Diversity of Dengue Virus Serotypes in Dhaka City: From 2017 to 2021

Rummana Rahim¹, Abu Hasan¹, Nazmul Hasan¹, Emi E. Nakayama², Tatsuo Shioda², Mizanur Rahman¹

¹Molecular Diagnostics Lab, Evercare Hospital Dhaka, Bashundhara R/A, Dhaka, Bangladesh.
²Research Institute for Microbial Diseases, Osaka University, Japan.

Abstract
This is a retrospective observational study aimed to search the alteration of circulating dengue virus (DENV) serotypes in Dhaka City for five consecutive years (2017-2021) along with disease outcome.

Methods: Routine dengue NS1 or PCR positive dengue samples from patients who visited Evercare Hospital Dhaka (ex. Apollo Hospitals Dhaka) were selected for serotype determination by serotype specific real time reverse transcriptase PCR (RT-PCR).

Results: In 2017, predominant serotype was DENV-2 (91.3%) with less than 5% of DENV-1 and DENV-3 among 161 cases. In 2018, among 127 cases, DENV-2 was the predominant serotype (40.95%) followed by DENV-3 (33.07%) and DENV-1 (25.98%). In 2019, predominance of DENV-2 was totally replaced by DENV-3 (91.86%) and DENV-1 (8.14%). In 2021, only DENV-3 serotype was detected among 178 samples. Regarding serotype association with disease outcome, more severe cases (Dengue Hemorrhagic Fever/Dengue Shock Syndrome) were observed from 2019 with notable shifting of serotype dominance to DENV-3 from DENV-2 in previous years. In our cohort, the prevailing age group was 1-20 years which is analogous with many studies in Asia.

Conclusion: Dominance of DENV serotype shifted to DENV-3 in 2019 from prolonged persistence of DENV-2 and DENV-1. Continuous surveillance for circulating DENV serotype is needed for preparedness of potential outbreaks and occurrence of severe cases.

Keywords: Dengue, DENV serotype, DHF, Diversity, DENV-3.

Introduction
Dengue is a mosquito borne systemic acute viral disease, a major public health concern in urban areas of tropical and sub-tropical countries. The disease is endemic in more than 100 countries and about half of the global population is at risk of infection with this arbovirus. According to World Health Organization (WHO) estimation, about 390 million of cases are detected each year, of which average 96 million manifests clinically¹.

Dengue virus (DENV) belongs to the genus Flavivirus, of family Flaviviridae, with a single-stranded positive-sense RNA of approximately 11kb long. The DENV genomic RNA has a single open reading frame (ORF) that encodes ten proteins, consisting of three structural proteins (C, prM and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5)². DENV comprises four distinct serotypes (DENV1-4) and each serotype demonstrates strain variation based on the evolution and molecular epidemiology and further classified into genetically distinct groups called genotypes³. Within each serotype of DENV, four to six geographically distinct genotypes have been reported. DENV-1 includes genotypes I, II, III (sylvatic), IV, V, and VI; DENV-2 includes Asian-I, Asian-II, Asian/American, American, Cosmopolitan, and sylvatic; DENV-3 includes genotypes I, II, III, IV, and V; DENV-4 includes genotypes I, II A, II B, III, and sylvatic⁴-¹⁰.

Correspondence:
Dr. Mizanur Rahman
Senior Consultant, Molecular Diagnostics Lab
Evercare Hospital Dhaka
Plot-81, Block-E, Bashundhara R/A, Dhaka-1229, Bangladesh.
Email: mizanur.rahman@evercarebd.com
In Bangladesh, DENV-3 was isolated for the first time from a patient in 196411 and was found to be the main circulating serotype during the 2000 to 2002 outbreaks12. Thereafter, until 2012 serotype data was not available. During the years 2013-2016, DENV-1 and DENV-2 were the predominant circulating serotypes in Bangladesh13.

Rapid and accurate serotyping of DENV is important for epidemiologic surveillance, preparedness and control of dengue outbreaks and transmission blocking strategies targeting the vector, as well as for development of vaccines and antivirals.

