

*Original Research***Studies on Diagnosis of Foot and Mouth Disease by ELISA and Reverse Transcription Polymerase Chain Reaction in Bovines**Arbind Singh^{1*}, Mahesh Kumar², Amit Kumar Verma¹ and Snehal Nirwan³¹Livestock Farm Complex (Veterinary Medicine), College of Veterinary & Animal Sciences, SVPUA&T, Modipuram, Meerut, Uttar Pradesh, INDIA²Veterinary Medicine, College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar, Uttarakhand, INDIA³Veterinary Officer, Animal Husbandry Department, Uttar Pradesh, INDIA***Corresponding author:** arbindsingh80279@gmail.com

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Abstract

Rapid and accurate identification of infected animals, those with either clinical or subclinical disease as well as with persistent infection, is essential for maintaining an efficient control programme of Foot and mouth disease. During the study period (2009-10), surveillance of Foot and mouth disease was conducted in the villages/towns/dairy farms/gaushalas affected with Foot and mouth disease outbreaks in Uttar Pradesh. A total of 25 tongue epithelia were collected from suspected cases of FMD and subjected to indirect sandwich ELISA and Reverse Transcription-PCR assays for the diagnosis. Out of 25 vesicular epithelium samples, 17 were found positive for the presence of FMD virus by ELISA (the overall type ability = 68.00%). Out of 25 samples, amplicons could be amplified from 20 samples (12 serotype O and 8 serotype A) using RT-PCR. However, serotype 'C' and 'Asia-1' could not be recovered from these samples. In the scenario of overall distribution pattern of FMD virus types, serotype 'O' (60.00%) was the predominant type, followed by serotype 'A' (40.00%). The findings indicate PCR assay as a simple, rapid, sensitive, reliable, reproducible and an additional confirmatory method for the identification of FMDV that will aid in surveillance, prevention and control of this pathogen.

Key words: Foot and Mouth Disease, RT-PCR, Sandwich ELISA, Tongue Epithelium, Uttar Pradesh**How to cite:** Singh, A., Kumar, M., Verma, A., & Nirwan, S. (2019). Studies on Diagnosis of Foot and Mouth Disease by ELISA and Reverse Transcription Polymerase Chain Reaction in Bovines. International Journal of Livestock Research, 9(6), 130-135. doi: 10.5455/ijlr.20190325112442**Introduction**

Foot and mouth disease virus (FMDV) has a worldwide distribution and is of major importance for the animal industry in India (Chakraborty *et al.*, 2014; Verma *et al.*, 2012, 2017). The disease is particularly a problem for countries where animals and meat contribute significantly to the national economy through export markets. Foot and mouth disease (FMD) is enzootic in many parts of India (Singh *et al.*, 2008;

Verma *et al.*, 2017). Therefore, rapid and accurate diagnosis of FMD is essential for the success of FMD control programme (Verma *et al.*, 2008). A suitable and reliable test must be able to detect FMDV circulating in animal population, as well as a low number of viral particles. The reverse transcription polymerase chain reaction (RT-PCR) has been shown to be a useful tool in the diagnosis of FMD (Marquardt *et al.*, 1995; Raies *et al.*, 2009; Verma *et al.*, 2010), as a part of the viral genome can be detected with a very high sensitivity in less than 24 h in a wide range of samples (Meyer *et al.*, 1991). The detection of structural protein antigen of FMD by ELISA was shown to be rapid and simpler to perform (Ferris and Dawson, 1988) and considered as the primary test for FMD diagnosis in the regionally located FMD diagnostic laboratories in the country (Anon, 2010). In this study, RT-PCR was used for screening the vesicular epithelium samples collected from cattle and buffaloes from some districts of Uttar Pradesh state for the diagnosis of different serotypes of FMD virus.

Materials and Methods

Sample Collection

A total of 25 vesicular epithelia (tongue epithelium) samples were collected from 19 cattle and 6 buffaloes showing clinical signs *viz.* vesicle formation on the mucous membranes of the tongue, interdigital spaces, salivation, anorexia from different districts of Uttar Pradesh. These samples were preserved in 50% phosphate buffered saline (PBS) glycerol, pH 7.4 till further test.

