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Research Articles

On the risk of severe dengue during secondary infection: A systematic review coupled with mathematical modeling

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ABSTRACT

Background & objectives: The present study aimed to systematically quantify the well known risk of severe dengue during secondary infection in literature and to understand how epidemiological mechanisms of enhancement during the secondary infection influence the empirically estimated risk of severe dengue by means of mathematical modeling.

Methods: Two conditional risks of severe dengue, i.e. symptomatic illness and dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), given secondary infection were explored based on systematically searched prospective studies. A two-strain epidemiological model was employed to simulate the transmission dynamics of dengue and to identify the relevant data gaps in empirical observations.

Results: Using the variance-based weighting, the pooled relative risk (RR) of symptomatic illness during secondary infection was estimated at 9.4 [95% confidence interval (CI): 6.1–14.4], and similarly, RR of DHF/DSS was estimated to be 23.7 (95% CI: 15.3–36.9). A variation in the RR of DHF/DSS was observed among prospective studies. Using the mathematical modeling technique, we identified the duration of cross-protective immunity as an important modulator of the time-dependent behaviour of the RR of severe dengue. Different epidemiological mechanisms of enhancement during secondary infection yielded different RR of severe dengue.

Interpretation & conclusion: Optimal design of prospective cohort study for dengue should be considered, accounting for the time-dependence in the RR during the course of dengue epidemic. It is critical to statistically infer the duration of cross-protective immunity and clarify how the enhancement influences the epidemiological dynamics during secondary infection.

Key words Dengue; dengue hemorrhagic fever; enhancement; epidemiology; *Flaviviridae*; interference; mathematical model; susceptibility

INTRODUCTION

Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are caused by dengue virus (DENV) that belongs to the family of *Flaviviridae*, consisting of four closely related serotypes[1]. The virus is transmitted by mosquito vector *Aedes* spp including *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae), contributing to high disease burden in tropical and sub-tropical areas where the vectors are abundant[2]. Over the decades, the geographic area with ongoing DENV transmission has been magnified[3]. Although the pathogenesis of "severe dengue", namely, the symptomatic infection, DF, DHF and DSS (especially the last two) has yet to be fully clarified, a number of apparent risk factors for DHF and DSS including secondary infection with heterologous strain[4–5] and different virulence and susceptibility[6–8] has been reported in literature.

The elevated risk of severe dengue during secondary infection (as compared to primary infection) has been originally identified from epidemiological observations[9]. During the secondary infection, the so-called antibody dependent enhancement (ADE) is known to be observed and is considered responsible for the pathophysiological development of DHF or the vascular permeability syndrome[4]. While a number of prospective studies have been conducted to observe the elevated risk of severe dengue during secondary (and tertiary) infection, and also a review study has taken place elsewhere[10], we have yet to understand the link between ADE (or other biological mechanisms of severe dengue) and the data generating process of epidemiologically observed severe dengue. However, epidemiological mechanisms of severe dengue during secondary infection have yet to be clarified: it has not been fully understood how the enhancement influences the epidemiological dynamics, *e.g.* is severe den-

gue caused by: (i) simply increasing the conditional probability of severe clinical manifestations (given infection); (ii) increasing susceptibility of host who was previously exposed to other serotype(s); or (iii) delaying recovery from infection during secondary infection and extending infectious period?[11] To appropriately describe and interpret the epidemiology of severe dengue, epidemiological studies should be designed based on a firm understanding of the transmission dynamics.

Whereas, the causal relationship between DHF/DSS and secondary infection has been well demonstrated in literature, it is fruitful to understand how the secondary infection leads to an increase in severe dengue in an epidemiological manner. The purposes of the present study are two-folds. First, we aimed to quantitatively analyze the known "risks" of severe dengue during secondary infection by systematically reviewing prospective observational studies at a population level, thereby characterizing the elevated risks of severe dengue in literature. Second, we exploited a simple mathematical model with two interacting serotypes (and enhancement), simulating epidemics caused by two serotypes and measuring differential impact of enhancement on the relative risk estimates of severe dengue during secondary infection.

MATERIAL & METHODS

The present study consists of two major steps, (i) a systematic review and meta-analysis of published literature on severe dengue; and (ii) mathematical modeling and scenario analysis that intend to clarify the data gaps and help consider appropriate study design in the future. As for the former, this systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[12].

Search strategy

Prospective studies containing data on enhancement were retrieved from the Medline (PubMed) and Web of Science electronic databases on 1 March 2013. We used the following free text search terms in 'All fields': 'dengue', 'enhancement' OR 'secondary infection', 'cohort' OR 'prospective study' and their combinations.

The search was limited to studies published after 1984, i.e. subsequent to the first long-term dengue prospective cohort study in Thailand[5]. Additional relevant studies identified by authors were manually retrieved from other databases.

Study selection

To select studies, all titles, identified by the search strategy, were screened by two authors independently (K.M. and H.N.). Subsequently, abstracts of only relevant titles were further screened for review of eligibility, and articles were selected for closer examination of full-text if it turns out to be a prospective (cohort) study of dengue. For clarity, retrospective and clinical studies (including prospective ones with recruitment only at medical facilities) were excluded. Eligible articles must explicitly define secondary infection (*e.g.* having been confirmed as immune to a single serotype before an epidemic of the other serotype) as well as primary infection (*e.g.* having been confirmed as fully susceptible before an epidemic). Multiple reports of the same dataset (i.e. the same geographic area at the same time) were jointly assessed.

