British Microbiology Research Journal
16(4): 1-13, 2016, Article no.BMRJ.28040
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular Epidemiology of Foot-and-Mouth Disease Viruses Circulated in Bangladesh from 2011–2014

M. Giasuddin[1*], M. Showkat Mahmud[1], S. M. S. Alam[2], M. A. Samad[1], M. R. Islam[1], M. D. Ahasan[1], M. H. Rahman[1], M. R. Karim[1], M. R. Islam[3], P. Acharjee[3] and M. A. Yousuf[1]

[1]Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh.
[2]Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh.
[3]Department of Microbiology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MG and MSM contributed during study design, collected and processed the samples, evaluated the data and helped in writing the manuscript. Authors MDA, MHR, MRI and PA contributed to collect and process the samples, performed RT-PCR and data analysis. Authors SMSA, MAS, MRI, MRK and MAY contributed to the study design and critically involved in writing the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/28040
Editor(s):
(1) Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.
Reviewers:
(1) Mona Zaki Zaghloul, Ain Shams University, Cairo, Egypt.
(2) Livia Garcia Bertolacci-Rocha, Universidade Federal de Goiás, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/15865

Original Research Article

Received 30th June 2016
Accepted 27th July 2016
Published 20th August 2016

ABSTRACT

Background: Foot-and-mouth disease (FMD) is one of the highly contagious and economically devastating diseases of cloven-hoofed animals in Bangladesh. The present study was undertaken to investigate the molecular epidemiology of circulating FMD virus (FMDV) in Bangladesh during the period of 2011 to 2014.
Methodology: A total of 134 samples from clinically FMD infected cattle were collected from 15 different outbreak areas of Bangladesh. All samples were subjected to RNA extraction and RT-PCR for detection and serotyping of FMDV in Bangladesh.

**Corresponding author: E-mail: mgiaso4@yahoo.com;*

Results: Out of 134 samples, 73% (98) samples were positive for FMDV. Three different serotypes (O, A, and Asia 1) of FMDV were found to be present in Bangladesh. Among the positive FMDV, serotype O and Asia 1 accounts for about 31% followed by A (7%) have been detected. Phylogenetic analysis of partial VP1 nucleotide sequences demonstrated that all BLRI/FMDV serotype O isolates were closely related to PanAsia strains, including those that originated from Bangladesh, Bhutan and India for the period of 2012–2014. Results of the sequencing of VP1 gene of FMDV serotype O revealed that there was slight divergence among BLRI isolated strains. BLRI/127 isolates of FMDV serotype A showed close resemblances with the isolates originated from India during 2000 to 2006. While BLRI/ FMDV serotype Asia 1 isolates were most closely related to other FMDV isolates collected in Bangladesh during 2013.
Conclusions: Assessment of genetic variation of FMD viruses in the field is useful for estimating the origin of outbreaks and provides valuable information applicable to control measures such as regulating animal movement and selecting appropriate vaccine strains.

Keywords: FMDV; serotypes; endemic; RT-PCR; VP1 gene; Bangladesh.

1. INTRODUCTION

Foot and mouth disease (FMD) is one of the most important transboundary and re-emerging infectious diseases of the ungulates [1]. It is an endemic disease of cloven-hoofed animals [2] that cause severe economic losses due to high morbidity and export trade restrictions imposed on affected countries [3,4]. FMD virus (FMDV), a member of the genus *Aphthovirus* and family *Picornaviridae*, is the etiologic agent of the diseases [5] that causes an acute disease characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats, with high morbidity and low mortality [6]. Transmission can take place by direct or indirect contact with infected animals and contaminated fomites; virus spread through inhalation of the aerosolized virus, contaminated feed, and the virus enters through skin abrasions or mucous membranes. Different routes of transmission vary with the species. Sheep and cattle were more susceptible to the aerosolized virus than other animals [7]. Many FMDV-infected animals clear the virus within 7–14 days. However, 50% of cattle can carry the virus asymptomatically and intermittently in oropharyngeal fluid beyond 28 days post-infection, and this is referred to as persistent infection and the animals as FMDV carriers [8-10]. FMD virus persists in the light zones of germinal centers in lymph nodes associated with the pharyngeal region, while these tissues did not develop infective virus [11].

FMDV has a single-stranded positive RNA genome that possesses high potential for genetic and antigenic variation. Seven recognized serotypes of FMDV (O, A, C, SAT1, SAT2, SAT3 and Asia1) and about 65 subtypes of FMDV have been defined [12,13]. Based on the phylogenetic analysis of the VP1 gene sequence, FMDV serotype A is classified into 10 major genotypes (I–X) [14,15], serotype O is classified into 10 topotypes [16] and serotype Asia 1 is grouped into 6 genotypes (I-VI) [17]. Topotypes of FMDV serotype O are designated as Europe-South America (Euro-SA), Middle East-South Asia (ME-SA), Southeast Asia (SEA), Cathay (CHY), West Africa (WA), East Africa 1 (EA-1), East Africa 2 (EA-2), East Africa 3 (EA-3), Indonesia-1 (ISA-1), and Indonesia-2 (ISA-2) [16]. All FMDV serotypes are immunogenically different and vaccination with one serotype does not develop immunity against other serotype or subtypes of a serotype [18]. Within the seven serotypes, serotype A displays the greatest number of newly occurring subtypes, which makes the control by vaccination very difficult [14].

FMDV is non-enveloped icosahedral particle with a smooth surface and a diameter of about 30 nm having sedimentation coefficient of 146S and the genome is polyadenylated at 3′ end and carries a small covalently linked protein, VPg at 5′ end [19]. The size of FMDV RNA genome is 8.5 Kb. The genome encodes four structural proteins (VP1, VP2, VP3, and VP4) and eight non structural proteins (L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D). The genome is encapsidated by sixty copies each of the four structural proteins of which VP1-3 are exposed outside of virion and VP4 is completely internal [20,21]. The genome of FMDV is subject to a high rate of mutation because the FMDV RNA-dependent RNA polymerase lacks proof reading ability.

