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Serological and biochemical analysis of some recent type A foot-and-mouth disease virus isolates from the Middle East*

A. R. SAMUEL, N. J. KNOWLES AND R. P. KITCHING

World Reference Laboratory for Foot-and-Mouth Disease, AFRC Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 ONF

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SUMMARY

In 1986 and 1987 foot-and-mouth disease virus (FMDV) serotype A was isolated from outbreaks of disease in Saudi Arabia and Iran. Selected virus isolates were antigenically distinct from the prototype A_{22} virus strain (A_{22}/Iraq/64), but were serologically related to each other. However, polyacrylamide gel electrophoresis showed that whilst the respective Saudi Arabian structural polypeptides were homogeneous, those from an Iran isolate were distinct. Direct sequencing of part of the P-1D (VP1) gene demonstrated considerable difference in nucleotide homology between the two groups of viruses; the Saudi Arabian viruses were closely related to each other but only distantly related to both the A_{22} prototype virus strain and the Iranian virus isolate. The latter viruses were only slightly more closely related to each other. Thus there appeared to be at least two distinct FMDV type A variants co-circulating in the Middle East, both of which differed considerably from the classical A_{22} subtype.

INTRODUCTION

Foot-and-mouth disease (FMD) is endemic in the Middle East. Since 1964 the prevalent type A viruses in this region have been related antigenically to the A_{22} subtype. A_{22} was originally identified by the World Reference Laboratory (WRL) from an outbreak among cattle in Mosul, Iraq in November 1964. It rapidly spread through the Middle East and Turkey during 1964–6 (Arrowsmith, 1975) and became established as the predominant type A strain in this region. In the ensuing years it showed little antigenic variation, as determined by complement fixation, from the parent strain. Some A_{22} variants appeared to occur in the early 1970s, notably the isolates A_{22}/EGY/1/72 and A_{22}/LEB/2/72 (Arrowsmith, 1975), but these strains did not become established. In 1973 and 1984 FMD viruses (FMDV) isolated from samples received from Saudi Arabia were identified as being antigenically related to A_{22}/Iraq/64 (WRL, unpublished results). However,

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in Iran FMDV type A variants were identified in June 1983 in Fars and 1984 in Azerbaijan (Firouzi-Bandpay *et al.* 1985).

In 1986 15 samples were received by the WRL from outbreaks of suspected FMD in Saudi Arabia. Virus isolation and serotyping showed serotype A to be responsible. In 1987 a further 12 samples containing FMD virus type A were received from Saudi Arabia, indicating the continued presence of disease due to this serotype. In April 1987 two samples of FMDV serotype A were received from an outbreak of disease at Mard Abad-Kardj, Iran (about 50 km west of Tehran), both of which were antigenically distinct from A_{22}/Iraq/64. In both cases strain characterization was immediately undertaken to identify suitable vaccines for disease control.

This paper describes the serological and molecular investigations undertaken to compare these virus isolates with reference FMDV type A strains and additionally with some recent type A isolates from Western India. The 1983–4 Iran isolates were not submitted to the WRL and therefore were not available for this study.

MATERIALS AND METHODS

Viruses

The viruses used in this study are listed in Table 1. They were isolated on primary bovine thyroid cells and then adapted to grow in BHK-21 cells with the minimum number of passages.

Antisera

All guinea-pig antisera used in the virus neutralization (VN) tests were produced against either live guinea-pig adapted virus or purified 146S virus. In some cases bovine convalescent or post-vaccinal serum was used.

Virus neutralization tests

Chessboard VN tests were used in the serological investigation (Rweyemamu *et al.* 1978). The majority were one-way tests, as homologous antiserum was only available to one of the field isolates (A/SAU/23/86). Relationships between field isolates and the reference strains were expressed as *r* values (heterologous serum titre divided by the homologous serum titre) and interpreted according to the criteria described by Pereira (1977).

Enzyme-linked immunosorbent assay (ELISA)

An ELISA was used to compare the titre of bovine convalescent sera with the homologous vaccine strain and field isolates of FMDV (Hamblin, Barnett & Hedger, 1986). Briefly, this assay measures the residual antigen remaining after overnight reaction between dilutions of serum and a pre-titrated mass of antigen. The serum titre obtained has been shown to correlate closely with that obtained using the conventional VN test, and *r* values can therefore be calculated and interpreted as described above.

