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Signature:

Miranda Chan

Date

Incubation Periods of Dengue Viruses

By

Miranda Chan
Master in Public Health

Epidemiology

Keith Klugman, MD, PhD
Committee Chair

Michael Johansson, PhD
Committee Member

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By

Miranda Chan

B.S.
Rutgers University
2007

Thesis Committee Chair: Keith Klugman, MD, PhD

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Abstract

Incubation Periods of Dengue Viruses By Miranda Chan

Dengue viruses are major contributors to illness and death throughout the tropical and subtropical regions of the world. Understanding the dynamics of dengue virus infection is critical to surveillance and control activities. Here we focus on the timing of two critical processes: the extrinsic incubation period (EIP), the time between a mosquito taking an infectious blood meal and becoming infectious to humans; and the intrinsic incubation period (IIP), the time between an infectious mosquito bite and the onset of illness in a human. We performed a literature review to identify data on the extrinsic and intrinsic incubation periods of dengue viruses in *Aedes aegypti* mosquitoes and humans, respectively. For the EIP, we also collected data on temperature as the effects of temperature on the EIP are well known if not well defined. We used these data for a statistical meta-analysis using Bayesian censored time-to-event models. The EIP model with the best fit, i.e. the lowest deviance information criterion (DIC) value, was the log-normal model with a median EIP estimate at 30°C of 13.5 days (95% credible interval of 11.3—16.2). The IIP model with the best fit was the Weibull model with the median estimate of 5.9 days (95% credible interval of 5.6—6.1 days). The results are robust estimates of incubation periods, their distributions, and their uncertainty. These should be useful in clinical diagnosis, outbreak investigation, prevention and control programming, and mathematical modeling of transmission.

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INTRODUCTION

Dengue viruses (DENV) are the most common arboviral cause of illness and death in the tropics and subtropics (1). Over the past 50 years, geographic expansion of areas where transmission occurs and increased transmission intensity has led to a 30 fold increase in the reported incidence of dengue globally (2). Now, nearly two fifths of the world's population is at risk of infection from one of the four DENV serotypes (DENV-1 to DENV-4) (2). Furthermore, the increased incidence has been accompanied by an increase in clinically severe disease (3, 4).

The four related, but antigenically distinct DENV serotypes belong to the *Flaviviridae* family. DENV infections are often asymptomatic, but many result in dengue fever, a flu-like illness with fever, headache, joint and muscle pain, and rash, and some result in severe disease which may include extensive bleeding, plasma leakage, and death (2). The more severe manifestations of dengue are associated with secondary DENV infections of heterologous serotypes (5, 6). Infection severity likely also depends on specific strains or genotypes within each serotype (5, 7).

In this paper, we focus on the extrinsic and intrinsic incubation periods of DENV infections. These time periods are the viral incubation periods from infection of the mosquito or human to the mosquito becoming infectious or the human becoming sick, respectively. Though not well characterized, they are important determinants of DENV transmission dynamics as they are critical for clinical diagnosis, outbreak investigation,

implementation of prevention and control programming, and mathematical modeling of DENV transmission.

The extrinsic incubation period (EIP) begins with a mosquito taking an infectious blood meal from a viremic human host. DENV present in the blood meal then invades the midgut, replicates, and eventually disseminates throughout the mosquito which becomes infectious once virus reaches the salivary glands (8). The EIP for DENV, like other flaviviruses (9-11), is dependent on ambient temperature. At higher temperatures within the viable temperature range of the vector, DENV replicates faster and the EIP is shorter (12, 13). While variability in the EIP is widely recognized, the temperature-sensitivity has not been well characterized and the EIP is most frequently referred to a static range of 8–12 days (2, 14).

The intrinsic incubation period (IIP) is the time between an infectious mosquito bite and the onset of disease in the human host. Though not temperature-sensitive, the IIP does vary and is regularly cited in the literature as ranges such as 2–7 days by the World Health Organization (2) or 3–10 days by the Centers for Disease Control (14).

Despite the importance of the EIP and IIP, simple ranges poorly define the expected duration of each incubation period which can be more formally defined using statistical distributions. In the only similar work we are aware of, Nishiura and Halstead (15) analyzed data from two previous studies to fit a log-normal distribution of the IIP. Here we expand upon this work by collecting relevant data from a greater variety of previously

published studies and using Bayesian meta-analysis to define and parameterize multiple time-to-event models for the temperature-dependent EIP and the IIP.

