



Case report

Understanding dengue outbreaks in Rajshahi district, Bangladesh: A comprehensive case study

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ABSTRACT

Background and aims: The 2023 dengue outbreak in Bangladesh marked an unprecedented increase in fatalities, particularly in Dhaka, and demonstrated extensive prevalence nationwide, including Rajshahi district. Dengue fever remains a significant public health challenge in Southeast Asia, with complex epidemiological patterns. Previous research has mainly focused on den serotypes in Dhaka. Therefore, this study aims to identify serotypes in the Rajshahi district under endemic conditions

Methods: Blood samples from suspected dengue patients were collected at Rajshahi Medical College Hospital. Initial rapid detection of dengue-positive cases was performed using (Nonstructural Protein 1 L) NS1, (Immunoglobulin G) IgG, and (Immunoglobulin M) IgM tests. Upon confirmation of dengue positivity, viral RNA was extracted for molecular testing. The dengue serotype was identified using real-time reverse transcription-polymerase chain reaction (rRT-PCR).

Results: The study revealed that 93.3 % of the patient were infected with (Dengue virus type 2) DENV2 and rest 6.7 % of the patient were (Dengue virus type 3) DENV3 among 30 dengue positive patients. Demographic observations show the distribution of dengue over nine upazilas. In Paba upazila, we found two DENV3 alongside DENV2.

Conclusion: The study concludes that the 2023 dengue outbreak in Rajshahi district, Bangladesh, predominantly involved the DENV2 serotype. Geospatial analysis underscores the importance of understanding regional distribution patterns to enhance targeted interventions against dengue fever in endemic areas.

Introduction

Dengue is an intermittent and infectious disease which leads to the conditions like dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome with following onset symptoms such as high fever, headaches, muscle and joint pains, discomfort behind the eyes, rapid breathing, weakness, and a marked decrease in white blood cells. It is transmitted primarily by daytime bites of infected female *Aedes aegypti* and *Aedes albopictus* mosquitoes. [1]. The first dengue outbreak in Bangladesh was recorded in 1964 with the DENV3 serotype, followed

by eight more outbreaks [2]. The 2023 outbreak was notable for its high fatalities and widespread impact. Significant dengue cases were rare until the first major outbreak in 2000 [3].

According to the Directorate General of Health Services (DGHS) press release (<https://old.dghs.gov.bd/index.php/bd/home/5200-daily-dengue-status-report>), on December 28, 2023, Bangladesh reported 320,945 confirmed dengue cases and 1701 deaths. Dhaka was particularly affected, especially during August, September, and October, with September alone recording 79,598 cases and 396 deaths. Rajshahi district reported 862 cases with no deaths. Historical records indicate

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Dhaka as the focal point of the outbreak, with most studies centered on the city [4]. Dengue infections are caused by five serotypes: DENV1, DENV2, DENV3, DENV4, and the novel DENV5, identified in Malaysia [5]. In Bangladesh, four serotypes have been detected. Until 2016, DENV1 and DENV2 were dominant. DENV3 became the most prevalent during the 2019 outbreak. By 2023, DENV2 had re-emerged as the dominant serotype [6]. The Institute of Epidemiology, Disease Control, and Research (IEDCR) (<https://old.iedcr.gov.bd/images/files/dengue/dengue%20serotypes.pdf>) found that DENV2 and DENV3 were prevalent in Dhaka in June 2023. In contrast, DENV3 was prevalent in 2021 and DENV4 in 2022.

Historical records from 2013 to 2018 show DENV2 as the most common in Dhaka, followed by DENV1, with occasional co-detection of DENV3 [7]. In 2019, DENV3 became dominant. This pattern has been consistent since 2000. Dengue cases have been a yearly occurrence in all major urban areas of Bangladesh [8]. Whereas DENV3 contains genotypes I, II, and III, DENV2 contains six genotypes (Asian/American, Asian I, Asian II, Cosmopolitan, American, and Sylvatic) and is linked to more severe dengue illnesses. The DENV1 and DENV4 variants are less severe than the DENV2 and DENV3 variants [9]. Infection with one serotype provides long-term immunity, but consecutive infections with different serotypes increase risk [10]. In 80 % of cases, the infection is mild and flu-like. Identifying the specific dengue serotype is crucial for effective treatment, preparedness, and disease management.

