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Viral Kinetics of Primary Dengue Virus Infection in Non-Human Primates: A Systematic Review and Individual Pooled Analysis

Benjamin M. Althouse[1,*], **Anna P. Durbin**[2], **Kathryn A. Hanley**[3], **Scott B. Halstead**[4], **Scott C. Weaver**[5], and **Derek A. T. Cummings**[2]

[1]Santa Fe Institute, Santa Fe, NM

[2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[3]New Mexico State University, Las Cruces, NM

[4]5824 Edson Lane, Bethesda, MD 20852, USA

[5]University of Texas Medical Branch, Galveston, TX

Abstract

Viremia kinetics directly influence the clinical course and transmission dynamics of DENV, but many aspects of viral dynamics remain unknown. Non-human primates (NHP) have been used as a model system for DENV infection for decades. Here, we identify papers with experimentally-infected NHP and estimate the time to- and duration of viremia as well as estimate associations between these and serotype, inoculating dose, viremia assay, and species of NHP. We estimate the time to viremia in rhesus macaques to range from 2.63 to 3.32 days for DENV-2 and -1 and the duration to range from 3.13 to 5.13 days for DENV-4 and -2. We find no differences between non-human primates for time to viremia or duration, and a significant negative relationship between inoculating dose and duration of viremia. These results aide in understanding the transmission dynamics of sylvatic DENV non-human primates, an issue of growing importance as dengue vaccines become available.

Keywords

dengue virus; non-human primates; viral kinetics; within-host dynamics; sylvatic dengue

Introduction

Knowledge of the kinetics of dengue fever virus (DENV) within primate and non-primate hosts is key to understanding transmission dynamics and identifying populations at risk for infection [1]. Due to logistical and ethical obstacles, few studies have measured wildtype DENV viremia in humans over the course of an infection. Thus, non-human primates have

*To whom correspondence should be addressed. althouse@santafe.edu.

been the major model system for comparison of viral dynamics between DENV serotypes and strains as well as evaluation of dengue therapeutics. While non-human primates differ from humans in pathological responses to DENV infection, estimates of duration of viremia that exist appear to be similar [2, 3], albeit with lower viral replication and limitation of virus to a subset of those tissues infected in humans [4].

In addition to serving as a potential model for human diseases, insight in to the replication of DENV in non-human primates is important in its own right. Four serotypes of sylvatic DENV have been shown to circulate between non-human primates and arboreal *Aedes* mosquitoes in Southeast Asia [5] and sylvatic DENV serotype 2 is maintained in West Africa [6]. These sylvatic viruses are ancestral to the four serotypes of DENV that are currently transmitted between humans by domestic and peridomestic *Aedes* [7]. Populations living in areas surrounding sylvatic hotspots of DENV transmission are at risk of infection [8, 9] from a transmission process that is poorly understood [10]. Importantly, it has recently been discovered that sylvatic DENV infection in humans can produce the most severe manifestation of dengue disease – dengue hemorrhagic fever [8, 9]. In the light of recent advances in DENV vaccines [11, 12], sylvatic reservoirs may play a key role in maintaining transmission over long time scales and may continue to expose human populations to new, genetically distinct viruses after human endemic transmission is controlled [7].

Isolations of sylvatic DENV have occurred at roughly eight year intervals in Senegal over the past 50 years [6]. The key determinants of cycle length are largely unknown. As the natural history of a pathogen has direct influence on transmission dynamics [13] knowledge of the time to detectable viremia and the length of viremia in non-human primates will be useful in ecological models of transmission [14] and may generate hypotheses for the observed serotype-specific transmission patterns [15] and clinical manifestations [16, 17] observed across DENV serotypes.

It is the goal of the present study to examine the kinetics of DENV viremia in non-human primates through systematic review and individual pooled analysis. We conducted a literature review to identify experimental DENV infections of DENV-naïve monkeys. We find associations between time from inoculation to viremia and duration of viremia and several covariates of interest using mixed effects regression models. We report robust estimates of the time to detectable viremia and the duration of viremia using recently developed methods for handling doubly-interval censored data [18].

Methods

Systematic Review

We searched PubMed, Web of Science and Google scholar for articles containing the terms “dengue primate viremia infection”, “dengue viremia primates”, “dengue viremia monkey”, “dengue vaccine primates”, and “dengue infection primates”. We narrowed our focus to primary infections where details on the infecting virus were reported. Our inclusion criteria were:

1. The non-human primate must be DENV naïve at the time of experimental infection (including free of exposure to experimental vaccines),
2. The challenge serotype (DENV-1–4) and the specific virus strain (with passage number, if applicable) must be clearly identified and the dosage of virus stated (in plaque forming units [PFU]), and
3. The presence of viremia must be reported on a day-by-day basis, at at least two time points, either in a graph or table, and not in a summary statistic. This does not preclude monkeys bled sporadically (e.g., every other day).

Additional (unpublished) studies were identified through expert consultation. Abstracts were doubly reviewed (BMA, DATC).

Time to Event Data

A survival analytic approach was used to determine time-to-event (viremia or clearance). If more than one method for assessing viremia was used, the method with the higher sensitivity was reported (though multiple methods were compared). Data were classified as fully observed, single- or doubly-interval censored. Observations were fully observed if the non-human primates were bled and found not to be viremic before and after being found viremic. If non-human primates were found to be viremic on the first or last sample taken, then the data point was assumed single-interval censored. If non-human primates were viremic on both the first and last sample, then the data point was assumed doubly-interval censored imposing left and right boundaries of inoculation and 16 days (estimates are insensitive to this number, see Supplementary Material). Observations missing or negative surrounded by two viremic samples were assumed to be viremic.

