Biotechnology International 4 (2): 26-30, June 2011
ISSN 0974-1453
Published by Biotechnology Society Online www.bti.org.in

Reverse transcriptase PCR based diagnosis of Foot and Mouth Disease virus serotype O in clinical samples collected from Assam

Neeta Longjam* , Rajib Deb[1], Basavraj S. Mathapati [2] , Tilling Tayo[3] , Vijay Kumar Saxena[1] and A.K. Sarmah

Department of Veterinary Microbiology, College of Veterinary Science , Assam Agricultural University, Khanapara, Guwahati-781022; [1]Division of Animal Biotechnology, [2]Division of Veterinary Virology ,[3] Division of Animal Nutrition , Indian Veterinary Research Institute, Izatnagar, UP- 243122,
*corresponding author : neetavet@gmail.com

Summary: Foot-and-mouth disease (FMD) is one of the highly contagious diseases of domestic animals. In India, the disease is highly endemic and caused by the serotypes O, A, and Asia 1 in the recent years. Various molecular tools are used to detect the virus in clinical samples and Reverse transcriptase PCR (RT PCR) is one of the highly specific and sensitive tool. The present study was undertaken to detect foot-and-mouth disease virus serotype 'O' in clinical samples viz. tongue / feet epithelium and oral swabs by RT PCR isolated from Assam of North Eastern Region of India. By using type specific primer, FMD virus serotype 'O' were detected in 15 out of 18 clinical samples by RT PCR.

Key Words : FMD, RT PCR , diagnosis

Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease affecting *artiodactylae*, mostly cattle, swine, sheep, goats and many species of wild ungulates (Brooksby, 1982). *Office Of International des Epizooties* (presently World organization for Animal health) (OIE) ranks the disease first in its list A diseases and the virus as Risk Group 4 of transboundary importance (OIE, 2008 and 2009). Poor surveillance and diagnostic facilities as well as inadequate control programs are major problems in control of this disease in the country. FMD is still a leading cause of loss of livestock economy in India. Outbreaks are still being reported from time to time round the year (Tamilselvan *et al*., 2009). Out of the possible seven, only four serotypes, viz, 'O', 'A', 'C' and Asia 1 were ever recorded in India. Currently more than 1500 outbreaks are being reported throughout the year, and more than

75% of the outbreaks are attributed to serotype 'O', and the remaining 25 % of outbreaks are due to serotypes 'A' and Asia1. This indicates that serotype 'O' viruses are highly adapted to their host and environment to survive in the nature. The molecular epidemiological studies have established that the Pan-Asian strain is the major cause of outbreak of FMD involving serotype 'O' in India including North Eastern region like Assam.With the introduction of molecular techniques and the advent of Polymerase Chain Reaction (PCR) technique in the recent past has led to development of several reverse transcription PCR (RT-PCR) procedures for specific detection of FMDV RNA (Meyer *et al*., 1991; Laor *et al.*, 1992; Hoffman *et al*., 2006). The aim of our present study is to detect FMDV "O" serotype by Reverse transcription PCR (RT-PCR) from clinical samples originated from Assam.

Materials and Methods

Field samples of FMD: A total of 18 clinical materials were taken from the FMDV infected animals from different parts of Assam, India which were not vaccinated against FMDV. The clinical materials comprised of mouth/feet lesion i.e. tissue sample (10) and oral swab (8) (Table 1) . All the tissue materials were properly preserved in glycerol phosphate buffer and stored in -20^0 while the oral swabs are collected in maintenance media and stored at -20^0.The tissue samples preserved in transport medium containing equal amounts of glycerol and phosphate buffered saline (PBS pH 7.2–7.6) was used for preparing 10% tissue suspension (epithelium suspension) in PBS. Briefly, 200 mg of sample (infected epithelium) was taken and washed in the PBS and triturated in a sterile mortar and pestle using 2 ml PBS. The well triturated material was collected and transferred to centrifuge tube along with equal volume of chloroform. The content was centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and stored in sterile cryovial at -20^0C.

RNA extraction: The aliquots of supernatant were used for RNA extraction. An aliquot of supernatant was used for RNA extraction using the TRIzol reagent (Invitrogen) as per the manufacturer's instructions.

RT-PCR: Reverse transcription of the viral RNA was performed using RevertAid™ M-MuLV Reverse Transcriptase (MBI Fermentas (Vilnius). 25 µl of the reaction mixture contained 2.0 µl of oligodT primer (10 pmols/µl), 8.0 µl Template RNA, 2.0 µl of 10 mM deoxy nucleotide triphosphates (dNTPs), 5.0 µl 5X Reaction Buffer, 0.5 µl Reverse Transcriptase (200 units/µl), 0.2 µl of Ribonuclease Inhibitor (20 units/µl) and remaining adjusted with Nuclease free water. Template RNA and primer were first mixed and kept at 70^0C for 10 minutes to melt secondary structures in viral RNA. The tube was immediately cooled to 4^0C by placing in crushed ice in order to prevent reformation of secondary structures followed by the addition of the remaining reagents of the reaction mixture. The tube containing the final mixture was subjected to centrifugation to achieve thorough mixing of components and kept at 42^0C for 1 hour in a water bath for reverse transcription to occur. Before carrying out PCR with product, the tube containing RT product was subjected to heating at 95^0C for 5 minutes in a thermal cycler to inactivate M-MLV RT. After RT, cDNA products were stored at -40^0C after proper labeling. Amplification of O specific fragment from the each cDNA samples were prepared which contained 4 µl of each cDNA sample,

2.5 µl of 10x PCR buffer, 200 µM of each dNTPs and 50 pmol of each FMDV O specific primers (Table 2). Amplification mixture was subjected to 30 cycles of denaturation (94^0c, 1 min), annealing (55^0c, 45 sec) and extension (72^0c, 1 min) with a further final cycle for primer extension at 72^0c, 5 min. The PCR products obtained were subjected to electrophoresis in 2% agarose gel containing ethidium bromide.

