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Understanding the Molecular Relationship between Foot-and-Mouth Disease Virus Serotype O of Indian Vaccine Strain with Strains across the World by Phylogenetic Analysis

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A B S T R A C T

Foot-and-mouth disease is one of the important animal virus known to be highly infectious and causing a serious trade barrier. For effective control of FMD there is a need for in depth understanding of the virus epidemiology. The present study has been undertaken to understand the molecular relationship between the strains across world in comparison to Indian vaccine strain and virus isolate of 2010. The study is done by comparing the nucleotide sequence of VP1 protein, known to induce neutralizing antibodies by phylogenetic analysis. Based on phylogenetic analysis we found that the disease in India is mainly caused by transboundary evolution of virus but not due to the vaccine strain.

Introduction

Foot-and mouth-disease is an infectious and highly contagious viral disease of domestic and wild cloven hoofed animals causing a huge economic loss in agriculture worldwide (Kandeil *et al.*, 2013). As per the latest ICTV virus taxonomy release the etiological agent, FMD virus (FMDV) is grouped under the genus *Apthovirus* of family *Picornaviridae* (ICTV, 2015). FMD was first recognised by a Francisian monk Hieronymus Fracastorius (1546) in cattle, and Loeffler and Frosch (1897) demonstrated for the first time a filterable agent causing animal disease, FMD. FMD virus exists as seven different serologically distinct types namely, serotypes O, A (Valle and Carre, 1922), C (Waldmann and Trautwein, 1926), SAT 1, SAT2, SAT3 (Brooksby, 1958), Asia1 (Brooksby and

Roger, 1957) with no cross immune protection (Kitching, 1998). The disease plays an important role in global trade and is a priority disease among the list A diseases published by Office International des Epizootics (OIE).The disease is endemic in large areas of Africa, Asia, and South America and has the potency to cross international boundaries to create epidemics in non-infected areas (Knowles *et al.,* 2001; Alexandersen *et al.,* 2003). The 2001/2002 European outbreak explains the huge economic loss (6000 million Euros) caused by FMD (Domingo *et al.,* 2003). In India, FMD Serotype O is responsible for 80% of outbreaks confirmed (2150 outbreaks) whereas 12% accounts for Asia 1 and 8% by serotype A (Subramaniam *et al.,* 2013). The VP1 protein comprises of 213 amino acid residues (Acharya *et al.,* 1989) is concerned in formation of neutralizing antibodies and attachment to susceptible cells (Wild *et al.,* 1969). VP1 is the most studied FMDV protein due to its significance for virus attachment, entry, protective immunity and serotype specificity (Jackson *et al.,* 2003). The molecular characterization and understanding the epidemiology of the virus can give details into the control of FMD. Hence, in present study, we have retrieved the VP1 sequences from NCBI and phylogenetically analyzed the molecular relation of FMDV Indian vaccine strain against the FMDV strains across the world.

Materials and Methods

Retrieval of sequence data from NCBI

We searched GenBank for complete sequences of the FMDV VP1protein-coding gene of serotype O and recorded the geographic location and date of isolation. A total of 21 sequences of FMDV strains across the world including Indian vaccine strain were retrieved from the nucleotide database of NCBI.

Multiple sequence alignment

The sequences of 20 strains were aligned against the Indian vaccine strain and the amino acid sequence is compared for variable regions.

Phylogenetic analysis

The evolutionary distance matrix was constructed by calculating pairwise distance of the aligned sequences using MEGA 6. Further, the time tree and phylogenetic tree were constructed by neighbour joining statistical method in MEGA 6.

The bootstrap replications used are 500; model used is Tamura3-parameter model.