As predominant serotype of DENV changed over time in most of the countries, including Bangladesh, it is crucial to track and identify the circulating serotype of dengue virus at the beginning of every season for prediction of disease propagation and severity in coming season, so that it may contribute to early preparedness plan regarding dengue management. We have done a retrospective study to find out the alteration of circulating serotypes during 2017 to 2021. Here we report that the dominance of DENV-2 in 2017 and 2018 has shifted to DENV-3 in 2019 and till October, 2021, the only representing serotype was DENV-3 (100%) with no evidence of other serotypes.

Materials And Methods

Ethical approval

Dengue serotyping study proposal was approved by the Research and Ethical Practice Committee of Evercare Hospital Dhaka (ex. Apollo Hospitals Dhaka, approval number ERC 16/2018-3). De-identified stored RNA at -80°C was used with a different code for this research study. This study was exempt from obtaining participant’s consent since only leftover specimens were used after anonymization.

Patients and clinical specimens

Patients with clinical suspicion of dengue who visited Evercare Hospital Dhaka (ex. Apollo Hospitals Dhaka) from June 2017 to August 2021 were included in this study irrespective of their age group. Subsequently for serotype determination, patients with dengue PCR Ct value >34 cycles were excluded from this study. For routine assay, 3 ml whole blood sample from adult and 0.5 ml to 1 ml from pediatric patients having clinical suspicion of dengue were collected in plain vacutainer (red top). Serum was separated and stocked at -80°C until RNA was extracted.

RNA extraction and real time reverse transcriptase PCR

Viral RNA was extracted from 200 µl of serum following kit manufacturer’s protocol (QIAamp MinElute Virus Spin Kit, Qiagen, Germany for samples of 2017 to 2019 and MagMax Viral/Pathogen kit, Applied Biosystems, USA was used for samples of 2020 to 2021) and stored at -80°C.

For samples of 2017 to 2019, CE-IVD approved commercial one step reverse transcriptase real time PCR kit from FTD (Fast Track Diagnostics, Luxembourg) was used for the detection of dengue virus. 15 µl PCR master mix containing 12.5 µl buffer, 1.5 µl primer-probe mix and 1 µl enzyme was prepared for each sample, negative control and positive control and then 10 µl of the extracted RNA from samples, nuclease free water as negative and synthetic DNA as positive control was added, respectively in 0.1ml PCR tube. PCR amplification was done by Rotor Gene Q (Qiagen, Germany). According to kit manufacturer’s instruction thermocycler was programmed which was as follows: 50°C for 15 min, 94°C for 1 min, 40 cycles of 94°C for 8 s, 60°C for 1 min. Signal was acquired at 60°C, and analysis was performed on the linear scale. Thresholds were set manually on each run. For target, any exponential curve crossing this threshold was considered positive. Fluorescence detected in green channel was for the amplification of dengue virus and red channel was for the internal control.

For samples of 2020 to 2021, CE-IVD approved commercial one step reverse transcriptase real time PCR kit from TRUPCR, India was used for the detection of dengue virus. 15 µl PCR master mix containing 10 µl multiplex master mix, 2.65 µg dengue primer-probe mix, 2 µl internal control primer probe mix and 0.35 µl enzyme mix was prepared for each sample, negative and positive control and then 10 µl of the extracted RNA from samples, negative and positive control was added, respectively, in 0.2 ml PCR strip tube. QuantStudio 5 Dx platform (Applied Biosystems, USA) was used for PCR amplification according to kit manufacturer’s instruction which was programmed as follows: 50°C for 20 min, 94°C for 10 min, 45 cycles of 94°C for 15 s, 55°C for 30 s and 72°C for 30 s. Signal was acquired at 55°C, and analysis was performed on the linear scale. Thresholds were set manually on each run. Fluorescence detected in FAM channel was for amplification of Dengue virus and ROX channel was for amplification of internal control.
Diversity of Dengue Virus Serotypes in Dhaka City: From 2017 to 2021

Diversity of Dengue Virus Serotypes in Dhaka City: From 2017 to 2021

Detection of Dengue NS1 antigen
Kits from SD Bioline, Korea were used for detection of NS1-antigen of Dengue virus. Three drops (100 µl) of serum sample were added to the well “S” and result reading was done within 15-20 minutes. Result was given after comparison with the positive control line in the device. The presence of two-color line (“C” and “T”) in the result window indicates that the specimen is positive for dengue NS1 antigen, and the presence of only control line (“C”) indicates negative.

