olds, we observe up to a two-fold difference in the 241 probability of being naive, depending on the year of the study.

242

243 Finally, our results can guide the design of cohort studies aiming to characterize 244 transmission. Studies typically use a four-fold rise in titers against any serotype as 245 evidence of infection, regardless of the timing of sample collection. Using our titer 246 trajectories, we find that if blood draws are every 90 days, a four-fold cut-point on 247 measured titers has a specificity of >99% and a sensitivity of 87% (Figure 4C, Figure 248 S12). The sensitivity is reduced to 77% when blood is taken every six months and 62%249 when blood is taken annually, although it may be higher in seasonal settings when 250 samples are taken at the season's end. Using an alternative approach that uses the mean 251 titer across the four serotypes and a 1.6-fold cut-point, the sensitivity of the assay

252	improves to 96% when samples are taken every six months and to 90% for annual bleeds
253	(specificity >95%) (Figure S13). We provide the optimum cut-point and estimated
254	sensitivity for these approaches and a theoretical one where titers are on a continuous
255	scale (such as PRNT) (Figure S14) and where a minimum specificity of >99% is required
256	(Figure S13).

257

258 We demonstrate through simulation that our framework can recover the true number of 259 subclinical infections and parameters when only 30% of infections are symptomatic 260 (Table S5). Our approach is also robust to a scenario where there are differential rises in 261 titers for symptomatic and non-symptomatic infections (Table S6) and where we 262 incorporate school specific force of infection parameters (Table S7). In addition, we find 263 the timing (Figure S15A) and the serotype (Figure S15B) of undetected infections cluster 264 in the same locations as symptomatic infections. This provides strong support of our 265 modeling framework by suggesting that the model can correctly identify spatio-temporal 266 clustering of otherwise undetected infections. These findings also support focal transmission, irrespective of disease outcome^{13,30,31}. The approach presented here will be 267 268 applicable across disease systems where longitudinal titer data exists, allowing a wide 269 range of insights into fundamental questions of disease ecology and risk.

270 Figure 1



272 Figure 1. (A) Measured (dots) and model fit (lines) for three example individuals. Each 273 dot represents the mean titer across the four serotypes. The pink shaded regions are 274 periods of active surveillance. The solid blue arrows represent confirmed symptomatic 275 dengue infections. The open blue arrows represent estimates of timing of subclinical 276 infections from an augmented dataset. During the active surveillance windows, these 277 augmented infections represent subclinical infections whereas outside the surveillance 278 window, it is unknown if the individual had symptoms. (B) Serotype distribution of PCR confirmed symptomatic infections (DENV1 - green, DENV2 - blue, DENV3 - maroon, 279 280 DENV4 – orange, unknown serotype – black). The grey bars represent the estimated 281 distribution of infections not detected from active surveillance. The periods of active 282 surveillance are in pink (5.5 months per year). (C) Model fit (lines) and observed (dots) 283 titers pre and post infection for primary infections (infecting serotype in blue, non-

infecting serotypes in red) and post-primary infections (green). (**D**) Mean difference

- between observed log2-titer at different time points following infection with that at 1 year
- for all augmented and observed infections (average of 1,421 total infections across 100
- reconstructed datasets) with 95% confidence intervals. (E) Titer ratio of the infecting to
- the mean of the three non-infecting serotypes before and after symptom onset with 95%
- 289 confidence intervals for the 217 individuals with symptomatic infections where infecting
- serotype detected (N=3,366 total titer measurements).
- 291



Figure 2. Annualized probability of (A) infection, (B) developing any symptoms, (C) being hospitalized and (D) developing DHF as a function of the mean measured antibody titer across all serotypes at the time of exposure across all study subjects (N=3,451). The open circles on the left represent primary infections (i.e., those with no detectable titers to any serotype prior to exposure). The shaded regions represent 95% bootstrap confidence intervals.

301 **Figure 3**



303 Figure 3. Risk of subsequent infection and disease following an infection event (from 304 average of 1,420 infections across 100 reconstructed datasets). The probability of 305 survival from subsequent infection (irrespective of disease outcome (A) and that lead to 306 DHF (C)) as calculated from Kaplan-Meier for those with setpoint antibody titers of ≤ 3 307 (red) and >3 (blue) with 95% confidence intervals. The annualized probability of a 308 subsequent infection (irrespective of disease outcome (B) and that lead to DHF (D)) at 309 different time points following infection for those with setpoint antibody titers of ≤ 3 (red) 310 and >3 (blue).

311



314 Figure 4. (A) Proportion of study participants who have titers in risk zone (defined 315 detectable log2-titers \leq 3) over the study period for different birth-cohorts (colored lines) 316 and overall (black). The epidemic curve of all infections is in grey. (B) Proportion of 317 study participants with titers in risk-zone as a function of age for different birth-cohorts 318 (colored lines) and overall (black). (C) Performance of current assay testing protocol 319 where infection events are defined as a rise above a cut-point in any serotype across two 320 blood draws. (D) Relationship between PRNT titer and HI titer where both assays were 321 performed (N=1,771 samples). The boxplots show 2.5, 25, 75 and 97.5 quantiles as well 322 as the mean. Superimposed are the results from the Denvaxia vaccine for previously 323 seronaive (blue) and seropositive (red) prior (open symbols) and post (filled symbols) 324 vaccination.

325

326 Methods

327 1. Cohort study design

328 Individuals attending 12 different schools in Kamphaeng Phet district, a rural region of 329 Northern Thailand were recruited into a dengue cohort study that ran between 1998 and 2003 as previously described³². All individuals were between seven and 13 years old. 330 331 Blood samples were taken four times a year (in January, June, August and November) 332 with an average of 91 days between blood draws. In addition, from the start of June to 333 mid November each year, active surveillance was conducted through school-based 334 surveillance. Children who missed school due to febrile illness had additional acute and 335 convalescent blood draws. Dengue infection was confirmed using RT-PCR on the acute 336 sample, with the infecting serotype also recorded or through antibody detection (IgM 337 ELISA values >40 or HI rises of over four times between acute and convalescent blood 338 draws), in which case the infecting serotype was not known. The date of symptom onset, 339 whether or not the child was hospitalized and whether or not they developed DHF was 340 also recorded. Note that the cohort study was conducted prior to 2009 when the WHO 341 provided new guidance of the characterization of different levels of dengue severity. 342

342

343

344 2. Antibody measurements

For each individual's blood draw, antibody titers to each of DENV1, DENV2, DENV3, and DENV4 were measured using a hemagglutination inhibition assay. The following two-fold dilutions were used: 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560. We translated each titer onto a log₂ scale such that 1:10 was given a value of 1,

349 1:20 of value of 2 and so on. Undetectable titers (those with a titer of <1:10) were given a 350 value of 0. For a subset of 800 individuals, 1,771 samples were also tested using plaque 351 reduction neutralization tests (PRNTs). These samples were either paired samples from 352 individuals with symptomatic confirmed infection with one sample taken from a time 353 point prior to symptom onset and one sample from post symptom onset (N=75 pairs) or 354 randomly chosen sequential blood samples from individuals without a detected 355 symptomatic infection between the blood draws. 356 357 3. Characterizing how titers change following symptomatic infection 358 We can understand how titers to both the infecting serotype and to non-infecting 359 serotypes change over time prior to and following symptom onset. For all individuals that 360 experienced a symptomatic illness where the infecting serotype was identified, we 361 identify all titer measurements within each 10-day window from 100 days prior to 362 symptom onset to 600 days *post* symptom onset. For each window, we calculate the mean 363 titer to the infecting serotype and the average mean titer to the other three serotypes

across all individuals that had a blood draw within that window.

365

366 4. Modeling the dynamics of dengue antibody titers

367 Previous efforts in malaria have used hidden Markov models to include undetected

368 infections in estimates of the transmission intensity using presence/absence of specific

antibodies in longitudinal data³³. While these efforts are able to improve estimates on the

370 force of infection within a community compared to using symptomatic individuals, they

do not incorporate the changing dynamics of antibody titers over time. By specifically

including titer dynamics, we can help understand a wide range of issues, including assayerror, measures of protection and risk and cohort design.