Previous studies on dengue serotype analysis have primarily focused on Dhaka and other large cities in Bangladesh. However, no comprehensive studies have been conducted in the Rajshahi district, despite its relevance for understanding regional differences in dengue serotype prevalence. This study aims to fill this gap by analyzing dengue serotypes in the Rajshahi district, specifically across its 9 upazillas with a sample size of 30. This research is crucial for developing precise treatment strategies and improving public health responses in Rajshahi, where such data has been previously unavailable.

The dengue virus poses significant global public health challenges due to the lack of specific antiviral treatments, relying on supportive care and making early detection crucial. Prompt identification and intervention can reduce severity and mortality [11]. Effective vector control is essential, as *Aedes* mosquitoes transmit the virus. Prior infection with one serotype does not confer immunity against others and may lead to more severe forms like dengue hemorrhagic fever. This study examines dengue serotype occurrence in Rajshahi district patients during the 2023 epidemic in Bangladesh, highlighting the need for awareness, mosquito control, and rapid response [12].

Methodology

Sample collection

Samples were collected randomly from each of the nine upazilas (sub-districts) patients at Rajshahi Medical College, Rajshahi, who presented with acute onset of symptoms including high fever, headache, myalgia, arthralgia, ocular discomfort, tachypnea, and fatigue. And we exclude the patients who were negative in the rapid test (NS1, IgG, and IgM) initially, and furthermore, we exclude those who showed positive results in the rapid test but were negative in the rRT-PCR test. These samples were obtained with informed consent at the time of admission. Alongside the samples, we collect additional data, including the patient's name, age, gender, address, contact details, and the date of sample collection, to enhance the effectiveness of our findings. 5 ml of collected blood samples were centrifuged and the supernatant serum was separated.

Rapid detection

For the rapid detection of dengue, samples underwent NS1, IgG, and IgM tests by using ACCURATE (Rapid Card Test) kits made in the USA.

Effective for early detection (NS1) and differentiation between primary and secondary infections (IgG/IgM) with high sensitivity and specificity. NS1 antigen detection, IgM and IgG for antibody detection. The NS1 antigen test is an immunochromatographic assay that detects the non-structural protein 1 (NS1) in patient serum, indicative of acute infection [13]. Supernatant serum applied to the test device along with the buffer, where the presence of a colored line in the test region signifies a positive result. The IgM antibody test, using enzyme-linked immunosorbent assay (ELISA), identifies dengue-specific IgM antibodies that appear within the first week of infection. Serum samples are incubated in antigen-coated wells, followed by washing and adding enzyme-conjugated secondary antibodies; the resulting color change is measured spectrophotometrically to determine positivity. Lastly, the IgG antibody test, also an ELISA, detects IgG antibodies indicating past exposure or secondary infection. Serum is incubated in antigen-coated wells, followed by washing, adding enzyme-conjugated secondary antibodies, and substrate to produce a detectable signal, with results read spectrophotometrically.

rRT-PCR analysis

Viral RNA is extracted using the Chemagic Viral NA/gDNA kit, adhering to the protocols provided by PerkinElmer, MA, USA. This RNA was then subjected to a one-step real-time RT-PCR for both screening and serotyping of the dengue virus [14]. The initial screening uses DENV-specific consensus primers and probes to detect the presence of dengue virus RNA. Subsequently, all DENV-positive RNA samples underwent serotyping using serotype-specific primers and probes in a single-plex real-time RT-qPCR setup. The reactions were conducted in 96-well plates using the Azure CIELO Real-Time PCR system (Azure Biosystems). Detection of DENV serotypes was achieved through the FAM, HEX, Texas red, and Cy5 channels, respectively. A sample was considered positive if it produced a threshold cycle (CT) value of 37 or less.

Results

Demographic Analysis

Sample sites are illustrated through QGIS (version 3.30) to better understand dengue prevalence and dengue serotype distribution over the past six months (Fig. 1).

Demographic characteristics and clinical manifestations

In July-December 2023, out of 30 patients from different locations in Rajshahi, all of whom have tested positive for dengue virus, with the majority (93 %) being infected by DENV2 and only two cases (7 %) attributed to DENV3. The age range of the patients spans from 10 to 74 years, affecting both males and females. Common symptoms reported across these cases include high fever, body ache, muscles cramping, loss of appetite, and nausea, with variations in severity and additional symptoms such as vomiting, abdominal pain, and rapid breathing. All individuals tested positive across three diagnostic methods: NS1 antigen tests, IgM, and IgG antibody tests, confirming acute dengue virus infection. The specificity of the qRT-PCR test enabled the identification of the infecting serotype, which is crucial for understanding the epidemiology and managing the outbreak (Table 1).