Serotype specific real-time reverse transcriptase PCR
For dengue serotype identification we used commercial Genesig one step reverse transcriptase real time PCR kit from Primer design, UK. Four Dengue subtype specific primer and probe mixes are provided in a single tube and detected through the four different channels as described in the kit contents. The primer and probe mixes provided exploit the TaqMan® principle. Briefly, 5 ul RNA was taken in 0.2 ml PCR tube and then added to 10 ul Oasig master mix, 1 ul dengue primer probe mix and 4 ul nuclease free water. Reverse transcription was done in Rotor Gene Q and QuantStudio 5 Dx platform at 55°C for 10 minutes followed by enzyme activation at 95°C for 2 minutes and finally 50 cycles of denaturation at 95°C for 10 seconds and annealing and extension together at 60°C for 60 seconds. Then different dengue serotypes were detected in different channels according to the kit manufacturer’s instruction.

Results And Discussion

Serotype diversity and shifting of dominance
Dengue outbreaks occurring in many countries of the world, including Bangladesh, is considered a major public health concern. As Bangladesh is surrounded by dengue-endemic neighbors like India and Myanmar, there is always a risk of virus importation and transmission from neighboring countries. Over the past several decades, the rising trend of dengue incidence is due to increased travel, climate change, population growth, urbanization, and poor implementation of effective control measures. Frequent changes of serotypes were observed in almost every year, so, monitoring of DENV serotypes in early season of every year is important for proper management of mass population. In this study, we have detected the prevailing dengue serotype of five consecutive years (2017-2021) in Dhaka city to track the diversity of circulating serotypes [Table-1].

Table 1: Serotype distribution in five consecutive years (2017-2021)

| Years | Serotype done | Serotype positive | DENV1 N (%) | DENV2 N (%) | DENV3 N (%) | DENV4 N (%) |
|-------|--------------|-------------------|-------------|-------------|-------------|-------------|
| 2017  | 181          | 161               | 7 (4.35)    | 147 (91.30) | 7 (4.35)    | 0           |
| 2018  | 167          | 127               | 33 (25.98)  | 52 (40.95)  | 42 (33.07)  | 0           |
| 2019  | 116          | 86                | 07 (8.14)   | 0           | 79 (67.95)  | 0           |
| 2020  | 30           | 1                 | 0           | 0           | 1 (33.33)   | 0           |
| 2021  | 221          | 178               | 0           | 0           | 178 (100)   | 0           |

Samples for serotype determination were selected on routine NS1 or PCR positivity from suspected patients visiting our hospital. Routine PCR positive samples with Ct value ≤30 was preferred, but up to 34 Ct was considered for serotyping PCR as serotyping PCR often showed negative result when routine PCR Ct value >34.

In 2017, total 181 dengue positive cases were selected for serotyping and among them serotyping of 161 were successful. Serotyping PCR of 20 samples were unsuccessful probably due to low viral amount. Out of 161 we found 7 (4.35%) DENV-1,147 (91.3%) DENV-2, and 7 (4.35%) DENV-3.

The predominance of DENV-2 was also found in Dhaka city during the year 2013-2016 [13]. Dominance of dengue serotype in Dhaka city has been shifted to DENV-2 from DENV-3 found in first dengue epidemic in 2000-200214-16. Dominance of DENV-2 serotype was reported in Pakistan in 201717. However, in India DENV-1 was predominant in 201718.

In 2018, we could serotype 127 samples and out of those we found 33 (25.98%) DENV-1, 52 (40.95%) DENV-2, and 42 (33.07%) DENV-3. This result shows that in 2018, percentage of DENV-2 decreased but was still dominant over other serotypes and DENV-1 and DENV-3 increased significantly. A similar study in 2018 was reported from IEDCR of Bangladesh where DENV-2, DENV-3 and DENV-1 was found 41%, 31% and 9%, respectively19.

