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Molecular analysis of foot-and-mouth disease type O viruses isolated in Saudi Arabia between 1983 and 1995

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SUMMARY

Partial nucleotide sequence of the capsid polypeptide coding gene 1D (VP1) was determined for 68 serotype O foot-and-mouth disease viruses isolated between 1983 and 1995 from outbreaks occurring in Saudi Arabia. The sequences were compared with previously published sequences: 14 viruses of Middle Eastern origin (isolated between 1987 and 1991); and with four vaccine virus strain sequences, three originating from the Middle East (O_1/Turkey/Manisa/69, O_1/Sharquia/Egypt/72 and O_1/Israel/2/85) and one from Europe (O_1/BFS 1860/UK/67). The virus isolates from Saudi Arabia and the Middle East vaccine virus strains formed a related genetic group distinct from the European O_1 virus. Within this large group 12 distinct genetic sublineages were observed.

INTRODUCTION

Foot-and-mouth disease (FMD) virus (family *Picornaviridae*, genus *Aphthovirus*) causes an economically important vesicular disease of cattle and other cloven-hoofed animals. There are seven serologically distinct serotypes (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1) and within each serotype considerable antigenic variation occurs.

Foot-and-mouth disease is present in most of South America, Africa and Asia. Serotype O is enzootic throughout the Middle East whilst types A and Asia 1 cause sporadic outbreaks of disease. Epizootics due to types C and SAT 1 have occurred in the Middle East but they have been either infrequent or geographically isolated cases.

In Saudi Arabia, outbreaks of FMD due to type O virus have been recorded in the following years: 1971–3, 1978, 1980–95. Type A occurred in 1973, 1976, 1984, 1986–7 and 1991–5; type Asia 1 in 1980, 1992 and 1994; type C in 1984; and type SAT 1 in 1962 and 1970.

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The Saudi Arabian dairy industry is one of the most productive in the Middle East. In spite of an environment and climate significantly more hostile than that traditionally associated with milk production, high-yielding European breeds are now successfully reared and milked. The herds are zero-grazed and kept in large units of up to 25000 cattle. The fodder is grown either off-site or in adjacent irrigated areas, which are fenced off from the surrounding uncultivated land. Disease control is essential in a country in which many major veterinary diseases are still enzootic. The situation is made more acute by the high susceptibility of the imported cattle to these diseases, and the metabolic stress imposed upon them by high productivity. The herd owners accept that it is impossible to guarantee complete isolation of dairy herds from indigenous stock belonging to nomadic people, and therefore rely heavily on vaccines for disease control. However, vaccination by itself is insufficient to control FMD, and whilst every attempt is made to keep the dairy, stock yards and forage production units separated from indigenous sheep, goats and cattle, it is not

