Antigenic variation of foot-and-mouth disease virus serotype A

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The current measures to control foot-and-mouth disease (FMD) include vaccination, movement control and slaughter of infected or susceptible animals. One of the difficulties in controlling FMD by vaccination arises due to the substantial diversity found among the seven serotypes of FMD virus (FMDV) and the strains within these serotypes. Therefore, vaccination using a single vaccine strain may not fully cross-protect against all strains within that serotype, and therefore selection of appropriate vaccines requires serological comparison of the field virus and potential vaccine viruses using relationship coefficients (r_1 values). Limitations of this approach are that antigenic relationships among field viruses are not addressed, as comparisons are only with potential vaccine virus. Furthermore, inherent variation among vaccine sera may impair reproducibility of one-way relationship scores. Here, we used antigenic cartography to quantify and visualize the antigenic relationships among FMD serotype A viruses, aiming to improve the understanding of FMDV antigenic evolution and the scope and reliability of vaccine matching. Our results suggest that predicting antigenic difference using genetic sequence alone or by geographical location is not currently reliable. We found co-circulating lineages in one region that were genetically similar but antigenically distinct. Nevertheless, by comparing antigenic distances measured from the antigenic maps with the full capsid (P1) sequence, we identified a specific amino acid substitution associated with an antigenic mismatch among field viruses and a commonly used prototype vaccine strain, A_{22}/IRQ/24/64.

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly transmissible vesicular disease of cloven-hoofed animals with a high morbidity rate in adult livestock. In addition to causing production losses, FMD can have a severe economic impact in endemic countries through, among other things, restriction of trade with FMD-free countries. Incursion from endemic countries into disease-free areas also carries high animal welfare and economic costs (Thompson et al., 2002).

Current measures to control the disease in FMD-free countries are driven largely by international trade regulations and include vaccination, movement controls and slaughter of infected and susceptible animals. In endemic countries, and where movement restrictions and slaughter are not always feasible, vaccination often plays a central role in FMD control. One of the difficulties in controlling FMD through vaccination arises due to the extent of broad intra- and inter-serotype antigenic diversity that exists for the seven serotypes of FMD virus (FMDV) (Kitching et al., 2005). Currently, inactivated vaccines do not protect against all strains within a particular serotype (Doel, 2003). This is particularly problematic for FMDV serotype A, which is one of the most antigenically diverse serotypes (Knowles et al., 2009). Therefore, accurate antigenic matching between field viruses and potential vaccine viruses is essential to ensure effective vaccination.

FMDV belongs to the genus Aphthovirus in the family Picornaviridae. There are seven serotypes within FMDV (serotypes O, A, SAT1, SAT2, SAT3, C and Asia 1) and each of these is split into topotypes and lineages. Virus strains are placed into different topotypes based on geographical location and on having approximately 85 % identity in the VP1 protein (for SAT viruses this is 20 %). Topotypes are further classified into lineages based on sequencing, vaccine matching and geographical regions. The P1 region of the genome encodes four capsid proteins: VP1, VP2, VP3 and VP4. The first three (VP1, VP2 and VP3) make up the virus outer surface and tolerate a significant proportion of amino acid changes without obvious functional impairment (Haydon et al., 2001). VP1 is the most accessible protein for the host immune system and contains the binding site (RGD motif), which attaches to the integrin receptor on epithelial cells and is located on a highly mobile G–H loop (Logan et al., 1993; Jackson et al., 2003, 2004). The G–H loop is the most variable site on the capsid and, along with other surface-exposed regions of VP1, VP2 and VP3, constitutes the five antigenic sites of FMDV serotype A strains that have been mapped using mAbs (Thomas et al., 1988; Baxt et al., 1989; Bolwell et al., 1989; Crowther et al., 1993).

Although sequencing the viral capsid genes is straightforward and rapid, predicting antigenic variation using sequence data alone has been challenging (Maree et al., 2011), with some limited success using regression models to predict neutralization values from sequence and structural data for serotype SAT1 (Reeve et al., 2010). Recent work focusing on using structure-based B-cell epitope prediction programs to determine antigenic residues has also shown some success, especially when multiple programs are used (Borley et al., 2013). The challenge is in part because certain amino acid substitutions may have a disproportionate antigenic effect. Therefore, serological tests are routinely used to estimate which vaccine strains may protect against field viruses using relationship coefficients (r_1 values) (Booth et al., 1978; Hamblin et al., 1986). These tests provide an estimation of the antigenic similarity between field and vaccine viruses, but they do not show relationships among FMDV strains and are dependent on the sera used (Mattion et al., 2009).

