International Journal of Natural and Social Sciences
Print: 2617-6637 < ISSN > Online: 2313-4461

Molecular detection and serotyping of Foot and Mouth disease viruses (FMDV) from cattle in Bangladesh

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ARTICLE INFO

Article history

Received: 25 February
Accepted: 24 March

Keywords

Cattle, FMDV, qRT-PCR, sero-type O, sero-type A

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ABSTRACT

Foot and Mouth Disease (FMD) is a highly contagious, enzootic and economically important viral disease of cattle in Bangladesh. Despite regular vaccination this disease is appearing every year among the indigenous and cross-breed population of Bangladesh and is responsible for huge economic losses. The aim of this study was to detect the sero-types of Foot-and-Mouth disease viruses (FMDV) circulating in the cattle population of Feni and Lakshimpur districts during 2022 in Bangladesh. A total of 50 samples (tongue epithelium) were collected from 50 affected cattle for the detection and sero-typing of the circulating viruses using real time qRT-PCR. Out of 50 samples, 40 were positive of which 37 were sero-type O and 3 samples were sero-type A of FMDV. Findings of this study clearly indicated that realtime qRT-PCR is a highly sensitive and rapid method for early and confirmatory detection of FMDV from field samples. This study also indicates that off the two sero-types (O and A) the predominant sero-type of FMDV is sero-type O circulating in cattle population of the most affected farms of Feni and Lakshimpur districts of Bangladesh.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease that affects cloven-hoofed animals such as cattle, pigs, sheep, goats, and deer (Brooksby, 1982). The virus that causes FMD belongs to the genus Aphthovirus within the family Picornaviridae, is a single-stranded, positive-sense RNA virus and there are seven serotypes of the virus that have been identified (O, A, C, SAT1, SAT2, SAT3, and Asia 1). FMD is endemic in many parts of the world, particularly in Africa, Asia, and South America, and occasional outbreaks occur in other regions (Grubman et al., 2004).

The clinical signs and symptoms of FMD can vary depending on the species of animal that is infected and the strain of the virus that is causing the infection. In general, the disease is characterized by the development of vesicular lesions, or blisters, on the mouth, feet, and teats of infected animals (Knowles et al., 2003). These lesions can be painful and can cause animals to become lame, have difficulty eating or drinking, and experience weight loss. In cattle, FMD can also cause fever, increased salivation, and the development of vesicular lesions on the tongue and oral mucosa.

FMDV is transmitted through various routes, including direct contact, contaminated fomites, and airborne transmission. Direct contact is the primary route of FMDV transmission. Infected animals shed the virus in their saliva, nasal secretions, and feces, and healthy animals become infected by coming into contact with these secretions. Contact between infected and healthy animals can occur through nose-to-nose contact, licking, and mutual grooming. The virus can also be transmitted through contaminated feed and water, as well as through the environment, such as soil, manure, and equipment. The virus can remain viable in the environment for several days, and contaminated fomites can transmit the virus to

How to cite this article: Hayat S, Azad HMJ, Sadekuzzaman M, Chowdhury MGA and Islam MA (2022). Molecular detection and serotyping of Foot and Mouth disease viruses (FMDV) from cattle in Bangladesh. International Journal of Natural and Social Sciences, 9(4): 27-32. DOI: 10.5281/zenodo.7941704

susceptible animals. Airborne transmission of FMDV occurs when the virus is aerosolized and can travel through the air over long distances (Grubman et al., 2004, Patonet al., 2015).

The disease has a significant impact on the livestock sector in Bangladesh, which is an essential source of food and income for millions of people. FMD is endemic in Bangladesh, and outbreaks occur regularly throughout the country. The disease has a severe economic impact on the livestock sector, which contributes to over 3% of the country's Gross Domestic Product (GDP). According to a study by Ahmed et al. (2019), the economic losses due to FMD in Bangladesh were estimated at USD 1.5 billion over a ten-year period from 2005 to 2015. The losses were attributed to decreased milk production, weight loss, mortality, and the cost of disease control measures.