Table 1. *Designation and origin of foot-and-mouth disease viruses*

WRL ref. no.	Geographical location	Date collected	Animal
O_1/BFS 1860/UK/67	Wrexham,	UK06/01/67	Cattle
O_1/Manisa/TUR/69	Manisa, Turkey	01/04/69	Cattle
O_1/Sharquia/EGY/72	Sharquia Governate, Egypt	1972	Cattle
O/IND/53/79	Tamil Nadu, India	1977	Cattle
O/ISR/2/85	Geshur, Israel	05/85	Cattle
O/NYE/10/87	Sana'a, Yemen	23/07/87	Cattle
O/SYR/1/87	Duma, Syria	01/03/87	Cattle
O/ISR/1/88	Dalton, Zefat, Israel	24/06/88	Cattle
O/TUR/8/88	Oguzeli, Gaziantep, Turkey	11/05/88	Cattle
O/BAR/2/91	Bahrain	11/03/91	Cattle
O/BUL/1/91	Stefan Karadjevo, Bulgaria	26/07/91	Cattle
O/OMN/58/91	Muscat, Oman	01/06/91	Cattle
O/TUR/13/91	Odemis, Izmir, Turkey	28/06/91	Cattle
O/SAU/2/83	Al-Kharj, Saudi Arabia	1983	Cattle
O/SAU/4/83	Al-Kharj, Saudi Arabia	04/05/83	Cattle
O/SAU/2/84	Riyadh, Saudi Arabia	19/01/84§	Cattle
O/SAU/34/84	Al-Kharj, Saudi Arabia	30/11/84§	Cattle
O/SAU/11/85	Al-Kharj, Saudi Arabia	26/11/85	Cattle
O/SAU/17/86	Al-Kharj, Saudi Arabia	07/09/86	Cattle
O/SAU/1/87	Al-Jouf, Saudi Arabia	03/02/87§	Sheep
O/SAU/1/88	Quatif, Saudi Arabia	19/01/88	Cattle
O/SAU/8/88	Al-Kharj, Saudi Arabia	28/09/88	Cattle
O/SAU/15/88	Al-Kharj, Saudi Arabia	12/10/88	Cattle
O/SAU/20/88	Riyadh, Saudi Arabia	10/10/88	Cattle
O/SAU/30/88	Riyadh, Saudi Arabia	10/10/88	Cattle*
O/SAU/33/88	Durma, nr Riyadh, Saudi Arabia	15/12/88	Cattle
O/SAU/2/89	Al-Kharj, Saudi Arabia	04/01/89	Cattle
O/SAU/3/89	Al-Kharj, Saudi Arabia	22/01/89	Cattle
O/SAU/38/89	Al-Kharj, Saudi Arabia	22/03/89	Cattle
O/SAU/54/89	Todhia, Saudi Arabia	25/08/89	Cattle
O/SAU/56/89	Todhia, Saudi Arabia	25/08/89	Cattle
O/SAU/17/90	Al-Jouf, Saudi Arabia	05/06/90§	Cattle
O/SAU/18/90	Afif, Saudi Arabia	05/06/90§	Cattle
O/SAU/19/90	Quatif, Saudi Arabia	05/06/90§	Cattle
O/SAU/25/90	Thadiq, Saudi Arabia	29/07/90	Cattle
O/SAU/26/90	Al Hair, Saudi Arabia	26/11/90	Cattle
O/SAU/30/90	Riyadh, Saudi Arabia	11/09/90	Cattle†
O/SAU/35/90	Al-Majmaa, Saudi Arabia	05/12/90	Sheep‡
O/SAU/36/90	Riyadh, Saudi Arabia	10/12/90	Cattle
O/SAU/3/91	Al-Medyan, Saudi Arabia	19/02/91	Cattle
O/SAU/7/91	Al-Kharj, Saudi Arabia	05/01/91	Cattle†
O/SAU/16/91	Al-Kharj, Saudi Arabia	30/06/91	Cattle
O/SAU/24/91	Al-Medyan, Saudi Arabia	18/04/91	Cattle†
O/SAU/33/91	Riyadh, Saudi Arabia	04/05/91	Cattle
O/SAU/40/91	Quasim, Saudi Arabia	10/08/91	Cattle
O/SAU/43/91	Madina, Saudi Arabia	10/08/91	Cattle‡
O/SAU/44/91	nr Damman, Saudi Arabia	16/08/91	Cattle‡
O/SAU/45/91	Quassim, Saudi Arabia	28/08/91	Cattle‡
O/SAU/47/91	Madina, Saudi Arabia	05/10/91	Cattle‡
O/SAU/46/91	Hail, Saudi Arabia	26/09/91	Cattle
O/SAU/49/91	Wadi Dwasar, Saudi Arabia	12/10/91	Cattle
O/SAU/1/92	Maghateer, Saudi Arabia	20/01/92	Sheep
O/SAU/31/92	Gizan, Saudi Arabia	20/11/92§	Cattle
O/SAU/34/92	Al-Mazahmia	13/12/92	Cattle
O/SAU/29/93	200 km north of Jouf, Saudi Arabia	26/01/93	Sheep
O/SAU/66/93	Haradh, Saudi Arabia	06/04/93	Sheep

Table 1—*cont*.

