Duration of short-lived cross-protective immunity against a clinical attack of dengue: A preliminary estimate

Hiroshi Nishiura#

Theoretical Epidemiology, University of Utrecht, Yalelaan 7, 3584 CL, Utrecht, The Netherlands

Abstract

It is believed that primary infection with a single serotype of dengue virus elicits short-lived cross-protective immunity against other heterologous serotypes; however, the duration of cross-protection has not been explicitly estimated using epidemiological data. To offer an empirical estimate of the duration, the present study re-analysed historical cohort data of multiple clinical attacks of dengue among American soldiers in the Philippines from 1922–24. In the original study, the historical cohort of 299 cases with a first clinical attack of dengue were closely surveyed; 99 (33.1%) experienced a second attack, while the remaining 200 returned to the United States without further attacks. The time intervals from first to second attack among the 99 cases, and from first attack to departure to the United States among the 200 soldiers, were used for estimating the duration of cross-protective immunity based on a simple mathematical model. Employing an exponential distribution or Kronecker’s delta function as the loss function of cross-protection against a second clinical attack, the mean duration of cross-protective immunity since the first clinical attack was estimated as 6.90 (4.87, 11.83) days and 7.52 (4.88, 16.38) days, respectively. The force of infection, which was jointly estimated with the duration of cross-protection, reasonably explained the other observed epidemiological information in the data, supporting the finding of a short cross-protection period. Even though the estimates suggested that the first clinical attack most likely elicited cross-protective immunity, the length of cross-protection lasted only 1–2 weeks, far shorter than previously believed.

Keywords: Dengue; Epidemiology; Immunity; Serotype; Statistical model.

Introduction

Dengue fever (DF) is a vector-borne disease caused by four closely related dengue viruses (DENV-1-4)\textsuperscript{[1-2]}. It is distributed in most tropical and subtropical areas where \textit{Aedes aegypti} and/or \textit{Aedes albopictus} are abundant\textsuperscript{[3]}. Infection with DENV can also cause dengue haemorrhagic fever (DHF), which is a clinical syndrome characterized by increased vascular permeability, plasma leakage, hypovolemia and shock\textsuperscript{[4,5]}. Although the pathogenesis of DHF has yet to be fully clarified, several risks have been reported; these include secondary infection with heterologous serotypes\textsuperscript{[6,7]},...
primary infection in infants born to dengue-immune mothers\cite{8}, differing virulence of a strain\cite{9} and differing human susceptibility according to race or genetic factors\cite{10,11}.

Although the epidemiological risks of DHF have been explored for more than 30 years, many aspects of the transmission dynamics of dengue remain to be clarified\cite{12}. The transmission dynamics, especially of the interactions between two or more serotypes, have been explored using mathematical models\cite{13-22}. Despite a recent increase in the number of relevant studies, only those based on epidemiological observations in the field have provided detailed insights into the pathogenesis of DHF or interactions between dengue transmission and disease\cite{13-16}. There is a general lack of field data complete with serotype, time, age and space measurements that would allow scientists to investigate and model dengue at a population level. Questions that could be clarified with modeling exercises include: (i) more specific information on the mechanisms of innate dengue viral virulence (if any); (ii) the result of infections with any two specific heterologous serotypes; and (iii) the mechanisms and duration of protective immunity.

Among these unknowns, the present study focuses on cross-protective immunity among those who have experienced primary infection against further infection caused by a heterologous serotype. The duration of acquired cross-protective immunity has never been explicitly estimated and various epidemiological models have employed a number of different and unsupported assumptions. For instance, Ferguson et al.\cite{14} assumed the absence of cross-protection, although a historical study conducted by Sabin\cite{23} suggests that the presence of cross-protective immunity for a short time-period is plausible following primary infection. The presence of transient cross-protective immunity was once supported by explicit data analyses by Adams et al.\cite{15}, but the data were from DHF cases in Thailand in a time-series (with serotype-specificity) that required a number of other epidemiological assumptions. Other studies have assumed differing mechanisms of cross-protective immunity following an exposure to a heterologous serotype shortly after primary infection (e.g. exposure to the heterologous serotype results either in infection or sero-conversion\cite{20} and/or permits developing immunity against the heterologous serotype\cite{16,19}). Even though the presence of cross-protective immunity seems likely\cite{15,23}, and although the majority of previous studies have acknowledged the critical importance of short-lived cross-protective immunity in describing the oscillatory transmission dynamics of dengue, the actual duration remains unknown. An implicit suggestion has been that the duration is 2–9 months\cite{23}.

