Typing of Foot and Mouth Disease Virus Circulating in Bangladesh by Reverse Transcription Polymerase Chain Reaction


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Abstract

Foot and Mouth Disease (FMD) is one of the highly contagious diseases of domestic and wild animals of many countries of the world including Bangladesh. The present study was undertaken to detect currently circulating FMD virus (FMDV) in Bangladesh using BHK-21 cell line, and the FMDV were typed using RT-PCR. The viruses induced characteristics cytopathic effects in BHK-21 cell lines, for examples, rounding, swelling, breaking down of intercellular bridge and cell death. Out of 151 clinical samples collected from different districts of Bangladesh, 85 (56.29%) were successfully adapted in BHK-21 cell line, and were analyzed using FMDV type specific primers. Among these 85 BHK-21 adopted virus, 71 (83.52%) were found as positive for FMD virus by RT-PCR; of which, 31 (36.47%) were positive for type A, 26 (30.58%) for type O, and 10 (11.76%) for type Asia 1. Only 4 (4.70%) samples were found as positive for mixed infection having Type A and Asia 1 FMDV. All the FMDV originated from Tangail (n=7) and Rajshahi (n=6) districts were found as positive for FMDV while 71.42% (n=14/36) samples of Mymensingh district were positive for FMDV. The FMDV identified in this study could now be used for effective vaccine development to control the disease in Bangladesh.

Keywords: FMD, clinical sample, BHK-21 cell line, typing, RT-PCR.
Introduction

Foot and Mouth disease virus (FMDV) is a non-enveloped, single stranded and positive sense RNA virus of the genus Aphthovirus belonging to the family Picornaviridae (Mumford, 2007). There are seven serotypes of FMDV namely O, A, C, Asia 1 SAT 1, SAT 2, and SAT 3. Infection with one serotype does not confer immunity against another (OIE, 2009). Foot and mouth disease (FMD), usually called Apthus fever, is an acute, febrile, highly contagious, and sometimes fatal viral disease of all cloven hoofed domestic animals e.g., cattle, buffalo, sheep, goat, swine etc. Also, the virus affects more than 70 species of wild animals including deer, antelope etc. (Fenner et al., 1993).

FMD is a serious threat for livestock population throughout the world due to its high contagiousness, rapid multiplicity of the types and sub-types, wide species of animal susceptibility, short lived immunity, and the existence of the virus as persistent or carrier state in the susceptible and carrier animals (Gurhan et al., 1993; Rodriguez et al., 1994; Saiz and Domingo, 1996; Bergmann et al., 1996; Olabode et al., 2014). The virus could be detected from vesicular fluid, oral epithelial tissue, esophageal–pharyngeal sample etc. Diagnosis may also be done by the detection of FMDV in the blood, heart or other organs of fatal cases. A myocarditis may be seen macroscopically (the so-called “tiger heart”) in a proportion of fatal cases particularly in calf (OIE, 2009).

FMD is one of the major constraints for the development of livestock population in Bangladesh (Rahman, 2011). Outbreak of this disease causes severe economic loss to the livestock industries in terms of loss of draft power, meat and milk production, infant and adult animal mortality. The morbidity due to FMD could be 100% in cattle, 23% in buffaloes, and 5% in goats and sheep (Chowdhury et al., 1993). Mortality rate, especially in calves, had been found to be about 51% in outbreak areas. Annual loss due to FMD in Bangladesh has been estimated at about US$62 million (FAO/OIE, 2012). Sero-epidemiological investigation of FMDV in cattle population indicated that four different types (A, O, C and Asia-1) of FMDV were prevalent in Bangladesh since 1960 (Ameen, 1964; Chowdhury et al., 1996; Islam et al., 2000). Recently, the country has faced outbreaks of FMDV types O and A, which are closely related to the virus types that are active in India and Nepal (Nandi et al., 2013).

Despite vaccination, the disease appears every year across the country. Recent report from FAO has recommended that surveillance and reporting of FMD needs to be improved throughout the country. Furthermore, all suspected clinical cases of FMD should be confirmed by laboratory examination (Mondal and Yamage, 2014). Therefore, the present study was undertaken to identify the types of FMDV that are currently circulating in Bangladesh, so that the findings of the study could be used to adopt effective disease management and control strategies, including appropriate vaccination development in Bangladesh.

Materials and Methods

A total of 151 samples (e.g., tongue epithelia and foot tissues) were collected from FMD suspected cattle during the outbreak of FMD in Bangladesh during 2013. The location of sampling sites is presented in Fig 1. The samples were immediately transported to the Department of Microbiology and Hygiene, Bangladesh Agricultural University for analysis using virus transport media maintaining cool chain. The samples were homogenized with mortar and pestle separately, and 10% suspensions were prepared by adding sterile phosphate buffered saline (PBS). The suspension was then centrifuged at 5,000 rpm for 1 hour and then filtered with 0.2μm filter. Sterility of the inoculum was tested in fresh blood agar media and propagation into BHK-21 cell culture. The cells those formed complete and confluent monolayer in the culture flask within 24 hour of incubation were selected for infection with FMDV.
Fig. 1: A map of Bangladesh showing the sampling sites and the types of Foot and Mouth Disease virus detected in those areas.

Fig. 2: Uninfected (Normal/healthy) BHK-21 cells (200x).

