

Article

Molecular detection and adaptation of FMD virus serotype type O in BHK-21 cell line by RT-PCR

Md. Ahsan Ullah¹, Md. Najmol Hoque^{2*} , Khondoker Md Nasiruddin³, Md. Bahanur Rahman⁴  and Farjana Rauf⁵ 

¹Department of Agricultural Extension, The Government of the People's Republic of Bangladesh

²Department of Biochemistry and Molecular Biology, Khulna Agricultural University, Khulna-9202, Bangladesh

³Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

⁴Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

⁵Department of Seed Science and Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

*Corresponding author: Md. Najmol Hoque, Department of Biochemistry and Molecular Biology, Khulna Agricultural University, Khulna-9202, Bangladesh. E-mail: mdnajmol.hoque@ndsu.edu

Received: 24 February 2023/Accepted: 26 April 2023/Published: 04 May 2023

Copyright © 2023 Md. Ahsan Ullah *et al.* This is an open access article distributed under the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract: One of the viral diseases affecting both domestic and wild animals in many countries around the world is foot and mouth disease (FMD). The livestock sector has been suffering significant financial losses for many years as a result of the disease. The present research work was focused on the isolation, documentation and serotyping of the Foot and Mouth Disease (FMD) virus from different locations. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was used to detect the FMD virus (FMDV) serotype-O that is currently circulating in the BHK-21 cell line. All the tested samples were positive for FMDV and specific for serotype-O was 64.67-52.67% from tongue epithelium tissues and 30.67-16.67% from foot tissues respectively based on locations. In BHK-21 cell lines, the viruses caused cytopathic effects such as rounding, enlargement, intercellular bridge breakdown, and finally, death of cells, which shows the presence of the FMD virus under an inverted microscope. The BHK-21 cell culture fluid was found positive for FMDV in cell culture and also was positive based on CPE. Once more, the resulting inoculum was used to serotype the virus using RT-PCR. Based on the result of the RT-PCR method, the conclusion reached was that the serotype of the FMD virus was 'Type O'.

Keywords: FMD virus; serotype type O; cattle disease; RT-PCR; Bangladesh

1. Introduction

A highly contagious disease is Foot-and-mouth disease (FMD) which affects both local and wild cloven-hoofed animal species (Gortázar *et al.*, 2022). Foot-and-mouth disease virus (FMDV) is a tiny, non-enveloped, proactive, single-stranded RNA that belongs to the genus Aphthovirus under the Picornaviridae family (Malik *et al.*, 2017). Seven immunologically unique FMDV serotypes such as A, O, C, Asia1, SAT-1, SAT-2, and SAT-3 are found worldwide (Ouagal *et al.*, 2018). Except from Asia1, the other six FMDV serotypes were present in the African continent from 1993 to 2012 (Lloyd-Jones *et al.*, 2017). Due to outbreaks, the Asia 1 serotype of

FMDV has been restricted to Asia, whereas SAT serotypes are widespread in the South African region (Bo *et al.*, 2019).

The disease also has both direct and indirect economic impacts because of restrictions on international traffic of animal products and animals from the afflicted nations (Kass *et al.*, 2022). The disease is common in many regions of the world, resulting in significant economic losses due to lower milk and meat production, increase livestock mortality, losing weight, miscarriages, late conception, and failure of draught capacity in animals, which often requires a lengthy recovery time after the sickness (Qasem, 2015; Soumya *et al.*, 2022). FMD is a deadly, destructive, extremely infectious, and clinically acute disease caused by multiple serotypes of FMD viruses in cloven-hoofed animals like cattle, pigs, lamb, goats, and additionally more than 70 other wild animal species (Wren *et al.*, 2022; Cecco *et al.*, 2022).

FMD was previously eliminated in certain developed nations such as the USA, Canada, Australia, England, and Japan through vaccination or by combusting infected animals, however, its reemergence poses a severe danger to both domestic and wild wildlife in such countries (Foxell, 2003; Neumann, 2012). As a result, FMD is now regarded as the most dangerous epizootic threatening illness in the globe (Lazarus *et al.*, 2015; Singh *et al.*, 2019). Bangladesh, India, Burma, Nepal, Pakistan, Cambodia, Laos, Vietnam, and Thailand are still affected by FMDV serotypes O, A, and Asia 1, where serotype 'O' has been a prevalent serotype in Southeast Asia for many years, particularly in Bangladesh (Shahiduzzaman *et al.*, 2016; Brito *et al.*, 2017; Blacksell *et al.*, 2019; Arju *et al.*, 2022).

