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IDENTIFICATION OF FOOT AND MOUTH DISEASE (FMD) VIRUS FROM RECENTLY OUTBREAK CROSSBRED CATTLE IN RAJBARI DISTRICT OF BANGLADESH

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ABSTRACT

Foot and mouth disease (FMD) is a devastating viral disease and endemic in nature in Bangladesh that causes huge economical losses. The present research work was aimed to determine the prevalence of FMD outbreaks and molecular detection of FMDV serotypes by uRT-PCR and gsRT-PCR test, respectively from crossbred cattle in the Rajbari district of Bangladesh during the period from January to June 2018. A total of 16 tongue epithelial samples were collected from clinically FMD suspected 2 to 3 years old crossbred cattle. 14 samples were positive by uRT-PCR. The detection rate of FMDV by uRT-PCR was 87.50%. Then uRT-PCR positive samples were serotype by gsRT-PCR. Serotype based prevalence of FMDV was 42.86%, 100%, 21.43% and 21.43% in O serotype, Asia-1 serotype, A serotype and mixed infection with Asia-1 and A, respectively. Considering the age, the prevalence of confirmed FMD outbreak was 42.86%, 35.71% and 21.43% at the age of 2, 2.5 and 3 years, respectively. Serotype A, O and Asia-1 is circulated in Rajbari district and required trivalent vaccine for prevention and control of FMD in that area.

Keyword: Cattle, FMD, PCR, Prevalence, Serotype.

INTRODUCTION

Foot and mouth disease (FMD) is, one of the world's most important contagious viral diseases, responsible for large-scale epidemics, huge global losses of livestock production and trade (Knowles and Sameul, 2003; Kitching et al., 2005; Samuel and

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Knowles, 2001; OIE, 2019). FMD is a highly contagious disease of cloven-footed animals like domesticated cattle, buffalo, pigs, sheep, goats, and deer (Kitching et al., 2005). FMD is now a threat to the livestock population throughout the world including Bangladesh due to its high contagiousness, a rapid multiplicity of the type and subtypes, wide species of animal's susceptibility, short-lived immunity and the existence of persistent or carrier state (Bachrach, 1968; Saiz and Doming, 1996; Sevilla and Domingo, 1996). Crossbreed cattle are highly susceptible to FMD in Bangladesh (Rahman et al., 2015). In Bangladesh, annual loss due to FMD has been estimated at US $125 million per year (Sil and Taimur, 2000). The virus has seven serotypes (0, A, C, Asia-1, SAT-I, SAT-II, and SAT-III) and more than 65 subtypes (Bachrach, 1968). Cross protection has not been observed between serotypes and even between some subtypes within a serotype. Out of seven serotypes, serotypes 0, A, C and Asia1 have been detected in Bangladesh (Freiberg et al., 1999; Marquardt et al., 2000; Loth et al., 2011; Jannat et al., 2019). The disease is characterized by a short-lasting fever, epithelial lesions on the tongue, dental pad and inner mouth area leading to excessive salivation and drooling. Lesions on the feet cause lameness. Secondary infection of epithelial lesions can significantly increase the severity of the disease (Rhyan et al., 2008). Asia 1, O and A serotypes were circulating in Bangladesh (Jannat et al., 2019). For control of FMD outbreaks or complete elimination of the virus, reliable, rapid and confirmatory diagnosis of the disease is important and outbreaks should be detected at an early stage and persistent infections should also be recognized to prevent further transmittance. The present research has been designed to determine the prevalence of FMD virus from a recent outbreak of crossbreed cattle and characterizing the serotypes of FMD virus circulating in naturally infected crossbred cattle by uRT-PCR and gs RT-PCR, respectively.

MATERIALS AND METHODS

Ethical Approval

This research work was approved by the institutional animal experimental and ethical committee of Bangladesh Livestock Research Institute, Savar, Dhaka. However, it was properly concerned with the Dept. of Microbiology, HSTU and DLS authority of Rajbari district.

Study area

The study was carried out in the Rajbari district (Fig. 1) of Bangladesh and the laboratory tests were conducted in the Foot and Mouth Disease (FMD) Research Laboratory, Animal Health Research Division, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341 during the period of January- June 2018.