METHODS

Data

Relevant literature was collected by searching the Pubmed, Ovid, and the Armed Forces Pest Management Board Literature Retrieval System databases using search terms including *Aedes aegypti*, dengue, experiment, import, incubation, transmission, temperature, and travel. Further material was found by reviewing references from identified papers.

Studies used for the EIP analysis included all instances in which *Ae. aegypti* were fed on viremic humans or non-human primates and were later tested for their ability to transmit DENV at a known time interval. The maximum EIP for each mosquito was defined as the time from the infectious blood meal (the earliest in the case of multiple blood meals) to the first successful transmission of DENV. If transmissibility was tested and never successful, the maximum EIP is unknown. The minimum EIP was zero if the first transmission attempt was successful or, if not, the time from the last infectious blood meal to the last unsuccessful transmission experiment. Average ambient temperature data were recorded for each mosquito when available or estimated from the Climate Research Unit 30-year mean climatology dataset (CL 2.0) (16) based on geographic location and the time of year of the study.

The IIP analysis was restricted to events in which humans became sick after being experimentally infected by *Ae. aegypti* or after exposure to DENV by travelling into or out of an area with ongoing dengue transmission. Transmission events involving

inoculation of humans and/or mosquitoes with dengue virus through injections and mosquitoes that fed on animal blood or artificial media were excluded, as these modes do not reflect natural transmission. In some cases, the IIP was directly observed. In other cases, the maximum and minimum IIP were defined as the time from the first and last exposures, respectively, to the onset of illness.

Statistical Analysis

The EIP and IIP data were both analyzed using censored time-to-event models. For the IIP observations with a single exposure and a known time of illness onset, the data are uncensored. For observations of EIP or IIP defined by an interval, the event is interval-censored, i.e. it is assumed that the event occurred sometime between the minimum and maximum times defined by the observations. Observations with only a minimum time are treated as right-censored data.

For each incubation period, we analyzed four common time-to-event models: exponential, gamma, log-normal and Weibull. The specific formulations of each are available in Table 1. To estimate the temperature dependence of the EIP, we incorporated a linear covariate for temperature assuming multiplicative hazards. The analyses were performed in WinBUGS Version 1.4 (17). Parameters were estimated based on 500,000 samples after a burn-in of 10,000 samples. Models were checked for convergence and fit was assessed by comparing the relative deviance information criterion (DIC) for each model (18).

RESULTS

Data

The EIP data included 201 observations, 114 interval-censored and 87 right-censored collected from 8 studies (13, 19-25). The publication year of the studies ranged from 1905 to 1987. The average observed ambient temperature ranged from 14.9 to 35.0°C with a median temperature of 26°C. The serotype representation included 55 (27.4%) DENV-1, 91(45.3%) DENV-2, zero DENV-3, 33 (16.4%) DENV-4, and 22 (10.9%) DENV of unknown serotype. For the IIP, 189 observations were collected including 121 uncensored observations, 52 interval-censored observations, and 16 right-censored observations. These data were obtained from 35 studies published between the years 1903 to 2010 (19, 21-54).

Extrinsic Incubation Period

To characterize the EIP, we fit four time-to-event statistical models (exponential, gamma, log-normal and Weibull) with temperature as a covariate. The median estimates of the EIP at 30°C ranged from 12.2 days in the Weibull model to 15.5 days in the exponential model (Table 2). Increased temperature was associated with decreased EIP. This association was statistically significant in all but the exponential model (Table 2). The model with the best fit, i.e. the lowest DIC, was the log-normal model. The median estimate for the EIP at 30°C was 13.5 days with a 95% credible interval of 11.3—16.2 days. The distribution of the mean EIP at a 30°C is shown in Figure 1.

Intrinsic Incubation Period

Exponential, gamma, log-normal and Weibull models were also fit to the IIP data. The median IIP estimates ranged from 5.9 days in the gamma and Weibull models to 6.5 days in the exponential model (Table 3). The model with the best fit was the Weibull model with the median IIP estimate of 5.9 days and a 95% credible interval of 5.6—6.1 days (Table 3).

