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Evaluating the Suitability of Dengue Viraemia as a Surrogate Efficacy Outcome for Phase III Trials of Dengue Antivirals: A Systematic Review

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Evaluating the Suitability of Dengue Viraemia as a Surrogate Efficacy Outcome for Phase III Trials of Dengue Antivirals: A Systematic Review

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Abstract

Background: Dengue cases reached record levels in 2024 alongside geographic expansion into Europe and Africa. The low rate of progression to severe dengue presents a challenge for powering phase III dengue antiviral trials. Viraemia is used as a virological outcome in phase II trials, but whether treatment-induced reductions reliably predict clinical benefit remains uncertain. This systematic review assessed the association between dengue viraemia and disease severity and its suitability as a surrogate efficacy outcome for phase III antiviral trials.

Methods: We searched Ovid MEDLINE, Ovid Embase, Scopus, and Web of Science on 20 June 2025 for studies indexed since 2017, extending a previous systematic review. We included clinical studies of laboratory-confirmed dengue measuring quantitative serum viral load by RT-PCR at enrolment and at least one subsequent timepoint and reporting severe dengue as a clinical outcome. The review was registered with PROSPERO (CRD420251077148) and followed PRISMA 2020 reporting guidelines.

Results: We screened 3303 records and reviewed 60 full texts; 11 studies met the inclusion criteria, comprising predominantly hospitalised paediatric and younger adult cohorts from the Asia-Pacific. Seven studies reported higher early viraemia (typically during illness days 1–5) in patients with more severe disease, whereas three found no significant association and one provided insufficient evidence. Two studies assessing viraemia kinetics found that faster viral clearance was associated with lower risk of severe dengue. Heterogeneity in sampling timing, severity classification, and adjustment for host and viral factors limited interpretation. Certainty of evidence was very low.

Conclusions: Evidence for an association between dengue viraemia and disease severity was inconsistent and limited by methodological heterogeneity. Most studies assessed severity around the time of hospital admission and could not establish if viraemia predicted subsequent progression from non-severe to severe disease. None were designed to determine whether treatment-induced reductions in viraemia translate into clinical benefit. Dengue viraemia should therefore be regarded as a pharmacodynamic biomarker and supportive virological outcome for early-phase antiviral development, rather than as a

validated surrogate efficacy outcome for phase III trials, for which clinically meaningful patient-centred outcomes remain necessary.

Introduction

Dengue is a mosquito-borne viral infection caused by dengue virus (DENV), a single-stranded RNA flavivirus with four distinct serotypes (DENV-1 to DENV-4) transmitted by *Aedes* mosquitoes. Over recent decades, dengue incidence has increased due to rapid urbanisation, population growth, climate variability, and resulting expansion of vector habitats [1–3]. In 2024, dengue cases and deaths reached the highest levels ever recorded globally, with continued expansion from Asia and Latin America into Africa, North America, and Europe [4]. Important advances have been made in dengue prevention, including live-attenuated vaccines (LAVs) and vector-control strategies such as Wolbachia-infected mosquitoes; however, important gaps remain in prevention and access to effective dengue-specific interventions [5]. LAVs are contraindicated in specific populations such as pregnant and immunocompromised individuals, and do not have universal licensing across age groups and countries [6], while vector control interventions can be challenging to deploy at scale during large outbreaks because of resource and operational constraints [7]. Consequently, there remains a need for antiviral therapeutics, particularly for individuals at higher risk of severe and potentially fatal dengue, and for those in whom vaccines are contraindicated, less effective, or unavailable.

Dengue infection, when symptomatic, ranges from a self-limiting febrile illness to severe manifestations characterised by plasma leakage, bleeding, shock, and organ dysfunction [8]. Although most infections are non-severe, severe dengue is associated with substantial morbidity and mortality, placing considerable strain on healthcare systems during outbreaks [4,9]. The low absolute rate of progression to severe disease presents a major challenge for the evaluation of dengue therapeutics, as phase III efficacy trials powered on clinical outcomes such as severe dengue, hospitalisation, or mortality require large sample sizes and substantial resources.

Dengue disease severity classification has evolved over time (Table 1 & Supplementary Table 1). The World Health Organization (WHO) 1986 and 1997 classifications distinguished dengue fever (DF), dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) [10,11]. This was largely based on paediatric cases in Southeast Asia and was found to underestimate dengue severity, particularly in adults [12,13]. The WHO 2009 classification introduced dengue with and without warning signs as well as severe dengue, aiming to better reflect clinical management needs in all age groups [14]. The WHO 2025 clinical management guidelines classify patients with high-risk disease features warranting hospitalisation (including those with dengue with warning signs in the WHO 2009 classification) as having severe dengue whereas those suitable for outpatient treatment are classified as having non-severe dengue [15]. However, heterogeneity in classification across studies remains a challenge for comparative research [16].

Table 1: WHO dengue disease severity classifications.

1986 [10] **& 1997** [11]	**2009** [14]	**2025** [15]
Dengue Fever	Non-severe Dengue without Warning Signs	Non-severe Dengue Suitable for Outpatient Management
Dengue Haemorrhagic Fever (DHF grades 1 & 2)	Non-Severe Dengue with Warning Signs	Severe Dengue with Clinical Need for Hospitalisation
Dengue Haemorrhagic Fever with Dengue Shock Syndrome (DHF Grades 3 & 4)	Severe Dengue	

Dengue diagnosis in clinical practice relies on molecular (reverse transcription polymerase chain reaction [RT-PCR] detection of viral RNA), antigen-based (NS1 antigen detection), and serological methods (IgM and IgG antibody measurement) (Figure 1). RT-PCR can be used to quantify viraemia during the early acute febrile phase (typically illness days 1-5) [17], while NS1 antigen detection can help extend the diagnostic window up to 9 days after symptom onset for primary infections and up to 6 days in secondary infections [18]. Serological assays are often used to confirm infection in the later stages of illness and to distinguish primary from secondary dengue infection based on IgM and IgG antibody levels to infer immune status. Increasing access to quantitative RT-PCR has enabled detailed characterisation of the kinetics of dengue viraemia, which typically peaks before or around the onset of fever and declines rapidly over the first few days of illness [19,20].