Antigenic cartography enables reliable quantitative and visual interpretation of pathogen binding assay data with the benefit of incorporating multiple serum–virus relationships when measuring antigenic distances among viruses. This method was first applied to human influenza A (H3N2) viruses (Smith et al., 2004a) and has since been applied to other viruses including swine influenza virus (de Jong et al., 2007), human influenza A (H1N1) virus (Lorusso et al., 2011), equine influenza virus (Lewis et al., 2011), lyssaviruses (Horton et al., 2010) and enterovirus A71 (Huang et al., 2009). Here, using antigenic cartography, we quantified the antigenic relationships among a panel of FMD serotype A viruses, representing the global diversity within the serotype. Antigenic relationships among field viruses were compared with differences in their full capsid P1 sequences. This identified a single amino acid substitution associated with an immunologically important antigenic change.

RESULTS

Phylogenetic analysis

The maximum-likelihood (ML) phylogenetic relationships inferred from the capsid sequences highlighted the three topotypes within serotype A (Fig. 1) (Knowles & Samuel, 2003). Viruses grouped mostly according to geographical regions and by the year in which they were isolated. European/South American isolates appeared to share a common ancestor with the Asian topotype. A strain from Libya, A/LIB/14/2009, was an exception, as, although isolated from Africa, it clustered here with the Asian topotype as part of the A/ASIA/Iran-05 lineage.

Antigenic analysis

Antigenic relationships among the panel of 46 viruses are illustrated in a three-dimensional antigenic map (Fig. 2a). Colouring the viruses by year, topotype and geographical origin revealed no distinct clustering of viruses with time, distance or genetic relationship (Fig. 2). This was particularly true for the sublineage A/ASIA/Iran-05[BAR-08], for which there was large intra-lineage antigenic variation; for example, there were 2.81 antigenic units (AU) between A/IRN/23/2009 and A/LIB/14/2009 (1 AU equals a twofold dilution). The antigenic map shown by year also highlights the antigenic diversity of strains seen even within the same
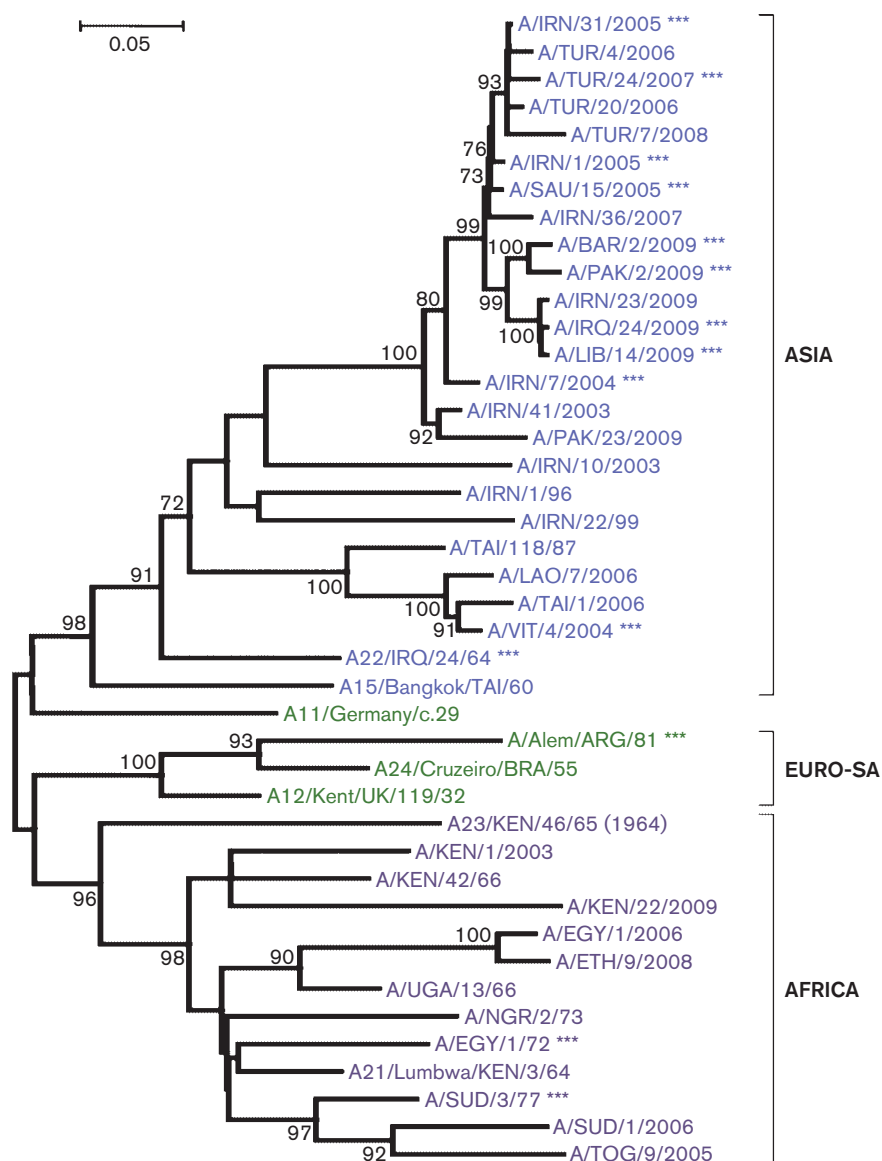
Fig. 1. Unrooted ML tree of 40 full P1 capsid nucleotide sequences using GTR+G+I in MEGA 5.10. Bootstrap replicates (1000) were performed, and only bootstrap values above 70 % are shown. The three different topotypes have been coloured: Asia (blue), Africa (purple) and Europe/South America (EURO-SA; green). *** indicates viruses that contained a proline at position 149. Bar, nucleotide substitutions per site.