Accordingly, it would be important to offer an empirical estimate of the duration using existing informative data. The present study aims to estimate the duration of cross-protective immunity against a second clinical attack of dengue as a function of the time since the first attack. For the estimation, historical cohort data from American soldiers in the Philippines from 1922–24 are re-analysed.

**Methods**

The historical data of DENV infection originated from a well-known and rigorous study by Joseph Franklin Siler, Milton Weston Hall and Arthur Parker Hitchens that took place in 1924–25\cite{24}. The study was originally published in the Philippine Journal of Science\cite{24} and was reprinted with appendices by the Bureau of Printing, Manila\cite{25}. Further details of the publication were revisited by Nishiura and Halstead in a recent study\cite{26}. The experimental transmission of DENV-4 in human volunteers
recruited from US Army personnel is widely known\[26,27\]. Siler et al. also conducted an epidemiological study of the natural infection of dengue among American soldiers in the Thirty-first Infantry from 1922–24\[24,25\]. This investigation revisits that study.

As well as the results from the transmission experiment in human volunteers, Siler et al.\[24,25\] hoped that the study would achieve further insights into the frequency of infection, acquired immunity and recurrence of dengue in the average American soldier in Manila under natural conditions. Accordingly, an epidemiological survey of the clinical attack of dengue was attempted among American soldiers; multiple clinical episodes of dengue in each individual were recorded. After obtaining the preliminary results of the epidemiological observations, the authors noted serious technical problems in interpreting the data and precisely estimating the frequency of infection at a population level. The issues included: (i) varying time-intervals between the first attack of dengue and the end of military duty (i.e. some cases experienced the first attack at a time close to the end of duty, and thus were unlikely to experience a second attack); (ii) the duration of military service was variable and some soldiers left for home during the period of observation while others remained in the Philippines; and (iii) mild attacks were less likely to be recorded compared with severe cases. To resolve these technical problems, Siler et al. conducted further epidemiological observations in the Thirty-first Infantry; subjects were limited to those who had their initial attack of dengue between 1 July 1922 and 30 June 1923. All of the enrolled soldiers started their duty on or after 1 January 1922 and left the Philippines no earlier than 31 December 1923. The usual length of duty in the Philippine Islands was 2 years. The period of observation ended 31 December 1924, a time by which the soldiers had been closely monitored for any possible signs or symptoms of dengue.

Individuals with irregular military assignments or transfer were excluded because of the above-mentioned epidemiological problems. Except for a few individuals, all of the included subjects were men from the United States who could be assumed to be fully susceptible at the beginning of their tour of duty in the Philippines, or at least were stated as “could not have been exposed to dengue for more than six months before.” Unfortunately, the severity of the cases was not well detailed, and it is unknown if there was any indication of DHF among those with clinical attacks.