374

375 *4.1. Notation*

We consider an individual *i*. We denote $n_i^I(t)$ the number of times the individual was 376 377 infected prior to time t. Each dengue infection of individual i is labeled by the index $\psi = l \dots n_i^l(t)$. We denote $\tau_{i,\psi}^l$ the time of infection number ψ of individual *i* and $s_{i,\psi}$ the 378 infecting serotype of infection number ψ of individual *i*. The history of infection (i.e., the 379 380 timing and serotype of all infections since birth) of individual *i* up to time *t* is labeled $H_i(t)$. We denote N_i^A the total number of times the individual had blood taken during the 381 study. Each blood draw of individual *i* is labeled by the index $\pi = 1 \dots N_i^A$. We denote $\tau_{i,\pi}^A$ 382 the time of blood draw π for individual *i*. We denote $A_{i,s,\pi}$ the true antibody titer (see 383 Section 4.3) and $A_{i,s,\pi}^*$ the measured antibody titer for individual *i* for serotype *s* at blood 384 draw π . $\Lambda_i(t)$ represents the cumulative force of infection exerted on individual *i* prior to 385 386 time *t*. The parameter vector is denoted by θ .

387

388 *4.2. Hierarchical structure of the model*

389 We can break down the probability of a measured antibody titer into three components:

$$P(A_{i,s,\pi=k}^{*}|A_{i,s,\pi=k})$$

$$\cdot P(A_{i,s,\pi=k}|\tau_{i,\psi=1}^{I}, ..., \tau_{i,\psi=n_{i}^{I}(t=\tau_{i,\pi=k}^{A})}^{I}, s_{i,\psi=1}, ..., s_{i,\psi=n_{i}^{I}(t=\tau_{i,\pi=k}^{A})}^{I}, A_{i,s,\pi=1}, ..., A_{i,s,\pi=k-1})$$

$$\cdot P(H_{i}(t=\tau_{i,\pi=k}^{A})|\{\lambda\}_{t})$$

390 The first part represents the 'measurement model', the second part the 'antibody391 dynamics model' and the third part the 'infection model'.

392

393 *4.3. Measurement model*

394 We model the underlying antibody levels on a continuous scale, however, the 395 hemagglutination inhibition assay is a discrete assay, such that in a situation of no 396 measurement error or systematic biases, a true antibody titer between any two dilutions 397 would be measured as the lower of the two dilutions. So for example, a true titer of 2.7 398 would be measured as 2 (assuming there are dilutions performed at 0, 1, 2, 3...). In 399 addition, there is also likely to exist measurement error and there may be underlying 400 differences by serotype (i.e., serotype-specific biases) in the assay that will impact all measurements of antibodies against a particular serotype. We consider a 'true titer' to 401 402 represent the underlying (but unmeasured) titer on a continuous scale. A 'measured titer' 403 is the value that is actually measured by the assay. Conditional on an individual's history 404 of infection, we assume independence between the measurements of the different 405 serotypes. This seems a reasonable assumption as assays are performed separately for each serotype. The probability of the measured titers, $A_{i,s,\pi=k}^*$ is: 406

$$P(A_{i,s,k}^*|A_{i,s,k}) = \int_{A_{i,s,k}^*}^{A_{i,s,k}^*+1} f(u) du$$

407 where f(u) is the density for a normal distribution with mean $A_{i,s,k} + \chi_s$ and a standard 408 deviation parameter, σ . Where:

409
$$\chi_s = 0 \text{ if } s = DENV1$$

410
$$\chi_s = \chi_2 \text{ if } s = DENV2$$

411
$$\chi_s = \chi_3 \text{ if } s = DENV3$$

- 412 $\chi_s = \chi_4 \text{ if } s = DENV4$
- 413
- 414 *4.4. Antibody dynamics model*
- 415 If an individual *i* was never infected by dengue, we assume they will have titers of 0

416 against the four serotypes (this assumes any maternal antibodies have disappeared and

417 there is no impact of infections by other flaviviruses). At each time point that the

418 individual becomes infected, their antibody titers will rise. We assume that the rise can be

419 broken down into a permanent increase (representing antibodies that will continue to

420 circulate, long after the infection has passed) and a temporary increase (representing the

421 short-lived antibodies generated upon infection).

422

423 *4.4.1. Permanent rise in titers*

424 The permanent rise in titers $Q_{i,s}(\psi)$, for serotype *s* from infection number ψ in individual 425 *i* is modeled as:

$$Q_{i,s}(\psi) = \omega_{i,\psi} \cdot K(\psi, s)$$

426 where $\omega_{i,\tau}$ is a random effect that is gamma distributed with mean parameter ω_m and 427 variance parameter ω_v and $K(\psi, s)$ allows differential antibody response by serotype for 428 primary infections: $K(\psi, s) = \eta$ if it is a primary infection (i.e., $\psi=1$) and *s* is the 429 infecting serotype; $K(\psi, s) = 1$ otherwise. 430

431 *4.4.2. Temporary rise in titers*

432 We assume that temporary antibody responses will decay exponentially over time:

$$R_{i,s}(t|H_i(t)) = \gamma_{i,\psi=n_i^I(t)} \cdot \exp\left(-\left(t - \tau_{i,\psi=n_i^I(t)}^I\right) \cdot \delta_{i,\psi=n_i^I(t)}\right) \cdot K(\psi = n_i^I(t), s)$$

where $\gamma_{i,\psi=n_i^I(t)}$ is a random effect that captures the instantaneous rise in temporary 433 antibody titers following the most recent infection (infection $n_i^I(t)$) prior to time t that 434 comes from a gamma distribution with mean parameter γ_m and variance parameter γ_v ; 435 $\delta_{i,\psi=n_i^I(t)}$ is the rate of decay of the temporary antibodies and comes from a gamma 436 distribution with mean parameter δ_m and variance parameter δ_v . As with the permanent 437 rise in titers, $K(\psi = n_i^I(t), s)$ allows differential antibody responses for primary 438 439 infections: $K(\psi, s) = \eta$ if it is a primary infection (i.e., $\psi=1$) and s is the infecting serotype; $K(\psi, s) = 1$ otherwise. Additional work is needed to understand if alternative 440 441 functional forms for the rise and decay in antibody titers may further refine how 442 antibodies behave following infection.

443

444 *4.4.3. Overall trajectory of antibody titers*

445 Under these assumptions, and an additional linearity assumption that the temporary and
446 permanent rises are additive, antibody titers at blood draw *k* for serotype *s* in individual *i*447 is:

$$A_{i,s,\pi=k} = Q_{i,s}(\psi = 1) + \dots + Q_{i,s}\left(\psi = n_i^I(t = \tau_{i,\pi=k}^A)\right) + R_{i,s}\left(t = \tau_{i,\pi=1}^A | H_i(t = \tau_{i,\pi=k}^A)\right) + \dots + R_{i,s}\left(t = \tau_{i,\pi=k}^A | H_i(t = \tau_{i,\pi=k}^A)\right)$$

448

449 4.5. Infection history model

450 We first assume that both the number of infections and the timing of infections are

451 known. This assumption will subsequently be relaxed. We assume that each individual

452 can get infected up to four times (once by each serotype). An individual's history of

453 infection depends on seasonality in dengue transmission and differences in the force of

454 infection across years. For a particular time *t*, the force of infection is assumed to be:

$$\lambda(t) = \bar{\lambda} \cdot \beta_{|t|} \cdot \left(1 + \delta \cdot \cos\left(\zeta + \frac{2\pi t}{365}\right)\right)$$

455 where $\bar{\lambda}$ is a parameter that represents the mean daily force of infection in 1998 (the first 456 year of the study) and $\beta_{|t|}$ is the mean force of infection in year |t| as compared to that in 457 1998.

458

For an individual *i*, the contribution to the likelihood for periods prior to any infection the probability of their infection history can be broken down into periods of infection and periods without infection. Individuals only contribute to the likelihood during their time in the study.

463

464 For each infection that occurs at time *t*, the contribution to the likelihood is:

 $\log\left(1 - \exp(-\lambda(t))\right)$

465 For each individual, each day during which no infection occurs, the contribution to the466 likelihood in respect of serotype *s* is:

467 $\exp(-\lambda(t))$ - where more than 90 days have passed since an infection by any 468 serotype and the individual has not previously been infected by

469 serotype s

470 0 - otherwise, including periods when the individual is not part of the
471 study

The presence of the 90-day window where no infection can take place avoids there being more than one infection event between two blood draws. This period is substantially shorter than the estimated period of cross-protection between serotypes of 2 years³⁴.

