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

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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.

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

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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 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.

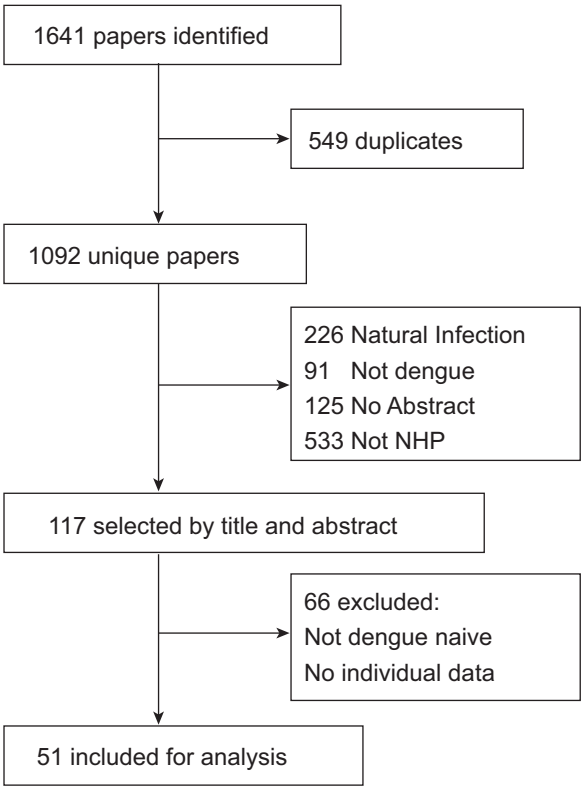

Fig. 1. Flow chart of systematic review.

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).