Table 1. *Viruses used in this investigation*

Strain	Abbreviation (WRL ref. no.)	Date of isolation	Comments
A_5/Allier/France/60	A_5/Allier	—.—.60	WRL ref. no. A_5/FRA/1/68
A_5/Parma/Italy/62	A_5/Parma	—.—.62	
A_{22}/Mosul/Iraq/64	A_{22}/Iraq	—.—.64	WRL ref. no. A_{22}/IRQ/24/64
A_{22}/Mahmatli/Turkey/65	A_{22}/Mahmatli	—.iii.65	
A_{22}/Alexandria/Egypt/72	A_{22}/EGY/1/72	—.—.72	
A_{22}/Bsarma/Lebanon/72	A_{22}/LEB/2/72	—.—.72	
A_{24}/Cruzeiro/Brazil/55	A_{24}/Cruzeiro	17.v.55	
A/Tamil Nadu/India/78	A/IND/57/79	24.iii.78	Vaccine strain
A/Gujarat/India/80	A/IND/7/82	—.—.80	Vaccine strain
A/Rio Grande do Sul/Brazil/79	A/BRA/79	—.—.79	
A/Argentine/81	A/ARG/81	—.—.81	
A/Riyadh/Saudi Arabia/84	A/SAU/19/84	—.—.84	
A/Durma/Saudi Arabia/86	A/SAU/12/86	24.viii.86	
A/Al Kharj/Saudi Arabia/86	A/SAU/16/86	—.ix.86	
A/King Saud Univ./Saudi Arabia/86	A/SAU/23/86	22.xi.86	Vaccine strain
A/King Saud Univ./Saudi Arabia/86	A/SAU/29/86	22.xi.86	
A/Al Kharj/Saudi Arabia/87	A/SAU/11/87	—.ii.87	
A/Al Kharj/Saudi Arabia/87	A/SAU/13/87	—.iii.87	
A/Al Kharj/Saudi Arabia/87	A/SAU/20/87	—.iii.87	
A/Al Kharj/Saudi Arabia/87	A/SAU/25/87	13.viii.87	
A/Mard Abad Kardj/Iran/87	A/IRN/1/87	11.iii.87	
A/Mard Abad Kardj/Iran/87	A/IRN/2/87	11.iii.87	
A/Jamnagar/India/86	A/IND/4/87	—.xii.86	
A/Banaskantha/India/87	A/IND/5/87	—.i.87	

—, Unknown date.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Preparations of FMDV were grown on BHK-21 cells in six-well tissue-culture plates (Falcon, Becton Dickinson Ltd) and labelled with [^{35}S]methionine (Du Pont) as described by Robson *et al.* (1979). Cellular debris was removed by centrifugation at 3000 g for 10 min and the virus partially purified by centrifugation through a viscosity–density gradient (Barzilai, Lazarus & Goldblum, 1972; Knowles & Hedger, 1985). SDS–PAGE was carried out using a 10% polyacrylamide gel containing 8 M urea as described by Laemmli (1970), with the modifications described by Knowles & Hedger (1985).

Primers

Two oligonucleotide primers were synthesized on an Applied Biosystems (Foster City, CA, U.S.A.) 381A machine and used either directly or following purification on a 20% polyacrylamide/8 M urea gel. The sequences of the primers and their location on the virus genome (numbering according to the A_{10}/Argentine/61 nucleotide sequence, starting from the translation initiation codon; Carroll, Rowlands & Clarke, 1984) were dTTCATGCGCACGAG (nucleotides 2698–2711) and dACGTCTCCCGCCAACTT (nucleotides 2827–2843). The former is located

Table 2. *Relationship (r) values obtained between the field isolates and reference strains by virus neutralization tests or ELISA*