475

476 *4.5.1. Context of full observation*

477 In the context of full observation, the probability of the history of infection for individual478 *i* can be given as:

$$P(H_i(t = \tau_{i,\pi=k}^A) | \{\lambda\}_t)$$

$$= \prod_{k=1}^{n_i^I(T_i)} \exp\left(-\int_{\tau_{i,k-1}^I}^{\tau_{i,k}^I} \lambda(u) \, du\right) (1 - \exp\left(-\lambda(\tau_{i,k}^I)\right))$$

$$\cdot \exp\left(-\int_{\tau_{i,n_i^I(T_i)}^I}^{T_i} \lambda(u) \, du\right)$$

479 where $\tau_{i,0}^{I}$ represents the time of birth and T_{i} the time point at which individual *i* leaves 480 the study (defined as the day of their final blood draw). We assume the same $\lambda(t)$ for all 481 serotypes.

482

483 *4.6. Situation of imperfect observation*

484 In practice, we do not know the infection history of all individuals. Many infections will

485 have occurred before individuals entered the study. In addition, there are likely to be

486 many subclinical infections that would not have been detected through active

487 surveillance. In addition, active surveillance only operated 5.5 months of every year.

488 Infections outside these periods would also have been missed (irrespective of symptoms).

490 4.6.1. Unobserved infections prior to recruitment

491 For the infection history of individuals before they enter into the study, we estimate a baseline titer $A_{i,s}(t_0)$ that represents the titer to serotype s one year prior to the first blood 492 493 draw. As we assume linearity, such that the temporary and permanent titers of successive 494 historic infections sum up to give the titer at a moment in time, this estimated baseline 495 titer allows us to incorporate the impact of historic infection events up one year prior to 496 enrollment but means we do not need to infer infection events before that time. 497 Individuals that are naïve at baseline (defined as those with no measured titers to any 498 serotype at the first blood draw) are given a baseline titer of 0. For an individual with no infection events during the study period, $A_{i,s}(t) = A_{i,s}(t_0)$ for all t. 499 500 501 4.6.2. Use of data augmentation for undetected infections or serotype during study 502 In the context of full observation during the study period, each individual would have the 503 serotype and time from each infection, $\{s_{i,\psi}, \tau_{i,\psi}\}$, known. In the setting of undetected 504 infections or detected infections but infecting serotype is unknown (such as when 505 symptomatic infections are only detected through IgM ELISA and therefore the serotype 506 is unknown), we can use a Bayesian data augmentation framework. In this framework, 507 the incompletely observed $\{s_{i,\psi}, \tau_{i,\psi}\}$ pairs are incorporated and considered as nuisance 508 parameters. The joint posterior distribution of the parameters and the augmented data is 509 explored via reversible-jump MCMC sampling.

511 If we call
$$y = \{s_{i,\psi}^*, \tau_{i,\psi}^*\}_{i=1,\dots,N,\psi=1,\dots,n_i^*(t=\infty)}$$
 the observed data,

512 $z = \{s_{i,\psi}, \tau_{i,\psi}\}_{i=1,\dots,N,\psi=1,\dots,n_i(t=\infty)}$ the full data (made up of the observed data and the

513 augmented data), the joint posterior is:

 $P(z, \theta|y) \propto P(y|z) \cdot P(z|\theta) \cdot P(\theta)$

514 P(y|z) represents the observation model, $P(z|\theta)$ is the titer model outlined above and

515 $P(\theta)$ gives the prior distribution of the parameters.

516

517 The observation model makes sure that the augmented datasets are consistent with the

518 observed data by having a value of 1 (if consistent) or 0 (if inconsistent). Consistent

519 augmented data have the following characteristics:

- 520 (i) No individual is infected during the study period by the same serotype more521 than once
- 522 (ii) No individual is infected more than once during a 90 day period
- 523

524 Note that, as DENV-titer responses to non-DENV flaviviruses such as Zika and Japanese

525 encephalitis are likely to be smaller that to DENV infections, such exposures are unlikely

526 to be detected by our model and incorporated as measurement uncertainty instead.

527

528 4.6.3. Date of symptom onset, date of infection and date of titer rise

529 For all detected (symptomatic) infections, we only detect the date of symptom onset and

- not the date of infection. To obtain the day of infection for symptomatic cases we subtract
- 531 a fixed period of 7 days from the day of symptom onset, representing the median
- 532 incubation period for dengue³⁵. Titers may also not rise on the day of symptom onset (due

533 to recall bias in when symptoms started or individual level variability). For symptomatic 534 infections, we approximate the true, unobserved day of titer rise using augmentation, 535 where we define consistent augmented data for which the day of titer rise is within ten 536 days of the reported date of symptom onset. For augmented (undetected) infections, we 537 assume that the day of titer rise following infection always occurs 11 days after the day of 538 infection, which represents an approximate estimate of the time between infection and 539 day of titer rise: calculated as the sum of the median incubation period for dengue (seven 540 days) and the median time between symptom onset and titer rise for the detected 541 infections (four days).

542

543 4.6.4. Impact of uneven data collection through time

544 This cohort used a rolling recruitment approach, which maintained an approximately 545 constant sized population and constitutes an important strength compared to cohorts 546 whose size may be strongly affected by participant dropout. As individuals only 547 contributed to the likelihood for their period of inclusion in the cohort and dropout is not 548 expected to depend on the history of infection, we do not expect that the turnover of 549 participants in the cohort will bias parameter estimates. This was demonstrated in a 550 simulation study where we were able to recover true parameters for a simulated cohort 551 with a similar design (see Section 4.8).

552

553 4.6.5. Prior distributions

554 We use a log-normal distribution with log-mean 0 and log-variance of 1 for the

parameters: mean and variance in the permanent rise in log₂-titers (ω_m , ω_v), mean and

556	variance in the temporary rise in \log_2 -titers (γ_m , γ_v), mean and variance in the decay in
557	log ₂ -titers per day (δ_m , δ_v), difference in rise for infecting vs. non infecting serotype
558	(primary infection only) (η), measurement error (σ), DENV2-4 bias (χ_2, χ_3, χ_4), daily force
559	of infection in 1998 per serotype (λ), relative force of infections versus 1998 for 1997
560	(β_0) and 1999-2002 $(\beta_2$ - $\beta_5)$ and the two seasonality parameters (δ and ζ).
561	

. . . .

• • • • • •

562 4.7. Estimation using MCMC

. . .

563 We develop a Markov chain Monte Carlo approach to explore the joint posterior

564 distribution of parameters and the augmented data with the following steps:

565 (i) Metropolis-Hastings update for the model parameters θ in turn with the 566 updates performed on a logarithmic scale. The step size of the proposals was 567 adjusted to obtain an acceptance probability of 20-30%. As the vast majority 568 of infections are undetected, when updating the six parameters that determine 569 the rise and decay of antibodies (namely $\omega_m, \omega_v, \delta_m, \delta_v, \gamma_m, \gamma_v$), we calculate 570 the likelihood using only the titers from one month prior to and year post the 571 symptomatic (and therefore detected) infections. This approach assumes that 572 the rise and fall in titers from all infections come from the same distributions, 573 irrespective of symptom status. More work is needed to understand if whether 574 or not an infection leads to symptoms changes the titer dynamics following 575 that infection.

576 (ii) For the symptomatic cases, as the day of titer rise may not fall exactly at the 577 recorded day of symptom onset we use an independence sampler to update the 578 day of titer rise. At each iteration, the day of the titer rise was updated for 100

- 579 randomly chosen symptomatic infections. Candidate values were chosen using
 580 a uniform distribution between 10 days prior to and 10 days post the recorded
 581 date of symptom onset.
- 582 (iii) Independence sampler for the identity of the infecting serotype for the 62
 583 symptomatic infections where the serotype was not identified. At each
 584 iteration, the serotype for each of these infections is updated with equal
 585 probability across the four serotypes.
- (iv) Independence sampler for the identity of the infecting serotype for the
 undetected infections. At each iteration, the serotype for 500 randomly chosen
 undetected infections is updated with equal probability across the four
 serotypes.
- (v) Independence sampler for the dates of titer rise for undetected infections. At
 each iteration, the day of infection is updated for 1000 randomly chosen
 undetected infections. For each infection, the proposal is a uniform
 distribution between one year prior to entry into the study and the day of the
 final blood draw.
- (vi) Independence sampler for the baseline titers for each individual. At each
 iteration, the baseline titer for one serotype is updated for 1000 randomly
 chosen individuals. The proposal distribution is a random uniform distribution
 between 0 and 10. All individuals that are naïve at baseline (i.e., those with no
 titers to any serotype at the first blood draw) are forced to have a baseline titer
 to 0 for all four serotypes.

