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Article

Molecular detection of foot and mouth disease virus serotype A in goats (*Capra hircus*)

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Abstract: Foot and mouth disease (FMD) is a highly contagious, transboundary, and re-emerging viral disease that mostly affects cloven-hoofed animals specially cattle, goats, swine, sheep, etc. resulting in significant economic losses. Despite regular vaccination, outbreaks of the disease have become a yearly occurrence across the country. RT-PCR was used to determine the prevalence and molecular detection of serotype A of FMDV in clinically affected goats in Goat research farm, BLRI, Savar, Dhaka, Bangladesh during August 2018. A total of 9 samples were taken from 1 to 2 years old Black Bengal goats suspected to have FMD. FMDV was detected in 55.56% (5/9) of the suspected samples using RT-PCR. The serotype of positive samples was also determined using gsRT-PCR. However, FMDV serotype A was prevalent in 100% (5) positive samples. Additionally, considering the age, the prevalence of confirmed FMD outbreak was 40% (2), 40% (2), and 20% (1) at the age of 1, 1.5 and 2 years, respectively. It was found that young goats are more susceptible to FMDV than adults. However, it can be concluded that suspected goats were infected with FMDV serotype A and trivalent FMD vaccine is suggested for prevention and control of FMD outbreak.

Keyword: FMD; goat; PCR; prevalence; serotype

1. Introduction

Goat is a member of the genus Capra, of the mammalian order Ungulata. There are about 300 breeds and varieties of goats domesticated in this subcontinent number of goats is about 30.33 million in Bangladesh (Banglapedia, 2021). More than 90% of the goats in Bangladesh are kept by rural people. The goat ranks second in position in terms of milk production and fourth position in terms of number and meat production (Banglapedia, 2021; Bary *et al*., 2018; DLS, 2019). Goat also called poor man's cow in Bangladesh (Sayeed *et al.*, 2020; Kashem *et al.*, 2011). The surroundings of our country is extremely favorable to cause various diseases of the animal (Munsi *et al.*, 2018; Ali *et al.*, 2019). Foot and mouth disease (FMD) is a most important contagious, transboundary and re-emerging viral disease affecting artiodactyl, mostly cattle, swine, sheep, goats and many species of wild ungulates (Ur-Rehman *et al.*, 2014; Forman *et al.*, 2009; Brooksby, 1982) and its local name is "Khura Rog" in Bangladesh (Ali *et al.*, 2019). Its etiologic agent is FMD virus (FMDV) is a member of Aphthovirus genus and family Picornaviridae which poses with single-stranded and positive-sense RNA genome (Ali *et al.*, 2019; Esmaelizad *et al.*, 2011) and the disease is characterized by fever, lameness and

vesicular lesions on the feet, tongue, snout and teats, with high morbidity and low mortality (Depa *et al.*, 2012; Domingo, 2003). Lameness is usually being the first sign of FMD in sheep and goats and then develops fever, reluctant to walk and separate itself from the rest of the flock (Kitching and Hughes 2002). Small ruminants (sheep and goats) are susceptible to foot-and-mouth disease (FMD), while studies with due emphasis on their role in the disease epidemiology have been meagre (Rout *et al.*, 2016; Ali *et al.*, 2020). In Turkey, 18.5% of the total FMD cases reported in 1996 were linked with small ruminants (Taylor and Tufan 1996). On the basis of serology, there are seven recognized serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia1) and about 65 subtypes of FMDV (Kitching *et al.*, 2005; Domingo *et al.*, 2003; Knowles and Samuel 2003; Brown *et al.*, 1991). All FMDV serotypes are immunogenically different and vaccination with one serotype does not develop immunity against other serotype or subtypes of a serotype (Paton *et al.*, 2005). Serotypes 0, A, C and Asia-1 have been circulating in Bangladesh (Islam *et al.*, 2021; Jannat *et al.*, 2019; Ali *et al.*, 2019; Nandi *et al.*, 2015; Loth *et al.*, 2011; Marquardt *et al.*, 2000). Our bordering countries also circulated the same FMD serotypes (Mahapatra *et al.*, 2017). Serotype A is considered to be the most diverse of the Eurasian serotypes both genetic and antigenic (Mohapatra *et al.*, 2011). Within the seven serotypes, serotype A displays the greatest number of newly occurring subtypes, which makes the control by vaccination very difficult (Kitching, 2005). So, this study was conducted for molecular detection of FMD Virus Serotype A in non-vaccinated goats.