Dengue Serotypes

The overall proportion of DENV2, when considering the total percentage of patients across all age groups, is approximately 93.2 %. The overall proportion of DENV3, similarly considered, is about 6.8 % (Table 1). Table 2 suggests that DENV2 is significantly more prevalent than DENV3 across all age groups. The highest percentage of patients

Dengue Sample Site Map of Rajshahi District

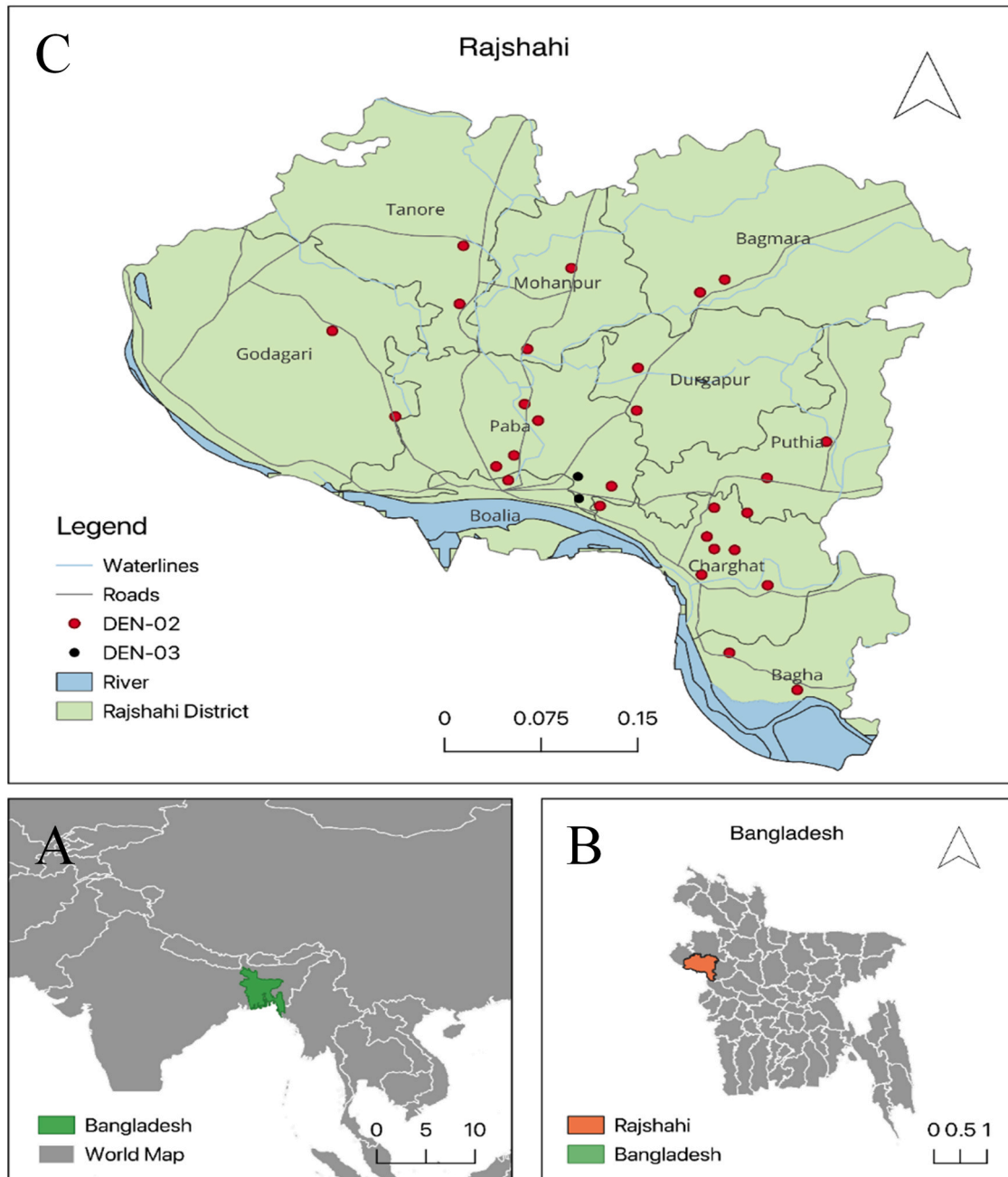


Fig. 1. : The following maps show the locations of Bangladesh and Rajshahi district from where samples were collected: A) Bangladesh in Southeast Asia; B) Rajshahi district in Bangladesh; and C) Rajshahi district map with highlighted roads, sub-district waterlines, and notable locations especially for DENV2 and DENV3 patients, respectively.

falls within the 21–40 age group, indicating that this demographic group may be the most affected by DENV infections among the groups studied (Table 2).