601	(vii)	Reversible jump –MCMC to add/remove unobserved infection events. As
602		$H_i(t_j)$ is unobserved, we use a Bayesian data augmentation approach that
603		treats it as a nuisance parameter. Rather than attempting to definitively
604		identify whether an infection occurred or not, these approaches allow us to
605		incorporate the uncertainty of the presence and timing of these events. We use
606		reversible jump MCMC (RJ-MCMC) to add and remove infection events.
607		Each step to add undetected infections proceeds as follows:
608		a. Randomly draw individual.
609		b. Draw a candidate date for the infection event using a uniform distribution
610		from 1 year prior to their first blood draw to the day of their final blood
611		draw.
612		c. Draw a candidate serotype of infection with the probability of each
613		serotype being 0.25.
614		d. Update the number, date and serotype of infections for that individual.
615		For the removal of undetected infections, we use a similar approach:
616		a. Randomly draw individual.
617		b. If that individual has undetected infections, randomly select one of their
618		infections with equal probability (if they have no infections move to the
619		next individual).
620		c. Update the number, date and serotype of infections for that individual by
621		removing that infection.
622		

623 4.8. Evaluation of model using simulated data

624	In order to evaluate the ability of the model to accurately estimate the parameters in a
625	scenario when only a minority of infections are observed, we use the same modelling
626	framework on a random subset of 1,000 individuals from the study with subsequent
627	changes in titers, We include the actual start date and the end date for these individuals
628	(i.e., when they entered and left the cohort). We simulate infections in these individuals
629	based on known parameters. We then randomly 'unobserve' 70% of infections to reflect
630	undetected infections. We then estimate the parameters using our framework and
631	compare them to the underlying true parameters.
632	

633 4.9. Sensitivity analysis using school-specific force of infection parameters

The force of infection exerted on individuals may differ across schools, resulting in nonindependence between individuals attending the same school. To assess the impact of any such correlation on our parameters, we performed a sensitivity analysis where we included a separate force of infection parameter for each school. In this model the force of infection exerted on an individual that attends school *sch* is:

$$\lambda(t, sch) = \bar{\lambda} \cdot \beta_{|t|} \cdot \beta_{|sch|} \cdot \left(1 + \delta \cdot \cos\left(\zeta + \frac{2\pi t}{365}\right)\right)$$

639 where $\bar{\lambda}$ is a parameter that represents the mean daily force of infection in 1998 in school 640 1, $\beta_{|t|}$ is the mean force of infection in year |t| as compared to that in 1998 and $\beta_{|sch|}$ is 641 the mean force of infection for school *sch* as compared to school 1.

642

643 4.10. Alternative functional forms for the decay in titers

644 Alternative functional forms for the decay in antibody titers exist. In particular, biphasic

645 models that model both short-term antibody decay and longer-term antibody decay with

different exponential decay rates have been shown to work well in other systems, such as
 malaria³⁶. The biphiasic form is captured by:

$$\text{Titer}_{t} = \theta_{1} \cdot (\theta_{2} \cdot \exp(-\theta_{3}t) + (1 - \theta_{2}) \cdot \exp(-\theta_{4}t))$$

where θ_1 , θ_2 , θ_3 and θ_4 capture the decay of the titers. To explore whether this biphasic 648 649 form may further refine how antibodies behave following infection here, we fitted both 650 exponential decay and biphasic models to the observed infections using the observed 651 titers following detected PCR-confirmed infections and the dates of symptom onset. We 652 found largely consistent results in the two models (Figure S1). As exponential decay is 653 the more parsimonious model, we retained this form for the final analysis. Nevertheless, 654 structural uncertainty in the model used for the analysis remains, which will not be 655 represented within the confidence intervals for the parameters.

656

657 *4.10. Estimation of impact on titers on infection and disease*

658 *4.10.1. Estimation of impact of mean titers on infection*

659 We use the augmented times and serotypes of infection from 100 model iterations to 660 reconstruct the antibody titer trajectories for each individual. For each augmented dataset 661 we extract the mean titer across all four serotypes for each day and whether they got 662 infected in the following day or not. Person-time in individuals who were considered not 663 susceptible (i.e., had been infected in the prior 90 days) was excluded. To explore the 664 relationship between mean titer and the probability of infection we conducted logistic 665 regression where we used a polynomial spline of order 2 for the mean titer (determined as 666 the optimal model through comparison of different polynomial models by AIC). To 667 account for sampling uncertainty, in each reconstructed dataset we use a bootstrap

approach to sample all individuals with replacement and then re-perform the logistic
regression each time. We present the mean and 95% confidence intervals from the
resultant distribution of the logistic model estimates of the probability of infection for
each titer obtained from across the model iterations.

672

673 *4.10.2. Estimation of impact of mean titers on disease outcome*

674 We explore the relationship between mean titer and the probability of having different 675 disease outcomes. We consider three different outcomes: symptomatic infection 676 (irrespective of severity), hospitalization and DHF. We use the same approach as in 677 Section 4.9.1. but only consider titers during the active surveillance windows and 678 whether or not individuals had an infection the following day that led to the outcome of 679 interest. For each outcome, we conduct logistic regression where we use a polynomial 680 spline of order 2 for the mean titer (consistently determined as the optimal model through 681 comparison of different polynomial models by AIC). We use a bootstrap approach to 682 sample all individuals with replacement and then re-perform the logistic regression each 683 time and identified the mean and 95% confidence intervals from the resultant distribution 684 for the estimates of the probability of having an infection that led to the outcome of 685 interest for each titer obtained from across the model iterations.

686

687 4.10.3. Estimation of impact of mean titers on disease outcome, conditional on being688 infected

689 For those that became infected during the active surveillance windows, we fit logistic

690 models to the mean titers and whether or not the disease outcome occurred. We looked at
691 three outcomes: any symptomatic illness, hospitalization and DHF. For each of the three 692 outcomes, we compare an intercept only model with models with a polynomial spline up 693 to order 2. To account for sampling uncertainty, in each reconstructed dataset we use a 694 bootstrap approach to sample all individuals who had an infection during the surveillance 695 windows with replacement and then re-perform the logistic regression each time. We 696 present the mean and 95% confidence intervals from the resultant distribution of the 697 logistic model estimates of the probability of infection for each titer obtained from across 698 the model iterations.

699

4.10.4. Estimation of impact of mean PRNT titers on disease outcome, conditional on
being infected

702 PRNT titers are available for a subset of 1,771 blood draws. For those that became 703 infected during the active surveillance windows and PRNT titers are available in the six 704 months window prior to infection, we fit logistic models to these mean PRNT titers from 705 that six-month time frame and whether or not the disease outcome occurred. We looked 706 at three outcomes: any symptomatic illness, hospitalization and DHF. For each of the 707 three outcomes of interest, we compare an intercept only model with models with a 708 polynomial spline up to order 2. To account for sampling uncertainty, in each 709 reconstructed dataset we use a bootstrap approach to sample all individuals who had an 710 infection during the surveillance windows with replacement and then re-perform the 711 logistic regression each time. To account for the fact that individuals and serum samples 712 may not have been completely selected at random for PRNT testing (e.g., preferential

- testing of those with symptomatic disease), we adjusted our estimate for the probability
- of sampling conditional on the outcome of interest.
- 715
- From the logistic regression described above, we can extract the probability of the
- outcome of interest given a particular PRNT titer and that a PRNT was conducted. Using
- 718 Bayes rule we can write down:

$$P(outcome | titer, PRNT \ done \) = \frac{P(PRNT \ done | outcome, titer)P(outcome | titer)}{P(PRNT \ done | titer)}$$

as the PRNT titer (or the HI titer) was not taken into account in the section process for

720 choosing whether or not a PRNT was done, this becomes:

$$P(outcome | titer, PRNT done) = \frac{P(PRNT done | outcome)P(outcome | titer)}{P(PRNT done)}$$

As we are interested in *P*(*PRNT done*|*outcome*), we can reorder this equation to:

$$P(PRNT \ done | outcome) = \frac{P(outcome | titer, PRNT \ done \)P(PRNT \ done)}{P(outcome | titer)}$$

722 We therefore multiply our logistic model outcomes by the following adjustment factor:

$$adj.factor = \frac{P(PRNT \ done)}{P(PRNT \ done|outcome)}$$

723 *P(PRNT done)* is calculated as the proportion of all infection events where a PRNT was

conducted in the prior 6 months from the infection and *P(PRNT done/outcome)* is

calculated as the proportion with the outcome of interest where PRNTs were conducted

- in the prior 6 months. We present the mean and 95% confidence intervals from the
- resultant distribution of the logistic model estimates of the probability of infection for
- each titer obtained from across the model iterations.
- 729

730 4.10.5. Estimation of impact of year and age on mean titers on disease outcome