Antisera	Viruses							
	A/SAU 12/86	A/SAU 16/86	A/SAU 23/86	A/SAU 29/86	A/SAU 11/87	A/IRN 1/87	A/IND 4/87	A/IND 5/87
A_5/Allier/France/60	< 0·1	< 0·1	< 0·1	< 0·1	—	—	—	—
A_5/Parma/Italy/62	< 0·1	< 0·1	—	—	—	—	—	—
A_{22}/Iraq/64	0·5	0·3	0·1	0·2	0·4	< 0·1*	—	—
A_{22}/Iraq/64†	—	—	0·1	0·2	—	—	1·0*	1·0*
A_{22}/Mahmatli/Turkey/65	—	—	< 0·1	< 0·1	—	—	—	—
A_{24}/Cruzeiro/Brazil/55	0·1	< 0·1	< 0·1	< 0·1	—	< 0·1*	—	—
A/Brazil/79	—	—	< 0·1	< 0·1	—	—	—	—
A/Argentine/81	—	—	< 0·1	< 0·1	—	—	—	—
A/SAU/23/86‡	—	—	1·0	—	0·5	1·0	0·7*	0·8*

* Results obtained by ELISA. † Bovine convalescent sera. ‡ Bovine post-vaccine sera.

176 nucleotides into the P-1D (VP1) gene and the latter 19 nucleotides into the P-2A gene.

Nucleotide sequencing

Virus RNA was prepared from a single 175 cm^2 flask of FMDV-infected BHK-21 cells. The clarified harvest was pelleted through a 30 % (w/v) sucrose cushion by high-speed ultracentrifugation and the virus RNA extracted directly from the pellet in a procedure similar to that described by Rico-Hesse *et al.* (1987) for polioviruses. The dideoxy-sequencing procedure for RNA templates described by Zimmern & Kaesberg (1978) and modified by Palmenberg *et al.* (1984) and Xie *et al.* (1987) was used with minor modifications.

Nucleotide and amino acid sequences were analysed on an Archimedes 440 microcomputer (Acorn Computers Ltd, Cambridge) using a program written by one of the authors (N.J.K.). All pairwise comparisons were performed by assigning each base substitution an equivalent statistical weight. Deletions were counted as substitutions and ambiguities were ignored. A dendrogram was constructed according to sequence relatedness across the interval of nucleotides 361–639 of the P-1D gene. In some cases a slightly lesser number of nucleotides were compared.

RESULTS

Serology

The VN results are shown in Table 2. Two early isolates, A/SAU/12/86 and A/SAU/16/86, showed some relationship with A_{22}/Iraq/64 ($r = 0{\cdot}5$ and 0·3, respectively), but later isolates. A/SAU/23/86 and A/SAU/29/86, showed little antigenic relationship with the A_{22} reference strain or with the European and South American reference virus strains ($r = 0{\cdot}2$ or less). A/SAU/11/87 had some antigenic relationship with both A/SAU/23/86 ($r = 0{\cdot}5$) and A_{22}/Iraq/64 ($r =$

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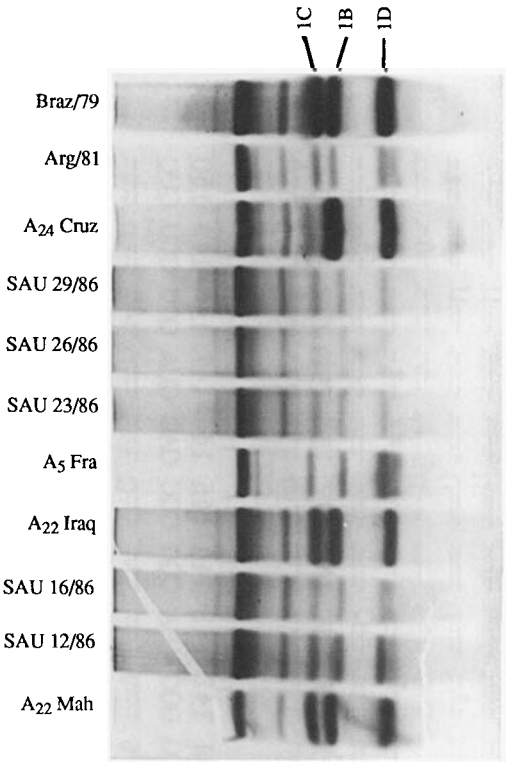

Fig. 1. Comparison of the 1986 Saudi Arabian isolates and different foot-and-mouth disease virus type A reference strains by SDS–PAGE. Strains are as defined in Table 1, and 1B, 1C and 1D designated structural polypeptides.

0·4). A/IRN/1/87 also showed a close antigenic relationship with A/SAU/23/86 ($r = 1·0$), but had no antigenic relationship with A_{22}/Iraq/64 or A_{24}/Cruzeiro/55 ($r = <0·1$).

