

SHORT COMMUNICATION

## Detection of Mumps Virus of Genotype G in Bangladeshi Children Suffering from Encephalitis

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### ABSTRACT

Although mumps virus (MuVi) is an important agent of encephalitis, however, mumps vaccine has not yet been included in the national immunization programme of Bangladesh. Furthermore, the genotype distribution of this virus in Bangladesh is unknown. Cerebrospinal fluid samples collected from 97 children with encephalitis from April 2009 to March 2010 were subjected to polymerase chain reaction (PCR) test to determine the causative agents. MuVi was detected in two samples, these samples were further subjected to conventional PCR using specific primers, then amplicons were sequenced, and genotype was determined as genotype G. Phylogenetic analysis showed that these strains were clustered with strains from Nepal, India, the UK, Thailand, and the USA. By Bayesian inference, we also determined that the ancestor of Bangladeshi and Indian MuVi were same and segregated only about two decades back. These results will help future surveillance and the detection of invading MuVi strains from other countries.

### INTRODUCTION

Mumps is a viral infection which affects normally young children. The main clinical features are fever and swelling of the parotid glands. Orchitis, oophoritis, transient deafness, meningitis, and encephalitis are serious complications associated with mumps. Encephalitis occurs in up to 0.1% of mumps cases (World Health Organization, 2012). MuVi only infects humans and

belongs to *Paramyxoviridae* family, subfamily *Paramyxovirinae* and genus *Rubulavirus*. The genome size of MuVi is 5,384 nucleotides, composed of single-stranded, negative-sense RNA (Jin et al., 2005). These encoding two surface glycoproteins; fusion (F) and haemagglutinin-neuraminidase (NH) and, four core proteins; nucleoprotein (NP), phosphoprotein (P), matrix (M), large protein (L), and the membrane-associated small hydrophobic (SH) protein (Jin et al., 2005). Among these protein-encoding genes, the most variable segment of the MuVi genome is the SH gene (Jin et al., 2005). Among the twelve genotypes of MuVi, wildtype of genotype A is no longer seen worldwide from 1990 (Jin et al., 2015). Strains of genotype G of MuVi are identified in the countries of Europe and the Americas. Whereas genotypes J and F are detected in the countries of Asia and the Pacific region. Genotype H is found in the Middle East where mumps vaccine containing Jeryl Lynn strain (genotype A) is mainly used and vaccines containing Urabe-AM9 and the Leningrad-Zagreb strains (genotype B) are used in some extent (Rubin et al., 2012). Therefore, molecular characterization of MuVi is an essential part of mumps surveillance strategy because it can lead to the identification of the spread routes of the MuVi, besides it will discriminate between natural and vaccine strains (Jin et al., 2005). However, limited data are available on the global distribution of MuVi genotypes; of 194 countries only 38 have reported the MuVi genotypes distribution in their territories (World Health Organization, 2012). Bangladesh is among countries where MuVi genotype distribution is unknown. We are afraid that the influx of Rohingya refugees from Myanmar with mumps (Mair et al., 2020) into Bangladesh may risk Bangladeshi children into unknown genotypes. Therefore, it becomes essential to identify the genotype of the prevailing Bangladeshi MuVi, their relatedness with similar strains circulating in other areas of the world as well as the timeline of evolution. Overall, understanding genetic diversity might help in selecting vaccine strains.

## MATERIALS AND METHODS

During a study to determine the causative agents, cerebrospinal fluid (CSF) was collected from 97 Bangladeshi children with encephalitis (Mori et al., 2017). The study was done in the hospital of the Institute of Child and Mother Health, Matuail, Dhaka, Bangladesh from April 2009 through March 2010. Genomic RNA was extracted from CSF using a commercial kit (Mori et al., 2017). A battery of viral family-specific conventional PCR tests was done, among these samples, only two were positive for *Paramyxovirinae* (Mori et al., 2017). The nucleotide sequencing of these amplicons confirmed the presence of MuVi. These samples were used in the present study to perform conventional PCR using the MuVi gene-specific primers as written below. Detailed patient information is shown in Table 1.

