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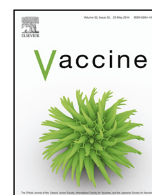

Genetic and antigenic characterisation of serotype A FMD viruses from East Africa to select new vaccine strains

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ABSTRACT

Vaccine strain selection for emerging foot-and-mouth disease virus (FMDV) outbreaks in enzootic countries can be addressed through antigenic and genetic characterisation of recently circulating viruses. A total of 56 serotype A FMDVs isolated between 1998 and 2012, from Central, East and North African countries were characterised antigenically by virus neutralisation test using antisera to three existing and four candidate vaccine strains and, genetically by characterising the full capsid sequence data. A Bayesian analysis of the capsid sequence data revealed the viruses to be of either African or Asian topotypes with subdivision of the African topotype viruses into four genotypes (Genotypes I, II, IV and VII). The existing vaccine strains were found to be least cross-reactive (good matches observed for only 5.4–46.4% of the sampled viruses). Three bovine antisera, raised against A-EA-2007, A-EA-1981 and A-EA-1984 viruses, exhibited broad cross-neutralisation, towards more than 85% of the circulating viruses. Of the three vaccines, A-EA-2007 was the best showing more than 90% *in-vitro* cross-protection, as well as being the most recent amongst the vaccine strains used in this study. It therefore appears antigenically suitable as a vaccine strain to be used in the region in FMD control programmes.

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1. Introduction

Foot-and-mouth disease (FMD) causes serious production losses and has an enormous impact on trade. It is costly and difficult to control because of the diversity of the viruses involved, the multiple host species affected (both domestic and over 30 wildlife animal species) and the speed and different routes of transmission. It is caused by FMD virus (FMDV), a small non-enveloped RNA virus belonging to the genus *Aphthovirus* in the family *Picornaviridae*. The virus exists as seven immunologically distinct serotypes: O, A, C, Asia 1, Southern African Territory (SAT)-1, SAT-2 and SAT-3. Each serotype has a spectrum of antigenically distinct subtypes due to a high mutation rate [1]. The viral genome is about 8.3 kb long and enclosed in a protein capsid. The capsid comprises 60 copies each of the four structural proteins (VP1-VP4); the VP1-3 proteins are located on the surface, while VP4 is internal.

All FMDV serotypes produce a clinically indistinguishable disease but immunity to one serotype does not confer protection against another due to the antigenic diversity. The role of humoral antibodies as the principal component of FMD vaccine-induced protection is well established [2]. Traditionally, monoclonal antibody (mAb) resistant (mar) mutant studies and sequencing of their capsids have been used to identify critical amino acid (aa) residues for neutralisation [3–8]. There are four known neutralising antigenic sites located on the three exposed capsid proteins of serotype A. Site 1 (G-H loop of VP1) is linear and trypsin-sensitive, whereas other sites are conformational and trypsin-resistant [5]. Crystallographic studies have identified that most neutralising epitopes have been found on surface oriented interconnecting loops between structural elements [9]. Mutation in an interconnecting loop may also cause distant effects by perturbation of loop stability [10]. The location of antibody binding sites (epitopes) or escape from binding can also be inferred from correlating the antibody cross-reactivity of viruses to their capsid sequence similarities [11]. Epitopes can also be predicted, in the absence of antibody recognition data, using different epitope prediction programmes using

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viral crystal structure [12]. However, there are no reports for analysis of epitopes or vaccine strain selection studies using serotype A isolates originating from East Africa.

Most FMD outbreaks in East Africa have been caused by serotype O, followed by serotype A and SAT-2 [13–15]. The serotype A viruses are present in all areas of the world where FMD has been reported and are diverse both antigenically and genetically. More than 32 subtypes [16] and 26 genotypes of serotype A FMDV have been reported [17]. Control of FMD mainly depends on the availability of matching vaccines that can be selected based on three criteria: epidemiological information, phylogeny of the gene sequence for evolutionary analysis and serological cross-reactivity of bovine post-vaccinal serum (bvs) with circulating viruses [18,19]. Mono-, bi- and quadri-valent vaccines are currently in use in East African countries for FMD control [20–22]. These vaccines are mainly produced in vaccine production plants located in Ethiopia and Kenya using relatively historic viruses and regular vaccine matching tests to select the best vaccine for use in the region are rarely carried out. Hence, the existing vaccines may not provide optimal protection against recently circulating FMD viruses. This study was, therefore, designed to characterise recently circulating FMD viruses in the region both antigenically and genetically and recommend matching vaccine strains for use in FMD control program in East African countries.

