



# Viral kinetics of primary dengue virus infection in non-human primates: A systematic review and individual pooled analysis

Benjamin M. Althouse<sup>a,\*</sup>, Anna P. Durbin<sup>b</sup>, Kathryn A. Hanley<sup>c</sup>, Scott B. Halstead<sup>d</sup>, Scott C. Weaver<sup>e</sup>, Derek A.T. Cummings<sup>b</sup>

<sup>a</sup> Santa Fe Institute, Santa Fe, NM, USA

<sup>b</sup> Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

<sup>c</sup> New Mexico State University, Las Cruces, NM, USA

<sup>d</sup> 5824 Edson Lane, Bethesda, MD 20852, USA

<sup>e</sup> University of Texas Medical Branch, Galveston, TX, USA

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## 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 aid in understanding the transmission dynamics of sylvatic DENV non-human primates, an issue of growing importance as dengue vaccines become available.

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## Introduction

Knowledge of the kinetics of dengue fever virus (DENV) within primate and non-primate hosts is a key to understanding transmission dynamics and identifying populations at risk for infection (Simmons et al., 2012). 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 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 (Halstead et al., 1973; Koraka et al., 2007), albeit with lower viral replication and limitation of virus to a subset of those tissues infected in humans (Zompi and Harris, 2012).

In addition to serving as a potential model for human diseases, insight into 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 (Rudnick et al., 1986) and sylvatic DENV serotype 2 is maintained in West Africa (Diallo et al., 2003). These sylvatic viruses are ancestral to the four serotypes of DENV that are currently transmitted between humans by domestic and peridomestic *Aedes* (Vasilakis et al., 2011). Populations living in areas surrounding sylvatic hotspots of DENV transmission are at risk of infection (Cardosa et al., 2009; Franco et al., 2011) from a transmission process that is poorly understood (Vasilakis and Weaver, 2008). Importantly, it has recently been discovered that sylvatic DENV infection in humans can produce the most severe manifestation of dengue disease – dengue hemorrhagic fever (Cardosa et al., 2009; Franco et al., 2011). In the light of recent advances in DENV vaccines (Guy et al., 2010; Durbin and Whitehead, 2010), 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 (Vasilakis et al., 2011).

Isolations of sylvatic DENV have occurred at roughly 8 year intervals in Senegal over the past 50 years (Diallo et al., 2003). The key determinants of cycle length are largely unknown. As the natural history of a pathogen has a direct influence on transmission dynamics (Keeling and Rohani, 2008) knowledge of the time to detectable viremia and the length of viremia in non-human

\* Corresponding author.

E-mail address: [althouse@santafe.edu](mailto:althouse@santafe.edu) (B.M. Althouse).

primates will be useful in ecological models of transmission (Althouse et al., 2012) and may generate hypotheses for the observed serotype-specific transmission patterns (Nisalak et al., 2003) and clinical manifestations (Balmaseda et al., 2006; Halsey et al., 2012) 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 (Reich et al., 2009).

## Results

### Literature

Literature searches returned 1092 unique papers (Fig. 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 nancymae*), 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 effect models were fit with a random effect for study and were universally preferred over linear fixed effect 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 effect models assume non-human primates are exchangeable within studies, and account for heterogeneity between studies. Mixed effect 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 effect 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 Fig. 2).

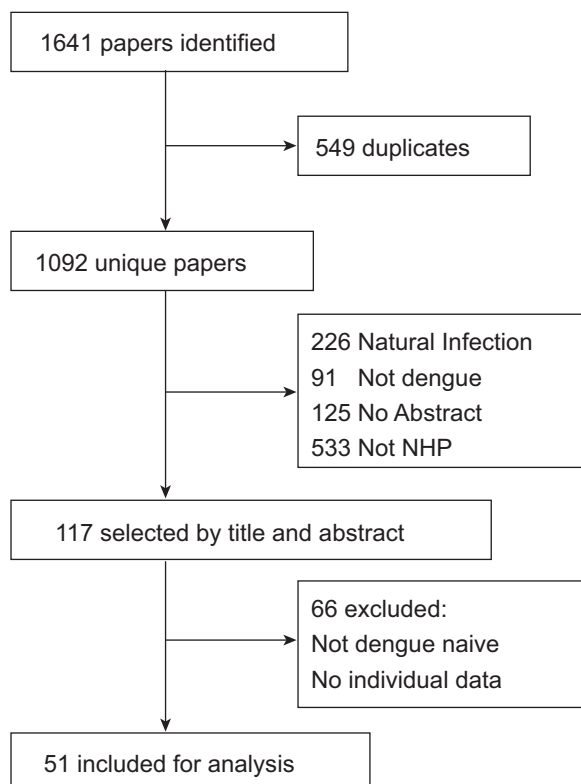


Fig. 1. Flow chart of systematic review.