FMD is recognized as a significant epidemic disease threatening the cattle industry since the sixteenth century and till date it is a major global animal health problem [22]. Because of its

associated economic impact and the difficulties in its effective control, *Office of International des Epizooties* (OIE) ranks the disease first in its list A diseases and the virus as Risk Group 4 of transboundary importance [23,24]. FMD is endemic in Bangladesh and one of the major constraints for livestock development in Bangladesh. Three of the seven FMDV serotypes (serotypes O, Asia 1, and A) are prevalent throughout the Bangladesh [25,26]. Outbreak of this disease causes severe economic losses to the livestock industries in terms of loss of draft power, meat and milk production, infant and adult animal mortality. Economic losses is incurred about 60-150 million USD per year due to only the outbreak of FMD in Bangladesh [2]. Beside the significant economic consequences, the disease also has a serious impact upon food security and rural people livelihood in Bangladesh. An integral part of any viral disease control strategy is the epidemiological tracing of virus transmission together with conventional field investigations.

Therefore, if Bangladesh wishes to ensure food security, increase rural people livelihood and access the lucrative markets of the developed world for her livestock and livestock products, control of FMD will need to be addressed more aggressively and effectively. For RNA viruses with high evolutionary rates, an integral part of disease control strategy is the epidemiological tracing of virus transmission together with conventional field investigations. Epidemiological data, genetic characterization of circulating field FMDV and continuous monitoring is necessary in order to control FMD outbreak in Bangladesh. In consideration of these factors, the study was undertaken to investigate molecular epidemiology, genotyping and employ phylogenetic analysis to determine the relationship of FMDV serotypes circulating in Bangladesh.

2. MATERIALS AND METHODS

2.1 Clinical Sample Collection

A total of 134 clinical samples were collected from FMD suspected outbreaks of fifteen different areas of Bangladesh for confirmatory diagnosis. During 2011 – 2014, all clinical samples were collected from FMD suspected cattle showed lameness, loss of appetite, fever, anorexia, salivation. Clinical samples include tongue and hoof (interdigital) epithelial tissue samples, saliva and milk were collected. Samples were immediately transported to the laboratory on ice with medium containing equal volumes of glycerol and phosphate-buffered saline (PBS) (pH 7.2-7.6) and 2% antibiotic-antimycotic.

2.2 Inoculum Preparation and RNA Extraction

A piece of the epithelial tissue (0.5 g) was removed from the glycerol buffer, blotted dry on absorbent paper to reduce the glycerol content and was ground in pestle and mortar with an equal weight of the sterilized sand and 10 ml of PBS. Ground suspension of each of the sample was centrifuged at 3000 rpm for 10 minutes. The supernatant of each of the samples were taken for further processing according to OIE manual. RNA extraction was carried out from FMDV inoculums by using the QIAamp® Viral RNA kit (Qiagen, Germany) according to the manufacturer's protocol. Total RNA was extracted from non-infected BHK cells to prepare negative controls.

2.3 Conventional Reverse Transcription Polymerase Chain Reaction (RT-PCR)

The target in the genome was amplified by one step RT-PCR using the primer set are shown in Table 1. The amplification was performed in a thermal cycler (Stratagene PCR machine, USA) with One Step RT-PCR kit (Qiagen, Germany) with one cycle of reverse transcription conditions of 50°C for 30 min and 95°C for 10 min and followed by 30 cycles of 94°C for 1 min (Universal FMDV) and 35 cycles of 94°C for 15 sec (FMDV type specific), annealing temperature 55°C for 1 minute and 72°C for 1 min and finally one cycle of final extension of 72°C for 10 min (For universal FMDV) and 60°C for 6 min (For FMDV type specific). The amplified product was visualized on 2% agarose gel containing 0.6 mg/ml ethidium bromide and documented with Alpha Imager Mini System Protein Simple, USA.

2.4 Sequencing of VP1 gene of FMDV

The Qiaquick PCR purification kit (Qiagen, Germany) was used for the purification of RT-PCR products to remove the residual oligonucleotide primers, dNTPs and enzyme as per the manufacturer's protocol. These purified PCR products were analyzed with serotype specific primer on an automated DNA sequencer (3130x1 Genetic Analyzers, Applied Biosystems, USA). Analysis of the sequence identity,

divergence and phylogenetic relationship were performed using the clustal X method with the weighted residue table provided in the MegAlign program (DNASTAR, Inc. Madison, WI, USA). The partial VP1 gene sequences which obtained in this study and displayed within the phylogenetic tree had been deposited in the GenBank database under the accession numbers: KP119754, KP119755, KP119756, KP119757, KP119758, KP119759, KP119760, KP119761, KP119762 and KP119763.

2.5 Phylogenetic Analysis

VP1 nucleotide sequences of each isolate used in this study were submitted to GenBank, and the accession numbers are listed in Table 2. Isolates name have been abbreviated using the following format: country/organization/location/isolate no. The three-letter country codes were designated as outlined by the World Reference Laboratory for FMD (WRLFMD). Alignments and contigs of nucleotide sequence data were assembled using chromas software. VP1 sequences from other sources were downloaded from the Entrez Nucleotide database, National Center for Biotechnology Information and from the WRL, Pirbright, UK. Alignments were performed using Clustal W software. Phylogenetic relationships were reconstructed utilizing maximum likelihood (ML) method using MEGA. Bootstrap analysis was performed on MP generated trees (1,000 replicates). In order to provide perspective to the phylogenetic trees, Bovine enterovirus and rhinovirus B strain were used as the out-group.

The evolutionary history was inferred using the Neighbor-Joining method [27]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method [28] and are in the units of the number of base differences per site. Evolutionary analyses were conducted in MEGA5 software [29].