Methods for analyzing doubly-interval censored data have been developed previously [18]. We estimate the time to detectable viremia and the duration of viremia, both of which we assume are log-normally distributed (see Supplementary Material). We stratify by DENV serotype and compute bootstrap confidence intervals.

Associations with Time to Viremia and Duration

To explore the potential association between length of time to detectable viremia and duration of viremia, linear and random effects models were fit with time to viremia or duration as the outcome, a random effect for study and serotype, inoculating dose, viremia assay, and species of non-human primate as potential covariates of interest [19]. As these models do not directly take into account the effects of censoring, we test for differences censoring between covariates. Linear and mixed effects models are compared using the Akaike information criterion (AIC) [20].

Results

Literature

Literature searches returned 1092 unique papers (Figure 1). Of these, 117 (11%) described dengue infection in non-human primates, 226 (21%) described observational/naturally occurring dengue infection in humans and not non-human primates, 91 (8%) were about

another disease, 125 (11%) had no abstracts, and 533 (49%) described experimental studies involving humans and animal models (not involving NHP).

Fifty one published studies and three unpublished studies met the criteria for inclusion and were included in the analysis (Table 1). Thirty six included rhesus macaque (*Macaca mulatta*), 7 cynomolgus macaques (*Macaca fascicularis*), 4 each with green monkeys (*Chlorocebus aethiops sabaeus*) and owl monkeys (*Aotus nancymaae*), 3 chimpanzee (*Pan troglodytes*), 2 each with spider monkey (*Ateles geoffroyi*) and pig-tailed macaques (*Macaca nemestrina*), and 1 each with common marmoset (*Callithrix jacchus*), patas (*Erythrocebus patas*), squirrel monkey (*Saimiri sciureus*), and White Handed Gibbon (*Hylobates lar*). The bulk of the studies were vaccine trials/challenge studies (34/51, 67%) the rest were experimental challenge trials (18/51 35%). 59 unique DENV genotypes were represented. 72 (10%) non-human primates were infected with DENV-4 4328S, 43 (6.1%) with DENV-2 S16803, and 40 (5.6%) with DENV-1 WP74 (see Supplementary Material). Table 2 reports numbers of non-human primates by DENV serotype.

Associations with Time to Viremia and Duration

Mixed effects models were fit with a random effect for study and were universally preferred over linear fixed effects models by AIC (see Supplementary Material). Intraclass correlation coefficients indicated strong heterogeneity by study (0.48, 95% CI: 0.37, 0.60) which could be due to differences among laboratories and assays employed. Mixed effects models assume non-human primates are exchangeable within studies, and account for heterogeneity between studies. Mixed effects models employed here do not take into account censoring, however only DENV-2 ($p = 0.001$) and common marmoset samples ($p = 0.03$) were associated with more censoring.

Tables 3 and 4 report the associations for serotype, $\log_{10}$ inoculating dose, assay, and species of non-human primate with length of time to detectable viremia and duration of viremia in mixed effects models. Both univariate (with only the covariate of interest included) and multivariate (with all covariates included) models were fit. The multivariate models accounting for study heterogeneity indicated the time to detectable viremia for DENV-1 was statistically significantly longer than for DENV-4 and DENV-2 and -3 were not significantly different from DENV-4. Time to detectable viremia was statistically significantly longer in patas monkeys and marginally significantly shorter in spider monkeys than rhesus macaques; and time to detectable viremia was significantly shorter in those non-human primates assayed by Immunofluorescence assays (IFA). Increasing log dose of inoculum was statistically significantly associated with shorter times to detectable viremias (Table 3). Large study heterogeneity was present, with the variance of the random intercept equal to 1 day.

Duration of viremia was statistically significantly longer for DENV-1 and -2 as compared to DENV-4 after accounting for study heterogeneity. Duration for DENV-3 was not significantly different from DENV-4 (Table 4). Adjusting for study, species, assay, and dose increased the difference in durations between DENV-1 and -2 and DENV-4. Changing the reference serotype to DENV-2 shows DENV-1, -3, and -4 to have statistically significantly shorter durations of viremia than DENV-2 (see Supplementary Material). Significantly

longer durations of viremia were observed when assayed by RT-PCR and IFA compared to plaque-forming assays, adjusting for study, species, assay, and dose. No significant differences in viremia duration were observed across species, besides a significant shortening in patas monkeys (however, only 3 patas monkeys were tested) and a marginally significant shortening in green monkeys from rhesus monkeys. Duration of viremia was negatively associated with dose of inoculum, with durations decreasing by 0.44 days (95% CI: 0.18, 0.7) per $\log_{10}$ increase in dose. Again, the variance of the random intercept was quite large (2.32 days).

Estimates of Time to Detectable Viremia and Duration of Viremia

In rhesus macaques the median time to detectable viremia of DENV was 3.32 days (95% CI: 3.01, 3.65), 2.63 days (95% CI: 2.40, 2.89), 3.02 days (95% CI: 2.71, 3.34), and 3.23 days (95% CI: 2.99, 3.47) for DENV-1, -2, -3, and -4, respectively (Table 5 and Figure 2). The median duration of viremia was 4.67 days (95% CI: 4.27, 5.12), 5.13 days (95% CI: 4.82, 5.48), 3.22 days (95% CI: 2.83, 3.72), and 3.13 days (95% CI: 2.86, 3.46) for DENV-1, -2, -3, and -4, respectively. As no significant differences were observed in duration of viremia between species (see above), estimates of duration were pooled across all species. The median time to detectable viremia of DENV was 3.23 days (95% CI: 3.00, 3.45), 2.44 days (95% CI: 2.22, 2.65), 2.89 days (95% CI: 2.67, 3.11), and 3.17 days (95% CI: 2.98, 3.37) for DENV-1, -2, -3, and -4, respectively (see Supplementary Material). The median duration of viremia was 4.33 days (95% CI: 4.03, 4.67), 4.84 days (95% CI: 4.52, 5.15), 3.34 days (95% CI: 3.01, 3.68), and 3.24 days (95% CI: 3.01, 3.51) for DENV-1, -2, -3, and -4, respectively.