Ascertainment of primary infection and secondary infection

During the systematic review, we focused on two risks of severe dengue during secondary infection, including the conditional risk of symptomatic illness and the conditional risk of DHF (inclusive of DSS), both given DENV infection. Relative risk (RR) was calculated as the proportion of severe dengue during secondary infection divided by the same proportion during primary infection. DENV infection was identified by virologic testing or serologic evidence of infection. Primary and secondary infections have been defined as infections among those with previous sero-negative and sero-positive states prior to the infection event, respectively. For simplicity, we discarded the serotype information and the sequence of infections with different serotypes.

Data extraction

We extracted the information regarding primary/secondary infection, asymptomatic/symptomatic infection and DHF/DSS. If reported, we also analyzed additional results from the serological survey (including the timing of the survey relative to the epidemic time), absenteeism survey, and attendance to fever clinic. All the datasets were summarized in a standardized form.

Meta-analysis

RRs of the above mentioned two outcomes were extracted from each eligible study, and subsequently, the pooled estimate was obtained. We used the inverse of the variance of RR as the weight of each study when calculating the pooled estimate. As an alternative method, we

also computed the pooled effect size by employing the random effects model. Statistical heterogeneity was assessed by Q statistic as well as I^2 statistic (representing the extent of the degree of variation). All statistical data were analyzed using a statistical software JMP version 9.0.0 (SAS Institute Inc., Cary, NC, USA).

Modeling method

As a second part of the present study, we employed a deterministic epidemiological model that describes the time-dependent transmission dynamics of dengue with two distinct serotypes. Specifically, we tackled two questions: (i) how the timing of observation influences the estimate of effect size (i.e. RR of severe dengue during secondary infection) during the course of epidemics, and (ii) how different epidemiological scenarios with different mechanisms of enhancement influence the RR estimate. As for the former, we computed the RR of severe dengue during secondary infection by varying the mean duration of cross-protective immunity and the time-lag between the invasion of one serotype and that of the other serotype. For the latter question, we compared the dynamics under three hypothetical scenarios, i.e. the scenario in which: (a) the conditional risk of severe dengue is elevated given secondary infection, (b) susceptibility during secondary infection is increased, and (c) the duration of infectious period during secondary infection is extended.

Figure 1 shows the compartment that we used to compute the epidemiological dynamics of dengue. Each state of infection is represented by single compartment in Fig. 1 with arrows indicating the direction and rate of transition. Since, we considered epidemics in a short-time scale, the background demography of human host, like birth and death, was ignored. First and second letters for each compartment stand for the state of infection with

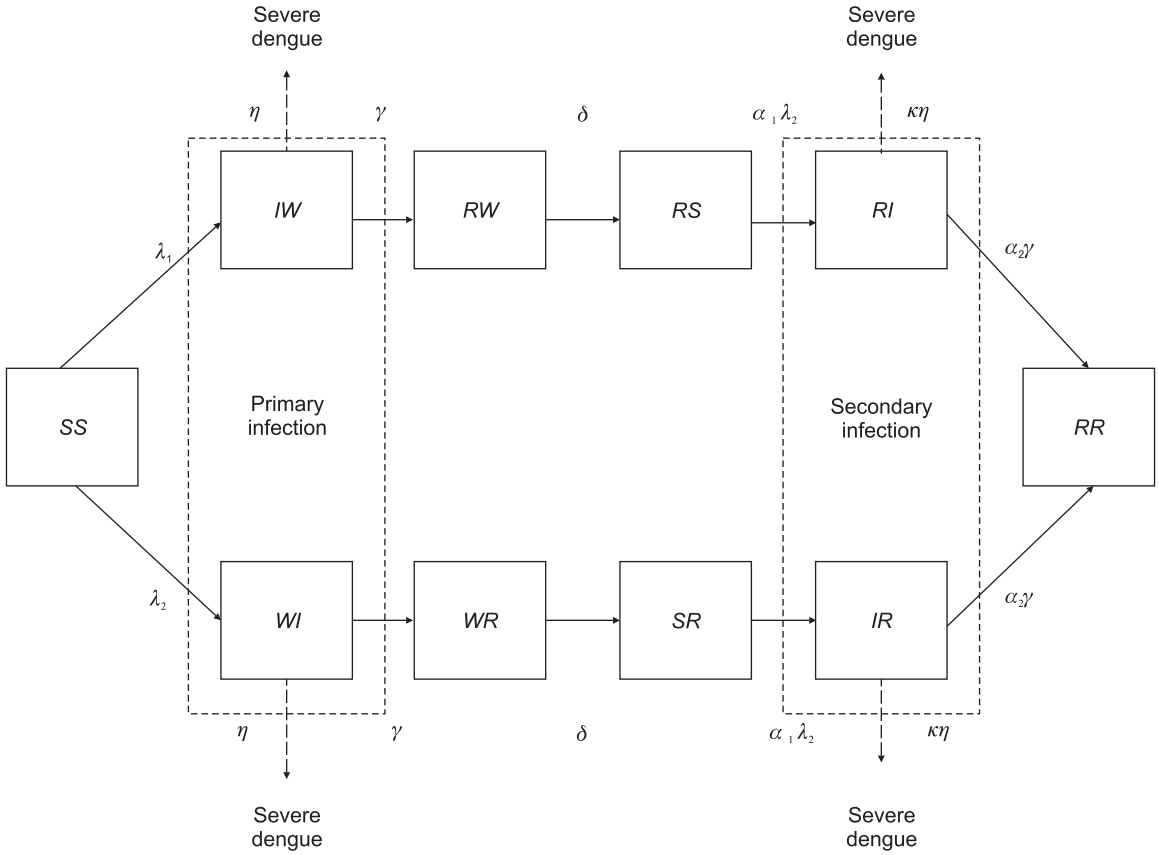

Fig. 1: Transmission dynamics of dengue with two serotypes. A compartmental model is employed. First and second letters for each compartment stand for the state of infection with respect to serotype 1 and serotype 2, respectively. *S* stands for susceptible, *I* stands for infectious, *R* represents recovered and immune, and *W* represents protected state due to cross-protective immunity. Thus, for example, *RI* represents the host who recovered from infection with serotype 1 while being infectious with serotype 2. The transition rates are written on the arrows. λ_i, the force of infection with serotype i (for $i = 1, 2$); $1/\gamma$, the mean duration of infectious period; $1/\delta$, the mean duration of cross-protective immunity; η, the conditional hazard of severe dengue given dengue virus infection; α_1, relative susceptibility during secondary infection compared to primary infection; $1/\alpha_2$, relative infectious period during secondary infection compared to primary infection; κ, relative conditional hazard of severe dengue during secondary infection given dengue virus infection.

