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Molecular Characterization of Foot and Mouth Disease Virus Serotype A from Mizoram

Satyabrat Dutta[1], Parimal Roychoudhury[1], Tapan Kumar Dutta[1], Nayanmoni Konwar[1], Richa Sarkar[1], Prasant Kumar Subudhi[1]

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ABSTRACT

Background: Foot and mouth disease virus serotype A has been reported from different states of India and is considered to be the third most commonly encountered serotype besides serotype O and Asia1. Serotype A viruses are most diverse in nature and have been globally grouped into 26 different types.

Methods: Foot and mouth disease virus serotype A could be detected from an outbreak in Mizoram that occurred in April 2020. The virus could be confirmed by performing a polymerase chain reaction targeting the 5′ UTR region and subsequent serotype-specific multiplex PCR. The full-length VP1 capsid protein gene of the virus could be amplified, cloned and sequenced.

Result: Sequence analysis revealed the virus belonging to genotype VII of serotype A. Deduce amino acid sequence comparison it has been observed that the ^{144}R-G-D^{146}tripeptidein the G-H loop remains conserved. However, the present sequence (OL419371), when compared with the Indian vaccine strain of serotype-A(HM854025) as well as the 2001 Mizoram outbreak (AF390659) revealed 11 amino acid differences with the Indian vaccine strain and almost 36 amino acid differences with Mizoram 2001 isolate in different amino acid positions. This indicates the diverse nature of the virus within a geographical area.

Key word: Foot and mouth disease virus, Phylogenetic analysis.

INTRODUCTION

Most of the marginal farmers in India depend upon the livestock sector for their livelihood. FMD outbreaks usually result in a huge amount of loss for them due to low output from herds and limitations of export in exaggerated areas. (Kitching and Hughes, 2002; Perry and Rich, 2007). There are records of about 5,000-6000 outbreaks occurring in our country yearly affecting nearly 3-3.5 lakh animals with a calculated economic loss of Rs. 4,300 cores. (Mukhopadhyay,1992). The virus belongs to the genus *Aphthovirus* of the *Picornaviridae* family and exists as 7 clinically indistinguishable serotypes: O, A, Asia-1, South African Territory-1 (SAT-1), (SAT-2), SAT-3 and C, almost all of them having various subtypes (Domingo *et al*. 2003). Natural Infection and clinical recovery or immunization with one serotype are generally unable to defend the host animal against infection from other serotypes. (Ferris and Dawson, 1988; Knowles and Samuel, 1995; Giridharan *et al*., 2005). As cross-protective antibodies are not able to be developed by alternative serotypes and subtypes which results in diminished strategies of mass vaccination programs for controlling and preventing the disease (Zhang *et al*., 2015; Beskawy *et al*., 2016). In the North-Eastern states of India, the incidence of FMD is highest with type “O” which is usually followed by other serotypes including the type “Asia-1” and “A”. Sarma *et al*. (1992). It has been reported that serotype O is responsible for most of the outbreaks/cases(80%) in India followed by Asia1(12%) and A (8%), respectively. Subramaniam *et al.* (2013) and Tosh *et al*. (2002) reported based on VP1 sequence analysis that, serotype A can be distributed into 10 major genotypes (designated as I-X) and the majority of the Indian strain belonged to the genotype I, IV, VI and VII. Tosh *et al*. (2002). So, it is very important to characterize circulating field viruses to develop potential immunotherapeutic and multivalent vaccines. The present study was conducted to identify the molecular type of FMDV from an outbreak in Mizoram that occurred in April, 2020.

[1]Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Aizawl-796 014, Mizoram, India.

Corresponding Author: Satyabrat Dutta, Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Aizawl-796 014, Mizoram, India. Email: satyabrat498@gmail.com

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MATERIALS AND METHODS

Location

The study was carried out at the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram.

Samples

Total 10 numbers of Fresh Tissue samples (Tongue epithelium and leg scar) were collected from the outbreaks of FMDV in Mizoram during April, 2020 and samples were selected for the present study. A total of 3 samples were processed for isolation and molecular detection of the foot and mouth disease virus. One positive control was used for comparison during molecular detection.

Virus detection

Confirmation of the FMD virus was done by reverse transcriptase polymerase chain reaction (RT-PCR) by targeting 5′-UTR [8] and serotype-specific multiplex PCR [2]. Total viral RNA was extracted from 3 tissue samples (Tongue epithelium and leg scar) from the clinically infected animals using QIAamp Viral RNA Mini Kit (Cat. No. 52904, QIAGEN) as per the manufacturer's protocol. For the synthesis of cDNA, Maxima H Minus First Strand cDNA Synthesis Kit (Cat. No. K1651, Thermo Scientific) was used as per the manufacturer's protocol.

