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Genetic basis of antigenic variation in foot-and-mouth disease serotype A viruses from the Middle East☆

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ABSTRACT

Foot-and-mouth disease viruses (FMDV) from serotype A exhibit high antigenic diversity. Within the Middle East, a strain called A-Iran-05 emerged in 2003, and subsequently replaced the A-Iran-96 and A-Iran-99 strains that were previously circulating in the region. Viruses from this strain did not serologically match with the established A/Iran/96 vaccine, although most early samples matched with the older A22/Iraq vaccine. However, many viruses from this strain collected after 2006 had poor serological match with the A22/Iraq vaccine necessitating the development of a new vaccine strain (A/TUR/2006). More recently, viruses from the region now exhibit lower cross-reactivity with the A/TUR/2006 antisera highlighting the inadequacy of the serotype A vaccines used in the region. In order to understand the genetic basis of these antigenic phenotypes, we have determined the full capsid sequence for 57 Middle Eastern viruses isolated between 1996 and 2011 and analysed these data in context of antigenic relationship (r_1) values that were generated using antisera to A22/Iraq and A/TUR/2006. Comparisons of capsid sequences identified substitutions in neutralising antigenic sites (1, 2 and 4), which either individually or together underpin these observed antigenic phenotypes.

1. Introduction

Foot-and-mouth disease (FMD) remains a globally important livestock disease affecting cloven-hoofed animals. It remains 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 wide spectrum of antigenically distinct subtypes.

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 entirely internal. Crystallographic studies have identified the structure of the FMDV capsid [1,2] 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 [3–9]. 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. Equivalent neutralising antigenic sites (except site 3) have also been identified for serotype A, with critical residues present in equivalent positions [3–6,9].

Serotype A viruses are present on all continents where FMD is reported, and is antigenically diverse [10] often exhibiting poor cross-protection [11]. In the Middle East (ME), a new variant, A-Iran-05, was identified in samples collected from Iran in 2003 and subsequently spread to neighbouring countries [10] and North Africa [12]. This genotype replaced the A-Iran-96 and A-Iran-99 genotypes that were previously circulating in the region; did not cross-react with A/Iran/96 vaccine antisera and shared a closer antigenic relationship with the older A22/Iraq vaccine strain (v/s) [10]. However, many samples isolated after 2006 did not even match with A22/Iraq v/s and so a new v/s, A/TUR/2006 was introduced. From sequence data, Jamal and colleagues indicated candidate

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amino acid (aa) substitutions in the capsid that might have contributed to these antigenic changes [13]. More recently, there is evidence that viruses from the region now exhibit lower cross-reactivity with the A/TUR/2006 antisera. The aim of this study was to investigate the molecular basis of the antigenic variation in these viruses using capsid sequences and their corresponding antigenic relationship (r_1) values.

2. Materials and methods

2.1. Cells and viruses

Fifty-seven serotype A viruses from the ME submitted to the Food and Agriculture Organisation's World Reference Laboratory for FMD (WRLFMD) at the Pirbright Institute were used in this study (Supplementary table). Two are the v/s A22/IRQ/24/64 (A22/Iraq) and A/TUR/2006 that were originally isolated in Iraq and Turkey, in 1964 and 2006 respectively; the 55 other viruses were isolated over a fifteen year period (1996–2011). These viruses were from seven ME countries, Turkey ($n=17$), Iran ($n=26$), Iraq ($n=2$), Pakistan ($n=5$), Afghanistan ($n=4$), Saudi Arabia ($n=1$) and Jordan ($n=2$) (Supplementary table). These samples were derived from cattle epithelial tissues (except one of ovine origin), and 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.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.08.102.

2.2. Polyclonal serum

Antisera were prepared against serotype A FMD viruses (A22/Iraq and A/TUR/2006) by immunising five cattle per v/s with inactivated, purified 146S FMD virus particles in ISA-206 adjuvant. Bulk blood was collected on 21 day post-vaccination for preparation of sera. For each antigen, a pool of sera from five animals was used in the serological tests. The A22/Iraq and A/TUR/2006 antisera exhibited equivalent homologous titres ($\log_{10}$ 2.43 and 2.54, respectively) 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 sera following established methodology [14]. 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 [15].

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

The sequences of the entire capsid coding region (P1) of selected viruses were generated. RNA extraction from the cell culture grown viruses and reverse transcription (RT) were performed as described [16]. 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 program [17] and the predicted aa sequences were translated using BioEdit 7.0.1 [18]. Alignments were used to construct distance matrices using the Kimura 2-parameter nucleotide substitution model [19] as implemented in the programme MEGA 4.0 [20].

2.5. Bayesian phylogenetic analysis

The complete P1 sequence of the viruses belonging to the A-Iran-05 strain ($n=51$) were aligned and subjected to jModelTest 0.1.1 [21]. The general time reversible (GTR) model for substitution model with combination of gamma distribution and proportion of invariant sites (GTR + I + G) was found to be the best model for the Bayesian analysis of the sequence dataset. Analysis was performed using the BEAST software package v1.5.4 [22] 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 as 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 [23]. 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 Nei and Gojobori [24] using the SNAP program (www.hiv.lanl.gov). The aa variability of the capsid region of the A-Iran-05 viruses was determined as described by Valdar [25]. Statistical analyses used Minitab release 12.21 software.

3. Results and discussion

The A-Iran-05 viruses, first detected in Iran [10], spread to neighbouring countries in the ME [10,12,13], and spawned sub-lineages over the next seven years. Most sub-lineages died out, whereas a few persisted and became dominant, and some are still circulating. In this study, we have focussed mainly on three sub-lineages, namely ARD-07, AFG-07 and BAR-08. ARD-07, first detected in Ardahan, Turkey in August 2007 was the main circulating strain in Turkey during 2007–2010. However, it has not been detected in samples received in WRLFMD, Pirbright from Turkey during 2011–2012. AFG-07, first isolated from a bovine sample in Afghanistan in 2007 has spread to other neighbouring countries such as Bahrain, Iran, Pakistan and Turkey. BAR-08, first detected in a bovine sample in the Manama region of Bahrain in 2008 has spread to other countries such as Iran, Pakistan and Turkey. This sub-lineage has also jumped to North African countries, such as Libya in 2009 [12] and Egypt in 2010 and 2011 (http://www.wrlfmd.org), probably because of trade links with ME countries. Evolution of the serotype A viruses in the ME has resulted in the appearance of further sub-lineages like HER-10 and SIS-10. These sub-lineages have gained dominance over the others and