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Molecular Diagnosis of Dengue Virus in Various Diagnostic Centers of Dhaka City, Bangladesh

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Dengue, the most dominant mosquito-borne infectious disease of humans that recurring global health problem. In Bangladesh, dengue outbreaks are on the increase despite the efforts of the government, and it is not clear what the understanding of the general Dhaka population towards dengue fever is. This study aims to assess the prevalence of dengue virus infection among suspected cases through laboratory-based molecular methods. Here, 93 samples from suspected patients at different diagnostic centers in Dhaka City, Bangladesh, were reported. Suspected samples were analyzed by the RT-PCR technique. Of the 93 samples tested, 20.43% were positive for dengue virus, while 79.57% were negative. It was observed that among various age major number of positive cases were male compared to females. In addition, the most affected age group was 41–60 years (63.16%), followed by 21–40 years (31.58%), while only 5.26% of cases were observed in individuals aged 18 years or below. This pattern may reflect a male predominance over females, increased outdoor activity, or occupational exposure among adults, particularly middle-aged groups, which could contribute to a higher infection risk. These findings highlight the importance of RT-PCR based accurate detection of dengue as well as awareness and prevention strategies, especially for high-risk demographic groups.

Keywords: Dengue, RT-PCR, Molecular, Methods, Prevalence, Bangladesh

INTRODUCTION

Dengue, the mosquito-borne infectious disease, has become a major global public health concern caused by one of four main dengue virus (DENV) serotypes, primarily spread by Aedes aegypti—a mosquito species that thrives in human environments, breeding in water-related structures like household containers, gutters, drains, and sewage systems (1-3). Over the past few decades, dengue cases have risen sharply worldwide, making it the most rapidly spreading insect-borne vector disease. Dengue has been classified as one of the top ten worldwide public health threats by the World Health Organization (WHO) in 2019 (4). As of March 2025, more than 1.4 million dengue cases and over 400 related deaths have been reported across 53 countries or territories within the WHO Regions of the Americas (PAHO), South-East Asia (SEARO), the Western Pacific (WPRO), the Eastern Mediterranean (EMRO), and Africa (5). About 52% of the world's dengue-at-risk population resides in South-East Asia, where the first dengue virus infection was discovered (6). The first dengue outbreak in Bangladesh was documented in 1964 under the name "Dhaka/Dacca fever," despite the fact that dengue disease was first described in 1780 (7). Dhaka, the capital of Bangladesh, has emerged as a dengue fever (DF) hotspot (8). In addition, dengue cases surged significantly after 2000, with a particularly high number of deaths reported during the major outbreak in 2019 (9). A study claimed that the huge migration of dengue-infected people from Dhaka to other cities around the country caused this major outbreak in 2019 (8). In 2020, based on the Directorate General of Health in Bangladesh, a total of 1026 confirmed cases were reported during the pandemic (10). According to the Directorate General of Health Services (DGHS), 20 people have died while 2,586 others have been hospitalized as of 2nd May from dengue so far this year (11). Based on some studies, an acute dengue outbreak in the city might be caused by a high population density, rapid unplanned urbanization, Inadequate dengue surveillance systems, Neglect of dengue prevention practices, and climate change (12, 13).

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Dengue fever (DF), caused by dengue virus serotypes (DENV-1, 2, 3, or 4), can lead to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (14). The classification of these four serotypes is based on the envelope protein's antigenicity, which is important for understanding the dynamics of infection and epidemics. When DENV connects to host cell receptors during infection, the envelope protein merges with the host cell and enters the cell. It produces certain neutralizing and protective antibodies, which boosts the host immune response (15). All four serotypes (DEN-1, 2, 3, or 4) have been documented in Dhaka city since the beginning of the notable outbreak in 2000, with the DEN-3 serotype having a higher prevalence in 2003. The DEN-3 serotypes reemerged in 2017 following a 2013–2016 hiatus, and was reportedly the most commonly detected serotype during the 2019 outbreak. Therefore, the dengue burden is quite spatially heterogeneous (for instance, across major metropoles and smaller cities or between neighborhoods), although it is not obvious which socio-environmental factors are most important in causing these risk differences. (3, 16,17,18). Although there isn't a vaccine that can effectively prevent DF, healthy habits like following genuine counsel, keeping a good outlook, and doing the right things could help eradicate the disease (19). Dengue is frequently linked to urban settings, which offer favorable microclimates and high densities of Aedes breeding habitat (3, 18). Changes in community and individual behavior, along with appropriate government assistance for DF, can help reduce the rising dengue incidence in Dhaka and, by extension, the entire nation.