year. Viruses A/IRN/1/2007 and A/IRN/36/2007 were both isolated in Iran during the same year and, with only 3.93 % nucleotide differences in the VP1 region, are considered to be the same strain. However, they were 2.21 AU apart.

Comparison of antigenic and genetic data

Using antigenic cartography, the antigenic distances among all viruses were calculated (Table S1, available in JGV Online), and these distances were used to compare antigenic and genetic data. Quantitative comparison of antigenic distance with the number of amino acid substitutions in P1 showed a significant but low correlation ($r=0.42$, confidence interval (CI) 0.36–0.48, $P<2.2 \times 10^{-16}$) (Fig. 3). A linear regression model applied to the antigenic and genetic data predicted a mean value of 42 (5.7 %) amino acid changes having to occur for every change in AU.

Antigenic effect depends on the position and nature of amino acid substitutions. Therefore, the sequence and antigenic data were investigated further to detect evidence for any amino acid substitutions with large antigenic effects. Amino acid sequences for all 46 viruses on the antigenic map were aligned and systematically analysed for specific substitutions common to all sequences in each antigenic group on the map. Specific attention was paid to
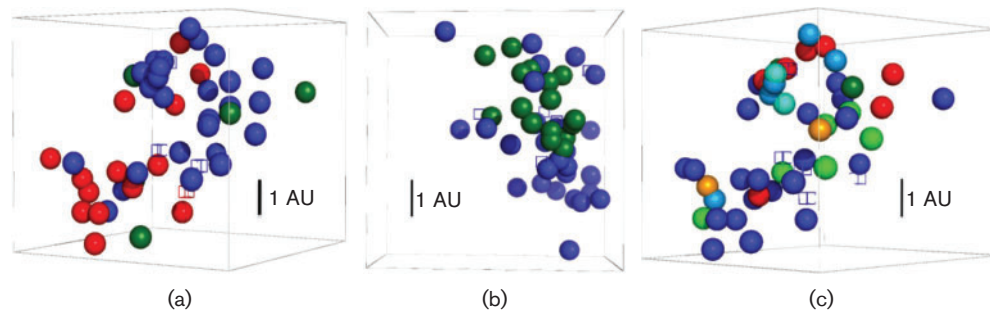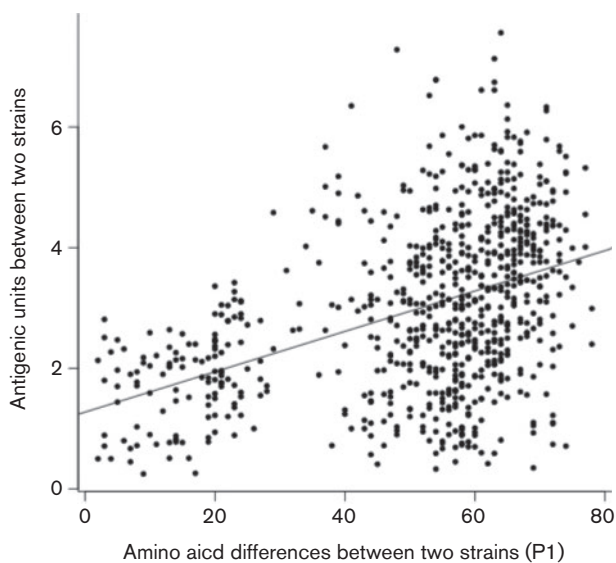
Fig. 2. Three-dimensional antigenic maps of the 46 FMDV serotype A viruses representing the global diversity of serotype A FMDV. Viruses (spheres) and antisera (open cubes) were positioned such that the distance from each serum to each virus is determined by the neutralization titre. Multidimensional scaling was used to position both sera and viruses relative to each other, so orientation of the map within the axes is free. Bar, 1 AU (equivalent to a twofold dilution in antibody titre). All views are shown at a different orientation to highlight the patterns seen (viewed using Pymol; DeLano Scientific LLC). (a) Viruses coloured according to different topotypes: Africa (red), Asia, (blue) and Europe/South America (green). (b) Viruses coloured to highlight the A/ASIA/Iran-05 lineage (green). (c) Viruses coloured by year: up to and including 2003 (blue), 2004 (turquoise), 2005 (light blue), 2006 (green), 2007 (dark green), 2008 (orange) and 2009 (red).