2. Materials and Methods

2.1. Study area

In August 2018, the FMD outbreaks in goats were suspected in Goat research farm of BLRI in Savar Upazila of Dhaka district. It is located geographically at 23.8887^0N and 90.2739^0N with average annual rainfall was 1854 mm and temperature that ranges from 15 to 36^0C. Semi-intensive method was performed for all goats and goats were grazing for 7 hours in the selected land of BLRI from 8 AM to 3 PM. Concentrate feeds were given as per standard feeding schedule. During this study no history of FMD vaccination to the goats.

2.2. Clinical examination

After being informed about the presence of FMD in goat, clinical examination was done by an expert team on several herds in Goat research farm of BLRI. All clinical signs were recorded after taking the case history of the diseases and the vaccination programs.

2.3. Preparation of virus transport media (VTM)

The VTM prepared by equal volumes of glycerol and phosphate-buffered saline (PBS) (pH 7.2-7.6) with 2% antibiotic-antimycotic (Giasuddin *et al.*, 2016).

2.4 Clinical sample collection

A total of 9 clinical samples were collected from FMD suspected goats of BLRI Goat research farm during August, 2018. All clinical samples were collected from FMD suspected goats showed lameness, loss of appetite, fever, anorexia, salivation. Clinical samples include tongue and interdigital space epithelial tissue samples and saliva were collected (Figure 1). The tissue samples were collected according to the guideline of OIE Terrestrial Manual (OIE, 2010). Samples were immediately transported to the FMD Research laboratory of Animal Health Research Division, BLRI, Savar, Dhaka in VTM on a cool box containing ice.

Figure 1. Vesicles in disruption of interdigital space epithelial tissue, gum and tongue epithelia of affected goat.

2.5. Inoculum preparation and RNA extraction

A piece of the epithelial tissue was removed from the VTM, 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 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. RNA was quantified by spectrophotometric analysis. PCR mastermix volume per reaction was 25 µl was given below in Table 1 and List of Oligonucleotide primers used for universal FMDV and serotyping of FMDV by RT-PCR was given below in Table 2.

Table 1. RT-PCR mastermix reaction.

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

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		
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	Callens and De Clercq, 1997
	P33	AGCTTGTACCAGGGTTTGGC	2B		

2.6. 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 2. 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 secs (type A) 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 trans illuminator (AlphaImager®Mini System, USA).

3. Results

Among these 9 samples, 5 samples were positive and 4 samples were negative for FMDV by RT-PCR. After that, the RT-PCR positive samples (5 samples) were further subjected to gsRT-PCR for confirmation of serotypes of FMDV by using gene specific primers. Where all FMDV serotypes were identified as serotype A (Figure 2). The product amplicon size of gsRT- PCR was 732 bp for FMD serotype A (Figure 2).

The overall prevalence of FMDV was 55.56% in clinically infected goat. It may due to vesicular, eruption of hoof and tongue epithelia could be the major source of FMD virus shedding. However, considering the age the prevalence of confirmed FMD outbreak was 40% (n=2), 40% (n=2) and 20% (n=1) at the age of 1, 1.5 and 2 years respectively in goats from Goat research farm in BLRI, Savar, Dhaka shown in Table 3. It was seeming that young animals are more susceptible to FMD than adults.

Table 3. Prevalence of FMD according to age in goats.

Age (years)	RT-PCR positive samples (N =05)	Prevalence (%)
1	2	40
1.5	2	40
2	1	20