DISCUSSION

This study provides important insight into the transmission dynamics of dengue viruses by formally describing the statistical distributions of the extrinsic and intrinsic incubation periods. The models support the inverse relation of temperature to EIP.

The use of Bayesian analysis allowed us to describe and parameterize the statistical distributions of the DENV incubation periods. Historical data on the incubation periods were limited in the literature, as investigations of these events often required dangerous, expensive and time intensive experimental studies. Available studies were carefully examined to ensure the collected data were sufficient and relevant to our study. Some studies were missing critical information and thus, were excluded. In the case of missing temperature data, when geographic location and time of year of the study was available, temperature was estimated as described in the methods section. Due to the estimation of some temperature data, there is some uncertainty of the temperature sensitivity in the EIP models. Although incubation period data is difficult to measure and is scarce, the use of time-to-event models allowed the incorporation of time censored data into the analysis. The sample sizes were moderate (201 for EIP and 189 for IIP) and the use of Bayesian analysis does not require the assumption of large sample size for valid standard errors and confidence intervals as in classical statistical analysis.

The estimates from the IIP analysis provide clinically useful information for physicians as knowledge of the likely time frame of exposure to symptoms can include or exclude dengue as a potential diagnosis. Additionally, the time from infectious bite from a

mosquito to infectiousness in humans is important factor in dengue transmission.

However, we were not able to analyze this due to limited data. Knowledge of this time frame is significant, as infectious humans, who also include asymptomatic human cases, can infect mosquitoes and propagate epidemics.

Similarly, the EIP analysis provides detailed understanding of the distributions of this critical time frame in dengue transmission. However, the analysis does not include other important factors in the complex transmission of dengue, such as infectious dose. All mosquitoes in the studies used here were infected by natural feeding in which the infectious dose is difficult to measure. However, infective viral dose has been shown influence the EIP (8, 55). Mosquitoes feeding on blood meals with a low viral dose compared to a high viral dose may require a longer EIP, since fewer viruses are present to infect, replicate, and subsequently disseminate to the salivary glands in the mosquito (8).

Similar to previous findings with other flaviviruses (8, 11), the EIP of dengue viruses was inversely associated with temperature. The addition of temperature in the EIP models allowed for the quantification of the temperature sensitivity with EIP. The average lifespan of *Ae. aegypti* under optimal conditions is 30-50 days (56). At cool temperatures, the mosquito is less likely to survive in the environment and the EIP may be greater than the lifespan of the mosquito. Thus, at cooler temperatures, an infected mosquito has a greater potential to die before the EIP is completed and not perpetuate DENV transmission. At warm temperatures, completion of the EIP is likely to occur as EIP is shorter and a smaller proportion of the mosquito lifespan. Thus, warm

temperatures enhance DENV transmission as mosquitoes are able to infect humans earlier than at cool temperatures. However, at extreme hot temperatures the association of EIP with temperature is not valid due to the adverse effects of extreme hot temperatures on mosquito survival. Extreme temperatures outside of this range are likely to kill the mosquito before EIP is completed.

The analysis of the DENV incubation periods is valuable for clinical diagnosis, outbreak investigation and implementation of prevention and control programming, and mathematical modeling. The use of IIP estimates presented here can inform physicians of dengue as a possible diagnosis in patients, particularly in travel-related cases in which patients have visited areas with on-going dengue transmission. IIP can also be used to guide outbreak investigations by pointing toward likely locations and time of exposure from index cases. With this information, additional dengue cases may potentially be discovered or prevented. The temperature-sensitive EIP estimates can inform public officials in the decision process of vector control. When temperatures are cool, the use of insecticides may be more useful as mosquitoes are likely to die quicker from the control measures before completing the EIP. The IIP and EIP measures can also be used in mathematical models to inform and evaluate control measures. Additionally, the temperature-sensitive EIP estimates can be integrated into models of climate change to better assess the potential for climate change to influence future variations in dengue incidence.