WRL ref. no.	Geographical location	Date collected	Animal
O/SAU/69/93	Haradh, Saudi Arabia	06/04/93	Sheep
O/SAU/95/93	Riyadh, Saudi Arabia	21/10/93	Cattle
O/SAU/3/94	Abu-Sabah, Saudi Arabia	16/02/94	Cattle
O/SAU/12/94	Todhia, Saudi Arabia	10/03/94	Cattle
O/SAU/15/94	Al-Kharj, Saudi Arabia	22/03/94	Cattle
O/SAU/17/94	Al-Kharj, Saudi Arabia	29/04/94	Cattle
O/SAU/19/94	Al-Kharj, Saudi Arabia	29/04/94	Cattle
O/SAU/43/94	Al-Kharj, Saudi Arabia	16/04/94	Sheep
O/SAU/45/94	Riyadh, Saudi Arabia	16/04/94	Sheep
O/SAU/51/94	Al-Kharj, Saudi Arabia	06/06/94	Cattle
O/SAU/55/94	Riyadh, Saudi Arabia	11/06/94	Cattle
O/SAU/58/94	Al-Mazahmia, Saudi Arabia	12/06/94	Cattle
O/SAU/60/94	Al-Kharj, Saudi Arabia	14/06/94	Cattle
O/SAU/61/94	Al-Kharj, Saudi Arabia	14/06/94	Cattle
O/SAU/62/94	Gizan, Saudi Arabia	14/06/94	Sheep
O/SAU/65/94	Bahdaria, Saudi Arabia	05/07/94	Cattle
O/SAU/69/94	Al-Kharj, Saudi Arabia	21/07/94	Cattle
O/SAU/72/94	Al-Kharj, Saudi Arabia	21/07/94	Cattle
O/SAU/73/94	Al-Nakheel, Saudi Arabia	25/10/94§	Cattle
O/SAU/75/94	Al-Nakheel, Saudi Arabia	25/10/94§	Cattle
O/SAU/76/94	Al-Nakheel, Saudi Arabia	25/10/94§	Cattle
O/SAU/79/94	Riyadh, Saudi Arabia	18/10/94	Sheep
O/SAU/100/94	Al-Kharj, Saudi Arabia	18/12/94	Sheep
O/SAU/1/95	Al-Kharj, Saudi Arabia	20/02/95	Cattle
O/SAU/2/95	Al-Kharj, Saudi Arabia	07/01/95	Deer
O/SAU/5/95	Al-Kharj, Saudi Arabia	08/01/95	Cattle
O/SAU/8/95	Al-Jouf, Saudi Arabia	11/01/95	Sheep
O/SAU/14/95	Al-Jouf, Saudi Arabia	15/01/95	Sheep
O/SAU/20/95	Heradh, Saudi Arabia	21/01/95	Sheep
O/SAU/28/95	Al-Mazahmia, Saudi Arabia	14/02/95	Goat

* Nomadic animal.
† Carrier animal.
‡ Non-vaccinated animal.
§ Date sample received at the WRLFMD, Pirbright.

always successful. The situation is further complicated by the fact that Saudi Arabia annually imports over six million live animals for meat from many areas where FMD is enzootic. This clearly increases the possibility of 'exotic' strains of FMD virus being introduced.

The value of vaccination against FMD depends on the proper use of a potent inactivated vaccine containing strains of virus antigenically close to those likely to challenge the vaccinated animals. However, even with a potent vaccine, protective immunity is never complete throughout a herd [1] and, should those cattle that have inadequate immunity become infected, they become a potent source of infection which could overcome the immunity of others and possibly result in the emergence of antigenic variants.

The results of serological tests, including monoclonal antibody (Mab) profiling suggested the presence of two strains of FMD type O virus co-circulating within Saudi Arabia during 1988 and 1989 [2]. Virus isolates with similar MAb profiles to some of the Saudi Arabian isolates were also identified in Turkey (O/TUR/8/88) and Libya (O/LIB/6/88) in 1988 (A. R. Samuel, unpublished observations).

The work presented here was undertaken to determine the genetic diversity of FMD type O viruses isolated from outbreaks in Saudi Arabia. Virus isolates were chosen from all farms within Saudi Arabia from which samples had been received. Additionally, viruses recovered from oesophageal/pharyngeal scrapings taken from cattle on some of the farms were examined to determine if viruses from carrier animals were distinguishable from those isolated during outbreaks. Animals are considered as

Table 2. *Location and sequence of oligonucleotide primers*

Designation	Primer sequence (5′→3′)	Sense	Use	Location	
				Gene	Nt position
pNK10	GAAGGGCCCAGGGTTGGACTC	Negative	Sequencing	2A/2B	34-48/1-6
pNK61	GACATGTCCTCCTGCATCTG	Negative	RT; PCR	2B	58-77
pARS4	ACCAACCTCCTTGATGTGGCT	Positive	PCR	1C	124-144

carriers if it is possible to isolate FMD virus from oesophageal/pharyngeal scrapings for longer than 28 days after the initial infection. More detailed studies of carrier FMD viruses of Saudi Arabian origin are in progress (E. L. Woodbury and A. R. Samuel, unpublished observations). The sequence of approximately 170 nucleotides at the 3′ end of the gene coding for capsid protein 1D (VP1) of 68 FMD type O viruses was determined and their relationships compared with each other and with 18 previously published sequences.