previously identified antigenic sites and other surface-exposed residues. Using this approach, a single amino acid substitution, a proline at the third amino acid after the RGD domain of VP1, aa 149 (P149), defined a single cluster of antigenically related viruses (Fig. 4). The viruses in this cluster were from different geographical regions, spanning more than 30 years (Fig. 1). The closest vaccine virus to this cluster is prototype vaccine virus A_{22}/IRQ/24/64, which also contains a proline at this position. Measurement of antigenic distance from this vaccine virus

demonstrated that, for these 46 viruses, P149 was a strong predictor of antigenic match (and therefore presumed protection) to A_{22}/IRQ/24/64, and there were only three viruses that did not match this pattern: A/SAU/15/2005, A/IRQ/24/2009 and A/SUD/3/77.

To test this prediction on a larger dataset, 166 complete or partial VP1 sequences from a global panel of serotype A virus strains spanning 24 years (1987–2010) were analysed along with their corresponding r_1 value data against A_{22}/IRQ/24/64 [data from the World Reference Laboratory/European Union Reference Laboratory (WRL/EURL) for FMD]. A proline at position 149 remained a strong predictor of vaccine match to A_{22}/IRQ/24/64 (as determined by an r_1 value $\geqslant 0.3$), with only 15 % of vaccine matches not being predicted based on the amino acid at position 149 of VP1 (Fig. 5). Of the 53 matched samples (r_1 value $\geqslant 0.3$), only three did not have a proline at position 149 of VP1.

Fig. 3. Pair-wise antigenic and genetic distance (P1) between viruses. The antigenic distances were derived from the three-dimensional antigenic map of FMDV serotype A. Pearson's correlation coefficient was 0.42 (95 % CI is 0.36–0.48; $P < 2.2 \times 10^{-16}$). The Spearmans's rank correlation (ρ) was equal to 43 %.
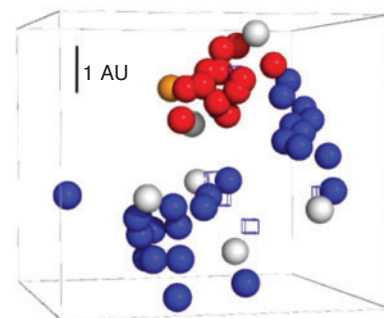
Fig. 4. Three-dimensional antigenic map of FMDV serotype A isolates (see Fig. 2). Viruses are coloured by amino acid at position 149: proline (red), A/IRQ/24/64 (orange), others (blue), not sequenced due to confidentiality agreement (white). The cubes indicate the different sera.
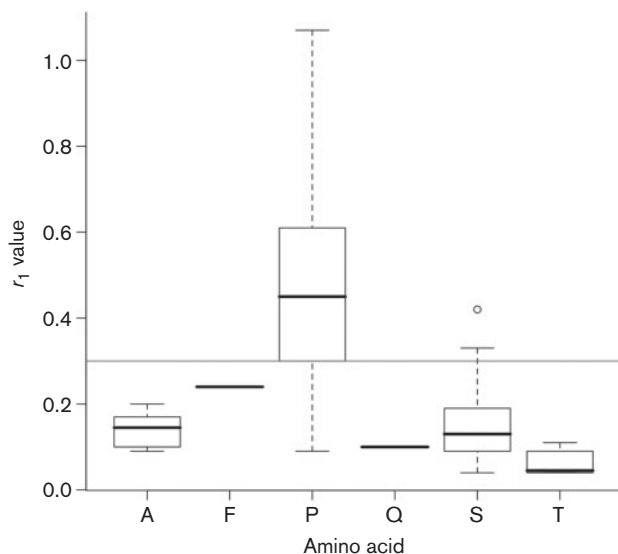
Fig. 5. Boxplot of 166 r_1 values obtained from the WRL/EURL for FMD against A_{22}/IRQ/24/64 vaccine virus. The amino acid at position 149 of VP1 and the resulting r_1 value are shown. An r_1 value $\geqslant 0.3$ is suggestive of a vaccine match against A_{22}/IRQ/24/64 (horizontal line).