In 2019, 86 samples were serotype positive from 116 selected samples. The result shows that predominance of DENV-2 is totally replaced by DENV-3 (91.86%) and
other serotypes were only few. A study by Riad et al conducted on Bangladeshi population showed that DENV-3 was the dominating serotype in 2019, which originated an extreme and unprecedented surge in the number of infections. About 100,000 cases were reported in 2019 which is more than double the number of combined cases in the previous 19 years\(^2\)\(^,\)\(^3\). Huge number of cases were documented in 2019, which may be due to mass awareness program by Government regarding disease severity caused by reemergence of DENV-3 after a prolong presence of DENV-2 (2003-2018). Other possibilities of this outbreak may be, the expanding transmission capability of the emerging serotype from humans to mosquitoes, and vice versa, vector competence, and other environmental factors. A study in Myanmar showed over the entire 3-year period (2017-2019), the most prevalent serotype in Myanmar was DENV-3 (46.2\%) followed by DENV-1 (30.1\%) & DENV-4 (19.8\%)\(^2\).

**In 2020, we received very limited number of dengue samples probably due to COVID-19 pandemic situation and only a single case of DENV-3 was found among 24 dengue NS1 positive cases and 6 routine PCR positive samples where Ct values were >32. This large number serotype negativity (29 among 30) may be the cause of low viral load. Similar situation was found in Guangzhou, China in 2020 where they reported few dengue cases (only two local cases) attributable to the effect of COVID-19 pandemic\(^2\). A discordant picture was seen in Singapore where dengue fever surge was observed with predominance of DENV-3 in 2020. They narrated the cause of this surge was due to focus on COVID-19 pandemic and lack of preventive measures to confine the spread of new and predominant DENV-3 serotype against which the population was not immune\(^2\).

**In 2021, we did serotype of 178 dengue positive samples and surprisingly all are found to be DENV-3 serotype (100\%).**

Bangladesh Council of Scientific and Industrial Research (BCSIR) also found only DENV-3 serotype in Dhaka city in 2021, although, with only small (20) number of samples\(^2\).

**Gender distribution and age prevalence**
The present study is showing male predominance, with 60.04\% male and 39.96\% female. Studies in Singapore, Myanmar and India showed similar male predominance\(^2\),\(^2\)\(^,\)\(^2\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^11\)\(^,\)\(^12\)\(^,\)\(^17\). However, this difference may not indicate more susceptibility of male to dengue infection, rather it is associated with the socio-economic status of the region studied. Females in these areas are less privileged and may not get equal opportunity to have treatment for fever. On the contrary, males, often being the only earning person to maintain family cost, get more attention from other members of the family to seek medical care. Reports from South America showed either equal proportions of male and female dengue cases or a greater proportion of female cases\(^2\),\(^9\)\(^,\)\(^10\). In three consecutive years (2017, 2018 & 2019), 1-10 years age group was the dominating age group whereas in 2021, it was 11-20 [Table-2]. Thus, dengue infection occurred mainly in children and adolescence where maximum positive cases (40.51\%) were among 1-20 years age group. Children are particularly vulnerable to the disease because their immune systems are weaker than adults and they try to play outside where there is less protection against the mosquitoes. Schools are a hotbed of dengue because many have open windows and lack mosquito repellents. Children and adolescents under 15 years of age are the most affected group in Asia, the hyperendemic area for dengue fever (DF) and dengue hemorrhagic fever (DHF)/ dengue shock syndrome (DSS), which corresponds with our findings\(^3\). The age distribution is different in America where these syndromes occur in all age groups, although most fatalities during epidemics occur in children\(^3\).