**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 nancymae*), 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	<a href="#">Anez et al. (2009)</a>
Angsubhakorn (1988)	4	Cynomolgus macaques, rhesus macaque	4	<a href="#">Angsubhakorn et al. (1988)</a>
Bernardo (2008)	4	Cynomolgus macaques, rhesus macaque	1	<a href="#">Bernardo et al. (2008)</a>
Bray (1996)	16	Rhesus macaque	1, 2, 4	<a href="#">Bray et al. (1996)</a>
Butrapet (2002)	2	Cynomolgus macaques	1	<a href="#">Butrapet et al. (2002)</a>
Chen (2007)	3	Cynomolgus macaques	1	<a href="#">Chen et al. (2007)</a>
Clements (2010)	3	Rhesus macaque	2	<a href="#">Clements et al. (2010)</a>
Durbin (2006), Unpub. <sup>a</sup>	6	Rhesus macaque	1, 2	Unpub.
Durbin (2007), Unpub. <sup>a</sup>	6	Rhesus macaque	1, 2	Unpub.
Durbin (2008), Unpub. <sup>a</sup>	6	Rhesus macaque	1, 2	Unpub.
Freire (2007)	26	Rhesus macaque	1, 2, 3	<a href="#">Freire et al. (2007)</a>
Galler (2005)	5	Rhesus macaque	2	<a href="#">Galler et al. (2005)</a>
Goncalvez (2007)	3	Rhesus macaque	4	<a href="#">Goncalvez et al. (2007)</a>
Guirakhoo (2000)	8	Rhesus macaque	2	<a href="#">Guirakhoo et al. (2000)</a>
Guzman (2003)	3	Cynomolgus macaques	4	<a href="#">Guzmán et al. (2003)</a>
Halstead (1973)	119	Green monkey, patas, rhesus macaque	1, 2, 3, 4	<a href="#">Halstead et al. (1973)</a>
Hanley (2004)	2	Rhesus macaque	4	<a href="#">Hanley et al. (2004)</a>
Harrison (1977)	6	Chimpanzee, rhesus macaque	2	<a href="#">Harrison et al. (1977)</a>
Hermida (2006)	3	Rhesus macaque	2	<a href="#">Hermida et al. (2006)</a>
Houng (2000)	3	Rhesus macaque	2	<a href="#">Houng et al. (2000)</a>
Kochel (2000)	5	Aotus	1	<a href="#">Kochel et al. (2000)</a>
Kochel (2005)	18	Aotus	1	<a href="#">Kochel et al. (2005)</a>
Lai (2007)	2	Rhesus macaque	4	<a href="#">Lai et al. (2007)</a>
Marchette (1973)	27	Rhesus macaque	1, 2, 3, 4	<a href="#">Marchett et al. (1973)</a>
Markoff (2002)	9	Rhesus macaque	1	<a href="#">Markoff et al. (2002)</a>
Martin (2009)	12	Green monkey	2	<a href="#">Martín et al. (2009b)</a>
Martin (2009)	6	Green monkey	2	<a href="#">Martín et al. (2009a)</a>
Maves (2011)	6	Aotus	1	<a href="#">Maves et al. (2011)</a>
Men (1996)	12	Rhesus macaque	4	<a href="#">Men et al. (1996)</a>
Men (2000)	4	Rhesus macaque	2	<a href="#">Men et al. (2000)</a>
Omatsu (2011)	20	Common marmoset	1, 2, 3, 4	<a href="#">Omatsu et al. (2011)</a>
Onlamoon (2010)	6	Rhesus macaque	2	<a href="#">Onlamoon et al. (2010)</a>
Pamungkas (2011)	14	Pig-tailed macaques ( <i>Macaca nemestrina</i> )	3	<a href="#">Pamungkas et al. (2011)</a>
Pletnev (2001)	4	Rhesus macaque	4	<a href="#">Pletnev et al. (2001)</a>
Price (1968)	17	Spider monkey	1, 3, 4	<a href="#">Price et al. (1968)</a>
Price (1973)	20	Spider monkey	1, 2, 3, 4	<a href="#">Price et al. (1973)</a>
Price (1974)	25	Chimpanzee, cynomolgus macaques, Rhesus macaque, squirrel monkey	1, 4	<a href="#">Price et al. (1974)</a>
Putnak (1996)	3	Rhesus macaque	2	<a href="#">Putnak et al. (1996)</a>
Putnak (2003)	9	Rhesus macaque	2	<a href="#">Putnak et al. (2003)</a>
Raviprakash (2000)	5	Rhesus macaque	1	<a href="#">Raviprakash et al. (2000)</a>
Raviprakash (2006)	5	Rhesus macaque	1, 2	<a href="#">Raviprakash et al. (2006)</a>
Raviprakash (2008)	24	Rhesus macaque	1, 2, 3, 4	<a href="#">Raviprakash et al. (2008)</a>
Rumyantsev (2006)	8	Rhesus macaque	4	<a href="#">Rumyantsev et al. (2006)</a>
Scherer (1978)	10	Chimpanzee	1, 2, 3, 4	<a href="#">Scherer et al. (1978)</a>
Schiavetta (2003)	15	Aotus	1, 2, 3, 4	<a href="#">Schiavetta et al. (2003)</a>
Simmons (2006)	4	Rhesus macaque	2	<a href="#">Simmons et al. (2006)</a>
Simmons (2010)	20	Rhesus macaque	1, 2, 3, 4	<a href="#">Simmons et al. (2010)</a>
Sun (2006)	20	Rhesus macaque	1, 2, 3, 4	<a href="#">Sun et al. (2006)</a>
Tarr (1976)	4	Rhesus macaque	2	<a href="#">Tarr and Lubiniecki (1976)</a>
Valdes (2009)	3	Green monkey	2	<a href="#">Valdés et al. (2009)</a>
Velzing (1999)	2	Cynomolgus macaques	2	<a href="#">Velzing et al. (1999)</a>
Whitehead, various <sup>b</sup>	84	Rhesus macaque	1, 2, 3, 4	<a href="#">Blaney et al. (2006)</a>
Whitehead (1970)	33	White handed gibbon	1, 2, 3, 4	<a href="#">Whitehead et al. (1970)</a>
Widjaja (2010)	16	Pig-tailed macaques ( <i>Macaca nemestrina</i> )	1, 2, 3, 4	<a href="#">Widjaja et al. (2010)</a>

<sup>a</sup> These studies are unpublished experiments by an author (APD) conducted in 2006, 2007, and 2008.

<sup>b</sup> These data were provided by Dr. S. Whitehead. The source studies are reviewed in [Blaney et al. \(2006\)](#).

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 ([Figs. 3 and 4](#)).

## 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. \(2011\)](#) reported a median duration of viremia of 6.2 days (IQR 5.8–7.2) for all serotypes and 6.8 days (IQR 6–7.3) for DENV-1. [Vaughn et al. \(2000\)](#) 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. [Murgue et al. \(2000\)](#) found a mean duration of 4.4 days for primary DENV infection in a cohort of French Polynesian children. 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 ([Halstead et al., 1973](#)). 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 ([Diallo et al., 2003](#)). 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 ([Nunn et al., 2000](#)).

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 ([Nisalak et al., 2003](#)) and in clinical manifestations ([Balmaseda et al., 2006](#); [Halsey et al., 2012](#)) 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. \(2010\)](#) found cases of dengue hemorrhagic fever (DHF) to be twice as likely in secondary DENV-2 and -3 infections than in secondary DENV-4. Conversely, we found DENV-2 to be statistically significantly longer than DENV-1, and -3, and -4. [Balmaseda et al. \(2006\)](#) found nearly double the odds of shock and internal hemorrhage with DENV-2 infection in outbreaks of DENV in Nicaragua. [Nisalak et al. \(2003\)](#) found DENV-3 to be associated with severe outbreaks of dengue in hospitalized cases in Bangkok, Thailand. [Fox et al. \(2011\)](#) found time to undetectable DENV-2 NS1 protein to be significantly longer than DENV-1. Extended durations of viremia for DENV-2 and -3 may be the cause of the increased severity of these infections and may be

**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 ( <i>Pan troglodytes</i> )	2	6	2	12
Common marmoset ( <i>Callithrix jacchus</i> )	1	17	1	1
Cynomolgus macaques ( <i>Macaca fascicularis</i> )	7	2	0	10
Green monkey ( <i>Chlorocebus aethiops sabaeus</i> )	1	23	1	0
Owl monkeys ( <i>Aotus nancymaeae</i> )	33	4	4	3
Patas ( <i>Erythrocebus patas</i> )	1	2	0	0
Pig-tailed macaques ( <i>Macaca nemestrina</i> )	4	4	18	4
Rhesus macaque ( <i>Macaca mulatta</i> )	97	155	75	139
Spider monkey ( <i>Ateles geoffroyi</i> )	8	5	12	12
Squirrel monkey ( <i>Saimiri sciureus</i> )	5	0	0	0
White handed gibbon ( <i>Hylobates lar</i> )	7	9	8	9

**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<sub>10</sub> inoculating dose and time to detectable viremia. Mixed effect 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 ( $\beta_0$ ) and variance of the random intercept are presented ( $\sigma$ ).