TABLES

Table 1: Time-to-event model distributions

Distribution	Probability Density Function $f(t)$	Parameters	Covariates
Exponential	$\lambda e^{-\lambda t}$	$\lambda = \text{rate}$	$\lambda(x) = 1/e^{\beta X}$
Gamma	$\frac{\lambda^v t^{v-1} e^{-\lambda t}}{\Gamma(v)}$	$\lambda = \text{rate}$ $v = \text{shape}$	$\lambda(x) = v/e^{\beta X}$
Weibull	$v\lambda t^{v-1} e^{-\lambda t^v}$	$\lambda = \text{rate}$ $v = \text{shape}$	$\lambda(x) = e^{\beta X}$
Log-normal	$\frac{\sqrt{\tau}}{\sqrt{2\pi}} \cdot \frac{e^{-\tau(\ln(t)-\mu)^2/2}}{t}$	$\mu = \text{mean}$ $\tau = \text{precision}$	$\mu(x) = e^{\beta X}$

Where for the extrinsic incubation period (EIP) models: $\beta X = \beta_o + \beta_T T$

Table 2:**Extrinsic incubation period of dengue viruses: Parameter estimates and goodness-of-fit measures for time-to-event models with temperature sensitivity**

Model	Notation	Constant parameter		Covariate coefficients				Mean extrinsic incubation period at 30°C (95% CI*)		DIC**
		Value (95% CI*)		β_0 (95% CI*)		β_T (95% CI*)				
Exponential	-	-	-	3.6 (1.5-5.5)		-0.029 (-0.097-0.052)		15.5 (11.5-22.0)		226
Gamma	shape (ν)	3.6 (2.4-5.5)		4.1 (3.0-5.3)		-0.0512 (-0.0961 to -0.0082)		12.7 (10.5-15.2)		203
Weibull	shape (ν)	2.1 (1.7-2.6)		-9.2 (-13.0 to -5.9)		0.130 (0.022-0.240)		12.2 (10.1-14.8)		200
Log-normal	precision (τ)	3.3 (2.1-5.0)		1.34 (0.99-1.69)		-0.0013 (-0.029 to -0.0013)		13.5 (11.3-16.2)		195

*95% credible interval

**deviance information criterion

Table 3:**Intrinsic incubation period of dengue viruses: Parameter estimates and goodness-of-fit measures for time-to-event models**

Model	Constant parameter			Parameter			Intrinsic incubation period		DIC**
	Notation	Value	(95% CI*)	Notation	Value	(95% CI*)	Mean	(95% CI*)	
Exponential	-	-	-	rate (λ)	0.16 (0.13-0.18)		6.5 (5.5-7.6)		766
Gamma	shape (ν)	14.4 (11.3-18.0)		rate (λ)	2.4 (1.9-3.1)		5.9 (5.7-6.2)		536
Weibull	shape (ν)	3.9 (3.5-4.5)		rate (λ)	0.00071 (0.00024-0.00064)		5.9 (5.6-6.1)		520
Log-normal	precision (τ)	12.5 (9.6-15.7)		mean (μ)	1.75 (1.70-1.79)		6.0 (5.7-6.3)		525