We used a logistic regression approach to explore the impact of year of infection and the age at the time of infection. To explore the impact of year, we take each augmented dataset in turn and sample all the individuals with replacement to incorporate sampling uncertainty. We then regress the year of infection (as a categorical variable) on whether the outcome $Y_{i,t}$ occurred:

$$logit(Y_{i,t}) = \beta_0 + \beta_1 \cdot Year_{i,t}$$

where $Year_{i,t}$ is the year (1998, 1999, 2000, 2001 or 2002) within which day t occurred 736 737 for individual *i*. We conducted separate regression where the outcome was an infection 738 event (irrespective of whether the infection led to symptoms), symptomatic infection 739 events (irrespective of disease severity), hospitalization and development of dengue 740 hemorrhagic fever. For the last three models we only considered data during the active 741 surveillance windows, as we do not know the symptom status of infections outside these 742 windows. To explore the impact of age, we dichotomized the age of individuals as being 743 less than or greater than 9 (the Sanofi Pasteur vaccine is not recommended for individuals 744 under 9). We then performed the regression:

$$logit(Y_{i,t}) = \beta_0 + \beta_1 \cdot Age_{i,t}$$

where separate models for the same four outcomes, $Y_{i,t}$, were performed. Finally, we built multivariable models that also accounted for mean titer using a polynomial of order 2:

$$logit(Y_{i,t}) = \beta_0 + \beta_1 \cdot Age_{i,t} + \beta_2 \cdot Titer_{i,t} + \beta_2 \cdot Titer_{i,t}^2$$

747

748 4.11. Impact of titer on outcome using cox proportional hazard models

749 In the context of small probabilities of an event occurring and short time intervals 750 between readings, logistic regression will give consistent results with that from cox 751 proportional hazards models that specifically takes the non-independence of titer observations from the same individuals into account¹⁷. To demonstrate the consistency of 752 753 the two approaches we estimate the impact of titer on our four outcomes (infection, 754 symptomatic infection, hospitalized infection and DHF infection) using a time-varying 755 cox proportional hazards model, specifically incorporating clustering of observations by individual³⁷. We used 100 augmented datasets. For each augmented dataset we extract the 756 757 mean titer across all four serotypes for each day and whether they got the outcome of 758 interest in the following day or not. For the disease specific outcomes (any symptomatic 759 disease, hospitalized infection and DHF infection), we only used time points during the 760 surveillance windows. We then calculated the impact of the mean titer (polynomial of 761 order 2) on the relative hazard of infection, incorporating a clustering id per individual using the *survival* package in R^{37} . We then calculate the mean effect of titer on the 762 763 outcome of interest by averaging the estimates across the reconstructed datasets. 764

To compare our results using logistic regression, we multiply the annualized estimate of a titer *x* on the risk of the outcome (calculated as 1 - exp(-365x)) by the estimated baseline hazard for those with a measured titer of 0 (calculated as the proportion of infections in time points with a measured titer of 0). We find that the results are almost identical (Figure S6). As the logistic model approaches allow us to directly estimate the underlying probability of the outcome, it is preferred.

771

772 5. Survival analysis

5.1. Annualized probability of infection using titer data only

Over 100 reconstructed datasets, we initially identify all individuals who experienced aninfection (irrespective of disease severity). We then identify the setpoint antibody load

for that infection as the mean titer 1 year following infection as predicted by our model.

Individuals were divided into two groups, those with a setpoint antibody load ≤ 3 and

those with a load >3. For each individual in each titer group, we use the logistic model

from 4.9.1 to predict the daily probability of a subsequent infection based on the mean

titers each day following the initial infection. We also calculated the daily probability of

experiencing an infection that leads to DHF using the logistic model from 4.9.2. We

annualize the predicted probabilities of subsequent infection by using the conversion 1-

exp(-365x) where x is the daily probability of infection. We present the mean annualized

784 probabilities across all individuals and over all the reconstructed datasets.

785

776

786 5.2. Kaplan-Meier analysis

For individuals who experienced an infection, we calculate Kaplan-Meier survival curves for experiencing a subsequent infection (both irrespective of disease outcome and for DHF only). Over 100 reconstructed datasets, we identify all individuals who experienced an infection event. We then identify the setpoint antibody load for that infection as the mean titer 1 year following infection as predicted by our model. Individuals were divided into two groups, those with a setpoint antibody load ≤ 3 and those with a load >3. To incorporate sampling uncertainty we resample all individuals with replacement. For each

group we then calculate Kaplan-Meier survival curves. We present the mean and 2.5 and

795 97.5 quantiles from the resultant distribution.

796

797 6. Prediction of DHF outcome using mean titer

798 We assess the ability of our logistic model to discriminate between those who developed

799 DHF and those who did not using leave one out cross validation.

800

801 6.1. DHF outcome among all cohort participants

802 For each reconstructed dataset, taking each DHF case in turn, we initially identified all

803 individuals who were in the cohort at the same time as the DHF infection with detectable

titers who did themselves not have a DHF infection within a 1-year period. We then

805 randomly selected one of those individuals and used the titer from that day. Once we had

selected a matched control for each DHF case, we calculated the ROC using leave one

807 out cross validation. To do this we removed each individual in turn from the dataset

808 (including both the cases and the controls) and recalculated the relationship between

809 mean HI titer and DHF infection using all the remaining titer readings. We then predicted

810 the probability that the held-out case had a DHF infection. The ROC was calculated using

these probabilities across individuals. We present the mean ROC from across 100

812 reconstructed datasets.

813

814 6.2. DHF outcome among all infections

815 We assessed the ability of our model to discriminate between those who did and did not

816 develop DHF following infection. For a reconstructed dataset, we identified all

817	individuals with detectable titers prior to infection who had a DHF infection and those
818	that did not have a DHF infection (i.e., those with an infection during the surveillance
819	windows that did not develop DHF). For each infection event, we identified the mean
820	titer the day before infection. We then used leave one cross validation as described above
821	to assess our ability to identify those that went on to develop DHF from those that did
822	not. We present the mean ROC from across 100 reconstructed datasets.
823	

• • • • •

824 7. Clustering of infections by school

825 For additional model validation, we explore whether augmented infections occurred in 826 the same schools at around the same time as observed cases, despite no information on

- 827 location being provided to the model.
- 828

829 7.1. Clustering of subclinical infections within schools

830 To explore the clustering of subclinical with symptomatic infections in schools, we use the tau clustering statistic ^{31,38} to calculate the odds of observing an subclinical infection 831 832 (irrespective of serotype and infection parity) within a set time period (t1, t2) of a 833 symptomatic infection within the same school relative to the odds of observing an 834 subclinical infection in a different school within the same time window.

$$\hat{\tau}(t_1, t_2) = \frac{\hat{\pi}(t_1, t_2)}{\hat{\pi}(\infty)}$$

835 where:

$$\hat{\pi}(t_1, t_2) = \frac{\sum_{i=1}^{N_{symp}} \sum_{j=1}^{N_{asymp}} I(sch_{ij} = 1, t_1 < |s_{ij}| < t_2)}{\sum_{i=1}^{N_{symp}} \sum_{j=1}^{N_{asymp}} I(sch_{ij} = 0, t_1 < |s_{ij}| < t_2)}$$

where N_{symp} and N_{asymp} are the number of symptomatic and subclinical infections within any model iteration, sch_{ij} is equal to one if individuals *i* and *j* go to the same school and 0 otherwise, s_{ij} is the time between infections. We varied the time window between 0-90 days, 90-180 days and greater than 180 days.

840

841 7.2. Clustering of serotypes within schools

842 We explore whether the augmented serotypes that were assigned to subclinical primary

843 infections (serotypes could not reliably be assigned in post primary infections due to

844 cross reaction) were consistent with the serotypes of the symptomatic infections of

845 individuals within the same school for different periods of time.

846

For augmented primary infections that are consistently of the same serotype (defined as >50% of augmented datasets have a primary infection in the same individual caused by the same serotype in the same six-month time window), we calculated the odds that an augmented primary infection that occurs in the same school and within a fixed time window of a PCR-confirmed case is of the same serotype relative to the odds that an augmented primary infection that occurs within the same time window in a different school is of the same serotype.

