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. result 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 O, 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°N and 90.2739°N with average annual rainfall was 1854 mm and temperature that ranges from 15 to 36°C. 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 manufacturer’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)AT TGACCT(G:A:T:C)ATGCA (G:A:T:C) AC (G:A:T:C) CAC | 1D       | 732               | Callens and De Clercq, 1997 |
|          | P33         | AGCGTTGTACCAGGTTTGGC        | 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 (AlphaImage®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.

References
Abu-Elzein EME, FMT Housawi, Y Bashareek, AA Gameel, AI Al-Afaleq and EC Anderson, 2004. Severe PPR infection in Gazelles kept under semi-free range conditions in Saudi Arabia. J. Vet. Microbiol., 51: 68-71.
Alam MA, M Rahman, ML Hossen, S Ahmed, S Parvej, MFR Khan and MB Rahman, 2015. Reverse transcription polymerase chain reaction (RT-PCR) based detection and serotyping of FMD Virus from field samples of Gazipur, Bangladesh, and adaptation of the virus in BHK-21 cell. J. Adv. Vet. Anim. Res., 2: 291-295.
Ali MZ, G Carlile and M Giasuddin, 2020. Impact of global climate change on livestock health: Bangladesh perspective. Open Vet. J., 10: 178-88.
Ali MZ, E Islam and M Giasuddin, 2019. Outbreak investigation, molecular detection, and characterization of foot and mouth disease virus in the Southern part of Bangladesh. J. Adv. Vet. Anim. Res., 6: 346-354.
Bary MA, AZI Ali, S Chowdhury, A Mannan, M Nur e Azam, MM Moula, ZA Bhuiyan, MT Shaon and MA Hossain, 2018. Prevalence and molecular identification of haemoprototozoan diseases of cattle in Bangladesh. Adv. Anim. Vet. Sci., 6: 176-82.
Brooksby JB, 1982. Portraits of viruses: foot-and-mouth disease virus. Intervirology., 18: 1-23.
Brown CC, HJ Olander and RF Meyer, 1991. A Preliminary Study of the Pathogenesis of Foot-and-mouth Disease Virus using in situ hybridization. Vet. Pathol., 28: 216-222.
Callens M and K De Clercq, 1997. Differentiation of the seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. J. Virol. Methods, 67: 35-44.
Depa PM, U Dimri, MC Sharma and R Tiwari, 2012. Update on epidemiology and control of foot and mouth disease - A menace to international trade and global animal enterprise. Vet. World, 5: 694-704.
Domingo E, C Escarmis, E Baranowski, CM Ruiz-Jarabo, E Carrillo and JI Núñez, 2003. Evolution of foot-and-mouth disease virus. Virus Res., 91: 47–63.
Esmaeïzad M, S Jelokhani-Niaraki, K Hashemnejad, M Kamalzadeh and M Lotfi, 2011. Molecular characterization of amino-niacin 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., 12: 363-371.
Forman S, F Le Gall, D Belton, B Evans, JL Francois, G Murray, D Shesley, A Vandersmissen and S Yoshimura, 2009. Moving towards the global control of foot and mouth disease: An opportunity for donors. Rev. Sci. Tech., 28: 883-896.
Giasuddin M, MZ Ali, MA Sayeed and E Islam, 2020. Financial loss due to foot and mouth disease outbreak in cattle in some affected areas of Bangladesh. Bang. J. Livest. Res., 27: 82-94.
Giasuddin M, MS Mahmud, MA Al Asari and S Akter, 2017. Occurrence of Foot and Mouth Disease (FMD) during 2014-2016 in cattle of Sirajganj district, Bangladesh. Jahangirnagar Uni. J. Biol. Sci., 6: 45-49.
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Giasuddin M, MS Mahmud, SM Alam, MA Samad, MR Islam, MD Ahasan, MH Rahman, MR Karim and P Acharchee, 2016. Molecular epidemiology of foot-and-mouth disease viruses circulated in Bangladesh from 2011–2014. Br. Microbiol. Res. J., 16: 1-3.

Giridharan P, D Hemadri, C Tosh, A Sanyal and SK Bandyopadhyay, 2005. Development and evaluation of a multiplex PCR for differentiation of foot-and-mouth disease virus strains native to India. J. Virol. Methods, 126: 1-11.

Hossen ML, S Ahmed, MFR Khan, MT Rahman, S Saha, KHMNH Nazir, MA Islam and MB Rahman, 2014. Typing of foot and mouth disease virus circulating in Bangladesh by reverse transcription polymerase chain reaction. J. Vet. Adv., 4: 778-785.

Islam MR, MR Akter, MZ Hassan, MH Rahman, E Islam, MA Khan, A Chakrabarty and M Giasuddin, 2021. Identification of Foot and Mouth Disease (FMD) virus from recently outbreak crossbred cattle in Rajbari District of Bangladesh. SAARC J. Agric., 19: 201-210.