FMD disease is an ongoing global danger to cattle health and production. It is critical to developing innovative methods of controlling this condition. In-vitro transfection tests in BHK-21 cells demonstrated that the viral structure was expressed in FMDV-specific antigen while not overproducing it (Dar *et al.*, 2021). FMD represents one of the key barriers to the progress of Bangladesh's cattle population. FMD-related yearly losses in Bangladesh have been estimated to be \$62 million (FAO/OIE, 2012). This virus, together with the other viral factors' constant change in attributes, makes it challenging for specialists in this sector to battle the disease (Rahman *et al.*, 2022). According to RT-PCR, FMDV serotypes O, A, and Asia-1 are actively prevalent in Bangladesh (Islam *et al.*, 2017). Combined infection of Type A and Asia-1 FMDV was also discovered. From 1960 to 1990, a seroepidemiological examination of this virus in the cattle population discovered that four different varieties (A, O, C, and Asia-1) of FMDV were common in Bangladesh (Islam *et al.*, 2017). FMDV serotypes A and O were prevalent in this country between 1996 and 2000 (Islam, 2001). According to a recent study, three distinct types (A, O, and Asia-1) were widespread in Bangladesh from 2007 to 2008 (Sarker *et al.*, 2015). Among these, serotype 'O' is associated with 82% of disease outbreaks, whereas Asia1 and A were responsible for 18% combinedly (Siddique *et al.*, 2018). The study of geographic locations and time revealed variable incidence rates. For instance in India, the Eastern area is infected by 43%, the Southern 31.5%, the Northeast by 11.6%, the Capital by 5%, the Western part by 4.4%, and the Northern part incidence is 4% of this serotype 'O' (Allen, 2016). On the other hand, the month with the most outbreaks is September, while the fewest is June (Yang *et al.*, 2023). Although FMD vaccinations are administered all through the country, still the disease continues to spread in many places of the country. FAO recently suggested that FMD surveillance and documentation have increased across the country, furthermore, all the suspected animals should be brought by under laboratory analysis. Therefore, this study's main objective was to isolate, identify and molecularly detect the Foot and Mouth Disease virus serotype "O" from tongue epithelial tissues of FMD-infected cattle, as well as its adaptation strategy in the BHK-21 cell line, to develop appropriate disease management approaches, along with effective vaccination progress in Bangladesh.

2. Materials and Methods

2.1. Ethical approval

No ethical approval is needed as samples were collected from living cattle tongue epithelial and foot tissues of the FMD-suspected animals.

2.2. Sample collection and preparation

A total of 600 samples (Baby hamster kidney cell- BHK-21 line) were collected from the FMD-suspected cattle from Gazipur [tongue epithelium (n=150); foot samples (n=150)] and Mymensingh [tongue epithelium (n=150); foot samples (n=150)] areas of Bangladesh and stored at - 80 °C. These specimens were utilized to look for FMD "O" type virus by one-step RT-PCR (Promega, USA). Subsequently, after confirmation of the serotype by RT-PCR, inocula were used to infect the BHK-21 cell culture for the observation of the cytopathic effect and isolation of the foot and mouth disease virus.