serotype 1 and serotype 2, respectively. Although, there are four distinct serotypes of dengue virus in reality, we considered only two theoretical serotypes in modeling exercise, because our outcome (i.e. RR of severe dengue during secondary infection) for a two-serotype setting mirrors that of a realistic four-serotype setting. *S* stands for susceptible, *I* stands for infectious, *R* represents recovered and immune state, and *W* represents a protected state due to a short-lasting cross-protective immunity (i.e. when a host experiences infection with a single serotype, it is assumed that the infected host is protected from infection with other serotypes for a short period of time[13–14]). Thus, for example, *RI* represents the host who recovered from infection with serotype 1 while being infectious with serotype 2. It should be noted that we ignored the dynamics of mosquito vector in the following model (while incorporating the delay in secondary transmission due to the extrinsic incubation period into the model by extending the generation time), because the detailed population dynamics of mosquitoes is out of the scope of the present study and we focus on the natural history of dengue in the human host.

Let $1/\gamma$ be the mean duration of infectious period and $1/\delta$ be the mean duration of cross-protective immunity. It should be noted that for simplicity, we assumed that all parameters are identical between serotypes (i.e. the dynamics is symmetric). The transmission dynamics is described by the system of ordinary differential equations:

$$\begin{aligned}
&\frac{dSS}{dt} = -(\lambda_1 + \lambda_2)SS, \quad \frac{dWI}{dt} = \lambda_2 SS - \gamma WI,\\
&\frac{dIW}{dt} = \lambda_1 SS - \gamma IW, \quad \frac{dWR}{dt} = \gamma WI - \delta WR,\\
&\frac{dRW}{dt} = \gamma IW - \delta RW, \quad \frac{dSR}{dt} = \delta WR - \alpha_1\lambda_1 SR, \quad \text{... (1)}\\
&\frac{dRS}{dt} = \delta RW - \alpha_1\lambda_2 RS, \quad \frac{dIR}{dt} = \alpha_1\lambda_1 SR - \alpha_2\gamma IR,\\
&\frac{dRI}{dt} = \alpha_1\lambda_2 RS - \alpha_2\gamma RI, \quad \frac{dRR}{dt} = \alpha_2\gamma(RI + IR),
\end{aligned}$$

where, λ_i represents the force of infection with serotype i (for $i = 1$ or 2) (or the rate at which susceptible individuals acquire infection with serotype i) calculated as:

$$\begin{aligned}
\lambda_1 &= \beta(IW + IR),\\
\lambda_2 &= \beta(WI + RI),
\end{aligned} \quad \text{... (2)}$$

where, β scales the rate of transmission. Other two parameters in system (1) during secondary infection, α_1 and $1/\alpha_2$ reflect the enhancement in epidemiological dynamics, each interpreted as follows: α_1, the relative susceptibility during secondary infection compared to primary infection, and $1/\alpha_2$, the relative infectious period during secondary infection compared to that during primary infection. Let η be the conditional hazard of severe dengue given infection. Cumulative numbers of severe dengue during primary and secondary infections are calculated as:

$$\begin{aligned}
\frac{dX_1}{dt} &= \eta(IW + WI),\\
\frac{dX_2}{dt} &= \kappa\eta(RI + IR),
\end{aligned} \quad \text{... (3)}$$

where, κ is the conditional relative hazard of severe dengue given secondary infection. Let $Y(t)$ represent the number of hosts who have experienced only primary infection, i.e.

$$Y(t) = N - SS(t) - RI(t) - IR(t) - RR(t), \quad \text{... (4)}$$

where, N represents the total population size which is assumed to be a constant. Considering that an epidemiological study samples the data from the population at the most recent time T, the relative risk estimate that we obtain is calculated as:

$$r(T) = \frac{X_2(T)\,[N - SS(T)]}{X_1(T)\,[RI(T) + IR(T) + RR(T)]}. \quad \text{... (5)}$$

The parameter values that we used for numerical solutions are shown in Table 1[13,15–17].

We examined the sensitivity of RR in (5) to model parameters and initial conditions for two different scenarios. In both scenarios, we consider an epidemic in the population of $N = 1{,}000{,}000$ individuals in which N-1 are initially fully susceptible and 1 is newly infected with serotype 1 at time $t = 0$. In the first scenario, it is assumed that secondary infection induces only the elevated risk of severe clinical manifestations at 10 times as compared with that during primary infection, and the mean duration of cross-protective immunity is allowed to vary from 0 to 360 days. During the course of the epidemic, an infected individual with serotype 2 is introduced to the community at variable timings; before the epidemic peak (at Day 50), nearby the epidemic peak (Day 72) and during the declining phase of the epidemic (Day 100). We can then measure how $r(t)$ varies as a function of epidemic time. In the second scenario, the epidemiological mechanism of enhancement is varied, while the time to introduce a single infected individual with serotype 2 is fixed at Day 72. We can measure $r(t)$ as a function of time for