The Thirty-first Infantry numbered 1086 personnel, among which there were 562 potential episodes of dengue. Because of missing observations of 28 potential cases, the authors proportionally decreased the total sample (n = 1032). The first attack of dengue was clinically assessed and detailed clinical records were obtained for 421 cases. Furthermore, strictly applying the exclusion criteria to satisfy the authors’ concern regarding epidemiological problems (especially, to meet the condition of the time of assignment being on or after 1 January 1922), only 299 cases (71.0% of those with clinical records) were selected for further analyses. Again, proportionally decreasing the total sample size (n = 733), the authors concluded that a clinical attack of dengue was observed at least once among 40.8% of the soldiers. Of the 299 cases that had a first attack, 99 (33.1%) experienced a second attack while the remaining 200 left the Philippines without any further clinical attacks. For all of the included subjects, the time interval between their arrival in the Philippines and the first attack was recorded. Moreover, the time from the first attack to departure to the United States was recorded among the 200 cases without a second attack. The time interval from the first to the second attack as well as the time interval from the second attack to departure back to the United States was recorded among the 99 cases with
a second clinical attack. In the original publication, the data for those with only a first clinical attack were reported as a group (i.e. given as just summary tables) by discrete time intervals and there was no individual information (such as time from arrival-to-attack and attack-to-departure), but the data for those with a second attack \((n = 99)\) were recorded for each individual. Although third and fourth clinical attacks were observed among 14 (14.1\%) and 1 (1.0\%) cases among the 99 experiencing a second attack, the information was discarded in the present study for simplicity.

Using the historical cohort data of those who experienced at least a first clinical attack of dengue, the present study estimates the duration of cross-protective immunity against a second clinical attack as a function of time since the first attack. Cases, both with and without a second clinical attack \((n = 99 \text{ and } 200)\), are analysed. First, the descriptive statistics of the time intervals were examined. The time intervals between arrival and the first attack and between the first attack and departure to the United States were compared between those who did and those who did not experience a second clinical attack. For these comparisons, a t-test and the Welch analysis of variance (ANOVA) were employed following the use of a F-test\(^{28}\). Subsequently, a mathematical model was developed and applied to estimate the duration of cross-protective immunity against a second clinical attack of dengue.

Figure 1 shows a schematic diagram of a mathematical model that describes the cohort episode of first and second clinical attacks of dengue. Let \(t\) denote the time since the first clinical attack. Moreover, \(I_1(t)\), \(S(t)\) and \(I_2(t)\) denote, respectively, the fractions of those who experienced a first attack and are still immune to another clinical attack, who are susceptible to another clinical attack caused by a heterologous serotype, and who experienced a second clinical attack, at time \(t\) since the first attack of dengue. Supposing that the rate to lose cross-protective immunity and the force of infection (i.e. the rate at which susceptible individuals experience infection) are \(\delta\) and \(\lambda\) (per day), respectively, the model for the observed intervals is given by

\[
\begin{align*}
\frac{dI_1(t)}{dt} &= -\delta I_1(t) \\
\frac{dS(t)}{dt} &= \delta I_1(t) - \lambda S(t) \\
\frac{dI_2(t)}{dt} &= \lambda S(t)
\end{align*}
\]

\((1)\)

**Figure 1:** Compartmental model to describe the time interval between first and second clinical attacks of dengue in the Thirty-first Infantry in the Philippines from 1922-24

[Although infected soldiers are assumed as transiently immune against other serotypes immediately after the first clinical attack, they loose the cross-protective immunity at rate \(\delta\) and become susceptible to other heterologous serotypes. The susceptible individuals experience infection at rate \(\lambda\) and experience a second clinical attack.]
It should be noted that a constant force of infection $\lambda$ assumes an endemic equilibrium in the Philippines (see discussion on seasonality). Since $I_1(0) = 1$ and $S(0) = I_2(0) = 0$, the probability density and the cumulative distribution of the second clinical attack at time $t$ since the first attack, $f(t)$ and $F(t)$, respectively, are

$$f(t) = \frac{dI_2(t)}{dt} = \frac{\delta \lambda}{\lambda - \delta} \left[ \exp(-\delta t) - \exp(-\lambda t) \right]$$

$$F(t) = I_2(t) = \frac{\delta \lambda}{\lambda - \delta} \left[ \frac{1 - \exp(-\delta t)}{\delta} - \frac{1 - \exp(-\lambda t)}{\lambda} \right]$$

(2)