Hence, this study aims to detect and analyze the presence of dengue virus using molecular diagnostic methods in clinical samples collected from various diagnostic centers across Dhaka City, Bangladesh.

MATERIALS AND METHODS

Ethical approval: As this is a retrospective study the ethical approval was not required.

Study time: This study was done from 1st July to 31st December, 2024.

Inclusion criteria: Suspected dengue cases with positive RT-PCR results were included. (Fever with any two of nausea/vomiting, rash, aches/pain, leucopenia, any warning sign, or positive tourniquet test) were screened.

Exclusion criteria: RT-PCR negative samples were excluded from the study despite the fact that patients produced dengue-like symptoms.

Sample/ specimen collection: A total of 93 samples were collected from a renowned diagnostic center in Dhaka city. A blood sample was collected and processed as instructed by the manufacturer following standard protocol (21).

RNA extraction and real-time RT-PCR: DENV genomic RNA was amplified using a One-Step RT-PCR kit (Qiagen, Hilden, Germany) with serotype-specific primers. The thermal cycling protocol consisted of: reverse transcription at 50°C for 30 min; initial denaturation and reverse transcriptase inactivation at 95°C for 15 min (which also activated the HotStarTaq DNA Polymerase); 35 cycles of denaturation at 94°C for 30 sec, annealing for 1 min (55°C for DENV-1, 46°C for DENV-2, or 49°C for DENV-3), and extension at 72°C for 2 min 30 sec; followed by a final extension at 72°C for 10 min. The resulting amplicons were subsequently used as a template for a nested RT-PCR, performed with primers and according to the manufacturer's instructions (23).

RESULTS

Percentage (%) of positive and negative dengue cases: The test results of patients presenting with symptoms, as well as those without such symptoms, suggestive of dengue virus infection, were collected and thoroughly analyzed. Out of the total 93 tested samples, 20.43% were found to be reactive, indicating a positive result for dengue virus infection. In contrast, the remaining 79.57% of samples were non-reactive, suggesting the absence of detectable dengue virus at the time of testing.

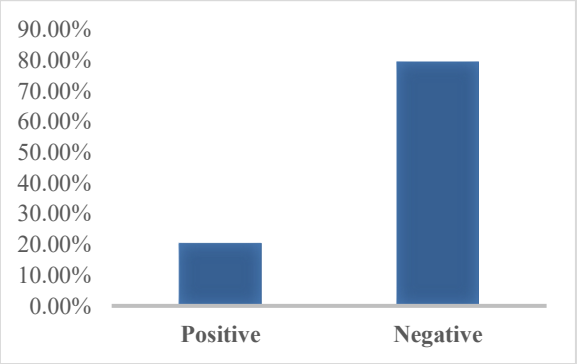

Figure 1: Percentage (%) of positive and negative dengue cases.

Distribution of dengue cases based on gender: The test results were determined based on the RT-PCR test. A total of 93 samples were analyzed, and it was observed that various age number was included in this study. Of the confirmed dengue cases, 66% positive cases found male and 34% were female. This study found predominance of males over females.

Distribution of dengue cases based on age: Among the 93 collected samples of dengue-suspected patients, individuals from various age groups were represented. The most affected age group was 41–60 years, accounting for 63.16% of the total cases. This was followed by the 21–40 years age group, comprising 31.58%, while the youngest group, aged 18 years or below, made up only 5.26% of the cases.

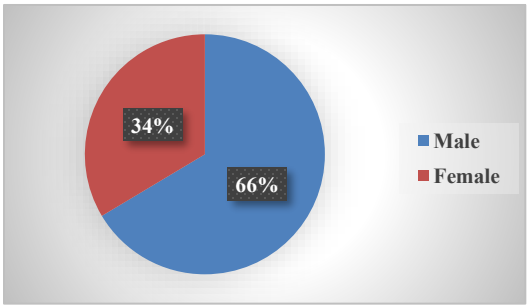

Figure 2: Gender distribution of dengue positive test results.

