Acta virologica **55**: 349 – 352, 2011 doi:10.4149/av_2011_04_349

Genetic characterization of vaccine and field strains of serotype A foot-and-mouth disease virus from India

J. K. MOHAPATRA[1], S. S. PAWAR[1], C. TOSH, S. SUBRAMANIAM, R. PALSAMY, A. SANYAL, D. HEMADRI, B. PATTNAIK[*]

Project Directorate on Foot and Mouth Disease (ICAR), Mukteswar, Nainital 263 138, Uttarakhand, India

Received June 29, 2011; accepted October 24, 2011

Summary. – Extreme antigenic and genetic heterogeneity of serotype A foot-and-mouth disease virus (FMDV) population has resulted in change of vaccine strains in India twice in the last decade. In such a situation, complete characterization of the vaccine strains is imperative. With regard to the frequent outbreaks of this disease, FMDV field strains are also of interest. Therefore three vaccine strains and two field strains of type A FMDV from India were completely sequenced and the obtained sequences were subjected to sequence and phylogenetic analyses. Based on the complete coding region, all the Indian strains clustered in the Asia topotype and exhibited a more than 11% nt divergence from the other Asian strains. The 5'-UTR of some Indian strains revealed block deletions of 43 and 86 nt corresponding to the pseudoknot region. Amino acids S44 in VP2 and F164 in VP1 were found to be the exclusive signatures for the Asia topotype. The vaccine strains differed at 65 aa positions in the capsid region, 13 of them antigenically critical. Variability at such positions is likely to affect the antigenic profile of these strains. Complete genome sequences of the vaccine strains presented here could serve as the reference for any comparative genomics in future.

Keywords: foot-and-mouth disease virus; vaccine strains; field strains; India

Introduction

Foot-and-mouth disease (FMD) is one of the most contagious transboundary animal diseases, which can cripple a country's economy due to substantial loss in livestock output and trade restrictions. The causative agent, FMDV is the prototype member of the genus *Aphthovirus* in the family *Picornaviridae* and exists in the form of seven distinct serotypes. Among the three serotypes prevalent in India (O, A, and Asia 1), type A is reported to be the most unstable population both genetically and antigenically (Jangra *et al.,* 2005). VP1 coding region (1D) based molecular phylogeny has shown circulation of four {2 (I), 10 (IV), 16 (VI) and 18 (VII)} out of the twenty six global genotypes (showing more than 15% nt divergence among them at 1D region) in India (Mohapatra *et al.,* 2011). Quasispecies dynamics accompanied by immune selection in the context of sparse vaccination practiced in an endemic region is thought to have resulted in a complicated phenomenon of rapid genotype/lineage turnover in India (Mohapatra *et al.,* 2009). Moreover, antigenic profiling of circulating field strains and vaccine matching have demonstrated poor intergenotypic antigenic cross-match. Based on such studies, the older vaccine strains have been replaced with the newer ones on two occasions in the last decade (Tosh *et al.,* 2003; Mohapatra *et al.,* 2008a). Complete genomic characterization of vaccine strains is vital in elucidating their genetic relationship with the circulating field strains, in detecting variation at the antigenically critical residues, in keeping track of any undesirable changes occurring in the vaccine strains upon cell culture propagation, which could compromise the overall

[*]Corresponding author. E-mail: pattnaikb@gmail.com; fax: +915942-286307.[1]These authors contributed equally to the paper.

Abbreviations: aLRT = approximate likelihood ratio test; *cre/bus* = cis-acting replication element/3B-uridylylation site; FMD = foot-and-mouth disease; FMDV = FMD virus; HKY85 = Hasegawa-Kishino-Yano 85; PK = pseudoknots; PhyML = phylogenetic estimation using maximum likelihood

antigenic and growth characteristics of the original seed virus, and also in solving any vaccine-related outbreaks with high degree of authenticity.