Let the time interval from the first to the second attack of case $i$ be $t_i$ (where $i$ belongs to the 99 cases with a second attack), and let the time interval from the first attack to the return to the United States of case $j$ be $t_j$ (where $j$ belongs to the 200 cases without a second attack). The observed $t_j$ among the 200 without a second clinical attack are dealt with as censored data. That is, the likelihood of not observing a second clinical attack for $t_j$ days is given by $1 - F(t_j)$. Accordingly, the total likelihood is given by

$$L(\delta, \lambda) = \prod_i f(t_i) \prod_j (1 - F(t_j))$$

(3)

The parameters, $\delta$ and $\lambda$, were estimated by minimizing the negative logarithm of equation (3). Profile likelihood confidence intervals were computed. In addition to the exponentially distributed immune-loss function in model (1), Kronecker’s delta function was also employed as the loss function of cross-protective immunity. Delta function assumes that the duration of cross-protection does not differ between individuals (i.e. is constant), and yields an estimate of the duration that can be regarded as maximum. Under the alternative assumption, $f(t)$ and $F(t)$ are replaced by

$$f(t) = \lambda \exp(-\lambda(t - \delta))$$

$$F(t) = 1 - \exp(-\lambda(t - \delta))$$

(4)

which was also applied to the data using the likelihood function (3).

It should be noted that the following assumptions were made for inference: (i) all included subjects were fully susceptible to dengue at the beginning of their military duty in the Philippines; (ii) the first clinical attack elicited life-long immunity against the causative homologous serotype; (iii) the force of infection was independent of time, and seasonality was ignored because of the absence of adequate data; (iv) multiple serotypes were co-circulating during the period of observation in the Philippines with identical transmission potential (though the exact number of co-circulating serotypes does not have to be known); and (v) the second clinical attack does not have to reflect secondary infection, and the assumed loss of immunity reflected the waning of cross-protection against a second ‘clinical’ attack. Although the results in the present study are deemed preliminary because of these simplistic assumptions, it is critically important to validate the realism of these assumptions (especially, iii, iv and v) to appropriately interpret the results. Thus, these points are later discussed in more detail (see Discussion).

**Results**

Figure 2A shows the distribution of time from arrival in the Philippines to the first attack for 299 cases. The mean (and standard deviation (SD)) and median (and lower-upper quartiles) were 153.9 (115.1) and 144 (48-213) days, respectively. The mean (SD) intervals from arrival to the first attack among those who did or did not experience a second clinical attack were 124.7 (103.6) and 168.4 (118.0) days; significantly different by a t-test ($t$ ratio $= -3.27$, $p < 0.01$). Figure 2B shows the distribution of time from the first to the second attack of dengue among 99 cases. The mean (SD) and median (lower-upper quartiles) were 216.9 (142.4) and 213 (92-279) days, respectively.
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**Figure 2:** Frequency distributions of the time from arrival to the first attack and the time from the first to the second attack in the Thirty-first Infantry in the Philippines from 1922-24

[A. The time since arrival in the Philippines to the first clinical attack of dengue (n = 299). B. The time interval between the first and second attacks among 99 American soldiers. The other 200 soldiers did not experience a second clinical attack.]

![Figure 2](image1)

**Figure 3:** Comparison of the time from first attack to departure between those with and those without a second attack of dengue in the Thirty-first Infantry in the Philippines from 1922-24

[Departure denotes the end of military service in the Philippines (soldiers then returned to the United States)].

![Figure 3](image2)
Figure 3 compares the time from the first attack to departure to the United States between those with and those without a second clinical attack. The mean (SD) and median (lower-upper quartiles) lengths for the entire samples (n = 299) were 574.2 (141.0) and 575 (475-675) days, respectively. The mean (SD) intervals from the first attack to departure for those who did and those who did not experience a second attack were 600.8 (107.7) and 561.0 (153.4) days, respectively. Since the F-test revealed a significant difference in variance (F-ratio = 2.03, p < 0.01), a Welch ANOVA was subsequently employed for the comparison. This showed that the time from the first attack to departure among those with a second attack was significantly longer than those without (F-ratio = -6.75, p = 0.01). All of the above-mentioned time intervals among those with a second attack were given as individual data, permitting an estimation of the total length of stay in the Philippines. The mean (SD) length of stay was 725.5 (100.2) days, roughly corresponding to 2 years as described in the original study.\textsuperscript{[24,25]}