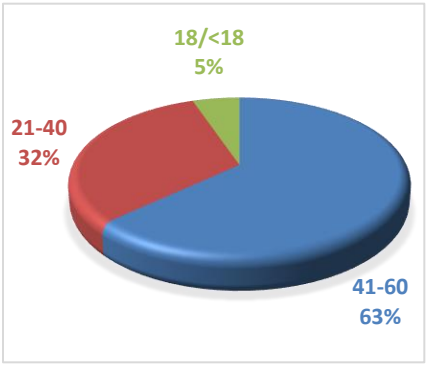

Figure 3. Age distribution of dengue positive results.

DISCUSSION

Dengue is a major health problem in Bangladesh. It is one of the major health threats to the people living in Dhaka. Over the last 70 years infection rate has increased dramatically. But the case has increased 9-fold within the last three years (24). It can be assumed that the reason behind the high infection rate is negligence about the cleanliness of the nearby environment. The government has been warning the people about the consequences of this alarming rate, even knowing that these people are not taking the necessary steps to keep their houses, terraces and open places. In our study, to detect whether the described clinical presentations are solely caused by infection with the dengue virus, samples from all patients were initially diagnosed by RT-PCR from the patients. Overall, there were nearly 20.4% RT-PCR positive cases of dengue virus infection. The highest numbers of cases were recorded in the age group 41-60 years, and the infection rate between males and females differs significantly.

The finding of Rahim *et.al.*, 2021, states that less than 5% of DENV-1 and DENV-3 among 161 cases were positive (22). In contrast to our study, Hossain et.al., 2025 found 320 confirmed cases out of 364 suspected cases, where quantitative RT-PCR was used (57.5%) (25). During the first epidemic in 2000, more than 80% of cases were adults (> 18 years of age); the peak number of cases occurred between 18 and 33 years of age. Likewise, the majority (62%) of the confirmed cases belonged to the 16–30 age group, with a mean age of 29 years in the 2002 outbreak (1). In this study, the most affected age group was 41-60 years (63.16%), followed by 21-40 years (31.58%), and 18 years or younger (5.26%). Older adults are more vulnerable to severe dengue due to factors including a weakened immune system, increased risk of comorbid conditions like diabetes and hypertension, and a higher likelihood of a severe outcome from secondary dengue infection. Also, diagnosis of dengue in older people may be challenging because of atypical presentations (26).

The present study shows male predominance, with 62.48% male and 31.58% female. Similar to our study, Rahim *et.al.*, 2021, found male predominance, with 60.04% male and 39.96% female (22). In three consecutive years (2017, 2018 & 2019), the 1-10 years age group was the dominating age group, whereas in 2021, it was 11-20 (27). These findings suggest that dengue predominantly affects males than females due to exposure patterns and healthcare-seeking behaviors (28, 29). It is important to note that females can experience a higher case fatality rate in certain demographics (30). The perceived male predominance can be influenced by factors like increased outdoor activity and work-related exposure among males, as well as potential underreporting or under-hospitalization of severe cases in females, particularly in some developing countries. Since there is no definitive cure for dengue, ongoing surveillance
and proactive preparedness strategies are essential each year to reduce dengue-related deaths and illnesses. This study was conducted at a single center in Dhaka, limiting population diversity. To gain deeper insights, a large-scale, multi-center investigation focusing on serotype prevalence is needed. It is high time dengue must be controlled in order to save millions of lives. A comprehensive approach involving environmental management, community participation, and health system preparedness is essential for sustainable dengue prevention in Bangladesh (31).

CONCLUSIONS

The molecular diagnosis of dengue virus in Dhaka's diagnostic centers reveals a critical landscape. While conventional methods like serology remain widely used, RT-PCR testing demonstrably offers superior accuracy, enabling earlier and more specific detection of active dengue infections. This precision is vital for timely patient management and effective surveillance. However, a significant insufficiency in RT-PCR technology availability and accessibility persists across many centers in Bangladesh, limiting the realization of these diagnostic benefits. This gap hampers optimal case identification and public health response. Therefore, it is imperative that stakeholders, including government health agencies, private healthcare providers, and international partners, prioritize investment in and widespread implementation of RT-PCR-based dengue detection. Enhancing RT-PCR capacity is essential to improve diagnostic accuracy, strengthen outbreak control, and ultimately reduce the dengue burden in Dhaka and beyond.