MATERIALS AND METHODS

Viruses

The origins of the 84 FMD virus isolates studied are listed in Table 1. They were isolated on primary bovine thyroid (BTy) cells and adapted to grow in either IB-RS-2 or BHK-21 cells for the sequencing studies. The nucleotide sequences of the following viruses have previously been determined: O_1/BFS 1860/UK/67 [3], O_1/Manisa/Turkey/69, O_1/Sharquia/Egypt/72, O/ISR/2/85, O/NYE/10/87, O/SYR/1/87, O/ISR/1/88, O/SAU/8/88, O/SAU/33/88, O/TUR/8/88, O/SAU/3/89, O/SAU/26/90, O/SAU/35/90, O/BAR/2/91, O/OMN/58/91, O/SAU/3/91, O/SAU/7/91 and O/TUR/13/91 [4].

Virus RNA preparation

Virus RNA was prepared from FMD virus infected IB-RS-2 or BHK-21 cells grown in 175 cm^2 flasks. The harvest was clarified and then partly purified by pelleting through a 30% (w/v) sucrose cushion by high speed ultracentrifugation [5]. RNA was extracted directly from the pellet using the procedure previously described for polioviruses [6].

Primers

Oligonucleotide primers were synthesized on an Applied Biosystems (Foster City, CA, USA) 381 A machine and used either directly or after purification in a 20% polyacrylamide/8 M urea gel. The location and sequences of the three primers used are shown in Table 2.

Direct RNA sequencing

The sequence of approximately 170 nucleotides at the 3′ end of the capsid protein 1D (VP1) gene was determined for 56 of the 84 isolates by direct RNA sequencing using the dideoxy chain-termination method [7, 8] with some modifications [9]. It was not always possible to determine the identity of all nucleotides due to either the occurrence of a mixed population of RNA species or to strong secondary structures in the RNA template.

Reverse transcription and polymerase chain reaction amplification

For 28 of the 84 isolates studied the vRNA, extracted as described above, was used for reverse transcription (RT) and the resultant cDNA as the target for polymerase chain reaction (PCR) amplification.

The RT reaction was carried out as follows: 2·5 μg of the extracted RNA and 3·5 μl (70 pmol) of primer pNK61 were added to a 0·5 ml Eppendorf tube and heated to 95 °C for 2 min and then incubated at 72 °C for 10 min to pre-anneal the primer. The tube was allowed to cool to room temperature over 15 min and 2·5 μl of 10 mM dNTP's, 50 units (2·5 μl) AMV reverse transcriptase (Northumbria Biologicals, UK), 60 units (6 μl) RNase inhibitor (Boehringer Mannheim, UK Ltd), 4 μl of 10 × RT buffer (Northumbria Biologicals, UK) and diethyl pyrocarbonate (DEPC)-treated water were added to give a final volume of 40 μl. The mixture was incubated in a 42 °C water bath for 1 h.

A 1301 bp PCR product was amplified using primers pNK61 and pARS4. Each 50 μl reaction mixture consisted of 2·5 μl 10 mM dNTP's, 5 μl of 5 × Taq buffer, 20 pmol pNK61, 20 pmol pARS4, 1 μl cDNA and 3·5 units (0·7 μl) Taq DNA polymerase (Boehringer Mannheim UK Ltd) and the volume

