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Original Article

Co-circulation of Three Dengue Virus Serotypes in 2017 in Dhaka city: First report from Bangladesh

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ABSTRACT: Dengue is a major public health problem in Bangladesh similar to many other tropical regions of the world. There are four serotypes of dengue virus and circulation of all four serotypes were reported in our neighbor countries. Disease severity depends on serotype of dengue virus and serotype changes. So, serotype determination each year and observation of the change of serotype is important. However, yearly basis serotype data in Bangladesh is not available although dengue is endemic for more than decades. In an attempt to unveil the circulating serotype in Dhaka city, during July to December 2017, RNA from 181 reverse transcriptase PCR (RT-PCR) confirmed cases of dengue were screened for serotypes by serotype specific real time RT-PCR. DENV-1-3 were detected in 161 (88.95%) samples, of which 7 (4.34%) as DENV-1, 147 (91.3%) as DENV-2 and 7 (4.34%) as DENV-3 indicating DENV-2 was the predominant serotype of the outbreak. This is the first report of co-circulation of 3 dengue serotypes in a season in Dhaka city and warrants further surveillance of dengue serotypes for monitoring serotype changes and public health preparedness accordingly.

Keywords: Co-circulation, dengue, serotype, Bangladesh

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INTRODUCTION

Dengue virus infection is a major, growing public health problem with an estimated 2.5 billion people at risk of infection [1]. Globally, dengue virus transmission has expanded in recent years, and all four dengue virus serotypes are now circulating in Asia, Africa, and the Americas. Each serotype has several subtypes or genotypes. DENV-1 has three, DENV-2 has two, and DENV-3 and DENV-4 each have four. Each serotype has unique characteristics and can present with severe manifestations in a particular population depending upon its interaction with the host response [2]. However, the epidemiology of dengue is very complex and ever changing. Serotype specific clinical feature [3-4] and disease severity is reported in some studies [5-6]. Patients can be infected with more than one serotype of dengue virus in their lifetime. Secondary infection with heterologous serotypes is more severe than primary infection, which may be explained by the antibody-dependent enhancement (ADE) theory [7]. As the severity of dengue infection had been found to be affected by the dengue serotypes involved and also the interval between the primary and secondary infections, these factors need to be considered when clinical prediction of the severity of dengue patients is being made. Moreover, as certain serotypes resulted in higher percentage of severe cases, such as secondary infection by South East Asian (SEA) DENV-2, DENV-3, DENV-4, and non-SEA DENV-2, DENV-3

or primary infections by DENV-3 from SEA: these serotypes require proper clinical attention [8]. Since DENV-4 was found to cause the lowest percentage of the sample size; serotype-specific antiviral treatments may be more focused on the other serotypes, DENV-1, DENV-2 and DENV-3. Besides, as DENV-2 and DENV-4 had been more associated with secondary infection, patients with a history of past dengue infection should take extra precautions during outbreaks of DENV-2 and DENV-4 infections [8]. So, serotype determination each year and observation of the change of serotype is very important. Unfortunately, dengue serotype is not tracked well on yearly basis though dengue has become serious public health problem in the country and endemic since 2000 and there are only few reports about serotype. DENV-3 was isolated for the first time from patients in Bangladesh in 1964 and again DENV-3 was found as the main circulating serotype during 2000 to 2002 outbreaks [9-11]. Thereafter serotype data is not available till 2012 except a report of high (80%) and wide spread seroprevalence of dengue virus infection in Dhaka in 2012 [12]. Recent reports show DENV-1 and DENV-2 was the circulating serotypes in 3 major cities in the country including Dhaka city during the year 2013-2016 [13]. We have performed serotyping of routinely tested 181 RT-PCR confirmed dengue viruses by serotype specific real time PCR and here, for the first time, we report co-circulation 3 serotypes DENV-1, DENV-2 and DENV-3 in Dhaka city with the high predominance of DENV-2 in 2017.