In this study, we determined complete genome sequences of three type A FMDV vaccine strains including two older strains (IND 17/1977 of genotype 10 and IND 17/1982 of genotype 16) and the current strain (IND 40/2000 of genotype 18) and two field strains (IND 258/1999 of genotype 16 and IND 81/2000 of genotype 18). These sequences along with others from the database (for GenBank Acc. Nos, see Fig. 1) were employed for phylogenetic analyses.

Materials and Methods

Viruses. Five serotype A FMDV strains in the form of infected BHK-21 cell culture supernatant (between passage level 3 and 6) were obtained from the 'National FMD Virus Repository' maintained at the Project Directorate on FMD, Mukteswar, India.

Sequencing. Viral RNA was extracted from the infected cell culture supernatant using RNeasy Mini Kit (Qiagen). Reverse transcription was performed using MMLV RT (Promega) and oligo $d(T)_{15}$ primer. The complete genome was amplified in seven overlapping fragments (SF1F-SF370R, LF1F-MG39, L463R-NK61, 1C562-MG8, MG33-CTLV10, MG15-MG16 and 3D1081-Anchored oligo dT) using *pfu* DNA polymerase (Fermentas). The full-length nt sequence excluding the original poly(C) tract and the poly(A) tail was resolved on ALF Express II automated DNA sequencer (Amersham Pharmacia Biotech). The details of the primers used here are essentially as described earlier (Tosh *et al.,* 2003; Mohapatra *et al.,* 2008b), except for a sequencing primer {3D1234 (5′CTCTCCTTTGCACGCCGTGG) in place of 3D1081}, which was employed for resolving the 3′-UTR sequence.

Sequence and phylogenetic analyses. The nt and aa sequences were aligned using ClustalX version 1.83. (Thompson *et al.,* 1997) and the maximum likelihood phylogeny was inferred from the complete coding region sequences using PhyML version 3.0 (Guindon and Gascuel, 2003). For this analysis, the HKY85 nucleotide substitution model and the approximate likelihood-ratio test (aLRT) for branches were selected. For prediction of two-dimensional RNA secondary structures of pseudoknots (PK), the pknotsRG tool was employed (Reeder and Giegerich, 2004).

Results and Discussion

In the maximum likelihood tree, all the taxa clustered in the three geographically restricted continental topotypes such as Asia, Africa and Euro-South America (Knowles and Samuel, 2003) with high aLRT (>0.8) values (Fig. 1). The overall topology of the tree reconstructed based on the complete coding region displayed congruence with that for the 1D region (data not shown). Divergence in more than 14% of nt in the complete coding region was noticed between the topotypes. The five Indian strains clustered in the Asia topotype and appeared to share the most recent common ancestor with the strains from the Middle East and Pakistan. However, the Indian sequences differed from those strains by more than 11% of nucleotides, indicating their distinct genetic identity. Among the Indian strains, nt and aa divergence varied from 0.7 to 10.2% and 0.6 to 4.6%, respectively. The three vaccine strains revealed 9.4 to 10.2% nt divergence among themselves. The current vaccine strain (IND 40/2000) grouped closely with a field strain (IND 81/2000) with just 0.7% nt difference, suggesting their close epidemiological link. Both these strains were recovered within a month's time from the same outbreak area, but from different hosts i.e., clinically affected cattle and buffalo.

For all five Indian strains, the complete coding region was found to be 6999 nt long, encoding 2333 aa without any insertions or deletions. The length of the 5′-UTR (considering only 6 'C' residues of the poly(C) tract) varied from 1003 to 1090 nt. The small fragment of 5′-UTR was of uniform length (370 nt) in all the strains, while the redundant PK region in the large fragment immediately following the poly(C) tract revealed block deletions of 43 and 86 nt in IND 17/1982 of genotype 16 and in IND 40/2000 and IND 81/2000 of genotype 18, respectively. Such large deletions disrupted prediction of one or two PKs (data not shown). But a minimum of two PKs in all five sequences were maintained, probably because of some hitherto unknown evolutionary constraint against any further deletions. Such deletions in the PK region do not seem to follow any genotype specific trend as IND 258/1999 of genotype 16 did not reveal any deletion, while IND 17/1982 of the same genotype did so. The 3′-UTR (excluding the poly(A) tract) varied in length between 94 and 101 nt.