Dengue serotypes in Bangladesh: comparisons and trends

Analyzing current Dengue data in Rajshahi District alongside historical records reveals noteworthy trends. By comparing the number, severity, and geographic spread of cases over time, patterns emerge, offering insights into the cyclical nature of Dengue (Fig. 2). On the basis of available data from the Bangladesh government's public health and literature review, from 2000 to 2023, spanning 23 years, Bangladesh

has faced four different serotypes of dengue virus, and all four serotypes were seen in 2000 [15]. In 2002, only the DENV3 serotype was found; from 2013 to 2016, DENV1 and DENV2 serotypes of that virus were detected [6,16]. In 2017–2019, DENV1, 2, and 3, three different serotypes, were identified, but we followed the predominance of DENV2 and DENV3 [16,17]. Then, in the next two years, 2020 and 2021, just DENV3 were found in our country [16]. Surprisingly, in 2022, DENV4 was recirculated with a small case of number in the predominance of DENV3 [7]. In the last year of 2023, according to the World Health Organization (WHO) (<https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON481>), DENV2 and DENV3 were more prevalent.

Table 1
Details of dengue patients of this study (n = 30).

Lab ID	Age	Sex	Onset symptoms	NS1	IgG	IgM	rRT-PCR	Number of patients suffered with a particular symptom (%)
RMD172	30	Male	High fever, extreme fatigue, body ache	Positive	Positive	Positive	DENV2	
RMD220	20	Female	Fever, abdominal pain, muscles cramping	Positive	Positive	Positive	DENV2	High fever (43.33 %)
RMD226	20	Male	Fever, rapid breathing, persistent vomiting, body ache	Positive	Positive	Positive	DENV2	Fever (56.66 %) Fatigue (16.67 %)
RMD259	28	Male	High fever, restlessness, loss of appetite, Severe stomach pain	Positive	Positive	Positive	DENV2	Nausea (23.34) Vomiting (23.34)
RMD315	45	Male	Fever, muscles cramping, loss of appetite	Positive	Positive	Positive	DENV2	Body ache (30 %)
RMD471	30	Female	Fever, pain in the eye socket, extreme fatigue	Positive	Positive	Positive	DENV2	Abdominal pain (6.67 %)
RMD530	17	Male	High fever, headache, muscle and joint pain, nausea	Positive	Positive	Positive	DENV3	Pain in the eye socket (10 %) Muscle cramping and joint pain (26.67 %)
RMD539	38	Male	High fever, muscles cramping	Positive	Positive	Positive	DENV2	Rapid breathing (20 %)
RMD572	30	Female	High fever, nausea, loss of appetite, severe stomach pain	Positive	Positive	Positive	DENV2	Loss of appetite (36.67 %) Rashes (3 %)
RMD781	40	Male	Fever, Vomiting, loss of appetite, Fatigue	Positive	Positive	Positive	DENV3	Restlessness (13.34)
RMD954	35	Male	High fever, muscles cramping, fatigue and restlessness, loss of appetite	Positive	Positive	Positive	DENV2	
RMD1072	45	Male	Fever, rapid breathing, persistent vomiting, body ache	Positive	Positive	Positive	DENV2	
RMD1098	34	Male	High fever, abdominal pain and rashes, muscles cramping	Positive	Positive	Positive	DENV2	
RMD1162	35	Male	Fever, pain in the eye socket, muscles cramping	Positive	Positive	Positive	DENV2	
RMD1252	20	Male	High fever, muscles cramping, restlessness, loss of appetite	Positive	Positive	Positive	DENV2	
RMD1360	50	Female	Fever, nausea, headache	Positive	Positive	Positive	DENV2	
RMD1948	50	Female	Fever, body ache, persistent vomiting, loss of appetite	Positive	Positive	Positive	DENV2	
RMD2023	74	Male	Fever, rapid breathing, loss of appetite	Positive	Positive	Positive	DENV2	
RMD2265	60	Male	High fever, muscles cramping, headache	Positive	Positive	Positive	DENV2	
RMD2268	50	Male	Fever, nausea, muscles cramping, abdominal pain, rashes	Positive	Positive	Positive	DENV2	
RMD2369	35	Female	Fever, nausea, severe stomach pain, rapid breathing	Positive	Positive	Positive	DENV2	
RMD2491	10	Female	Fever, loss of appetite, severe stomach pain	Positive	Positive	Positive	DENV2	
RMD2528	28	Male	High fever, headache, muscle and joint pain, nausea	Positive	Positive	Positive	DENV2	
RMD2642	20	Male	Fever, nausea, body ache, loss of appetite	Positive	Positive	Positive	DENV2	
RMD2647	35	Male	High fever, fatigue, rapid breathing, vomiting	Positive	Positive	Positive	DENV2	
RMD2874	18	Male	Fever, nausea, pain in the eye socket, body ache	Positive	Positive	Positive	DENV2	
RMD2911	11	Female	High fever, vomiting, loss of appetite	Positive	Positive	Positive	DENV2	
RMD3502	60	Male	Fever, rapid breathing, persistent vomiting, body ache	Positive	Positive	Positive	DENV2	
RMD3505	17	Female	Fever, body ache, persistent vomiting, loss of appetite, Restlessness	Positive	Positive	Positive	DENV2	
RMD3600	22	Male	Fever which persists, nausea, pain in the eye socket, body ache	Positive	Positive	Positive	DENV2	