FMDV is considered one of the most economically important animal diseases due to its impact on trade and food security. Rapid and accurate detection of FMDV is essential for controlling the spread of the disease. There are various methods for the detection of FMDV, including virus isolation, serology, and molecular methods. Virus isolation is the gold standard method for the diagnosis of FMDV, but it is time-consuming and requires a laboratory with high biosecurity measures.

Molecular methods, such as real time reverse transcription-polymerase chain reaction (RT-PCR), have become the preferred method for the detection of FMDV due to their sensitivity, specificity, and speed (Reid et al., 2003). RT-PCR has been used for the detection of FMDV in various sample types, including serum, saliva, nasal swabs, and tissue samples (Marquardt et al., 1995).

Several studies have reported the successful detection of FMDV using RT-PCR. For example, a study conducted by Parida et al. (2006) developed a real-time RT-PCR assay for the detection and quantification of FMDV RNA in clinical samples. The assay was able to detect all seven serotypes of FMDV and had a sensitivity of 0.1 $TCID_{50}$ (50% tissue culture infectious dose) per reaction.

The aim of this study was to use a RT-PCR for the detection of FMDV in clinical samples obtained from FMD symptomatic cattle in Bangladesh.

MATERIAL AND METHODS

Description of the study areas

This research work was implemented in Feni and Lakshmipur districts, which are located in south-estern part of Bangladesh. Feni is Located at an elevation of 12 meters (39.37 feet) above sea level, Feni has a Tropical monsoon climate (Classification: Am). The district's yearly temperature is 26.71ºC (80.08ºF) and it is -1.03% lower than Bangladesh's averages. Feni typically receives about 139.3 millimeters (5.48 inches) of precipitation and has 156.54 rainy days (42.89% of the time) annually. Located at an elevation of 9.05 meters (29.69 feet) above sea level, Lakshmipur has a Tropical monsoon climate (Classification: Am). The district's yearly temperature is 26.0ºC (78.8ºF) and it is -1.74% lower than Bangladesh's averages. Lakshmipur typically receives about 135.6 millimeters (5.34 inches) of precipitation and has 152.38 rainy days (41.75% of the time) annually. Total number of cattle was 2,39,477 in Lakshmipur and 1,42,191 in Feni district (Official website)

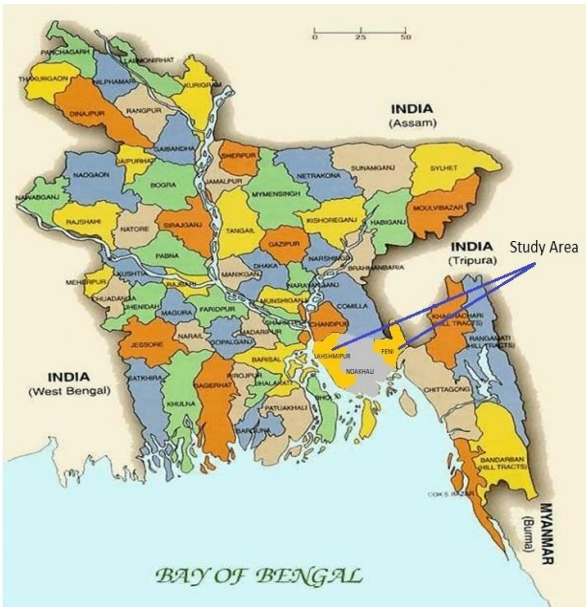

Figure 1: Sample collection location

Sample collection

The animals those exhibited clinical signs of FMD infection such as salivation, ulceration, erosion on the tongue and mouth. Tongue epithelium was collected using sterile forceps or a tongue scraper; gently scrape the affected area on the tongue. The tissue samples were placed immediately into sterile transport media and all the tubes were labeled clearly with the animal's identification number, sample collection date, and other relevant information.