Discussion

The results of our meta-analysis indicate that the median time to detectable viremia and duration of viremia of DENV was not statistically significantly different between non-human primate species. In rhesus macaques (*Macaca mulatta*), median times to detectable viremia ranged from 2.63 (95% CI: 2.40, 2.89) days for DENV-2 to 3.32 (95% CI: 3.01, 3.65) days for DENV-1 and median duration of viremia from 3.13 (95% CI: 2.86, 3.46) days for DENV-4 to 5.13 (95% CI: 4.82, 5.48) days for DENV-2. These estimates are shorter than those previously reported in humans. Tricou et al. reported a median duration of viremia of 6.2 days (IQR 5.8 to 7.2) for all serotypes and 6.8 days (IQR 6 to 7.3) for DENV-1 [21]. Vaughn et al. reported a mean duration of viremia in humans of 5.5 days for primary DENV-1 infection and 4.6 days for primary DENV-3 infection [22]. Murgue et al. found a mean duration of 4.4 days for primary DENV infection in a cohort of French Polynesian children [23]. However, all three of these studies estimate the duration of viremia in individuals hospitalized with dengue, and thus likely not on the first day of viremia. This would tend to underestimate the true duration of viremia. Additionally, due to selection of dengue cases based on severity (i.e. hospitalized patients) the cases included in these studies may not be representative of all dengue infections. The non-human primate studies identified here skirt these two problems directly.

We found no statistically significant differences in time to- or duration of viremia between the 11 species of non-human primates studied here save for patas monkeys. Patas monkeys

were found to have significantly longer times to detectable viremia and shorter duration of viremia, however, only 3 patas were infected in one study [2]. More measurements in patas monkeys would be an important contribution as it is one of the few species from which sylvatic DENV has been isolated [6]. Similarly, spider monkeys had a marginally significantly shorter time to detectable viremia ($p = 0.04$) than rhesus monkeys. Interestingly, the monkeys examined here included several species of old and new world non-human primates, otherwise expected to exhibit differing physiologic and immune responses [24].

Interestingly, the duration of DENV-4 viremia was significantly shorter than DENV-1 and -2 after adjusting for study, species, assay and dose of inoculum. There has been clear demonstration of differences in transmission patterns [15] and in clinical manifestations [16, 17] across the four serotypes of DENV. Shorter duration of DENV-4 viremia may account for the reduced severity observed in this serotype. Fried et al. found cases of dengue hemorrhagic fever (DHF) to be twice as likely in secondary DENV-2 and -3 infections than in secondary DENV-4 [25]. Conversely, we found DENV-2 to be statistically significantly longer than DENV-1, and -3, and -4. Blamaseda et al. found nearly double the odds of shock and internal hemorrhage with DENV-2 infection in outbreaks of DENV in Nicaragua [16]. Nisalak et al. found DENV-3 to be associated with severe outbreaks of dengue in hospitalized cases in Bangkok, Thailand [15]. Fox et al. found time to undetectable DENV-2 NS1 protein to be significantly longer than DENV-1 [26]. Extended durations of viremia for DENV-2 and -3 may be the cause of the increased severity of these infections and may be the reason sylvatic DENV-2 is the only serotype to have emerged in Africa from southeast Asia.

We found significantly longer durations of viremia when assayed using RT-PCR or immunofluorescence as compared to plaque-forming assays. This is most likely due to a higher sensitivity of RT-PCR as compared to other, older methods for determining viremia such as plaque counting and inoculation of suckling mice. Though some of this may be due to detection of viral RNA, and not actively replicating virus. More modern methods such as ELISA and focus-forming assays were not found to be significantly different from plaque-forming assays, but this could be due to small sample sizes. The effect estimates for ELISA and FFA were 1.34 and 0.27 days longer, respectively, adjusting for study heterogeneity, serotype, species, and inoculating dose. Importantly, these differences in detection of viremia were robust to adjustment for study heterogeneity, which was considerable. Intraclass correlation coefficients indicated nearly half of the observed variance was due to differences between studies. This underlines the importance of using random effects models to account for differences between studies, and using care when interpreting results of viremia assays.

Surprisingly, increasing doses were associated with both shorter times to detectable viremia and shorter durations of viremia. This phenomenon was observed by Martin et al. in green monkeys [27], in yellow fever virus (YFV) infections in rhesus monkeys [28], chimeric YF-DENV vaccine in cynomolgus macaques [29] and in humans receiving a live, attenuated Japanese Encephalitis vaccine (ChimeriVax-JE) [30, 31] and a live, attenuated West Nile virus vaccine [32]. It could be that a large inoculating dose causes a rapid initial rise in

viremia inducing a stronger innate immune response leading to quicker clearance. Studies in humans have found that higher peak viremia titers were positively associated with more severe disease [21, 22, 33]; however the evidence for an association between the magnitude and duration of viremia remains inconclusive. Vaughn et al. found the time from peak viremia to clearance was more rapid in DHF cases than in DF cases [22], while Fox et al. found no difference in rate of clearance between DHF and DF [26].

Not all studies included in this meta-analysis reported daily levels of viremia. Additional studies examining directly the relationship between inoculating dose, peak viremia and duration of viremia are necessary, as well as studies investigating the effects of preexisting immunity on time to viremia and duration.