3. RESULTS

A total of 134 clinical samples from cattle of different ages (1 month to 7 years) in fifteen different areas such as Savar, Joydevpur, Munshiganj, Kaligonj, Kapasia, Sirajgonj, Kurigram, Dinajpur, Gaibandha, Kamrangichar, Rajshahi, Tangail, Nilphamari, Comilla and Chittagong of Bangladesh were investigated (Table 3 and Fig. 1).

It was found that out of 134 suspected clinical samples, 98 (73%) isolates were found positive for FMDV in RT-PCR using 5' UTR specific primer. All universal FMDV positive isolates were performed RT-PCR with FMDV serotype specific primer (Table 1). Among all FMDV isolates, serotype O and Asia 1 were found to be the most prevalent (31%) followed by serotype A (7%) in Bangladesh. Interestingly, the result showed that 9% samples had mixed serotypes exhibited serotype O and A (2%); and O and Asia (7%). In this observation, FMDV serotype C was absent among the tested clinical samples. There were 21% of FMDV positive isolates did not typing with serotype specific primer with RT-PCR.

The other aspect of this study was determination of the magnitude of prevalence of FMDV outbreak in respect of area wise distribution. Clinical samples of Savar area had the highest prevalence of (44%) FMDV positive than those of Kapasia (16%) followed by Gaibandha (15%), Dinajpur (7%), Kaligonj (6%), Sirazgonj (6%), Kurigram (3%), Joydevpur (2%), Chittagong (2%). Along with three FMDV serotypes, high frequency of Asia 1 observed at Savar area, O type at Kapasia, Dinajpur, Gaibandha and A type at Savar area from clinical samples.

As regard to year wise distribution, this study was conducted from the period of 2011-2014 to detect circulating FMDV serotypes in Bangladesh. The highest prevalence of FMDV found during 2014 (82%) followed by 75% in 2013, 67% in 2012 and 57% in 2011. Among the three different serotypes of FMDV, O was the dominant (60%) during 2013, Asia 1 (41%) in 2014 and A serotype showed at the lower frequency for the period of 2011 to 2014 from the clinical samples (Fig. 2).

3.1 Phylogenetic Analysis of FMDV Types O, Asia 1 and a Isolates

Phylogenetic analyses further defined genetic relationships of these viruses with available VP1 nucleotide sequences of viruses circulating in this region. Results of sequence analysis of VP1 gene of FMDV type O revealed that FMDV type O of BLRI isolates Kapasia/44, Dinajpur/61, Gaibandha/70, Kurigram/82 were closely related to each other and also shared 99 to 99.5% similarity at the nucleotide level.

Table 1. List of primers used for RT-PCR for serotyping of FMDV

FMDV serotype	Primer name	Sequence (5' to 3')	Location	PCR Products (bp)	Reference
All serotypes	1F	GCCTGGTCTTTCCAGGTCT	5'UTR	328	[38]
	1R	CCAGTCCCCTTCTCAGATC	5'UTR		
O	P38	GCTGCCTACCTCCTTCAA	1D	402	[39]
	P33	AGCTTGTACCAGGGTTTGGC	2B		
C	P40	GTTTCTGCACTTGACAACACA	1D	596	
	P33	AGCTTGTACCAGGGTTTGGC	2B		
Asia 1	P74	GACACCACTCAGGACCGCCG	1D	292	
	P33	AGCTTGTACCAGGGTTTGGC	2B		
A	P110	GT(G:A:T:C)ATTGACCT(G:A:T:C)ATGCA (G:A:T:C) AC (G:A:T:C) CAC	1D	732	[40]
	P33	AGCTTGTACCAGGGTTTGGC	2B		

Table 2. List of isolates used in phylogenetic analysis

Isolate	Host species	GenBank accession No.	Isolate	Host species	GenBank accession no.
O/BAN/BLRI/38/2013	Bovine	KP119758	Asia-1/ PAK_030/2002	Bovine	JF749849.1
O/BAN/BLRI/44/2013	Bovine	KP119759	Asia-1/PAK/L2810/2009	Bovine	JN006720.1
O/BAN/61/2013	Bovine	KP119760	Asia-1 /MAY/9/99	Bovine	HQ632774.1
O/BAN/BLRI//70/2013	Bovine	KP119761	Asia-1/ BAN/187/2013	Bovine	KJ175186.1
O/BAN/BLRI/82/2013	Bovine	KP119762	Asia-1/BAN/06/2012	Bovine	KJ175175.1
O/MAY/002/2004	Bovine	JF749852.1	Asia1/Vietnam/QuangTri/2007	Bovine	GQ452295.1
O/BAN/156/2013	Bovine	KF985189.1	Rhinovirus B strain MI402/2008	Bovine	KF427762.1
O/BAN//186/2013	Bovine	KJ175185.1	A/BAN/BLRI/127/2013	Bovine	KP119763
O/AFG/41/2011	Bovine	KJ606977.1	A /BAN/197/2013	Bovine	KJ754939.1
O/ISL/PAK/L1573/2009	Bovine	HQ113232.1	A /BAN/14/2012	Bovine	KC795950.1
O/Pak/Fsd1/2007	Bovine	AM942747.1	A /IND/ 43/2006	Bovine	HQ832586.1
O/Nepal/46/95	Bovine	AJ0044652.1	A/IND/40/00	Bovine	HM854025.1
O/IRN/073/2001	Bovine	JF749851.1	A/IND /17/2009	Bovine	HQ832592.1
O/BHU/1/2013	Bovine	KJ206908.1	A/IRN/34/2001	Bovine	EU414526.1
O/o5india /iso34/2004/1962	Bovine	AY593828.1	A/JAW/AFG/L1437/ 2009	Bovine	HQ439276.1