The Indian isolates A/IND/4/87 and A/IND/5/87 showed a close antigenic relationship with A_{22}/Iraq/64 ($r = 1·0$) and with A/SAU/23/86 ($r = 0·7$ and 0·8, respectively).

SDS–PAGE

The migration patterns of the structural polypeptides (1B, 1C and 1D) of the selected 1986 Saudi Arabian isolates were indistinguishable from each other but different from A_{22}/Iraq/64, A_{22}/Mahmatli/65, A_5/Allier/60, A_{24}/Cruzeiro/55, A/Brazil/79 and A/Argentine/81 (Fig. 1). Differences were seen between the migration patterns of the structural proteins 1C and 1D of the selected 1986 and 1987 Saudi Arabian isolates. Comparison between the Saudi Arabia 1984, 1986 and 1987 isolates and A_{22}/Iraq/64, A_{22}/LEB/2/72 and A_{22}/EGY/1/72 showed that the migration patterns for each year and country were distinct. The migration patterns of A/IRN/1/87 and A/IRN/2/87 were identical to each other, and showed differences in all three capsid polypeptides compared with the 1986 and 1987 Saudi Arabian viruses. A/IND/4/87 and A/IND/5/87 showed

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	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135
$A_{2\,2}$/Iraq/64	GCG	CCA	CAC	CGC	GUG	UUG	GCA	ACA	GUG	UAC	AAC	GGG	ACG	AGC	AAG
$A_{2\,2}$/USSR/64	---	---	---	---	--A	---	--G	---	---	---	---	---	---	---	---
$A_{2\,2}$/CCCP	---	---	---	---	--A	---	--G	---	---	---	---	---	---	G--	---
A/SAU/12/86											---	---	--U	---	---
A/SAU/23/86	---	-G*	---	---	---	---	---	---	---	---	---	---	--U	---	---
A/SAU/25/87	---	-G*	---	---	---	---	-*-	---	---	---	---	---	--U	---	---
A/IRN/1/87												--	---	-A-	---
A/Iran/87					---	---	--U	---	---	---	-**	-*-	---	-A-	---
A_5/Allier/60	---	---	---	---	---	---	---	--C	---	---	---	---	---	-A-	---
$A_{2\,4}$/Cruzeiro/55	---	--G	---	--U	---	-C-	---	---	---	---	---	---	---	--U	---
A/Argentine/79	--A	---	---	--U	---	C--	---	---	---	---	---	---	--A	---	--A

	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
$A_{2\,2}$/Iraq/64	UAC	UCC	GCA	GGU	GGU	ACG	GGC	AGA	CGG	GGC	GAC	CUA	GGG	CCU	CUC
$A_{2\,2}$/USSR/64	---	---	---	---	---	-U-	---	---	---	---	---	---	-A-	---	---
$A_{2\,2}$/CCCP	---	---	---	---	---	-U-	---	---	---	---	---	---	-A-	---	---
A/SAU/12/86	-*-	---	-AG	AC-	*-*	*G-	C-U	GU-	---	--*	---	---	--A	-A*	A--
A/SAU/23/86	---	---	--G	AC-	*--	**-	*AU	GU-	---	--U	---	---	--A	-AG	A--
A/SAU/25/87	---	---	-AG	AC-	*--	**-	C-U	GU-	---	--*	---	---	--A	-AG	A--
A/IRN/1/87	---	G-*	---	AC*	---	G-U	:::	---	---	--*	---	-**	---	*--	--*
A/Iran/87	---	G-U	---	ACC	---	*-U	:::	---	---	--*	---	---	---	U--	*--
A_5/Allier/60	---	---	A-G	-AC	---	C--	:::	---	--A	-A-	---	A-G	---	U-G	-C-
$A_{2\,4}$/Cruzeiro/55	--U	G-U	-UG	---	---	U-A	---	---	--U	---	---	A-G	---	U--	---
A/Argentine/79	---	A--	-UG	---	---	U-A	---	--G	--U	--U	---	A-G	---	U-C	---