Results and Discussion

By aligning the amino acid sequences of different FMDV strains (20) in comparison to Indian vaccine strain we found the later has three changes incorporated in it. From Figure 1 the changes of threonine to phenylalanine at position 4 (T4P), phenylalanine to serine at position 140 (P140S), serine to asparagine at position 197 (S197N) can be observed. Figure 2 shows the evolutionary divergence between the sequences of FMDV Indian vaccine strain and Indian isolate 2010 as 0.113, FMDV Indian vaccine strain and FMDV O Manisa as 0.096. From the results presented in Figure 3 the FMDV Indian vaccine strain showed closest relatedness with the KRG isolate of 2006 with 80% sequence identity, FMDV Indian isolate 2010 showed closest relatedness with the BAN and NEP isolate of 2009 with 100% sequence identity, FMDV O Manisa showed closest relatedness with the IRN isolate of 2007 with 100% sequence identity. From the results presented in Figure 4 the FMDV Indian vaccine strain showed lowest divergence with the KRG isolate of 2006 with 0.01 divergence time, FMDV Indian isolate

2010 showed lowest divergence with the BAN and NEP isolate of 2009 with 0.00 divergence time, FMDV O Manisa showed lowest divergence with the IRN isolate of 2007 with 0.01 divergence time but shown >0.2 divergence time with others.

Fig.1 Amino acid sequence alignment showing the differences in FMDV Indian vaccine strain in comparison with other 20 strains generated by multiple sequence alignment by Clustal ω

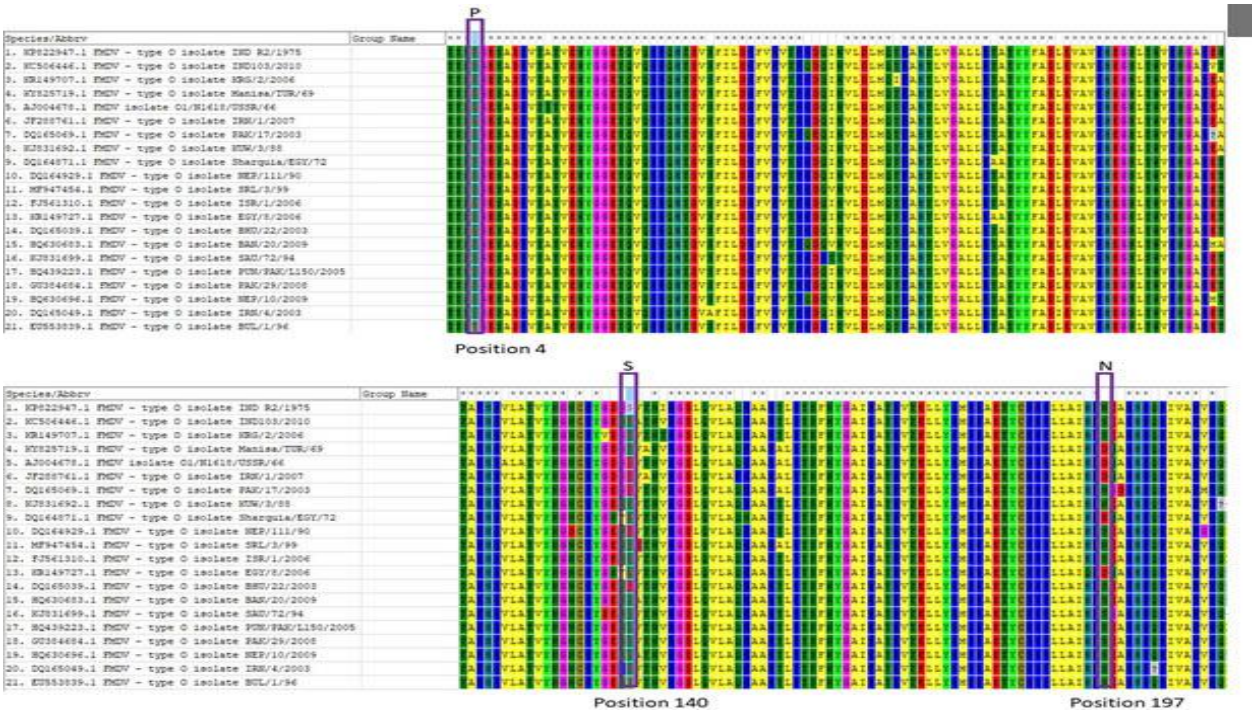


Fig.2 Estimates of evolutionary divergence between sequences. The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Tamura 3-parameter model (Tamura, 1992). The rate variation among sites was modelled with a gamma distribution. The analysis involved 21 nucleotide sequences. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013)