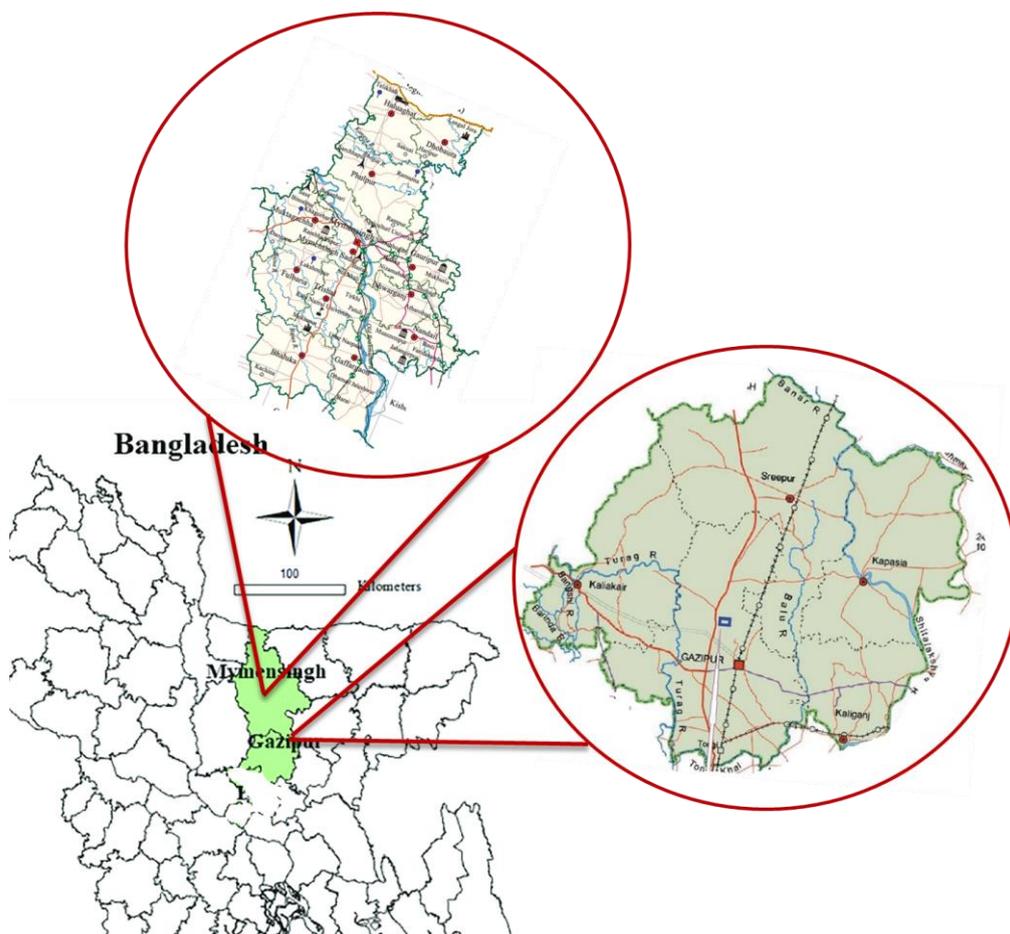


Figure 1. FMD viral samples collection areas viz. Mymensingh and Gazipur districts of Bangladesh.

2.3. Reagents and culture media preparation

Minimum Essential Medium (Sigma, Germany) and analytically graded inorganic reagents L- glutamine solution, 0.25 % Trypsin, Antibiotic-antimycotic solution, 6.3% sodium bicarbonate solution, Freezing medium (Caisson, USA), 70% Ethyl alcohol, Phosphate buffered saline (PBS), Fetal bovine serum (Bio-chrome, Sigma, Germany) were added to prepare culture media (Table 1).

About 9 gm of Dulbecco's powder was added to 1000 ml distilled water. Then it was heated until boiling and then it was autoclaved at 121 °C and 15 lbs pressure for 15 minutes and stored at 4 °C to 8 °C in the maintain a cool temperature until used. One hundred millilitres (100 ml) of 10x MEM was mixed with 100 ml of 10% fetal bovine serum (FBS), 20 ml L-glutamine solution, 6.6 ml of 6.3% NaHCO₃ and 10 ml of 200 mM 10X antibiotic-antimycotic solution. Finally autoclaved distilled water was added to up to 1000 ml in a sterile flask. This is the 1x MEM for the BHK-21 cell culture (Table 2).

Table 1. Ingredients of tissue culture media (Growth media, 1000 ml and pH: 7.2).

Sl	Ingredients	Amount
1.	Sterile Liquid MEM 10x (Minimum Essential Medium)	100
2.	Fetal bovine serum (FBS)	100
3.	L-glutamine, 200mM solution	20
4.	Antibiotic-antimycotic 100X solution	10
5.	Sodiumbicarbonate 6.3% solution	6.3
6.	Sterile deionized water	763.7
7.	Total	1000

Table 2. 1x MEM Preparation for FMDV infection/transportation: (1000 ml, pH: 8.2).

SI	Ingredients	Amount
1	10x Sterile Liquid MEM (Minimum Essential Medium)	100
2	Fetal bovine serum (FBS) (heat inactivated)	100
3	200mM L-glutamine, solution	20
4	100X Antibiotic-antimycotic solution	10
5	6.3% Sodium bicarbonate solution	12
6.	Sterile deionized water	758.4
	Total	1000

2.4. Subculture of BHK-21 cell culture

Before usage, the medium and reagents should all be kept at room temperature. After removing the growth medium from the flask containing the BHK-21 cell culture, the monolayer of cells were washed twice with sterile 1x PBS. To extract the cell from the flask, 1 ml of trypsin was added, mixed with the cell, and the flask was placed in the incubator for 5 minutes. About 20-30 ml of the conservation media was added to a 25 cm² flask. 8 ml of media containing cells was poured into a 25 cm² flask and 20 ml of maintenance media was poured into a 75 cm² flask and returned to the incubator. The flask was left for incubation at 37°C for 24 hours.