Table 1. Parameter values for an epidemiological model of dengue

Parameter's interpretation	Notation	Baseline value	Reference
Mean duration of cross-protective immunity	$1/\delta$	180 (days)	[13]
Relative susceptibility during secondary infection compared to primary infection	α_1	≥1 (See main text)	[assumed]
Relative infectious period during secondary infection compared to primary infection	$1/\alpha_2$	≥1 (See main text)	[assumed]
Mean duration of infectious period	$1/\gamma$	10 (days)	[15]
Basic reproduction number of serotype i	R_i	3*	[16–17]
Relative conditional hazard of severe dengue during secondary infection given infection	κ	≥1 (See main text)	[assumed]
Conditional hazard of severe dengue given infection	η	1.2×10^{-2}	[assumed]
Force of infection with serotype i	λ_i	(As calculated in the main text)	

*The transmission coefficient, β was calculated from the relationship $\beta_i = R_i\gamma$. We ignored the serotype-specificity (*e.g.* difference in γ) in simulations.

three possible types of enhancement, i.e. (a) $\kappa = 10$, (b) $\alpha_1 = 10$, or (c) $1/\alpha_2 = 10$. We also examined the same types of enhancement with different level— (a) $\kappa = 5$, (b) $\alpha_1 = 5$, or (c) $1/\alpha_2 = 5$. Subsequently, we investigated how $r(t)$ is scaled by κ, α_1 and $1/\alpha_2$ at the end of the epidemics of both serotypes. A differential equation solver, Berkeley Madonna version 8.0.1 (Robert Macey and George Oster, CA, USA) was used to numerically simulate the epidemics.

RESULTS

Reviewed literature

Of the 46 titles that were initially identified, 37 abstracts were assessed for eligibility, of which 22 were excluded. In total, 15 full length articles were assessed for eligibility (Fig. 2). Of these, eight studies were determined to be eligible and included in this systematic review (Table 2)[5, 18–25]. One of the eight studies was split into two original research articles in a single volume of a periodical[21–22], and thus, hereafter we combined two articles into a single study. Of the excluded 22 abstracts, 20 articles were clinical studies (*e.g.* conducted at healthcare facilities), one article was retrospective study, and one article was a mathematical modeling study. Of the excluded seven full length reports, six articles were clinical studies enrolling participants at hospital settings only[26–31] and one article was a retrospective study[32].

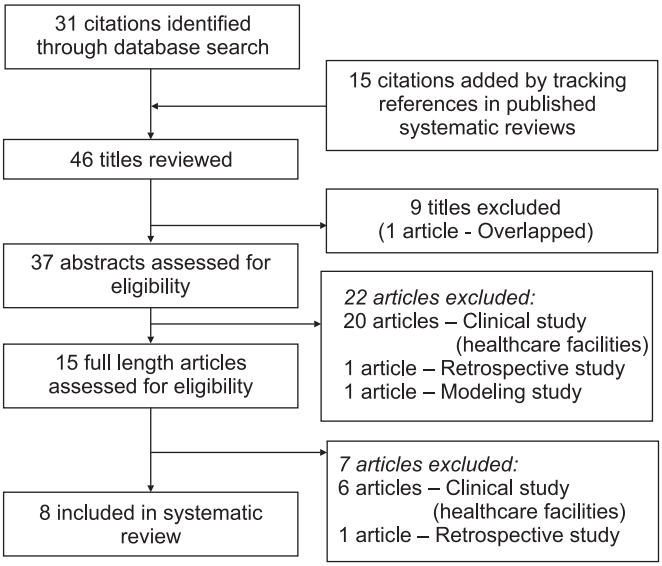

Fig. 2: Flow diagram of study selection.

Secondary infection and severe dengue in literature

Of the included eight cohort studies, five studies reported RR of symptomatic illness and DHF/DSS during secondary infection compared to primary infection. The reported estimates are summarized in Fig. 3 with the RR of symptomatic illness and DHF/DSS ranging from 1.9 to 14.3 and from 1.4 to 58.1, respectively. There have been multiple reports that measured the association between secondary infection and DSS, but DSS during primary infection was too scarce and did not permit precise calculation of RR. For this reason, we grouped DHF and DSS into a single category in all of the following results. Using the variance-based method, the pooled estimate of RR of symptomatic illness during secondary infection was 9.4 [95% confidence interval (CI): 6.1–14.4], and similarly, RR of DHF/DSS was 23.7 (95% CI: 15.3–36.9). The pooled estimates using the random effects model were not significantly different: RR of symptomatic illness and DHF/DSS were 5.9 (95% CI: 0.99–35.7) and 15.2 (95% CI: 1.7–137.1), respectively, although the lower bound for symptomatic illness was slightly below unity. A very little heterogeneity was identified for the symptomatic

Table 2. Available epidemiological information other than symptomatic illness and dengue hemorrhagic fever and the timing of serological survey in eight selected prospective studies of dengue virus infection

Country	First author reference	Absenteeism*	Attendance to fever clinic**	Relative timing†	Duration‡
Thailand	Sangkawibha *et al*[5]	No	Yes	After epidemic	Seemingly < 3 months
Thailand	Burke *et al*[18]	Yes	Partly yes	During and after epidemic	Seemingly < 3 months
Myanmar	Thein *et al*[19]	No	Yes	After epidemic	NA
Indonesia	Graham *et al*[20]	No	Yes	During epidemic	0 month
Thailand	Endy *et al*[21–22]	Yes	Partly yes	During and after epidemic	Seemingly < 3 months
Indonesia	Porter *et al*[23]	Yes	No	During and after epidemic	< 3 months
Nicaragua	Balmaseda *et al*[24]	Yes	Partly yes	After epidemic	Seemingly < 3 months
Thailand	Endy *et al*[25]	Yes	Partly yes	During and after epidemic	Seemingly < 3 months

*Conducted the survey of children's absenteeism during dengue epidemic; **Conducted the survey of febrile individuals (*e.g.* enforcing people to visit outpatients upon onset of fever); †The timing of serological sampling relative to the epidemic; ‡The time lag between the end of epidemic and serological survey.

illness (Fig. 3a) with $Q = 3.8$ and $I^2 = 0\%$, while a variation in the RR of DHF/DSS was seen (Fig. 3b) with $Q = 13.1$ and $I^2 = 69.4\%$.