Fig. 2. For legend see page 585

	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165
A_{22}/Iraq/64	GCG	GCG	AGG	GUC	GCC	GCU	CAG	CUU	CCU	GCU	UCU	UUC	AAC	UUU	GGU
A_{22}/USSR/64	---	---	---	---	---	---	---	---	---	A--	---	---	---	---	---
A_{22}/CCCP	---	---	---	---	---	---	---	---	---	A--	---	---	---	---	---
A/SAU/12/86	---	--A	--A	--U	A-G	C-*	*--	---	---	--C	--C	---	---	---	--C
A/SAU/23/86	-*-	*-A	--A	C-U	--G	C-C	*--	---	---	--C	--C	---	---	---	--C
A/SAU/25/87	--A	--A	--A	--U	--G	C-C	*--	---	---	--C	--C	---	---	---	--C
A/IRN/1/87	---	--A	---	---	--*	--G	--A	--C	---	U--	--C	-*-	---	---	--G
A/Iran/87	---	--A	---	---	---	--G	--A	--C	---	U--	--C	---	---	---	--G
A_5/Allier/60	---	---	C--	-C-	--G	AAA	--A	---	---	--C	---	--U	---	-AC	---
A_{24}/Cruzeiro/55	---	---	C-A	---	-UG	AAA	---	---	---	---	--A	--U	---	-AC	---
A/Argentine/79	---	--A	C-A	---	--G	AAA	--A	---	---	---	--A	---	---	-A-	---

	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
A_{22}/Iraq/64	GCC	AUC	CAA	GCC	ACG	ACC	AUC	CAC	GAG	CUC	CUC	GUG	CGC	AUG	AAG
A_{22}/USSR/64	--A	--U	---	---	---	---	---	---	---	---	---	---	---	---	---
A_{22}/CCCP	--A	--U	---	---	---	---	---	---	---	---	---	---	---	---	---
A/SAU/12/86	--A	---	-GG	---	--U	---	---	---	---	--U	---	---	---	---	---
A/SAU/23/86	--A	---	-GG	---	--U	---	---	--U	---	--U	---	---	---	---	---
A/SAU/25/87	--A	---	-GG	---	--U	---	---	---	---	--U	---	---	---	---	---
A/IRN/1/87	-*A	--U	*GG	---	---	---	---	---	---	--U	--*	*-*	*--	---	-G-
A/Iran/87	--A	--U	-GG	---	---	---	---	---	---	--U	---	---	---	---	-G-
A_5/Allier/60	--A	---	AGG	---	GAC	---	---	---	---	--U	---	---	---	---	--A
A_{24}/Cruzeiro/55	--A	---	A-G	---	GAC	G--	---	---	--A	--U	---	---	---	---	--A
A/Argentine/79	--A	--U	A-G	---	--C	G--	---	---	---	---	---	---	---	---	--A

Fig. 2. (*cont.*) For legend see page 585

	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
	CGU	GCC	GAA	CUC	UAC	UGC	CCC	AGA	CCA	CUG	UUG	GCA	GUG	GAG	GUG
A22/Iraq/64	---	---	---	---	---	---	---	---	---	---	---	---	---	-U-	---
A22/USSR/64	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A22/CCCP	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A/SAU/12/86	---	---	--G	---	---	---	--G	--*	---	C--	--G	---	---	---	---
A/SAU/23/86	---	---	--G	---	---	---	--G	---	---	C--	--G	---	---	---	---
A/SAU/25/87	---	---	--G	---	---	---	--G	---	---	C--	--G	---	---	---	---
A/IRN/1/87	--C	---	--G	---	---	--*	--G	--G	--G	---	C--	---	A--	---	---
A/Iran/87	--C	---	--G	---	---	---	--G	--G	--G	---	C--	---	A--	---	---
A5/Allier/60	--G	--A	--G	---	---	---	---	--G	---	--A	---	---	A-A	---	---
A24/Cruzeiro/55	--G	---	--G	---	---	---	---	---	--G	---	C--	---	A-A	---	---
A/Argentine/79	--G	---	---	---	---	--U	---	--G	---	--C	C--	--G	---	--A	---