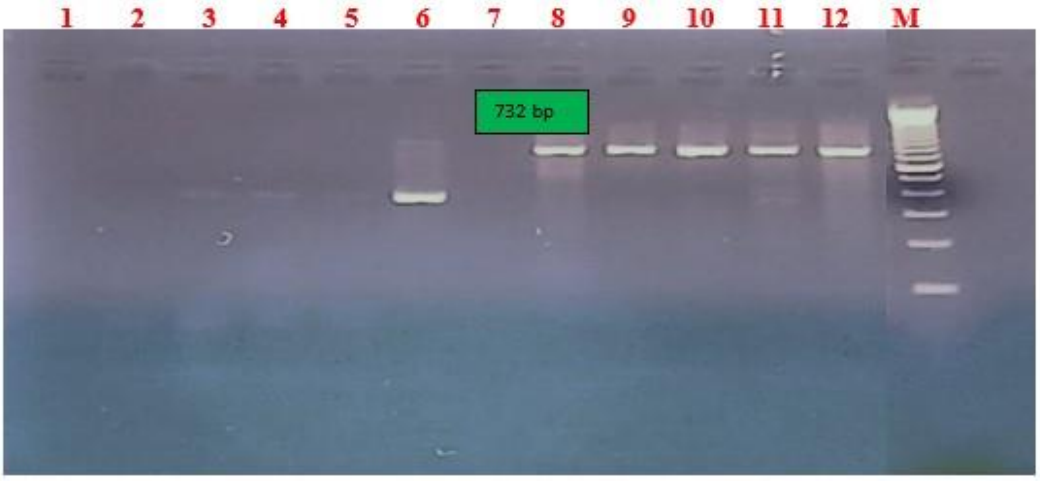

Figure 2. Showing the specific band of gsRT-PCR products of representative field samples in 2% agarose gel electrophoresis. Lane: 1, 2, 3 = negative control (RNase-free water), Lane: 4, 5. 6, 7 = negative samples, Lane: 8, 9, 10, 11, 12 = serotype A, Lane- M = 100 bp DNA ladder (Invitrogen, USA).

4. Discussion

Foot and mouth disease (FMD) is one of the most economically demoralizing diseases of ruminants all over the world (Rodriguez *et al.,* 2011). Bangladesh has been considered as an endemic country of FMD. It causes massive economic impact to the livestock population and economy in this country (Giasuddin *et al*., 2020). According to Nelson *et al*. (2017), the FMD outbreak should be handled at an early stage in order to implement effective control measures and avoid further transmission. Because of its airborne transmission style, the FMD virus can spread quickly from the commencement of shedding to adjacent locations. 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 RT-PCR were used to confirm the FMDV genome from clinical samples of different outbreaks in farm animals. For the RT-PCR, 328 bp DNA fragment was amplified for any serotypes of FMDV. Among the 9 samples, 5 were found positive by RT-PCR. Detection rate of FMDV by RT-PCR was 55.56% which is become close to Islam *et al.* (2021), Ali *et al.* (2019) and Giasuddin *et al*. (2017). Although the causes of failure of detection of FMDV from 4 (44.44%) field samples of this study is not very clear but this may be due to sample collection from recovered animals or might be treated with an antiviral agent before sampling.

There are three serotypes of FMDV circulating in cattle of Bangladesh including serotype A, O and Asia 1 (Giasuddin *et al.,* 2016; Ali *et al*., 2019). Serotyping confirmation of FMDV was done by gsRT-PCR using gene specific primers. In this study, 5 RT-PCR positive samples were tested to differentiate into serotypes. All the samples were identified as FMD serotypes A. The prevalence rate of serotypes was 100% for serotype A in Goat research farm, BLRI, Savar, Dhaka. These results are partially similar to the findings of Nandi *et al.* (2015), Alam *et al.* (2015), Hossen *et al.* (2014) 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 67.56% positive for serotype A and 20.00% for Asia-1 in Pabna district. Considering the age, the prevalence of FMD was 40% (n=2), 40% (n=2) and 20% (n=1) at the age of 1, 1.5 and 2 years respectively in goats which nearly similar to the findings of Islam *et al.,* (2021). Results of the present study indicated that FMDV serotypes A was prevailing in goats of Goat research farm, BLRI, Savar, Dhaka.

5. Conclusions

This research work was conducted during the start of the outbreak of FMDV in goats at BLRI Goat Research Farm, Savar, Dhaka. The results were 100% positive to serotype A which means it is the predominant infective serotype in goats at our study area. It may help in production of the vaccine to make a preventive plan for the disease. However, results show that young goats are more susceptible to FMD than adults. The investigation could not conclude the source of the outbreak and its need for further epidemiological study.

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Conflict of interest

None to declare.

Authors' contributions

Md Habibur Rahman, Sonia Akther, Md Zakir Hassan, Md Zulfekar Ali and Md Giasuddin are equally contributed for sample collection, processing and molecular detection of FMDV in goats. Md Habibur Rahman wrote the first draft of this article and all other authors were carried out to review and submit according to guidelines. All authors have read and approved the final manuscript.

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