N Jannat, MS Rahman, E Islam, NA Rumi, M Giasuddin, M Hasan, MR Islam and MZ Hassan, 2019. Seroprevalence and molecular detection of FMDV in cattle at Savar in Bangladesh. SAARC J. Agric., 17: 67-78.

Kashem MA, MA Hossain, SU Ahmed and MA Halim, 2011. Prevalence of diseases, morbidity and mortality of Black Bengal Goats under different management systems in Bangladesh. Uni. J. Zool. Rajshahi Uni., 30: 1-4.

Kitching RP and GJ Hughes, 2002. Clinical variation in foot and mouth disease: sheep and goats. Revue scientifique et technique (International Office of Epizootics). 21: 505-12.

Kitching RP, 2005. Global epidemiology and prospects for control of foot-and-mouth disease. Curr. Top. Microbiol. Immunol., 288: 133-148.

Kitching RP, AM Huber and MV Thrusfield, 2005. A review of foot and mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease. Vet. J. 169: 197–209.

Knowles NJ and AR Samuel, 2003. Molecular epidemiology of foot-and mouth disease virus. Virus Res. 91: 65–80.

Loth L, MG Osmani, MA Kalam, RK Chakraborty, J Wadsworth, NJ Knowles and C Benigno, 2011. Molecular characterization of foot-and-mouth disease virus: implications for disease control in Bangladesh. Transbound. Emerg. Dis., 58: 240-246.

Mahapatra M, S Upadhyay, S Aviso, A Babu, G Hutchings and S Parida, 2017. Selection of vaccine strains for serotype O foot-and-mouth disease viruses (2007–2012) circulating in Southeast Asia, East Asia and Far East. Vaccine, 35: 7147–53.

Mittal M, C Tosh, D Hemadri, A Sanyal and S Bandyopadhyay, 2005. Phylogeny, genome evolution, and antigenic variability among endemic foot-and-mouth disease virus type A isolates from India. Arch. Virol. 150: 911-928.

Mohapatra JK, S Subramaniam, LK Pandey, SS Pawar, A De, B Das, A Sanyal and B Pattnaik, 2011. Phylogenetic structure of serotype A foot-and-mouth disease virus: global diversity and the Indian perspective. J. Gen. Virol., 92: 873-9.

Momtaz S, A Rahman, M Sultana and MA Hossain, 2014. Evolutionary analysis and prediction of peptide vaccine candidates for foot-and-mouth-disease virus types A and O in Bangladesh. Evol. Bioinfom., 10: 187.

Nandi SP, MZ Rahman, S Momtaz, M Sultana and MA Hossain, 2015. Emergence and distribution of foot-and-mouth disease virus serotype A and O in Bangladesh. Transbound. Emerg. Dis., 62: 328–31

Nelson N, DJ Paton, S Gubbins, C Colenutt, E Brown, S Hodgson and JL Gonzales, 2017. Predicting the ability of preclinical diagnosis to improve control of farm-to-farm foot-and-mouth disease transmission in cattle. J. Clin. Microbiol., 55: 1671-81

Office International des Epizooties (OIE), 2010. Foot-and-mouth disease, in Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals 2010

Paton DJ, JF Valarcher, I Bergmann, OG Matlho, VM Zakharov, EL Palma and GR Thomson, 2005. Selection of foot and mouth disease vaccine strains--a review. Rev Sci Tech., 24: 981-993

Reid SM, NP Ferris, GH Hutchings, AR Samuel and NJ Knowles, 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J. Virol. Methods, 89: 167-76.

Rodriguez LL and CG Gay, 2011. Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert Rev. Vaccines, 10: 377-387.

Rout M, S Subramaniam, JK Mohapatra and B Pattnaik, 2016. Clinico-molecular diagnosis and phylogenetic investigation of foot-and-mouth disease in small ruminant population of India. Small Rumin. Res., 144: 1-5.
Sayeed MA, MS Khatun, MS Bari, AK Dash, PK Haldar and BK Sarker, 2020. Prevalence of gynecological disorders of goat and pattern of drug used at Chuadanga, Bangladesh. Agr. Sci. Digest-A Res. J., 40: 424-429.

Taylor MN and M Tufan, 1996. Detailed investigations using farmer interviews to assess the losses caused by FMD outbreaks in Turkey. In: Report of Turkish-German Animal Health Information Project (GTZ), Ministry of Agriculture and Rural Affairs, Republic of Turkey, Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) GmbH-German Technical Cooperation, Eschborn, Germany.

Ur-Rehman S, M Arshad, I Hussain and Z Iqbal, 2014. Detection and seroprevalence of foot and mouth disease in sheep and goats in Punjab, Pakistan. Transbound. Emerg. Dis., 61: 25-30.

Vangrysperre W and K De Clercq, 1996. 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., 141: 331-44.