2.5. Adaptation of FMDV in BHK-21 cell culture

Within 24 hours of incubation, the cells in the culture flask that produced completely having confluent monolayers were chosen for viral infection. After removing the growing medium from the flask containing the BHK-21 cells, the monolayer cells had washed twice with sterile 1X PBS. In order to promote interaction, the cultured monolayer was infected with 1 ml of propagules made from 10% culture in PBS using the collected samples. The inoculation was spread throughout the cell sheet by rotating for 45–60 minutes. A 25 cm² flask containing 1*MEM supplemented with 2% heat-inactivated fetal calf serum was then filled with 10 ml of the maintenance medium, and the vessel was placed back in the incubator to incubate at 37 °C. As soon as the cells began to exhibit FMDV's characteristic cytopathic effect (CPE), they were inspected twice-daily under an inverted microscope.

2.6. Sterility test of inoculums

To remove bacterial and fungal contamination, all the collected samples were analyzed by adding 1ml of antibiotic antimycotic solution 100X (Sigma, Germany) in the required proportion. To determine the incidence of any kind of bacteria within the collected samples for the sterility test, a slight amount was placed into a bacteriological medium (BA).

2.7. Isolation of FMDV by cell culture

A subculture of BHK-21 monolayer cell culture was injected with an inoculum made from foot tissues and tongue epithelia that were removed from calves suspected of having FMD. The appearance of several types of distinctive changes CPE on to each field sample-infected cell culture allowed researchers to determine whether the virus was replicating there or not. The infectious fluid) containing FMDV was harvested after 48 to 72 hrs of post-infection and was kept at -20 °C until further use for molecular studies.

2.8. Molecular techniques for FMDV detection

Reverse Transcription Polymerase Chain Reaction (RT-PCR) for the detection of different serotypes 'O' of foot and mouth disease virus from the BHK-21 cell infected infectious fluid.

2.8.1. Reverse transcription-polymerase chain reaction (RT-PCR)

Viral RNA was extracted from the BHK-21 cell infected infectious fluid for molecular detection of the serotypes by RT-PCR, which involved extraction of RNA using RNA extraction Kit (Promega, USA), RT-PCR amplification of desired nucleic acid strands and analysis of RT-PCR products by gel electrophoresis. Every procedure was carried out in a very sterile environment. Precautions were taken to avoid cross-contamination and bacterial contamination.

The SV total RNA Isolation System was used to extract the RNA. A nuclease-free autoclaved eppendorf tube was filled with 1.175 µl of RNA lyses buffer, which was then completely mixed by inversion. RNA dilution buffer of 350 pl was then added, well mixed, and incubated at 70 °C for 3 minutes. After 10 minutes of

centrifugation at 12000 rpm, the combination generated a clear lysate that was transferred into a fresh tube. Following adding 200 μ l of 95% ethanol to the clear lysate and thoroughly mixing it with a gentle pipette, the eluate was centrifuged for one minute at 12000 rpm and discarded. The RNA Wash Solution was added to 600 μ l, centrifuged for 1 minute at 12000 rpm, and the eluate was discarded. Following that, a DNase incubation mixture was made using 40 μ l of yellow core buffer, 5 μ l of MnCl₂, and 5 μ l of DNase I. Pipetting was used to gently mix the fluid without creating a vortex. The membrane received 50 μ l of the DNase mixture, which was then incubated for 15 minutes at room temperature. After that, centrifugation was conducted for one minute at 12000 rpm using 200 μ l of DNase Stop Solution (DSA) and 600 μ l of RNA Wash Solution (Soumya *et al.*, 2022). The membrane was filled with nuclease-free water, centrifuged for one minute to extract the RNA, and then stored at -70°C. Viral RNA was extracted from the BHK-21 cell-infected infectious fluid for the purpose of molecular serotype identification by one-step RT-PCR. This study used RT-PCR system (Promega, USA) and made the reaction mixture, as per the instruction of the manufacturers. The FMDV oligonucleotide primer is used to detect FMDV and their serotyping were selected from published literature (Marupanthorn *et al.*, 2013). The employment of three sets of primers for the detection of FMDV respective of serotypes (Table 3). For serotype identification, three serotype-specific forward primers and reverse primers were selected (Table 3). A reference FMDV sample was used as the positive control. For FMDV types O, A, and Asia 1, the product lengths for each sample were verified and displayed at molecular weights of 1301, 866, and 911 bp, respectively.

Table 3. List of the primers used for the detection of FMD virus serotypes.