Table 2 shows additional information of our interest during secondary infection in eight cohort studies. Of the eight studies, routine surveillance of absenteeism was conducted in five studies (62.5%), while the attendance to fever clinic was observed in seven studies (87.5%). As for the timing of serological survey relative to the epidemic, four studies (37.5%) obtained samples "during and after epidemic" and three studies (37.5%) did so "after epidemic" only. The time lag from the epidemic to serological survey was most commonly <3 months in five studies (62.5%).

Simulated epidemics with two serotypes

Figure 4 shows the relative risk estimates (of severe dengue which can be interpreted as symptomatic illness, DHF/DSS or other form of severe manifestation) by varying the time to introduce serotype 2 during the course of the serotype 1 epidemic and also by varying the mean duration of cross-protective immunity. It is remarkable that the RR varies as a function of time, and it reaches to the stable state only after the end of epidemics of both serotype 1 and 2. Figures 4 (a), (c) and (e) indicate that the timing to introduce serotype 2 has only limited impact on the time-dependency of the RR. However, Figs. 4 (b), (d) and (f) clearly demonstrate that the time-dependent behaviour of RR, $r(T)$ is sensitive to the duration of cross-protective immunity.

In Fig. 5, we examined $r(T)$ for three possible types of enhancement, i.e. when (a) the conditional risk of severe illness was elevated, (b) susceptibility to secondary infection was elevated, and (c) the infectious period during secondary infection is extended. Three different aspects are notable. First, as can be expected from Fig. 4, the time-dependent behaviour of $r(T)$ is sensitive to the duration of cross-protective immunity. Second, although the enhancement types (a) and (c) lead to similar equilibrium at the end of the epidemic of both serotypes, it is remarkable that the enhancement in susceptibility finds a rather different equilibrium value. That is, although the increase in susceptibility has been a common assumption (or interpretation) for describing the epidemiological mechanism of enhancement in various published studies[11, 33], the elevated susceptibility does not lead to an increase in $r(T)$ and even leads to the relative risk below unity. Third, for all three types of enhancement, the equilibrium value of the relative risk is different from the scale of the enhancement in the corresponding parameter. For instance, Figs. 5 (a), (c) and (e) magnified one of parameters by 10 times, but the equilibrium $r(T)$ value takes approximately 5 for (a) and (c), and 1 for (b), respectively. Figures 5 (b), (d) and (f) multiply 5 to one of the parameters, but $r(T)$ is stable approximately at 3.5 for (a) and (c), and 1 for (b), respectively. Figure 6 shows the relationship between $r(T)$ value at an equilibrium (i.e. at the end of epidemics of both serotypes) and the relative change in one of the parameters that were assumed to govern the enhancement during secondary infection. Again, it is remarkable that an increase in susceptibility does not lead to an observation of increase in the relative risk of severe dengue during secondary infection.