CONFLICTS OF INTEREST

The authors have declared that no competing interests exist.

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REFERENCES

1. Hossain MS, Noman AA, Mamun S and Mosabbir AA. 2023. Twenty-two years of dengue outbreaks in Bangladesh: epidemiology, clinical spectrum, serotypes, and future disease risks. Tropical Medicine and Health, 51: 1-14.
2. Brady OJ and Hay SI. 2020. The global expansion of dengue: how Aedes aegypti mosquitoes enabled the first pandemic arbovirus. Annual Review of Entomology, 65: 191-208.
3. Kolimenakis A, Heinz S, Wilson ML, Winkler V, Yakob L, Michaelakis A et al. 2021. The role of urbanisation in the spread of Aedes mosquitoes and the diseases they transmit—A systematic review. PLOS Neglected Tropical Diseases, 15(9): e0009631.
4. Zhang W-X, Zhao T-Y, Wang C-C, He Y, Lu H-Z, Zhang H-T et al. 2025. Assessing the global dengue burden: Incidence, mortality, and disability trends over three decades. PLOS Neglected Tropical Diseases, 19(1): e0012932.
5. Dengue worldwide overview. European Centre for Disease Prevention and Control. Dengue worldwide overview 2025. https://www.ecdc.europa.eu/en/dengue-monthly.
6. Saghir MA, Omer B-S, Hatem AOB, Al-Harthy HA, Sumait TIB, Alamoudi S et al. 2025. Knowledge, attitude and preventive practices of the community towards dengue fever in Seiyun city, Yemen: a cross-sectional study. Discover Public Health, 22: 154.
7. Rahman MM, Islam ARMT, Khan SJ, Tanni KN, Roy T, Islam MR et al. 2022. Dengue fever responses in Dhaka City, Bangladesh: a cross-sectional survey. International Journal of Public Health, 67: 1604809.
8. Hossain MS, Siddiqee MH, Siddiqi UR, Raheem E, Akter R and Hu W. 2020. Dengue in a crowded megacity: Lessons learnt from 2019 outbreak in Dhaka, Bangladesh. PLOS Neglected Tropical Diseases, 14(3): e0008349.
9. Kayesh MEH, Khalil I, Kohara M and Tsukiyama-Kohara K. 2023. Increasing dengue burden and severe dengue risk in Bangladesh: an overview. Tropical Medicine and Infectious Disease, 8: 32.
10. Hasan MJ, Tabassum T, Sharif M, Khan MAS, Bipasha AR, Basher A et al. 2021. Comparison of clinical manifestation of dengue fever in Bangladesh: an observation over a decade. BMC Infectious Diseases, 21: 1113.
11. Alam H. 2025. Rain sparks fresh fear of dengue surge. The Daily Star.
12. Mutsuddy P, Tahmina Jhora S, Shamsuzzaman AKM, Kaisar SG and Khan MNA. 2019. Dengue situation in Bangladesh: an epidemiological shift in terms of morbidity and mortality. Canadian Journal of Infectious Diseases and Medical Microbiology, 2019: 3516284.
13. Rahman MS, Karamehic-Muratovic A, Baghbanzadeh M, Amrin M, Zafar S, Rahman NN et al. 2021. Climate change and dengue fever knowledge, attitudes and practices in Bangladesh: A social media–based cross-sectional survey. Transactions of The Royal Society of Tropical Medicine and Hygiene, 115(1): 85-93.
14. Zerfu B, Kassa T and Legesse M. 2023. Epidemiology, biology, pathogenesis, clinical manifestations, and diagnosis of dengue virus infection, and its trend in Ethiopia: a comprehensive literature review. Tropical Medicine and Health, 51: 11.
15. Jiang L, Liu Y, Su W, Liu W, Dong Z, Long Y et al. 2023. Epidemiological and genomic analysis of dengue cases in Guangzhou, China, from 2010 to 2019. Scientific Reports, 13: 2161.