Serotype	Primer Designation	Sequence (5'-3')	PCR products size	Reference
O	FMDO F	ACC AAC CTC CTT GAT GTG GCT	1301	Marupanthorn <i>et al.</i> , 2013
	FMDO R	GAC ATG TCC TCC TGC ATC TG		

2.8.2. Gel electrophoretic documentation of the PCR product

PCR products were then electrophoresed at 100V for 30 minutes in TAE buffer on a 2% agarose gel, ethidium bromide (0.6 mg/ml) was applied to the gel for 10 minutes. In order to capture the images, the gel was carefully removed from the ethidium bromide, gently cleaned under running water, and then set up on the UV-transilluminator (Bio-Rad, USA).

3. Results and Discussion

FMD is a severe threat to Bangladesh's cattle population. Although the country undertakes regular vaccination programs against FMD year after year, the occurrence of this disease continued and spread in many places in Bangladesh (Hossen *et al.*, 2014). The present research was carried out with 600 FMD suspected cattle samples collected from two major cattle farming areas of the country for FMDV serotype 'O' identification with RT-PCR a recent concerning serotype using the BHK-21 cell line. All the collected samples were successfully proliferated and adapted for the FMDV virus. These samples demonstrated FMDV-specific cytopathic effect (CPE), such as rounding, thickening, cell aggregating to form giant cell and intercellular bridge breakdown (Figures 2 and 3), and lastly, cell death, which is an indicator of FMDV-infected cells. The finding of cell culture following infection with FMDV was correlated with the other findings (Nandi *et al.*, 2015; Ali *et al.*, 2019). The FMD-affected samples were then tested for FMDV serotype 'O' using an RT-PCR technique.

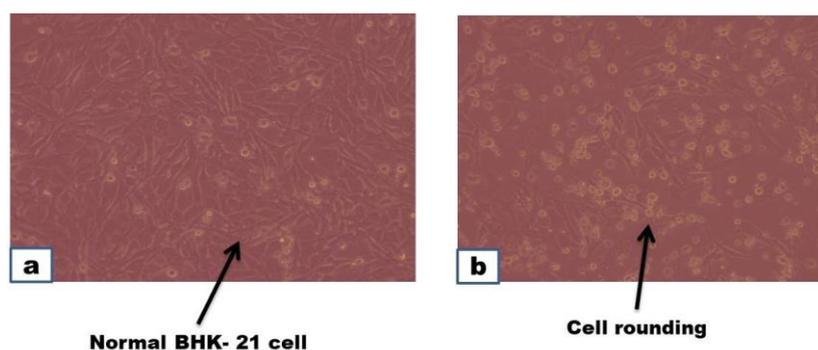


Figure 2. FMDV dissemination in BHK-21 cell line. a) Uninfected (healthy) BHK-21 cell line (elongated shape) b) Infected BHK-21 cell line (round and flat).

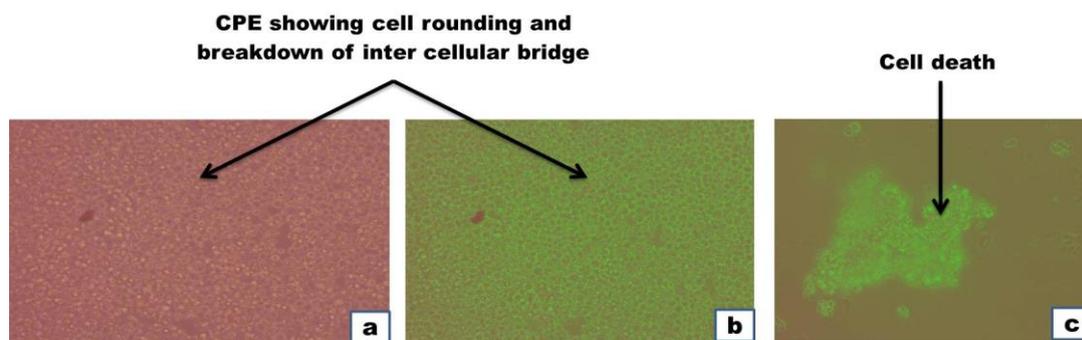


Figure 3. FMDV infected BHK-21 cell. a) 1st passage: initiation of infection and cell rounding started after 16 hours of infection; b) 2nd passage: almost 95% cell infected, after 36 hours of infection; c) 3rd passage: almost 100% cell infected, after 48 hours of infection (observed under 40x).