Isolate	Host species	GenBank accession No.	Isolate	Host species	GenBank accession no.
O/IND/45/98	Bovine	AF390732.1	A/PAK/76/2009	Bovine	GU384686.1
Rhinovirus B strain	Bovine	KF427762.1	A/PAK5/2006	Bovine	EF494488.1
Asia-1/BAN/BLRI/40/2013	Bovine	KP119754	A/BHU/41/2002	Bovine	EU414525.1
Asia-1/BAN/BLRI/78/2013	Bovine	KP119755	A/BHU/27/2003	Bovine	FJ755013.1
Asia-1/BAN/BLRI/107/2011	Bovine	KP119756	A/NEP/21/84	Bovine	FJ755081.1
Asia-1/BAN/BLRI/116/2014	Bovine	KP119757	Outgroup_Bovine enterovirus	Bovine	NC_001859.1
Asia-1/ IND /139/02	Bovine	DQ989322.1			
Asia-1 /IND /97/03	Bovine	DQ989323.1			
Asia1/BAM/AFG/L-590/2009	Bovine	HQ113233.1			

Table 3. Area wise distribution of FMDV with three serotypes in Bangladesh

Region/ District	Total sample	Positive isolates	Serotyping			Mixed infection		Non typing FMDV	% of positivity
			O type	A type	Asia1	O + Asia 1 type	O + A type		
Savar	52	39	4	5	16	-	-	14	75
Joydevpur	3	2	1	-	1	-	-	-	67
Munshiganj	6	6	-	-	2	4	-	-	100
Kaliganj	5	5	3	-	-	-	2	-	100
Kapashia	17	14	8	1	3	-	-	2	82
Sirajganj	9	7	1	-	4	2	-	-	78
Kamrangichar	2	0	-	-	-	-	-	-	-
Kurigram	5	3	2	-	-	-	-	1	60
Rajshahi	2	0	-	-	-	-	-	-	-
Dinajpur	6	6	6	-	-	-	-	-	100
Tangail	1	0	-	-	-	-	-	-	-
Gaibandha	15	13	6	1	2	-	-	4	87
Nilphamari	4	0	-	-	-	-	-	-	-
Chittagong	5	2	-	-	2	-	-	-	40
Comilla	2	1	-	-	-	1	-	-	50
Total	**134**	**98**	**31 (31%)**	**7 (7%)**	**30 (31%)**	**7 (7%)**	**2 (2%)**	**21 (21%)**	**73%**