**Table 1** Clinical features, data on laboratory investigations and demographic information of patients with mumps virus-associated encephalitis

Variables	Patient ID No. 34	Patient ID No. 43
Sample collection date	May 30, 2012	July 22, 2012
Gender	Female	Male
Weight (nutritional status)	8.8 kg (low body weight)	11 kg (low body weight)
Fever	Yes	No
Parotid gland enlargement	Absent	Absent
Runny nose	Yes	Yes
Body rash	No	No
Glasgow coma scale	Normal	Normal
Residence	Village	Urban slum
Vaccination covered by EPI	None	None
Similar illness in the neighbourhood	No	No
First medical consultation	Direct to hospital	Direct to hospital
Time interval between the onset of infections and hospitalization	<24 h	<24 h
Duration of hospitalization	10 days	2 days

CSF tests		
Colour	Clear	Clear
WBC ( $\mu$ l)	2	280
PMN (%)	0	30
Lymphocytes (%)	100	70
Protein (mg/dl)	60	160
Glucose (mg/dl)	50	40
Blood tests		
WBC ( $\text{mm}^3$ )	11,720	10,500
PMN (%)	85.4	51.0
Lymphocytes (%)	14.2	44.0
Eosinophils (%)	0.0	2.0
Monocytes (%)	1.2	1.3
Basophils (%)	0.1	0.5
Haemoglobin (mg/dl)	10.4	12.0
ESR (mm)	40	25
CRP (mg/dl)	4.8	12.0
Treatment given	Ceftriaxone, Acyclovir, Quinine	Ceftriaxone, Acyclovir
Outcome	Discharged	Discharged on request

**Notes:**

EPI: Expanded Programme of Immunization  
 Haemoglobin: considered anaemic when the level is less than 11 g/dl in children (6 months to 6 years old)  
 ESR: Erythrocyte Sedimentation Rate normal up to 20 mm  
 CRP: C-reactive protein  
 Reference value: Less than 0.2 mg/dl  
 WBC: White Blood Cell  
 PMN: Polymorphonuclear leukocytes  
 Mumps IgM or IgG antibody: Not determined

Using these samples, we performed reverse transcription-PCR to determine the genotype of MuVi (Jin et al., 1999). Briefly, for the first round of nested PCR, primers SH1 (5'AGTAGTGTGCGATGATCTCAT-3') and SH2R (5'GCTCAAGCCTTGATCATTGA-3') were used to amplify a 639-bp fragment covering the entire SH gene, then primers SH3 (5'-GTCGATGATCTCATCAGGTAC-3') and SH4R (5'-AGCTCACCTAAAGTGACAAT-3') were used for the second round of PCR.

The amplicons were sequenced and the nucleotide sequences were analyzed by BLAST (Altschul et al., 1990) to identify the