854

$$\hat{\tau}_2(t_1, t_2) = \frac{\hat{\pi}_2(t_1, t_2)}{\hat{\pi}_3(t_1, t_2)}$$

- / 、

855 where:

$$\widehat{\pi_{2}}(t_{1}, t_{2}) = \frac{\sum_{i=1}^{N_{symp}} \sum_{j=1}^{N_{asymp}} I(sch_{ij} = 1, t_{1} < |s_{ij}| < t_{2}, ser_{ij} = 1)}{\sum_{i=1}^{N_{symp}} \sum_{j=1}^{N_{asymp}} I(sch_{ij} = 1, t_{1} < |s_{ij}| < t_{2}, ser_{ij} = 0)}$$

$$\widehat{\pi_{3}}(t_{1},t_{2}) = \frac{\sum_{i=1}^{N_{symp}} \sum_{j=1}^{N_{asymp}} I(sch_{ij} = 0, t_{1} < |s_{ij}| < t_{2}, ser_{ij} = 1)}{\sum_{i=1}^{N_{symp}} \sum_{j=1}^{N_{asymp}} I(sch_{ij} = 0, t_{1} < |s_{ij}| < t_{2}, ser_{ij} = 0)}$$

where ser_{ij} is equal to 1 if *i* and *j* go to the same school and 0 otherwise. We varied the time window between 0-90 days, 90-180 days and greater than 180 days.

859

860 7.3 Uncertainty

861 To incorporate sampling uncertainty into our estimates, for each model iteration we

randomly selected all infection events with replacement before calculating the tau

863 estimates. Ninety-five percent confidence intervals were calculated from the 2.5% and

the 97.5% quantiles of the resultant distribution across all model iterations.

865

866 8. Different approaches to identify infections using simple cut-points

- 867 To assess the sensitivity and specificity of the current approach to identify infections
- 868 based on titer differences across two blood draws, we simulated titer trajectories where
- 869 infections did and did not take place.

- 871 8.1. Simulated titers where infections did take place
- 872 We used the following algorithm:
- 873 (i) Randomly draw MCMC iteration
- 874 (ii) Randomly divide the population of individuals who had at least one infection
- in two: 'model fit' individuals and 'held out' individuals.
- 876 (iii) Of the model fit individuals, randomly draw an individual i

877	(iv)	Identify the parameters for the antibody dynamics for the first infection for
878		that individual (i.e., $\psi_{i,\tau=1}, \gamma_{i,\tau=1}, \omega_{i,\tau=1}$) and the baseline titer $A_{i,s}(t_0)$ from
879		that MCMC iteration. The true titer for each serotype will be $A_{i,s}(t_0)$.
880	(v)	Calculate the measured titer for each serotype using a random draw from a
881		normal distribution with mean $A_{i,s}(t_0)$ and standard deviation σ , where σ
882		represents the measurement error for the assay. Under scenarios of a discrete
883		assay, the measured titer is also rounded down to the nearest integer.
884	(vi)	Draw an infection time point using a uniform distribution between 0 and t_{max}
885		where t_{max} represents the time of the second blood draw.
886	(vii)	Calculate the true titer at t_{max} for each serotype, $A_{i,s}(t_{max})$
887	(viii)	Calculate the measured titer using a random draw from a normal distribution
888		with mean $A_{i,s}(t_{max})$ and standard deviation σ . Under scenarios of a discrete
889		assay, the measured titer is also rounded down to the nearest integer.
890		
891	8.2. Simul	ated titers where infections did not take place
892	(i)	Randomly draw MCMC iteration
893	(ii)	Randomly divide the population of individuals who had at least one infection
894		in two: 'model fit' individuals and 'held out' individuals.
895	(iii)	Of the model fit individuals, randomly draw an individual <i>i</i>
896	(iv)	Identify the baseline titer $A_{i,s}(t_0)$ from that MCMC iteration. The true titer for
897		each serotype will be $A_{i,s}(t_0)$.
898	(v)	Calculate the measured titer for each serotype using a random draw from a
899		normal distribution with mean $A_{i,s}(t_0)$ and standard deviation σ , where σ

900		represents the measurement error for the assay. Under scenarios of a discrete
901		assay, the measured titer is also rounded down to the nearest integer.
902	(vi)	Calculate a second measured titer using a random draw from a normal
903		distribution with mean $A_{i,s}(t_0)$ and standard deviation σ . Under scenarios of a
904		discrete assay, the measured titer is also rounded down to the nearest integer.
905		
906	8.3. Differ	ent assays
907	8.3.1. Cur	rent approach
908	The current	nt approach is to see whether there is a four-fold rise between blood draws in
909	any of the	four serotypes using the discrete HI assay.
910		
911	8.3.2. 'Me	an' approach
912	This appro	each is to first calculate the mean across the four serotypes at each time point
913	and then c	ompare the mean titers across two time points to identify whether infections
914	have occu	rred or not.
915		
916	8.3.3. 'Co	ntinuous assay' approach
917	Some assa	ys give titers on a continuous scale (and not discretized like the HAI assay). In
918	this approa	ach, as with the 'Mean' approach, we initially calculate the mean titer across
919	the four se	protypes at each time point and then compare the mean titers across two time
920	points to i	dentify whether infections have occurred or not.
921		
922	8.4. Asses	sment of the different assays by time between blood draws and error in assay.

923 Using the simulation approaches set out above we obtained 10,000 individuals with pairs 924 of measured titers (with one titer for each serotype) where an infection did take place in 925 between the titer measurements and a further 10,000 individuals with pairs of 926 measurements where no infection took place. We varied the time between blood draws 927 (t_{max}) between 10 days and 400 days and the error in the assay (σ) between 0.1 and 1. For 928 each resultant dataset we used the held-out dataset (i.e., those individuals not included in 929 the model fitting) to calculate the sensitivity and specificity under each of the approaches 930 in 6.3. Each time, we also identified the cutpoint that maximized the sensitivity while 931 maintaining at least 95% specificity. We performed a separate analysis where we identify 932 cutpoints to maximize sensitivity while maintaining 99% specificity.

933

934 9. Comparison between PRNT and HI titers

935 For 1,771 blood draws, both plaque reduction neutralization tests and HIs were

936 conducted. We compare the mean PRNT log titer across the four serotypes with the mean

HI log titer from the four serotypes and fit a line through the two using linear regression.

We compared different polynomial models up to order 2 and used the best fitting one as

939 determined by AIC.

940

941 10. Comparison with Sanofi Pasteur vaccine titers

942 To explore the potential impact of the Sanofi vaccine we extracted the geometric mean

943 PRNT titers following vaccination for both seronegative and seropositive individuals who

944 were vaccinated in Latin America²¹. The extracted values for PRNT titer, 28 days after

945 the second injection are (see Table S8 in 21) are shown below:

	Seronegative at baseline		Seropositive at baseline	
	Pre vaccination	Post vaccination	Pre vaccination	Post vaccination
DENV1	5	26	278	912
DENV2	5	69	306	1050
DENV3	5	71	261	907
DENV4	5	73	73	353
Mean	5	60	228	806

947

948 The values 28 days after the third injection are also available and are 81 for those

seronegative prior to vaccination and 658 for those seropositive prior to vaccination²¹. 949

950 We plot these values on a plot of the relationship between HI titer and PRNT titer from

951 our assays (Figure 4D).

952

953 11. Ethical approval

954 The cohort protocol was approved by the institutional review boards of the Thai Ministry

955 of Public Health, the Office of the US Army Surgeon General, and the University of

956 Massachusetts Medical School. Informed consent was obtained from participants and

957 their parents/guardians. No personally identifiable information was available to the

958 researchers for the presented analysis.

959

960 12. Code availability statement

961 c++ code is available from the corresponding author on request.

962

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1064		
1065		

1066	Extended data is linked to the online version of the paper at www.nature.com/nature
1067	

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1070

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1073 D.E., L.M., I-K.Y., R.J., S.T., A.R., ran, collected and stored the cohort study results, I.R-

1074 B., J.L. and L.K. aided in interpreting results. All authors commented on and edited the

1075 paper.

1076

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1078 www.nature.com/reprints. The authors declare no competing financial interests.

1079 Correspondence and requests for materials should be addressed to

1080 henrik.salje@pasteur.fr. De-identified data used in this project is available as part of this

submission. This requires the removal of all date information. Individuals interested in

1082 accessing a full dataset with identifying information should contact the first author to

1083 obtain the necessary IRB approval.

1084

1085 **Disclaimer**: Material has been reviewed by the Walter Reed Army Institute of Research.

1086 There is no objection to its presentation and/or publication. The opinions or assertions

1087 contained herein are the private views of the author, and are not to be construed as

1088 official, or as reflecting true views of the Department of the Army or the Department of

- 1089 Defense. The investigators have adhered to the policies for protection of human subjects
- 1090 as prescribed in AR 70–25.