**Table 2: Age and gender distribution of study population**

| Age (Years) | Male (%) | Female (%) | Male (%) | Female (%) | Male (%) | Female (%) | Male (%) | Female (%) | Male (%) |
|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|
| <1      | 0.2      | 0.2         | 1.8      | 1.8         | 2.2      | 2.2         | 0.6      | 0.6         | 0.6      |
| 1-10    | 0.8      | 0.8         | 1.8      | 1.8         | 1.8      | 1.8         | 0.6      | 0.6         | 0.6      |
| 11-20   | 0.3      | 0.3         | 0.4      | 0.4         | 0.4      | 0.4         | 0.6      | 0.6         | 0.6      |
| 21-30   | 0.6      | 0.6         | 0.8      | 0.8         | 0.8      | 0.8         | 0.6      | 0.6         | 0.6      |
| 31-40   | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      |
| 41-50   | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      |
| 51-60   | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      |
| >70     | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      | 0.6         | 0.6      |
| Total   | 6.7      | 6.7         | 6.7      | 6.7         | 6.7      | 6.7         | 6.7      | 6.7         | 6.7      |

**Disease severity and serotype**
We compared diseases severity (classical/severe dengue) in admitted patients in our hospital each year during the span of 2017 to 2021 and clinically more severe cases (34.41\%) were found in 2019 followed by 31.19\%, 26.32\%, 18.55\%, 11.22\% in 2021, 2020, 2018, 2017
respectively. Dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), dengue with severe thrombocytopenia were considered as severe dengue. Most affected age group was 1-10 years with male predominance [Table-4]. We also correlated disease severity with different serotypes and a greater number of severe cases (40.7%) were observed in 2019 compared to 13.7%, 8.7%, 5% in 2021, 2018, 2017 respectively. We ignored the single case of 2020 [Table-3]. Although clinically diagnosed cases were huge in 2019 but serotype was done with 116 cases, out of which 86 were serotype positive. Among these 86, clinically severe cases were 35 and non-severe cases were 51. All 35 severe cases were DENV-3 and among non-severe 51 cases, 44 (86.3%) were DENV-3 and 7 cases (13.7%) were DENV-1. In 2019, most of the non-severe cases were routinely tested by NS1 only. As it was a retrospective study and huge number of samples were requested for routine dengue PCR, we didn’t include NS1 positive samples for serotyping. For this reason, higher percentage of DENV-3 and severity data may be influenced by lower number of serotyped non-severe cases. From 2017 the reemergence of DENV-3 was noted and seen to replace the dominance of DENV-2 and in 2019, DENV-3 was the flourishing serotype without any evidence of DENV-2. As during 2013-2016 Bangladesh population was exposed by DENV-1 and DENV-2 serotype mainly, reemergence of DENV-3 in 2017 and onwards, caused serotype specific cross-reactivity that may be related with disease severity and fatality. Moreover, DENV-3 has so far been reported to be the most pathogenic serotype; DENV-1 and DENV-2 have been noticed to be nearly similar in their pathogenic traits, whereas DENV-4 is probably the least pathogenic serotype.32

Table 3: Severity correlation with different serotypes (2017-2021)

| Year (n=101) | DENV1 (%) | DENV2 (%) | DENV3 (%) | Total | DENV1 (%) | DENV2 (%) | DENV3 (%) | Total | Severity percentage |
|-------------|-----------|-----------|-----------|-------|-----------|-----------|-----------|-------|---------------------|
| 2017 (n=101) | 6 (3.9)   | 14 (9.5)  | 42 (26.6) | 62    | 0         | 7 (7.5)   | 1 (12.5)  | 18    | 8                   |
| 2018 (n=127) | 32 (27.3) | 46 (37.9) | 38 (32.7) | 116   | 1 (9.1)   | 6 (5.4)   | 4 (36.4)  | 11    | 11.8                |
| 2019 (n=86)  | 7 (13.7)  | 0 (0)     | 44 (63.1) | 51    | 0         | 0         | 35 (100)  | 35    | 46.7                |
| 2020 (n=1)   | 0         | 0         | 0         | 0     | 0         | 0         | 100%      | 1     | 100%                |
| 2021(n=178*) | 0         | 0         | 82 (90.1%)| 82    | 0         | 0         | 13 (100)  | 13    | 13.5                |

Conclusion
Identification of circulating DENV serotype at the beginning of every season is important as changing serotype is allied with disease intensity and fatality. We observed serotype shift of DENV-3 from prolonged persistence of DENV-2 and DENV-1 which resulted in more severe outcome of infection particularly in children and adolescents in recent years. As there is no specific treatment for dengue, continuous surveillance and early preparedness plan regarding dengue management is required to prevent and control dengue related mortality and morbidity in each year. It is a single centered study in Dhaka and there is a lack of diversity of population. A large-scale multi-centric study of serotype prevalence along with circulating genotype determination is recommended for further evaluation.

