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Antigenic and genetic comparison of foot-and-mouth disease virus serotype O Indian vaccine strain, O/IND/R2/75 against currently circulating viruses

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ABSTRACT

Foot-and-mouth disease (FMD) virus serotype O is the most common cause of FMD outbreaks in India and three of the six lineages that have been described are most frequently detected, namely Ind2001, PanAsia and PanAsia 2. We report the full capsid sequence of 21 serotype O viruses isolated from India between 2002 and 2012. All these viruses belong to the Middle East–South Asia (ME–SA) topotype. The serological cross-reactivity of a bovine post-vaccination serum pool raised against the current Indian vaccine strain, O/IND/R2/75,was tested by virus neutralisation test with the 23 Indian field isolates, revealing a good match between the vaccine and the field isolates. The cross reactivity of the O/IND/R2/75 vaccine with 19 field isolates from other countries (mainly from Asia and Africa) revealed a good match to 79% of the viruses indicating that the vaccine strain is broadly cross-reactive and could be used to control FMD in other countries. Comparison of the capsid sequences of the serologically non-matching isolates with the vaccine strain sequence identified substitutions in neutralising antigenic sites 1 and 2, which could explain the observed serological differences.

1. Introduction

Foot-and-mouth disease (FMD) remains a globally important livestock disease affecting cloven-hoofed animals. It is enzootic in many regions, especially in developing countries where it imposes a trade barrier upon livestock and their products. The causative agent, FMD virus (FMDV) has a rapid mutation rate and exists in seven immunologically distinct serotypes, O, A, C, Asia 1, SAT (Southern African Territories) 1, 2 and 3, each with a spectrum of antigenically distinct strains.

FMDV is a single-stranded, positive-sense RNA virus (Genus *Aphthovirus*, family *Picornaviridae*). The viral genome is about 8.3 kb long, enclosed within a protein capsid. The capsid is composed of 60 copies each of four different structural proteins (VP1–4); VP1–3 are surface exposed while VP4 is internal. Crystallographic studies have identified the structure of the FMDV capsid [1–4] and immunological epitopes have been mostly found on surface-oriented interconnecting loops between structural elements. Studies employing monoclonal antibodies (mAb) have identified antigenic sites by sequencing mAb neutralisation resistant (mar) mutants [5–10]. Of the five antigenic sites reported so far for the most extensively studied serotype O, site-1 (G-H loop) is linear and trypsin-sensitive whereas the others are conformational and trypsin-resistant.

Serotype O virus is present in all continents where FMD is reported, and is antigenically less diverse [11] often exhibiting good cross-protection between strains. India has a large population of FMD-susceptible livestock (approximately 528 million) and FMD is endemic, although starting to be brought under control [12]. In the Indian sub-continent three FMDV serotypes (O, A and Asia1) are currently circulating [13], the majority of the outbreaks (~80%) being caused by serotype O virus [13–15]. Two serotype O vaccine strains (v/s), O/TNN 24/84 and O/IND/R2/75 were incorporated

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in the vaccine used for regular vaccination purposes in India until October 2003 when the use of O/TNN/24/84 v/s was discontinued [16]. Currently only O/IND/R2/75 is being used for vaccination purposes throughout the country to control serotype O outbreaks. In this study we report the suitability of the O/IND/R2/75 v/s for use in the country and also provide evidence that this vaccine could provide protection against isolates from other countries. In addition, for the first time, we present detailed analysis of the full capsid sequence data of contemporary serotype O Indian isolates.

2. Materials and methods

2.1. Cells and viruses

Twenty-six serotype O viruses from the Indian sub-continent were used in this study (Supplementary Table 1A). One is the current v/s O/IND/R2/75 originally isolated from India in 1975; the other twenty five viruses were isolated over an eleven year period, 2002 to 2012. These samples were derived from bovine epithelial tissues except one each of caprine and porcine origin. In addition, 19 serotype O viruses submitted to the Food and Agriculture Organisation's World Reference Laboratory for FMD (WRLFMD) at the Pirbright Institute were also used in this study (Supplementary table 1B). Two are the v/s O1/BFS and O1/Manisa that were originally isolated in the UK and Turkey in1967and 1969 respectively; the 17 other viruses were isolated over an eleven year period (2001 to 2011). These viruses were from three continents; Asia ($n = 13$), Europe ($n = 2$) and East Africa ($n = 4$) (Supplementary Table 1B). These samples were mostly derived from bovine epithelial tissues except three of porcine origin and one of caprine origin. All the viruses were initially grown in primary bovine thyroid cells with subsequent passage in either BHK-21 or IB-RS2 cells. Stocks of virus were prepared by infecting IB-RS2 cell monolayers and were stored as clarified tissue culture harvest at −70 °C until required.