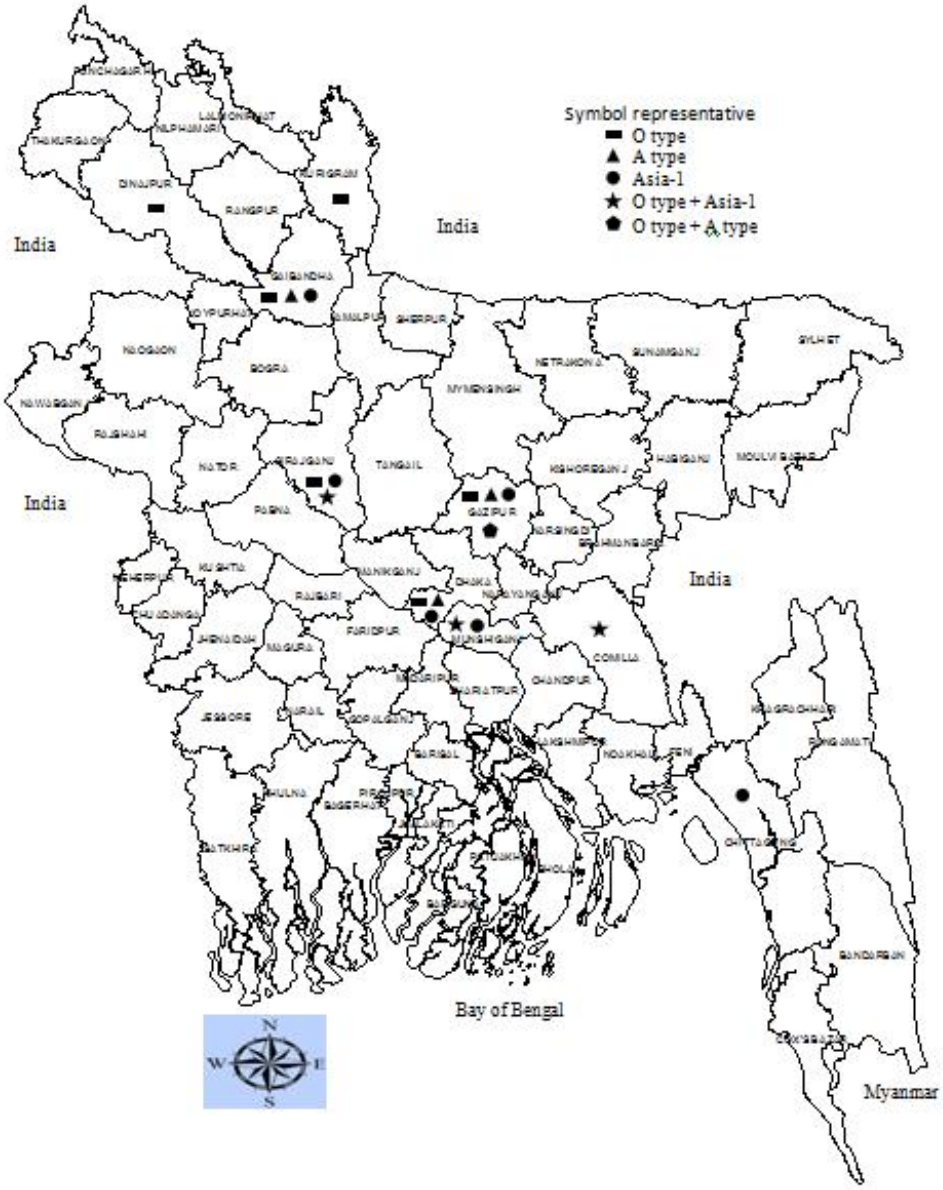


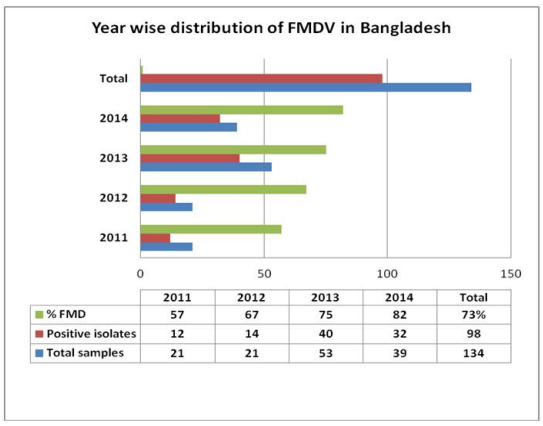
Fig. 1. Distribution of FMDV serotypes in Bangladesh during the period of 2011-2014

A total of 134 clinical samples from cattle in fifteen different areas of Bangladesh were investigated for typing of FMDV identification using conventional RT-PCR

There was significant divergence rates observed among Kapasia, Dinajpur, Gaibandha, Kurigram) strain. Phylogram of serotype O also indicating that Kapasia, Dinajpur, Gaibandha, Kurigram FMDV serotype O strains have close relation with Assam of India (IND/2014/KJ825807). Bhutan isolates (BHU/2013/KJ206908.1) of serotype O also originated from same branch of the phylogenetic tree (Fig. 3).

Phylogenetic tree constructed with FMDV serotype A isolates showed that BLRI isolate Savar/127 was the most nearly isolate to other FMDV serotype A of India, Bangladesh, Bhutan, Nepal. BLRI/Savar/127 may be originated from A /IND/ 43/2006 and A/IND/40/2000. Much divergent rates between BLRI/Savar/127 A strains and the other international FMDV strains (Fig. 4). FMDV serotype Asia 1 isolated strains showed that BLRI isolates Kap/40 and CTG/116 clustered at the same branch with 99.4% identity were most closely related to isolates collected from the Gazipur area (Asia 1 BAN/187) of Bangladesh during 2013. Whereas analyses of

VP1 gene sequences suggested that BLRI isolates GAI/78 and SAV/107, have maximum identity of 99.4%, and were most closely related to isolates collected in India (Asia-1/ IND /139) during 2002 (Fig. 5). Significant divergence rates observed between Kapasia, Chittagong strains and Gaibandha and Savar for Asia 1 strains of FMDV.



	2011	2012	2013	2014	Total
% FMD	57	67	75	82	73%
Positive isolates	12	14	40	32	98
Total samples	21	21	53	39	134

Fig. 2. Year wise distribution of FMDV in Bangladesh

The three serotypes (O, A and Asia 1) of FMDV were circulated in fifteen different areas of Bangladesh from the period of 2011-2014

4. DISCUSSION

From fifteen different selected areas of Bangladesh, a total of 134 clinical samples was collected over a span of four years and analyzed for the evaluation of specific serotypes. All samples were subjected to RT-PCR with FMDV universal gene and serotype specific genes. Three different FMDV serotypes (O, A and Asia 1) were detected in Bangladesh and serotype O was found predominant followed by serotype Asia 1 and serotype A. It was found that 73% (98) of suspected samples were positive for FMDV in RT-PCR using universal primers 1F and 1R. In FMDV positive samples, 31% (31), 31% (30) and 7% (7) were O, Asia-1 and A, respectively during this study (Table 1). From the data, it was revealed that three serotypes were prevailing in Bangladesh. Three serotypes were co-circulating at the same time in Savar and Kapasia. Among these, serotype O was circulating highly and followed by Asia-1 and A. Molecular analysis of FMDV isolates also proof that the circulating FMDV in Bangladesh during 2011 to 2014 may be transmitted from the border of India.

The previous study also revealed that in Bangladesh, out of 7 serotypes of FMDV, three serotypes of FMMDV were prevalent such as O, A and Asia 1 where serotype-O accounts for about 85% of the outbreaks followed by types A and Asia1. It was reported that FMDV serotype A, O, C, Asia1 and subtype A_{22} were prevalent in Bangladesh. The occurrence of FMDV serotype O was prevalent in Bangladesh during the period of 2000-2010 [25]. Further study also confirmed the emergence of two distinct serotypes A and O with an abundance of serotype A in Chittagong and Gazipur, and serotype O in Pabna and Faridpur district in Bangladesh during 2012 [26]. In 2014, two serotypes of FMDV such as O and Asia 1 were identified, whereas type C and A were absent in Sylhet district of Bangladesh. In Table 3, showed 21 FMDV isolates were not performed typing. Since virus may mutate at that region from where serotype specific primer was selected, that's why 21% virus were not detected.

Phylogenic analysis of the VP1 region of FMD viruses has been used extensively to identify and characterize FMDV isolates, and investigate the molecular epidemiology of the disease worldwide [30-32]. These techniques have assisted in studies of the genetic relationships between different FMD virus isolates, geographical distribution of lineage and genotype and the establishment of genetically and geographically linked topotypes and tracing the source of virus during outbreaks [33]. A total of 10 BLRI isolates of FMDV (5 isolates for O type, 4 isolates for Asia 1 and for A type) of FMDV were selected on the basis of different areas and sequenced at VP1 coding region and subjected to phylogenetic analysis. A phylogenetic tree based on the VP1 (1D) region of FMDV is widely used for genetic characterization because of its significance for virus attachment and entry, protective immunity, and serotype specificity [34,35].