	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
	UCG	UCU	CAA	GAC	AGA	CAC	AAA	CAG	AAG	AUC	AUU	GCA	CCU	GCA	AAA
A22/Iraq/64	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A22/USSR/64	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A22/CCCP	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A/SAU/12/86	-U-	--G	---	---	---	---	---	--A	---	--U	---	---	---	---	---
A/SAU/23/86	---	--G	---	---	---	---	---	--A	---	--U	--C	---	---	---	---
A/SAU/25/87	---	--G	---	---	---	---	---	--A	---	---	---	---	---	---	---
A/IRN/1/87	---	G-G	G--	-G-	---	---	---	---	---	---	---	---	---	---	--G
A/Iran/87	---	G-G	G--	-G-	---	---	---	---	---	---	---	---	---	---	--G
A5/Allier/60	--U	--A	---	---	--G	---	--G	--A	-G-	---	---	---	---	---	--G
A24/Cruzeiro/55	--U	--G	---	---	--G	---	--G	--A	---	---	---	---	--A	---	--G
A/Argentine/79	---	--A	---	---	C-G	---	---	---	---	--U	---	---	---	---	---

Fig. 2. (*cont.*) For legend see page 585

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	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225
A_{22}/Iraq/64	CAA	CUU	UUG												
A_{22}/USSR/64	---	---	---												
A_{22}/CCCP	---	---	---												
A/SAU/12/86	--G	--C	C**												
A/SAU/23/86	--G	--C	C-*												
A/SAU/25/87	--G	--C	C-*												
A/IRN/1/87	--G	---	C-*												
A/Iran/87	--G	---	C-*												
A_5/Allier/60	--G	U-G	C--												
A_{24}/Cruzeiro/55	--G	---	C--												
A/Argentine/79	--G	---	---												

Fig. 2. Nucleotide sequences of the 3′ half of the P-1D (VP1) gene of some of the foot-and-mouth disease viruses studied. Codon numbering has been used. *nucleotide not conclusively determined; —identical to A_{22}/Iraq/64 sequence; :deletion compared to other sequences; space = region not sequenced. The following sequences were previously determined: A_{22}/Iraq/64 (C. Bolwell and colleagues, unpublished observations), A_{22}/CCCP (Onishchenko *et al.* 1986), A/Iran/87 (Ahl & Marquardt, 1987; O. Marquardt, personal communication, 1988), A_5/Allier/60 (FRA/1/68) (Beck & Strohmaier, 1987), A_{22}/USSR/64, A_{24}/Cruzeiro/55 and A/Argentine/79 (Weddell *et al.* 1985).

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                        130         140         150         160         170         180
A22/Iraq/64     APHRVLATVYNGTSKYSAGGTGRRGDLGPLAARVAAQLPASFNFGAIQATTIHELLVRMK
A22/USSR/64     --------------------M------E----------T--------------------
A22/CCCP        ------------G------M------E----------T--------------------
A/SAU/12/86           -----*-ET**RV-----*I----TP*----------R----------
A/SAU/23/86     -R----------------T***V-----QI**-L-P*----------R----------
A/SAU/25/87     -R----*----------ET**RV-----QI-----P*----------R----------
A/IRN/1/87            *-N--A-T-A:----*-*----------S-*---*-*--------**-R
A/Iran/87         ------**-N--A-T-*:------S*---------S-------R----------R
A5/Allier/60    ------------N---TD-P:--D-M-SP---A-K-------Y---R-D---------
A24/Cruzeiro/55 -----S----------AV--S-----M-S-----VK-------Y---K-DA--------
A/Argentine/79  ----------------TV--S-----M-S------K-------Y---K--A--------