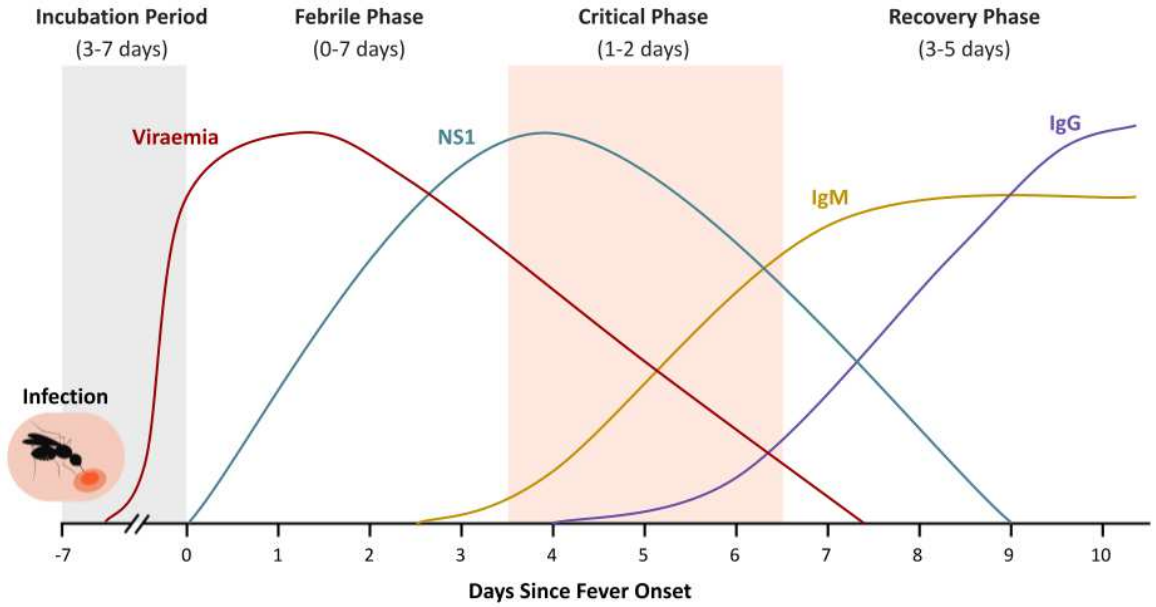


Figure 1: Dengue disease phases and evolution of circulating viral RNA, antigen, and antibody levels following dengue infection. Adapted from [18].

Dengue disease severity arises through complex interactions between viral replication, host immune and inflammatory responses, and other patient-specific factors (e.g. pre-existing organ dysfunction) rather than through viraemia alone (Figure 2). Following infection, dengue virus replication stimulates innate and adaptive immune responses that contribute both to viral clearance and, in some patients, immunopathology [21]. Although antiviral therapy acts directly to suppress viral replication and consequently viraemia, progression to severe disease involves downstream host immune and inflammatory responses, with the direct and indirect effects of circulating virus potentially also contributing [21]. Host factors, including previous dengue infection, dengue vaccination history, age, comorbidities, and immunosuppression, influence viraemia, host immune and inflammatory responses, and the risk of severe clinical outcomes through multiple pathways [22–24]. Similarly, viral factors such as infecting serotype and genotype influence viral kinetics, immune activation, and dengue disease severity [19,25–27]. The interaction between host and viral factors is exemplified by secondary heterotypic infection (with a different dengue virus serotype), which has been associated with increased risk of severe disease. Antibody-dependent enhancement has been proposed as one mechanism linking pre-existing dengue immunity to increased viral replication and subsequent immunopathology [19,28]. Likewise, infecting serotype influences

both viraemia magnitude and the probability of developing severe disease, with DENV-2 and DENV-3 associated with greater disease severity than DENV-1 or DENV-4, although this relationship may vary according to population and immune status [25,26,29,30]. Age and comorbidities further modify susceptibility to severe disease through altered immune function and reduced physiological reserve [22–24]. Consequently, observed associations between viraemia and severe dengue may reflect shared determinants rather than a direct causal relationship, complicating evaluation of whether treatment-induced viraemia reductions will translate into meaningful clinical benefit.

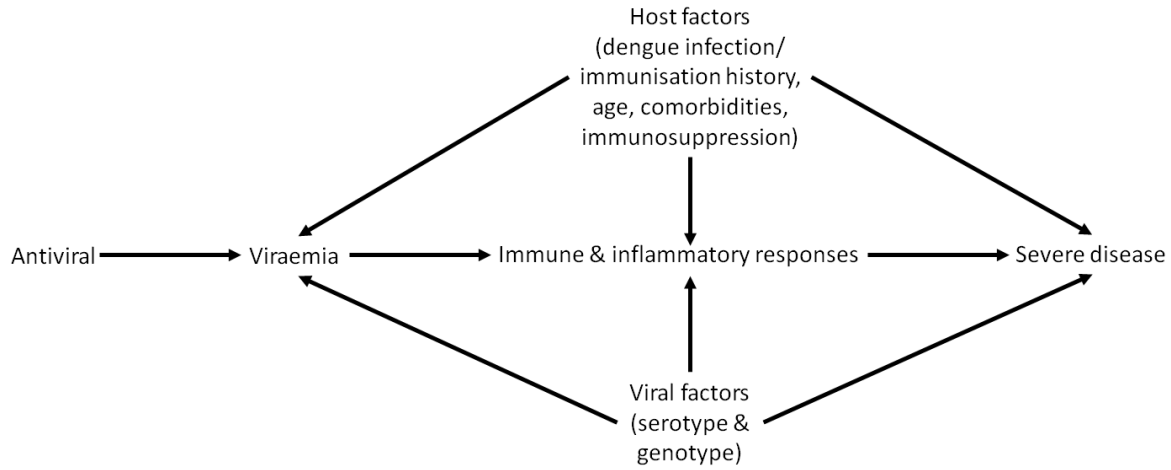


Figure 2: Factors influencing the relationship between dengue viraemia and severe disease. Causal diagram of relationships between antiviral therapy, dengue viraemia, host immune and inflammatory responses, and severe disease. Host and viral factors influence severe disease through direct and indirect pathways mediated through viraemia and host responses.