Of the 15 % of serotype A viruses for which the vaccine matching could not be predicted based on the amino acid at position 149 of VP1, nearly half (10/22) were from one sublineage within the genetic lineage A/ASIA/Iran-05, i.e. BAR-08. A phenylalanine at aa 66 of VP1 and a glutamic acid at aa 64 of VP2 differentiated the three A/ASIA/Iran-05[BAR-08] isolates from all other viruses. If the A/ASIA/Iran-05[BAR-08] sublineage was not included in the analysis, the P149 substitution correctly predicted vaccine virus matching to A22/IRQ/24/64 for 92 % of the 166 viruses analysed.

DISCUSSION

Here, we have for the first time both quantified and visualized the antigenic variability among FMDV serotype A strains, allowing the detection of an important amino acid substitution possibly responsible for the antigenic match to a widely used vaccine strain, A_{22}/IRQ/24/64. An advantage of using antigenic cartography over r_1 values is the use of multiple neutralization values, rather than against one homologous serum as used for r_1 values to infer antigenic relationships. This allows antigenic relationships among all field viruses and multiple vaccine viruses to be assessed simultaneously. Antigenic maps made using a specifically generated body of virus neutralization test (VNT) data showed that there were discrepancies between antigenic, genetic and geographical relationships of viruses.

Antigenic cartography assumes that multiple epitopes or multiple antibodies against the same epitope can be represented as a single point. If the viruses were most parsimoniously represented by multiple points, corresponding to the different antigenic epitopes, then we would expect to see a large error associated with the position of the viruses and poor prediction of titres from the map.

Comparing the antigenic data with P1 capsid sequence data suggested that P149 is strongly associated with a match to vaccine A_{22}/IRQ/24/64. Even in the one lineage (A/ASIA/Iran-05[BAR-08]) with low r_1 values against A_{22}/IRQ/24/64 despite the amino acid at position 149, P149 was still associated with a discernible antigenic effect. This is probably due to multiple epitopes contributing to the overall antigenicity of the virus. In addition, this residue is in close vicinity to the VP1 aa 148, which has been reported to be critical in the A_{24} strain by mAb escape mutant studies (Mahapatra et al., 2011). It is possible that substitutions in this area, especially from a proline, a known helix breaker, may be responsible for the change in overall conformation of the protein leading to antigenic variation in these viruses. This lineage highlights the importance of continuous monitoring of the antigenic variation in circulating viruses and the need to understand the interactions and contributions among antigenic sites on the FMDV capsid.

What effect this proline has on the structure of FMDV serotype A strains is not known, as this region of VP1 is too flexible to be structurally modelled reliably (Fry et al., 2005). However, this region has been suggested previously to be part of antigenic site one for serotype A (Thomas et al., 1988; Baxt et al., 1989). For FMDV serotype O, where this region has been modelled using a reduced structure of the G–H loop and crystallography, there is an α-helix in this region (Logan et al., 1993). This work on serotype O would suggest that a proline at aa 149 of VP1 might interfere with a similar α-helix on serotype A viruses, resulting in this antigenically distinct group.

The antigenic map allows comparison between existing and new vaccine viruses. Using the antigenic map, we identified a cluster of viruses closely related to vaccine virus A/IRQ/24/64. A_{22}/IRQ/24/64 has been one of the most widely used vaccine viruses in the Middle East, but recently a new lineage of FMDV (A/ASIA/Iran-05) has emerged (Knowles et al., 2009), and viruses isolated from this lineage (A/IRN/2005 and A/TUR/2006) have been incorporated into new vaccines. The A/IRN/2005 vaccine virus is 2.26 AU away from the vaccine virus A_{22}/IRQ/24/64, and the two A/TUR/2006 vaccine viruses are 2.81 AU and 3.05 AU away from A_{22}/IRQ/24/64. There are limited data and no sequences available for the A/IRN/2005 vaccine virus, due to confidentiality agreements. The A/TUR/2006 vaccine viruses group phylogenetically with the other A/ASIA/Iran-05 viruses.

