

Occurrence of Foot and Mouth Disease (FMD) during 2014-2016 in cattle of Sirajganj district, Bangladesh

Md. Giasuddin¹, Mahmud MS¹, Md. Abu Musa Al Asari and Akter S*

Department of Microbiology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Key words: Foot and mouth disease, prevalence, age, sex, breed, season.

Foot and mouth disease (FMD) is a severe, highly contagious, and economically devastating viral disease which affects domestic cloven-hoofed cattle, pigs, sheep, goats as well as more than 70 species of mammals (Barnett & Cox, 1999; Brown, 2003; Grubman & Baxt, 2004; Du *et al.*, 2007; Giasuddin *et al.*, 2016). FMD is prevalent in many parts of the world and notorious for its ability to severely affect and indeed disrupt regional and international trade in animals and animal products. FMD is very common in many parts of the world, particularly in developing countries of Asia, Africa, the Middle East, and some parts of Europe. The occurrence of FMD in these countries results in severe economic losses. In FMD-endemic countries, usually the developing countries, the disease threatens food security and the livelihoods of smallholders. This disease causes an economic loss of US\$60–150 million annually in Bangladesh (Momtaz *et al.*, 2014).

The etiological agent of Foot and Mouth Disease Virus (FMDV) is a small non-enveloped virus and belongs to the genus *Aphtho* virus of the family Picornaviridae. FMDVs are classified into 7 serological types based on antigenic properties of the capsid proteins. The serotypes are O, A, C, Asia-1, SAT-1, SAT2 and SAT3 (Mittal *et al.*, 2005). Presently, 6 serotypes of FMDV (O, A, Asia-1, SAT-1,-2, and -3) are circulating worldwide, and serotype C has not been recorded since 1995 (Biswal *et al.*, 2012). Distribution of different serotypes of FMDV varies region to region. Serotypes O, A, and C mostly occur in Europe, South America, Africa, and Asia; SAT 1, SAT 2, and SAT 3 are normally limited to sub-Saharan Africa; and Asia1 occurs only in Asia (Ansell *et al.*, 1994).

In a previous study, sequence analysis revealed that serotype O and Asia1 is more prevalent while serotype A has very low frequency in Bangladesh. It was also found that the VP1 sequences of FMDV of the study samples were quite similar to sequence of FMDV isolated from animals in India and other neighboring countries (Giasuddin *et al.*, 2016). These outcomes imply the possibility of virus transmission during cross-country import of animals, which lacks strict policy for animal health check.

¹ Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh

* Corresponding Author. Email: salma.akter025@gmail.com

Moreover, some other environmental factors may play crucial role in transmission and progression of the disease in animal host. Despite vaccination, the disease appears every year across the country due to high evolutionary rates. Therefore, if Bangladesh needs to ensure food security, increase livelihood of rural people and get access to the lucrative markets for her livestock and livestock products, the control of FMD need to be addressed more effectively. Therefore, the present study was undertaken to identify environmental factors and host factors that influence the FMDV outbreak in Bangladesh. Besides we also aim to investigate molecular epidemiology, genotyping and employ phylogenetic analysis to determine the relationship of FMDV serotypes circulating in Bangladesh. Therefore this study was performed to detect the factors (both environmental and host) responsible for FMD outbreak and occurrence of FMD to highly susceptible area (Sirajganj District).

Total 68 samples were collected from clinically FMD-positive animals (cattle) from Sirajganj, Bangladesh. Samples preferably included mucosal epithelium (Tongue epithelium), tissue from the inter-digital space (Hoof tissue), saliva and fluid from the ruptured vesicles. Samples were collected in the viral transport medium (VTM) containing 0.04 M phosphate buffer (pH 7.2-7.6), 1% phenol red, antibiotics (penicillin 100 U/ml and streptomycin 100 µg/ml), and equal volume of glycerol. All of the available information (such as date of sample collection, age, sex and breed of the cattle, disease history etc.) for every sample were recorded on the FMD sample submission form provided by Bangladesh Livestock Research Institute (BLRI). The collected samples were mixed with PBS 5 times of the volume of sample and centrifuged at 3000 rpm for 10 minutes maintaining temperature at 4°C. The supernatant was collected in 50ml Falcon tube, marked properly and stored at -80°C till further processing.

