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Antigenic and Genetic Analyses of Foot and Mouth Disease Virus Isolates from 2005 to 2009 in Saudi Arabia for Selection of Candidate vaccine strains

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Abstract

Foot and mouth disease (FMD) is considered one of the enzootic animal diseases in Saudi Arabia. During the period 2005-2009, Large numbers of FMD type O outbreaks were reported and related to FMDV ME-SA Pan Asia II. FMD type A virus was only detected in December 2005. Its phylogenetic analysis confirmed that this type A virus was a member of the ASIA topotype. Our results of molecular-biological studies revealed that O Manisa appears to have only a moderate match against some isolates of the currently circulating type O PanAsia strain while FMD type A virus was most closely related to the Iranian A/Iran /2005. The authors recommend using a tetravalent vaccine containing type O FMDV PanAsia topotype, type A Iran 2005, Asia1 and Sat2 for FMD prevention in the large Saudi Arabian dairy farms. In addition to, bivalent vaccine containing type O FMDV PanAsia topotype and type A Iran 2005 for the routine vaccination campaigns all over the country.

*Keywords:*Epidemiology; Foot and mouth disease; Phylogenetic analysis; Saudi Arabia; Vaccine.

Introduction

Foot and mouth disease (FMD) is an extremely contagious, acute viral disease of all cloven hooved animals and is characterized by fever and vesicular eruption in the mouth and on the feet and teats (Radostits *et al*., 2000). The etiological agent of foot and mouth disease is a non enveloped icosahedral virus of genus *Aphthovirus*, family *Picornaviridae* with a single stranded positive sense RNA molecule of about 8200 nucleotides, within an icosahedral capsid made of 60 copies each of four proteins VP1, VP2 , VP3, and VP4 (Racaniello, 2001). The virus has a high mutation rate and may change, on a random basis, 1-8 nucleotides per replication cycle (Knowles *et al*., 2005). At the antigenic level, FMDV isolates sampled worldwide have been classified into seven distinct serotypes named serotype O, A, C, Asia1, SAT1, SAT2 and SAT3 with multiple subtypes within each serotype, the antigenic variation within a serotype can be such that vaccines must be carefully matched to outbreak strains to ensure efficacy (Pereira, 1977; Samuel and Knowles, 2001). The antigenic variation of FMDV is owing to spontaneous mutations, which occur during replication of the single-stranded RNA genome of positive polarity (Domingo *et al*., 1992).It has been shown that VP1 is the most variable among the capsid polypeptides and is considered to be the major immunogenic protein, since it contains a linear antigenic site able to induce neutralizing antibodies sufficient to protect animals against the disease (Bittle *et al*., 1982; Di Marchi *et al*., 1986). Nucleotide sequencing of part or all of the genome region coding for the outer capsid polypeptide VP1 was first used to study the epidemiology of FMD by Beck and Strohmaier (1987), who investigated the origin of outbreaks of types O and A in Europe over a 20-year period.

FMD is considered one of the enzootic animal diseases in Saudi Arabia (Salah, 1961; Yasin, 1963; Al-Mezaini *et. al*., 1985; Aidros, 2002) and causes severe economic losses (Hafez *et al*., 1994). Samuel *et al.* (1997) first noted the arrival of a new FMDV type O lineage in Saudi Arabia in 1994.

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Knowles *et al*. (2000) considered this lineage to be part of the PanAsia strain. Abdel Baky *et al*., (2005) recorded that the field isolates during FMDV outbreaks isolated from different regions in Saudi Arabia between July 1999 and Jan.2002 were closely related to O1 manisa strain of FMDV serotype O. Doel (2003) and Abdel Baky *et al*. (2005) stated that in 2000, serotype SAT2 invaded Saudi Arabia where it caused major problems due in part to the fact that none of the vaccines in use in the country at that time contained a SAT2 component.

The choice of a vaccine strain, however, is not determined exclusively by a single FMD outbreak. It is motivated principally by the necessity of large scale immunological coverage. For this purpose, thorough epidemiological background information is necessary from the all country involved in the FMD control programme (Fargeaud, 1995). So, Antigenic analysis of the field isolates in relation to the vaccine strains is significant for testing the appropriateness of the existing vaccine strain as well as for selection of new vaccine strains, if required (Jangra *et al*., 2005).