Covariate	Univariate	95% CI	<i>P</i>	Multivariate	95% CI	<i>P</i>
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 ( <i>Macaca mulatta</i> )	Ref.					
Chimpanzee ( <i>Pan troglodytes</i> )	−0.17	(−1.10, 0.76)	0.730	−0.30	(−1.22, 0.63)	0.483
Common marmoset ( <i>Callithrix jacchus</i> )	−1.14	(−3.15, 0.86)	0.214	−0.61	(−2.75, 1.53)	0.493
Cynomolgus macaques ( <i>Macaca fascicularis</i> )	0.66	(−0.18, 1.50)	0.132	0.74	(−0.12, 1.60)	0.096
Green monkey ( <i>Chlorocebus aethiops sabaeus</i> )	0.61	(−0.32, 1.53)	0.301	1.23	(0.12, 2.35)	0.095
Owl monkeys ( <i>Aotus nancymaeae</i> )	−0.21	(−1.30, 0.89)	0.671	−0.20	(−1.47, 1.08)	0.777
Patas ( <i>Erythrocebus patas</i> )	3.64	(2.13, 5.15)	< 0.001	3.89	(2.40, 5.39)	< 0.001
Pig-tailed macaques ( <i>Macaca nemestrina</i> )	−0.94	(−2.40, 0.52)	0.162	−0.50	(−2.15, 1.15)	0.446
Spider monkey ( <i>Ateles geoffroyi</i> )	−1.29	(−2.74, 0.15)	0.056	−2.49	(−5.20, 0.22)	0.040
Squirrel monkey ( <i>Saimiri sciureus</i> )	−0.35	(−1.73, 1.03)	0.620	−0.96	(−2.36, 0.43)	0.162
White handed gibbon ( <i>Hylobates lar</i> )	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
RT-PCR	−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 <sub>10</sub> dose	−0.15	(−0.32, 0.01)	0.069	−0.21	(−0.39, −0.04)	0.021
Intercept ( $\beta_0$ )				2.68	(1.63, 3.74)	< 0.001
Random effect ( $\sigma$ )				0.99	(0, 2.93)	



**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<sub>10</sub> inoculating dose and duration of viremia. Mixed effect 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<sub>10</sub> dose. Estimates of the fixed intercept ( $\beta_0$ ) and variance of the random intercept are presented ( $\sigma$ ).

Covariate	Univariate	95% CI	<i>P</i>	Multivariate	95% CI	<i>P</i>
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 ( <i>Macaca mulatta</i> )	Ref.					
Chimpanzee ( <i>Pan troglodytes</i> )	0.16	(−1.26, 1.57)	0.836	0.26	(−1.06, 1.59)	0.624
Common marmoset ( <i>Callithrix jacchus</i> )	−0.15	(−4.05, 3.76)	0.936	−1.80	(−5.09, 1.49)	0.185
Cynomolgus macaques ( <i>Macaca fascicularis</i> )	0.23	(−1.09, 1.55)	0.732	0.00	(−1.24, 1.24)	0.941
Green monkey ( <i>Chlorocebus aethiops sabaeus</i> )	−1.36	(−2.84, 0.12)	0.062	−1.48	(−3.08, 0.12)	0.050
Owl monkeys ( <i>Aotus nancymae</i> )	−0.46	(−2.54, 1.62)	0.637	−1.29	(−3.23, 0.66)	0.125
Patás ( <i>Erythrocebus patas</i> )	−2.07	(−4.21, 0.06)	0.057	−2.35	(−4.45, −0.25)	0.026
Pig-tailed macaques ( <i>Macaca nemestrina</i> )	−0.40	(−3.21, 2.42)	0.764	−0.64	(−3.16, 1.88)	0.514
Spider monkey ( <i>Ateles geoffroyi</i> )	−1.11	(−3.91, 1.69)	0.400	0.34	(−3.77, 4.46)	0.834
Squirrel monkey ( <i>Saimiri sciureus</i> )	−0.72	(−2.72, 1.29)	0.470	−1.39	(−3.36, 0.59)	0.170
White handed gibbon ( <i>Hylobates lar</i> )	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
RT-PCR	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
log <sub>10</sub> dose	−0.40	(−0.65, −0.14)	0.002	−0.44	(−0.69, −0.18)	< 0.001
Intercept ( $\beta_0$ )				5.00	(3.47, 6.53)	< 0.001
Random effect ( $\sigma$ )				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 <i>n</i> = 97	5th	1.43 (1.20, 1.68)	2.02 (1.73, 2.44)
	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 <i>n</i> = 155	5th	0.98 (0.82, 1.18)	2.24 (1.98, 2.59)
	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 <i>n</i> = 75	5th	1.30 (1.07, 1.57)	1.03 (0.84, 1.33)
	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 <i>n</i> = 139	5th	1.44 (1.32, 1.58)	1.10 (0.95, 1.31)
	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)

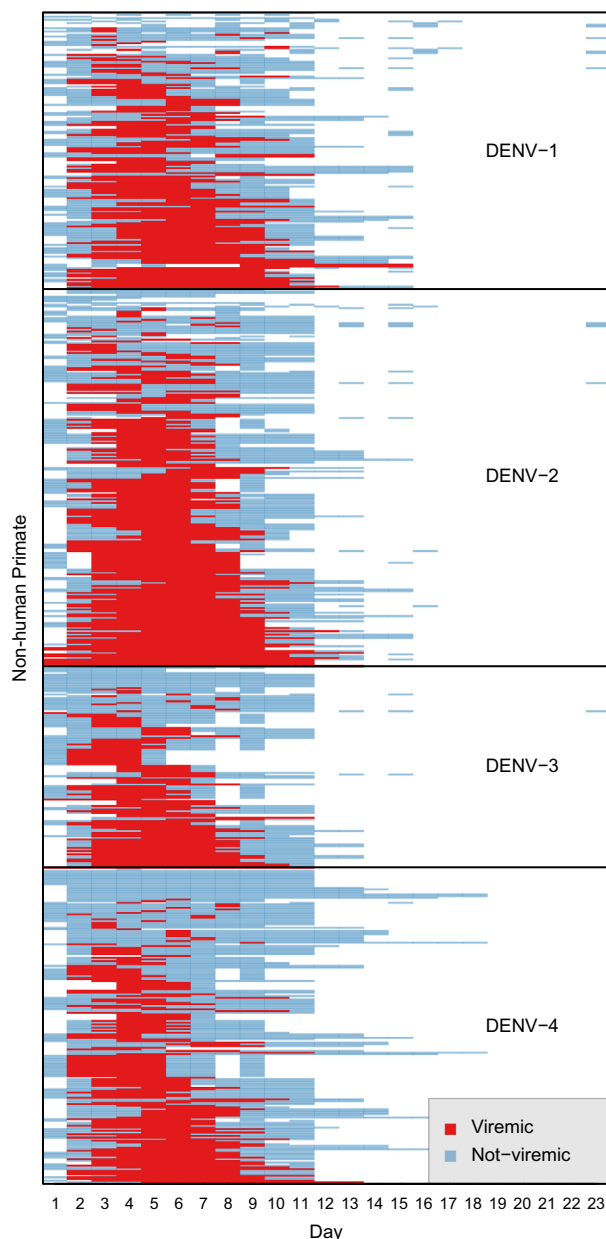
the reason for sylvatic DENV-2 which is the only serotype to have emerged in Africa from southeast Asia.