A total of 518 aa positions (22.2%) revealed variability in the complete coding region sequence alignment for the global dataset. Unsurprisingly, VP4 followed by 3C, 2B and 3D (in that order) revealed maximum proportion of invariant aa positions, whereas VP1 followed by 3A and L revealed maximum variable positions. Amino acids S44 in VP2 and F164 in VP1 were identified as signatures for Asia topotype. All established catalytic sites in the polymerase, proteases, the critical motifs in the IRES domains and the '*cre/bus*' element were found conserved in the Indian sequences (for references see Carrillo *et al.,* 2005).

The three vaccine strains differed at 65 aa positions in the capsid region (Table 1). Further, 13 out of those 65 positions have been shown to be antigenically critical in type A virus in earlier studies (for references see Tosh *et al.,* 2003). Hence, the variability at such positions is likely to affect the antigenic profile of these vaccine strains, as evident from the data generated on their one-way antigenic relatedness with the field viruses in microneutralization tests (Jangra *et al.,* 2005). The maximum proportion of the variability in the dataset was concentrated around the βB-βC (aa 40–60) and βG-βH (aa 140–160) loops of VP1 protein. Although presence of neutralization-relevant

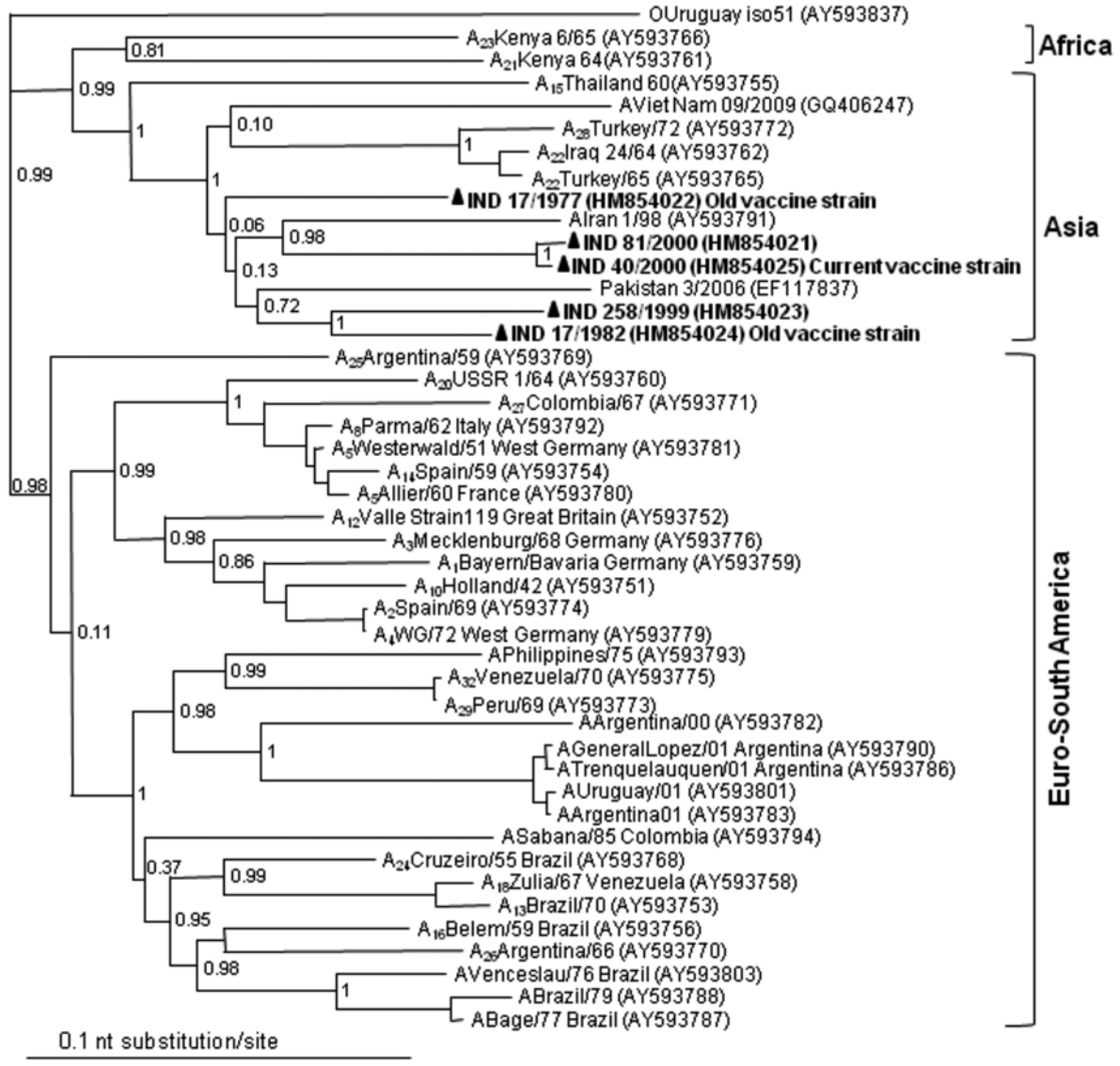

Fig. 1

Phylogenetic tree of serotype A FMDV strains based on the entire coding region

The aLRT supports are indicated at the nodes. Type O Uruguay iso51 (a type O strain isolated during 1963 in Uruguay) sequence was used as the outgroup in tree reconstruction for better resolution of the topology. Indian virus sequences that were determined in this study are shown in bold face with solid triangles, and topotypes are indicated with brackets.

