

## Effect of Antibodies Raised in Bovine and Guineapigs on The Genetic Characters of Serotype Asia1 FMD Virus

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**Abstract:** In the present work, the effect of antibodies (raised in Bovine against IND 63/72 and in Guineapigs against IND 63/72 and IND 491/97) on foot-and-mouth disease serotype Asia1 vaccine strain IND 63/72 was compared by analyzing the data obtained in nucleotide sequencing. The Nr viruses (neutralization resistant viruses) which were selected by growing them in the presence of antibodies (Bovine Vaccinate Serum, BVS and Guineapig Serum, GPS) showed certain characteristic features like common changes in the structural protein coding P1 region of this virus. This shows that the virus responds equally to the antibodies raised in both bovine (natural host) and guineapig (experimental host). The result here extends the finding that selection exerted by host antibody also plays a major role in the rapid evolution of FMD Virus serotype Asia1.

**Key words:** FMD virus, antibodies, genetic characters

### INTRODUCTION

Foot-and-Mouth Disease (FMD) is a highly contagious disease affecting *Artiodactylae*, mostly cattle, sheep, goats and several species of wild ungulates and elephants<sup>[1]</sup> and is responsible for the most economically important viral disease of all cloven-hoofed animals. Foot-and-Mouth Disease Virus (FMDV) is the prototype member of the genus *Aphthovirus* in the family *Picornaviridae*<sup>[2]</sup>. The virus exists in the form of seven different serotypes; O, A, C, Asia1 and South African Territories 1 (SAT1), SAT2 and SAT3 and a large number of subtypes have evolved within each serotype<sup>[3,4]</sup>.

All populations of RNA viral genomes examined to date exist not as molecules with identical sequences but as molecules exhibiting some microheterogeneity. This phenomenon, referred to as the "quasispecies" nature of RNA viral populations, is closely related to the error rate of the RNA polymerases, involved in replication of RNA genomes<sup>[5,6]</sup>. This property endows RNA viruses with a high potential for viral variation and adaptation. FMDV has high mutation rate and spontaneous mutants may be readily isolated in the laboratory<sup>[7]</sup>. Genetic and antigenic heterogeneity of FMDV populations, as well as high rates of fixation of mutation have been observed in populations derived from cloned viruses upon a limited number of acute and persistent infections in cell culture [48]. It has been proposed that selection by antibodies or other

immune mechanisms may play a major role in the rapid evolution of the virus<sup>[8,9]</sup>.

Neutralization resistant viruses can readily be obtained both *In-vivo* and *in-vitro* indicating that such a selection is a common and rapid response of FMDV to antibody pressure<sup>[10]</sup>. Genetic and antigenic variations were reported earlier in the neutralization resistant variants of FMDV strain A24 Cruzeiro<sup>[10]</sup>, O1 Caseros<sup>[11]</sup> and C3 Resende<sup>[12]</sup> selected *in-vitro* in the presence of immune pressure. The present work reports the characterization of neutralization resistant viruses of FMDV type Asia1 vaccine strain IND 63/72 selected in the presence of different Guineapig Serum (GPS) and Bovine Vaccinate Serum (BVS) and to compare the effect of antibodies raised in bovine as well as guineapigs on the genetic characters of this serotype virus.

### MATERIALS AND METHODS

**Cells, viruses, anti-sera:** BHK-21 clone 13 cells and FMDV Asia1 vaccine strains IND 63/72, IND 491/97 available in the Project Directorate on FMD were employed throughout this study. The reference antisera against the purified and inactivated whole virus (146S) particles of IND 63/72 was raised in hill bulls and against IND 63/72, IND 491/97 in guinea pigs.

**Selection of Nr viruses:** Parental virus (cloned P0) was serially passaged in the presence of independent serum

(IND 63/72 GPS, IND 63/72 BVS and IND 491/97 GPS) starting from the highest serum dilution (i.e., the dilution which neutralize 100 TCID<sub>50</sub> of the virus). The Nr viruses resistant to different sera were selected as reported earlier<sup>[12]</sup>. The selective cycle was continued for 30 times under identical conditions (IND 63/72+ IND 63/72 GPS P30; IND 63/72+ IND 63/72 BVS P30; IND 63/72+ IND 491/97 GPS P30) still it grows in the presence of undiluted serum. The Nr viruses thus selected were subjected to nucleotide sequence analysis.

