

Dengue and Chikungunya virus co-infection in fever cases attending a tertiary care hospital in Dhaka city

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Abstract

Background and objectives: Dengue virus (DENV) and chikungunya virus (CHIKV) are important Aedes-borne arboviruses that frequently co-circulate in tropical regions, posing diagnostic and public health challenges because of their overlapping clinical features. This study investigated the prevalence and distribution pattern of dengue, chikungunya, and their co-infection among clinically suspected arboviral febrile cases attending a tertiary care hospital in Dhaka city, Bangladesh.

Materials and methods: Patients with acute fever attending both the in and out-patient departments of a tertiary care hospital in Dhaka city between July and September 2025 were included in the study. Serum samples were tested for DENV (dengue virus), CHIKV (chikungunya virus) and ZIKV (zika virus) by real time multiplex RT-PCR. Demographic data of patients were collected from the hospital register.

Results: A total of 970 acute febrile cases were included in the study. CHIKV was detected in 557 (57.4%), DENV in 71 (7.3%), and DEN-CHIK co-infection in 15 (1.5%) patients, while 327 (33.7%) patients tested negative. CHIK infection predominated during July and August, whereas DEN infection increased significantly in September. Co-infection was uncommon but peaked in August. Male patients had significantly higher rates of CHIK infection and co-infection than females, while no significant age-related differences were observed.

Conclusions: The low co-infection rate of DENV and CHIKV in our study is comparable to reports from other endemic countries, suggesting limited temporal overlap of DENV and CHIKV transmission despite their co-circulation. These findings highlight the value of integrated arboviral surveillance for accurate diagnosis, timely outbreak detection, and effective public health interventions in Bangladesh.

Introduction

Dengue virus (DENV) and chikungunya virus (CHIKV) are two major Aedes-borne arboviruses

that have emerged as important public health threats across tropical and subtropical regions, particularly in South Asia. Both infections are

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transmitted primarily by *Aedes aegypti* and *Aedes albopictus*, share overlapping ecological niches, and often circulate during the same monsoon and post-monsoon periods [1]. As a result, outbreaks of DENV and CHIKV may occur simultaneously, creating diagnostic, clinical, and public health challenges.

The clinical manifestations of dengue (DEN) and chikungunya (CHIK) infections frequently overlap, especially in the acute febrile phase. Fever, headache, myalgia, rash, arthralgia, and malaise are common to both diseases, making syndromic differentiation difficult without laboratory confirmation. However, the clinical implications differ—dengue may progress to plasma leakage, hemorrhage, organ involvement, and shock, whereas CHIK infection is more characteristically associated with severe and often prolonged arthralgia and post-viral disability [1]. In settings where both viruses co-circulate, co-infection can occur when an individual is infected with both pathogens concurrently or within a short interval. Although reported less commonly than mono-infection, dengue–chikungunya co-infection is epidemiologically important because it may confound diagnosis, influence symptom profile and severity, and complicate patient management and surveillance interpretation.

A systematic review and meta-analysis including 83 studies and 43,341 participants estimated a global pooled prevalence of DEN-CHIK coinfection of 2.5% (95% CI: 1.8–3.4) using a random-effects model. The frequency of coinfection was found similar in males and females. Regionally, Asia reported the highest pooled prevalence (3.3%; 95% CI: 2.3–4.6), whereas North America had the lowest (0.8%; 95% CI: 0.3–2.4). Considerable geographic variation was observed, with the highest prevalence reported in Colombia (37.4%), followed by Madagascar (18.2%), Laos (12.5%), Maldives (4.5%), and Thailand (3.7%) [2].

Bangladesh provides a highly suitable ecological setting for the co-circulation of these arboviruses. Rapid urbanization, high population density, inadequate water management, unplanned construction, monsoon rainfall, and widespread vector breeding opportunities have facilitated

repeated *Aedes*-associated outbreaks. Dengue has been recognized in Bangladesh since the major nationwide epidemic of 2000 and has since evolved from an episodic urban disease into a recurrent and increasingly widespread national public health problem [3]. Large DENV surges have been documented in 2018, 2019, 2021, 2022, and especially 2023, when Bangladesh experienced its largest recorded DEN outbreak [1]. The 2025 DENV outbreak in Bangladesh reflects a notable epidemiological shift, with continuous transmission occurring throughout the year rather than being confined to the usual June–November season. The case fatality rate (CFR) in 2025 was 0.40% (412 deaths among 102,562 cases) [4].