Acknowledgement
We express our sincere gratitude to Mr. Abu Sobhan Murad and Malay Biswas, Molecular Lab, Evercare Hospital Dhaka for their continuous support.

Disclosure Statement
The authors declare no conflicts of interest with this article’s content.

Funding
This research was funded by the Japan Agency for Medical Research and Development under grant numbers JP19fm0108003, 20wm0225010h0101, and 21wm0225010h0102.

Author Contributions
All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

References
1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr; 496(7446): 504-7.
2. Henchal EA, Putnak JR. The dengue viruses. Clinical microbiology reviews. 1990 Oct;3(4):376-96.
3. Rico-Hesse R. Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. Virology. 1990 Feb 1;174(2):479-93.
4. Goncalvez AP, Escalante AA, Pujol FH, Ludert JE, Tovar D, Salas RA, et al. Diversity and evolution of the envelope gene of dengue virus type 1. Virology. 2002 Nov 10;303(1):110-9.
5. Pyke AT, Moore PR, Taylor CT, Hall-Mendelin S, Cameron JN, Hewitson GR. Highly divergent dengue virus type 1 genotype sets a new distance record. Scientific reports. 2016 Feb 29;6(1):1-2.
6. Añez G, Morales-Betouille ME, Rios M. Circulation of different lineages of dengue virus type 2 in Central America, their evolutionary time-scale and selection pressure analysis. PLOS one. 2011 Nov 4;6(11):e27459.

7. Khan MA, Ellis EM, Tissera HA, Alvi MY, Rahman FF, Masud F, et al. Emergence and diversification of dengue 2 cosmopolitan genotype in Pakistan, 2011. PLOS One. 2013 Mar 8;8(3):e56391.

8. Twiddy SS, Farrar JJ, Chau NV, Willi B, Gould EA, Grisuen T, et al. Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. Virology. 2002 Jun 20;298(1):63-72.

9. Wittke V, Robb TE, Thu HM, Nisalak A, Nimmannitya S, Kalayanarooj S, et al. Extinction and rapid emergence of strains of dengue 3 virus during an interepidemic period. Virology. 2002 Sep 15;301(1):148-56.

10. AbuBakar S, Wong PF, Chan YF. Emergence of dengue virus type 4 genotype IIA in Malaysia. Journal of general virology. 2002 Oct 1;83(10):2437-42.

11. Russell PK, Buescher EL, McCown JM, Ordoñez J. Recovery of dengue viruses from patients during epidemics in Puerto Rico and East Pakistan. The American journal of tropical medicine and hygiene. 1966 Jul 1;15(4):573-9.

12. Aziz MM, Hasan KN, Hasanat MA, Siddiqui MA, Salimullah M, Chowdhury AK, et al. Predominance of DEN-3 genotype during the recent dengue outbreak in Bangladesh. Southeast asian journal of tropical medicine and public health. 2002 Mar 1;33(1):42-8.

13. Muraduzzaman AK, Alam AN, Sultana S, Siddiqua M, Khan MH, Akram A, et al. Circulating dengue virus serotypes in Bangladesh from 2013 to 2016. Virus disease. 2018 Sep 29(3):303-7.

14. Rahman M, Rahim R, Hasan A, Murad AS, Biswas M. Co-circulation of three dengue virus serotypes in 2017 in Dhaka city: first report from Bangladesh. Bioresearch communications-(BRC). 2019 Jan 1;5(1):637-41.

15. Podder G, Breiman R, Azim T, Thu HM, Velathanthiri NI, Mai LQ, et al. Origin of dengue type 3 viruses associated with the dengue outbreak in Dhaka, Bangladesh, in 2000 and 2001. American journal of tropical medicine and hygiene. 2006;74(2):263-5.