antigenic sites on the βG-βH loop of VP1 has been demonstrated in type A virus, no report on the involvement of βB-βC loop residues in the neutralization of the virus infectivity is available so far. Considering the degree of variability observed in the VP1 βB-βC loop of field strains of different antigenic make-up, it is presumed that this surface-exposed region might have implications for extending the antigenic diversity of type A FMDV population similar to other serotypes.

We resolved the full-length genome sequence of vaccine strains and field strains of serotype A FMDV from India and demonstrated their phylogenetic grouping in the Asia topotype. Considerable variability was shown to be concentrated around the βB-βC and βG-βH loop of VP1 protein, indicating their probable involvement in widening the antigenic repertoire of the virus. The analysis determined two Asia topotype-specific aa signatures as S44 in VP2 and F164 in VP1. The complete

Table 1. Variability in the capsid proteins of the Indian vaccine strains

Strains	VP2 aa																	
	39	68	71	**79**	85	88	131	134	149	154	189	190	191	193	195	**196**	201	207
IND 17/1977	G	N	P	**E**	N	T	E	M	S	M	T	S	T	S	G	**E**	A	H
IND 17/1982	A	D	T	**A**	T	K	K	P	S	M	T	S	N	G	Q	**Q**	V	H
IND 40/2000	G	D	A	**E**	T	K	E	T	N	T	N	A	G	G	T	**Q**	A	Y

Strains	VP3 aa											
	8	37	**59**	65	**70**	92	94	**139**	**175**	197	204	216
IND 17/1977	S	Y	**D**	V	**A**	S	L	**Q**	**V**	Q	V	I
IND 17/1982	S	Y	**N**	E	**E**	A	I	**K**	**T**	H	V	V
IND 40/2000	A	F	**N**	E	**E**	S	I	**K**	**T**	N	A	I

Strains	VP1 aa																	
	4	22	24	28	39	41	42	43	44	45	46	48	55	57	59	60	**83**	84
IND 17/1977	T	T	V	Q	F	R	I	T	A	V	S	T	R	H	H	G	**E**	G
IND 17/1982	T	V	A	H	S	K	V	N	A	V	S	I	Q	P	N	T	**T**	D
IND 40/2000	A	T	V	H	F	K	I	G	T	T	N	T	Q	H	H	G	**S**	G

