**The reappearance of Chikungunya virus in Bangladesh, 2024**

**Abstract:**

The world is currently witnessing the largest recorded arbovirus outbreak in history, with Bangladesh being one of the country’s most severely affected by the dengue epidemic. In Bangladesh, the Chikungunya virus (CHIKV) caused a significant outbreak in 2017 but subsequently nearly disappeared from the country. This study reports an outbreak of CHIKV in Dhaka and its surrounding areas, detailing the clinical characteristics of the virus and the disease it causes.

The Institute of Epidemiology, Disease Control and Research (IEDCR) enrolled patients through event-based surveillance between 19 October and 31 December 2024. Following enrolment, patients were contacted via telephone for follow-up between 21 and 28 days to record updates on their condition.

A total of 138 confirmed CHIKV patients were identified through RT-PCR, and none travelled internationally. The majority were male (71%) and aged over 30 years (57%). Most patients (98%) resided within the Dhaka City Corporation area. Common clinical symptoms included fever (100%), arthralgia (98%), myalgia (94%), and headache (68%). Of the 71 patients with follow-up data, 56% (n=40) reported persistent symptoms. No fatalities were recorded; however, 20 patients (30%) required hospitalization, with an average hospital stay of 5.6 days (range: 2–18 days). On average, patients lost 10.1 working days (range: 2–30 days) due to the illness causing an estimated loss of $68.00 per family considering the national daily per capita income. Disease severity was associated with older age >30 years (1.14, 95% CI: 1.02-1.28), and severe clinical signs (IRR: 0.87, 95% CI: 0.77-0.98). Phylogenetic analysis of the CHIKV E gene revealed a mutation in the xxx gene.

CHIKV is likely to re-emerge in Bangladesh, amidst the ongoing and severe dengue outbreak. The country may face a significant CHIKV outbreak in 2025 or 2026. Strengthened efforts to control the dengue virus are critical for managing arboviruses, and hospitals must be prepared to handle a surge in patients effectively.

**Introduction:**

Chikungunya virus (CHIKV) is a member of the Alphavirus genus of the family Togaviridae transmitted by *Aedes* mosquitoes, primarily *Ae. aegypti* and *Ae. albopictus(1)*. CHIKV was first identified in Tanzania in 1950s (2) and initially caused sporadic outbreaks in Africa and Asia until 2004 (3). However, a significant outbreak in Kenya in 2004 marked the beginning of a resurgence of CHIKV, resulting in extensive spread to the Indian Ocean islands, including the Comoros, Seychelles, Mauritius, and the French territories of Mayotte and La Réunion(3). The epidemiology and transmission patterns of CHIKV shifted notably during the 2005–2006 outbreaks on La Réunion, where *Aedes albopictus* mosquitoes were identified as the primary vector (3,4). The global spread of CHIKV has been partially attributed to its adaptation to this mosquito species, facilitated by a mutation in the envelope protein 1 gene (E1-A226V)(3). This mutation enhanced the ability of *Aedes albopictus* mosquitoes to transmit the virus to humans(3). After this adaptation CHIV has transmitted to more than 100 countries worldwide between 2014 and 2019(1). The CHIK virus infects approximately 3 million people annually, with an estimated 1.3 to 2.7 billion people currently residing in areas at risk of CHIKV transmission (5).

Chikungunya virus was first reported in Bangladesh in December 2008 in two adjacent north-western districts, Rajshahi and Capai Nowabgang. Subsequently, outbreaks were reported in 2009, 2011 and 2012 (6,7). In 2017, Bangladesh experienced the largest CHIKV outbreak with 13,176 clinically confirmed cases in 17 of 64 districts of the country (8). A modelling study predicted a peak prevalence of 47 cases per 1,000 people in Dhaka city during 2017 outbreak(9). These estimates are significantly higher than the official report of 13,176 total cases between April and September (9). The study also estimated a very high basic reproduction number of CHIKV (4.20) during 2017 outbreak (9). Nationwide surveillance conducted between 2015 and 2016 reported a seroprevalence of 2.4% and predicted 4.99 million people to be infected with CHIKV before the 2017 major outbreak in Bangladesh(10). However, after 2017, CHIKV had almost disappeared from Bangladesh with no case detected anywhere in the country. This study reports an outbreak of CHIKV in Dhaka and its surrounding areas, detailing the clinical characteristics of the virus and the disease it causes.