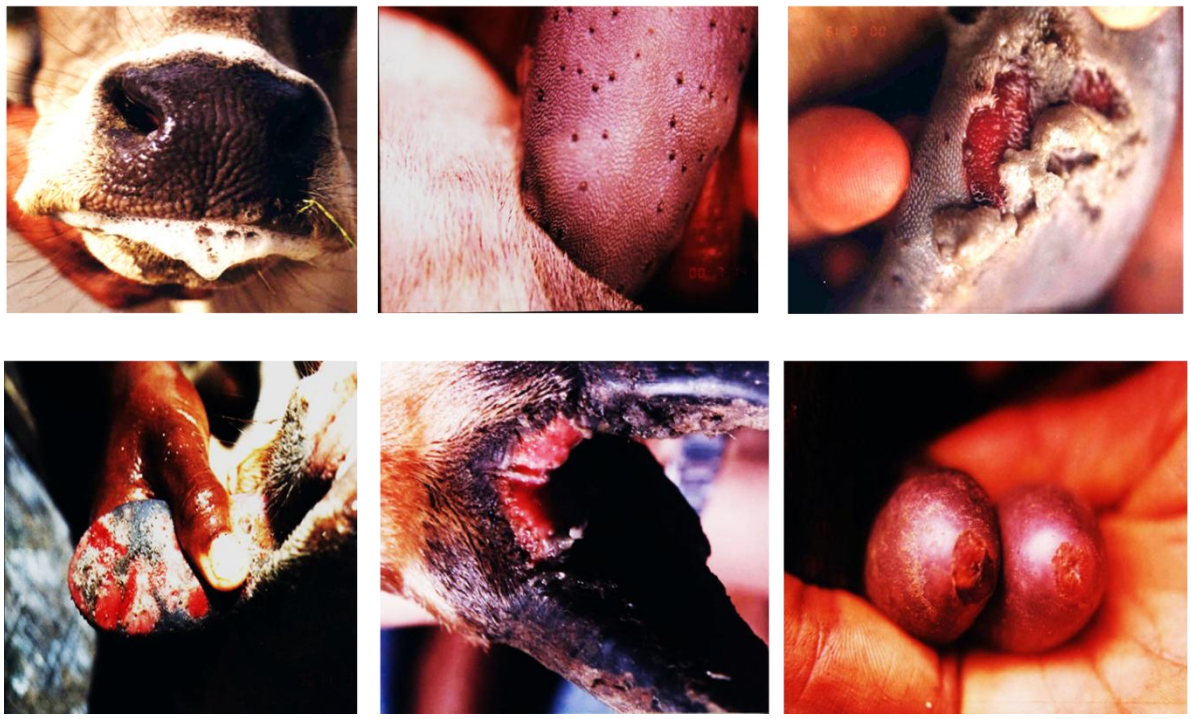

Figure 2: Clinical signs of FMD affected cattle

During the outbreaks in 2022, a total of 50 diagnostic samples (tongue epithelium) were collected from clinically infected cattle of Feni and Lakshmipur districts of Bangladesh and transported to Central Disease Investigation Laboratory (CDIL), Dhaka, Bangladesh for molecular detection.

Sample preparation

A 10% (wt/vol) suspension of each tissue sample was prepared with 0.04 M phosphate buffer for real-time qRT-PCR analysis.

Extraction of viral RNA

Automated systems were used to generate nucleic acid (RNA) template from each sample for real-time qRT-PCR. Viral RNA was extracted using MagMAX™ viral RNA isolation kit in a King Fisher (Thermo Fisher scientific ™) automated nucleic acid extraction machine according to the manufacter's protocol. Following extraction of viral RNA from tissue samples, RNAs were preserved at – 80 °C freezer for further use.

Real time qRT-PCR amplification

In this assay, AgPath-ID™ One-Step RT-PCR Kit, was used in which 25 µl qRT-PCRs contained 2 µl of each primer, 1µl of TaqMan® probe, 12.5 µl of master mix and 5 µl of extracted viral RNA from the clinical sample and control samples. One-step

reverse transcription and PCR amplification (Bio-Rad CFX 96™) was performed using the following cycling program: 60°C for 30 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Serotype-specific primers and probes for serotypes O, A and Asia-1 FMDVs were used as described early by Belsham and Jamal (2015).

Table 1: Primer and probe sequences for real time qRT-PCR (Belsham and Jamal, 2015).