1. KP822947.1 FMDV - type O isolate IND R2/1975																				
2. KC506446.1 FMDV - type O isolate IND103/2010	0.113																			
3. KR149707.1 FMDV - type O isolate KRG/2/2006	0.079	0.136																		
4. KY825719.1 FMDV - type O isolate Manisa/TUR/69	0.096	0.126	0.097																	
5. AJ004678.1 FMDV isolate O1/N1618/USSR/66	0.092	0.129	0.087	0.034																
6. JF288761.1 FMDV - type O isolate IRN/1/2007	0.098	0.129	0.100	0.002	0.036															
7. DQ165069.1 FMDV - type O isolate PAK/17/2003	0.115	0.117	0.133	0.111	0.126	0.114														
8. KJ831692.1 FMDV - type O isolate KUW/3/88	0.109	0.093	0.134	0.117	0.115	0.120	0.098													
9. DQ164871.1 FMDV - type O isolate Sharquia/EGY/72	0.107	0.138	0.113	0.078	0.054	0.080	0.158	0.131												
10. DQ164929.1 FMDV - type O isolate NEP/111/90	0.111	0.076	0.124	0.110	0.112	0.112	0.112	0.068	0.118											
11. MF947454.1 FMDV - type O isolate SRL/3/99	0.118	0.076	0.137	0.113	0.120	0.115	0.114	0.090	0.131	0.052										
12. FJ561310.1 FMDV - type O isolate ISR/1/2006	0.109	0.095	0.129	0.117	0.124	0.119	0.138	0.114	0.133	0.080	0.098									
13. KR149727.1 FMDV - type O isolate EGY/8/2006	0.110	0.138	0.116	0.080	0.056	0.083	0.159	0.134	0.006	0.121	0.131	0.136								
14. DQ165039.1 FMDV - type O isolate BHU/22/2003	0.109	0.091	0.136	0.105	0.117	0.107	0.123	0.089	0.128	0.054	0.087	0.064	0.131							
15. HQ630683.1 FMDV - type O isolate BAN/20/2009	0.127	0.018	0.141	0.124	0.126	0.126	0.119	0.096	0.140	0.082	0.078	0.106	0.141	0.097						
16. KJ831699.1 FMDV - type O isolate SAU/72/94	0.123	0.095	0.129	0.129	0.126	0.131	0.111	0.093	0.143	0.037	0.068	0.106	0.143	0.075	0.101					
17. HQ439223.1 FMDV - type O isolate PUN/PAK/L150/2005	0.129	0.088	0.138	0.135	0.119	0.121	0.121	0.091	0.137	0.058	0.082	0.068	0.140	0.058	0.082	0.086				
18. GU384684.1 FMDV - type O isolate PAK/29/2008	0.127	0.086	0.133	0.116	0.119	0.119	0.119	0.089	0.135	0.056	0.080	0.066	0.138	0.056	0.084	0.083	0.002			
19. HQ630696.1 FMDV - type O isolate NEP/10/2009	0.127	0.016	0.144	0.128	0.131	0.131	0.126	0.102	0.140	0.082	0.078	0.106	0.140	0.097	0.008	0.101	0.082	0.084		
20. DQ165049.1 FMDV - type O isolate IRN/4/2003	0.127	0.105	0.126	0.123	0.126	0.126	0.124	0.101	0.142	0.065	0.096	0.085	0.145	0.094	0.111	0.065	0.100	0.098	0.113	
21. EU553839.1 FMDV - type O isolate BUL/1/96	0.117	0.096	0.135	0.133	0.126	0.136	0.129	0.097	0.132	0.044	0.075	0.099	0.132	0.073	0.103	0.030	0.087	0.085	0.103	0.075

Fig.3 Evolutionary relationships of 21 strains of FMDV serotype O. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Tamura 3-parameter method (Tamura, 1992) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution. The analysis involved 21 nucleotide sequences. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013)