The confluent monolayer was allowed to infect with 1ml of inoculum prepared from 10% suspension in PBS with field sample, and the inoculum was spread over the cell sheet by tilting for about 45-60 minutes for better adsorption. Then 10 ml of the maintenance media was added in a 25 cm² flask (1×MEM supplemented with 2% heat inactivated fetal calf serum), and the vessel was
returned to the incubator. Virus added flask was allowed to incubate at 37°C. The cells were examined twice daily under inverted microscope (Carl Zeiss, Germany) until showing characteristic cytopathic effects (CPE). Presence of cell rounding, swelling, breaking down of intercellular bridge and finally cell death in cell culture indicated the presence of FMDV in the sample. The infectious fluid containing FMDV was harvested after 48 h to 72 h of post infection and viral RNA was extracted by SV total RNA isolation System® (Promega, USA) for molecular detection of the serotypes by RT-PCR according to the instructions of the manufacturers. Detection of virus and its typing were carried out by RT-PCR using FMDV type specific primers listed in Table 1. The RT-PCR was carried out using Access RT-PCR system® (Promega, USA) as per instruction of the manufacturers.

### Table 1: Foot and Mouth Disease virus type specific primers used for RT-PCR.

<table>
<thead>
<tr>
<th>Name of primers</th>
<th>Primer sequence (5'-3')</th>
<th>Specificity (Type)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMDO F</td>
<td>5'-ACC AAC CTC CTT GAT GTG GCT-3'</td>
<td>O</td>
<td>Reid et al., (2000)</td>
</tr>
<tr>
<td>FMDO R</td>
<td>5'-GAC ATG TCC TCC TGC ATC TG-3'</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>FMDAs1F</td>
<td>5'-TAC ACT GCT TCT GAC GTG GC-3'</td>
<td>Asia1</td>
<td>Gurumurthy et al., (2001)</td>
</tr>
<tr>
<td>FMDAs1R</td>
<td>5'-GAA GGG CCC AGG GTT GGA CTC-3'</td>
<td>Asia1</td>
<td></td>
</tr>
<tr>
<td>FMDA F</td>
<td>5'-TAC CAA ATT ACA CAC GGG AA-3'</td>
<td>A</td>
<td>Reid et al., (2000)</td>
</tr>
<tr>
<td>FMDA R</td>
<td>5'-GAC ATG TCC TCC TGC ATC TG-3'</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

### Results and Discussion

Foot and Mouth disease is a serious threat for the cattle population of Bangladesh. Although every year vaccination is carried out throughout the country against FMD, still the disease outbreak occurs in many parts of Bangladesh (Sarker et al., 2011). In the present study, 151 clinical samples were collected and subjected to tissue culture screening in BHK-21 cell line for FMDV. For the propagation of FMDV, several cell lines have been used in different laboratories around the world. Among these cell lines, BHK-21 is one of the commonly used cell lines for propagation and adaptation of FMDV (Zabal and Fondevila, 2013). Here, we used the BHK-21 cell lines successfully for the propagation and adaptation of FMDV virus from the clinical samples. Among these 151 samples, 85(56.29%) showed the CPE specific for FMDV e.g., rounding, swelling, clumping of the cells and broken down of intercellular bridge etc (Fig 3, 4 and 5). These 85 samples were further analyzed for FMDV typing using RT-PCR protocol.
RT-PCR is a reliable, rapid, highly sensitive and specific tool for the molecular detection of infectious agents including FMDV (Mehran et al., 2006; Alexandersen et al., 2000; Reid et al., 2000; Farag et al., 2004). RNA was extracted from the 85 tissue cultured fluid that were found positive for FMDV in BHK-21 cell line. Using FMDV type specific primers, among the 85 samples, 71 (85.88%) were found positive for FMDV by RT-PCR (Figure 6). Among these 71 samples, 31 (36.47%) were positive for type A, 26 (30.58%) for type O, 10 (11.76%) type Asia 1, and 4 (4.70%) for mixed infection (Type A and Asia 1) (Table 2). On district basis, all the samples originated from Tangail and Rajshahi were 100% positive for FMDV, while, the samples originated from Mymensingh had lowest in number (71.42%) in terms of positive by RT-PCR. On type basis, FMDV type A was mostly prevalent (36.47%) followed by Type O (30.58%) and Type Asia 1 (11.76%). In this study serotypes O, A and Asia 1 was found as the currently circulating FMDV in
Bangladesh. Similar finding has been reported by Chowdhury et al., (1996) and Zinnah et al., (2010).

Similarly, Loth et al., (2011) detected serotype O in Bangladesh. Recently, Nandi et al., (2013) detected serotype O and A as the currently circulating FMDV in Bangladesh. Moreover, FAO reported serotypes O, A and Asia 1 as the circulating FMDV prevalent in Bangladesh, Bhutan, India, Nepal, and Sri Lanka, supporting the findings of the present study. Chowdhury et al., (1996) identified serotype C in Bangladesh in addition to type O, A and Asia 1. However, after 1996, type C could not be detected in Bangladesh (FAO, 2014a). Our present study identified serotype A as marginally dominant type of FMDV in Bangladesh as compared to type O and Asia 1. However, in earlier, serotype O has been identified as the dominant type of FMDV (FAO, 2014b). Similarly in India (neighboring country of Bangladesh), serotype O has been identified as the dominant type of FMDV (Bhattacharya et al., 2005). This variation in the type of dominates FMDV serotype might be linked with different geographic locations of origin, and sample size.

**Conclusion**

Present study confirms type O, A and Asia1 as the currently circulating FMDV in Bangladesh as revealed by RT-PCR. Mixed infection having Type A and Asia 1 FMDV was also detected. The isolated virus now could be used for the development of effective vaccine.

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References


foot-and-mouth disease virus prevalent in Bangladesh.