At present, there are no licensed antiviral therapies for dengue and clinical management remains supportive. Phase II dengue antiviral trials have often used reductions in viraemia, measured by RT-PCR, as primary or secondary efficacy outcomes [31–33]. However, the validity of viraemia as a surrogate outcome for phase III trials requires careful evaluation. A surrogate outcome must consistently capture a sufficient proportion of the treatment effect on the clinical outcome to reliably predict clinical benefit. Severe dengue involves complex host immune and inflammatory responses, which may become increasingly dissociated from viraemia after the early acute febrile phase. Consequently, the timing of sample collection is

critical because observed viraemia declines rapidly as severe disease emerges. Furthermore, host and viral factors influence viraemia, host immune and inflammatory responses, and disease severity through multiple pathways, further complicating interpretation of treatment-induced reductions in viraemia as evidence of clinical benefit [19,22–27,30,34–36] (Figure 2).

A prior systematic review and meta-analysis by Morsy et al. reported that higher early viraemia was associated with greater disease severity, but highlighted substantial heterogeneity in study designs, disease severity definitions, and viraemia measurement methods, concluding that the evidence was insufficient to support viraemia as a reliable predictor of severe dengue [37]. Given the growing interest in dengue antivirals, driven by the escalating global burden, limitations of existing preventive strategies, and the logistical challenges of conducting phase III trials, it is timely to reassess the relationship between dengue viraemia and clinical severity. An updated review is therefore needed to incorporate recent evidence and consider the implications of the relationship between viraemia and disease severity in the context of dengue antiviral development. This systematic review aims to assess the consistency of the association between dengue viraemia and severe disease, and to evaluate the extent to which the available evidence supports the potential use of viraemia as a surrogate outcome in phase III dengue antiviral trials.

Methods

This systematic review was registered prospectively on the PROSPERO database (CRD420251077148) and adheres to PRISMA 2020 reporting guidelines [38].

Search Strategy

An information specialist (EH) searched Ovid MEDLINE, Ovid Embase, Scopus, and the Web of Science Core Collection on 20 June 2025 for published studies from 2017 onwards about dengue and viraemia, informed by the search strategy of the previous systematic review [37]. The full set of strategies are available in the supplementary appendix (Supplementary Methods). No language limits were applied. All references identified through our literature search, together with the 30 studies included in the previous systematic review irrespective

of publication date [37], were exported to Covidence for deduplication and screening against eligibility criteria. Reference lists of the eligible records were also checked for additional relevant studies.

Eligibility Criteria

We included published primary clinical observational studies or randomised controlled trials of patients of any age group, including pregnant women, with naturally-acquired acute laboratory-confirmed (RT-PCR, NS1 antigen, or serology) dengue infection according to WHO laboratory diagnostic guidance [18], measuring circulating dengue viral load using quantitative RT-PCR at enrolment as well as at least one subsequent timepoint, and reporting severe dengue as defined by WHO classification systems (allowing for regional and study-specific adaptations) as a clinical outcome (Table 1). Pooled individual participant-level data from eligible prospective clinical cohorts were included where the underlying cohorts were not otherwise represented. No restrictions were applied regarding healthcare setting (community or hospital), geographic location, country income level, or type of healthcare system. Studies of patients who had co-infections with more than one arbovirus were excluded, as were case reports of individual patients and studies with only animal, *in vitro*, or *in silico* data. Secondary analyses based solely on aggregate data or previously reported cohorts were also excluded.

Data Extraction

We extracted data on study characteristics (location, period of enrolment, design, health facility setting, size), patient characteristics (age, sex, pregnancy, comorbidities, immunocompromise, dengue immunisation status, previous dengue infection status), viral characteristics (DENV serotype), viraemia measurement methodology (time points relative to illness day with day 1 defined as day of symptom onset), and severe dengue classification (WHO 1986, WHO 1997, WHO 2009, WHO 2025, and regional or study-specific adaptations) [10,11,14,15]. The viraemia time points and endpoints, statistical methods (including adjustment for potential confounders), and clinical endpoints used for each study's analysis of the association between dengue viraemia and severe dengue were also extracted (Supplementary Methods).

Data Analysis

A priori scoping during protocol development identified substantial heterogeneity in study designs, population characteristics, reporting of comorbidities and immune status, viraemia measurement time points, and outcome measure definitions, precluding meaningful quantitative synthesis. All extracted data were therefore summarised in tabular format according to pre-specified variables of interest and synthesised narratively.

Risk of Bias Assessment

The Newcastle-Ottawa Scale (NOS), adapted for cohort studies, was used to assess the quality of included cohort studies [39]. The NOS was adapted to reflect the review question, including definitions of exposure, outcome, confounders, and study quality (Supplementary Methods). RCTs evaluating intervention effects that could influence viraemia or disease severity were assessed using the Cochrane Risk of Bias 2 (RoB 2) tool [40].