RT-PCR is a dependable, fast, accurate, and specific method for molecular characterization of infectious pathogens such as FMDV (Bachanek-Bankowska *et al.*, 2016). RNA was isolated from FMD-detected tissue culture that confirmed positive for FMDV in the BHK-21 cell line. This research did PCR after generating cDNA from FMDV viral extract RNA and discovered positive bands at 1301 bp following 2% agarose gel electrophoresis that was particular to FMDV serotype "O" (Figure 4). Utilizing FMDV serotype 'O' specific primers, RT-PCR revealed that out of 600 samples, tongue epithelium tissues from Gazipur (n=150) and Mymensingh (n=150) affected 97 (64.67%) and 79 (52.67%), respectively, where foot samples [Gazipur (n=150) and Mymensingh (n=150)] affected 46 (30.67%) and 25 (16.67%) respectively FMDV serotype 'O' (Table 4).

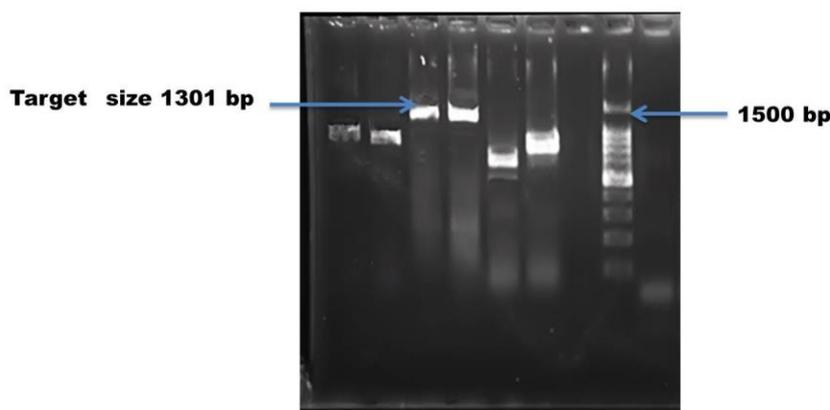


Figure 4. RT-PCR products in 2% agarose gel electrophoresis: Image: Lane 1-2 (positive control), Lane 3-4 (samples), Lane 5-6 (other samples), Lane 7 (negative control), Lane 8 (1000 bp DNA marker) where only Lane 3, 4 = positive PCR product of FMD virus serotype type O which showed the light band at 1301 bp.

Table 4. Detection of FMDV serotypes ‘O’ by one-step RT-PCR.

Location	Sources	No. of positive sample	Percentages (%)
Gazipur	Tongue epithelium (n=150)	97	64.67
	Foot samples (n=150)	46	30.67
Mymensingh	Tongue epithelium (n=150)	79	52.67
	Foot samples (n=150)	25	16.67

By analyzing all of the specimens collected from the two districts of Gazipur and Mymensingh, it was discovered that, in terms of positive RT-PCR results, Gazipur was more vulnerable to FMD Serotype O' than Mymensingh. The highest percentage of FMDV serotype O was detected in the BHK-21 cell line between the two types of field samples. The occurrence at Gazipur was 64.67% from tongue epithelia, which can be compared to earlier results (Islam *et al.*, 2001), who found that tongue epithelia samples tested positive for the virus in 51.43% of cases while foot tissue samples tested positive in only 30.67% of cases. In the case of the FMDV sample from Mymensingh, a similar variance was observed. The system for collecting samples, the area where samples were collected, and the seasons might all contribute to this difference. Reduced amounts of viral antigens may result from sample being exposed to higher temperatures, an inappropriate pH, or rancidity of the material (Saeed *et al.*, 2015). The present findings are comparable to those of Marupanthorn *et al.* (2013), who isolated viral RNA. For FMDV serotypes O, A, and Asia 1, the component lengths were verified and exhibited at molecular weights of 1301, 866, and 911 bp, respectively. Thus, using an RT-PCR-based molecular approach, we identified FMDV type O' from the collected viral sample. Moreover, this research also investigated tongue epithelia tissue more prominent for FMD type O' exposure than foot tissue.

4. Conclusions

The present research confirmed the type of FMDV currently circulating in the Gazipur and Mymensingh districts of Bangladesh as confirmed by RT-PCR. The RT-PCR methodology, accompanied by viral adaption in BHK-21 cells a very precise technique for FMDV isolation and detection that may be used to detect FMD viral serotype O'. In the future, the latest findings might be utilized to develop a vaccination against FMD serotype O' and determine the efficiency of a vaccine at the field level.

Acknowledgements

The authors want to express their sincere gratitude to the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, for providing laboratory facilities.

Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

None to declare.