Table 1. Clinical sample collected from bovines from different parts of Assam

Sample No.	**Place of collection**	**Date of collec-tion**	**Samples type**
1	Dist.Golaghat, Assam	02-05-07	Tissue
2	Jorhat, Assam	25-07-07	Tissue
3	Dist. Golaghat, Assam	26-07-07	Tissue
4	Dist.Kamrup, Assam	24-09-07	Tissue
5	Dist. Kamrup, Assam	24-09-07	Tissue
6	Dist. Morigoan, Assam	29-10-07	Tissue
7	Dist. Morigoan, Assam	30-10-07	Tissue
8	Dist. Kamrup, Assam	03-11-07	Tissue
9	Dist. Kamrup, Assam	03-11-07	Tissue
10	Dist. Nagaon. Assam	03-11-07	Tissue
11	Dist.B arpeta, Assam	08-11-07	Oral Swab
12	Dist. Barpeta, Assam	08-11-07	Oral Swab
13	Dist.B arpeta, Assam	08-11-07	Oral Swab
14	Dist. Kamrup, Assam	16-11-07	Oral Swab
15	Dist. Kamrup, Assam	02-01-08	Oral Swab
16	Dist. Kamrup, Assam	05-02-08	Oral Swab
17	Amerigog, Guwahati, Assam	26-02-08	Oral Swab
18	Dist. Golaghat, Assam	01-03-08	Oral Swab

Table 2: FMDVO specific primer set used for the present study

Name of the primers	**Sequences (Accession No. HQ632770.1)**
Forward primer FMDVO	5’CGGCCTACCACAAGGCACCG3’
Reverse primer FMDVO	5’AGTGGCCGGGGGCAGTATGT3’

Results and Discussion

Out of the 18 samples subjected to RT PCR, 15 (84%) samples were found to be positive for FMDV serotype 'O' (249bp) (Figure 1), while the rest 3 (16%) samples did not reveal presence of FMDV serotype 'O' Among the 15 samples positive in RT PCR , 16 belonged to ES and only two oral swabs were found to have gene for FMDV serotype 'O' . The result of the present study have shown that RT PCR is efficient for detection of FMDV serotypes from clinical samples as it could detect around 84% type 'O' serotype from 18 samples .It might be possible that the remaining three samples of infected animals might possess other serotypes of FMD virus which can be further diagnose by using serotype specific primers.

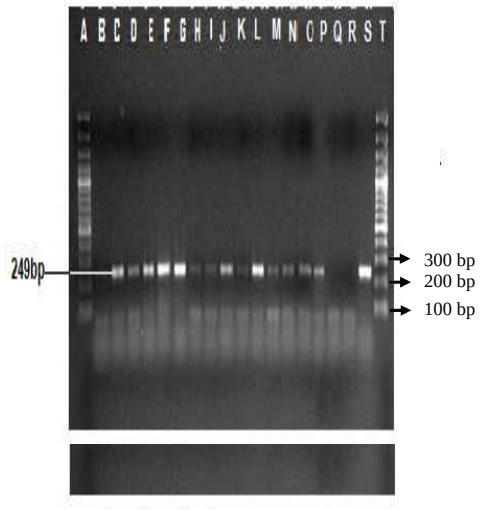

Fig.1.Agarose gel electrophoresis of amplified gene products of clinical materials showing serotype 'O' specific band
Lane A&T: DNA Molecular Marker; B-S: PCR products

Acknowledgement

Authors are thankful to the Director, Project Directorate on Foot and Mouth Disease, Indian Veterinary Research Institute, campus, Mukteswar, Uttarakhand for providing all the facilities to carryout the studies.

References

Brooksby J B (1982). Portraits of viruses: foot-and-mouth disease virus. Intervirology., 18: 1-10.

Office Of International des Epizooties, OIE (2009). OIE Terrestrial Manual 2009. Foot and mouth disease. Chapter 2.1.5. pp: 1-29.

Office Of International des Epizooties, OIE (2008). OIE Terrestrial Manual 2008. Foot and mouth disease. Chapter 2.1.5. pp: 190-217.

Tamilselvan RP, De Sanyal A, Pattnaik B (2009). Genetic transitions of Indian serotype O Foot and Mouth Disease Virus isolates responsible for field outbreaks during 2001-2009: A brief note: OIE/FAO Reference laboratories network meeting: 23rd to 27th November, 2009. New Delhi. pp 11- 13.

Laor O, Torgersen H, Yadin H, Becke Y (1992). Detection of FMDV RNA amplified by the polymerase chain reaction (PCR). J. Virol. Methods., 36: 197-208.

Meyer RF, Brown CC, House C, House JA. Molitor TW (1991). Rapid and sensitive detection of foot-and-mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene. J. Virol. Methods, 34: 161–172.

Hoffmann B, Depner K, Schirrmeier H, Beer M (2006). A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. J. Virol. Methods., 136: 200–209.