Table 2
Dengue patients by age group (n = 30).

Age (years)	Patients (%)	DENV2 (%)	DENV3 (%)
0-20	9 (30.0)	8 (89)	1 (11)
21-40	13 (43.3)	12 (92)	1 (8)
41-60	7 (23.3)	7 (100)	0
>60	1 (3.3)	1 (100)	0

Discussion

This study offers analysis of the dengue fever outbreak that profoundly impacted Bangladesh in 2023, with a focused examination on the Rajshahi district. It highlights the persistent threat of dengue fever in Bangladesh, exacerbated by the favorable breeding conditions for *Aedes* mosquitoes. The study's revelation of an unprecedented outbreak in 2023, characterized by high fatality rates and widespread transmission, underscores the escalating challenge of managing dengue in the region. The historical perspective provided on the evolution of dengue outbreaks, dating back to the first recorded outbreak in 1964, contextualizes the current public health crisis and emphasizes the cyclical nature of dengue epidemics in Bangladesh [2].

The finding that the majority of cases were DENV2, with only 2 cases

of DENV3. This is a notable discovery given the regional focus of most prior studies on Dhaka. Our findings contrast with those reported in Dhaka, where several studies have documented a higher prevalence of DENV3 [18]. For instance in Dhaka found that DENV3 was the predominant serotype in over 60 % of the cases, whereas our study in Rajshahi identified DENV2 as the dominant serotype. Similarly, a multi-city study reported that DENV3 was more prevalent in urban centers like Chittagong and Khulna [19]. In contrast, our results align more closely with a recent study conducted in the smaller city of Sylhet, which also reported a higher prevalence of DENV2 [20]. This suggests that regional variations in serotype distribution may exist, potentially influenced by factors such as local vector populations, climatic conditions, and human movement patterns.

The difference in serotype distribution between Rajshahi and Dhaka might be due to several factors. Dhaka, being a major urban center, has different socio-environmental dynamics compared to Rajshahi, which might affect vector breeding and virus transmission. Additionally, the higher mobility and population density in Dhaka could facilitate the spread of different serotypes more than in less populated areas.