In this study, we describe the antigenic and genetic analysis of FMDV field isolates between 2005 and 2009 in order to provide basic molecular epidemiology information about FMDV causing outbreaks and review the suitability of vaccine strains that could provide antigenic coverage to FMDV co-circulating in Saudi Arabia.

Materials and methods

Samples

Routine diagnostic samples (detached epithelial, vesicular fluids) from cattle, sheep and goats were collected from FMD suspected outbreaks during the period 2005-2009 by the district field veterinarians from all regions of the country (Fig. 1). All of these samples were placed in a transport medium composed of equal amounts of glycerol and 0.04M phosphate buffer, pH 7.2-7.5 and submitted to the Central Veterinary Diagnostic Laboratory in Riyadh for FMDV detection and serotyping by ELISA test. The clinical samples were grinded with sterile sand and prepared 10% suspension in phosphate buffer solution. Selected positive field samples from FMD outbreaks were submitted to the

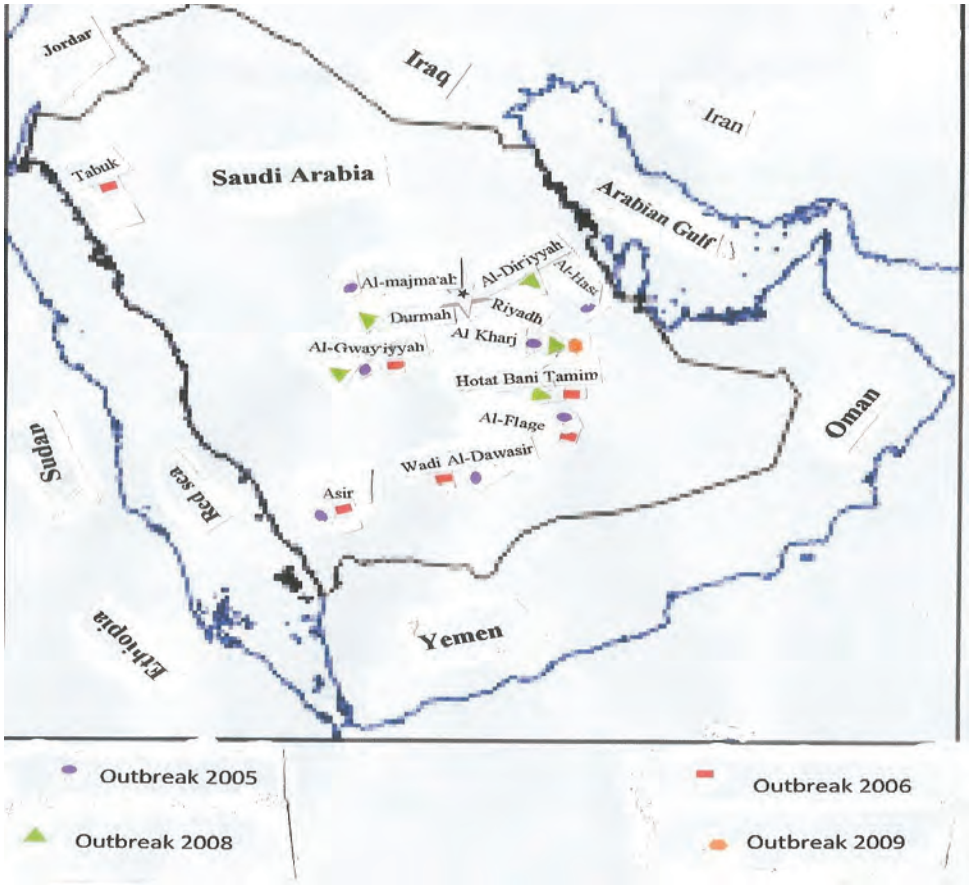

Fig. 1. Map of Saudi Arabia showing the location of provinces in which FMD field viruses were isolated between 2005 to 2009.