Our methods separately accounted for the two largest potential sources of bias: random effects models accounted for study heterogeneity and doubly-interval censored survival analysis accounted for the large amount of censoring (right-, left- and both) present in reported days of non-human primate viremia. While the random effects model did not take into account the effects of censoring, the amount of censoring only differed in DENV-2 and common marmoset samples, and inferences drawn from them are useful for examining associations between covariates of interest and the time to detectable viremia and duration of viremia. Even though the random effects model accounts for most of the heterogeneity between studies, some caution must still be used when interpreting the results of the associations as some residual confounding may exist from remaining heterogeneity between studies. Finally, while all efforts were made to find all studies reporting non-human primate viremia, it is possible that some studies were missed, or that some data were not published [34].

Our study provides estimates of the times to detectable viremia and durations of DENV-1–4 viremia in multiple non-human primate species, both Old World and New World, and identifies how these differ across serotype, viremia assay, non-human primate species and inoculating dose. Few if any studies have directly compared DENV infection in multiple non-human primates. Our results further understanding of within host DENV replication kinetics which are especially important in how they influence transmission dynamics. In the light of new dengue vaccine trials [35, 36], sylvatic DENV infection in non-human primates could provide a source of infectious introductions. An accurate and thorough understanding of the sylvatic cycle of dengue, including the roles of the various non-human primate species in transmission, may allow prediction of epidemics within non-human primates and thereby lessen the impact of spillover on humans living in areas of overlap with non-human primate hosts. Our results also are important in parameterizing dynamic models of dengue [14], and further understanding of DENV transmission dynamics in general, including differences in serotype-specific cycles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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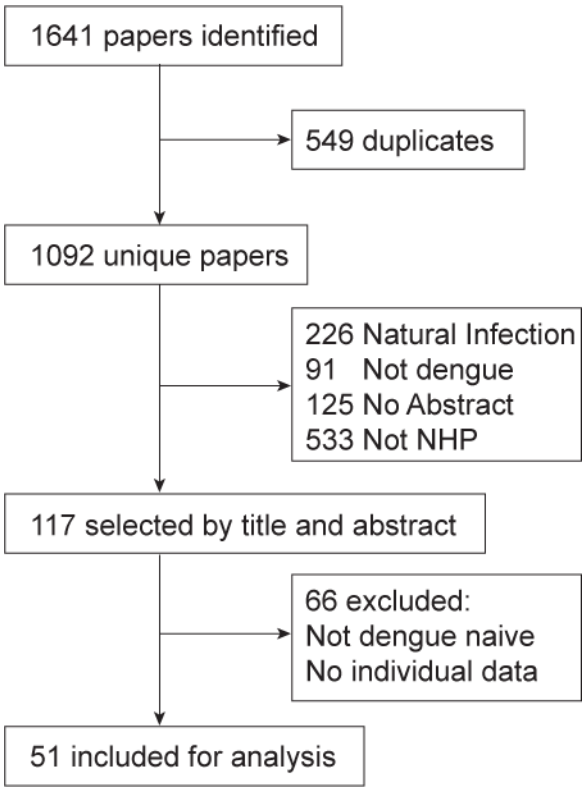

Figure 1.
Flow Chart of Systematic Review

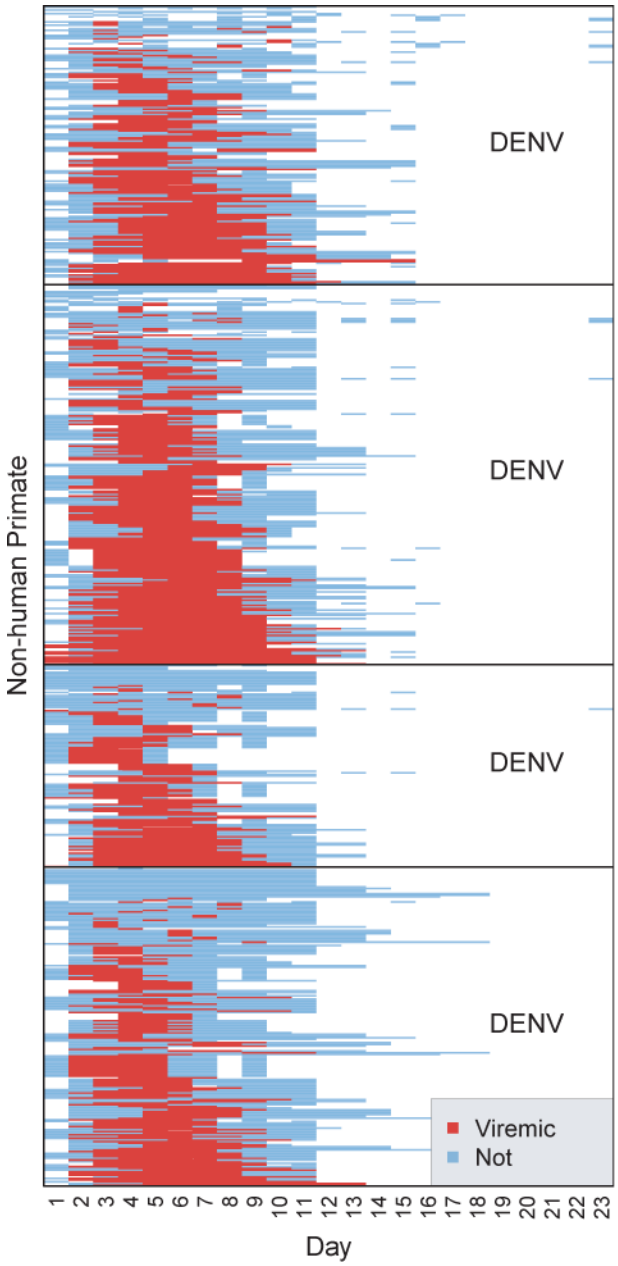

Figure 2. Days of Viremia for Primates

Figure shows the days of viremia for each non-human primate stratified by DENV serotype. Blue bars indicate DENV negative blood samples and red indicates positive samples. White indicates no samples. Data were sorted by DENV serotype, then by days of viremia.