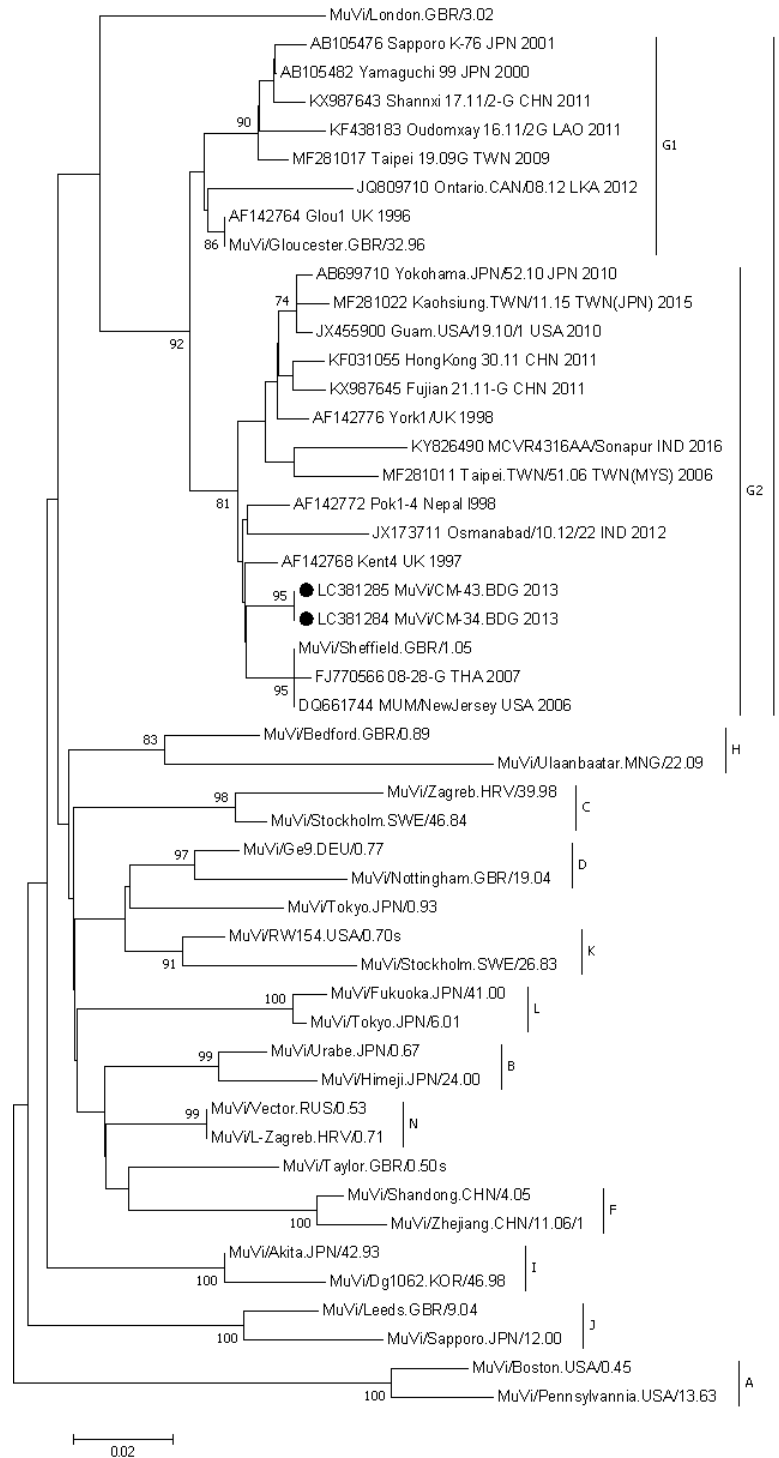
viruses and genotypes (Mori et al., 2017). For phylogenetic analysis, nucleotide sequences of at least two SH gene of standard MuVi strains representing each genotype and subtype were extracted from GenBank. Multiple sequence alignment was conducted using ClustalW. The phylogenetic analysis was performed by the neighbour-joining method using MEGA7.0 software (Kumar et al., 2016). To test the reliability of the branching pattern of the phylogenetic tree a bootstrap analysis of 1,000 replicates was performed.

Evolutionary analysis was performed using the SH gene sequences. By using the Bayesian Markov chain Monte Carlo method accessible in BEAST (Drummond & Rambaut, 2007) version 1.6.1, we inferred maximum clade credibility phylogenetic tree. A relaxed (uncorrelated lognormal) molecular clock and HKY+ $\Gamma$  model of nucleotide substitution were used for analysis. All chains were run for  $9 \times 10^7$  generations and sampled every 3,000 steps. The posterior densities were calculated with 10% burn-in and checked for convergence by using Tracer version 1.5 available in BEAST.

The study was approved by the ethics committee of the Institute of Child and Mother Health, Matuail, Dhaka, Bangladesh.