FMD World Reference Laboratory, Pirbright, England for vaccine matching tests by studing the antigenic relationship (r-value) and VP1 gene sequencing of these field isolates in comparison with many reference FMD vaccinal strains and other field strains isolated from countries neighboring Saudi Arabia. The antigenic relationship (r-value) of the field isolates to the reference strains was is expressed as the ratio between heterlogous/homologous serum titer. The criteria of Samuel *et al*. (1990) and the World Organization for Animal Health (OIE) (2008) were applied for interpreting the antigenic relationships; an r-value range of 0.40-1.00 indicates that the existing vaccine strain provides enough protection; while in the range of 0.20-0.39 indicated a need for a more potent vaccine .However, r-values below 0.20 stipulate the necessity for a new vaccine strain.

Enzyme linked immunosorbant assay

ELISA has been established as a routine method for the diagnosis and serotyping of FMD in the Central Veterinary Diagnostic Laboratory, Riyadh. Commercial indirect sandwich ELISA kit produced by FMD World Reference Laboratory (WRL), Pirbright, UK was used .The kit is based on a standard indirect sandwich ELISA technique to determine the presence of FMDV antigens in tissue samples as described by Roeder and Le Blanc Smith (1987); Ferris and Dawsonsn (1988). Briefly, rabbit antisera specific for the deferent serotypes of FMDV (trapping antibodies) are passively added to polystyrene microwells (Nunc®, Denmark).With the addition of test samples ,antigen(if present) is trapped by the immobilized antibodies .Specific guinea pig anti-FMDV detecting antibodies are then added which react with the trapped antigen. The bound guinea pig antibodies are detected by means of the rabbit anti-guinea pig conjugated to horse radish peroxidase .Extensive washing is carried out between each stage to remove unbound reagents. A color reaction on the addition of substrate/chromogen solution indicates a positive reaction. With strong positive reactions this will be evident to the naked eye, but results can also be read spectrophotometrically at 492 nm.

Results

The results of FMDV typing from the laboratory of central veterinary diagnostic laboratory, Riyadh, and WRL, Pirbright are the same, with FMD type O virus in 2005,2007,2008,2009 and FMD type A virus in 2005 being detected in both laboratories. In 2006, 2007 several FMD epidemics in different parts of KSA (Tabouk, Wady Al dawaser, Aseer, Hotate bny Tamime,Al kueyah and Al sulail) have been identified as Type (O) in the Central Veterinary Laboratory ,Riyadh.Table (1) revealed that during the period 2005-2009, large numbers of FMD type O outbreaks were reported .These virus isolates were related to ME-SA Pan Asia II. FMD type A virus was detected in Bny Tamim province, December 2005. Its phylogenetic analysis confirmed that this type A virus was a member of the ASIA topotype.

Discussion

Foot and mouth disease vaccines commonly contain more than one strain of the virus reflecting the epidemiological situation in the customer's country. In the Arabian peninsula, there is the potential threat from serotypes prevalent in Africa, elsewhere in the Middle East ,and India, and vaccines containing four serotypes (O, A, Asia, SAT2), including several distinct strains within the O and A serotypes (Doel, 2003). FMD control and eradication strategy in Saudi Arabia depend on restriction of animal movement and regular mass vaccination of dairy cattle farms with good quality septavalent vaccine and vaccination of sheep and goats in the vicinity of dairy cattle farms with monovalent type O vaccine (Knowles and Samuel, 2003). Currently, there are two vaccines in use in Saudi Arabia: 1-hexavalent vaccine containing O Manisa, O 3039, A Iran 05, A Saudi 95, Asia1 &Sat2 for large dairy cattle farms; 2-Monovalent type O Manisa vaccine for vaccination of sheep and goats in the vicinity of dairy cattle farms. While, trivalent vaccine (O, A 22, Asia 1) is commonly implemented in most of Middle Eastern countries: Bahrain, Iran, Iraq, Lebanon, Oman, PAT and Turkey. Syria is using trivalent vaccines with O India 53/73, A Iran 96 and Asia 1, twice a year on cattle. Egypt, Jordan, Turkey and Yemen are using also bivalent vaccines (A and O Manisa). Kuwait, UAE and Qatar are using tetravalent vaccines (O, A, Asia 1 and SAT 2), (OIE-ME, 2009). Phylogenetic analysis of the virus protein (VP) 1 region of FMD viruses has been used extensively to investigate the molecular