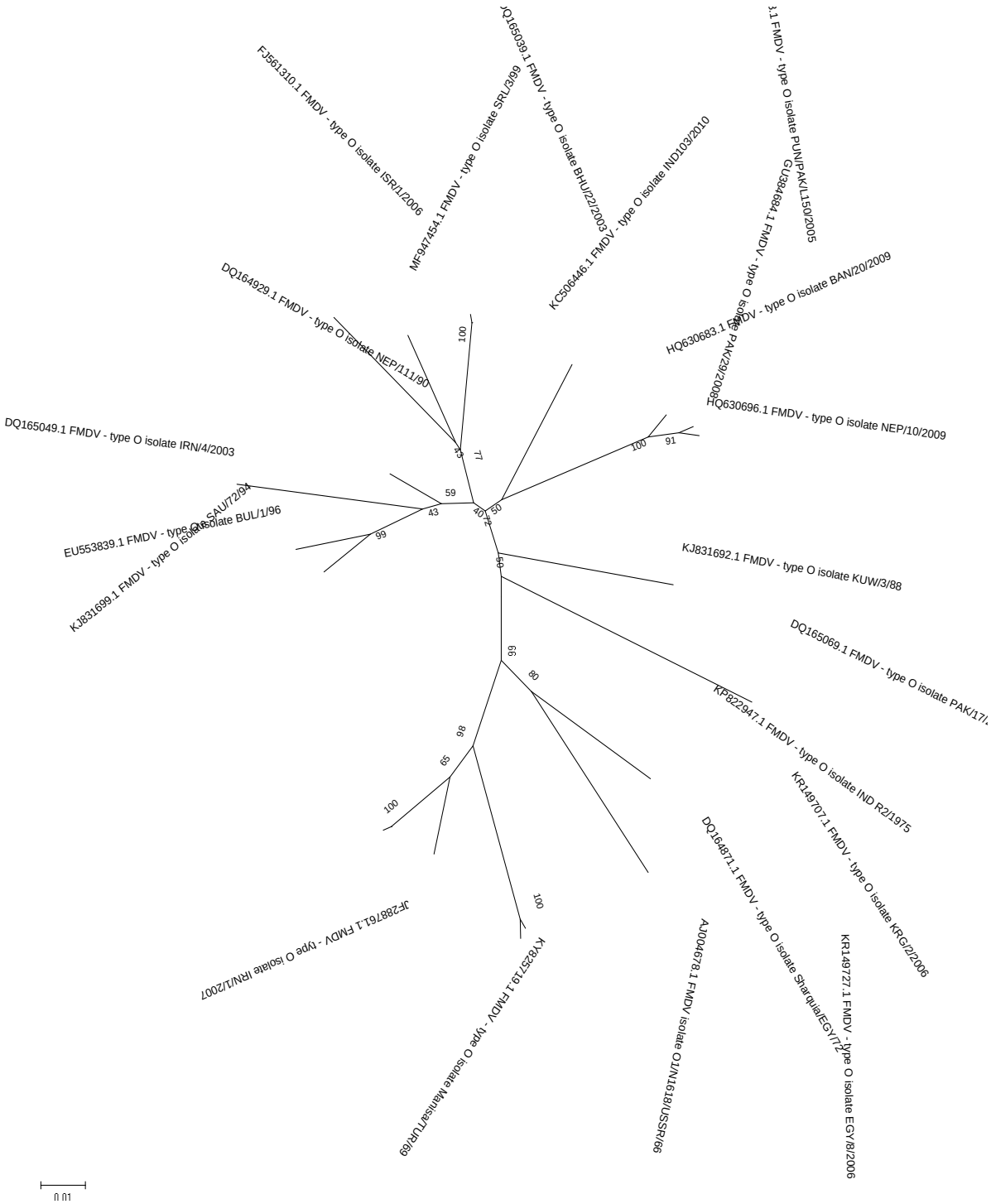
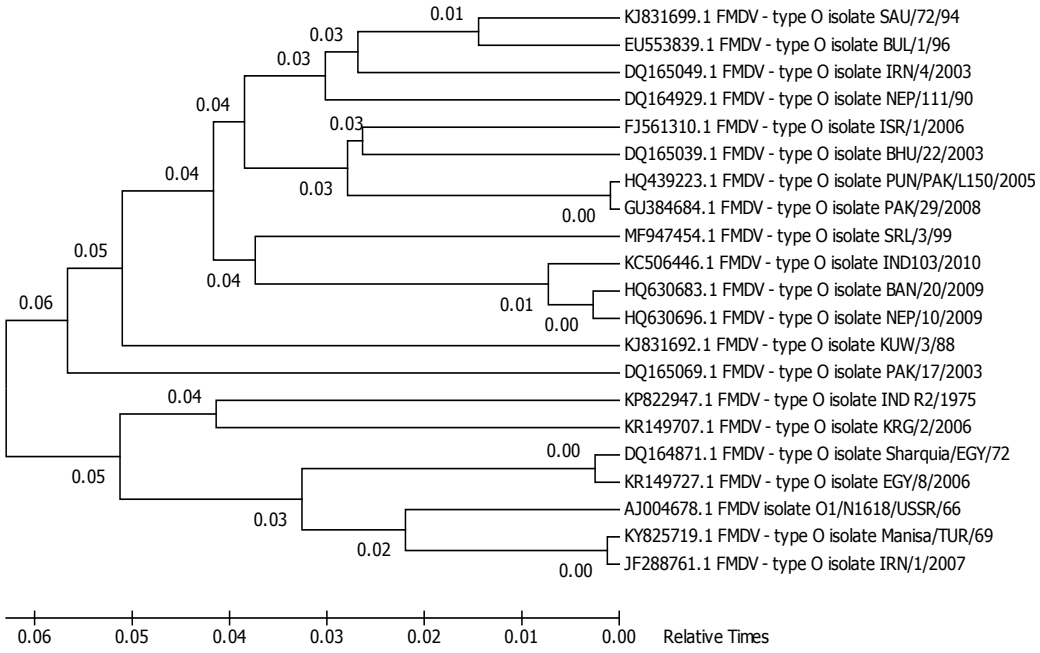