Quality Assessment

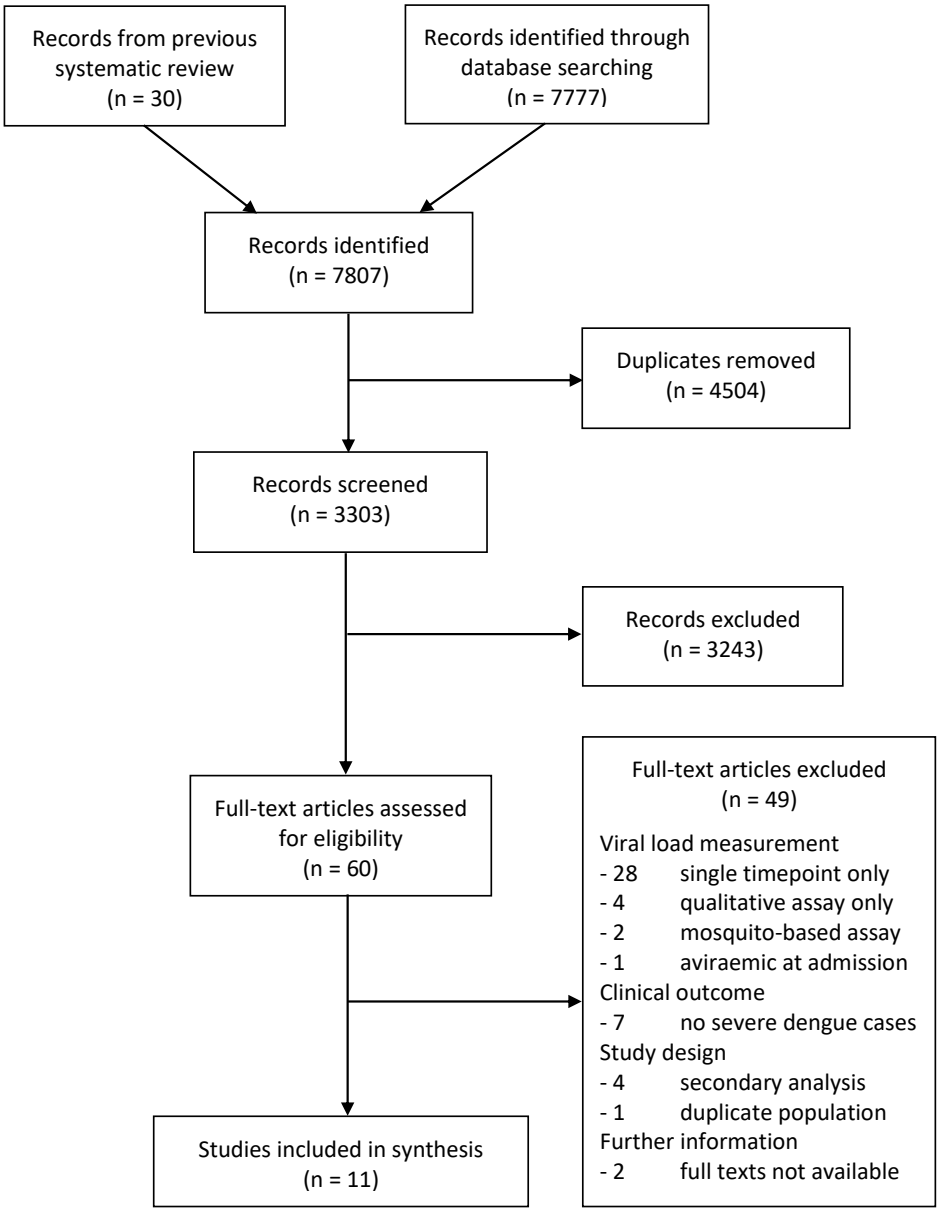
Certainty of evidence was assessed using the GRADE framework [41]. Assessment was conducted at the outcome level for severe dengue across the domains of risk of bias, inconsistency, indirectness, imprecision, and publication bias, based on the overall body of evidence.

Review Team and Tools

At least two independent reviewers (JMG, YNW, XHSC) performed screening (titles and abstracts, followed by full texts), discussed study eligibility, extracted data, and undertook risk of bias and quality assessment using Covidence. Each record was assessed independently by two reviewers, with decisions compared in Covidence and discrepancies resolved through discussion and consensus.

Results

Figure 3: PRISMA flow chart.



After deduplication, 3,303 total records were screened for titles and abstracts, of which 60 full texts were reviewed. Eleven studies met the inclusion criteria (Figure 3).

Study Characteristics

Table 2: Characteristics of included studies and population demographics.

First Author (Year Published)	Years Study Conducted	Location	Study Design	Healthcare Facility Setting	Cohort Size (n)	Population Type	Age (years): mean ± SD	Male (%)	Pregnant Women (n)	Patients with Comorbidities (n)	Patients with Immunosuppression (n)	Dengue Infection Type (%)	Dengue Serotypes Present (n)
Arragain (2016) [42]	2012-2013	New Caledonia	Prospective cohort	Inpatient	19	Adult & Paediatric	NR	NR	10	NR	NR	Primary (94.7); Secondary (5.3)	DENV-1 (19)
Bhatt (2024) [43]	2019-2021	India	Ambispective cohort	Inpatient	126	Adult	34.2 ± 15.4	73.8	NR	NR	0	Primary (57.1); Secondary (42.9)	DENV-1 (37); DENV-2 (34); DENV-3 (10); DENV-4 (3)
Matangkasombut (2020) [44]	2012-2015	Thailand	Prospective cohort	Inpatient	279	Adult & paediatric	NR	48.8	NR	NR	NR	Primary (7.9); Secondary (74.6); Indeterminate (17.6)	DENV-1 (39); DENV-2 (38); DENV-3 (89); DENV-4 (96)
Murgue (2000) [45]	1996-1997	Tahiti	Prospective cohort	Inpatient	49	Paediatric	7.1 ± 3.3 (estimated)	71.4	NR	NR	NR	Primary (42.9); Secondary (57.1)	DENV-2 (49)
Nainggolan (2023) [46]	2010-2011	Indonesia	Prospective cohort	Inpatient	48	Adult & paediatric	24.3	50.0	0	0	0	Primary (31.3); Secondary (68.8)	DENV-1 (14); DENV-2 (19); DENV-3 (12); DENV-4 (3)
Singla (2016) [47]	2012-2014	India	Prospective cohort	Inpatient & outpatient	97	Paediatric	8.8 ± 2.6	52.6	NR	NR	NR	Primary (40.2); Secondary (59.8)	DENV-1 (3); DENV-2 (84); DENV-3 (1); two serotypes (2)
Suputtamongkol (2021) [33]	2014-2015	Thailand	RCT	Inpatient	203 (Placebo: 103 Ivermectin: 100)	Adult	24.8 ± 9.8 (estimated)	57.6	0	NR	0	Primary (6.9); Secondary (93.1)	DENV-1 (19); DENV-2 (29); DENV-3 (37); DENV-4 (117)
Tricou (2011) [48]	2007-2008	Vietnam	Prospective cohort (secondary observational analysis of RCT)	Inpatient	248	Adult	22.1 ± 6.7 (estimated)	67.3	NR	NR	NR	Primary (13.3); Secondary (86.7)	DENV-1 (142); DENV-2 (51); DENV-3 (39); DENV-4 (7)
Vuong (2024) [49]	2000-2009; 2011-2016	Vietnam	Pooled prospective cohorts	Inpatient & outpatient	2340	Adult & paediatric	13.7 ± 5.9 (estimated)	60.0	NR	NR	NR	Primary (20.3); Secondary (69.2) Indeterminate (10.6)	DENV-1 (310); DENV-2 (848); DENV-3 (1137); DENV-4 (45)
Wang (2006) [50]	2002	Taiwan	Prospective cohort	Inpatient	103	Adult	51.5 ± 12.8	33	NR	NR	NR	Secondary (100)	DENV-2 (103)
Wijesinghe (2020) [51]	NR	Sri Lanka	Prospective cohort	Inpatient	79	Adult	NR	NR	NR	NR	NR	Primary (16.5); Secondary (83.5)	DENV-1 (22); DENV-2 (57)