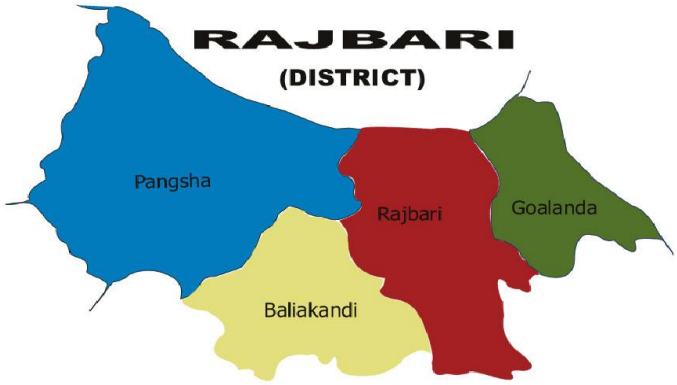

Figure 1. Map showing the sampling areas.

Sample Collection

A total of 16 field samples (tongue epithelium) were aseptically collected from clinically FMD suspected crossbred cattle having age between 2 to 3 years approximately (Fig. 2) within virus transport media (VTM) and immediately transported to the laboratory for identifications and serotyping.

Figure 2. Sample collection from tongue epithelia of crossbred cattle

Inoculum Preparation and RNA Extraction

A piece of the epithelial tissue was removed from the glycerol buffer, blotted dry on absorbent paper to reduce the glycerol content. Approximately 1-2 gm tissue was weighted by an electric balance and homogenized by grinding with sterilized mortar and pestle. Then 20% suspension was prepared by adding phosphate buffer saline (PBS). The suspension of each of the samples was then centrifuged at 3,000 rpm for 10 minutes maintaining the temperature at 4°C. The supernatant of each of the

samples was taken for further processing according to the OIE-2004 manual. For the sterility test, a small number of inoculums was inoculated into bacteriological media to identify the presence of any type of bacteria. RNA extraction was carried out from FMD inoculums by using the QIAamp® Viral RNA kit (Qiagen, Germany) according to the manufacture's protocol. Mastermix volume per reaction was 25 µl was given below in Table-01.

Table 1. Mastermix table for PCR

Reaction Component	Volume Per Reaction (µl)
Nuclease free water	4.5
2×RT-PCR Buffer Kit lot	12.5
25×RT PCR Enzyme Mix Kit lot	1
Forward Primer (100 pmol/µl)	1
Reverse Primer (100 pmol/µl)	1
Total Volume	20
Template RNA	5
Total	**25**

Conventional Reverse Transcription Polymerase Chain Reaction (RT-PCR)

The target in the genome was amplified by one-step RT-PCR using the FMD universal and serotype specific primer (Reid et al., 2000). Primer details were mentioned in the Table-02.

Table 2. List of Oligonucleotide primers used for universal FMDV and serotyping of FMDV by RT-PCR

Serotype	Primer name	Primer sequence (5' to 3')	Location	PCR Products (bp)	Reference
Universal	1F	GCC TGG TCT TTC CAG GTCT	5′UTR	328	Vangrysperre and De Clercq, (1996)
	1R	CCA GTC CCC TTC TCA GATC	5′UTR		
O	P38	GCTGCCTACCTCCTTCAA	1D	402	
	P33	AGCTTGTACCAGGGTTTGGC	2B		
Asia 1	P74	GACACCACTCAGGACCGCCG	1D	292	
	P33	AGCTTGTACCAGGGTTTGGC	2B		
A	P110	GT(G:A:T:C)ATTGACCT(G:A:T:C)A TGCA (G:A:T:C) AC (G:A:T:C) CAC	1D	732	Callens and De Clercq, (1997)
	P33	AGCTTGTACCAGGGTTTGGC	2B		

The amplification was performed on a thermal cycler 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, 55°C for 1 sec (type A), 55°C for 30 sec (type O) and 72°C for 1 min and finally one cycle of final extension of 72°C for 10 min. After PCR, the amplified products were visualized by agarose gel electrophoresis using 2% agarose gel containing 0.6 mg/ml ethidium bromide at 100V in 1X tris borate EDTA (TBE) buffer. At the end of electrophoresis, the gel was documented on a UV transilluminator (AlphaImager®Mini System, USA).

RESULTS AND DISCUSSION

All the collected samples initially subjected to uRT-PCR followed by gsRT-PCR for the detection of serotypes. All of the 16 field samples of tongue epithelia were subjected to uRT-PCR for the detection of FMDV by yielding an amplicon size of 328 bp through 2% agarose gel electrophoresis (Figure-03). Among these 16 samples, 14 samples were positive and 2 samples were negative for FMDV by uRT-PCR (Table 3).