16. Lee SA, Economou T, de Castro Catão R, Barcellos C and Lowe R. 2021. The impact of climate suitability, urbanisation, and connectivity on the expansion of dengue in 21st century Brazil. PLOS Neglected Tropical Diseases, 15(12): e0009773.
17. Kache PA, Santos-Vega M, Stewart-Ibarra AM, Cook EM, Seto KC and Diuk-Wasser MA. 2022. Bridging landscape ecology and urban science to respond to the rising threat of mosquito-borne diseases. Nature Ecology & Evolution, 6: 1601-1616.
18. Gao P, Pilot E, Rehbock C, Gontariuk M, Doreleijers S, Wang L et al. 2021. Land use and land cover change and its impacts on dengue dynamics in China: A systematic review. PLOS Neglected Tropical Diseases, 15(6): e0009879.
19. Coudeville L, Baurin N and Shepard DS. 2020. The potential impact of dengue vaccination with, and without, pre-vaccination screening. Vaccine, 38: 1363-1369.
20. Khosavanna RR, Kareko BW, Brady AC, Booty BL, Nix CD, Lyski ZL et al. 2020. Clinical symptoms of dengue infection among patients from a non-endemic area and potential for a predictive model: a multiple logistic regression analysis and decision tree. The American Journal of Tropical Medicine and Hygiene, 104(1): 121-131.
21. Verberk IM, Misdorp EO, Koelewijn J, Ball AJ, Blennow K, Dage JL et al. 2022. Characterization of pre‐analytical sample handling effects on a panel of Alzheimer's disease‐related blood‐based biomarkers: Results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. Alzheimer's & Dementia, 18: 1484-1497.
22. Rahim R, Hasan A, Hasan N, Nakayama EE, Shioda T and Rahman M. 2021. Diversity of dengue virus serotypes in Dhaka city: from 2017 to 2021. Bangladesh Journal of Medical Microbiology, 15: 23-29.
23. Suzuki K, Nakayama EE, Saito A, Egawa A, Sato T, Phadungsombat J et al. 2019. Evaluation of novel rapid detection kits for dengue virus NS1 antigen in Dhaka, Bangladesh, in 2017. Virology Journal, 16: 102.
24. Yang X, Quam MB, Zhang T and Sang S. 2021. Global burden for dengue and the evolving pattern in the past 30 years. Journal of Travel Medicine, 28: taab146.
25. Hossain F, Ghosh P, Chowdhury FR, Basher A, Ahsan HMN, Khan AH et al. 2025. Evaluating a rapid molecular assay in a mobile laboratory for improved diagnosis of dengue in Bangladesh. International Journal of Infectious Diseases, 150: 107299.
26. Ng WY, Ngim CF, Chow KY, Goh SX, Zaid M and Dhanoa A. 2022. Clinical manifestations, laboratory profile and outcomes of dengue virus infection in hospitalised older patients. Transactions of The Royal Society of Tropical Medicine and Hygiene, 116: 545-554.
27. Rahim R, Hasan A, Hasan N, Nakayama EE, Shioda T and Rahman M. 2021. Diversity of dengue virus serotypes in Dhaka city: from 2017 to 2021. Bangladesh Journal of Medical Microbiology, 15: 23-29.
28. Akter M, Araf Y, Akter S and Hossain MG. 2024. Dengue in Bangladesh: A Gendered Perspective on Infection and Fatality Rates amidst Global Epidemiological Trends. Journal of Arthropod-Borne Diseases, 18(3): 281-284.
29. Debes MS, Tayeb MA, Nassani MF, Basaeed AA and Dwaima A. 2016. Dengue Fever in Adults, a Retrospective Study. American Journal of Internal Medicine, 4(6): 93-100.
30. Roney M and Aluwi MFF. 2024. Dengue Death Rate Higher Among Women than Men in Bangladesh. Bangladesh Journal of Infectious Diseases, 11(1): 79-82.
31. Hasan MJ, Tabassum T, Sharif M, Khan MAS, Bipasha AR, Basher A et al. 2021. Comparison of clinical manifestation of dengue fever in Bangladesh: an observation over a decade. BMC Infectious Diseases, 21(1): 1113.