Although a new vaccine virus A/ASIA/Iran-05 has been developed, vaccine virus A_{22}/IRQ/24/64 appears still to protect against some new virus strains. Therefore, it would appear that there is co-circulation of multiple antigenic strains occurring in the Middle East. This implies that new vaccine strains that are developed in response to antigenic shift away from current vaccine viruses cannot fully replace

the use of older vaccines. Also, currently there is incomplete information available from most endemic countries as to what vaccines are used, where and how often vaccination takes place and what species are targeted. Importantly, if there is co-circulation of multiple antigenic strains, a co-ordinated vaccination regime to eliminate disease locally will require scientific and strategic planning and implementation.

Predicting vaccine match using the number of amino acid substitutions alone is not accurate (Fig. 3). It appears that FMDV serotype A is able to sustain a large number of amino acid changes that do not affect the antigenicity of the virus. Also, the opposite appears to be true, where a single amino acid can cause a proportionally significant structural change resulting in the virus escaping the host's humoral immunity.

The future use of FMDV antigenic cartography may include choosing a field strain as a potential new vaccine virus. However, although the position of a vaccine virus relative to field viruses will give an indirect guide to vaccine coverage, the position of a homologous serum against a vaccine is more appropriate to determining whether the vaccine virus is a good candidate, as we are interested in the antigenic response of cattle (see Table S1 for differences between virus-to-virus and virus-to-serum distances). This adds complexity, as the homologous serum is not always at the same location on the map as the vaccine virus. Finding a suitable vaccine may therefore require choosing more than one virus until the desired position of serum is achieved, as the position of the sera on the map may be distant from the group of field viruses for which the vaccine virus was intended. Individual animals will have a slightly different immunological response to a vaccine virus, but experience with influenza antigenic maps suggests that the sera are located in approximately the same area.

Antigenic cartography allows the antigenic evolution to be visualized and compared with the genetic evolution. This study has been shown to be useful not only in describing the antigenic variation but also in discriminating the antigenic and genetic relationships. This is particularly true for vaccine matching with A_{22}/IRQ/24/64, which can be predicted from a single substitution at position 149 of VP1.

METHODS

Virus propagation. Forty-six FMDV serotype A isolates from the WRL/EURL for FMD (Pirbright, UK) were used in this study (Table 1). Once received from the WRL/EURL, the majority of viruses were propagated once or twice in a pig kidney cell line (IB-RS-2) with the maximum number of propagations being four passages. However, some viruses had been propagated prior to being obtained for use in this study. This was done in order to obtain a virus titre above 10^3 TCID$_{50}$. Tissue-culture supernatant was harvested when more than 85 % cytopathic effect was noted, and the viruses were stored in glycerol (50 %, v/v) at -20 °C for the working virus stock or -80 °C for long-term use.

Sequencing and phylogenetic analysis. For phylogenetic analyses, full capsid sequences were obtained for 40 of the 46 viruses used for

antigenic interpretation. Eleven viruses had been sequenced previously (Carrillo et al., 2005; Klein et al., 2007), 29 viruses were sequenced as part of this study from the same stock viruses used for VNT and four viruses could not be sequenced due to confidentiality agreements with pharmaceutical companies (Table 1).

Total RNA from the virus working stocks was extracted using an RNeasy kit (Qiagen). Reverse transcription was performed using a SuperScript III reverse transcriptase kit (Invitrogen). PCR was then carried out using a KOD Hot-start Polymerase kit (Novagen) according to the manufacturer's instructions with primer pairs L463F (5′-ACCTCCRACGGGTGGTACGC-3′) and NK72R (5′-GAAGGGCCCAGGGTTGGACTC-3′) or L463F and EUR2B52R (5′-GACATGTCCTCCTGCATCTGGTTGAT-3′), resulting in an amplified product of approximately 2500 bp. These PCR amplicons were purified using an Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare) according to the manufacturer's instructions. Internal sequencing primers were then used to sequence the entire P1 region on both strands (primer sequences available on request). Cycle sequencing was carried out using an ABI 3730 sequencer (Applied Biosystems). Sequences were checked manually, and consensus sequences were determined from at least one forward and one reverse sequence for all regions. Consensus nucleotide sequences of the viruses were aligned using the CLUSTAL W multiple sequence alignment program (Thompson et al., 1994). The predicted amino acid sequences were translated using BioEdit version 7.0.1 (Hall, 1999).

The optimum evolutionary molecular model for these data was determined to be the general time reversible model with gamma shape parameter and a proportion of invariant sites (GTR+G+I; jMODELTEST) (Guindon & Gascuel, 2003; Posada, 2008). MEGA version 5.10 (Tamura et al., 2011) was used to construct the ML tree with the topology optimized using subtree pruning-regrafting (SPR level 5). Bootstrap resampling (1000 cycles) was also carried out to assess the robustness of individual nodes.