Authors' contribution

Md. Ahsan Ullah: Conceptualization, Data curation, Writing–original draft. Md. Najmol Hoque: Data curation, Writing & editing –original draft. Md. Bahanur Rahman: Investigation, Writing – review & editing. Farjana Rauf: Resources, Formal analysis. Khondoker Md Nasiruddin: Investigation, Writing – review & editing. All authors have read and approved the final manuscript.

References

- 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.
- Allen B, 2016. The impact of the foot and mouth disease control pathway on milk production in India. PhD thesis, Purdue University ProQuest Dissertations Publishing.
- Arju S, S Nahar, MR Ara, MSA Sardar, ME Haque, MF Hossain, P Basak and MZ Hossain, 2022. Determination of TCID50 of O, A, Asia-1 serotypes foot-and-mouth disease virus to the production of trivalent vaccine in bioreactor. *Asian J. Med. Biol. Res.*, 8: 63-68.
- Bachanek-Bankowska K, HR Mero, J Wadsworth, V Mioulet, R Sallu, GJ Belsham, CJ Kasanga, NJ Knowles and DP King, 2016. Development and evaluation of tailored specific real-time RT-PCR assays for detection of foot-and-mouth disease virus serotypes circulating in East Africa. *J. Virol. Methods*, 237: 114-120.
- Blacksell SD, J Siengsan-an-Lamont, S Kamolsiripichaiporn, LJ Gleeson and PA Windsor, 2019. A history of FMD research and control programmes in Southeast Asia: lessons from the past informing the future. *Epidemiol. Infect.*, 147: 1–13.
- Bo LL, KS Lwin, S Ungvanijban, NJ Knowles, J Wadsworth, DP King, R Abila and Y Qiu, 2019. Foot-and-mouth disease outbreaks due to an exotic serotype Asia 1 virus in Myanmar in 2017. *Transbound. Emerg. Dis.*, 66: 1067-1072.

- Brito BP, LL Rodriguez, JM Hammond, J Pinto and AM Perez, 2017. Review of the global distribution of foot-and-mouth disease virus from 2007 to 2014. *Transbound. Emerg. Dis.*, 64: 316-332.
- Cecco BS, IR Santos, FA Molossi, CW Canal, CS Barros, D Driemeier, L Sonne and SP Pavarini, 2022. Viral diseases of sheep in Brazil: a review and current status. *Ciência Rural*, 53: e20220218.
- Dar PA, K Ganesh, G Nagarajan, S Sarika, GR Reddy and VV Suryanarayana, 2012. Sindbis Virus Replicase-based DNA Vaccine Construct Encoding FMDV-specific Multivalent Epitope Gene: Studies on its Immune Responses in Guinea Pigs. *Scandinavian J. Immunology*, 76: 345-353.
- FAO/OIE, 2012. FMD virus pools and the regional programmes Virus Pool 2- South Asia.
- Foxell JJ, 2003. The terrorist threat to US food security. *American foreign policy interests*, 25: 99-126.
- Gortázar C, P Barroso, R Nova and G Cáceres, 2022. The role of wildlife in the epidemiology and control of Foot-and-mouth-disease and similar transboundary (FAST) animal diseases: a review. *Transbound. Emerg. Dis.*, 69: 2462-2473.
- Hassan AI, 2016. Effect of different culture systems on the production of foot and mouth disease trivalent vaccine. *Vet. World*, 9: 32.
- Hossen ML, S Ahmed, MF Khan, MT Rahman, S Saha, KH Nazir, M Rahman, 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 MS, MA Habib, MR Islam, MS Mahmud, PC Saha, T Ruba, PM Das and MA Khan, 2017. Clinicopathological investigation of foot and mouth disease and serotype identification of the viruses in cattle of Bangladesh. *Immunol. Infect. Dis.*, 5: 16-23.
- Islam MA, MM Rahman, KH Adam and O Marquardt, 2001. Epidemiological Implications of the Molecular Characterization of Foot-and-mouth Disease Virus Isolated between 1996 and 2000 in Bangladesh. *Virus Genes*, 23: 203-210.
- Kass M, A Viltrop, J Prakofjewa, R Sõukand and R Kalle, 2022. Control of foot-and-mouth disease in a closed society: A case study of Soviet Estonia. *Front. Vet. Sci.*, 9: 828583.
- Lazarus DD, YS Wungak, MI Adah, JO Ibu, JN Abenga, PS Ekong and HG Ularamu, 2015. Serological response of commercial dairy cattle to inactivated foot-and-mouth disease vaccine (type-O & A) in Nigeria. *Asian J. Med. Biol. Res.*, 1: 163-168.
- Lloyd-Jones K, M Mahapatra, S Upadhyaya, DJ Paton, A Babu, G Hutchings and S Parida, 2017. Genetic and antigenic characterization of serotype O FMD viruses from East Africa for the selection of suitable vaccine strain. *Vaccine*, 35: 6842-6849.
- Malik N, A Kotecha, S Gold, A Asfor, J Ren, JT Huiskonen, TJ Tuthill, EE Fry and DI Stuart, 2017. Structures of foot and mouth disease virus pentamers: Insight into capsid dissociation and unexpected pentamer reassociation. *PLoS Pathogens*, 13: e1006607.
- Marupanthorn K, N Nittayapongchai, B Krachangwong and N Dungdee, 2013. Development of a multiplex PCR assay for the typing of foot and mouth disease in pigs and cattle. In: *Proceedings of World Academy of Science, Engineering and Technology*, 76: 241.
- 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: 328-331.
- Neumann EJ, 2012. Disease transmission and biosecurity. *Diseases of Swine*. 10th ed. Chichester, UK: John Wiley & Sons Ltd, 141-64.
- Ouagal M, E Brocchi, P Hendriks, D Berkvens, C Saegerman, S Grazioli, BY Adel, S Keith, D Kiram, and A Oussiguere, 2018. Study on seroprevalence and serotyping of foot and mouth disease in Chad. *Revue Scientifique et Technique. Rev. Sci. Tech. Off. Int. Epiz.*, 37: 937-947.
- Qasem JR, 2015. Prospects of wild medicinal and industrial plants of saline habitats in the Jordan valley. *Pak. J. Bot.*, 47: 551-570.
- Rahman MH, S Akther, MZ Hassan, MZ Ali and M Giasuddin, 2022. Molecular detection of foot and mouth disease virus serotype A in goats (*Capra hircus*). *Asian J. Med. Biol. Res.*, 8: 30-36.
- Sarker YA, AH Miah, N Sharif, MH Himel, S Islam, RC Ray, TK Paul, MT Islam and MH Sikder, 2015. A retrospective study of common diseases at veterinary teaching hospital, Bangladesh Agricultural University, Mymensingh. *Bangladesh J. Vet. Med.*, 13: 55-61.
- Saeed A, M Abubakar and O Kul, 2015. Modification of animal products for fat and other characteristics. *The Role of Biotechnology in Improvement of Livestock: Animal Health and Biotechnology*, Edited by: Abubakar, M., Saeed, A., Kul, O. (eds) *The Role of Biotechnology in Improvement of Livestock*. Springer, Berlin, Heidelberg pp. 55-89.