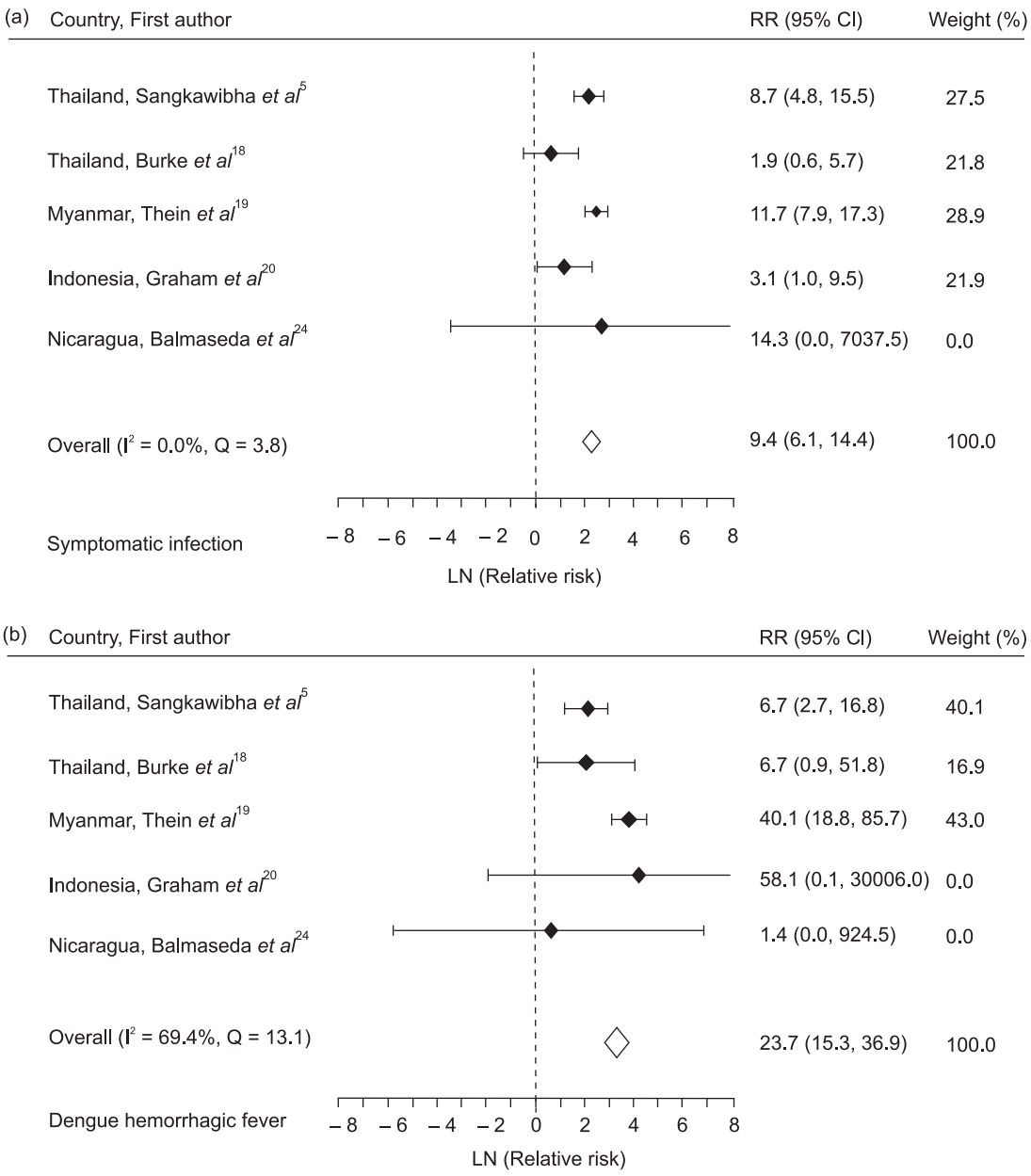

Fig. 3: Relative risk of: (a) symptomatic illness, and (b) dengue hemorrhagic fever or dengue shock syndrome during secondary infection. Note that logarithmic scale is employed for the horizontal axis, i.e. relative risk =1 corresponds to LN (relative risk = 0). The whiskers extend out to lower and upper 95% confidence intervals. Pooled estimate using the inverse of the variance of RR is shown as the diamond with both ends extending to the lower and upper 95% confidence intervals.

DISCUSSION

The present study combined two epidemiological approaches, i.e. systematic review and mathematical modeling, to understand known epidemiological risks of severe dengue during secondary infection and clarify relevant data gaps and pitfalls in measuring the RR of severe dengue during secondary infection by means of mathematical modeling. In the systematic review and meta-analysis, we examined two common outcomes of severe dengue, namely the RR of symptomatic illness and DHF/DSS during secondary infection. A variation in the estimated RR of DHF/DSS was observed between studies. In addition, we identified that many serological surveys took place during or shortly after an epidemic season. Using an epidemiological model, we identified the duration of cross-protective immunity as an important modulator of the time-dependent behaviour of the RR of severe dengue. Differently assumed epidemiological mechanisms of enhancement during secondary infection