Assuming an exponentially distributed immune-loss function, the maximum likelihood estimates (and the corresponding lower and upper 95% CI) of δ and λ were 0.14 (0.08, 0.21) and $7.52 \times 10^{-4}$ (6.13 $\times$ 10$^{-4}$, 9.12 $\times$ 10$^{-4}$) per day, respectively. The mean length of cross-protective immunity against a second clinical attack is given by $1/\delta$, i.e., 6.90 (4.87, 11.83)

\textbf{Figure 4: Estimated duration of cross-protective immunity against a second clinical attack of dengue}

(The estimated fraction of those still protected against a second clinical attack, from a heterologous serotype, is shown as a function of time since the first attack. Two different models, exponential distribution (thick line with dotted-and-dashed 95% confidence intervals) and Kronecker’s delta function (thin line with dotted 95% confidence intervals), were assumed as the survival function of cross-protective immunity. The 95% confidence intervals were derived from profile likelihood.)
days. Similarly, $1/\lambda$ details the mean length of time to experience a second attack after a complete loss of cross-protective immunity, which was estimated as 3.64 (3.00, 4.47) years. Similarly, assuming Kronecker’s delta function for the loss of cross-protective immunity, the maximum likelihood estimates (and 95% CI) of $\delta$ and $\lambda$ were 0.13 (0.06, 0.20) and $7.53 \times 10^{-4}$ ($6.89 \times 10^{-4}$, $7.95 \times 10^{-4}$) per day, respectively, indicating that the mean length of cross-protective immunity was 7.52 (4.88, 16.38) days and the mean length of time from complete loss of cross-protection to a second attack was 3.64 (3.44, 3.98) years. Figure 4 shows the estimated survivorship functions of those who would still have possessed cross-protective immunity as a function of time since the first attack. Both distributional assumptions yielded similar mean lengths of the cross-protective immunity.

**Discussion**

Despite the critical importance of cross-protective immunity for understanding the epidemiological dynamics of dengue, there has been no previous determination of the duration of cross-protection against a heterologous serotype; thus, the present study re-analysed historical case cohort data among US Army personnel in the Philippines from 1922-24. As it was observed from the experimental transmission of dengue in human volunteers\(^{26,27}\), another original data set of Siler et al.\(^{25}\) also yielded critically important information on the time intervals of exposure and transmission events, permitting empirical assessment of the length of acquired cross-protective immunity against a second clinical attack. The most important conclusion drawn from the simple exercise undertaken in the present study is that the duration of transient cross-protective immunity was estimated as short as 1 or 2 weeks, which is far shorter than has been implicitly suggested (i.e. 2-9 months)\(^{23}\). The finding of short-lived cross-protective immunity can explain Sabin’s note in which mild systemic inflammation was observed by inoculating subjects who had been thought to be cross-protected\(^{23}\).

Although it was not possible to quantify the degree (or strength) of cross-protection, to the best of the author’s knowledge, the present study is the first to explicitly estimate the duration based on empirical epidemiological data. The role of cross-protective immunity has been recognized as critical for describing the transmission dynamics of dengue, and especially, oscillatory epidemiological patterns\(^{15,20}\). Despite the successful quantification of the short length of cross-protection, it should be noted that the estimated duration reflected cross-protection against a second clinical attack. Considering that DENV infection involves a substantial fraction of sub-clinical infections\(^{28,29}\), cases with a second attack could have been infected before their second clinical attacks were observed. Considering that experimental infection of animals with silent sero-conversion has been observed\(^{30}\), the sub-clinical secondary infection is indeed plausible. Nevertheless, the possible presence of sub-clinical secondary infection indicates that the duration of cross-protective immunity against infection (rather than clinical attack) is shorter than the estimated duration in the present study. This supports the main conclusion of the present study, i.e. that the duration of cross-protection is extremely short. Considering the fact that infection with a second heterologous serotype tends to enhance the severity of a secondary infection\(^{6,7}\), the duration of cross-protective immunity against infection with a second heterologous serotype may be reasonably close to the estimate in the present study, and may be slightly shorter than the estimated duration against a second clinical attack.
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Since the present study was intended to present preliminary results of estimates based on a simple model structure, the mathematical model employed a number of unrealistic assumptions, among which (v), the interpretation of a second ‘clinical’ attack, was discussed above. The remaining two important issues, (iii) the constant force of infection, and (iv) equal frequency of co-circulation among heterologous serotypes, are discussed here. Although the force of infection could vary as a function of time in reality (e.g. reflecting seasonal ecological dynamics of the vector population), during the study period from July 1922 to December 1924, there was no month without a clinical attack of dengue. Moreover, the largest difference in incidence (i.e. highest minus lowest incidence) was as small as 1560 per 1000 per annum among the entire population of American soldiers, indicating that the seasonal forcing was not critical quantitatively. That is, even though assumption (iii) could have influenced the precision of the estimate, the main conclusion of an extremely short duration of cross-protection is still deemed valid.