                        190         200         210
A22/Iraq/64     RAELYCPRPLLAVEVSSQDRHKQKIIAPAKQLL
A22/USSR/64     ------------V------------------
A22/CCCP        --------------------------------
A/SAU/12/86     --------------L---------------*
A/SAU/23/86     --------------------------------
SAU25/87        --------------------------------
A/IRN/1/87      ------------M---AEG-------------
A/Iran/87       ------------M---AEG-------------
A5/Allier/60    ------------I----------R---------
A24/Cruzeiro/55 ------------I------------------
A/Argentine/79  --------------------------------
```

Fig. 3. Deduced amino acid sequences of the C-terminal end of the capsid protein P-1D (VP1) of some of the foot-and-mouth disease viruses studied. *amino acid not determined; —identical to A_{22}/Iraq/64 sequence; :deletion compared to other sequences; space = region not sequenced.

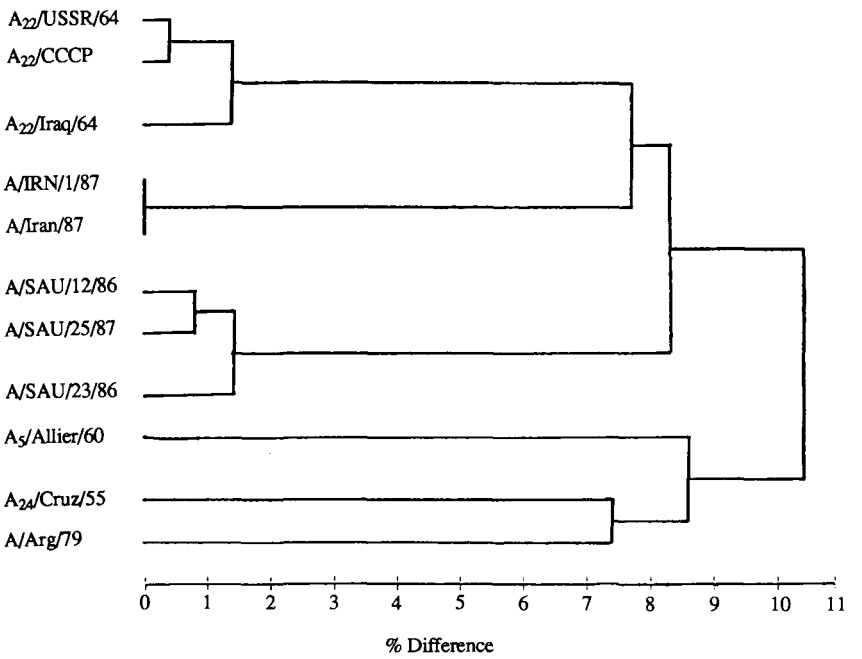

Fig. 4. Dendrogram depicting genetic relationships between some of the foot-and-mouth disease type A viruses studied. The percentage sequence divergence between any two strains is twice the distance along the abscissa to the connecting node.

differences in 1B, 1C and 1D from each other on SDS–PAGE and could also be distinguished from A_{22}/Iraq/64 and the 1986–87 Middle East isolates (data not shown).

Nucleotide sequencing

It was not possible to determine the identity of all nucleotides, presumably due either to the occurrence of a mixed population of RNA species or to strong secondary structures in the RNA template.

Fig. 2 shows the nucleotide sequences found in part of the 1D gene of some of the field isolates studied and compares them with published data: A_{22}/Iraq/64 (C. Bolwell and colleagues, unpublished observations); A_{22}/Azerbaijan/USSR/64 (N550), A_{24}/Cruzeiro/55 and A/Argentine/79 (Weddell *et al.* 1985); A_{22}/CCCP – an unidentified A_{22} virus strain recently reported by Onishchenko *et al.* (1986) which is probably A_{22}/Azerbaijan/USSR/64; A_5/Allier/60 (Beck & Strohmaier, 1987), and a 1987 isolate from Iran (Ahl & Marquardt, 1987). The latter sequence includes corrections and additions kindly provided by Dr O. Marquardt.

Fig. 3 shows the deduced amino acid sequences of the virus strains studied. There were a number of amino acid differences in the putative antigenic region (aa 140–160) between the A_{22} and Saudi Arabian viruses. There were few differences between A_{22} and the Iran viruses in this region; however, in the latter viruses there were three novel amino acid changes at positions 197–199. The Iranian isolates (from both Tubingen and Pirbright) had a single amino acid deletion (142) in a

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putative antigenic region and had identical nucleotide sequences. The A_5 viruses also had a single deletion at this position; however, since there is no direct relationship between these two groups this must have occurred independently.

The nucleotide sequence homologies and genetic relationships clearly show that the Saudi Arabian virus isolates are closely related to each other and are only distantly related to all the other viruses studied (Fig. 4). Despite the antigenic relationships observed, the Iran viruses also represent a distinct genetic group.

DISCUSSION

The results of VN tests using A/SAU/12/86 and A/SAU/16/86, collected in September 1986, indicated that a vaccine prepared from the reference strain A_{22} /Iraq/64 would be antigenically appropriate to give adequate protection in the field. However, the *r* values obtained with the A_{22}/Iraq/64 reference serum showed that although these isolates could still be considered as part of the A_{22} subgroup, there were some antigenic differences from the A_{22}/Iraq/64 strain. A/SAU/16/86, which was isolated from a sample collected on a farm in Al-Kharj, gave an *r* value of 0·3 with A_{22}/Iraq/64, whereas A/SAU/12/86 from a farm 80 miles north-west of Al-Kharj gave an *r* value of 0·5 with the same antiserum. Analysis by SDS–PAGE showed that the polypeptide migration patterns of these two isolates were identical to each other, but the migration patterns of the structural proteins 1B and 1D of both were different from the A_{22}/Iraq/64 and A_{22}/Mahmatli/65 reference virus strains. Although these changes cannot be directly related to antigenic similarity, they exclude the possibility of 100% homology to the A_{22}/Iraq/64 virus strain, the 'A' component in the quadrivalent vaccine used routinely before and in the face of the outbreaks (Anon, 1987).