Strains	VP1 aa																
	99	102	108	134	138	**139**	**141**	142	**143**	**149**	**154**	167	168	**170**	171	191	197
IND 17/1977	V	S	H	S	A	**P**	**A**	G	**R**	**P**	**T**	V	R	**T**	T	M	S
IND 17/1982	S	S	H	S	T	**A**	**I**	G	**R**	**A**	**V**	I	R	**T**	T	L	T
IND 40/2000	S	G	N	N	A	**A**	**G**	R	**T**	**Q**	**I**	I	K	**D**	A	L	S

Residues identified to be antigenically critical are shown in bold face.

genome sequence of vaccine strains generated here would serve as references for future phylogenomic investigations.

Acknowledgement. The authors thank the Indian Council of Agricultural Research for providing necessary facilities to carry out this work.

References

Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish GF, Rock DL (2005): Comparative genomics of foot-and-mouth disease virus. J. Virol. 79, 6487–6504. http://dx.doi.org/10.1128/JVI.79.10.6487-6504.2005

Guindon S, Gascuel O (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704. http://dx.doi.org/10.1080/10635150390235520

Jangra RK, Tosh C, Sanyal A, Hemadri D, Bandyopadhyay SK (2005): Antigenic and genetic analyses of foot-and-mouth disease virus type A isolates for selection of candidate vaccine strain reveals emergence of a variant virus that is responsible for most recent outbreaks in India. Virus Res. 112, 52–59. http://dx.doi.org/10.1016/j.virusres.2005.03.021

Knowles NJ, Samuel AR (2003): Molecular epidemiology of foot-and-mouth disease virus. Virus Res. 91, 65–80. http://dx.doi.org/10.1016/S0168-1702(02)00260-5

Mohapatra JK, Hemadri D, Rao TVS, Sreenivasa BP, Subramaniam S, Sanyal A, Periyasamy TR, Singh NK, Pattnaik B, Venkataramanan R (2008a): Assessment of suitability of two serotype A candidate vaccine strains for inclusion in FMD vaccine in India. Vet. Microbiol. 131, 65–72. http://dx.doi.org/10.1016/j.vetmic.2008.02.020

Mohapatra JK, Priyadarshini P, Pandey LK, Subramaniam S, Sanyal A, Hemadri D, Pattnaik B (2009): Analysis of the leader proteinase (Lpro) region of type A foot-and-mouth disease virus with due emphasis on phylogeny and evolution of the emerging VP359-deletion lineage from India. Virus Res. 141, 34–46. http://dx.doi.org/10.1016/j.virusres.2008.12.012

Mohapatra JK, Sanyal A, Hemadri D, Tosh C, Biswas S, Rasool TJ, Bandyopadhyay SK, Pattnaik B (2008b): Comparative genomics of serotype Asia 1 foot-and-mouth disease virus isolates from India sampled over the last two decades. Virus Res. 136, 16–29. http://dx.doi.org/10.1016/j.virusres.2008.04.010

Mohapatra JK, Subramaniam S, Pandey LK, Pawar SS, De A, Das B, Sanyal A, Pattnaik B (2011): Phylogenetic structure of serotype A foot-and-mouth disease virus: global diversity and the Indian perspective. J. Gen. Virol. 92, 873-879. http://dx.doi.org/10.1099/vir.0.028555-0

Reeder J, Giegerich R (2004): Design, implementation and evaluation of a practical pseudoknot floding algorithm based on thermodynamics. BMC Bioinformatics 5, 104. http://dx.doi.org/10.1186/1471-2105-5-104

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997): The CLUSTAL X windows interface: flexible strategies for mul- tiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876

Tosh C, Hemadri D, Sanyal A, Bandyopadhyay SK (2003): Genetic and antigenic analysis of two recently circulating genotypes of type A foot and mouth disease virus in India: evidence for positive selection in the capsid coding genes. Arch. Virol. 148, 853–869. http://dx.doi.org/10.1007/s00705-002-0968-2