1092 Extended data

Serotype	N (%)
DENV1	39 (14)
DENV2	100 (37)
DENV3	72 (26)
DENV4	6 (2)
Serotype unknown	55 (20)
Total	272 (100)

Table S1. Serotype distribution

Table S1. Serotype distribution (by PCR) of detected symptomatic cases.

1095 Table S2. Parameter estimates from model fit to all study subjects (N=3,451)

Parameter	Estimate (95% CI)
Mean permanent rise in log2-titers (ω_m)	1.33 (1.18-1.48)
Variance permanent rise in log2-titers (ω_v)	1.85 (1.42-2.47)
Mean temporary rise in log2-titers (γ_m)	5.39 (5.14-5.64)
Variance temporary rise in log2-titers (γ_v)	4.54 (3.72-5.64)
Mean decay in log2-titers per day (δ_m)	0.017 (0.015-0.019)
Variance decay in log2-titers (δ_v)	0.00020 (0.00015-0.00028)
Difference in rise for infecting vs. non infecting serotype	1.16 (1.15-1.18)
(primary infection only) (η)	
Measurement error (σ - standard deviation of log2-titers)	0.49 (0.49-0.50)
DENV2 bias (χ_2) (log2-titers)	-0.85 (-0.880.81)
DENV3 bias (χ_3) (log2-titers)	-0.19 (-0.220.16)
DENV4 bias (χ_4) (log2-titers)	0.06 (0.04-0.09)
Daily force of infection in 1998 per serotype $(\bar{\lambda})$	0.00018 (0.00016-0.00019)
1997 FOI vs 1998 (β ₂)	1.11 (0.97-1.27)
1999 FOI vs 1998 (β ₂)	0.88 (0.76-1.00)
2000 FOI vs 1998 (β ₃)	0.29 (0.23-0.35)
2001 FOI vs 1998 (β ₄)	1.01 (0.88-1.17)
2002 FOI vs 1998 (β ₅)	0.43 (0.34-0.53)
Seasonality parameter 1 (δ)	0.32 (0.24-0.39)
Seasonality parameter 2 (ζ)	4.0 (3.7-4.2)

1097 Table S3. Log-odds of symptoms, hospitalization and DHF for those infected during

1098	the surveillance windows (N	N=781) as a	function of year	r of infection.
------	-----------------------------	-------------	------------------	-----------------

	Any symptoms	Hospitalization	DHF	
Intercept	-0.65	-2.09	-2.66	
	(-0.950.37)	(-2.681.63)	(-3.262.09)	
1998	Ref	Ref	Ref	
1999	0.02	0.02	-0.01	
	(-0.40-0.42)	(-1.08-0.84)	(-0.96 – 0.76)	
2000	0.04	0.03	0.03	
	(-0.58-0.66)	(-1.32-0.88)	(-15.71-1.10)	
2001	0.04	-0.05	-0.05	
	(-0.36-0.36)	(-0.73-0.67)	(-0.81-0.85)	
2002	0.03	-0.02	-0.04	
	(-0.53-0.57)	(-1.20-0.89)	(-1.39-1.04)	

Table S3. Exponentiated coefficients from logistic regression models with 95%

1102 confidence intervals in parantheses.

1103 Table S4. Regression for log-odds of symptoms, hospitalization and DHF as a

function of with age and titer

	Any symptoms		Hospitalization		DHF	
	Simple	Multivariable	Simple	Multivariable	Simple	Multivariable
Intercept	-0.62	-0.67	-2.59	-3.17	-3.11	-4.65
	(-0.800.47)	(-1.000.31)	(-2.952.30)	(-4.112.48)	(-3.562.76)	(-7.213.38)
Age 8-9	-0.03	-0.02	0.43	0.48	0.21	0.33
	(-0.36 – 0.31)	(-0.36- 0.31)	(-0.15-1.00)	(-0.10-1.08)	(-0.58-0.94)	(-0.52-1.10)
Age >9	Ref	Ref	Ref	Ref	Ref	Ref
Titer	-	0.43	-	4.03	-	7.39
		(-0.70-1.63)		(1.67-7.43)		(3.11-14.99)
Titer ²	-	-0.69	-	-7.22	-	-10.53
		(-2.30-0.73)		(-13.402.56)		(-19.113.81)

Table S4. Exponentiated coefficients from logistic regression models with 95%

1108 confidence intervals in parantheses.

1109 Table S5. Simulated results

Parameter	True value	Estimated (95%
		confidence intervals)
Mean permanent rise in titers (ω_m)	1.4	1.3 (1.1-1.5)
Variance permanent rise in titers (ω_v)	2.0	1.5 (1.0-2.3)
Mean temporary rise in titers (γ_m)	5.5	5.2 (5.0-5.5)
Variance temporary rise in titers (γ_v)	2.0	1.4 (1.0-2.0)
Mean decay in titers (δ_m)	0.02	0.02 (0.02-0.03)
Variance decay in titers (δ_v)	0.0002	0.0005 (0.0002-0.0009)
Difference in rise for infecting vs. non infecting	1.20	1.18 (1.16-1.20)
serotype (primary infection only) (η)		
Measurement error	0.50	0.49 (0.49-0.50)
DENV2 bias (χ_2)	-0.8	-0.8 (-0.70.9)
DENV3 bias (χ_3)	-0.2	-0.2 (-0.1-0.3)
DENV4 bias (χ_4)	0.05	0.04 (-0.02-0.11)
Daily force of infection in 1998 $(\bar{\lambda})$	0.0009	0.0011 (0.0009-0.0014)
Year 2 FOI vs Y1 (β_2)	1.0	0.9 (0.7-1.1)
Year 3 FOI vs Y1 (β_3)	0.2	0.2 (0.1-0.2)
Year 4 FOI vs Y1 (β_4)	1.0	1.0 (0.8-1.3)
Year 5 FOI vs Y1 (β_5)	0.4	0.2 (0.1-0.5)
Seasonality parameter 1 (δ)	0.4	0.4 (0.3-0.5)
Seasonality parameter 2 (ζ)	4.0	3.8 (3.5-4.1)

	Number of subclinical infections	370	360 (350-370)
1110			

- 1112 Table S6. Simulated results in scenario with different rises for symptomatic and non-
- 1113 symptomatic infections

Parameter	True value	Estimated (95%
		confidence intervals)
Mean permanent rise in titers (ω_m)	1.4	1.4 (1.2-1.6)
Variance permanent rise in titers (ω_v)	2.0	1.3 (0.9-2.0)
Mean temporary rise in titers (γ_m)	5.5 for	5.2 (4.9-5.5)
	symptomatic	
	4.5 for	
	subclinical	
Variance temporary rise in titers (γ_v)	2.0	2.0 (1.4-2.8)
Mean decay in titers (δ_m)	0.02	0.024 (0.019-0.032)
Variance decay in titers (δ_v)	0.0002	0.0005 (0.0002-0.0011)
Difference in rise for infecting vs. non infecting	1.20	1.17 (1.16-1.20)
serotype (primary infection only) (η)		
Measurement error	0.50	0.50 (0.49-0.50)
DENV2 bias (χ_2)	-0.8	-0.76 (-0.690.83)
DENV3 bias (χ_3)	-0.2	-0.22 (-0.0.150.28)
DENV4 bias (χ_4)	0.05	0.08 (0.01 – 0.15)
Daily force of infection in 1998 $(\bar{\lambda})$	0.0009	0.001 (0.0009-0.0013)
Year 2 FOI vs Y1 (β_2)	1.0	0.9 (0.8-1.2)
Year 3 FOI vs Y1 (β_3)	0.2	0.2 (0.1-0.3)

Year 4 FOI vs Y1 (β_4)	1.0	1.2 (0.9-1.6)
Year 5 FOI vs Y1 (β_5)	0.4	(0.4 (0.2-0.7)
Seasonality parameter 1 (δ)	0.4	0.4 (0.2-0.5)
Seasonality parameter 2 (ζ)	4.0	3.8 (3.4-4.2)
Number of subclinical infections	340	320 (310-330)

Table S7. Parameter estimates when incorporating school-specific force of infection

¹¹¹⁷ parameters across all study subjects (N=3,451)