16. Islam MA, Ahmed MU, Begum N, Chowdhury NA, Khan AH, del Carmen Parquet M, et al. Molecular characterization and clinical evaluation of dengue outbreak in 2002 in Bangladesh. Japanese journal of infectious diseases. 2006 Apr 1;59(2):85.

17. Khan NU, Danish L, Khan HU, Shah M, Ismail M, Ali I, et al. Prevalence of dengue virus serotypes in the 2017 outbreak in Peshawar, KP, Pakistan. Journal of clinical laboratory analysis. 2020 Sep;34(9):e23371.

18. Murugesan A, Aridoss D, Senthilkumar S, Sivathanu L, Sekar R, Shankar EM, et al. Molecular Diversity of Dengue Virus Serotypes 1–4 during an Outbreak of Acute Dengue Virus Infection in Theni, India. Indian journal of medical microbiology. 2020 Nov 1;38(3-4):401-8.

19. Shirin T, Muraduzzaman AK, Alam AN, Sultana S, Siddiqua M, Khan MH, et al. Largest dengue outbreak of the decade with high fatality may be due to reemergence of DEN-3 serotype in Dhaka, Bangladesh, necessitating immediate public health attention. New microbes and new infections. 2019 May;29.

20. Riad MH, Cohnstaedt LW, Scoglio CM. Risk Assessment of Dengue Transmission in Bangladesh Using a Spatiotemporal Network Model and Climate Data. The American journal of tropical medicine and hygiene. 2021 Apr;104(4):1444.

21. Titir SR, Paul SK, Ahmed S, Haque N, Nasreen SA, Hossain KS, et al. Nationwide Distribution of Dengue Virus Type 3 (DENV-3) Genotype I and Emergence of DENV-3 Genotype III during the 2019 Outbreak in Bangladesh. Tropical medicine and infectious disease. 2021 Jun;6(2):58.

22. Soe AM, Ngwe Tun MM, Nabeshima T, Myat TW, Htun MM, Lin H, et al. Emergence of a Novel Dengue Virus 3 (DENV-3) Genotype-I Coincident with Increased DENV-3 Cases in Yangon, Myanmar between 2017 and 2019. Viruses. 2021 Jun;13(6):1152.

23. Jiang L, Liu Y, Su W, Liu W, Yang Z. Decreased dengue cases attributable to the effect of COVID-19 in Guangzhou in 2020. PLOS neglected tropical diseases. 2021 May 26;15(5):e0009441.

24. Singapore’s historic dengue outbreak year in 2020. News Desk. 2021 Jan 26: Animal diseases.
25. Dengue serotype-3 dominates in Dhaka. The Business Standard Report. 2021 Aug 29.

26. Ooi EE. Changing Pattern of Dengue Transmission in Singapore [Dengue Bulletin]. World Health Organization; 2001.

27. Sreejith MG, George P. Study on the diagnostic efficacy of Clinico-Laboratory Parameters in serologically diagnosed cases of Dengue Fever. International journal of scientific & technology research. 2014; 11(1):12-16.

28. Neff JM, Morris L, Gonzalez-Alcover RA, Coleman PH, Lyss SB, Negron H. Dengue fever in a Puerto Rican community. American journal of epidemiology. 1967 Jul 1;86(1):162-84.

29. Likosky WH, Calisher CH, Michelson AL, Correa-Corronas RA, Henderson BE, Feldman RA. An epidemiologic study of dengue type 2 in Puerto Rico, 1969. American journal of epidemiology. 1973 Apr 1;97(4):264-75.

30. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends in microbiology. 2002 Feb 1;10(2):100-3.

31. Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. Journal of clinical virology. 2003 May 1;27(1):1-3.

32. Tsang TK, Ghebremariam SL, Gresh L, Gordon A, Halloran ME, Katzelnick LC, et al. Effects of infection history on dengue virus infection and pathogenicity. Nature communications. 2019 Mar 18;10(1):1-9.