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 effect models to account for differences between studies, and using care when interpreting results of viremia assays.

Surprisingly, increasing dose of inoculum was associated with both shorter times to detectable viremia and shorter durations of viremia. This phenomenon was observed by [Martín et al. \(2009a\)](#) in green monkeys, in yellow fever virus (YFV) infections in rhesus monkeys ([Fox and Penna, 1943](#)), chimeric YF-DENV vaccine in cynomolgus macaques ([Guirakhoo et al., 2004](#)) and in humans receiving a live, attenuated Japanese Encephalitis vaccine (ChimeriVax-JE) ([Monath et al., 2003, 2002](#)) and a live, attenuated West Nile virus vaccine ([Monath et al., 2006](#)). 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 ([Tricou et al., 2011](#); [Vaughn et al., 2000](#); [Wang et al., 2003](#)); however the evidence for an association between the magnitude and duration of viremia remains inconclusive. [Vaughn et al. \(2000\)](#) found the time from peak viremia to clearance was more rapid in DHF cases than in DF cases, while [Fox et al. \(2011\)](#) found no difference in rate of clearance between DHF and DF.

Not all studies included in this meta-analysis reported daily levels of viremia. Additional studies examining directly the relationship among inoculating dose, peak viremia and duration of



**Fig. 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 bars indicate positive samples. White indicates no samples. Data were sorted by DENV serotype, then by days of viremia.

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 effect 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 effect 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 effect 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 (Dickerson et al., 1990).

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 (Halstead, 2012; Sabchareon et al., 2012), 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 (Althouse et al., 2012), and further understanding of DENV transmission dynamics in general, including differences in serotype-specific cycles.

## Methods

### Systematic review

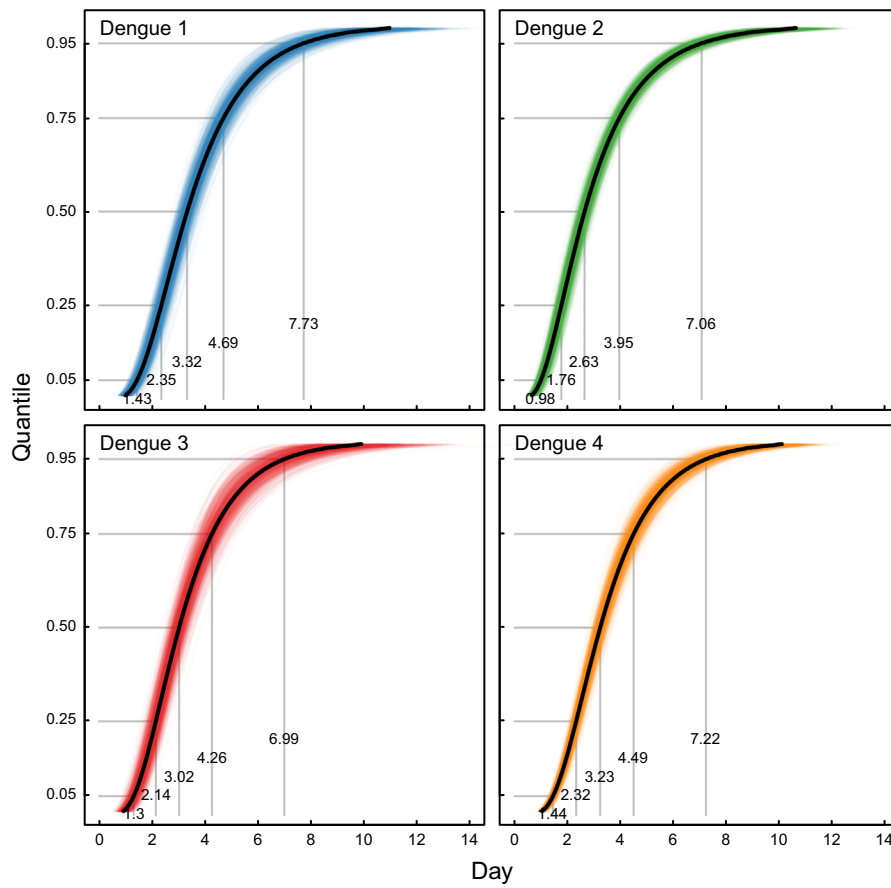
We searched PubMed, Web of Science and Google scholar for articles containing the terms “dengue primate viremia infection”, “dengue viremia primates”, “dengue viremia monkey”, “dengue vaccine primates”, and “dengue infection primates”. We narrowed our focus to primary infections where details on the 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 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).

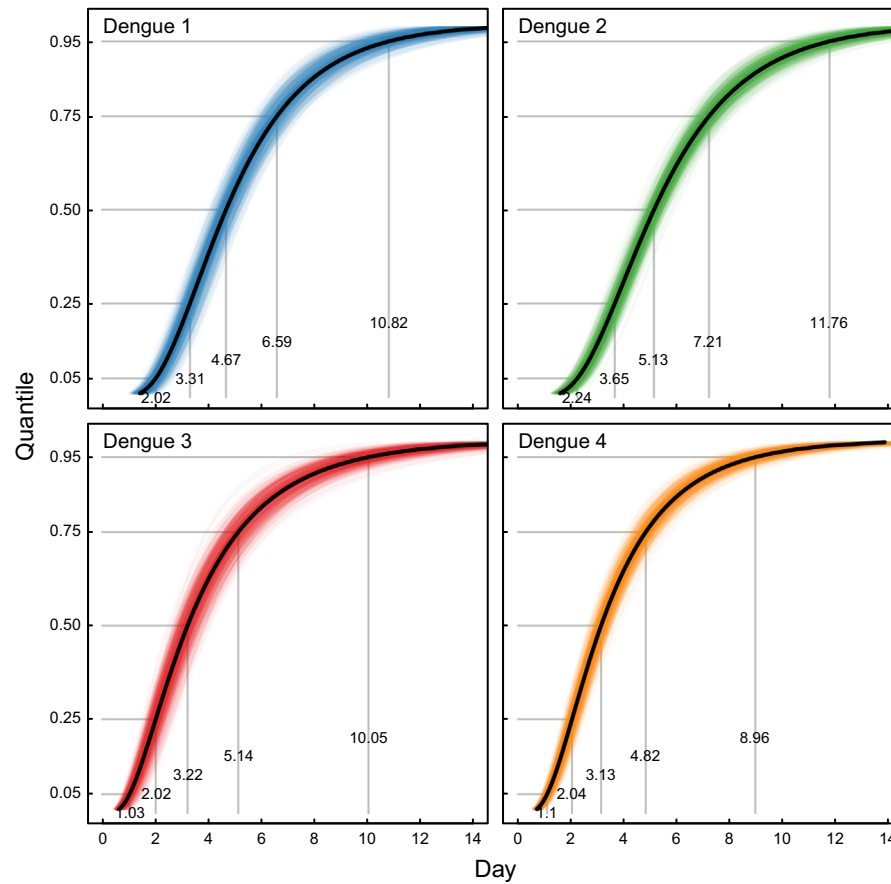
Additional (unpublished) studies were identified through expert consultation. Abstracts were doubly reviewed (BMA, DATC).