Sequence data from the BLRI isolates of FMDV in this study indicated that the three serotypes of FMDV circulating during the period of 2011–2014 were closely related to FMDV isolates of Bangladesh and surrounding countries. FMD outbreaks predicted agreed that animal movement between countries is generally unrestricted in this geographical region. Sequenced FMDV serotype O isolates collected on the northern borders of Bangladesh during 2013 that were closely related and shared the same genetic lineage suggesting a common origin (Fig. 3). From the results presented in this

study, it also became evident that all serotype O viruses sequenced from Bangladesh outbreaks belong to the Pan Asia topotype [26]. Viruses in this topotype occurred in various lineages and, based on the phylogenetic analysis, it can be concluded that sequenced viruses from the BLRI isolates may be transmitted from north border of India (Assam), Bhutan to Bangladesh (Kurigram). Moreover, it revealed that there was slight divergence (0.5% to 6.3%) among the BLRI isolated serotypes O strains. However, there was an obvious divergence (more than 12%) to the other compared FMDV strains. Previously published work indicates circulating serotype O in Bangladesh was genetically closely related to the India 2001 sublineage of Middle East–South Asia (ME-SA) topotype [26]. However, serotype O Ind 2001 sublineage of ME-SA topotype viruses were predominantly circulating in India since 1982 [36] and was detected to be homogenously distributed over the regions in Bangladesh.

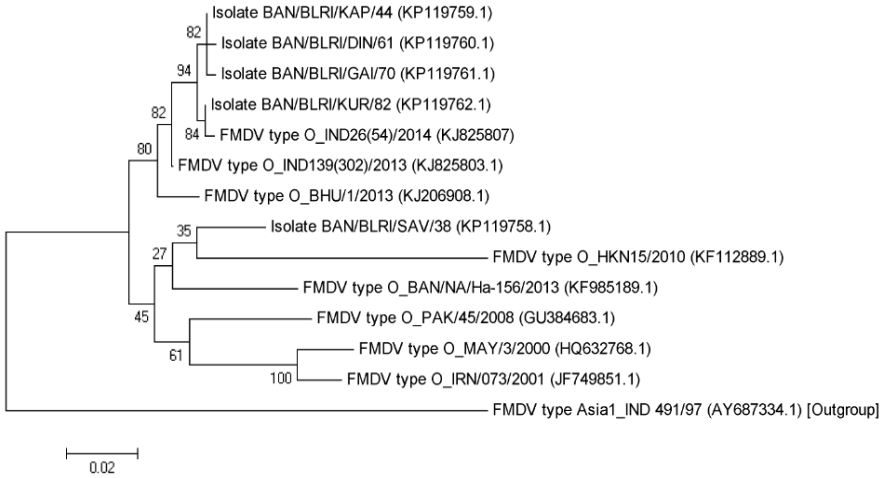


Fig. 3. Phylogenetic tree showing the relationships of FMDV serotype O isolates

The relationship is based on VP1 partial nucleotide sequences. Sequence analysis of VP1 gene of FMDV type O revealed that FMDV type O of BLRI isolates Kapasia/44, Dinajpur/61, Gaibandha/70, Kurigram/82 were closely related to each other and also shared 99 to 99.5% similarity at the nucleotide level

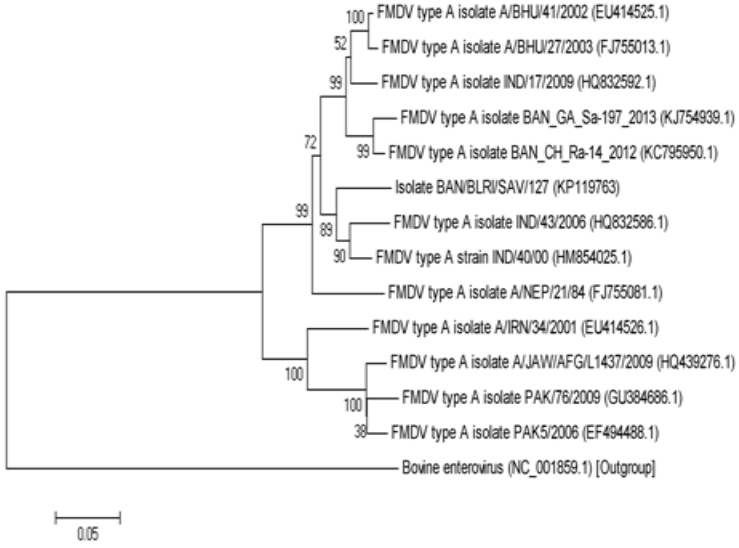


Fig. 4. Phylogenetic tree based on VP1 partial nucleotide sequences showing the relationships of FMDV serotype A isolates

BLRI isolate Savar/127 was the most nearly isolate to other FMDV serotype A of India, Bangladesh, Bhutan, Nepal. BLRI/Savar/127 may be originated from A/IND/43/2006 and A/IND/40/2000. Much divergent rates between BLRI/Savar/127 A strains and the other international FMDV strains

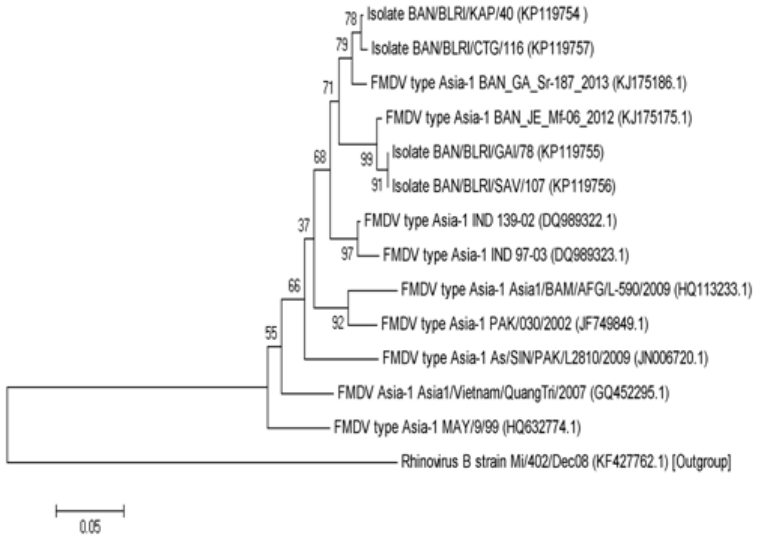


Fig. 5. Phylogenetic tree showing the relationships of FMDV serotype Asia1 isolates which based on VP1 partial nucleotide sequences