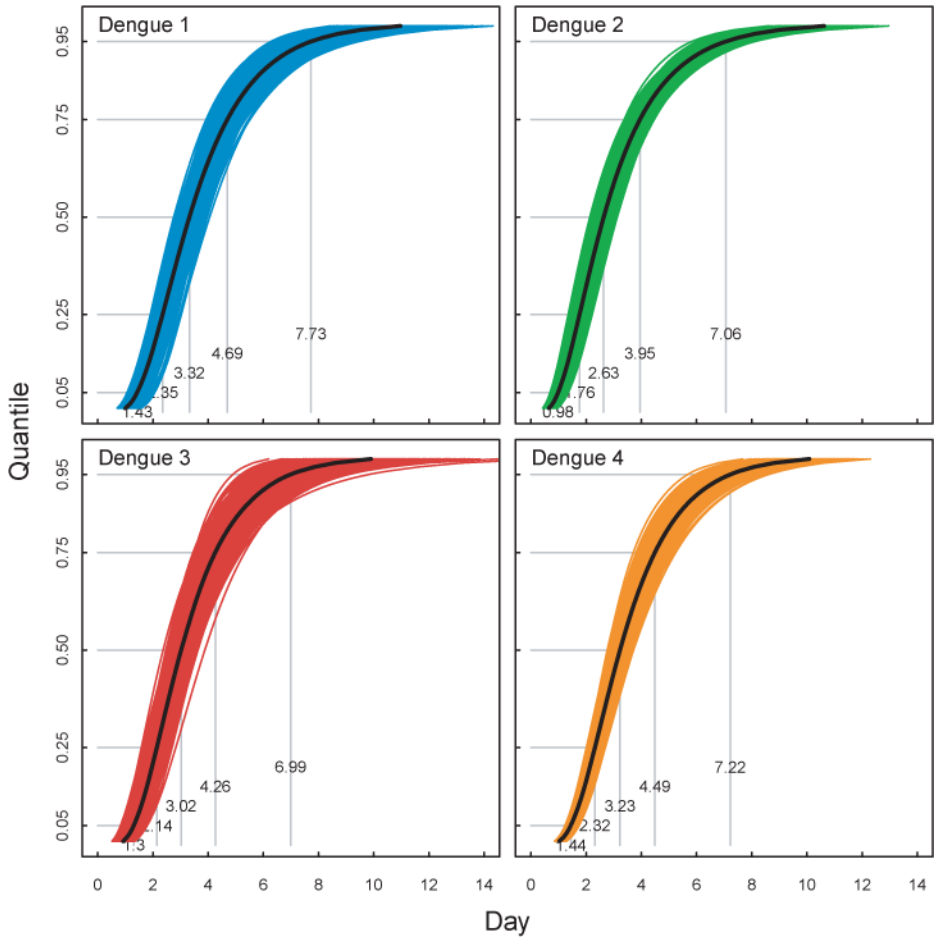

Figure 3. Time to Detectable Viremia for DENV in Rhesus Macaque

Figure shows estimates of the time to detectable viremia for DENV-1–4 in Rhesus primates. Black lines indicate estimates from full dataset and light colored lines indicate bootstrap replicates. Grey lines indicate the 5th, 25th, 50th, 75th and 95th quantiles.