**RNA extraction, RT-PCR and sequencing:** RNA was extracted from the infected cell culture fluid by guanidine isothiocyanate method using Rneasy Total RNA kit (Qiagen) following the manufacturer's recommendations. The structural protein-coding region (P1) was amplified using primers L01F (5'-GTGCCCCAGTTTAAAAAGCTT,<sup>[13]</sup> and NK61 (5'-GACATGTCCTCCTGCATCTG,<sup>[14]</sup> lying on 5'UTR and 2B gene, respectively using Superscript™ One-Step RT-PCR system (Life Technologies). The thermal conditions used for amplification: 1 cycle at 48°C for 30 min, 40 cycles at 94°C for 20 s, 53°C for 30s and 68°C for 3 min, followed by 1 cycle at 68°C for 10 min (Hybaid, UK). The amplified PCR products of ~3 kb size were identified by 1% agarose gel electrophoresis and ethidium bromide staining. Prior to sequencing the PCR products were purified using QIAquick gel extraction kit (Qiagen) following the recommendations of the suppliers. The nucleotide sequences were determined by direct sequencing of the PCR products using fmol DNA sequencing kit (Promega) using the Cy-5 labelled primers<sup>[15]</sup>. The sequencing reaction was resolved on an ALF Express II DNA analysis system (Amersham Pharmacia Biotech). Nucleotide and amino acids sequences were aligned using CLUSTAL W algorithm<sup>[16]</sup> available in OMIGA 2.0 package (Oxford Molecular Ltd., UK).

**RESULTS AND DISCUSSION**

Selection exerted by the host's antibody could play a major role in the evolution of FMDV<sup>[9,17]</sup>. In this process, antigenic variants are selected upon replication in immune or partially immune hosts<sup>[8,9]</sup>. In a country like India where the disease is endemic, it is expected that majority of the FMD susceptible animals will have antibodies against this virus and it is likely that the viruses circulating in this region are under immune pressure in some part of their evolutionary path<sup>[18]</sup>. This could lead to the selection and emergence of genetic as well as antigenic variants during field outbreaks<sup>[19-21]</sup> as well as when passaging in the

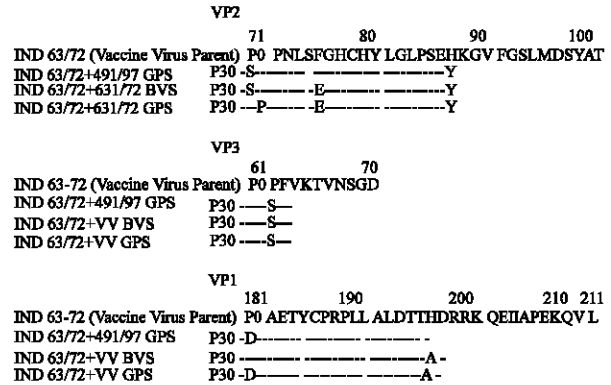


Fig. 1: Amino acid substitution in the Nr viruses of the vaccine strain IND 63/72 selected with antibodies raised both in bovine and guinea pig. P0 is the consensus sequence and the substitutions are bolded

experimental hosts. This clearly shows that FMDV is undergoing a rapid evolution in the field situation.

During the process of selecting polyclonal antibody resistant viruses, after a small number of passages under immune pressure a constant increase of serum was required to suppress the infectivity, thus suggesting that Nr viruses had been selected. In order to confirm the selection of polyclonal antibody resistant viruses. To study the effect of antibodies raised in Bovine and Guinea pigs at the nucleotide and amino acids level of the FMDV type Asia1, the structural protein coding P1 region was sequenced for all the Nr viruses selected from the vaccine strain.

The amino acid alignment of the variants selected from the vaccine strain is shown in (Fig. 1). The variants showed a total of 7 changes throughout this region. These are in VP2 [N<sub>72</sub>→S, S<sub>74</sub>→P, G<sub>82</sub>→E, T<sub>100</sub>→Y], VP3 [N<sub>67</sub>→S] and VP1 [E<sub>182</sub>→D and V<sub>210</sub>→A] and showed no characteristic change in VP4 protein.

The antigenic sites and the residues involved in the neutralization of Asia1 viruses have not been studied in detail. The variants showed a constant change at various critical residues that are reported to involve in the neutralization of other serotypes. At the carboxy terminal region of VP1 protein, the variants of IND 63/72 showed a change at residue 210. A single change in VP1 at residues 201 and 209 in the escape variants of type A10 and A12<sup>[22,23]</sup> showed complete resistance to neutralization. This shows that a single change in the complete variants at residue 210 in VP1 of serotype Asia1 can be of greater importance in terms of providing increased resistance to neutralization by antibodies.

A constant change at residue 67 of VP3 protein in all the variants of IND 63/72 was found. At residue 72 and 82

of VP2, all the three variants showed changes in relation to their parental viruses. The area of VP2 protein consisting of residues 70 to 100 is highly variable and some of the residues of this region were predicted to lie adjacent to the major antigenic loop of VP1<sup>[24]</sup>. This region is conformation dependent and some of the critical residues play important role in the biological properties and survival of the variants<sup>[23,25]</sup>.

### CONCLUSION

In conclusion, the results herein suggests the participation of the whole P1 region in the virus response to immunological pressure exerted by both bovine and guineapig antibodies *in-vitro*. And the immunological response of this Asial virus towards the antibodies raised in Bovine and Guineapigs were found to be similar. This finding using polyclonal sera add new information with respect to the antigenic variation in type Asial virus.

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