BLRI isolates Kap/40 and CTG/116 clustered at the same branch with 99.4% identity were most closely related to isolates collected from Gazipur area (Asia 1 BAN/187) of Bangladesh during 2013. Whereas analyses of VP1 gene sequences suggested that BLRI isolates GAI/78 and SAV/107, have the maximum identity of 99.4%, and were most closely related to isolates collected in India (Asia-1/ IND /139) during 2002

There was a limitation of FMDV serotype A isolation from different areas of Bangladesh because this serotype was co-circulated at the negligible level during the period of 2011 to 2014. So, it is difficult to determine the genetic comparison of this isolate throughout the country. However, the sequencing of VP1 gene of FMDV serotype A, BLRI/BAN/127 isolates showed close resemblances to isolates originated from India during 2000 to 2006 (Fig. 4). There was high divergence showed (1.1% to 20.7%) from the sequencing of VP1 gene of FMDV serotype A with BLRI and other isolates. Nandi et al. [26] reported the emergence of FMDV serotype A genotype VII virus were found in Bangladesh during the outbreaks of 2012. It is one of the 10 existing genotypes of FMDV serotype A worldwide and was previously isolated from India. These data coupled with the fact that the serotype A viruses are known to be the most antigenically variable among the four Eurasian FMDV serotypes [31,37] and also provide strong evidence that the viruses collected on the northern borders of Bangladesh as well as A /IND/ 43/2006 and A/IND/40/2000 share a recent common origin.

The FMDV serotype Asia 1 collected in Bangladesh were grouped in the same clade including two different genetic lineages. First lineage revealed Asia 1 shared the same origin with southern border of Bangladesh (BAN/BLRI/40/2013, BAN/BLRI/116/2014 and BAN/187/2013) and the second lineage relates with the northern part of Bangladesh (BAN/BLRI/78/2013, BAN/BLRI/107/2011 and BAN/06/2012). The next closest relatives (Asia/IND /139/2002 and Asia 1/IND /97/03) were largely divergent at the nucleotide level which reflecting the different temporal origin of these viruses (Fig. 5). The results of the sequencing of VP1 gene (1D) of FMDV serotype Asia 1 also showed minor divergence (0.6% to 6.3%) among the strains isolated in Bangladesh. While there were a noticeable divergence (more than 13.1%) between BLRI isolates and the other isolates that compared with FMDV serotype A strains. The close genetic relationship of these isolates, though collected in different distant geographic regions, indicated intra- and inter-country spread of the disease. Detection of circulating FMDV serotypes and disease monitoring of animals entering Bangladesh are crucial components for an effective national FMDV control program in Bangladesh [26].

5. CONCLUSION

This study provided an overview of the molecular epidemiology of FMDV in Bangladesh. Detected

three different FMDV serotypes O, A, and Asia 1, which are co-circulating in Bangladesh. Although only a limited number of ad-hoc samples were available for sequence analysis. It was concluded that serotype O and Asia 1 were more prevalent in Bangladesh than A serotypes of FMDV. Molecular analysis of FMDV isolated from Bangladesh also proof that the circulating FMDV serotype O and A during 2011 to 2014 may be transmitted from India. Additional surveillance and epidemiological studies in this region combined with proper phylogenetic analysis should aid in implementation and development of appropriate control measures.

ACKNOWLEDGEMENTS

The research was funded by the developing project of "Research on FMD and PPR in Bangladesh project" (Award no. 7080) of Ministry of Fisheries and Livestock, Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Forman S, Le Gall F, Belton D, Evans B, Francois JL, Murray G, Sheesley D, Vandersmissen A, Yoshimura S. Moving towards the global control of foot and mouth disease: An opportunity for donors. Rev Sci Tech. 2009;28(3):883-896.
2. Momtaz S, Rahman A, Sultana M, Hossain MA. Evolutionary analysis and prediction of peptide vaccine candidates for foot-and-mouth-disease virus types A and O in Bangladesh. Evol Bioinform Online. 2014;10:187-196.
3. FAO: Improved animal health for poverty reduction and sustainable livelihoods. FAO; 2002.
4. FAO: Foot-and-mouth disease. Situation worldwide and major epidemiological events in 2005-2006. Rome: FAO; 2007.
5. Esmaelizad M, Jelokhani-Niaraki S, Hashemnejad K, Kamalzadeh M, Lotfi M. Molecular characterization of amino acid deletion in VP1 (1D) protein and novel amino acid substitutions in 3D polymerase protein of foot and mouth disease virus subtype A/Iran87. J Vet Sci. 2011; 12(4):363-371.
6. Depa PM, Dimri U, Sharma MC, Tiwari R. Update on epidemiology and control of foot and mouth disease - A menace to international trade and global animal enterprise. Vet World. 2012;5:694-704.
7. Aftosa F. Foot and mouth disease the center for food security and public health. USA: IOWA State University; 2007.
8. Woodbury EL. A review of the possible mechanisms for the persistence of foot-and-mouth disease virus. Epidemiol Infect. 1995;114(1):1-13.
9. Salt JS. The carrier state in foot and mouth disease--an immunological review. Br Vet J. 1993;149(3):207-223.
10. Sutmoller P, McVicar JW, Cottral GE. The epizootiological importance of foot-and-mouth disease carriers. I. Experimentally produced foot-and-mouth disease carriers in susceptible and immune cattle. Arch Gesamte Virusforsch. 1968; 23(3):227-235.
11. Juleff N, Windsor M, Reid E, Seago J, Zhang Z, Monaghan P, Morrison IW, Charleston B. Foot-and-mouth disease virus persists in the light zone of germinal centres. PLoS One. 2008;3(10):e3434.
12. Brown F. The history of research in foot-and-mouth disease. Virus Res. 2003; 91(1):3-7.
13. Saiz M, Nunez JI, Jimenez-Clavero MA, Baranowski E, Sobrino F. Foot-and-mouth disease virus: Biology and prospects for disease control. Microbes Infect. 2002; 4(11):1183-1192.
14. Kitching RP. Global epidemiology and prospects for control of foot-and-mouth disease. Curr Top Microbiol Immunol. 2005;288:133-148.
15. Tosh C, Sanyal A, Hemadri D, Venkataramanan R. Phylogenetic analysis of serotype A foot-and-mouth disease virus isolated in India between 1977 and 2000. Arch Virol. 2002;147(3):493-513.
16. Knowles NJ, Samuel AR, Davies PR, Midgley RJ, Valarcher JF. Pandemic strain of foot-and-mouth disease virus serotype O. Emerg Infect Dis. 2005;11(12):1887-1893.