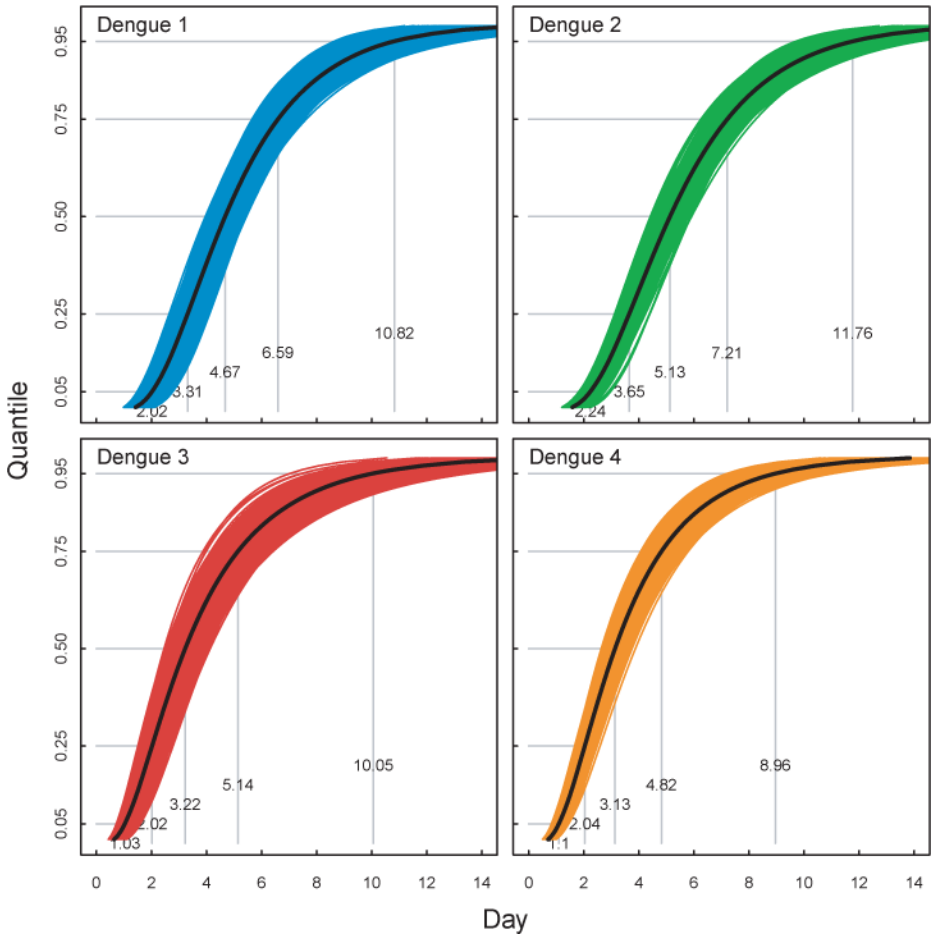

Figure 4. Duration of DENV Infection in Rhesus Macaque

Figure shows estimates of the duration of infection for DENV-1–4 in Rhesus primates. Black lines indicate estimates from full dataset and light colored lines indicate bootstrap replicates. Grey lines indicate the 5th, 25th, 50th, 75th and 95th quantiles.

Table 1

Summary of included studies. In table, primates studied are: rhesus macaque (Macaca mulatta), cynomolgus macaques (Macaca fascicularis), green monkeys (Chlorocebus aethiops sabaeus), owl monkeys (Aotus nancymaae), chimpanzee (Pan troglodytes), spider monkey (Ateles geoffroyi), pig-tailed macaques (Macaca nemestrina), common marmoset (Callithrix jacchus), patas (Erythrocebus patas), squirrel monkey (Saimiri sciureus), and White Handed Gibbon (Hylobates lar).

Study	No. of primates	Species	Serotype	Ref.
Anez (2009)	4	Rhesus macaque	4	Anez et al. (2009)
Angsubhakorn (1988)	4	Cynomolgus macaques, rhesus macaque	4	Angsubhakorn et al. (1988)
Bernardo (2008)	4	Cynomolgus macaques, rhesus macaque	1	Bernardo et al. (2008)
Bray (1996)	16	Rhesus macaque	1, 2, 4	Bray et al. (1996)
Butrapet (2002)	2	Cynomolgus macaques	1	Butrapet et al. (2002)
Chen (2007)	3	Cynomolgus macaques	1	Chen et al. (2007)
Clements (2010)	3	Rhesus macaque	2	Clements et al. (2010)
Durbin (2006), Unpub.[a]	6	Rhesus macaque	1, 2	Unpub.
Durbin (2007), Unpub.[a]	6	Rhesus macaque	1, 2	Unpub.
Durbin (2008), Unpub.[a]	6	Rhesus macaque	1, 2	Unpub.
Freire (2007)	26	Rhesus macaque	1, 2, 3	Freire et al. (2007)
Galler (2005)	5	Rhesus macaque	2	Galler et al. (2005)
Goncalvez (2007)	3	Rhesus macaque	4	Goncalvez et al. (2007)
Guirakhoo (2000)	8	Rhesus macaque	2	Guirakhoo et al. (2000)
Guzman (2003)	3	Cynomolgus macaques	4	Guzman et al. (2003)
Halstead (1973)	119	Green monkey, patas, rhesus macaque	1, 2, 3, 4	Halstead et al. (1973)
Hanley (2004)	2	Rhesus macaque	4	Hanley et al. (2004)
Harrison (1977)	6	Chimpanzee, rhesus macaque	2	Harrison et al. (1977)
Hermida (2006)	3	Rhesus macaque	2	Hermida et al. (2006)
Houng (2000)	3	Rhesus macaque	2	Houng et al. (2000)
Kochel (2000)	5	Aotus	1	Kochel et al. (2000)
Kochel (2005)	18	Aotus	1	Kochel et al. (2005)
Lai (2007)	2	Rhesus macaque	4	Lai et al. (2007)
Marchette (1973)	27	Rhesus macaque	1, 2, 3, 4	Marchett et al. (1973)
Markoff (2002)	9	Rhesus macaque	1	Markoff et al. (2002)
Martin (2009)	12	Green monkey	2	Martin et al. (2009b)
Martin (2009)	6	Green monkey	2	Martin et al. (2009a)
Maves (2011)	6	Aotus	1	Maves et al. (2011)
Men (1996)	12	Rhesus macaque	4	Men et al. (1996)
Men (2000)	4	Rhesus macaque	2	Men et al. (2000)
Omatsu (2011)	20	Common marmoset	1, 2, 3, 4	Omatsu et al. (2011)
Onlamoon (2010)	6	Rhesus macaque	2	Onlamoon et al. (2010)
Pamungkas (2011)	14	Pig-tailed macaques (Macaca nemestrina)	3	Pamungkas et al. (2011)

Study	No. of primates	Species	Serotype	Ref.
Pletnev (2001)	4	Rhesus macaque	4	Pletnev et al. (2001)
Price (1968)	17	Spider monkey	1, 3, 4	Price et al. (1968)
Price (1973)	20	Spider monkey	1, 2, 3, 4	Price et al. (1973)
Price (1974)	25	Chimpanzee, cynomolgus macaques,	1, 4	Price et al. (1974)
		Rhesus macaque, squirrel monkey		
Putnak (1996)	3	Rhesus macaque	2	Putnak et al. (1996)
Putnak (2003)	9	Rhesus macaque	2	Putnak et al. (2003)
Raviprakash (2000)	5	Rhesus macaque	1	Raviprakash et al. (2000)
Raviprakash (2006)	5	Rhesus macaque	1, 2	Raviprakash et al. (2006)
Raviprakash (2008)	24	Rhesus macaque	1, 2, 3, 4	Raviprakash et al. (2008)
Rumyantsev (2006)	8	Rhesus macaque	4	Rumyantsev et al. (2006)
Scherer (1978)	10	Chimpanzee	1, 2, 3, 4	Scherer et al. (1978)
Schiavetta (2003)	15	Aotus	1, 2, 3, 4	Schiavetta et al. (2003)
Simmons (2006)	4	Rhesus macaque	2	Simmons et al. (2006)
Simmons (2010)	20	Rhesus macaque	1, 2, 3, 4	Simmons et al. (2010)
Sun (2006)	20	Rhesus macaque	1, 2, 3, 4	Sun et al. (2006)
Tarr (1976)	4	Rhesus macaque	2	Tarr and Lubiniecki (1976)
Valdes (2009)	3	Green monkey	2	Valdés et al. (2009)
Velzing (1999)	2	Cynomolgus macaques	2	Velzing et al. (1999)
Whitehead, various[b]	84	Rhesus macaque	1, 2, 3, 4	Blaney et al. (2006)
Whitehead (1970)	33	White handed gibbon	1, 2, 3, 4	Whitehead et al. (1970)
Widjaja (2010)	16	Pig-tailed macaques (Macaca nemestrina)	1, 2, 3, 4	Widjaja et al. (2010)