NR = not reported; RCT = randomised controlled trial; SD = standard deviation

The included studies were published between 2000 and 2024 and reported data collected between 1996 and 2021 in the Asia-Pacific region, specifically South Asia, Southeast Asia, and French Polynesia. None of the studies were conducted in the Americas. Study sizes ranged from 19 to 2,340 participants. Eight studies were prospective observational cohorts [42,44–48,50,51]; one was a pooled individual participant-level data analysis of prospective cohorts [49], one randomised controlled trial [33], and one ambispective cohort study which included prospective (n=60) and retrospective (n=66) data [43]. Most studies enrolled hospitalised patients, although two included both inpatient and outpatient populations. Study populations were predominantly children and younger adults.

Reporting of pregnancy status, comorbidities, and immunosuppression was limited across studies, and no study reported dengue immunisation status. However, all studies reported dengue infection type (primary or secondary) and infecting DENV serotypes. Nine of the 11 studies [33,44–51] predominantly included participants with secondary dengue infections, with one of these studies consisting entirely of secondary infections [50]. All four dengue serotypes were represented across the included studies. DENV-2 was the most frequently reported serotype, being identified in 10 of the 11 studies [33,43–51], followed by DENV-1, DENV-3, and DENV-4 (Table 2).

Dengue Disease Severity Classification

Dengue severity was classified using WHO 1986, WHO 1997, WHO 2009, SEARO 2011 and other regional classifications (Table 3), with several studies applying more than one classification system.

Table 3: Viraemia measurement and association with dengue disease severity in included studies.

First Author (Year Published)	WHO Dengue Severity Classification	WHO 1986 or 1997 (n)	WHO 2009 (n)	Other Classifications (n)	Viraemia Sampling Timepoints	Viraemia Samples Used in Analysis	Viraemia Endpoints Assessed with Severity	Association Between Viraemia and Severity	Covariates Adjusted for
Arragain (2016) [42]	WHO 2009		DWoWS: 4 DWS: 12 SD: 3		Mothers (n=10): serial samples from symptom onset until viraemia undetectable Newborns (n=9): serial samples from birth until viraemia undetectable	Serial samples for two mothers: 7 samples from 96 hours before to illness day 9 Single sample for 8 mothers: from illness days 1 to 3	Serum viraemia level (copies/mL)	Descriptively, the one mother who died had a lower serum viraemia level at delivery than the other mothers.	None
Bhatt (2024) [43]	WHO 1997 & 2009	DF: 111 DHF: 13 DSS: 2	DWoWS: 93 DWS: 20 SD: 13		Prospective cases (n=60): daily from admission until discharge Retrospective cases (n=66): day 3 only	Serial samples spanning illness days 2 to 8	Median day 3 serum viraemia level (log10 copies/mL) Median peak serum viraemia level (log10 copies/mL) Mean serum viral clearance time (days)	Higher median day 3 viraemia in DHF/DSS than DF ($p<0.01$) and in SD than DWoWS ($p<0.001$) Among secondary dengue cases, higher median day 3 viraemia (n=47; $p<0.001$) and median peak viraemia (n=29; $p<0.05$) in DHF/DSS than DF. No significant difference in mean viral clearance time between DHF/DSS and DF. Day 3 viraemia (n=123) discriminated SD (AUC: 0.763; 95% CI: 0.635-0.890). Cut-offs evaluated were ≥7.055 log10 copies/mL (100% sensitivity, 36.4% specificity) and ≥8.719 log10 copies/mL (73.3% sensitivity, 61.7% specificity).	None
Matangkasombut (2020) [44]	WHO 1997	DF: 177 DHF: 102 DSS: 0			Admission and day of defervescence	Paired samples spanning hospitalisation days 1 to 7	Mean day 1 plasma viraemia level (log10 copies/mL) Plasma viraemia clearance rate (log10 copies/mL/day)	Generalised linear modelling found higher mean day 1 viraemia (n=222; RR=5.88, $p<0.001$) and faster viraemia clearance (n=183; RR=1.44, $p=0.004$) in DHF than DF.	Age Sex Serotype Day 1 viraemia (clearance rate only)
Murgue (2000) [45]	WHO 1986	DF: 42 DHF: 4 DSS: 3			Daily from admission until discharge	48/49: ≥3 serial samples spanning illness days 1 to 7 1/49: 1 sample	Mean daily plasma viraemia level (equivalent log10 TCID50/mL, i.e. calibrated against viral culture control) Mean plasma viraemia duration (days)	Mean viraemia levels over days 2 to 5 significantly higher in DHF than DF ($p=0.047$). Longer mean viraemia duration in DHF than DF (5.0 vs 4.3 days) but statistical significance not reported.	None
Nainggolan (2023) [46]	WHO 1997	DF: 28 DHF: 20 DSS: 0			Daily from illness days 1 or 2 until day 6	Samples spanning illness days 1 to 6	Median daily plasma viraemia level (copies/mL)	Higher median day 4 viraemia in DHF than DF ($p=0.024$) overall and among primary but not secondary infections ($p=0.010$).	None
Singla (2016) [47]	WHO 2009		DWoWS: 21 DWS: 30		Admission (n=97) 48 hours after admission (n=53)	Samples spanning illness days 1 to 10	Median admission whole blood viraemia level (log10 copies/mL)	Admission and 48-hour median viraemia levels did not differ significantly across clinical severity groups (DWoWS, DWS, and SD), even when	None