Sandwich ELISA Test

The samples were tested by indirect sandwich ELISA (Bhattacharya *et al.*, 1996).

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from all the 25 samples (tongue epithelium) with the modification of the acid guanidium phenol-chloroform methods (Callens *et al.*, 1998). Reverse transcription (RT) was carried out with 10 µl of RNA in 25µl of final reaction volume using MMLV reverse transcriptase and NK61 primer GACATGTCCTCCTGCATCTG (Knowles and Samuel, 1998). The reaction mixture was incubated at 48°C for 1 h. Additional incubation at 95°C for 5 min. was done to inactivate the enzyme. The PCR amplification of the entire VP1 genomic region from this cDNA was carried out using Hotstar PCR kit (Qiagen) and 20 pmol of each forward primer (Table 1) and NK61 reverse primer (Knowles and Samuel, 1998) as per the recommendations of the supplier.

Table 1: Primers used for amplification of FMDV

S. No	Primer	Primer Sequence (5'-3')	Sense	Gene	Product Length (bp)	Serotype
1	NK-61 (Reverse)	GACATGTCCTCCTGCATCTG	-ve	2B		
2	ARS-4 (Forward)	ACCAACCTCCTTGATGTGGCT	+ve	1C	1301	O
3	A-1C ₅₆₂ (Forward)	TACCAAATTACACACGGGAA	+ve	1C	863-866	A
4	As1-1C ₅₀₅ (Forward)	TACTGCTTCTGACGTGGC	+ve	1C	908-914	Asia-1

The thermal cycling condition employed for PCR amplification are shown in Table 2.

Table 2: Optimum PCR conditions for each primer sets

Serotype	Primer pair	No. of Cycles	Denaturation (94°C)	Annealing	Extension (72°C)
O	ARS4/NK61	40	1 min	45 sec at 60°C	2 min
A	A-1C ₅₆₂ /NK61	40	1 min	1 min at 55°C	2 min
Asia-1	As1-1C ₅₀₅ /NK61	40	1 min	1 min at 55°C	1.5 min

The PCR products (5 µl sample) were run on 1% agarose gel containing ethidium bromide and the DNA bands were visualized using a UV transilluminator.

Results and Discussion

Foot and mouth disease virus (FMDV) is the economically most important veterinary pathogen due to its highly infectious and contagious nature, ability to cause persistent infections and long term effects on the condition and productivity of the many animal species. Countries which have this disease have many trade restrictions placed upon them (Shanafelt and Perrings, 2018). FMD is endemic in India and occurs in all parts of country throughout the year. Rapid and accurate laboratory detection of FMDV plays a critical role in the implementation of control measures to eradicate FMD. In the present study, out of 25 vesicular epithelium samples (tongue epitheliums) collected from suspected cases of FMD, 17 were found positive for the presence of FMD virus by indirect sandwich ELISA. Several researchers from different countries have reported that sandwich ELISA is a good test in terms of sensitivity as well as specificity for serotyping of FMD virus (Alonso *et al.*, 1992; Chomczynski *et al.*, 1987; Mann *et al.*, 1998; Ouldrige *et al.*, 1987; Prasad *et al.*, 1992; Verma *et al.*, 2008). In the present study, the overall typeability was 68.00% using sandwich ELISA. The percentage was found comparable to international standards. The possible reasons for non-typing of virus from remaining samples may be either samples were not collected at the proper time of clinical manifestation of disease or were not suitably preserved (Pattnaik *et al.*, 1990). All the 25 tongue epithelium samples were also processed by reverse transcription (RT) using random hexanucleotide primer, followed by the polymerase chain reaction (PCR) (Fig. 1).