### 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



**Fig. 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. Gray lines indicate the 5th, 25th, 50th, 75th and 95th quantiles.



**Fig. 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. Gray lines indicate the 5th, 25th, 50th, 75th and 95th quantiles.

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 (Reich et al., 2009). 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 effect 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 (Fitzmaurice et al., 2011). As these models do not directly take into account the effects of censoring, we test for differences censoring between covariates. Linear and mixed effect models are compared using the Akaike information criterion (AIC) (Burnham et al., 2002).

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#### Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.virol.2014.01.015>.

#### References

- Althouse, B.M., Lessler, J., Sall, A.A., Diallo, M., Hanley, K.A., Watts, D.M., Weaver, S. C., Cummings, D.A., 2012. Synchrony of sylvatic dengue isolations: a multi-host, multi-vector sir model of dengue virus transmission in Senegal. *PLoS Negl. Trop. Dis.* 6 (11), e1928.
- Anez, G., Men, R., Eckels, K.H., Lai, C.-J., 2009. Passage of dengue virus type 4 vaccine candidates in fetal rhesus lung cells selects heparin-sensitive variants that result in loss of infectivity and immunogenicity in rhesus macaques. *J. Virol.* 83 (October), 10384–10394.
- Angsubhakorn, S., Yoksan, S., Bhamarapavati, N., Moe, J.B., Marchette, N.J., Pradermwong, A., Sahaphong, S., 1988. Dengue-4 vaccine: neurovirulence, viraemia and immune responses in rhesus and cynomolgus monkeys. *Trans. R. Soc. Trop. Med. Hyg.* 82 (5), 746–749.
- Balmaseda, A., Hammond, S.N., Pérez, L., Tellez, Y., Saborío, S.I., Mercado, J.C., Cuadra, R., Rocha, J., Pérez, M.A., Silva, S., Rocha, C., Harris, E., 2006. Serotype-specific differences in clinical manifestations of dengue. *Am. J. Trop. Med. Hyg.* 74 (March), 449–456.
- Bernardo, L., Izquierdo, A., Alvarez, M., Rosario, D., Prado, I., López, C., Martínez, R., Castro, J., Santana, E., Hermida, L., Guillen, G., Guzmán, M.G., 2008. Immunogenicity and protective efficacy of a recombinant fusion protein containing the domain III of the dengue 1 envelope protein in non-human primates. *Antivir. Res.* 80 (November), 194–199.
- Blaney, J., Durbin, A., Murphy, B., Whitehead, S., 2006. Development of a live attenuated dengue virus vaccine using reverse genetics. *Viral Immunol.* 19 (1), 10–32.
- Bray, M., Men, R., Lai, C.J., 1996. Monkeys immunized with intertypic chimeric dengue viruses are protected against wild-type virus challenge. *J. Virol.* 70 (June), 4162–4166.
- Burnham, K.P., Anderson, D.R., Burnham, K.P., 2002. *Model Selection and Multi-model Inference: A Practical Information-Theoretic Approach*, 2nd ed. Springer, New York.
- Butrapet, S., Rababert, J., Angsubhakorn, S., Wiriyarat, W., Huang, C., Kinney, R., Punyim, S., Bhamarapavati, N., 2002. Chimeric dengue type 2/type 1 viruses induce immune responses in cynomolgus monkeys. *Southeast Asian J. Trop. Med. Public Health* 33 (September), 589–599.
- Cardosa, J., Ooi, M.H., Tio, P.H., Perera, D., Holmes, E.C., Bibi, K., Abdul Manap, Z., 2009. Dengue virus serotype 2 from a sylvatic lineage isolated from a patient with dengue hemorrhagic fever. *PLoS Negl. Trop. Dis.* 3 (4), e423.
- Chen, L., Ewing, D., Subramanian, H., Block, K., Rayner, J., Alterson, K.D., Sedegah, M., Hayes, C., Porter, K., Raviprakash, K., 2007. A heterologous DNA prime-venezuelan equine encephalitis virus replicon particle boost dengue vaccine regimen affords complete protection from virus challenge in cynomolgus macaques. *J. Virol.* 81 (November), 11634–11639.
- Clements, D.E., Collier, B.-A.G., Lieberman, M.M., Ogata, S., Wang, G., Harada, K.E., Putnak, J.R., Ivy, J.M., McDonnell, M., Bignami, G.S., Peters, I.D., Leung, J., Weeks-Levy, C., Nakano, E.T., Humphreys, T., 2010. Development of a recombinant tetravalent dengue virus vaccine: immunogenicity and efficacy studies in mice and monkeys. *Vaccine* 28 (March), 2705–2715.
- Diallo, M., Ba, Y., Sall, A.A., Diop, O.M., Ndione, J.A., Mondo, M., Girault, L., Mathiot, C., 2003. Amplification of the sylvatic cycle of dengue virus type 2. Senegal, 1999–2000: entomologic findings and epidemiologic considerations. *Emerg. Infect. Dis.* 9 (March), 362–367.
- Dickersin, K., 1990. The existence of publication bias and risk factors for its occurrence. *JAMA* 263 (March), 1385–1389.
- Durbin, A.P., Whitehead, S.S., 2010. Dengue vaccine candidates in development. *Curr. Top. Microbiol. Immunol.* 338, 129–143.
- Fitzmaurice, G.M., Laird, N.M., Ware, J.H., 2011. *Applied Longitudinal Analysis*, Wiley Series in Probability and Statistics, 2nd ed. Wiley, Hoboken, NJ.
- Fox, J., Penna, H., 1943. Behavior of 17D yellow fever virus in rhesus monkeys relation to substrain and neural or extraneural inoculation. *Am. J. Epidemiol.* 38 (2), 152–172.
- Fox, A., Le, N.M.H., Simmons, C.P., Wolbers, M., Wertheim, H.F.L., Pham, T.K., Tran, T., H.N., Trinh, T.M.L., Nguyen, T.L., Nguyen, V.T., Nguyen, D.H., Farrar, J., Horby, P., Taylor, W.R., Nguyen, V.K., 2011. Immunological and viral determinants of dengue severity in hospitalized adults in Ha Noi, Viet Nam. *PLoS Negl. Trop. Dis.* 5 (3), e967.
- Franco, L., Palacios, G., Martinez, J.A., Vázquez, A., Savji, N., De Ory, F., Sanchez-Seco, M.P., Martín, D., Lipkin, W.I., Tenorio, A., 2011. First report of sylvatic DENV-2-associated dengue hemorrhagic fever in west africa. *PLoS Negl. Trop. Dis.* 5 (August), e1251.
- Freire, M.S., Marchevsky, R.S., Almeida, L.F.C., Yamamura, A.M.Y., Caride, E.C., Brindeiro, P.A., Motta, M.C.A., Nogueira, R.M.R., Kubelka, C.F., Bonaldo, M.C., Galler, R., 2007. Wild dengue virus types 1, 2 and 3 viremia in rhesus monkeys. *Mem. Inst. Oswaldo Cruz* 102 (May), 203–208.
- Fried, J.R., Gibbons, R.V., Kalayanaraj, S., Thomas, S.J., Srikiatkachorn, A., Yoon, I.-K., Jarman, R.G., Green, S., Rothman, A.L., Cummings, D.A.T., 2010. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in bangkok, thailand from 1994 to 2006. *PLoS Negl. Trop. Dis.* 4 (3), e617.
- Galler, R., Marchevsky, R.S., Caride, E., Almeida, L.F.C., Yamamura, A.M.Y., Jabor, A.V., Motta, M.C.A., Bonaldo, M.C., Coutinho, E.S.F., Freire, M.S., 2005. Attenuation and immunogenicity of recombinant yellow fever 17D-dengue type 2 virus for rhesus monkeys. *Braz. J. Med. Biol. Res.* 38 (December), 1835–1846.
- Goncalvez, A.P., Engle, R.E., St Claire, M., Purcell, R.H., Lai, C.-J., 2007. Monoclonal antibody-mediated enhancement of dengue virus infection in vitro and in vivo and strategies for prevention. *Proc. Natl. Acad. Sci. U. S. A.* 104 (May), 9422–9427.
- Guirakhoo, F., Weltzin, R., Chambers, T.J., Zhang, Z.X., Soike, K., Ratterree, M., Arroyo, J., Georgakopoulos, K., Catalan, J., Monath, T.P., 2000. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. *J. Virol.* 74 (June), 5477–5485.
- Guirakhoo, F., Pugachev, K., Zhang, Z., Myers, G., Levenbook, I., Draper, K., Lang, J., Ocran, S., Mitchell, F., Parsons, M., Brown, N., Brandler, S., Fournier, C., Barrere, B., Rizvi, F., Travassos, A., Nichols, R., Trent, D., Monath, T., 2004. Safety and efficacy of chimeric yellow fever-dengue virus tetravalent vaccine formulations in nonhuman primates. *J. Virol.* 78 (May), 4761–4775.
- Guy, B., Saville, M., Lang, J., 2010. Development of Sanofi Pasteur tetravalent dengue vaccine. *Hum. Vaccine* 6 (September).
- Guzmán, M.G., Rodríguez, R., Rodríguez, R., Hermida, L., Alvarez, M., Lazo, L., Muné, M., Rosario, D., Valdés, K., Vázquez, S., Martínez, R., Serrano, T., Paez, J., Espinosa, R., Pumariega, T., Guillén, G., 2003. Induction of neutralizing antibodies and partial protection from viral challenge in *Macaca fascicularis* immunized with recombinant dengue 4 virus envelope glycoprotein expressed in *Pichia pastoris*. *Am. J. Trop. Med. Hyg.* 69 (August), 129–134.
- Halsey, E.S., Marks, M.A., Gotuzzo, E., Fiestas, V., Suarez, L., Vargas, J., Aguayo, N., Madrid, C., Vimos, C., Kochel, T.J., Laguna-Torres, V.A., 2012. Correlation of serotype-specific dengue virus infection with clinical manifestations. *PLoS Negl. Trop. Dis.* 6 (5), e1638.
- Halstead, S.B., 2012. Dengue vaccine development: a 75% solution? *Lancet* 380 (9853) 1535–1536, [http://dx.doi.org/10.1016/S0140-6736\(12\)61510-4](http://dx.doi.org/10.1016/S0140-6736(12)61510-4).