- Siddique MA, MR Ali, AS Alam, H Ullah, A Rahman, RP Chakrabarty, MA Amin, SA Hoque, SP Nandi, M Sultana and MA Hossain, 2018. Emergence of two novel sublineages Ind2001 BD 1 and Ind2001 BD 2 of foot-and-mouth disease virus serotype O in Bangladesh. *Transbound. Emerg. Dis.*, 65: 1009-1023.
- Shahiduzzaman AN, ME Haque, MH Rahman, MF Khan and MB Rahman, 2016. Serotyping and adaptation of foot and mouth disease virus in BHK-21 cell line towards the development of vaccine candidate. *International Journal of Vaccines and Vaccination*, 3: 00060.
- Soumya NP, R Banerjee, M Banerjee, S Mondal, RL Babu, M Hoque, IJ Reddy, S Nandi, PS Gupta and PK Agarwal, 2022. Climate change impact on livestock production. *Emerging Issues in Climate Smart Livestock Production: Biological Tools and techniques*. Edited by: Sukanta Mondal, Ram Lakhan Singh, Academic Press, pp. 109-148
- Singh RK, GK Sharma, S Mahajan, K Dhama, SH Basagoudanavar, M Hosamani, BP Sreenivasa, W Chaicumpa, VK Gupta, A Sanyal, 2019. Foot-and-mouth disease virus: immunobiology, advances in vaccines and vaccination strategies addressing vaccine failures—an Indian perspective. *Vaccines*, 7: 90.
- Wren MW, D Petts, G Guthrie, S Clarke, BR Nation, L Peters, S Mortlock, I Sturgess, M Wright and C Burt, 2022. Pestilence, Plague and Pandemics: A Troubled History. *The Ulster Medical Journal*, 91: 143.
- Yang C, S An, B Qiao, P Guan, D Huang and W Wu, 2023. Exploring the influence of COVID-19 on the spread of hand, foot, and mouth disease with an automatic machine learning prediction model. *Environ. Sci. Pollut. Res. Int.*, 30: 20369–20385.