MATERIALS AND METHODS

Patients and clinical specimens

As a routine assay 3 ml whole blood sample from adult and 0.5 ml to 1 ml from pediatric patients having clinical suspicion of either chikungunya or dengue were collected in plain vacutainer (red top) by phlebotomist of Apollo Hospitals Dhaka, Bangladesh during June 29, 2017 to December 31, 2017. 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 ul of serum following kit manufacturer's protocol (QIAamp MinElute Virus Spin Kit, Qiagen, Germany) and stored at -80˚C if not used immediately. Then 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. 15ul PCR master mix containing 12.5 ul buffer, 1.5ul primer-probe mix and 1ul enzyme was prepared for each sample, negative control and positive control and then 10ul of the extracted RNA from samples, the extracted negative control and positive control was added, respectively. Then it was briefly mixed by up and transferred the amount in 0.1ml PCR tube and placed it on thermocycler, Rotor Gene Q (Qiagen, Germany). According to kit manufacturer's instruction thermocycler was programmed which was briefly as hold at 50˚C for 15 minutes, again hold 1 min at 94˚C, then 40 cycles of 8 second at 94˚C and 1 minute at 60˚C.

Each run was performed with a negative control (no template) and positive controls for CHIKV and DENV. Signal was acquired at 60˚C, and analysis was performed on the linear scale. Thresholds were set manually on each run. For both targets, any exponential curve crossing this threshold was considered positive. Fluorescence detected in the orange channel was for the amplification of chikungunya virus and fluorescence detected in the green channel was for the amplification of dengue virus and red channel was for the internal control.

Serotype specific real-time reverse transcriptase PCR

For dengue serotype identification we used commercial Genesig one step reverse transcriptase real time PCR kit from Primerdesign, UK. Four Dengue subtype specific primer and probe mixes are provided in a single tube, and this is detected through the four different channels as described in the kit contents. The primer and probe mixes provided exploit the TaqMan® principle. Briefly, 5ul RNA was taken in 0.1ml PCR tube and then added 15ul mixed having 10ul oasig master mix, 1ul dengue primer probe mix and 4ul nuclease free water. Reverse transcription was done in Rotor Gene Q 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 subtypes were detected in different channels according to the kit manufacturer's instruction.

Ethical Approval

Dengue serotyping study proposal was approved by the Research and Ethical Practice Committee of 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. Stored RNA was from serum of febrile patients visited at Apollo Hospitals Dhaka.

RESULTS AND DISCUSSION

Dengue is endemic in the country since 2000 and throughout the year cases are reported [12,14]. However, typical dengue period is considered during monsoon and post-monsoon period when the environment is most favorable for breeding of aedes mosquitoes, the vector for both the dengue viruses and chikungunya viruses. We started multiplex RT-PCR to detect and differentiate dengue and chikungunya virus as both the virus infections present overlapping clinical features and there was a huge outbreak of chikungunya in summer 2017. During June 29 to December 31 we

confirmed 268 cases of dengue out of 1651 febrile cases by RT-PCR from suspected patients visited mostly in Medicine and Pediatric clinic at Apollo Hospitals Dhaka. From 268 positive cases we randomly selected high titer viral RNA of 181 samples with Ct values <35 for serotype PCR.