- Halstead, S., Shotwell, H., Casals, J., 1973. Studies on pathogenesis of dengue infection in monkeys. 1. Clinical laboratory responses to primary infection. *J. Infect. Dis.* 128 (1), 7–14.
- Hanley, K.A., Manlucu, L.R., Manipon, G.G., Hanson, C.T., Whitehead, S.S., Murphy, B. R., Blaney, J.E., 2004. Introduction of mutations into the non-structural genes or 3' untranslated region of an attenuated dengue virus type 4 vaccine candidate further decreases replication in rhesus monkeys while retaining protective immunity. *Vaccine* 22 (September), 3440–3448.
- Harrison, V.R., Eckels, K.H., Sagartz, J.W., Russell, P.K., 1977. Virulence and immunogenicity of a temperature-sensitive dengue-2 virus in lower primates. *Infect. Immun.* 18 (October), 151–156.
- Hermida, L., Bernardo, L., Martín, J., Alvarez, M., Prado, I., López, C., Sierra, B.d.I.C., Martínez, R., Rodríguez, R., Zulueta, A., Pérez, A.B., Lazo, L., Rosario, D., Guillén, G., Guzmán, M.G., 2006. A recombinant fusion protein containing the domain III of the dengue-2 envelope protein is immunogenic and protective in nonhuman primates. *Vaccine* 24 (April), 3165–3171.
- Houng, H.H., Hritz, D., Kanasa-thasan, N., 2000. Quantitative detection of dengue 2 virus using fluorogenic RT-PCR based on 3'-noncoding sequence. *J. Virol. Methods* 86 (April), 1–11.
- Keeling, M.J., Rohani, P., 2008. *Modeling Infectious Diseases in Humans and Animals*. Princeton University Press, Princeton.
- Kochel, T.J., Raviprakash, K., Hayes, C.G., Watts, D.M., Russell, K.L., Gozalo, A.S., Phillips, I.A., Ewing, D.F., Murphy, G.S., Porter, K.R., 2000. A dengue virus serotype-1 DNA vaccine induces virus neutralizing antibodies and provides protection from viral challenge in Aotus monkeys. *Vaccine* 18 (July), 3166–3173.
- Kochel, T.J., Watts, D.M., Gozalo, A.S., Ewing, D.F., Porter, K.R., Russell, K.L., 2005. Cross-serotype neutralization of dengue virus in *Aotus nancymae* monkeys. *J. Infect. Dis.* 191 (March), 1000–1004.
- Koraka, P., Benton, S., van Amerongen, G., Stittelaar, K.J., Osterhaus, A.D.M.E., 2007. Characterization of humoral and cellular immune responses in cynomolgus macaques upon primary and subsequent heterologous infections with dengue viruses. *Microbes Infect.* 9 (July), 940–946.
- Lai, C.-J., Goncalves, A.P., Men, R., Wernly, C., Donau, O., Engle, R.E., Purcell, R.H., 2007. Epitope determinants of a chimpanzee dengue virus type 4 (DENV-4)-neutralizing antibody and protection against DENV-4 challenge in mice and rhesus monkeys by passively transferred humanized antibody. *J. Virol.* 81 (December), 12766–12774.
- Marchetti, N., Halstead, S., Falkner, W., Stenhouse, A., Nash, D., 1973. Studies on pathogenesis of dengue infection in monkeys 3. Sequential distribution of virus in primary and heterologous infections. *J. Infect. Dis.* 128 (1), 23–30.
- Markoff, L., Pang, X., Houng, H.S., Falgout, B., Olsen, R., Jones, E., Polo, S., 2002. Derivation and characterization of a dengue type 1 host range-restricted mutant virus that is attenuated and highly immunogenic in monkeys. *J. Virol.* 76 (April), 3318–3328.
- Martín, J., Hermida, L., Castro, J., Lazo, L., Martínez, R., Gil, L., Romero, Y., Puente, P., Zaragoza, S., Cosme, K., Guzmán, M.G., Cardosa, J., Guillén, G., 2009a. Viremia and antibody response in green monkeys (*Chlorocebus aethiops sabaues*) infected with dengue virus type 2: a potential model for vaccine testing. *Microbiol. Immunol.* 53 (April), 216–223.
- Martín, J., Hermida, L., Castro, J., Romero, Y., Cardosa, J., Guillén, G., 2009b. Viremia and the magnitude of the immune response upon infection of green monkeys with dengue virus type 2 are strain-dependent. *Curr. Microbiol.* 59 (December), 579–583.
- Maves, R.C., Oré, R.M.C., Porter, K.R., Kochel, T.J., 2011. Immunogenicity and protective efficacy of a psoralen-inactivated dengue-1 virus vaccine candidate in *Aotus nancymae* monkeys. *Vaccine* 29 (March), 2691–2696.
- Men, R., Bray, M., Clark, D., Chanock, R.M., Lai, C.J., 1996. Dengue type 4 virus mutants containing deletions in the 3' noncoding region of the rna genome: analysis of growth restriction in cell culture and altered viremia pattern and immunogenicity in rhesus monkeys. *J. Virol.* 70 (June), 3930–3937.
- Men, R., Wyatt, L., Tokimatsu, I., Arakaki, S., Shameem, G., Elkins, R., Chanock, R., Moss, B., Lai, C.J., 2000. Immunization of rhesus monkeys with a recombinant of modified vaccinia virus ankara expressing a truncated envelope glycoprotein of dengue type 2 virus induced resistance to dengue type 2 virus challenge. *Vaccine* 18 (July), 3113–3122.
- Monath, T.P., McCarthy, K., Bedford, P., Johnson, C.T., Nichols, R., Yoksan, S., Marchesani, R., Knauber, M., Wells, K.H., Arroyo, J., Guirakhoo, F., 2002. Clinical proof of principle for chimerivax: recombinant live, attenuated vaccines against flavivirus infections. *Vaccine* 20 (January), 1004–1018.
- Monath, T.P., Guirakhoo, F., Nichols, R., Yoksan, S., Schrader, R., Murphy, C., Blum, P., Woodward, S., McCarthy, K., Mathis, D., Johnson, C., Bedford, P., 2003. Chimeric live, attenuated vaccine against japanese encephalitis chimerivax-je: phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated japanese encephalitis antigen. *J. Infect. Dis.* 188 (October), 1213–1230.
- Monath, T.P., Liu, J., Kanasa-Thasan, N., Myers, G.A., Nichols, R., Deary, A., McCarthy, K., Johnson, C., Ermak, T., Shin, S., Arroyo, J., Guirakhoo, F., Kennedy, J.S., Ennis, F. A., Green, S., Bedford, P., 2006. A live, attenuated recombinant west nile virus vaccine. *Proc. Natl. Acad. Sci. U. S. A.* 103 (April), 6694–6699.
- Murgue, B., Roche, C., Chungue, E., Deparis, X., 2000. Prospective study of the duration and magnitude of viraemia in children hospitalised during the 1996–1997 dengue-2 outbreak in French Polynesia. *J. Med. Virol.* 60 (April), 432–438.
- Nisalak, A., Endy, T.P., Nimmannitya, S., Kalayanarooj, S., Thisyakorn, U., Scott, R.M., Burke, D.S., Hoke, C.H., Innis, B.L., Vaughn, D.W., 2003. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. *Am. J. Trop. Med. Hyg.* 68 (February), 191–202.