**RESULTS**

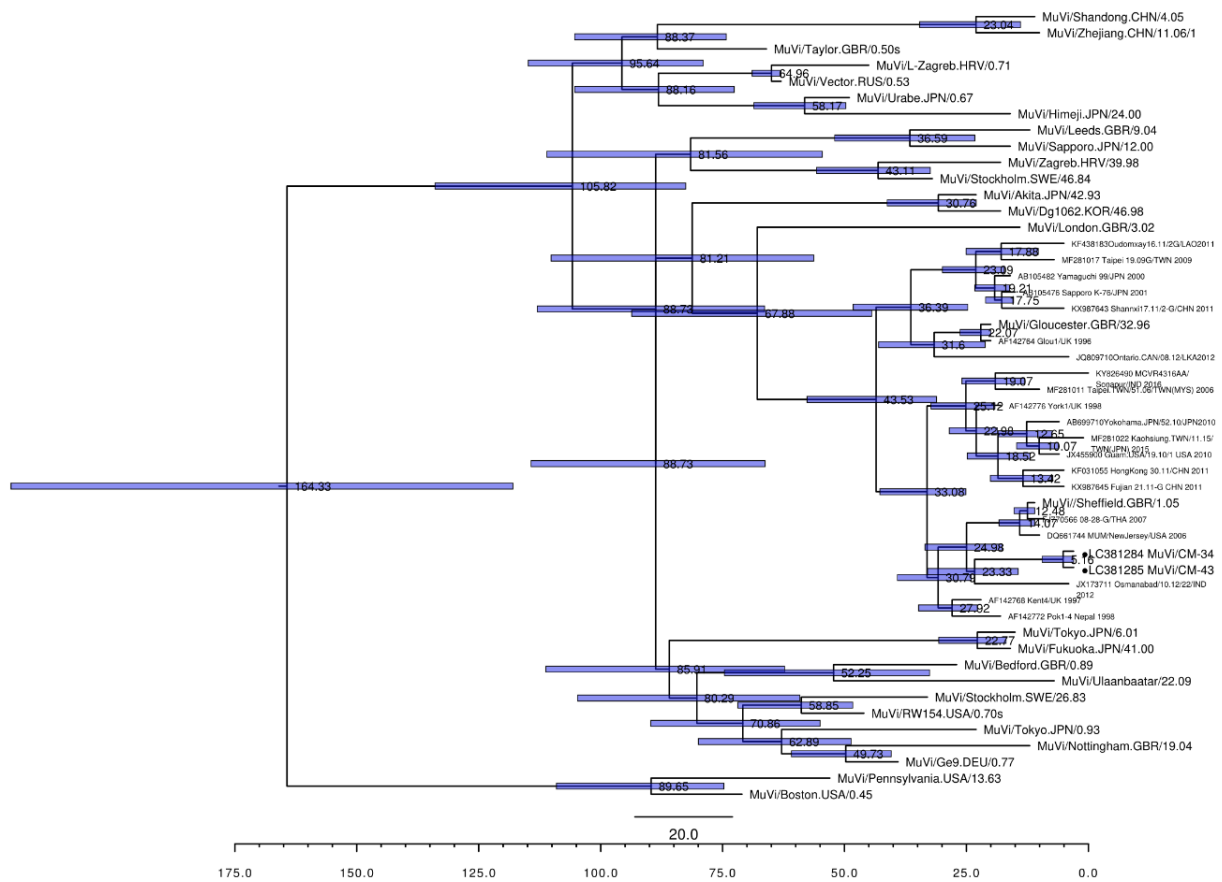
Of 97 patients, two were positive for MuVi making the proportion as 2.1%. In this study, our phylogenetic analysis revealed that MuVi strains from Bangladesh belonged to genotype G (Figure 1). Nucleotide sequences of Bangladeshi MuVi showed 100% homology among them and 94 – 98% to the prototype strain of genotype G. Phylogenetically Bangladeshi strains together with strains from Nepal, India, the UK, Thailand, and the USA belonged to G2 cluster. With these MuVi strains from different countries, Bangladeshi MuVi strains have 96 – 98% nucleotide identities.