The demographic analysis, which mapped the geographical distribution of cases and assessed the demographic characteristics of the affected population, provides valuable insights into the outbreak's impact across different segments of the Rajshahi district. This spatial and

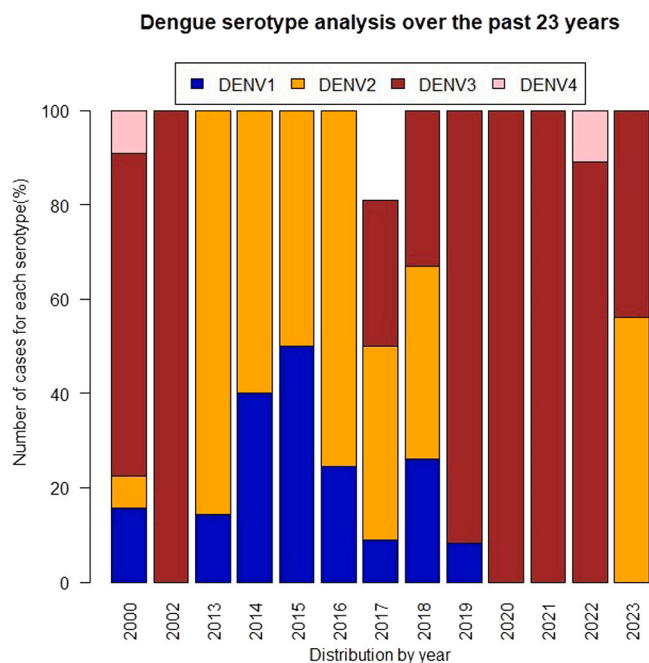


Fig. 2. Demographic information reflects the stereotypical data related to dengue over the past 23 years in Bangladesh.

demographic understanding is crucial for designing effective vector control and disease prevention strategies.

One of the significant limitations of this study is the restricted number of dengue samples collected from the Rajshahi region. This constraint is particularly relevant in the context of epidemiological studies where the incidence and prevalence of diseases can vary significantly across different populations and time periods. Furthermore, the absence of sequencing data represents a critical limitation in our ability to conduct an in-depth analysis of the dengue virus strains present in the region. Sequencing data would have enabled us to identify the genetic diversity of DENV, understand its evolutionary dynamics, and ascertain the presence of any novel or emerging strains.

Based on our findings, future research should focus on conducting larger-scale regional studies to validate the serotype distribution observed in Rajshahi and explore variations across Bangladesh. Longitudinal studies are needed to track changes in serotype prevalence over time. Investigating local vector dynamics and environmental factors, as well as the impact of human mobility on serotype spread, will provide deeper insights. Additionally, examining clinical outcomes associated with different serotypes and developing tailored public health strategies can improve dengue management and control efforts.

Ethical approval

Rajshahi Medical College Hospital Institutional Review Board granted approval for this study. The approval number for research-135 is July 20, 2023.

Consent

All authors have given consent to submit the case report in the journal 'IDCases'.

Authors' contributions

SKB developed the protocol, and methodology, conceived and coordinated the study, and reviewed the manuscript. MHS, MJ, and SRT interpreted laboratory data, cleaned and finalized the dataset,

performed the descriptive analyses, and prepared the first draft of the manuscript. MHS, MJI and SRT were involved in the laboratory work, and analysis of laboratory data and provided intellectual input to the manuscript. MAS prepared the geographical distribution map of the sampling sites. KFS and MJI built the questionnaires, and clinical data collection for study participants and supervised the specimen collection and recording. DKP, KFS and MR critically reviewed the manuscript and provided intellectual input. All authors reviewed subsequent drafts of the manuscript and approved the final version. All authors had full access to all the data in the study and accepted the responsibility for the integrity of the data, accuracy of the data analysis, and for publication.

Author contribution

SKB conceived the idea, design the first draft and coordinated the study. MHS, SRT, and MJI did the experiment. MHS and MAS Wrote the First draft and prepare figures and tables. SKB, DKP, KMFA, MJ and MR critically reviewed the manuscript and provided intellectual input. All authors reviewed subsequent drafts of the manuscript and approved the final version.

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CRediT authorship contribution statement

Selim Reza Tony: Methodology, Data curation. **Md. Johorul Islam:** Methodology, Data curation. **Dipak Kumar Paul:** Writing – review & editing. **Md Al Shaharia:** Software, Methodology, Formal analysis. **Khandker Md. Faisal Alam:** Writing – review & editing. **Mustafizur Rahman:** Writing – review & editing. **Sudhangshu Kumar Biswas:** Writing – review & editing, Methodology, Conceptualization. **Md. Mehedi Hasan Sumon:** Writing – original draft, Formal analysis, Data curation. **Mohammad Jubair:** Writing – review & editing.

Declaration of Competing Interest

All authors declare no competing interests.

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Informed consent

Informed consent was obtained from all subjects involved in the study.

Transparency statement

The corresponding author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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