Parameter	Base model	Extra school-specific
	(95% CI)	parameters (95%CI)
Mean permanent rise in log2-titers	1.33 (1.18-1.48)	1.33 (1.19-1.48)
(ω_m)		
Variance permanent rise in log2-titers	1.85 (1.42-2.47)	1.90 (1.46-2.54)
(ω_v)		
Mean temporary rise in log2-titers (γ_m)	5.39 (5.14-5.64)	5.42 (5.19-5.65)
Variance temporary rise in log2-titers	4.54 (3.72-5.64)	4.07 (3.35-5.12)
(γ_v)		
Mean decay in log2-titers per day (δ_m)	0.017 (0.015-0.019)	0.016 (0.015-0.018)
Variance decay in log2-titers (δ_v)	0.00020 (0.00015-	0.00020 (0.00015-
	0.00028)	0.00028)
Difference in rise for infecting vs. non	1.16 (1.15-1.18)	1.16 (1.15-1.17)
infecting serotype (primary infection		
only) (η)		
Measurement error (σ - standard	0.49 (0.49-0.50)	0.49 (0.49-0.50)
deviation of log2-titers)		
DENV2 bias (χ_2) (log2-titers)	-0.85 (-0.880.81)	-0.85 (-0.870.83)
DENV3 bias (χ_3) (log2-titers)	-0.19 (-0.220.16)	-0.19 (-0.220.18)
DENV4 bias (χ_4) (log2-titers)	0.06 (0.04-0.09)	0.06 (0.04-0.07)

Seasonality parameter 1 (δ)	0.32 (0.24-0.39)	0.32 (0.25-0.39)
Seasonality parameter 2 (ζ)	4.0 (3.7-4.2)	4.0 (3.8-4.3)
Number of augmented infections	1,149 (1,135-1,163)	1,151 (1,137-1,165)

1121 Figure S1





Figure S1. Biphasic and exponential decay curves fitted to HI antibody measurements





Figure S2. (A) Variability in titer responses. Violin plots showing median (black square),
25% and 75% quantiles (thick black line) and 95% distribution (in grey) of net titer rise at

1130 different time points following infection (N=1,420) (**B**) Estimated underlying differences

1131 across serotypes in the measurement of antibody levels by hemagglutination inhibition

assay over and above that attributable to infection (DENV1 is reference) with 95%

1133 credible intervals (fitted to data from 140,612 titer measurements). (C) Mean estimated

1134 error in the hemagglutination inhibition assay estimated with 95% credible intervals

using our model results (grey) and empirically derived (blue) from 795 repeated

1136 measurements on the same serum compared to that previously empirically derived

1137 estimated for plaque reduction neutralization tests (PRNTs) (blue).

1139 Figure S3. Serotype distribution for primary infections



1141 **Figure S3.** Distribution of serotypes by year comparing the detected symptomatic

1142 infections by PCR and the augmented primary infections where we could confidently

assign the serotype (>50% of model iterations inferring the same serotype). We could

1144 confidently assign the serotype in 60% of instances.

Figure S4



Figure S4. Serotype distribution for detected symptomatic primary infections and

augmented subclinical primary infections where the infecting serotype could be

1150 confidently assigned (>50% of model iterations inferring the same serotype).

1152 **Figure S5**



1153

Figure S5. Distribution of serotypes by year comparing the detected symptomatic

1155 infections by PCR and the augmented primary infections using a more stringent cutoff

1156 that >75% of model iterations infer the same serotype. In this scenario we could

1157 confidently assign the serotype in 32% of instances.



1160 Figure S6. Comparison of results using time varying cox proportional hazards model 1161 (dashed line) with that from logistic regression (solid line) for the annualized probability 1162 of (A) infection, (B) developing any symptoms, (C) being hospitalized and (D) 1163 developing DHF as a function of the mean measured antibody titer across all serotypes at 1164 the time of exposure using titer data from all study subjects (N-3,451). The open circles 1165 on the left represent primary infections (i.e., those with no detectable titers to any 1166 serotype prior to exposure). The shaded regions represent 95% bootstrap confidence 1167 intervals. To calculate probabilities, the relative hazards from the cox model are 1168 multiplied by the baseline hazard for those with measured titers of 0 (calculated as 1169 proportion of person-time with an infection time among those with measured titers of 0).

1171 Figure S7





1173 **Figure S7.** Ability of modelled relationship between measured HI titer and risk of DHF

1174 to identify those with DHF using those with DHF compared to randomly selected

1175 matched controls from individuals in the cohort who had detectable titers at the same

1176 time (N=36 with DHF with the same number of matched controls).

1178 **Figure S8**



1181 Figure S8. Probability of infection and disease as a function of mean titer across the four 1182 types at the time of infection. (A) Annualized probability of infection by mean antibody 1183 titer across all types at the time of infection (N=3,451). (B) For those infected during the 1184 surveillance windows, the probability of developing any symptoms as a function of mean 1185 titer (N=781). (C) For those infected during the surveillance windows, the probability of 1186 being hospitalized (N=781). (D) For those infected during the surveillance windows, the 1187 probability of developing DHF as a function of mean titer (N=781). The open circles on 1188 the left represent primary infections.


1190

Figure S9. Among those infected, relationship between PRNT titer and probability of outcome. For those infected during the surveillance windows (N=781), the probability of developing any symptoms as a function of mean PRNT titer. (**C**) For those infected, the probability of being hospitalized. (**D**) For those infected, the probability of developing

1195 DHF as a function of mean PRNT titer. The open circles on the left represent primary

1196 infections. The shaded region represents 95% confidence intervals.

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1204 Figure S10. (A) Proportion of cohort who are naïve as a function of time. (B) Proportion





Figure S11. Proportion of cohort with titers above risk zone (i.e., greater than 3) as a

1210 function of time (**A**) and age (**B**).

1213 **Figure S12. ROC**



Figure S12. The ROC for different assay approaches and time between blood draws calculated from 100,000 simulated titer responses. (A) Single serotype assay – if HIs are conducted for just a single serotype at two time points. (B) HIs conducted against all four serotypes. Infections are considered to occur if the ratio of any of the four titers at time point 2 versus time point 1 is greater than the threshold value. (C) HIs conducted against all four serotypes. Infections are considered to occur if the ratio of the mean of the four titers at time point 2 versus the mean at time point 1 is greater than the threshold value.





1225 Figure S13. Optimization of assays in detection of events where specificity is maintained 1226 at >95%. We explore the performance of three different assay testing protocols: current 1227 practice where infection events are defined as a rise above a cut-point in any serotype 1228 across two blood draws (A), 'mean approach' where the mean across all serotypes is first 1229 calculated before comparing across time points (**B**), 'mean approach' where titers are 1230 available on a continuous scale (C). For each protocol, we identify the optimal cut-point 1231 for a range of assay measurement errors from 100,000 simulated titers based on the fitted 1232 titer responses from infections in our study population, that maintains a specificity of 1233 >95% (top row). We then calculate the sensitivity of the approach for different time 1234 intervals between blood draws using 50% held out data (bottom row). 1235

1237



1238 Figure S14. Optimization of assays in detection of events where specificity is 1239 maintained at >99%. We explore the performance of three different assay testing 1240 protocols: current practice where infection events are defined as a rise above a cut-point 1241 in any serotype across two blood draws (A), 'mean approach' where the mean across all 1242 serotypes is first calculated before comparing across time points (**B**), 'mean approach' where titers are available on a continuous scale(C). For each protocol, we identify the 1243 1244 optimal cut-point for a range of assay measurement errors from 100,000 simulated titers 1245 based on the fitted titer responses from infections in our study population, that maintains 1246 a specificity of >99% (top row). We then calculate the sensitivity of the approach for 1247 different time intervals between blood draws using 50% held out data (bottom row). 1248



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1251 Figure S15. Clustering of symptomatic (N=274) and subclinical cases (mean N=507 1252 across 100 reconstructed datasets) by school by time and serotype. (A) Probability of 1253 observing an augmented subclinical infection (irrespective of serotype) occurs at different 1254 time intervals within the same school of a detected symptomatic case relative to the 1255 probability of observing an augmented subclinical infection occurring in a different 1256 school in that same time interval. (B) For augmented primary infections that are 1257 consistently of the same serotype (defined as >50% of augmented datasets have a primary 1258 infection in the same individual caused by the same serotype in the same six-month time 1259 window). Probability that an augmented primary infection that occurs within a fixed time 1260 window of a PCR-confirmed case and in the same is of the same serotype relative to the 1261 probability that an augmented primary infection that occurs within the same time window 1262 in a different school is of the same serotype. Note that the modelling framework can only 1263 allow differentiation of serotypes for primary infections. Cross-reaction prevents 1264 differentiation in post-primary infections. Overall, 60% of primary infections have a 1265 consistent serotype for a primary infection across augmented datasets. Each boxplot

- 1266 presents the 2.5%, 25%, 75% and the 97.5% quantiles of the distribution as well as the
- 1267 mean.