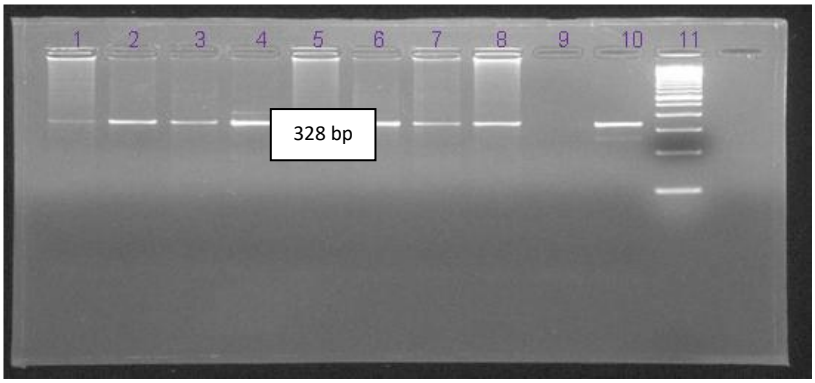

Figure 3. uRT-PCR products of representative field samples after 2% agarose gel electrophoresis, Lane1-8 = FMD Universal, Lane 9 = negative control (RNase-free water), Lane 10 = positive control, Lane 11 = 1 KB plus DNA ladder (Invitrogen, USA).

The uRT-PCR positive samples (14 samples) were further subjected to gsRT-PCR for confirmation of serotypes of FMDV by using gene specific primers. The FMDV serotypes were identified as O, A and Asia-1. The product amplicon size of gsRT-PCR were 402 bp for O type, 732 bp for A and 292 bp for Asia-1 respectively. Among the 14 uRT-PCR positive samples, 14 was positive for Asia-1, 6 was positive for type O, and 3 was positive for A which was mixed infection with Asia-1 in the circulating area (Fig. 4)

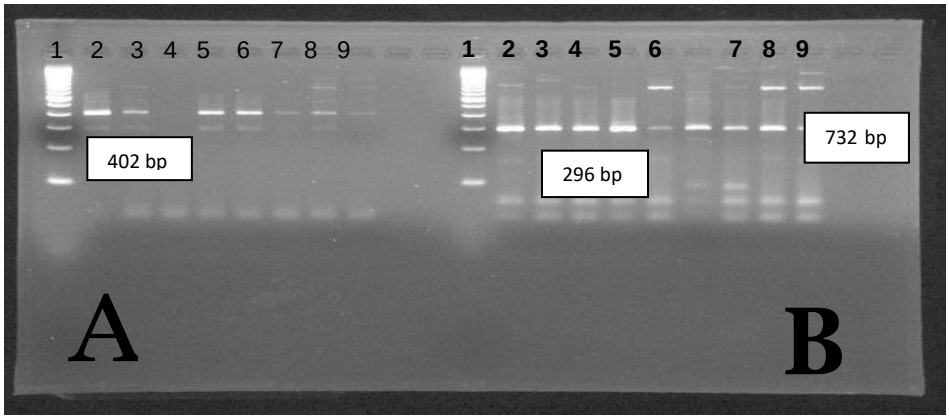

Figure 4. Showing the specific band of gsRT-PCR products of representative field samples in 2% agarose gel electrophoresis. (A) Lane -1: 1 KB plus DNA ladder (Invitrogen, USA), Lane 2=positive control for O type, Lane 3-9: (O type), Lane - 10: negative control (RNase-free water).(B) Lane-1: 1 KB plus DNA ladder (Invitrogen, USA), Lane-2-10: Asia-1, Lane- 6,9, 10 A and Lane-11: negative control (RNase-free water).

The overall prevalence of FMDV was 87.5% in clinically infected cross breed cattle. It may due to vesicular, eruption of tongue epithelia could be the major source of FMD virus shedding. However, considering the age the prevalence of confirmed FMD outbreak was 42.86% (n=6), 35.71% (n=5) and 21.43% (n=3) at the age of 2, 2.5 and 3 years respectively in cross breed cattle in Rajbari region that shown in Table 4. It was seems that young animals are more susceptible to FMD than adults. Furthermore, serotype-based prevalence of FMDV was 42.86% (n=6), 100 % (n=14), 21.43% (n=3) and 21.43% (n=3) in O serotype, Asia-1 serotype, A serotype and mixed infection with Asia-1 and A respectively that are circulating in the Rajbari district of Bangladesh that are shown in Table 5.