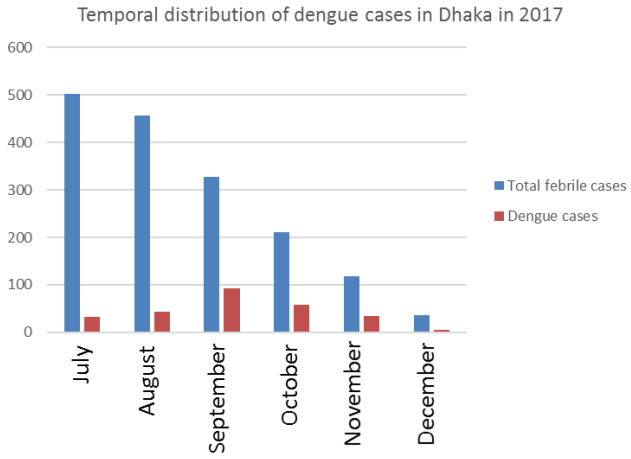

Figure 1. Temporal distribution of dengue cases in Dhaka in 2017

Temporal distribution (figure 1) of dengue cases diagnosed at Apollo Hospitals clearly showed its presence from the beginning of July and gradually increased and reached at peak appearance at September and then gradually decreased as the monsoon ends. Stored RNA from 181 RT-PCR confirmed dengue cases were screened for serotype identification by serotype specific real time PCR. Out of 181 patients 107 were male and 74 were female and the male:female ratio was 1.44. Large (150) number of samples were from adults and 31 were from children. From these 181 samples serotype was identified from 161 (88.95%) samples (table 1).

Table 1: Serotype distribution of Dengue virus in Dhaka in 2017 detected by serotype specific RT-PCR

Month (2017)	Sample tested No.	Dengue RT-PCR positive No.	Serotype PCR done No.	Serotype PCR positive No.	DENV-1 No.	DENV-2 No.	DENV-3 No.	DENV-4 No.
July	503	33	15	10	0	10	0	0
August	456	43	35	32	0	32	0	0
September	327	93	54	52	4	45	3	0
October	211	58	41	39	1	35	3	0
November	118	35	30	23	2	20	1	0
December	36	6	6	5	0	5	0	0
Total	1651	268	181	161	7	147	7	0

Serotype could not be determined of 20 samples, viral load of those were mostly low. 161 samples were identified with a single DENV serotype: 147 (91.3%) as DENV-2, seven (4.34%) as DENV-1, and seven (4.34%) as DENV-3. This data shows that serotype DENV-2 was the predominant serotype of the outbreak in 2017. The predominance of DENV-2 was also found in Dhaka city during the year 2013-2016 showed in a recent report (13). So, the dominance of dengue serotype in Dhaka city has been changed to DENV-2 from earlier dengue outbreaks in 2000-2002 where it was DENV-3 [10-11]. Moreover, in addition to this serotype change Muraduzumman et al. showed co-circulation of DENV-1 and DENV-2 in Dhaka city and in other two major metropolitan cities in the country during 2013-2016 (13). In addition to DENV-1, DENV-2 we found co-circulation of DENV-3 in Dhaka city in 2017. We did not find any DENV-4 in our study samples and there is no clear report of DENV-4 yet in the country though Aziz et al. (15) showed data of one case of DENV-4 as co-infection with DENV-3 during 2000 outbreak, gel electrophoresis data is not clear enough and it was not verified by sequencing. Further, we did not find any coinfections though many coinfections and co-circulation of all serotypes were reported in the surrounding countries (16).

Serotype changes of dengue virus were found in Delhi in 2008 where serotype 2 and serotype 3 were displaced by serotype 1 [17]. DENV-1, 2, and 4 serotypes were the common circulating strains from 2008 until 2010, after which DENV-3 serotype

infections rise and led to a massive dengue outbreak in Kolkata with increased numbers of DHF and DSS cases in 2012 [18]. In Myanmar in the 2013 outbreak, dengue virus serotype 1 predominated, while in the 2015 outbreak, serotypes 1, 2, and 4 were those mainly in circulation [19].

It has been postulated that concurrent infections by multiple DENV serotypes which is not yet found in our hospital during the study period may influence the clinical course of the disease. This is considered as a single major factor for the emergence of severe dengue, but larger studies are needed to prove this association. In a hyperendemic scenario, besides coinfections, the number of secondary infections also increases. Secondary infections with a different DENV serotype are major risk factors for severe diseases because of antibody-dependent enhancement mechanism [7, 20].

It is important to identify the circulating serotype of dengue virus at the beginning of every season for prediction of disease amplitude and severity of the disease in coming season. This attempt may contribute in early preparedness plan regarding management and containment of the disease at policy making level of the country. Continuous surveillance and detailed clinical analysis of dengue-confirmed patients would provide a further understanding of the impact of this serotype change in Dhaka city.

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