Name of Primer/probe	Orientation	Serotype	Sequence (5'– 3')
O-JB-F	Forward	O	GAGACAGCGTTGGAYAACACC
O-JB-R	Reverse	O	TGWGGTGCCGTGTAAGGCAG
O-JB-F-P	------	O	Fam—AATCCAACGGCTTACCACAAGGCACC—Tamra
A-JB-F	Forward	A	GCCACGACCATCCACGAGCT
A-JB-R	Reverse	A	GTCCTGYGACRACACTTCCAC
A-JB-F-P	------	A	Fam—CTCGTGCGYATGAAACGTGCYGAGCT—Tamra
As-JB-F	Forward	Asia-1	TGCCYACYTCXTTYAAYTACGG
As-JB-R	Reverse	Asia-1	CARAGGYCTRGGGCAGTATGT
As-JB-F-P	--------	Asia-1	Fam—CGTTTCATGCGRATYAAMAGCTCAGTGAT—Tamra

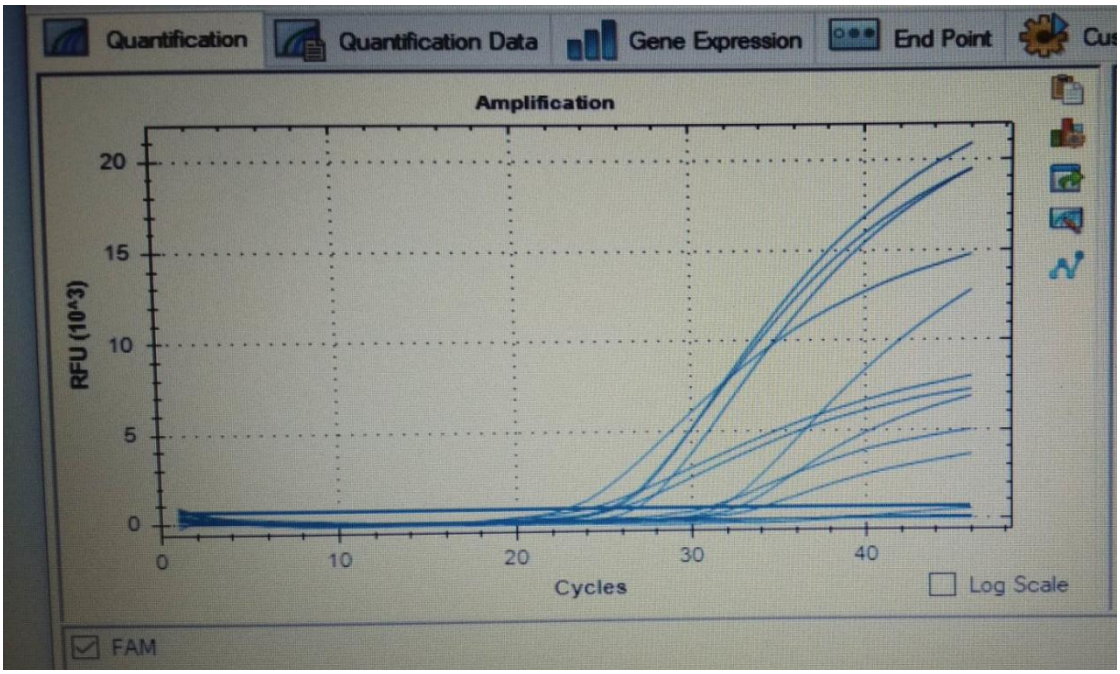

Figure 3: Real time RT-PCR amplification by serotype-specific primers and probes for serotypes O, A and Asia-1 FMDVs

RESULTS

Clinically positive 50 tissue samples (tongue epithelium) were evaluated by RT-PCR for the diagnosis of FMD with different pairs of universal, A and O serotype-specific primers. Unknown samples producing a curve above threshold value (ct) were considered positive (Figure 3).

Our results showed that the samples of Feni district were 83% and 87% of FMD PCR positive for the male and female cattle respectively. Almost

similar result observed with the samples of Lakshimpur district, which were 90% and 85% among the male and female cattle (Table 2).