			SD: 46				Median 48-hour whole blood viraemia level (log10 copies/mL)	stratified by illness day (days 1-3, 4-6, and 7-10) and by primary versus secondary infection. However, median 48-hour viraemia level was higher in secondary than primary infections (p=0.0074), despite clinical improvement.	
Suputtamongkol (2021) [33]	WHO 1997 & 2009	DF: 147 DHF: 56 DSS: 0	DWoWS: NR DWS: NR SD: 13		Every 12 hours from admission (median illness day 3) until discharge	Single admission sample	Plasma admission viraemia level (log10 copies/mL)	Multiple logistic regression found no statistically significant association between admission viraemia and DHF.	Serotype Platelets ALT NS1 Drug
Tricou (2011) [48]	WHO 1997	DF: 179 DHF: 69 DSS: 0			Admission then twice daily (9am and 3pm) for at least 5 further days and again 10 to 14 days after discharge	Serial samples spanning illness days 1 to 9	Median daily plasma viraemia level (log10 copies/mL) Median peak plasma viraemia level (log10 copies/mL)	Higher median day 3 viraemia (n=139, 0.01<p<0.05) and median peak viraemia (n=142, p=0.03) in DENV-1-infected patients with DHF than DF.	None
Vuong (2024) [49]	WHO 2009		DWoWS: NR DWS: NR SD: 65	Plasma leakage: 353	Daily from illness days 1 to 3 until days 7 to 10	Serial samples spanning illness days 1 to 7	Daily plasma viraemia level (log10 copies/mL) Plasma viraemia clearance rate (log10 copies/mL/day)	Multiple logistic regression found higher viraemia most strongly associated with SD on day 1 (OR 2.98, 95% CI 1.46-6.10) with attenuation by day 5 (OR=1.08, 95% CI: 0.75-1.54 on day 5). Faster viral clearance was associated with lower risk of SD (OR 0.14, 95% CI 0.06-0.33; p<0.001).	Age Sex Infection type Serotype Illness day
Wang (2006) [50]	WHO 1997	DF: 54 DHF: 49 DSS: 0			Serial samples between illness days 2 to 8	Serial samples spanning illness days 2 to 8	Mean daily plasma viraemia level (log copies/mL) Plasma viraemia clearance rate (log copies/mL/day) Mean plasma viraemia duration (days)	Higher mean viraemia levels on defervescence days -2 to +3 (p<0.05) in DHF than DF. In patients with ≥3 samples (n=26), slower viraemia clearance (−1.30 vs −2.01 log copies/mL/day; p=0.037) and greater mean viraemia duration (7.7 vs 6.2 days, p=0.02) in DHF than DF. On the day of defervescence, a viraemia threshold of >5.7 log RNA copies/mL had positive predictive value of 0.88 and negative predictive value of 0.95 for DHF.	None
Wijesinghe (2020) [51]	SEARO 2011 (adapted from WHO 2009) [52]			DF: 38 DHF:41	Tfh/plasmablast cohort (n=40): one acute (illness day 4 to 8) and one convalescent (day 21 to 30) sample Neut50 cohort (n=39): daily from admission until discharge	Tfh cell/plasmablast cohort: single acute sample Neut50 cohort: serial daily samples from illness day 3 onwards	Tfh/plasmablast cohort: Serum viraemia level (copies/mL) Neut50 cohort: Median serum viraemia level (copies/mL)	Tfh cell and plasmablast frequencies higher in DHF than DF (p<0.0001), while viraemia was inversely correlated with overall Tfh cell frequency (Spearman r=−0.63, p<0.0001) and plasmablast frequency in DHF (r=-0.51, p=0.02) but not DF (r=-0.29, p=0.26). In the Neut50 cohort, no differences in median viraemia between DHF and DF at any timepoint.	None

AUC=area under curve; ALT=alanine aminotransferase; CI=confidence interval; DF=dengue fever; DHF=dengue haemorrhagic fever; DSS=dengue shock syndrome; DWoWS=dengue without warning signs; Neut50=neutralising antibodies; NS1=non-structural protein 1; ROC=receiver operating characteristic; SD=severe dengue; SEARO=South-East Asia Regional Office; TCID50=50% tissue culture infectious dose; Tfh cells=T follicular helper; WHO=World Health Organization.

Enrolment Timing, Viraemia Sampling Strategy, and Analysis Approaches

Most studies enrolled hospitalised patients (Table 3), and severity comparisons were therefore frequently based on disease classification at admission rather than subsequent progression from non-severe to severe disease.

Sampling strategies varied substantially across studies (Table 3). Most studies began sampling on illness days 1-3, typically at hospital admission, continuing until defervescence or discharge, although one of the two Wijesinghe et al. cohorts sampled patients only later in illness (days 4-8) [51]. Across studies, viraemia measurements from illness days 1-7 were most commonly available. Consequently, early febrile-phase and peak viraemia were not consistently captured or specifically analysed, limiting comparability across studies.