Table 1. FMD field isolates sent to WRL used in the present study

Field isolate	Isolation date	Virus isolation place	Species animal	Serotyping results
SAU 5/2005	April 2005	Al-Hasa	Cattle	O
SAU 6/2005	April 2005	Al-Flage	Cattle	O
SAU 7/2005	April 2005	Asir	Sheep	O
SAU 8/2005	May 2005	Wadi Al-Dawasir	Cattle	O
SAU 9/2005	May 2005	Hotat Bani Tamim	Cattle	O
SAU10/2005	May 2005	Al-Gwayiyyah	Cattle	O
SAU11/2005	May 2005	Al-majma'ah	Cattle	O
SAU12/2005	June 2005	Asir	Cattle	O
SAU13/2005	August 2005	Al Kharj	Cattle	O
SAU15/2005	Dec .2005	Hotat BaniTamim	Cattle	A
SAU16/2005	Dec .2005	Hotat BaniTamim	Cattle	A
SAU 1/2007	May 2007	Nature reserve	Oryx	O
SAU 2/2007	May 2007	Nature reserve	Oryx	O
SAU 3/2008	Jan.2008	Al kharj	Cattle	O
SAU 4/2008	Jan.2008	Hotat BaniTamim	Cattle	O
SAU 5/2008	March 2008	Dhurma	Cattle	O
SAU 6/2008	October2008	Hotat BaniTamim	Cattle	O
SAU 7/2008	Jan.2008	Al-Gwayiyyah	Sheep	O
SAU 8/2008	March 2008	Al-Gwayiyyah	Sheep	O
SAU 9/2008	March 2008	Janadriah	Cattle	O
SAU10/2008	Jan .2008	Al-Diriyyah	Cattle	O
SAU 1/2009	August 2009	Al Kharj	Cattle	O
SAU 2/2009	August 2009	Al Kharj	Cattle	O

epidemiology of the disease worldwide. These techniques have helped define genetic relationships between FMDV isolates and geographic distribution of lineages and genotypes; they have also helped establish genetically and geographically linked topotypes and trace the source of outbreaks. Topotypes are defined as geographically clustered viruses that form a single genetic lineage generally sharing >85% (O, A, C, and Asia 1) or >80% (SAT 1, SAT 2, and SAT 3) nucleotide identity in the VP1-coding region (Samuel and Knowles, 2001; Knowles and Samuel, 2003).

Our results in tables (1,2) revealed in 2005, 2007, 2008, and 2009 that Serotype O was the most prevalent serotype. These isolates of serotype O collected from different parts in Saudi Arabia (as shown in table, 2) belonged to the PanAsia strain. These isolates were shown to have a very good matching (r1 value ≥ 0.68 and 88.4-89.9% identity) with O Manisa. In addition to Serotype O, there have also been reported outbreaks due to serotype A (Iran 05 lineage) in Hotate Bany Tamime and was closely related to serotype A circulating in Iran with 99.22% identity as shown in table (3). Our results also revealed no SAT outbreak reported in Saudi Arabia until 2010 since its first isolation in 2000 (Doel, 2003; Abdel-Baky *et al*., 2005). Saudi Arabia annually imports approximately 6.5 million livestock, mainly sheep and goats from Asia, Africa and Australia. Animals from Africa and Asia bring their own FMD serotypes which spread within the nomadic herds of Saudi Arabia and neighbouring countries, and put severe constraints onto the modern cattle industry (Aidaros, 2002).Three types of FMD viruses were detected in the Middle East region between 2004 an 2006. These are the PanAsia strain of serotype O, of which a number of variants have been found in Israel, Jordan, Saudi Arabia, Kuwait, Iran and Pakistan; serotype A (strain A22 and Iran 05) in Turkey, Jordan, Iran, Saudi Arabia and Pakistan; and serotype Asia1 in Iran, 2004 and Pakistan, 2005 (Paton *et al.*, 2007). Asia 1 FMD is

Table 2. Summary of antigenic characterization and comparative DNA sequencing results of Type (O (FMD field isolates by matching with vaccine strains