Chikungunya has also become an important re-emerging arboviral disease in Bangladesh. After the first documented outbreak in 2008, subsequent outbreaks and clusters were reported, culminating in the large 2017 epidemic that affected Dhaka and multiple other regions of the country [5]. As of June 2025, the Institute of Epidemiology, Disease Control and Research in Bangladesh reported an outbreak of CHIK in Dhaka city, with 337 cases reported between January and May 28, 2025 [6]. The temporal and spatial overlap between DENV and CHIKV transmission has heightened concern that Bangladesh is increasingly vulnerable not only to dual circulation, but also to laboratory-confirmed co-infection, particularly during peak *Aedes* breeding seasons.

Despite this growing risk, there remains limited epidemiologic evidence from Bangladesh characterizing the relative contribution of DEN, CHIK, and DEN-CHIK co-infection among acute febrile patients, and even fewer studies have examined how these distributions vary by age, sex, and seasonality. Such information is essential for addressing clinical suspicion, improving laboratory testing strategies, and informing vector-control planning.

Therefore, this study aimed to describe the distribution of RT-PCR–confirmed DEN, CHIK, and DEN-CHIK co-infection among clinically suspected arboviral cases in Bangladesh, and to examine their associations with demographic and seasonal variables.

Materials and methods

This retrospective, cross-sectional study was conducted at a tertiary care hospital of Dhaka city between July 2025 to September 2025. Patients with fever attending both the in and out-patient departments, from different locales, mostly within but also beyond Dhaka city, were included in the study.

Serum samples from fever cases were tested for DENV (dengue virus), CHIKV (chikungunya virus) and ZIKV (zika virus) by real time multiplex RT-PCR. Viral RNA was extracted from serum using a column-based viral nucleic acid extraction kit (Qiagen N.V, Germany) following the manufacturer's specifications. Viral RNA was amplified using Bioperfectus zika virus, dengue virus and chikungunya virus Real Time PCR kit (Jiangsu Bioperfectus Technologies Co. Ltd., China), in accordance with the thermal profile recommended by the manufacturer. The assay utilizes polyprotein gene region of ZIKV, 3'UTR region of DENV and NSP1 region of CHIKV as PCR targets, and cycle threshold (Ct) value ≤ 40 for any of the targets was considered as positive.

Demographic data of patients were collected from the hospital register. Data were analyzed with Statistical Product for Service Solutions (SPSS) software (IBM SPSS Statistics for Windows, version 20.0).

Results

A total of 970 febrile cases were included during the study period (July–September 2025), of which 557 (57.4%) were CHIK-positive, 71 (7.32%) DEN-positive, and 15 (1.55%) were positive for both CHIK and DEN infections (Table-1). Chikungunya accounted for nearly half to two-thirds of cases across all age groups, highest in 18–40 years (58.5%) and >40 years (58.1%), compared with <18 years (48.8%). Dengue represented a smaller proportion within each age group, peaking at 9% in those aged 18–40 years, while co-infection remained low ($\leq 1.7\%$). Differences across age groups were not statistically significant.

Significantly greater proportion of males had CHIK compared with females (59.6% vs 54.9%; $p=0.036$), and co-infection was also significantly higher

Table: Distribution of RT-PCR–confirmed chikungunya (CHIKV) and dengue (DENV) infections ($n=970$)