- Nunn, C.L., Gittleman, J.L., Antonovics, J., 2000. Promiscuity and the primate immune system. *Science* 290 (November), 1168–1170.
- Omatsu, T., Moi, M.L., Hirayama, T., Takasaki, T., Nakamura, S., Tajima, S., Ito, M., Yoshida, T., Saito, A., Katakai, Y., Akari, H., Kurane, I., 2011. Common marmoset (*Callithrix jacchus*) as a primate model of dengue virus infection: development of high levels of viraemia and demonstration of protective immunity. *J. Gen. Virol.* 92 (October), 2272–2280.
- Onlamoon, N., Noisakran, S., Hsiao, H.-M., Duncan, A., Villinger, F., Ansari, A.A., Perng, G.C., 2010. Dengue virus-induced hemorrhage in a nonhuman primate model. *Blood* 115 (March), 1823–1834.
- Pamungkas, J., Iskandriati, D., Saepuloh, U., Affandi, M., Arifin, E., Paramastri, Y., Dewi, F., Sajuthi, D., 2011. Dissemination in pigtailed macaques after primary infection of dengue-3 virus. *Microbiol. Indones.* 5 (2), 7.
- Pletnev, A.G., Bray, M., Hanley, K.A., Speicher, J., Elkins, R., 2001. Tick-borne Langat/mosquito-borne dengue flavivirus chimera, a candidate live attenuated vaccine for protection against disease caused by members of the tick-borne encephalitis virus complex: evaluation in rhesus monkeys and in mosquitoes. *J. Virol.* 75 (September), 8259–8267.
- Price, W.H., 1968. Sequential immunization as a vaccination procedure against dengue viruses. *Am. J. Epidemiol.* 88 (November), 392–397.
- Price, W.H., Casals, J., Thind, I., O'Leary, W., 1973. Sequential immunization procedure against Group B arboviruses using living attenuated 17D yellow fever virus, living attenuated Langat E5 virus, and living attenuated dengue 2 virus (new Guinea C isolate). *Am. J. Trop. Med. Hyg.* 22 (July), 509–523.
- Price, W.H., Casals, J., O'Leary, W., 1974. Studies on the sequential immunization against Group B arboviruses in squirrel monkeys, cynomolgus monkeys, rhesus monkeys, and chimpanzees. *Am. J. Trop. Med. Hyg.* 23 (January), 118–130.
- Putnak, R., Barvir, D.A., Burrous, J.M., Dubois, D.R., D'Andrea, V.M., Hoke, C.H., Sadoff, J.C., Eckels, K.H., 1996. Development of a purified, inactivated, dengue-2 virus vaccine prototype in vero cells: immunogenicity and protection in mice and rhesus monkeys. *J. Infect. Dis.* 174 (December), 1176–1184.
- Putnak, R., Fuller, J., VanderZanden, L., Innis, B.L., Vaughn, D.W., 2003. Vaccination of rhesus macaques against dengue-2 virus with a plasmid DNA vaccine encoding the viral pre-membrane and envelope genes. *Am. J. Trop. Med. Hyg.* 68 (April), 469–476.
- Raviprakash, K., Porter, K.R., Kochel, T.J., Ewing, D., Simmons, M., Phillips, I., Murphy, G.S., Weiss, W.R., Hayes, C.G., 2000. Dengue virus type 1 DNA vaccine induces protective immune responses in rhesus macaques. *J. Gen. Virol.* 81 (July), 1659–1667.
- Raviprakash, K., Apt, D., Brinkman, A., Skinner, C., Yang, S., Dawes, G., Ewing, D., Wu, S.-J., Bass, S., Punnonen, J., Porter, K., 2006. A chimeric tetravalent dengue DNA vaccine elicits neutralizing antibody to all four virus serotypes in rhesus macaques. *Virology* 353 (September), 166–173.
- Raviprakash, K., Wang, D., Ewing, D., Holman, D.H., Block, K., Woraratanadham, J., Chen, L., Hayes, C., Dong, J.Y., Porter, K., 2008. A tetravalent dengue vaccine based on a complex adenovirus vector provides significant protection in rhesus monkeys against all four serotypes of dengue virus. *J. Virol.* 82 (July), 6927–6934.
- Reich, N., Lessler, J., Cummings, D., Brookmeyer, R., 2009. Estimating incubation period distributions with coarse data. *Stat. Med.* 28 (22), 2769–2784.
- Rudnick, A., Lim, T., Ireland, J., 1986. Dengue fever studies in Malaysia. Institute for Medical Research, Kuala Lumpur, Malaysia.
- Rumyantsev, A.A., Chanock, R.M., Murphy, B.R., Pletnev, A.G., 2006. Comparison of live and inactivated tick-borne encephalitis virus vaccines for safety, immunogenicity and efficacy in rhesus monkeys. *Vaccine* 24 (January), 133–143.
- Sabchareon, A., Wallace, D., Sirivichayakul, C., Limkittikul, K., Chanthavanich, P., Suvannadabba, S., Jiwariyavej, V., Dulyachai, W., Pengsaa, K., Wartel, T.A., Moureau, A., Saville, M., Bouckennooghe, A., Viviani, S., Tornieporth, N.G., Lang, J., 2012. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* 380 (9853), 1559–1567.
- Scherer, W.F., Russell, P.K., Rosen, L., Casals, J., Dickerman, R.W., 1978. Experimental infection of chimpanzees with dengue viruses. *Am. J. Trop. Med. Hyg.* 27 (May), 590–599.
- Schiavetta, A.M., Harre, J.G., Wagner, E., Simmons, M., Raviprakash, K., 2003. Variable susceptibility of the owl monkey (*Aotus nancymae*) to four serotypes of dengue virus. *Contemp. Top. Lab. Anim. Sci.* 42 (September), 12–20.
- Simmons, M., Porter, K.R., Hayes, C.G., Vaughn, D.W., Putnak, R., 2006. Characterization of antibody responses to combinations of a dengue virus type 2 DNA vaccine and two dengue virus type 2 protein vaccines in rhesus macaques. *J. Virol.* 80 (October), 9577–9585.
- Simmons, M., Burgess, T., Lynch, J., Putnak, R., 2010. Protection against dengue virus by non-replicating and live attenuated vaccines used together in a prime boost vaccination strategy. *Virology* 396 (January), 280–288.
- Simmons, C.P., Farrar, J.J., Nguyen, v.V.C., Wills, B., Dengue, N., 2012. *Engl. J. Med.* 366 (April), 1423–1432.
- Sun, W., Nisalak, A., Gettayacamin, M., Eckels, K.H., Putnak, J.R., Vaughn, D.W., Innis, B.L., Thomas, S.J., Endy, T.P., 2006. Protection of rhesus monkeys against dengue virus challenge after tetravalent live attenuated dengue virus vaccination. *J. Infect. Dis.* 193 (June), 1658–1665.
- Tarr, G.C., Lubinicki, A.S., 1976. Chemically induced temperature-sensitive mutants of dengue virus type 2: comparison of temperature sensitivity in vitro with infectivity suckling mice, hamsters, and rhesus monkeys. *Infect. Immun.* 13 (March), 688–695.
- Tricou, V., Minh, N.N., Farrar, J., Tran, H.T., Simmons, C.P., 2011. Kinetics of viremia and NS1 antigenemia are shaped by immune status and virus serotype in adults with dengue. *PLoS Negl. Trop. Dis.* 5 (September), e1309.