Year	Field isolate	Reference Virus Strains								Topotype	Genotype/ strain
		O1 Manisa		BFS		O IND/R2/75		O/TAW/2/99			
		Identity%	r1 value	Identity%	r1 value	Identity%	r1 value	Identity%	r1 value		
2005	SAU/4/2005	89.67	0.78	80.28	-	88.58	-	95.31	-	ME-SA	PanAsia
	SAU/5/2005	89.83	-*	80.44	-	88.42	-	95.15	-	ME-SA	PanAsia
	SAU/7/2005	89.98	-	80.28	-	88.58	-	94.99	-	ME-SA	PanAsia
	SAU/8/2005	89.83	0.68	80.44	-	88.42	-	94.99	-	ME-SA	PanAsia
	SAU /9/2005	89.88	0.95	80.28	-	88.58	-	94.99	-	ME-SA	PanAsia
	SAU/1o/2005	89.84	0.83	80.59	-	88.42	-	94.84	-	ME-SA	PanAsia
	SAU/13/2005	89.84	>1.0	80.13	-	88.11	-	94.84	-	ME-SA	PanAsia
2007	SAU/1/2007	87.64	>0.94	79.75	-	88.58	-	94.05	-	ME-SA	PanAsia
	SAU/2/2007	87.64	>0.94	79.75	-	88.58	-	94.05	-	ME-SA	PanAsia
2008	SAU/1/2008	88.26	0.50	79.5	0.40	87.32	>0.80	92.96	-	ME-SA	PanAsia2
	SAU/4/2008	87.95	-	79.81	-	87.32	-	92.96	-	ME-SA	PanAsia2
	SAU/5/2008	88.11	-	79.34	-	87.48	-	93.11	-	ME-SA	PanAsia2
	SAU/6/2008	88.73	-	80.28	-	88.11	-	93.74	-	ME-SA	PanAsia2
	SAU/7/2008	88.58	-	80.13	-	87.95	-	93.27	-	ME-SA	PanAsia2
2009	SAU/1/2009	88.42	0.74	80.75	0.72	88.42	0.46	94.37	-	ME-SA	PanAsia2
	SAU/2/2009	88.42	0.72	80.75	o.66	88.42	0.35	94.37	-	ME-SA	PanAsia2

r_1 values were obtained by VNT.
(*) :not done

Table 3. Summary of Antigenic characterization and Comparative DNA sequencing results of Type (A) FMD field isolates by matching with vaccine strains

Year	Field isolate	Reference Virus Strains								Topotype
		A22		A IRN96		A SAU 23/86		A IRN 2005		
		Identity%	r1 value	Identity%	r1 value	Identity %	r1 value	Identity %	r1 value	
2005	SAU/15/2005	82	0.29	82.6	0.12	82.94	*-	99.22	-	Asia
	SAU/16/2005	82	0.25	82.6	0.12	82.94	-	99.22	-	Asia

:(*)not done

usually detected in the Indian subcontinent, from where it periodically spreads to the Middle East and occasionally Eastern Europe. However it should be noted that Asia 1 generally shows little antigenic variation, therefore existing vaccine strains (Shamir strain) usually provide good coverage (Valarcher *et al*., 2005; Schumann *et al*., 2008),

In terms of selecting vaccine strains, it is clearly not possible to include every strain that could threaten Saudi Arabia and therefore any decision on selection of strains in FMD vaccine must involve a compromise. It has been noted that O Manisa appears to have only a moderate match against some isolates of the currently circulating O PanAsia strain. So, to ensure good effect against strains currently circulating KSA, the authors recommend to use a tetravalent vaccine containing type O FMDV strain belonging to PanAsia topotype, type A Iran 2005, Asia1(Shamir) and Sat2 for FMD prevention in the large Saudi Arabian dairy farms instead of hexavalent vaccine containing O Manisa, O 3039, A Iran 05, A Saudi 95, Asia1 &Sat2 Also, It is advisable to include sheep and goat in the routine vaccination campaigns all over the country by bivalent vaccine containing type O FMDV strain belonging to PanAsia topotype and type A Iran 2005.

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