Although all studies measured viral load at enrolment and at least one additional timepoint in keeping with this review's eligibility criteria, analyses often used only a subset of the samples collected. Five studies, including the first (Tfh cell and plasmablast) cohort from Wijesinghe et al., analysed primarily single or limited viraemia measurements [33,42,43,47,51]. In contrast, seven studies, including the second (Neut50) cohort of Wijesinghe et al., incorporated repeated viraemia measurements into their severity analyses, allowing assessment of viraemia trends across illness [44–46,48–51].

As viraemia declines rapidly during early dengue infection, illness day at sampling is a critical determinant of observed viraemia. Host and viral factors also influence viraemia and disease severity. However, statistical adjustment for these factors was uncommon among included studies (Table 3). Despite universal reporting of infecting serotype and dengue infection type (Table 2), only one study adjusted simultaneously for illness day, DENV serotype, dengue infection type, age, and sex in multivariable analyses [49]. Most studies relied on univariable or stratified analyses, limiting assessment of whether observed associations reflected independent effects of viraemia or residual confounding (Table 3).

Association Between Dengue Viraemia and Dengue Disease Severity

Across the eleven included studies, evidence for an association between dengue viraemia and disease severity was mixed (Table 3). Seven studies reported higher viraemia levels and/or

slower viral clearance in patients with severe clinical outcomes [43–46,48–50], three found no significant association [33,47,51], and one maternal cohort had insufficient data for meaningful assessment [42].

Seven studies reported higher viraemia levels during early illness (typically between days 1-5) in severe dengue and DHF/DSS compared to non-severe dengue and DF although the timing, magnitude, and statistical robustness of the observed association varied substantially across studies [43–46,48–50]. The largest included study and the only study which adjusted for illness day, alongside other host and viral factors, in multivariable analyses, Vuong et al., further found that higher viraemia was independently associated with increased odds of severe dengue, with the strongest association occurring on illness day 1 (OR 2.98, 95% CI 1.46-6.10), which progressively weakened by illness day 5 (OR 1.08, 95% CI 0.75-1.54) [49].

Three studies found no statistically significant association between viraemia level and disease severity [33,47,51]. Singla et al. found no differences in median viraemia between WHO 2009 severity groups at admission or 48 hours later [47], while Suputtamongkol et al. found no association between admission (illness day 3) viraemia and DHF in multivariable analyses [33]. Likewise, the Neut50 cohort of Wijesinghe et al. did not have significant differences in median viraemia levels between patients with DF and DHF at any timepoint [51].

The Tfh cell and plasmablast cohort of Wijesinghe et al. provided indirect evidence of an inverse relationship between viraemia and frequency of Tfh cells, an immune cell population found to expand in DHF, but did not directly demonstrate differences in viraemia levels between severity groups [51]. Arragain et al. included only one severe maternal outcome and predominantly single maternal viraemia measurements, precluding meaningful assessment of the association of interest [42].

Two studies specifically assessed viraemia kinetics in relation to dengue disease severity [49,50]. Vuong et al. found that faster viraemia clearance was independently associated with reduced risk of severe dengue ($p<0.001$) [49], while Wang et al. reported a slower rate of viraemia clearance ($p=0.037$) in DHF compared to DF [50].

Two studies assessed the predictive performance of viraemia thresholds for severe dengue [43,50]. Bhatt et al. reported that illness day 3 viraemia demonstrated the best discrimination for severe disease (AUC 0.763, 95% CI 0.635-0.890), with thresholds of ≥7.055 log10 copies/mL and ≥8.719 log10 copies/mL evaluated [43]. Wang et al. identified a viraemia threshold of >5.7 log RNA copies/mL on the day of defervescence with a positive predictive value of 0.88 and a negative predictive value of 0.95 for DHF [50].

Risk of Bias Assessment

Using the Newcastle-Ottawa Scale adapted for this review, nine of the ten cohort studies were rated as moderate or high quality [43–51], with one study rated as low quality [42] (Supplementary Table 2). The main limitations identified across these ten cohort studies were uncertainty about whether viraemia measurement preceded development of severe dengue and limited accounting for potential confounders. The one randomised controlled trial which retained treatment allocation in the viraemia and disease severity analysis was assessed as having some concerns for bias because the analysis of interest was *post hoc* without an accessible pre-specified analysis plan [33] (Supplementary Table 3).

Certainty of Evidence

The certainty of evidence was judged to be very low, reflecting the predominantly observational nature of studies, risk of bias, inconsistency in study methodology and findings, indirectness, and imprecision around the magnitude of effect (Supplementary Results).

Discussion

In this systematic review, we evaluated the consistency of the association between dengue viraemia and severe dengue, and the degree to which the available evidence supports the potential use of dengue viraemia as a surrogate efficacy outcome for phase III dengue antiviral trials. Across 11 heterogeneous studies, none of which were designed to establish surrogate endpoint validity, we found mixed evidence, with overall very low certainty. While several studies reported statistically significant associations between higher early viraemia (typically during illness days 1-5) and subsequent dengue disease severity, others found no association. The strength of observed associations appeared to depend on the timing and frequency of

sampling, statistical adjustment for host and viral factors, and the dengue severity classification criteria used. Taken together, the available evidence does not yet support viraemia as a validated surrogate outcome for phase III antiviral trials.

Why the Association between Viraemia and Disease Severity is Inconsistent

The true time of infection is rarely known in natural dengue infections, and peak viraemia is thought to occur prior to or around fever onset, before declining rapidly and becoming undetectable from approximately illness day 7 [8,19]. Patients typically present days after symptom onset and the included studies generally enrolled patients only at hospital admission. Thus, by the time of enrolment, viraemia may already have substantially declined and patients may have already developed severe disease. This creates important temporal ambiguity as associations between admission viraemia, and disease severity may reflect disease that is already established rather than the ability of viraemia to predict subsequent progression. Although all included studies measured viraemia at enrolment and at least one subsequent timepoint, several analyses relied on a subset of available measurements, whereas studies incorporating serial measurements across early illness, such as through viral kinetics analyses, were better able to characterise the dynamic relationship between viral replication and disease progression.