Table 2: RT-PCR results based on age and sex

Location	Age (Years)	Sex	Samples No.	Positive cases
Feni	1-8	Male	12	83%
	1-8	Female	15	87%
Lakshmipur	1-8	Male	10	90%
	1-8	Female	13	85%

Our result demonstrated that among the tested samples from Feni district 92% were FMDV "O" and 8% were FMDV "A" positive. In Lakshimpur district, 95 % of the cases were FMDV "O" serotype and 5% of the samples were FMDV" A" serotype. But in both the districts there was no positive cases Asia -1 sero-type of FMDV in this study.

Table 3: FMDV serotyping

District	Sample tested	FMDV Serotype	Serotype percent
Feni	27	O	92%
		A	8%
		Asia-1	0%
Lakshimpur	23	O	95%
		A	5%
		Asia-1	0%

DISCUSSION

Various techniques are in use for diagnosis of FMD including antigen capture ELISA, virus isolation, conventional and real time qRT-PCR assays (Jamal et al., 2013). In order to control FMD outbreaks, an early and accurate identification of the causative FMDV is very important. Virus detection is usually carried out using pan-FMDV FMDV RT-qPCR assays that have been extensively evaluated for routine use (Callahan et al., 2002; Moniwa et. al., 2007; Reid et al., 2003). Simple detection of FMDV using this assays is, however, not sufficient in countries where prophylactic vaccination is used. Serotype identification is crucial to allow selection of the most appropriate vaccine, at least at the serotype level, as there is no cross-protection. Ideally the vaccine should be well matched to the outbreak virus but often there are practical limitations e.g. in terms of vaccine availability, and vaccine matching studies or sequence determination can take some time.

Real-time RT-PCR assay is widely used for the laboratory diagnosis of FMD and other important human and veterinary diseases. Being RNA in nature, FMD genome is very sensitive to degradation by RNAses and other degradative enzymes. If a sample is collected during the early phase of the infection (especially from vesicles), chances of viral amplification by RT-PCR are higher, but if the lesions get invaded by bacteria, or lesions start healing, the probability of obtaining the intact viruses from samples decreases drastically. Some samples were collected in the late phase of infection and the viral genome may have been degraded by bacterial RNAses and other degradative enzymes resulting in either weak or no detectable signals by RT-PCR. In the TaqMan RT-qPCR assays, the amount of amplified DNA is measured after each cycle of amplification via dyes that generate fluorescent signals, the magnitude of which is proportional to the amount of the amplicon generated. The amplification curves, generated by plotting the fluorescence against the number of cycles, represent the accumulation of product over the duration of the reaction.

The current report describes the performance of real-time RT-PCR during the outbreaks of FMD that occurred in Bangladesh 2022. In this study RT-PCR was performed on 50 clinical samples. In order to detect FMD viral RNA, regardless of their serotypes the primer-probe was used in the RT-PCR. This primer-probe was used in several international studies (Jamal et al., 2013). Our study found that serotype O was the predominant serotype in this country while serotype A was the second serotype. The present result is in agreement with the other local and international studies (Islam et al., 2017) which further confirm the presence of serotype O in Bangladesh.

The findings of this study revealed that RT-PCR assay is suitable for the reliable rapid and accurate detection of FMD virus in clinical samples in Bangladesh.

CONCLUSION

qRT-PCR, have become the preferred method for the detection of FMDV due to its sensitivity, specificity, and rapidity. This method has been successfully used for the detection of FMDV in various sample typing and has the potential to be used for the surveillance and control of FMDV outbreaks in Bangladesh. Adoption of this qRT PCR for rapid and sensitive diagnosis of FMDV in Bangladesh will definitely help the veterinary professional to take advantage of this modern technology for prevention and control of this economically devastating disease in Bangladesh.

Acknowledgements

The authors would like to extend their heartfelt gratitude and special thanks to the officers and staffs of Department of livestock services for their kind co-operation and help during collection, transportation and processing the field samples of FMD affected cattle of the two districts.

Conflict of Interest

The authors have no conflicts of interest to declare.

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