Samples received in 1986 from King Saud University, Riyadh (A/SAU/23/86 and A/SAU/29/86), gave *r* values of 0·2 or less with the A_{22}/Iraq/64 reference strain serum, and *r* of $< 0{\cdot}1$ with the other reference strains on VN tests. Values this low qualify for different subtype classification (Pereira, 1977), and suggest that none of the reference strains examined would be suitable as a vaccine. However, field observations (M.D. MacKendrick, personal communication, 1987) indicated that older cattle which had received multiple vaccinations containing the A_{22} vaccine strain were protected from FMD during the outbreaks, clinical disease being restricted to the yearling cattle and older calves. By SDS–PAGE analysis the migration pattern of the structural proteins of three isolates from King Saud University (A/SAU/23/86, A/SAU/26/86 and A/SAU/29/86) were indistinguishable from each other and from the two earlier isolates (A/SAU/12/86 and A/SAU/16/86).

The VN and SDS–PAGE results indicated that a new strain of FMDV type A was responsible for the outbreaks in Saudi Arabia. The earlier samples, A/SAU/12/86 and A/SAU/16/86, did show some antigenic relationship to A_{22}/Iraq/64, which suggests that they may have been derived from previous Middle East strains. However, analysis by SDS–PAGE indicated that the structural proteins differed from those of the 1984 isolate (A/SAU/19/84) and from those of the A_{22} variants A_{22}/EGY/1/72 and A_{22}/LEB/2/72.

The identification of type A FMDV in samples from Iran, which were also

antigenically distinct from the A_{22}/Iraq/64 strain and very closely related to A/SAU/23/86 antigenically, suggested a possible common origin between the two outbreaks. However, subsequent analysis by SDS–PAGE showed differences between the migration patterns of the structural proteins of these strains.

Although SDS–PAGE shows that there are differences between the two virus isolates from Western India, both show antigenic relationships with A_{22}/Iraq/64 and A/SAU/23/86. Therefore further studies using nucleotide sequencing are being performed to resolve these relationships.

The nucleotide-sequencing data clearly shows that A/SAU/12/86, A/SAU/23/86 and A/SAU/25/87 are genetically closely related, and differ considerably from both the A_{22} reference virus strains and the A/IRN/1/87 isolate. However, all these recent isolates are more closely related to the 'A_{22} group' as represented by A_{22}/Iraq/64 and A_{22}/Azerbaijan/64 than to either the European (A_5) or South American (A_{24}) groups. The nucleotide sequence of the A/IRN/1/87 isolate was identical to that of the isolate independently submitted to Tubingen. In separate investigations of the sequence of a recent type A FMDV from Turkey, distinct regions of nucleotide sequence homology with A/IRN/1/87 were found, suggesting that this group of viruses may be circulating outside Iran (N. J. Knowles and W. C. Carpenter, unpublished results).

In conclusion, the recent Saudi Arabian and Iranian virus isolates are considered to be genetically divergent from each other and from other type A FMDV, and to constitute an antigenically distinct group of viruses in the Middle East.

A study by other workers on the strains from outbreaks in Saudi Arabia in 1986 showed similar serological findings to our own. A vaccine incorporating both A_{22}/Iraq/64 and A/SAU/23/86 has been prepared, and has been reported to be controlling FMD due to the new type A strains in Saudi Arabia (Anon, 1987).

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