Molecular characterization of FMDV by VP1 gene sequencing

The full-length VP1 gene was amplified using designed primers (Table 1) and was cloned in a TA cloning vector and sequenced by outsourcing at the University of Delhi (South campus), New Delhi. A reaction mixture of 25 µl containing 2.5 µl of 10X Taq buffer with 20 mM $MgCl_2$, 2 µl of 10 mM dNTPs, 1 µl of each of 10 pM forward and reverse primers, 0.2 µl of 5 U/µl Taq polymerase and 2 µl of cDNA as template was prepared. Cycling condition was carried out as an initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 45 sec and extension at 72°C for 30 sec. A final extension was set at 72°C for 10 min. The amplified product was verified on 1.5% agarose gel electrophoresis and was gel eluted using GeneJET Gel Extraction Kit (Cat. No. K0691, Thermo Scientific) as per the manufacturer's protocol. The purified product was used for cloning using InsTAclone PCR Cloning Kit (Cat. No. K1213, Thermo Scientific) as per the manufacturer's protocol. The gel-eluted PCR product was ligated with pTZ57R/T cloning vector and transformation was performed in competent DH5á (mutant *Escherichia coli*). The transformed cells were plated in LB ampicillin and incubated for 18 hrs at 37°C. The clone was confirmed by colony PCR and subsequently sequenced by outsourcing. Phylogenetic analysis was carried out on the basis of a full-length VP1 sequence using MEGA10 software. A phylogenetic tree was generated by using the Neighbor-Joining method, keeping bootstrap consensus from 1000 replicates. Deduced amino acid sequences of VP1 were compared using BioEdit software, with sequences of FMDV type A from previous outbreaks retrieved from GenBank.

RESULTS AND DISCUSSION

Virus detection by PCR

RT-PCR targeting the 5′UTR region gives an amplification of 328 bp fragment, confirming the FMDV, however, it cannot determine the serotype of the virus. Serotype-specific multiplex PCR for the serotypes O, A, C and Asia-1 confirms the virus as serotype A with amplification of 376 bp fragment of 1D gene of FMDV. In an endemic area where more than one serotype is circulating among the animal population, only a serotype-specific multiplex PCR can provide more accurate and rapid detection of the virus in outbreaks. Serotype-specific multiplex PCR described by Giridharan *et al.* (2005) consists of serotype-specific forward primers DHP-9 (Asia-1), DHP-13 (O), DHP-15 (A), DHP-16 (C) and universal reverse primer NK-61R, which gives expected size of 537 bp, 249 bp, 376 bp and 627 bp respectively.

Molecular characterization of VP1 capsid protein gene of FMDV

Positive amplification of full-length VP1 of 639 bp was observed in all 3 samples along with positive control, water control and no template control after electrophoresis (Fig 1). One of the amplified genes was cloned and sequenced and also annotated and submitted to the GenBank and the accession number received is OL419371 for the present sequence. On the basis of the full-length VP1 gene, Phylogenetic analysis of the present isolate (OL419371) was carried out by comparing with 639 nucleotides of the coding region of VP1 sequences of different serotypes (O, A, C, Asia-1) of previous outbreaks in India as well as outbreaks of some countries. The same analysis was also carried out by comparing VP1 sequences of the present isolate (OL419371) with different isolates of only serotype A of previous outbreaks in India including the 2001 outbreak in Mizoram (AF390659). A phylogenetic tree was generated by comparing Serotype A (Taiwan, Pakistan, different Indian strains including Assam and Mizoram), Serotype O (Nepal, Bangladesh), Serotype Asia-1(Pakistan Baluchistan), Serotype C (Swidden, India) and Indian vaccine strain (HM854025) sequences were retrieved from GenBank data and included in the analysis (Table 2, Fig 2). Phylogenetic analysis reveals the present sequence belonging to serotype A of FMDV and within this serotype, there were several sub-clades indicating multiple genotypes within A serotype. Further, we compared the present isolate VP1 sequence

Table 1: Primer sequences used in the present study.

Primer name	Sequence	Amplicon size	Use
PRIMER1 F2	ACCACCACTGCCGGGGAGT	639 bp	Detection of VP1 of FMDV by RT-PCR
PRIMER R	GCTGTTTTGCTGGTGCAATG		

with important geno groups within serotype A, circulating in Asian countries showing a cluster within genotype VII (Fig 2, 3). The similarity of the sequence within the serotype cluster ranged between 36.9-91.7% and within serotype A it ranged between 79-91.7%. However, the sequence of the outbreak of FMDV in Mizoram during 2001(AF390659) was found in a different phylogenetic clade *i.e*., genotype VI. Sequence similarity with the Indian vaccine strain was 89.1% and with the 2001 isolate was found to be 82.4%. Sequence similarity of the present isolate was found at 67.4% with Asia-1 strains, 66.6% with Serotype O strains and only 36.9-38.1 with serotype C strains.