2.2. Polyclonal serum

Antisera were prepared against FMDV O Indian v/s O/IND/R2/75 by immunising five male calves of 12–15 months old with inactivated, purified 146S FMD virus particles in ISA-206 adjuvant (SEPIC, France). Bulk blood was collected on 21 day post-vaccination for preparation of sera. A pool of equal amounts of sera from these five animals was used in the serological tests. The pooled antiserum exhibited a homologous titre of $\log_{10}$ 2.48 by virus neutralisation test (VNT).

2.3. Two-dimensional micro neutralisation assay (2D-VNT)

The 2D-VNT was carried out using the 21-day post-vaccination serum pool following established methodology [17]. Antibody titres were calculated from regression data as the $\log_{10}$ reciprocal antibody dilution required for 50% neutralisation of 100 tissue culture infective units of virus ($\log_{10}SN_{50}$/100 $TCID_{50}$). The antigenic relationship of viruses based on their neutralisation by antibodies is given by the ratio: 'r_1' = neutralising antibody titre against the heterologous virus/neutralising antibody titre against the homologous virus. Differences in the r_1-values obtained by the polyclonal antiserum were evaluated according to standard criteria [18]. All the tests were carried out in duplicates and repeated at least twice and the average of the two tests was used in further analysis.

2.4. Nucleotide (nt) sequencing and analysis of the sequence data

The sequences of the entire capsid coding region (P1) of selected Indian type O viruses ($n = 21$) were generated in this study. RNA extraction from the cell culture grown viruses and reverse transcription (RT) were performed as described [19]. PCR was carried out using the "KOD hot-start DNA polymerase" kit (Novagen) as recommended by the manufacturer, using the forward primer L463F (5′-ACCTCCRACGGGTGGTACGC-3′) and one of the reverse primers NK72 (5′-GAAGGGCCCAGGGTTGGACTC-3′) or EUR2B52R (5′-GACATGTCCTCCTGCATCTGGTTGAT-3′). PCR products were purified using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) using the PCR primers and additional internal sequencing primers (sequences available on request). Sequences (from the ABI 3730 machine) were assembled and analysed using SeqMan II (DNAStar Lasergene 8.0). Nucleotide sequences of the viruses were aligned using the CLUSTAL X multiple sequence alignment programme [20] and the predicted aa sequences were translated using BioEdit 7.0.1 [21]. In addition to the 21 sequences generated in this study, another five sequences (Supplementary Table 1A) available in Gene Bank were also used in the analysis. Out of these five capsid sequences O/TNN/24/84, which was one of the old Indian serotype O v/s contained the sequence of only VP1–3 (Acess. no. JQ818555). Therefore sequences of VP1–3 of all the isolates used in this study were aligned and used in all subsequent analysis in this study. Alignments were used to construct distance matrices using the Kimura 2-parameter nucleotide substitution model [22] as implemented in the programme MEGA 5.1 [23].

2.5. Bayesian phylogenetic analysis

The VP1–3 sequences of the viruses belonging to the Indian serotype O strain ($n = 26$) were aligned and subjected to jModelTest 0.1.1 [24]. The general time reversible (GTR) model for nucleotide substitution was used with a combination of gamma distribution and proportion of invariant sites (GTR + I + G) as this was found to be the best model for the Bayesian analysis of the sequence dataset. Analysis was performed using the BEAST software package v 1.5.4 [25] with the maximum clade credibility (MCC) phylogenetic tree inferred from the Bayesian Markov Chain Monte Carlo (MCMC) method. The age of the viruses were defined by the date of sample collection. In BEAUti v1.5.4, the analysis utilised the GTR + I + G model to describe rate heterogeneity among sites. In order to accommodate variation in substitution rate among branches, a random local clock model was chosen for this analysis [26]. BEAST output was viewed with TRACER 1.5 and evolutionary trees were generated in the FigTree program v1.3.1.

2.6. Data analysis

The proportion of synonymous substitutions per potential synonymous site and the proportion of non-synonymous substitutions per potential non-synonymous site were calculated by the method of [27] using the SNAP programme (www.hiv.lanl.gov). The amino acid (aa) variability of the capsid region of the serotype O viruses was determined as described by [28]. Statistical analyses used Minitab release 12.21 software.

3. Results and discussion

The population of FMD-susceptible livestock in India is very large. The Government of India is undertaking an FMD control programme (FMDCP) and in their last (10th) five-year plan covered 54 districts in several states [12,29,30]. There were 16 rounds of vaccination in these 54 districts using an inactivated trivalent vaccine containing strains of serotype O, A and Asia 1. Even though the programme was successful in reducing the incidence of FMD, the disease still occurred due to movement of animals and the existence