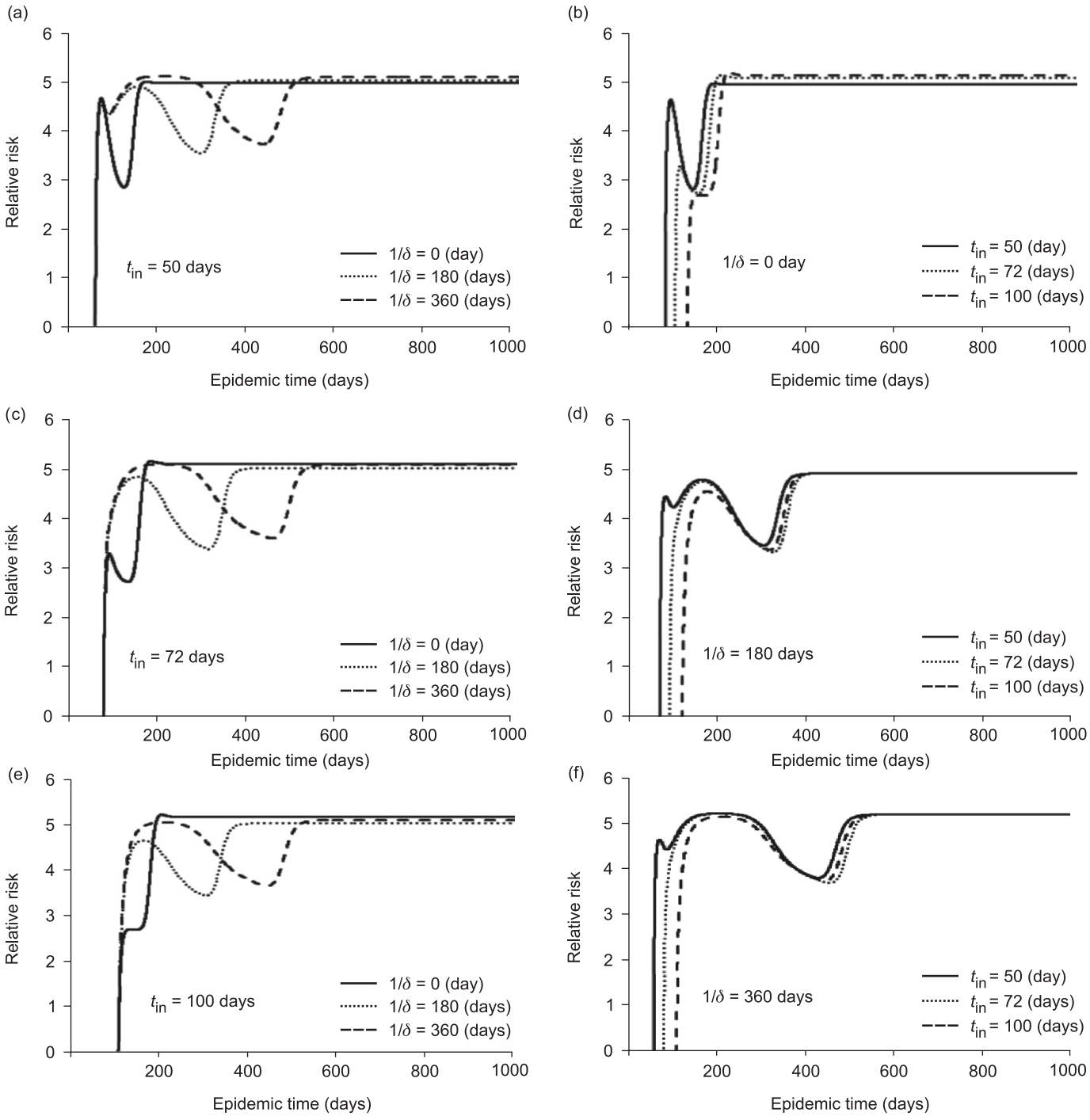

Fig. 4: Estimated relative risk of severe dengue during secondary infection as a function of time. Sensitivity of relative risk (RR) of severe dengue (*e.g.* DHF/DSS) to different lengths of cross-protective immunity following a primary infection ($1/\delta$) and different time lags between the invasion of 1 serotype and that of the other serotype (t_{in}). Figures (a), (c) and (e) examine the sensitivity of RR to different mean durations of cross-protective immunity ($1/\delta$ ranging from 0 to 360) with the introduction of second serotype at t_{in} = 50 days, t_{in} = 72 days and t_{in} = 100 days, respectively, from the start of the epidemic with serotype 1. Figures (b), (d) and (f) examine the sensitivity of RR to different time lags between the invasion of one serotype and that of the other serotype (t_{in} ranging from 50 to 100) with the fixed mean durations of cross-protective immunity at $1/\delta$ = 0 day, 180 days and 360 days, respectively. For all scenarios, it is assumed that secondary infection induces only the elevated risk of severe clinical manifestations at 10 times as compared with during primary infection).