The deduced amino acid order of VP1 protein of the present isolate of FMDV was compared with other sequences of serotype A of previous outbreaks in India including Mizoram (AF390659) and vaccine strain

Table 2: Sequence used for the analysis of the present isolate.

Accession number	Country	Year	Strain/isolate	Serotype
EU553877	Spain	1973	A24/Santander/SPA/1/73	Serotype-A
KU737534	Maharashtra, India	1995	IND199/1995	Serotype-A
MT981285	UAE	1995	A/SAU/24/1995	Serotype-A
AF390631	India	1996	A/IND/270/96	Serotype-A
AF204108	West-Bengal, India	1997	A22/India/17/77	Serotype-A
HM854025	India vaccine virus strain	1999	IND40/00	Serotype-A
AF390592	West-Bengal, India	2000	A/IND/104/2000	Serotype-A
AF390659	Mizoram, India	2001	A/IND/68/2001	Serotype-A
HQ832584	Assam, India	2006	IND 22/2006	Serotype-A
HQ832589	India	2006	IND 109/2006	Serotype-A
FJ798194	Pakistan	2007	PAK/73/2007	Serotype-A
HQ116353	Thailand	2009	A/TAI/10/2009	Serotype-A
HM854025	India vaccine virus strain	2010	IND40/00	Serotype-A
KY825722	Switzerland	1965	C1/Noville/SWI/65	Serotype-C
AF207522	Bombay, India	1964	C/Bombay/64	Serotype-C
KM981285	Rajasthan,India	1998	O/Rajasthan/98	Serotype-O
KM921862	Nepal	2014	NEP/2/2014	Serotype-O
HQ630676	Bangladesh	2009	BAN/1/2009	Serotype-O
DQ164929	Nepal	1990	O/NEP/111/90	Serotype-O
MT442715	Pakistan	2011	PAK/89/2011	Serotype-Asia1
JX435109	Pakistan	2011	Asia/BAL/PAK/iso-2/2011	Serotype-Asia1

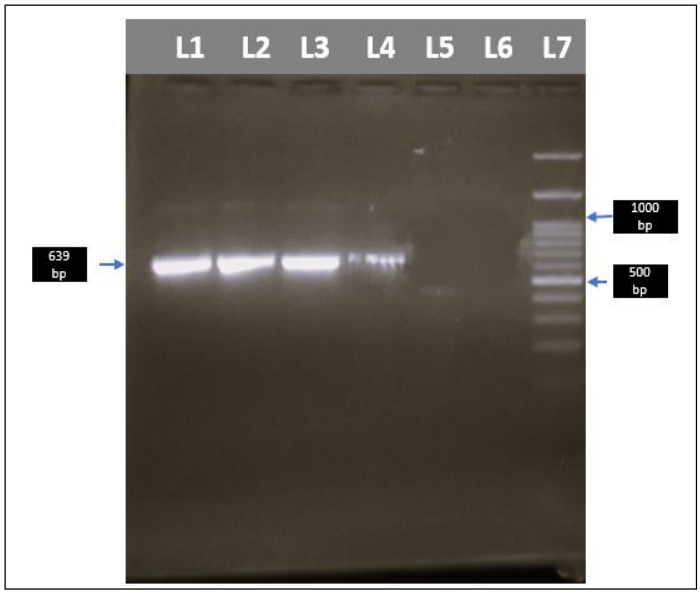

Fig 1: Full-length PCR amplification of VP1 of serotype A FMDV with specific primer.
L1-Positive control, L2- Sample1, L3-Sample2, L4-Sample3, L5-No template control, L6-Water control, L7-100 bp DNA Ladder.