Variable	RT-PCR positive for			
	Chikungunya	Dengue	Both (CHIKV+DENV)	Negative
Total case ($n=970$)	557 (57.4%)	71 (7.32%)	15 (1.55%)	327 (33.7%)
Age				
<18 years ($n=82$)	40 (48.8%)	6 (7.3%)	1 (1.2%)	35 (42.7%)
18–40 years ($n=354$)	207 (58.5%)	32 (9%)	5 (1.4%)	110 (31.1%)
>40 years ($n=534$)	310 (58.1%)	33 (6.2%)	9 (1.7%)	182 (34.1%)
Mean ($\pm$ SD) age year	44.55 $\pm$ 19.33	38.30 $\pm$ 15.78	45.73 $\pm$ 20.02	45.49 $\pm$ 21.15
p value	$p=0.233$	$p=0.412$	$p=0.920$	
Sex				
Male ($n=513$)	306 (59.6%)	40 (7.8%)	13 (2.5%)	154 (30.0%)
Female ($n=457$)	251 (54.9%)	31 (6.8%)	2 (0.4%)	173 (37.9%)
p value	$p=0.036$	$p=0.089$	$p=0.008$	
Months				
July ($n=163$)	98 (60.1%)	5 (3.1%)	0	60 (36.8%)
August ($n=404$)	275 (68.1%)	12 (3.0%)	11 (2.7%)	106 (26.2%)
September ($n=403$)	184 (45.7%)	54 (13.4%)	4 (1.0%)	161 (40.0%)
Significance	$p<0.001$	$p<0.001$	$p=0.030$	

Chi square test: chikungunya: July vs august $p=0.07$, august vs September $p<0.001$, July vs September $p=0.001$, Dengue: July vs august $p=0.951$, august vs September $p<0.001$, July vs September $p<0.001$, Both: July vs august $p=0.03$, august vs September $p=0.06$, July vs September $p=0.20$.

among males (2.5% vs 0.4%; $p=0.008$), whereas dengue proportions were similar between both sexes ($p=0.089$).

Majority of CHIK cases presented in July (60.1%) and peaked in August (68.1%) before declining in September (45.7%; $p<0.001$). In contrast, DEN infection represented a small proportion in July and August (~3%) but increased significantly in September (13.4%; $p<0.001$). Co-infection peaked in August (2.7%; $p=0.030$). Overall, temporal variation and sex, but not age, were significantly associated with infection distribution.

Among the 15 patients listed in the separate co-infection dataset, platelet counts were available for 13 patients (86.7%). Using a reference range of $150\text{--}450 \times 10^9/\text{L}$ for platelet count [7], 5 of the 13 patients (38.5%) had platelets counts below the lower reference limit. The remaining eight patients (61.5%) had platelet counts within or above the reference range. The lowest recorded count was $40 \times 10^9/\text{L}$, indicating marked thrombocytopenia in at least one co-infected patient.

Discussion

The occurrence of DEN-CHIK co-infection has been recognized for more than six decades. The earliest documented cases were reported in Thailand by Nimmannitya et al., who identified 4 co-infected patients among 150 individuals with either DEN or CHIK during 1962–1964, corresponding to a co-infection rate of 2.6% [8]. Since then, sporadic and outbreak-associated co-infections have been reported from several endemic countries. A large outbreak in Punjab, India, in 2009 reported a remarkably higher co-infection rate of 54%, reflecting intense simultaneous transmission of both viruses [9]. Outside Asia, co-infections have also been documented in Africa and the Middle East. During an outbreak in Gabon, 8 of 773 patients (1.0%) were confirmed to have concurrent DEN and CHIK infections [10]. Similarly, in Al Hudaydah, Yemen, 13 of 400 patients (3.3%) were diagnosed with DEN-CHIK co-infection during the 2012 outbreak [11]. These studies collectively demonstrate that although DEN-CHIK co-infection has been recognized for decades, its prevalence varies widely across geographical regions and

outbreaks, ranging from less than 1% to more than 50%, depending on local transmission dynamics, vector abundance, timing of epidemics, and the laboratory methods used for diagnosis.

In the present study, the prevalence of DEN-CHIK co-infection was 1.5% (15/970), which is comparable to the relatively low rates reported from Thailand (2.6%), Gabon (1.0%), Yemen (3.3%), and Vellore (0.2%) [8,10-11] but substantially lower than the unusually high prevalence observed during the Punjab outbreak [9]. The low frequency of co-infection in our study may be explained by the differing epidemiology of the two viruses during the study period. Although both viruses were circulating concurrently in Bangladesh, CHIKV predominated during July and August, whereas DEN cases increased sharply in September, resulting in only a limited period of overlapping transmission. Furthermore, differences in viral transmission dynamics, mosquito infection rates, seasonal variation, vector competence, host immunity, healthcare-seeking behaviour, and study design may have influenced the likelihood of simultaneous infection. The use of multiplex real-time RT-PCR in the present study also ensured laboratory confirmation of acute infections, minimizing misclassification and providing a more accurate estimate of the true burden of co-infection compared with studies relying solely on serological assays.