Fig.4 Evolutionary relationships of 21 strains of FMDV serotype O (timetree). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). Divergence times for all branching points in the topology were calculated with the RelTime method (Tamura *et al.*, 2012) using the branch lengths contained in the inferred tree. The analysis involved 21 nucleotide sequences. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013)



Foot-and-mouth disease virus is one of the most contagious viral pathogen that cause huge risk for the livestock economy with almost having a host range of among 70 species of cloven-hoofed mammals (Carrillo *et al.*, 2005). In India, FMD Serotype O is responsible most of the FMD out breaks (Subramaniam *et al.*, 2013), the serotype we used in the study. The VP1 protein on which the present study was performed comprises of 213 amino acid residues (Acharya *et al.*, 1989) with major antigenic sites of virus capsid located between amino acids 138 and 160 (Strohmaier *et al.*, 1982) was concerned in formation of neutralizing antibodies and attachment to susceptible cells (Wild *et al.*, 1969). Hence, in this study the phylogenetic analysis was conducted on VP1 protein to observe the genetic relatedness of FMDV Indian vaccine strain. The VP1 based sequence similarity tree shown FMDV Indian vaccine strain closely related with the KRG isolate of 2006, FMDV Indian isolate 2010 closely related with the BAN and NEP isolate of 2009, FMDV O Manisa closely related with the IRN isolate of 2007. The genome of FMDV is known to have a high mutation rate of about 1-8 nucleotides per replication cycle (Domingo *et al.*, 1995). In the present study, also we observed many nucleotide changes between 21 strains. Further, the comparison of amino acid sequence of 21 FMDV strains revealed three major amino acid changes in Indian vaccine strain, at position 4, 140, 197. The virus isolate from India during 2010 show large divergence from the vaccine strain as evident from the phylogenetic tree. For phylogenetic interpretations the FMDV that differ in 2-5% from each other are generally believed to originate from same enzootic (Samuel *et al.*, 1997). However, we found much more difference between vaccine strain

and 2010 isolate, which indicate that the virus isolate of 2010 from infected animals are not related to vaccine strain. This may hint that the cause for outbreak may be due to the entry of new virus strain transboundary.

The present study describes the requirement for implementation of strict control measures to stop the emergence of virus transboundary. Further, the study also hints the requirement of more advanced alternative vaccines that can protect against different strains of virus.

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