Table 3. Results of Collected sample by uRT-PCR

Upazila of Rajbari District	Union	Village	Collected Sample	Age (Aprx. Years)	RT-PCR positive
Rajbari sadar	Chandoni	Raypur	3	2	3
Kalukhali	Kalikapur	Sattajitpur	4	2	3
Kalukhali	Boalia	Hatgram	1	3	1
Kalukhali	Mrigi	Kolkolia	2	3	1
Pangsha	Pourosova	Dhekipara	2	2.5	2
Pangsha	Pourosova	Puraton bazar	1	3	1
Pangsha	Bahadurpur	Meghna	2	2.5	2
Pangsha	Machpara	Nivakrishnapur	1	2.5	1
Total sample collected			16		14

Table 4. Prevalence of FMD outbreak according to age

Age (Years)	RT-PCR Positive Samples (N =14)	Prevalence (%)
2	6	42.86
2.5	5	35.71
3	3	21.43

Table 5. Serotypes of FMDV positive by primer specific gsRT-PCR and its prevalence

Outbreak areas and species	Total samples	No.of positive samples	Overall prevalence	Serotypes of FMDV positive by RT-PCR and its prevalence			
				O	Asia-1	A	Mixed serotypes A+Asia-1
Rajbari Cross breed cattle	16	14	87.5%	n= 6 (42.86 %)	n=14 (100%)	n=3 (21.43%)	n=3 (21.43%)
Total		14		6	14	3	6

Bangladesh has been considered as an endemic country of FMD. It causes massive damage to the livestock population in this country. Rapid detection and their serotype confirmation are important to take rapid measure against the disease. In general, serotyping of FMDV is done using the antigen capture ELISA, but RT-PCR is more sensitive and specific than ELISA for differentiating the serotypes of FMDV from

clinical samples (Giridharan et al., 2005). The molecular biological technique is rapid, accurate, highly sensitive and only small quantities of material are needed to perform the test. In this study, the uRT-PCR/gsRT-PCR was used to confirm the FMDV genome from clinical samples of different outbreaks in farm animals (Crossbreed cattle). Among the 16 field samples, 14 were found positive by uRT-PCR. Detection rate of FMDV by uRT-PCR was 87.50%. Although the causes of failure of detection of FMDV from 2 (12.50%) field samples of this study are not very clear but, this may be due to sample collection of recovered animals or might be treated with an antiviral agent before sampling. Serotyping confirmation of FMDV was done by RT-PCR using gene specific primers. In this study, 14 uRT-PCR positive field samples were tested to differentiate into serotypes. The prevalence rate of serotypes was 37.50% for type O, 87.50% for type Asia-1, 37.50% for mixed infection (O+Asia-1) in the Rajbari district. These results are almost similar to the findings of Alam et al. (2015), Hossen et al. (2014), Nandi et al. (2013) and Loth et al. (2011). Alam et al. (2015) reported that out of the 12 samples, 10 (83.33%) were found positive for FMDV and all of those were of serotype O in Kapasia upazila under Gazipur district of Bangladesh. Hossen et al. (2014) found 60.00% positive for serotype O and 20.00% for Asia-1 in Gazipur district and 67.56% for serotype A, 8.10% for serotype Asia-1 and 10.81% for mixed infection (serotype A+ Asia-1) in Pabna district. The overall prevalence rate of serotypes of FMDV was 37.50% for type O, 87.50% for Asia-1, 37.50% for mixed infection (O and Asia-1) in the Rajbari district of Bangladesh. This result was partially similar to Hossen et al. (2014). Hossen (2014) et al. found 30.58% positive for type O, 36.47% for type A, 11.76% for type Asia-1 and 4.70% for mixed infection (serotype A +Asia-1). Results of the present study indicated that FMDV serotypes O, Asia-1 are prevailing in cattle in Rajbari district of Bangladesh and outbreaks in different regions of Bangladesh may be due to the circulation of these serotypes among susceptible animals.

CONCLUSION

Foot and Mouth Disease (FMD) is one of the most fatal endemic disease in Bangladesh and economical losses ascribed as 180 billion TK (BD) annually due to high calf mortality, production losses, medication cost, labor cost and others. Hence, This MS research work was conducted for the determination of prevalence of FMDV and identification of the serotypes of Foot and Mouth Disease Virus (FMDV) from the field samples (Tongue Epithelia) of the recent outbreak areas in Bangladesh during the period from January to June, 2018. The overall prevalence of FMDV was 87.5% in clinically infected cross breed cattle through molecular detection method (PCR). The prevalence rate of serotypes was 37.50% for type O, 87.50% for type Asia-1, 37.50% for mixed infection (O+Asia-1) in the Rajbari district of Bangladesh respectively. It is most interesting that 14 tongue epithelial samples were PCR positive out of 16 samples. This may due to tongue epithelia may be the first and predilection site for FMDV multiplication. However, further genetic and

immunological study is required for planning effective control strategies against FMDV in Rajbari district of Bangladesh.

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