The contention of short-lasting cross-protection (and validity of the model) is also supported by two other calculations, one of which is also relevant to the interpretation of the above-mentioned point (iv). First, using the estimated force of infection \( \lambda = 7.5 \times 10^{-4} \) per day, the fraction of those who experienced a clinical attack of dengue during 2 years of military service would be given by 1-exp(-2\( \times 365 \times \lambda \)) = 0.422, which gives an estimate very close to the observed cumulative incidence of first clinical attack by the end of the study period (i.e. 40.8%). Accounting for the possible presence of sub-clinical infection, \( \lambda \) could have been greater than the estimate, but, in fact, a greater \( \lambda \) supports the finding of a short duration of cross-protection (i.e. if \( \lambda \) is greater than 7.5\( \times 10^{-4} \) per day, the estimated mean duration of cross-protection, \( 1/\delta \), will be shorter than that in the present study). Second, if there were 2, 3 or 4 co-circulating serotypes, the assumption of identical transmissibility would yield the force of infections for all 2, 3 or 4 circulating serotypes as 15.0, 11.3 and 10.0\( \times 10^{-4} \), respectively, per day. The median time from arrival to first attack is then given by \(-\ln(0.5)/\lambda = 462, 616 \) and 693 days. Although a direct comparison between these estimates and the observed data cannot be made because of the absence of detailed data about those who avoided a clinical attack of dengue, the estimated median times from arrival to first attack are shorter than the observed median among those experiencing a first attack (144 days in Figure 2A), indicating that the force of infection could have been larger than that estimated, which again supports the finding of short-lived cross-protective immunity. Also, if the force of infection of a specific serotype was much greater than those of other serotypes, this could reasonably explain the discrepancy between the expected median time from arrival to first attack and the median estimate in Figure 2A. Assuming that the average life expectancy of the host was \( L = 50 \) years and exponentially distributed, and adopting an approximate estimator of the basic reproduction number (i.e. the average number of secondary cases generated by a single primary case in a fully susceptible population), \( R_0 = \pi L \) (where \( \pi \) is the serotype-specific force of infection), equal frequency of transmissibility among co-circulating 2, 3 or 4 serotypes yields \( R_0 \) of 9.13, 6.84 and 6.08, respectively. Obtaining a more precise estimate utilizing an extended modelling approach is a subject for future study.

In summary, the present study re-analysed the distribution of time intervals between first and second clinical attacks of dengue, jointly estimating the duration of cross-protective immunity against a second clinical attack and the force of infection among US Army personnel who experienced at least a first attack in the Philippines. Although transient cross-protective immunity most likely exists, the
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length is estimated to be just 1-2 weeks, far shorter than previously suggested. Considering that DENV infection involves a substantial number of sub-clinical infections, and because the present study reported preliminary results based on simplistic model assumptions, future improvements that address the presence of sub-clinical infection, adjust seasonal characteristics of infection, and obtain more precise estimate of the duration of cross-protection, are considered crucial.

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References


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