- Valdés, I., Hermida, L., Martín, J., Menéndez, T., Gil, L., Lazo, L., Castro, J., Niebla, O., López, C., Bernardo, L., Sánchez, J., Romero, Y., Martínez, R., Guzmán, M.G., Guillén, G., 2009. Immunological evaluation in nonhuman primates of formulations based on the chimeric protein p64k-domain III of dengue 2 and two components of *Neisseria meningitidis*. *Vaccine* 27 (February), 995–1001.
- Vasilakis, N., Weaver, S.C., 2008. The history and evolution of human dengue emergence. *Adv. Virus Res.* 72, 1–76.
- Vasilakis, N., Cardoso, J., Hanley, K.A., Holmes, E.C., Weaver, S.C., 2011. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat. Rev. Microbiol.* 9 (July), 532–541.
- Vaughn, D.W., Green, S., Kalayanaraj, S., Innis, B.L., Nimmannitya, S., Suntayakorn, S., Endy, T.P., Raengsakulrach, B., Rothman, A.L., Ennis, F.A., Nisalak, A., 2000. Dengue viremia titer, and virus serotype correlate with disease severity. *J. Infect. Dis.* 181 (January), 2–9.
- Velzing, J., Groen, J., Drouet, M.T., van Amerongen, G., Copra, C., Osterhaus, A.D., Deubel, V., 1999. Induction of protective immunity against dengue virus type 2: comparison of candidate live attenuated and recombinant vaccines. *Vaccine* 17 (March), 1312–1320.
- Wang, W.-K., Chao, D.-Y., Kao, C.-L., Wu, H.-C., Liu, Y.-C., Li, C.-M., Lin, S.-C., Ho, S.-T., Huang, J.-H., King, C.-C., 2003. High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. *Virology* 305 (January), 330–338.
- Whitehead, R.H., Chaicumpa, V., Olson, L.C., Russell, P.K., 1970. Sequential dengue virus infections in the white-handed gibbon (*Hylobates lar*). *Am. J. Trop. Med. Hyg.* 19 (January), 94–102.
- Widjaja, S., Winoto, I., Sturgis, J., Maroef, C., Listyaningsih, E., Tan, R., Pamungkas, J., Iskandriati, D., Blair, P., Sadjuti, D., et al., 2010. Macaca nemestrina and dengue virus infectivity: a potential model for evaluating dengue vaccine candidates. *Microbiol. Indones.* 4 (2).
- Zompi, S., Harris, E., 2012. Animal models of dengue virus infection. *Viruses* 4 (January), 62–82.