(HM854025) retrieved from the GenBank. The antigenic site in FMDV serotype A is located in at the (40-60) position, G-H loop (138-154), (143-147) and (168-173) position. Hemadri *et al*. (2000). Several mutations in the deduced amino acid sequence are seen in the signal peptide domain of these regions which are the most important immunodominant region of the virus. In the G-H loop, the R(Arginine), G(Glycine) and D(Aspartate) tripeptide (144-146) which is conserved in all the genotypes and are very important because it helps in the attachment of the virus with the host cell. Tosh *et al*. (2002). Before this RGD region *i.e*., at position number 143, amino acid R(Arginine) is usually constant in genotype IV and VI of serotype A FMDV. This RGD position of amino acids is very important for the virus which has a greater role in the attachment of the virus with the host cells. These amino acids usually remain constant in all the genotypes of serotype A, which is also conserved in our present isolate. Whether, in group genotype VII, this amino acid at 143 position R(Arginine) is always substituted by T (Threonine). Mittal *et al*. (2005). This change was not observed in the isolates of 2001 outbreaks in Mizoram (AF390659) which was seen in the sub-clade of genotype IV. However, the presence of threonine before RGD tripeptide could be observed in the present sequence which is a clear indication of antigenic variation. Our present isolate OL419371 was compared with the Indian vaccine strain (HM854025) of serotype-A as well as Mizoram outbreak 2001(AF390659) isolates which were founded that there is 11 amino acid difference with the Indian vaccine strain and almost 36 amino acid difference with Mizoram 2001 isolate in different amino acid positions. Capsid protein VP1 is the most immunogenic protein of the virus. As a result of the substantial variability of the VP1 region among different topotypes and lineage, antibodies with low cross-reactivity within the same serotype may be produced; in other words, VP1 variability contributes to the antigenic diversity of various isolates. Jamal and Belsham, (2013; Nishi *et al*. (2017). Serotype A of the FMDV virus includes the most diverse group of viruses and has been described under 26 global genotypes. Mohapatra *et al.* (2008). In a study carried out by considering virus isolates over a period of 24 years, the FMDV serotype A isolates of India were divided into ten primary genotypes (marked as I–X). Tosh *et al*. (2002). In recent years, Indian isolates have been found in four genotypes (I, IV, VI and VII), with at least two genotypes (VI and VII) co-circulating in various states of the country. The investigation also revealed differences in genotype geographic distribution, with some (genotypes I and VII) being retrieved from broad geographic areas, sometimes even spanning continents, implying that the viruses have moved beyond continental limits. Hemadri *et al*., (2000). Similar amino acid substitutions in the antigenic site in FMDV serotype A have been found by Hemadri *et al*., (2000) and Tosh *et al*., (2002) in the (40-60) position, G-H loop (138-

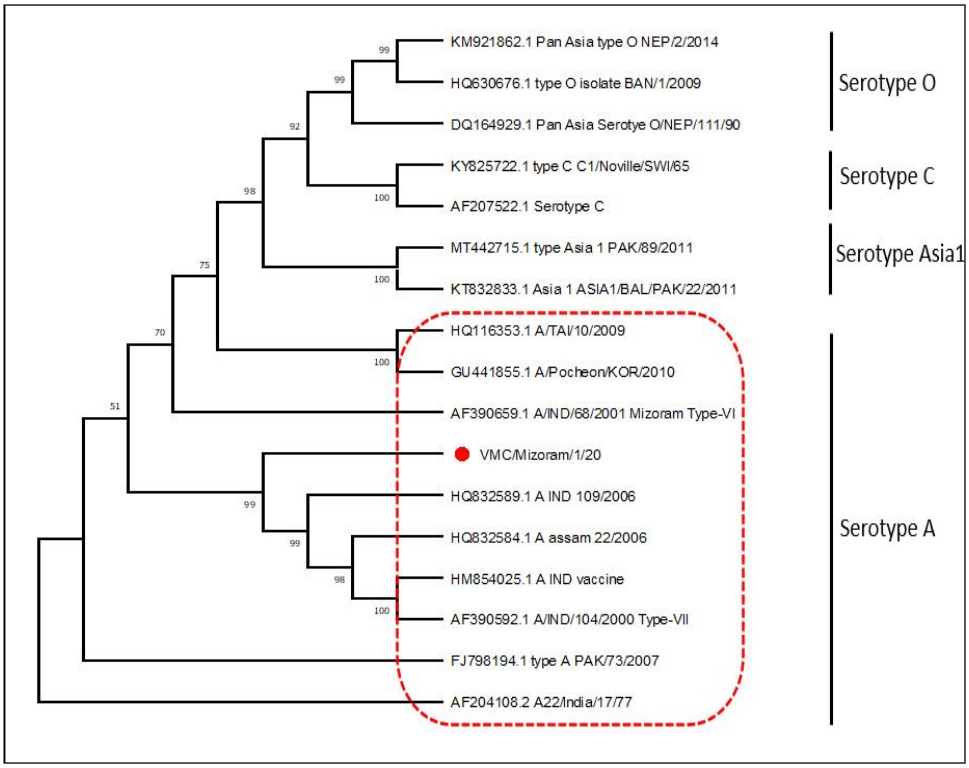

Fig 2: Phylogenetic tree generated on the basis of full-length VP1(639nt) of present isolate when compared with other serotype sequences of previous outbreaks in India and other countries using MEGA10 software.