**Figure 1** Using the nucleotide sequences of the small hydrophobic protein genes the phylogenetic tree was constructed. The strains from the present study are designated by filled circles. The bootstrap value is represented by the number adjacent to the node, values higher than 70% are shown. The scale bar at the bottom of the tree representing genetic distance expressed as nucleotide substitutions per site. Nucleotide sequences for Bangladeshi strains MuVi/CM-34.BDG/2013-G and MuVi/CM-43.BDG/2013-G appear in the nucleotide sequence databases of DNA DataBank of Japan, European Molecular Biology Laboratory, and GenBank with accession numbers LC381284 and LC381285, respectively.

The estimated mean rate of nucleotide substitution for the SH gene was  $8.1 \times 10^4$  substitutions/site/year (95% highest posterior density [HPD] values  $1.1 \times 10^3 - 5.2 \times 10^4$  substitutions/site/year). This rate of nucleotide substitution is comparable with previous findings (Cui et al., 2017). Approximately 164.3 years ago (95% HPD 118.0 – 220.9), circa 1848 (95% HPD range 1792 – 1894), the currently circulating MuVi strains diverged into different genotypes from the most recent

common ancestor (MRCA). Only 43.5 years ago (95% HPD 31.1 – 57.7 years) MuVi divided into G1 and G2 genotypes from the MRCA. Bangladeshi and Indian MuVi diverged from their MRCA, approximately 23.3 years ago (95% HPD 14.4 – 33.0 years), that is, in  $\approx 1989$  (95% HPD range 1979 – 1998). Approximately 5.2 years ago (95% HPD 3.0 – 9.5 years), that is, in  $\approx 2007$  (95% HPD range 2003 – 2009), strain CM-34 and CM-43 diverged from their MRCA in Bangladesh (Figure 2).



**Figure 2** The genealogy of mumps virus is represented by Bayesian maximum-credibility tree which was obtained by analyzing nucleotide sequences of the small hydrophobic protein gene. Nodes correspond to mean age at which lineages diverge from the most recent common ancestor (MRCA); 95% highest posterior density of the MRCA is represented by horizontal bars at nodes. Posterior values are represented by numbers at the main nodes. Time scales in years are represented by horizontal axis at bottom.

## DISCUSSION

The incidences and complications of mumps in Bangladeshi children are no less than those in other developing countries (Sultana et al., 2006). However, data are scarce on the prevalence of mumps, MuVi genotype distribution and immune status in Bangladeshi children against MuVi. Although mumps vaccine has been introduced in 122 member countries by the end of 2019 (World Health Organization, 2020), it is not yet included in the national immunization programme in Bangladesh.

The prevalence of mumps encephalitis in the present study was 2.6%. Although only two samples were tested, however, this is the first study to our knowledge reporting MuVi genotypes circulating among the children in Bangladesh and therefore, deserves importance. Although we found only G genotype in the present study, however, the redistribution of the genotypes of MuVi may occur over time with invasion of the virus from a foreign country (Hviid et al., 2008; Jin et al., 2015). Genotype C and G have been reported from neighbouring India (Mishra et al., 2013; Vaidya et al., 2016). There is a possibility of the existence of other genotypes in Bangladesh. By Bayesian inference, we also determined that the ancestor of Bangladeshi and Indian MuVi were same and segregated only about two decades back. The two Bangladeshi strains were from two different regions and they segregated from their common ancestor only about five years ago indicating that evolution is ongoing and spreading in different regions of Bangladesh. One of the limitations of this study is that it was performed on samples from one hospital only, therefore further study is needed to explore the full picture of MuVi genotype distribution in Bangladesh. The other limitation is that in the present study we used a partial length SH gene for determining the timeline of evolution, in future, a full-length SH gene should be used to verify the results.

## CONCLUSION

We have determined that genotype G is circulating MuVi genotype in Bangladesh children with encephalitis. The strain was phylogenetically related with MuVi genotype G from India. Monitoring the MuVi genotype in Bangladesh might be beneficial for the surveillance of mumps infection in the country.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicting interests in publishing this article.

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