2. Materials and methods

2.1. Cells and viruses

Fifty-six serotype A viruses from Africa submitted to the World Reference Laboratory for FMD (WRLFMD) at Pirbright were used in this study. These viruses were from five East African countries, Ethiopia ($n=8$), Eritrea ($n=9$), Sudan ($n=6$), Kenya ($n=6$), Tanzania ($n=7$) and from three neighbouring countries: Democratic Republic of Congo (COD, $n=5$), Egypt ($n=10$) and Libya ($n=5$). These samples are known to have been derived from cattle epithelial tissues except eight viruses from Egypt and one virus from Kenya where the host species is not known (Supplementary Table 1). All the samples were initially grown in primary bovine thyroid cells (BTY) with subsequent passage in either BHK-21 or IB-RS2 cells. The virus stocks were prepared by infecting cell monolayers and stored at −70 °C until use. Viruses are named according to a three letter code for the country of origin followed by the isolate number and the year of isolation, *e.g.* A-COD-02-2011. Candidate vaccine strains are designated by a two letter code for East Africa followed by the year of isolation, *e.g.* A-EA-2005.

2.2. Polyclonal sera

Seven anti-FMDV bovine post-vaccinal sera were used in the study. Two were against the two existing vaccine strains, A-KEN-05-1980 and A-ETH-06-2000 raised in Kenya and Ethiopia [21], respectively, by administering the commercially prepared vaccine. The animals vaccinated with A-KEN-05-1980 were bled on 21 day following vaccination. The animals vaccinated with A-ETH-06-2000 received a boost on 21-day post-vaccination and bled one week later. The rest five bvs were raised in cattle against one existing vaccine strain (A-ERI-1998) and four candidate vaccine strains (A-EA-1981, A-EA-1984, A-EA-2005 and A-EA-2007) following the method previously described [23]. The candidate vaccine strains were selected taking into account the genotypes currently circulating in the region. For each antigen, sera from four or five animals were pooled for use in the neutralisation test. The homologous neutralising antibody titres of each pooled serum are presented in Table 1a.

Table 1a
Homologous neutralisation serum titres of the seven bovine post-vaccinate sera.

Vaccine	Number of individual serum samples pooled	Mean serum titre (log_{10})
A-EA-2007	5	2.54
A-ERI-1998	5	2.49
A-EA-1984	5	2.40
A-EA-2005	4	2.36
A-KEN-05-1980	4	2.13
A-EA-1981	5	2.09
A-ETH-06-2000	5	1.99

The vaccines are arranged in decreasing order of serum titre.

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

The 2D-VNT test was carried out using the pooled post-vaccination bovine sera according to Rweyemamu and colleagues. [24]. 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 is given by the ratio: 'r_1' = neutralising antibody titre against the heterologous virus/neutralising antibody titre against the homologous virus. The significance of differences between 'r_1-values' obtained by the polyclonal antiserum was evaluated according to standard criteria [25].

2.4. Nucleotide sequencing and analysis of the sequence data

The sequences of the entire capsid coding region (P1) of the viruses were generated. RNA extraction from the cell culture grown viruses, reverse transcription (RT), polymerase chain reaction (PCR) to amplify the P1 region, sequencing, sequence analysis and assembling, and alignment were performed as described previously [26]. MEGA 5 [27] was used to determine nucleotide and aa variations. The aa variability of the capsid coding region of the type A viruses were determined as described by Valdar [28].

2.5. Genetic characterisation

The aligned, complete P1 nucleotide sequences were used to determine the most suitable nucleotide substitution model using jModelTest [29] and MEGA [27] resulting in the selection of a General time reversal (GTR) model with a combination of gamma distribution and proportion of invariant sites (GTR + G + I). Then, Bayesian analysis was performed using the BEAST software package v1.5.4 [30]. In BEAUti v1.5.4, the ages of the viruses were defined by the date of sample collection and the analysis used GTR + G + I model to describe rate heterogeneity among sites. Variations in substitution rate among branches were evaluated by comparing four different clocks in BEAST. The maximum clade credibility (MCC) phylogenetic tree was inferred using the Bayesian Markov Chain Monte Carlo (MCMC) method. Then, a Bayes factor analysis in TRACER version 1.5 [31] was used to determine the best-fit model that resulted in the selection of an uncorrelated exponential relaxed molecular clock. The tree was obtained using the Tree Annotator program in BEAST and the evolutionary trees were viewed in FigTree program 1.3.1.

2.6. Statistical analysis

The relationship between predicted protection (r_1-value ≥0.3) and changes in aa was analysed using a general linear model (GLM) with binomial error distribution. For this, a binomial variable 'protected/not protected' was created based on the estimated r_1-values ≥0.3 (protected), which was used as the response variable.

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