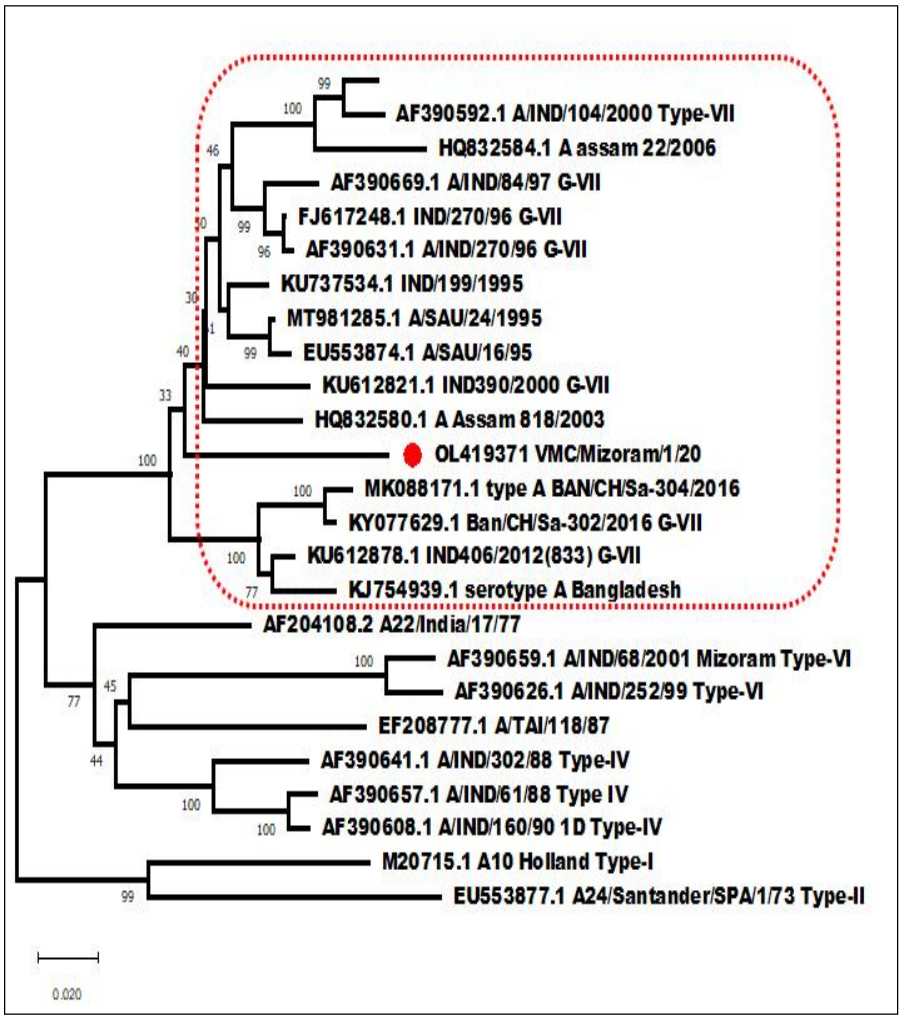

Fig 3: Phylogenetic tree generated on the basis of full-length VP1(639nt) of present isolate when compared with sequences of serotype A previous outbreaks in India including vaccine strains using MEGA10 software.

154), (143-147) and (168-173) positions. Diversity among the virus isolates of previous outbreaks, vaccine strains and the circulating viruses is critical in terms of immunization because vaccinated animals may not develop full immunity in response to the circulating virus strain. High variance strain in circulating isolates and vaccine strain indicates possibilities of low-level immune protection against vaccination.

CONCLUSION

Foot and mouth disease virus serotype A, genotype VII could be detected in an outbreak in Mizoram that occurred in April, 2020. The highest Sequence homology up to 91.7% has been observed when compared with serotype A sequences. Deduced amino acid analysis of VP1 protein when compared with other serotypes A sequences revealed that the ^{144}R-G-D^{146} motive present in the G-H loop of FMDV VP1 protein remains conserved. Overall,11 amino acid substitution was observed in the present sequence when compared with the Indian serotype A vaccine strain. A total of 36 amino acid substitutions were observed when compared with 2001 serotype A isolate of Mizoram in different major immunodominance amino acid positions of VP1 which may attribute to low-level immune protection against vaccination.

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Conflict of interest: None.

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