Total RNA was extracted from original epithelial suspensions using QIAamp® Viral RNA Extraction kit (Qiagen, Germany) following the recommendations of the suppliers. After elution in nuclease-free water, RNA samples were stored in aliquots at -80°C until required (Giasuddin *et al.*, 2016). Assays has been played an important role in detection of FMDV in clinical samples (Laor *et al.*, 1992; Meyer *et al.*, 1991). After RNA extraction, the target sequence was amplified by using QIAGEN® one step RT-PCR kit (QIAGEN Inc., Netherlands). The oligonucleotide primer for the detection of FMDV and FMDV serotypes was used from the 1D, 2B and 5' UTR regions of the viral genome as previously published (Callens *et al.*, 1998; Reid *et al.*, 2000). More specification of these primers is listed in the Table 1. All oligonucleotide primers were synthesized by Sigma, USA. The amplified PCR products of the expected length were subjected to electrophoresis in a 2% agarose gel and visualized by straining with 0.6 mg/ml ethidium bromide and documented with Alpha Imager Mini System Protein Simple, USA (Giasuddin *et al.*, 2016).

Table 1. Primers used in this study for the detection of specific genes in FMDV

Primer name*	Sequence (5' to 3')	Location	PCR Products (bp)	Reference
1F	GCCTGGTCTTTCCAGGTCT	5'UTR		Reid <i>et al.</i> (2000)
1R	CCAGTCCCCTTCTCAGATC	5'UTR	328	
P33	AGCTTGACCAGGGTTGGC	2B	131	Vangrysp erre and De Clercq, (1997)

*1F forward primer; 1R and P33 reverse primer

A total 68 suspected samples were collected from different areas of Sirajganj district, Bangladesh from 2014 - 2016. Out of these samples 48 samples were found FMDV positive. Highest number of suspected cases (31) and positive cases (22) were found in 2015 as shown in Table 2.

Table 2. FMDV detection by RT-PCR

Year	Suspected case	Positive case	% of positive case
2014	9	7	77.8
2015	31	22	70.9
2016	28	19	67.9
Total	68	48	70.6

The impact of seasonal variation on the outbreak of foot and mouth disease (FMD) in cattle was observed. During summer (March-June), monsoon (July-October), winter (November- February) 28, 18, 22 suspected samples were collected and among them 18, 16, 14 samples were found FMDV positive, respectively. In summer season the highest number 18 positive samples were identified. Animals were categorized into three age-groups and tested for the presence of FMD virus. Age groups were young, adult and old. FMD outbreaks were influenced by the age of host animals. Outbreak rate of FMD virus was varied among male and female cattle. Out of 31 male cattle, 20 were found positive, and 28 were positive in 37 female cattle, comprising 64.5% and 75.7% of total positive sample, respectively. Diagnosis of FMD outbreak also varied based on sample types. Results of RT-PCR detection technique varied between the sample types. Highest number of positive isolates was found from tissue sample that comprised 83.9% of total positive isolates. During the study period, out of 23 and 45 samples collected from indigenous and cross breed, 13 and 35 samples were found FMDV positive in respective groups. The susceptibility of various breed of cattle to FMD virus is shown in Table 3. It is found that, a significant variation of breed susceptibility was observed which affected mostly crossed breed cattle (72.9% of total positive sample) compared to indigenous cattle (27.1% of total positive sample).

Table 3. Variation of food and Mouth disease among breeds of the cattle

Name of breed	Suspected case	Positive case	% of positive case within group
Indigenous cattle	23	13	56.5
Crossed breed cattle	45	35	77.8

Chi square Test (P-value) =0.001496164

Susceptibility of the cattle to FMDV was variable and influenced by vaccination profile. In this study out of 28 and 40 samples from vaccinated and non-vaccinated cattle respectively, 17 and 31 samples were found FMDV positive in respective groups.