Polyclonal sera. Six bovine sera, collected 21 days post-vaccination, were supplied by the WRL/EURL for FMD for antigenic characterization. The vaccines (A/ERI/98, A/IRN/96, A/IRN/99, A/IRN/2005 and A/MAY/97) were inactivated and were oil adjuvant based (Merial). The prototype vaccine A_{22}/IRQ/24/64 was aqueous based and was prepared by WRL/EURL FMD and the Onderstepoort Veterinary Institute (South Africa). No significant difference could be detected between the log of the r_1 value generated using antisera raised against the aqueous-based and oil adjuvant-based A_{22}/IRQ/24/64 vaccine (paired t-test, $P=0.30$, $n=18$). Each serum was inactivated at 56 °C for 1 h prior to use in the VNT. All sera were stored at -20 °C.

VNT. For antigenic analyses, the ability of each serum to neutralize each virus was assessed using a VNT. The test was undertaken either using a two-dimensional (Rweyemamu et al., 1976) or a one-dimensional VNT technique as recommended by the Office International des Epizooties (OIE, 2008). The two-dimensional VNT was modified for diagnostic vaccine matching by the WRL/EURL using five virus doses instead of the nine described in earlier publications. Briefly, the vaccine antiserum was diluted twofold vertically in a 96-well plate. Five different virus doses were then added horizontally across the plate, resulting in the sera being titrated against all five virus doses. The five virus doses were calculated from the TCID$_{50}$ virus titre determined on a separate plate. For each of these virus doses, the 50 % end point serum neutralization titre was then calculated. The serum titre at a virus dose of 100 TCID$_{50}$ was then predicted using linear regression. The one-dimensional VNT technique suggests using one pre-aliquotted virus dose of 100 TCID$_{50}$ to neutralize anti-FMDV sera (OIE, 2008). Each test was repeated at least twice and all duplicates had to be within a twofold dilution of each other. Due to this modified method being faster, saving

Table 1. Serotype A FMDVs used in this study

Topotype	Lineage	Virus name	GenBank accession no.	Sequence reference
Asia	A_{15}	A_{15}/Bangkok/TAI/60	KF112918	This work
	A_{22}	A_{22}/IRQ/24/64	AY593763	Carrillo *et al.* (2005)
	Iran-05	A/IRN/41/2003	KF112906	This work
	Iran-05	A/IRN/2005	not available	-
	Iran-05	A/IRN/1/2005	EF494486	Klein *et al.* (2007)
	Iran-05	A/IRN/31/2005	KF112907	This work
	Iran-05	A/IRN/36/2007	Pending	Upadhyaya *et al.* (2013)
	Iran-05	A/IRN/7/2004	Pending	Upadhyaya *et al.* (2013)
	Iran-05	A/PAK/23/2009	KF112915	Upadhyaya *et al.* (2013)
	Iran-05	A/SAU/15/2005	Pending	Upadhyaya *et al.* (2013)
	Iran-05	A/TUR/20/2006	KF112923	This work
	Iran-05	A/TUR/24/2007	Pending	Upadhyaya *et al.* (2013)
	Iran-05	A/TUR/4/2006	KF112922	This work
	Iran-05[AFG-07]	A/BAR/2/2009	KF112900	This work
	Iran-05[AFG-07]	A/PAK/2/2009	KF112915	This work
	Iran-05[ARD-07]	A/TUR/7/2008	KF112924	This work
	Iran-05[BAR-08]	A/IRN/23/2009	KF112908	This work
	Iran-05[BAR-08]	A/IRQ/24/2009	KF112909	This work
	Iran-05[BAR-08]	A/LIB/14/2009	KF112913	This work
	Iran-96	A/IRN/1/96	KF152935	This work
	Iran-99	A/IRN/22/99	Pending	Upadhyaya *et al.* (2013)
	Unnamed	A/IRN/10/2003	KF112905	This work
	Unnamed	A/IRN/32/2001	Not available	–
	Sea-97	A/LAO/7/2006	Pending	Upadhyaya *et al.* (2013)
	Sea-97	A/May/97	Not available	–
	Sea-97	A/TAI/1/2006	KF112920	This work
	Sea-97	A/VIT/4/2004	KF112927	This work
	Thai-87	A/TAI/118/87	KF112919	This work
Africa	G-I	A/KEN/42/66	KF112910	This work
	G-I	A/KEN/1/2003	KF112911	This work
	G-I	A/KEN/22/2009	KF112912	This work
	G-II	A/EGY/1/72	KF112901	This work
	G-II	A/ETH/9/2008	KF112903	This work
	G-III	A_{21}/Lumbwa/KEN/3/64	AY593761	Carrillo *et al.* (2005)
	G-IV	A/SUD/3/77	KF112916	This work
	G-IV	A/ERI/3/98	Not available	–
	G-IV	A/SUD/1/2006	KF112917	This work
	G-IV	A/TOG/9/2005	KF112921	This work
	G-V	A/NGR/2/73	KF112914	This work
	G-VII	A/EGY/1/2006	KF112902	This work
	G-VII	A/UGA/13/66	KF112925	This work
	G-VIII	A_{23}/Kitale/KEN/64	AY593766	Carrillo *et al.* (2005)
Europe/South America	A_{11}	A_{11}/Germany/c.29 (AGB)	KF112904	This work
	A_{12}	A_{12}/Kent/UK/199/32	KF112926	This work
	A_{24}	A_{24}/Cruzeiro/BRA/55	AY593768	Carrillo *et al.* (2005)
	A-81	A/Alem/ARG/81	KF112899	This work