Another source of heterogeneity was the definition of dengue disease severity. Included studies applied five classification approaches (three global, one regional, and one study-specific), with none using the WHO 2025 system released after these studies had been published (Table 2). WHO 2025 guidance recognises hospitalised dengue as a high-risk state, acknowledging that admission decisions may be determined not only by established disease severity but also by evolving clinical features, comorbid risk, or capacity for outpatient monitoring [13,15]. This further complicates interpretation of studies in which severity was classified at admission, particularly when evaluating viraemia as a surrogate outcome for subsequent clinical outcomes. Under the WHO 2025 classification, only the outpatient sub-cohorts from two included studies would have been classified as having non-severe dengue at enrolment, permitting evaluation of progression to severe disease.

Interpretation of the relationship between dengue viraemia and disease severity was further complicated by incomplete reporting and inconsistent statistical adjustment for host and viral factors that influence both viraemia and clinical outcomes. Although all included studies reported infecting serotype, previous dengue exposure status, and age, reporting of other clinically relevant characteristics, including pregnancy, comorbidities, immunosuppression, and dengue vaccination status, was frequently incomplete, limiting assessment of population generalisability and precluding adjustment for potential confounders. Vuong et al. was the notable exception, finding higher early viraemia and slower viral clearance to be independently associated with severe dengue after comprehensive multivariable adjustment [49]. Most other studies relied on univariable or stratified analyses, making it difficult to distinguish independent effects of viraemia from residual confounding. As illustrated in Figure 2, viraemia is unlikely to directly determine severe disease, but rather represents one component within a causal pathway linking viral replication, host immune responses, and other patient-specific factors with downstream clinical manifestations. Thus, observed associations between early viraemia and severity may reflect shared determinants of viral kinetics and disease progression rather than a direct causal relationship. Even a consistent prognostic association with severe disease would therefore be insufficient to establish that treatment-induced reductions in viraemia translate into clinical benefit.

Implications for Outcome Selection for Clinical Trials of Dengue Treatments

Our findings are consistent with recent international normative guidance and expert consensus recommending clinically meaningful patient-centred efficacy outcomes for dengue therapeutics trials [53]. The WHO Target Product Profiles for Treatments for Dengue recommends symptom resolution and reduction of progression to severe dengue as the primary efficacy outcomes for treatments for non-severe dengue [54]. The Dengue Core Outcome Measurement Set research expert consensus similarly recognises the role of virological and biomarker outcomes in mechanistic studies and early phase evaluation while prioritising standardised clinical outcomes for late phase trials [55]. Together with the findings of this review, these recommendations support using viraemia as a pharmacodynamic biomarker rather than a validated surrogate efficacy endpoint for phase III dengue antiviral trials.

This distinction is reflected in the limited interventional evidence available. In the included randomised trial of ivermectin, treatment did not demonstrate benefit in either viraemia or clinical outcomes and therefore provided no evidence that a treatment-induced change in viraemia translated into clinical benefit [33]. Viraemia nevertheless remains useful for phase II antiviral trials, where virological endpoints such as viral clearance can demonstrate antiviral activity and inform dose selection [31–33]. Phase III trials, however, require outcomes that directly reflect clinical benefit across diverse patient populations and healthcare settings with viraemia used as a supportive biomarker alongside clinically meaningful outcomes rather than as a standalone primary efficacy outcome.

Strengths and Limitations

This review has several limitations. Meta-analysis was not possible because of substantial heterogeneity in viraemia sampling strategy, analytical approaches, and severity outcome definitions. Most evidence was observational and susceptible to bias arising from enrolment timing, incomplete reporting of host characteristics, and residual confounding. Our inclusion criteria were intentionally more restrictive than previous reviews to focus on studies with longitudinal viraemia measurements to enable evaluation of viraemia kinetics as a candidate surrogate endpoint for phase III dengue antiviral trials. Although this resulted in fewer included studies, this review provides, to our knowledge, the first systematic assessment of dengue viraemia as a potential surrogate efficacy outcome for phase III antiviral trials.

Future Research

Future research should move beyond documenting observational associations between viraemia and disease severity towards formally evaluating surrogate endpoint validity within antiviral trials. This would require prospective, standardised studies with frequent sampling from the earliest feasible point in the illness relative to treatment initiation, pre-specified virological endpoints, consistent severity definitions, comprehensive reporting of host and viral characteristics, and robust multivariable analyses. Individual patient-level data analyses across antiviral trials could help determine whether drug-induced changes in viraemia consistently predict improvements in clinically relevant outcomes and whether they satisfy accepted criteria for surrogate endpoint validation in addition to prognostic association.

Frequent virological sampling increases trial complexity, laboratory requirements, and cost, particularly in resource-limited dengue-endemic settings [56]. These feasibility considerations further reduce the attractiveness of viraemia as a phase III efficacy outcome compared to clinical outcomes such as hospitalisation, organ impairment, or bleeding which are more directly relevant to patients and more easily measured at scale during outbreaks.

Conclusion

This systematic review found inconsistent evidence for an association between dengue viraemia and severe dengue, with very low certainty of evidence. Higher early viraemia and slower viral clearance were associated with worse clinical outcomes in several studies, but these associations were influenced by sampling timing, disease severity classification, and limited adjustment for host and viral factors, and may therefore reflect shared determinants of viral kinetics and disease progression rather than a direct causal effect of viraemia. No included study was designed to determine whether treatment-induced reductions in viraemia translate into clinical benefit; therefore, even a consistent prognostic association between viraemia and severe dengue would be insufficient to establish surrogate endpoint validity. Dengue viraemia should therefore be regarded as a pharmacodynamic biomarker and supportive virological outcome for early-phase antiviral development, rather than a validated surrogate efficacy endpoint for phase III clinical trials, with clinically meaningful patient-centred outcomes remaining the basis for demonstrating efficacy in late-phase trials.

Author Contributions

XHSC conceived the study. EH conducted the literature search. JMG, XHSC, and YNW undertook screening, data extraction, and data analysis. JMG and XHSC wrote the first draft of the manuscript. All authors reviewed all versions of the manuscript.

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Competing Interests

The authors have no competing interests to declare.

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