**Methods:**

**Epidemiological Data collection**

In response to a reported chikungunya outbreak in Dhaka, the Institute of Epidemiology, Disease Control and Research (IEDCR) established a sample collection booth to facilitate testing for suspected chikungunya cases referred by physicians. A suspected chikungunya case was defined as any individual presenting with fever and arthralgia/arthritis not attributable to other medical conditions. Data were collected using a pre-designed questionnaire, and informed consent was obtained from all participants during sample collection to include them in the study. A total of 394 suspected cases were referred to IEDCR and subsequently enrolled in the investigation.

**Sample collection**

Following aseptic procedures, 3–4 mL of blood was collected from each participant into tubes containing a clot activator. The serum was then separated and stored at 4°C until further testing could be performed.

**RT-PCR testing**

Viral RNA was extracted from 140 µL of serum using the QIAamp Viral RNA Mini Kit (QIAGEN, Cat# 52906) following the manufacturer's instructions. The RNA was purified and eluted in a final volume of 30 µL.

Real-time reverse transcription PCR (RT-PCR) was performed using the Genesig Dengue, Zika, Chikungunya multiplex RT-PCR Kit (Primer Design, Cat# R00600) on an ABI QuantStudio 5 thermal cycler. The thermal cycling protocol included the following steps: Reverse Transcription at 550C for 10 mins, enzyme activation at 950C for 2 mins followed by 50 cycles of denaturation at 950C for 10 seconds and annealing & extension at 600C for 1 min. Fluorescence data were collected during the extension phase through the VIC (DENV), FAM (ZIKV), Cy5 (CHIKV), and ROX (internal control) channels. Post-PCR analysis involved evaluating amplification curves on a linear scale. Baseline thresholds were manually set for each run. Amplification curves with a cycle threshold (CT) value of <50 were considered as positive. Amplification of the internal control (IC) confirmed the absence of PCR reaction inhibition.

**E1 Gene Sequencing (needs to be updated)**

A total of ?? samples with cycle threshold (*CT*) values of <25 were selected for sequencing of the E1 gene. Viral RNA was extracted from 140 µL of serum using QIAamp Viral RNA mini Kit (QIAGEN, Cat#52906), converted into first-strand cDNA using LunaScript RT SuperMix (New England Biolabs, Cat#M3010L) according to the manufacturers instruction. The first-strand cDNA was amplified with Q5 Hot start High fidelity 2X Master mix (New England Biolabs, Cat# E3010) using the 3 sets of primers described previously (<https://doi.org/10.1371/journal.pone.0050476>). The primers produced a set of 3 overlapping amplicons of 756, 1014 and 839 bp size. This approach allowed the sequencing of the complete E1 gene. The final products were then purified with the ExoSAP-IT Express PCR product purification kit (Applied Biosystems, USA, Cat#). Cycle sequencing reactions were performed using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) using 3 sets of primers used for amplification of E1 gene, followed by purification of the cycle sequencing products with the BigDye XTerminator purification kit (Applied Biosystems). Automated capillary electrophoresis was performed with the purified cycle sequencing products in a SeqStudio Flex 8 Genetic Analyzer (Applied Biosystems).

**Results:**

We tested a total of 394 suspected CHIKV patients, of which 138 (35%) were confirmed positive**.** Among the 138 positive cases, none traveled internationally, the majority were male (n = 89, 64.5%) and aged ≥30 years (n = 115, 83.3%). The most common clinical symptoms included fever (100%), arthralgia (97.8%), myalgia (83.2%), headache (64.9%), and conjunctivitis (24.7%). Over 47% of patients (n = 65) had at least one comorbidity, with hypertension (26.3%, n = 36) and diabetes mellitus (26.3%, n = 36) being the most prevalent. Most patients were recruited during December (n=75), while the rest were in November and October (n=5) **(Fig. 1)**.

A graph of different types of data

Description automatically generated with medium confidence

Fig 1: The daily recruited confirmed chikungunya cases in Bangladesh between 16 October and 31 December. Most cases were enrolled in the month of December (n=75), while the rest were in November (n=58) and October (n=5).

Most of the patients were recruited from Dhaka city (72 from Dhaka South City corporation and 64 from Dhaka North City corporation) while only two were from outside Dhaka City, one being in Narayanganj district and another one in Keraniganj, a subdistrict of Dhaka **(Fig 2).** The maximum distance between the two cases was 12.21 kilometres.