Table 2
Summary of DENV infection by non-human primate species

Table reports numbers of non-human primate species by infection with DENV serotypes 1–4.

Species	DENV 1	DENV 2	DENV 3	DENV 4
chimpanzee (*Pan troglodytes*)	2	6	2	12
common marmoset (*Callithrix jacchus*)	1	17	1	1
cynomolgus macaques (*Macaca fascicularis*)	7	2	0	10
green monkey (*Chlorocebus aethiops sabaeus*)	1	23	1	0
owl monkeys (*Aotus nancymaae*)	33	4	4	3
patas (*Erythrocebus patas*)	1	2	0	0
pig-tailed macaques (*Macaca nemestrina*)	4	4	18	4
rhesus macaque (*Macaca mulatta*)	97	155	75	139
spider monkey (*Ateles geoffroyi*)	8	5	12	12
squirrel monkey (*Saimiri sciureus*)	5	0	0	0
white handed gibbon (*Hylobates lar*)	7	9	8	9

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Table 3

Associations with Time to Detectable Viremia

Table reports the results of the univariate and multivariate mixed effects regression calculating associations between serotype, species, viremia assay used, and $\log_{10}$ inoculating dose and time to detectable viremia. Mixed effects models included random effect for study. Univariate estimates are differences in days of viremia from the reference category of each model (denoted "ref."), and multivariate estimates are differences in days of viremia for each covariate from rhesus monkeys infected with DENV-4 assayed using plaque count. P-values calculated using likelihood ratio tests. Estimates of the fixed intercept (β_0) and variance of the random intercept are presented (σ).

Covariate	Univariate	95% CI	p	Multivariate	95% CI	p
DENV-4	ref.			ref.		
DENV-1	0.53	(0.20, 0.86)	0.002	0.55	(0.22, 0.89)	0.001
DENV-2	−0.09	(−0.41, 0.22)	0.558	−0.17	(−0.49, 0.14)	0.254
DENV-3	0.20	(−0.14, 0.54)	0.252	0.24	(−0.10, 0.57)	0.153
rhesus macaque (*Macaca mulatta*)	ref.					
chimpanzee (*Pan troglodytes*)	−0.17	(−1.10, 0.76)	0.730	−0.30	(−1.22, 0.63)	0.483
common marmoset (*Callithrix jacchus*)	−1.14	(−3.15, 0.86)	0.214	−0.61	(−2.75, 1.53)	0.493
cynomolgus macaques (*Macaca fascicularis*)	0.66	(−0.18, 1.50)	0.132	0.74	(−0.12, 1.60)	0.096
green monkey (*Chlorocebus aethiops sabaeus*)	0.61	(−0.32, 1.53)	0.301	1.23	(0.12, 2.35)	0.095
owl monkeys (*Aotus nancymaae*)	−0.21	(−1.30, 0.89)	0.671	−0.20	(−1.47, 1.08)	0.777
patas (*Erythrocebus patas*)	3.64	(2.13, 5.15)	< 0.001	3.89	(2.40, 5.39)	< 0.001
pig-tailed macaques (*Macaca nemestrina*)	−0.94	(−2.40, 0.52)	0.162	−0.50	(−2.15, 1.15)	0.446
spider monkey (*Ateles geoffroyi*)	−1.29	(−2.74, 0.15)	0.056	−2.49	(−5.20, 0.22)	0.040
squirrel monkey (*Saimiri sciureus*)	−0.35	(−1.73, 1.03)	0.620	−0.96	(−2.36, 0.43)	0.162
white handed gibbon (*Hylobates lar*)	0.62	(−1.35, 2.58)	0.508	−0.27	(−2.34, 1.80)	0.755
plaque count	ref.					
ELISA	−0.75	(−2.31, 0.82)	0.329	−1.53	(−3.51, 0.45)	0.161
FFA	0.02	(−1.49, 1.52)	0.974	0.22	(−1.41, 1.86)	0.746
IFA	−0.75	(−1.43, −0.07)	0.025	−0.89	(−1.74, −0.04)	0.016
RTPCR	−0.67	(−1.37, 0.03)	0.050	−0.51	(−1.39, 0.37)	0.170
suckling mice	−1.16	(−2.34, 0.02)	0.043	0.64	(−1.71, 2.99)	0.561
$\log_{10}$ dose	−0.15	(−0.32, 0.01)	0.069	−0.21	(−0.39, −0.04)	0.021

Covariate	Univariate	95% CI	p	Multivariate	95% CI	p
Intercept (β_0)				2.68	(1.63, 3.74)	< 0.001
Random Effect (σ)				0.99	(0, 2.93)	

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Table 4

Associations with Duration of Viremia

Table reports the results of the univariate and multivariate mixed effects regression calculating associations between serotype, species, viremia assay used, and $\log_{10}$ inoculating dose and duration of viremia. Mixed effects models included random effect for study. Univariate estimates are differences in days of viremia from the reference category of each model (denoted "ref."), and multivariate estimates are differences in days of viremia for each covariate from rhesus monkeys infected with DENV-4 assayed using plaque count. P-values calculated using likelihood ratio tests. Durations of DENV-1 and -2 viremia are significantly longer than DENV-4 after adjusting for study, species, assay and $\log_{10}$ dose. Estimates of the fixed intercept (β_0) and variance of the random intercept are presented (σ).