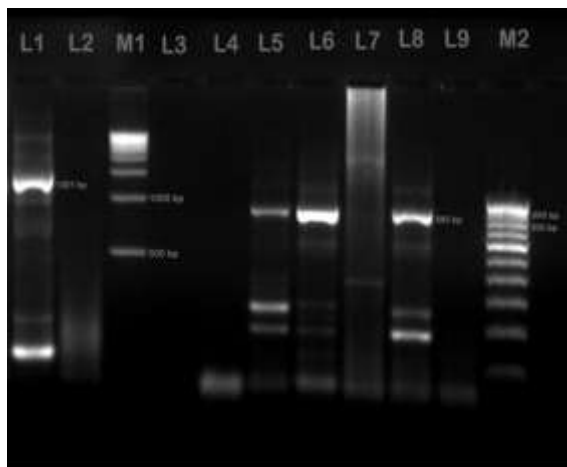


Fig. 1: Agarose gel electrophoresis of 1301 bp and 865 bp PCR products amplified from FMDV (**M1:** DNA marker 1 kb; **L 1:** Positive sample (Type O); **L 2-L4:** Negative sample; **L 3:** Negative sample; **L 4:** Negative control; **L 5:** Positive sample (Type A, UPI); **L 6:** Positive sample (Type A, BrI); **L 7:** Negative sample; **L 8:** Positive sample (Type A); **L 9:** Negative control; **M2:** DNA marker 100 bp)

Out of 25 samples, amplicons could be amplified from 20 samples (12 serotype O and 8 serotype A) using RT-PCR. However, serotype ‘C’ and ‘Asia-1’ could not be recovered from these samples. In the scenario of overall distribution pattern of FMD virus types, serotype ‘O’ (60.00%) was the predominant type, followed by serotype ‘A’ (40.00%), (Table 3).

Table 3: Distribution results of FMD virus types by RT-PCR

S. No.	Species	Total Samples	Virus Recovered	FMDV Serotypes			
				O	A	C	Asia-1
1	Cattle	19	16	10	6	-	-
2	Buffaloes	6	4	2	2	-	-
3	Total	25	20 (80.00)	12 (60.00)	8 (40.00)	-	-

Comparison of sandwich ELISA based typing and RT-PCR result revealed that 17 samples positive for FMDV by Sandwich ELISA were also positive with RT-PCR and amplicons of 1301 and 864 bp could be amplified. The known positive control RNA extract of FMDV also yielded similar amplicons. However 3 specimens negative in sandwich ELISA test were found to be positive in RT-PCR. It indicates that the RT-PCR is more sensitive than and sandwich ELISA test (Donn *et al.*, 1996; Reid *et al.*, 1999; Alexandersen *et al.*, 2000; Paprocka and Kesy, 2001; Paprocka *et al.*, 2002; Clavijo *et al.*, 2003; King *et al.*, 2006; Verma *et al.*, 2010, 2012). The higher sensitivity of RT-PCR may be because of its ability to detect very small number of virus as well as detection of RNA of non-viable FMD virus. The detection of viral genome, rather than live virus or viral proteins, has the advantage that viable virus or intact viral antigen is not required. Where subclinical infection is suspected or when samples are collected before the appearance of clinical sign (Marquardt *et al.*, 1995) or after the resolution of clinical disease or when processing saliva or

swabs, an enhanced RT-PCR technique (Callens and DeClercq, 1997) produces a detection system as sensitive and considerably more rapid than multiple passages on tissue culture. Moreover, it saves time and labour, as it takes only approximately 5-6h. to identify the presence of FMD virus compared to 10-16h. taken by the sandwich ELISA. These findings indicate PCR assay as a simple, rapid, sensitive, reliable, reproducible and an additional confirmatory method for the identification of FMDV that will aid in surveillance, prevention and control of this pathogen.

Conclusion

The results of this study provided the valuable data regarding the occurrence of FMD in bovines from Uttar Pradesh, India. The findings further indicated PCR assay as a simple, rapid, sensitive, reliable, reproducible and an additional confirmatory method for the identification of FMDV. Such outcomes proves to be very useful for accurate diagnosis of FMD and therefore, strengthens the strategies for prevention and control of this disease.

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