resources and giving similar results to the two-dimensional VNT, it was used for the majority of VNTs carried out in this study (WRL/EURL for FMD, unpublished data).

Calculation of serological relationship coefficient. The relationship coefficients (r_1 value) between the field virus and A_{22}/IRQ/24/64 were calculated for comparison with antigenic distances measured using antigenic cartography. These r_1 values were defined as the division of the heterologous (field virus) neutralization value by the homologous (vaccine virus) neutralization value. An r_1 value $\geqslant 0.3$

was considered indicative of a vaccine virus antigenically matched with the field virus (i.e. the vaccine virus can be expected to protect an animal from disease caused by infection with the field virus tested) (Rweyemamu *et al.*, 1976).

Antigenic cartography. The antigenic variation among FMDV serotype A isolates was both quantified and visualized using a method described previously (Smith *et al.*, 2004b). Briefly, neutralization titres between a virus and a serum were converted to a target distance by taking the difference between the $\log_2$ reciprocal neutralization titre

against a virus and the maximum $\log_2$ reciprocal titre achieved by that serum against any other virus. Thus, a serum having a high neutralization titre against a virus has a lower target distance to that virus than a serum with a lower neutralization titre. Calculating target distances in this way allowed quantification and visualization of the titre differences among viruses in twofold dilutions (denoted as 1 AU), no matter what the magnitude of the actual titre. All viruses and sera were then positioned on the map by minimizing the difference between the target distances and map distances using multidimensional scaling (Smith *et al.*, 2004b). The position of each virus and antiserum is therefore determined by the relationship and position of all other viruses and sera. To minimize local optima, multiple random restarts (100 repeats) of the conjugant gradient optimization method were used (Flannery *et al.*, 1988).

Mathematically, the antigenic maps were not limited to the second and third dimension, and Euclidean ('straight line') distances can be taken between points in any dimension. Therefore, blind prediction and self-consistency experiments were undertaken to determine the optimal dimension for visualizing these data. Antigenic maps were made with a random 10 % of the titres omitted. These omitted values were then predicted using maps in dimensions two to five. The mean prediction error was similar for each dimension [0.88 ± 0.16 AU (mean $\pm$ SEM) for the second dimension, 0.85 ± 0.15 AU for the third dimension, 0.95 ± 0.16 AU for the fourth dimension and 0.92 ± 0.17 AU for the fifth dimension], suggesting no discernible mean advantage in precision using higher dimensions. The mean difference between titres predicted by the antigenic map and the titre from the VNT was therefore less than a twofold dilution. The maximum difference seen between the predicted titre and the titre from the VNT was 2.61 AU for the second dimension and 1.17 AU for the third dimension, and therefore the third dimension was chosen.

Testing vaccine matching prediction using sequence. A larger dataset of r_1 values and VP1 sequences for 166 viruses tested against vaccine virus A$_{22}$/IRQ/24/64 was used to test predictions for vaccine matching. Neutralization data were generated by the WRL/EURL for FMD using a two-dimensional VNT with pooled sera from five cattle bled 21 days post-vaccination with an oil adjuvant-based A$_{22}$/IRQ/24/64 vaccine. Complete or partial VP1 sequences for all 166 were determined previously (Armstrong *et al.*, 1994; Knowles *et al.*, 2007; Schumann *et al.*, 2008; Valarcher *et al.*, 2008; Ayelet *et al.*, 2009; Knowles *et al.*, 2009; Abdul-Hamid et al., 2011; Habiela *et al.*, 2010; Waheed *et al.*, 2011; Knowles *et al.*, 2012).

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