Outbreak rate also fluctuated during different seasons and highest numbers of positive results were found in Monsoon (July- October). Female cattle were more susceptible than male cattle. Animals of young age were more vulnerable than other age groups. As vaccination could protect the emergence of foot and mouth disease in some cases, non-vaccinated cattle were more susceptible than vaccinated animals. Breeding patterns also influenced FMD outbreak, highest numbers of positive results were found in the animals having cross breed. The representative sequences of the positive isolates in this study and a previous study showed remarkable similarity with sequences of Bangladesh, Bhutan and India in 2012–2014 (Giasuddin *et al.*, 2016). This has implied that FMD is a trans-boundary disease. Therefore, we have to establish animal health check during the importation of animals and animal products from surrounding countries to minimize the emergence of foot and mouth disease in animal population.

Present study has confirmed that the occurrence of FMD in cattle of Sirajganj district is high and its association with variation of the seasonal effect, age, sex and breed of cattle is significant. Most risk group for outbreak was also identified. The results of this study could help to control the FMDV outbreak from this area.

Acknowledgement: This work was a part of M. Sc thesis, Department of Microbiology, Jahangirnagar University. The research works were supported by PPR & FMD research project in Bangladesh, Bangladesh Livestock Research Institute (BLRI) with a grant from Ministry of Fisheries and Livestock Research.

REFERENCES

- Ansell, D., Samuel, A., Carpenter, W. and Knowles, N. 1994. Genetic relationships between foot-and-mouth disease type asia 1 viruses, *Epidemiology and infection* **112**(01): 213-224.
- Barnett, P. and Cox, S. 1999. The role of small ruminants in the epidemiology and transmission of foot-and-mouth disease, *The Veterinary Journal* **158**(1): 6-13.
- Biswal, J. K., Sanyal, A., Rodriguez, L. L., Subramaniam, S., Arzt, J., Sharma, G. K., Hammond, J. M., Parida, S., Mohapatra, J. K. and Mathapati, B. S. 2012. Foot-and-mouth disease: global status and Indian perspective, *Indian J Anim Sci* **82**(2): 109-131.
- Brown, F. 2003. The history of research in foot-and-mouth disease, *Virus research* **91**(1): 3-7.
- Callens, M., De Clercq, K., Gruia, M. and Danes, M. 1998. Detection of foot-and-mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot-and-mouth disease, *Veterinary quarterly* **20**(sup2): 37-40.
- Du, J., Chang, H., Cong, G., Shao, J., Lin, T., Shang, Y., Liu, Z., Liu, X., Cai, X. and Xie, Q. 2007. Complete nucleotide sequence of a Chinese serotype Asia1 vaccine strain of foot-and-mouth disease virus, *Virus Genes* **35**(3): 635-642.
- Giasuddin, M., Mahmud, M. S., Alam, S., Samad, M., Islam, M., Ahasan, M., Rahman, M., Karim, M., Islam, M. and Acharjee, P. Molecular Epidemiology of Foot-and-Mouth Disease Viruses Circulated in Bangladesh from 2011–2014, *British Microbiology Research Journal* **16**(4): 1-13.

- Grubman, M. J. and B. Baxt 2004. Foot-and-mouth disease, *Clinical microbiology reviews* **17**(2): 465-493.
- Laor, O., Torgersen, H., Yadin, H. and Becker, Y. 1992. Detection of FMDV RNA amplified by the polymerase chain reaction (PCR), *Journal of virological methods* **36**(3): 197-207.
- Meyer, R., Brown, C., House, C., House, J. and Molitor, T. 1991. Rapid and sensitive detection of foot-and-mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene, *Journal of virological methods* **34**(2): 161-172.
- Mittal, M., Tosh, C., Hemadri, D., Sanyal, A. and Bandyopadhyay, S. 2005. Phylogeny, genome evolution, and antigenic variability among endemic foot-and-mouth disease virus type A isolates from India, *Archives of virology* **150**(5): 911-928.
- Momtaz, S., Rahman, A., Sultana, M. and Hossain, M. A. 2014. Evolutionary analysis and prediction of peptide vaccine candidates for foot-and-mouth-disease virus types A and O in Bangladesh, *Evolutionary bioinformatics online* **10**: 187.
- Reid, S. M., Ferris, N. P., Hutchings, G. H., Samuel, A. R. and Knowles, N. J. 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction, *Journal of virological methods* **89**(1): 167-176.