17. Valarcher JF, Knowles NJ, Zakharov V, Scherbakov A, Zhang Z, Shang YJ, Liu ZX, Liu XT, Sanyal A, Hemadri D, et al. Multiple origins of foot-and-mouth disease virus serotype Asia 1 outbreaks, 2003-2007. Emerg Infect Dis. 2009;15(7):1046-1051.
18. Paton DJ, Valarcher JF, Bergmann I, Matlho OG, Zakharov VM, Palma EL, Thomson GR. Selection of foot and mouth disease vaccine strains--a review. Rev Sci Tech. 2005;24(3):981-993.
19. Bachrach HL. Foot-and-mouth disease. Annu Rev Microbiol. 1968;22:201-244.
20. Mittal M, Tosh C, Hemadri D, Sanyal A, Bandyopadhyay SK. Phylogeny, genome evolution and antigenic variability among endemic foot-and-mouth disease virus type A isolates from India. Arch Virol. 2005; 150(5):911-928.
21. McCoy RJ, Sheppard M, Studdert MJ, Johnson MA. Genomic location and nucleotide sequence of a serotype 3 porcine adenovirus hexon gene. Arch Virol. 1999;144(6):1217-1227.
22. Longjam N, Deb R, Sarmah AK, Tayo T, Awachat VB, Saxena VK. A brief review on diagnosis of foot-and-mouth disease of livestock: Conventional to molecular tools. Vet Med Int. 2011;2011:905768.
23. OIE: OIE terrestrial manual. Foot and mouth disease. 2009;Chapter 2.1.5:1-29.
24. OIE: OIE terrestrial manual. Foot and mouth disease. 2008;Chapter 2.1.5:190-217.
25. Loth L, Osmani MG, Kalam MA, Chakraborty RK, Wadsworth J, Knowles NJ, Hammond JM, Benigno C. Molecular characterization of foot-and-mouth disease virus: Implications for disease control in Bangladesh. Transbound Emerg Dis. 2011;58(3):240-246.
26. Nandi SP, Rahman MZ, Momtaz S, Sultana M, Hossain MA. Emergence and distribution of foot-and-mouth disease virus serotype A and O in Bangladesh. Transbound Emerg Dis. 2015;62(3):328-331.
27. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4(4):406-425.
28. Nei M, Kumar S. Molecular evolution and phylogenetics. New York: Oxford University Press; 2000.
29. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24(8):1596-1599.
30. Klein J, Parlak U, Ozyoruk F, Christensen LS. The molecular epidemiology of foot-and-mouth disease virus serotypes A and O from 1998 to 2004 in Turkey. BMC Vet Res. 2006;2:35.
31. Knowles NJ, Samuel AR. Molecular epidemiology of foot-and-mouth disease virus. Virus Res. 2003;91(1):65-80.
32. Konig GA, Palma EL, Maradei E, Piccone ME. Molecular epidemiology of foot-and-mouth disease virus types A and O isolated in Argentina during the 2000-2002 epizootic. Vet Microbiol. 2007;124(1-2):1-15.
33. Mesfine S. Study on the genetic relationships of foot and mouth disease virus in East Africa. A thesis submitted in partial fulfillment of the requirement for the degree of doctor of philosophy in the Department of veterinary Tropical Disease. South Africa: University of Pretoria; 2004.
34. Burman A, Clark S, Abrescia NG, Fry EE, Stuart DI, Jackson T. Specificity of the VP1 GH loop of foot-and-mouth disease virus for alpha integrins. J Virol. 2006; 80(19):9798-9810.
35. Jackson T, King AM, Stuart DI, Fry E. Structure and receptor binding. Virus Res. 2003;91(1):33-46.
36. Yuvaraj S, Madhanmohan M, Nagendrakumar SB, Kumar R, Subramanian BM, Mohapatra JK, Sanyal A, Pattnaik B, Srinivasan VA. Genetic and antigenic characterization of Indian foot-and-mouth disease virus serotype O isolates collected during the period 2001 to 2012. Infect Genet Evol. 2013;13:109-115.
37. Pereira HG. Subtyping of foot-and-mouth disease virus. Dev Biol Stand. 1976; 35:167-174.
38. Reid SM, Ferris NP, Hutchings GH, Samuel AR, Knowles NJ. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J Virol Methods. 2000;89(1-2):167-176.

39. Vangrysperre W, De Clercq K. Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. Arch Virol. 1996;141(2):331-344.
40. Callens M, De Clercq K. Differentiation of the seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. J Virol Methods. 1997;67(1):35-44.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15865