Covariate	Univariate	95% CI	p	Multivariate	95% CI	p
DENV-4	ref.			ref.		
DENV-1	0.63	(0.17, 1.09)	0.008	0.74	(0.26, 1.21)	0.002
DENV-2	1.02	(0.58, 1.46)	< 0.001	1.21	(0.77, 1.65)	< 0.001
DENV-3	−0.34	(−0.81, 0.13)	0.151	−0.30	(−0.77, 0.17)	0.225
rhesus macaque (*Macaca mulatta*)	ref.					
chimpanzee (*Pan troglodytes*)	0.16	(−1.26, 1.57)	0.836	0.26	(−1.06, 1.59)	0.624
common marmoset (*Callithrix jacchus*)	−0.15	(−4.05, 3.76)	0.936	−1.80	(−5.09, 1.49)	0.185
cynomolgus macaques (*Macaca fascicularis*)	0.23	(−1.09, 1.55)	0.732	0.00	(−1.24, 1.24)	0.941
green monkey (*Chlorocebus aethiops sabaeus*)	−1.36	(−2.84, 0.12)	0.062	−1.48	(−3.08, 0.12)	0.050
owl monkeys (*Aotus nancymaae*)	−0.46	(−2.54, 1.62)	0.637	−1.29	(−3.23, 0.66)	0.125
patas (*Erythrocebus patas*)	−2.07	(−4.21, 0.06)	0.057	−2.35	(−4.45, −0.25)	0.026
pig-tailed macaques (*Macaca nemestrina*)	−0.40	(−3.21, 2.42)	0.764	−0.64	(−3.16, 1.88)	0.514
spider monkey (*Ateles geoffroyi*)	−1.11	(−3.91, 1.69)	0.400	0.34	(−3.77, 4.46)	0.834
squirrel monkey (*Saimiri sciureus*)	−0.72	(−2.72, 1.29)	0.470	−1.39	(−3.36, 0.59)	0.170
white handed gibbon (*Hylobates lar*)	0.00	(−3.87, 3.86)	0.998	0.09	(−3.09, 3.28)	0.932
plaque count	ref.					
ELISA	0.02	(−2.81, 2.85)	0.987	1.34	(−1.61, 4.30)	0.304
FFA	0.17	(−2.59, 2.93)	0.889	0.27	(−2.22, 2.75)	0.788
IFA	0.98	(−0.29, 2.24)	0.110	1.34	(0.05, 2.64)	0.018
RTPCR	1.89	(0.59, 3.18)	0.004	2.52	(1.19, 3.85)	< 0.001
suckling mice	−0.16	(−2.39, 2.06)	0.880	−0.37	(−3.92, 3.18)	0.807

Covariate	Univariate	95% CI	p	Multivariate	95% CI	p
$\log_{10}$ dose	−0.40	(−0.65, −0.14)	0.002	−0.44	(−0.69, −0.18)	< 0.001
Intercept (β_0)				5.00	(3.47, 6.53)	< 0.001
Random Effect (σ)				2.32	(0, 5.30)	

Table 5
Summary of DENV Virus Kinetics in Rhesus Macaques

Table reports median days to viremia and duration of viremia with 95% bootstrap confidence intervals for 5th, 25th, 50th, 75th, and 95th percentile.

Serotype	Percentile	Time to Viremia Days (95% CI)	Duration Days (95% CI)
1	5th	1.43 (1.20, 1.68)	2.02 (1.73, 2.44)
n = 97	25th	2.35 (2.08, 2.64)	3.31 (2.97, 3.76)
	50th	3.32 (3.01, 3.65)	4.67 (4.27, 5.12)
	75th	4.69 (4.28, 5.11)	6.59 (6.01, 7.18)
	95th	7.73 (6.89, 8.57)	10.82 (9.51, 12.12)
2	5th	0.98 (0.82, 1.18)	2.24 (1.98, 2.59)
n = 155	25th	1.76 (1.55, 2.00)	3.65 (3.35, 4.00)
	50th	2.63 (2.40, 2.89)	5.13 (4.82, 5.48)
	75th	3.95 (3.67, 4.22)	7.21 (6.73, 7.70)
	95th	7.06 (6.48, 7.64)	11.76 (10.48, 13.01)
3	5th	1.30 (1.07, 1.57)	1.03 (0.84, 1.33)
n = 75	25th	2.14 (1.85, 2.41)	2.02 (1.73, 2.41)
	50th	3.02 (2.71, 3.34)	3.22 (2.83, 3.72)
	75th	4.26 (3.78, 4.74)	5.14 (4.56, 5.84)
	95th	6.99 (5.91, 8.13)	10.05 (8.70, 11.58)
4	5th	1.44 (1.32, 1.58)	1.10 (0.95, 1.31)
n = 139	25th	2.32 (2.16, 2.51)	2.04 (1.83, 2.32)
	50th	3.23 (2.99, 3.47)	3.13 (2.86, 3.46)
	75th	4.49 (4.12, 4.84)	4.82 (4.38, 5.28)
	95th	7.22 (6.48, 7.89)	8.96 (7.96, 10.00)