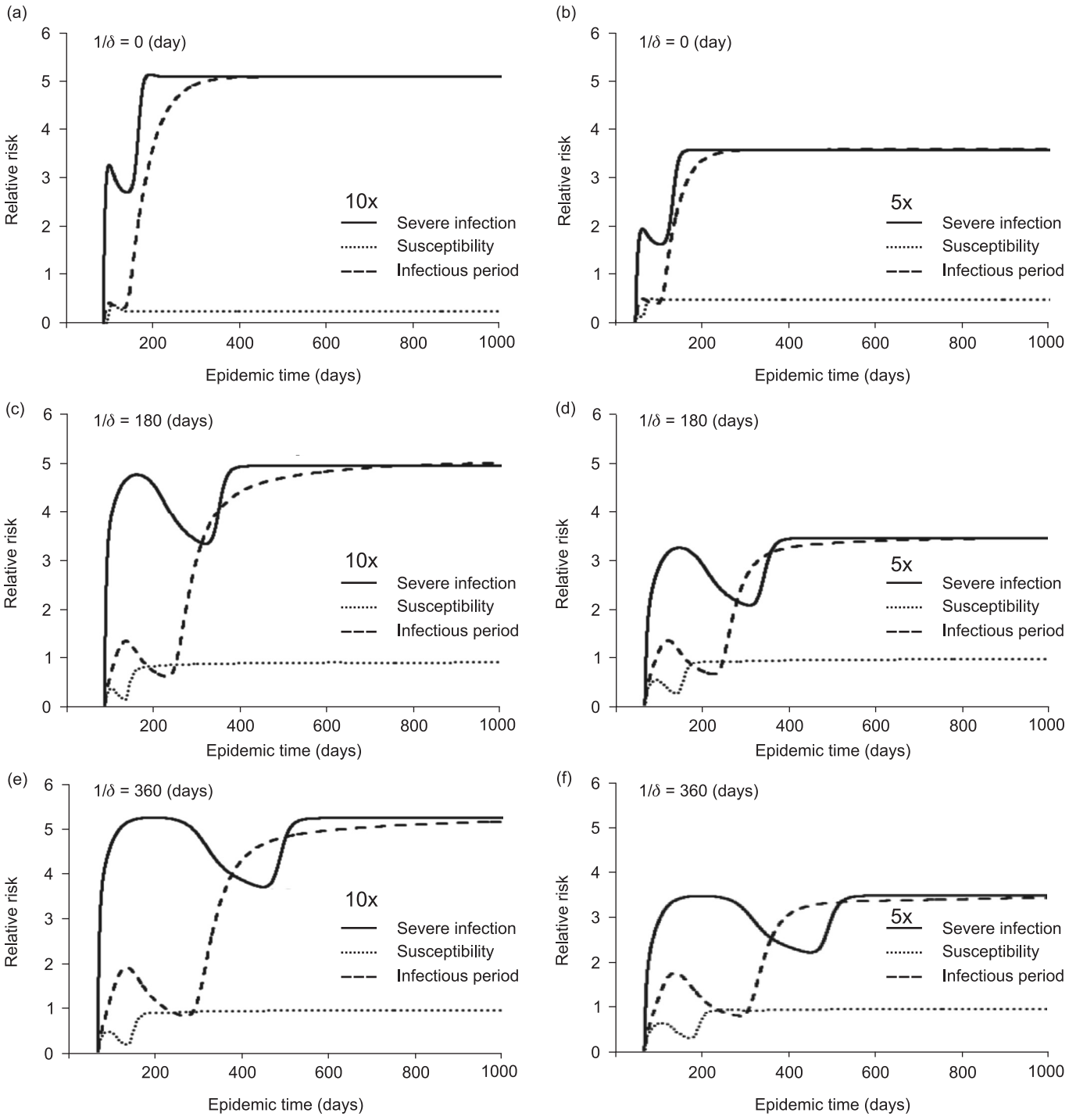

Fig. 5: Estimated relative risk of severe dengue during secondary infection with varying mechanisms of enhancement. Each Figure multiplies one of parameters that are considered to govern the enhancement by a factor of 10 [Figs. (a), (c) and (e)]; and 5 [Figs. (b), (d) and (f)]. In each Figure, there are three parameters to reflect the enhancement in epidemiological manners, i.e. the relative hazard of severe dengue given secondary infection ("Severe infection"), the relative susceptibility to secondary infection ("Susceptibility") and the relative duration of infectious period given secondary infection ("Infectious period"). The mean duration of cross-protective immunity ($1/\delta$) is also varied, i.e. $1/\delta = 0$ day (a and b), 180 days (c and d), and 360 days (e and f). The epidemic time (i.e. the time since introduction of a single index case infected with serotype 1) to introduce a single infected individual with serotype 2 is fixed at Day 72.

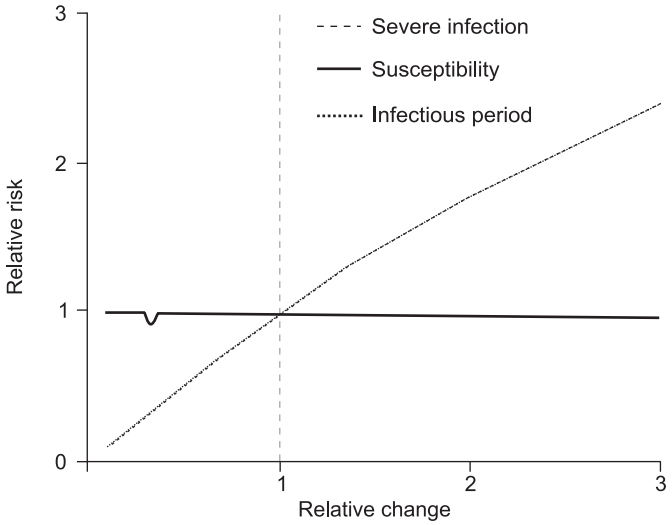

Fig. 6: Sensitivity of the relative risk of severe dengue at the end of epidemic to changes in the parameters governing enhancement. There are three parameters to reflect the enhancement in epidemiological manners, i.e. the relative hazard of severe dengue given secondary infection ("Severe infection"), the relative susceptibility to secondary infection ("Susceptibility") and the relative duration of infectious period given secondary infection ("Infectious period"). Varying one of the parameters from 0 to 3 and fixing other parameters, we examined the relative risk at the end of each epidemic. The epidemic time (i.e. the time since introduction of a single index case infected with serotype 1) to introduce a single infected individual with serotype 2 is fixed at Day 72. The mean duration of cross-protective immunity is fixed at 180 days.

yielded different estimates of the RR, and in particular, we observed an unexpectedly small RR when the susceptibility of once-infected host was elevated during secondary infection.

There are two important lessons that should be learnt from the present study. First, our finding indicates a critical need to consider the optimal design of prospective study, accounting for the time-dependent aspect of the risk during the course of epidemics. As we have shown, the duration of cross-protective immunity is likely a key epidemiological driver to regulate appropriate time frame of empirical observations, and the statistical inference of the duration as well as other natural history is deemed essential to understand the epidemiological dynamics of dengue[15, 34–35]. Second, it is critical to clarify how the enhancement mechanism during secondary infection influences the epidemiological dynamics. In particular, it should be noted that a common assumption of enhancement, i.e. the increased susceptibility, was demonstrated to be inconsistent with the elevated RR of DHF/DSS during secondary infection. The clarification on the mechanism of enhancement calls for a specialized design of empirical observations that permit us to measure the mechanism directly, *e.g.* by using individual-based observational research design with a possibility to deal with the conditional risk of enhancement given DENV infection (*e.g.* household-based study). While various mathematical models have been devised to discuss the dengue control including vaccination policy[36–39], it is vital to address the above mentioned point to bridge the gap between the empirically observed idea (*e.g.* elevated RR of DHF/DSS during secondary infection) and model assumptions.

Three limitations should be noted. First, our systematic review ignored strain-specific characteristics (*e.g.* different virulence) and we also combined the data ignoring all other aspects of observations including geographic location, ethnicity and background health status. The risks of symptomatic infection and severe dengue are known to depend on age[40–41]. Moreover, the diagnostic criteria of DF and DHF could also be different by study[42]. Nevertheless, these should not be regarded as a serious limitation, because the known risk factors have been already identified as independent predictors of severe dengue and would not alter our main results (i.e. accounting for these risk factors would only contribute to stratifying the risks by each individual attribute). Second, our mathematical model rested on simple assumptions with two serotypes only. Homogeneously mixing assumption was employed with a symmetric structure of natural history, and heterogeneous risks including mosquito dynamics were ignored. People were assumed to be fully susceptible at time 0, but usually it is the case that we observe cross reactions between different viruses in *Flaviviridae* (*e.g.* Japanese encephalitis virus). Third, while we identified the importance to account for the time-dependence and clarify the epidemiological mechanism of enhancement, the epidemiological determinants of dengue transmission have yet to be exhaustively identified, and epidemiologists will have to face many other regulators in empirical observations.

In summary, our study suggested that the duration of cross-protective immunity should be estimated from empirical datasets and also emphasized that the epidemiological dynamics of enhancement during secondary infection should be appropriately quantified. Clarifications on these points will help specify optimal study design of dengue in the future. Despite our simplistic approach, we believe that the combination of systematic review and mathematical modeling successfully identified an additional crucial element of severe dengue in epidemiological observations[43].

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Conflict of interests

The authors have declared that no conflict of interest exists.

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