*95% credible interval

**deviance information criterion

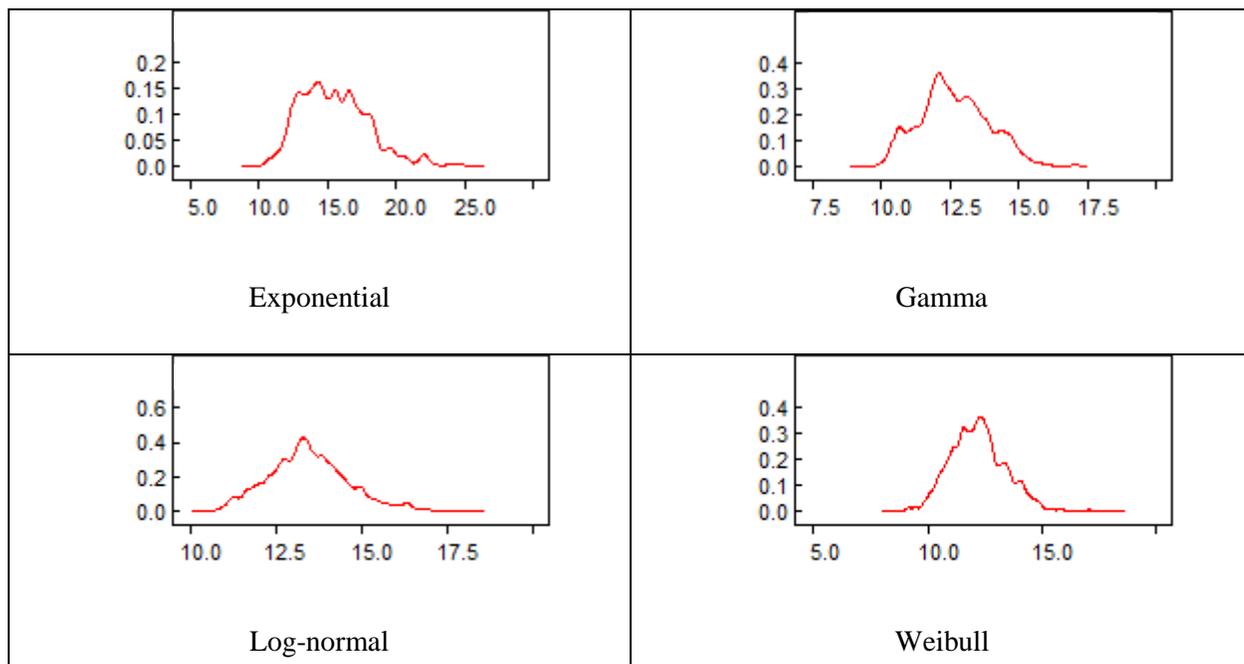
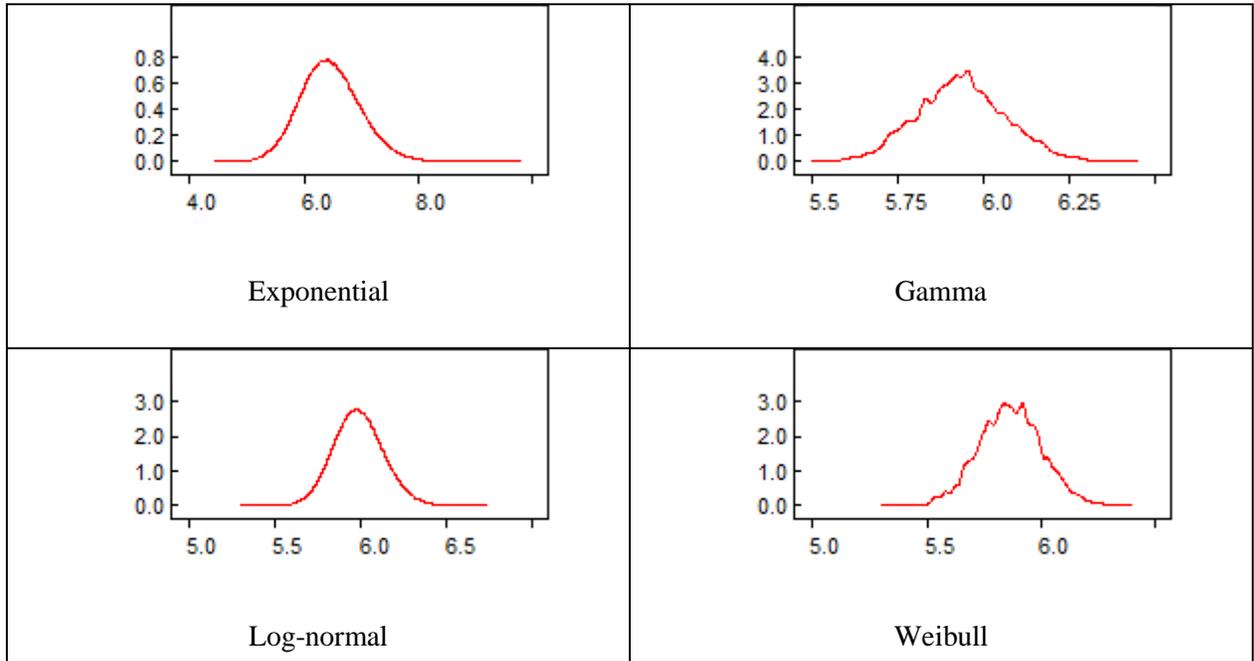
FIGURES**Figure 1: Extrinsic incubation period distributions at 30°C**

Figure 2: Intrinsic incubation period distributions

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