**Fig 2:** The geographical location of Chikungunya cases between 16 October 2024 and 31 December 2024 in Bangladesh.

We were able to follow up with 48 patients (34.7%) to assess their health outcomes between 21 and 28 days after the initial illness. No patient died, however, 40 patients (85.1%) reported persistent symptoms during the follow-up period, while only 7 patients (14.5%) had fully recovered. The most common persistent symptoms were joint pain (95%), joint swelling (22%), and fatigue (31%). On average, patients lost 10.1 working days (range: 3–30 days) due to CHIKV infection. Considering the daily per capita income of USD 6.98 in Bangladesh, the disease caused an average household income loss of $69.8 per patient.

Among the 138 CHIKV-positive cases 20 (14.5%) patients require hospitalization with a mean duration of 5.9 days of hospital stay. The mean (range) age of the hospitalized patients was 52 (15-78) years. The most common comorbidity of the patients who required hospitalization was diabetes Mellitus (45%), hypertension (45%), and arthritis (20%).

The hospitalization of the CHIK patients was associated with the older age group (>30 years) (Incidence rate ratio (IRR): 1.14 (95% Confidence interval: 1.02-1.28), and more than five clinical signs (IRR: 0.87, 95% CI: 0.77-0.98).

**Table 1: Factors associated with hospitalization of Chikungunya-infected patients in Bangladesh between 16 October and 31 December 2024, using a Modified Poisson regression model**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **IRR** | **95% CI** | **P-value** |
| **Age groups** | <30 | Reference |  |  |
|  | ≥30 | 1.14 | 1.02 - 1.28 | 0.027 |
| **Sex** | Female | Reference |  |  |
|  | Male | 1.08 | 0.94- 1.25 | 0.269 |
| **Employment Status** | No | Reference |  |  |
|  | Yes | 0.87 | 0.75 – 1.01 | 0.062 |
| **Clinical signs** | Low (median ≤4) | Reference |  |  |
|  | High (median >5) | 0.87 | 0.77 – 0.98 | 0.017 |
| **Delay in test** | Yes (median≤3) | Reference |  |  |
|  | No (median>3) | 1.03 | 0.90 - 1.18 | 0.665 |
| **Comorbidity** | No | Reference |  |  |
|  | Yes | 1.02 | 0.91 - 1.16 | 0.703 |

**Discussion:**

The re-emergence of the CHIKV in Dhaka, Bangladesh, came after a near disappearance of the virus following the 2017 outbreak highlighting a serious concern, especially in the context of the ongoing and large-scale dengue epidemic in Bangladesh, which continues to place significant strain on public health systems(11,12). The clinical characteristics observed in this study are consistent with prior reports of CHIKV infections, with fever, arthralgia, myalgia, and headache being the predominant symptoms (1). Importantly, more than half of the patients who were followed up reported persistent symptoms, underscoring the potential long-term health impact of CHIKV infections, even in the absence of fatalities.

The study also provides valuable insights into disease severity, which was found to be higher in individuals aged over 30 years and those presenting with more severe clinical signs. The need for hospitalization among 30% of patients and the loss of working days further emphasize the social and economic burden associated with the outbreak. This is particularly concerning given the strain that healthcare systems are already experiencing due to the ongoing dengue crisis.

Bangladesh provides a highly conducive and favorable environment for Aedes mosquito breeding, including factors such as rapid urbanization, extended rainfall, and numerous mosquito breeding sites. Additionally, the temperature in the country remains highly favorable for Aedes mosquitoes for approximately 9 out of the 12 months each year. The recent dengue outbreak has also demonstrated that rural areas are equally affected, suggesting that *Aedes albopictus* mosquitoes may be adapting to these regions. Furthermore, with a high basic reproduction number (R₀) for Chikungunya virus—estimated at 3.4 globally (13) and 4.2 during the 2017 outbreak in Bangladesh (9) —it is likely that CHIKV will cause a large outbreak in 2025 or 2026. In 2023, Bangladesh experienced the largest dengue outbreak in its history, leading to a national crisis involving shortages of intravenous saline solutions and hospital beds(11). This article serves as a crucial alert to prepare for a potential large-scale outbreak of CHIKV in the near future.

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