The higher proportion of CHIKV in July–August followed by a rise in DENV in September suggests a classical seasonal pattern with sequential dominance, where ecological conditions during early monsoon may favor CHIKV transmission, and later conditions (e.g., increased vector density, viral amplification, or herd immunity shifts) facilitate DENV predominance. This indicates that co-circulating arboviruses do not necessarily peak simultaneously but may alternate in dominance within the same season. This is biologically plausible in Bangladesh, where monsoon rainfall, temperature, vector density, and urban breeding conditions strongly influence the transmission of *Aedes* mosquito [3]. However, in case of DENV, a recent reporting of 24,535 cases in November and 8,165 in December months, typically considered low-risk months, suggests while peak transmission

remains seasonally structured, DENV transmission is no longer strictly confined to the mid-monsoon period only (mid- June to mid-August). Instead, there appears to be a prolonged tail of transmission extending into traditionally low-risk months [3]. Although co-infection was infrequent, its detection is epidemiologically important. Since DENV and CHIKV share the same principal vectors, *Aedes aegypti* and *Aedes albopictus*, co-infection is expected wherever both viruses co-circulate [3]. Furuya-Kanamori et al. revealed that reported co-infection likely underestimates the true burden because many suspected arboviral cases are tested for only one pathogen [12]. The said article reported that 13 out of 98 countries or territories have been identified to report DEN and CHIK co-infection [12].

In this study, age was not significantly associated with infection distribution, indicating that exposure was relatively broad across age groups. By contrast, males had a significantly higher proportion of CHIK and DEN-CHIK co-infection, which may reflect differences in mobility, occupational exposure, and daytime contact with *Aedes* mosquitoes, which are predominantly day-biting [3]. Similar sex-related variation has been inconsistently reported elsewhere, suggesting that this may be influenced by local epidemiologic and behavioral factors rather than a universal pattern [1].

The present dataset showed that 38.5% of patients with DEN-CHIK co-infection had thrombocytopenia. The finding is consistent with reports from other countries that thrombocytopenia can occur frequently during concurrent DEN and CHIK infection, although its reported frequency varies considerably between studies. A study from India reported thrombocytopenia in all 13 patients with DEN-CHIK co-infection, compared with 94.1% with DEN monoinfection and 33.3% of those with CHIK monoinfection [13]. Another Indian study reported thrombocytopenia in 77% of co-infected patients, while a more recent study found it in 71.4% of co-infected patients, compared with 62.3% of DEN monoinfection and 54.8% of CHIK monoinfection, but these differences were not statistically significant [14,15]. The lower proportion observed in the present dataset (38.5%) may reflect differences in patient selection, timing of platelet measurement, and clinical characteristics. However,

the small sample size of the present study limits direct comparison with larger studies.

The findings support the need for an integrated *Aedes*-borne disease surveillance approach in Bangladesh. During monsoon and post-monsoon periods, clinicians should consider dual testing in acute febrile patients with overlapping symptoms such as fever, rash, myalgia, arthralgia, and thrombocytopenia. Early and coordinated vector control measures are also essential, particularly before the peak transmission period.

However, the current research has several limitations. It was based on RT-PCR-tested suspected cases and therefore reflects the tested patient population rather than the total community burden. The small number of co-infected cases limits subgroup interpretation, and the study did not assess clinical severity, virologic typing, or entomologic correlates. Nonetheless, the use of molecular confirmation is a major strength and provides a reliable estimate of overlapping arboviral transmission.

In conclusion, this study shows that CHIK predominated early in the season, DEN increased later, and DEN-CHIK co-infection, though uncommon, was clearly present. These findings underscore the need to consider DEN-CHIK co-infection in arboviral diagnosis, surveillance, and outbreak preparedness in Bangladesh.

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