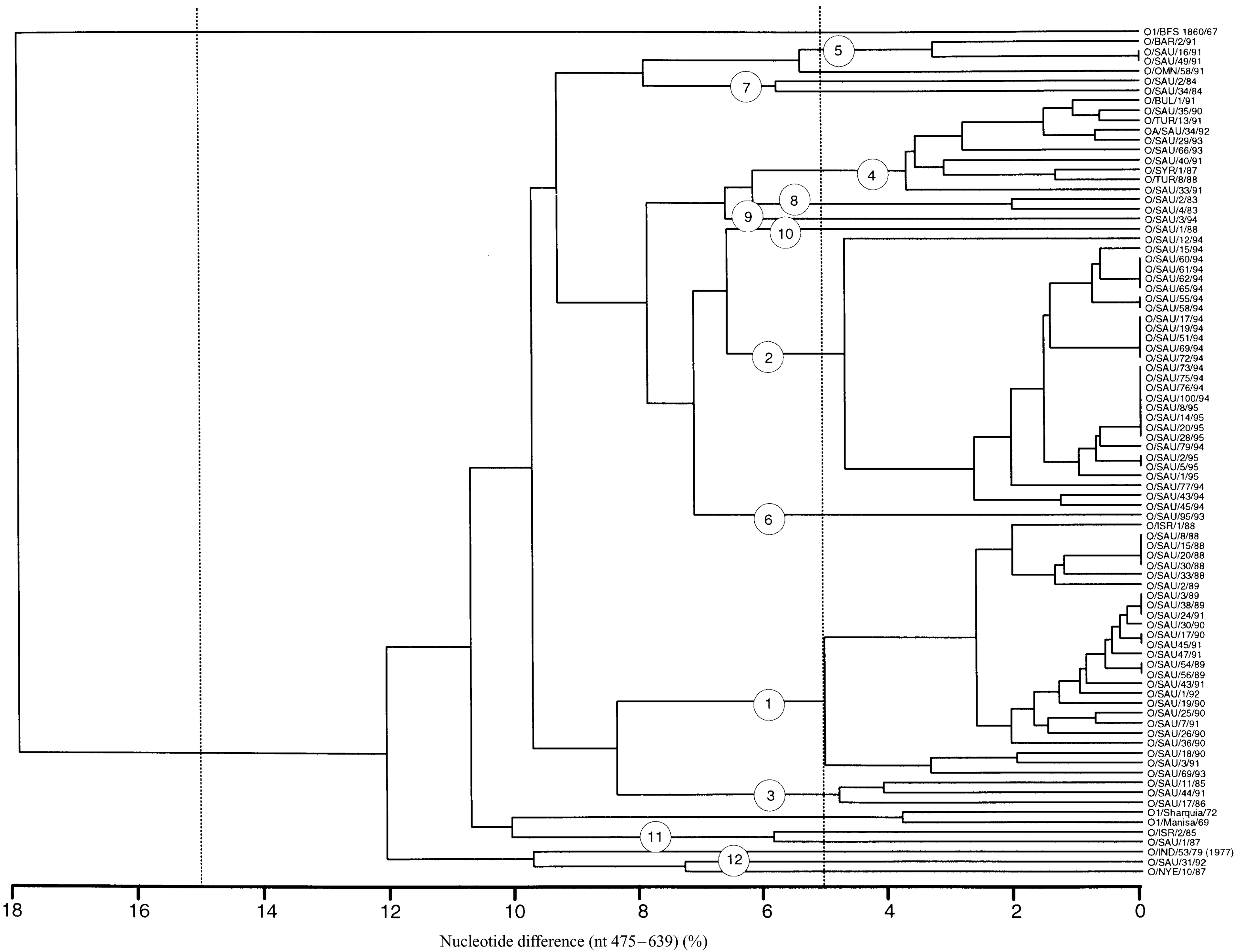

Fig. 1. Dendrogram showing the genetic relationships between foot-and-mouth disease type O viruses isolated in Saudi Arabia and selected reference virus strains.

made up to 50 μl with DEPC-treated water. The mixture was overlaid with 20 μl mineral oil and the tubes transferred to a thermal cycling block where the following thermal profile was applied to the samples: 94 °C for 1 min, 60 °C for 45 s, 72 °C for 3·5 min for 30 cycles then a 10 min soak cycle at 72 °C. After completion 5 μl samples of the PCR products were analysed on a 1% agarose gel run at 100 V for 30 min and stained with ethidium bromide (1 μg/ml). The PCR products were visualized by illumination with UV light.

Cycle sequencing

Approximately 80 fmol of the PCR product was used as a target for cycle sequencing using a f-mol® sequencing kit (Promega, UK). Oligonucleotide primer pNK10 (20 pmol) was end-labelled with [^{32}P]γ-ATP as described in the kit protocol. Sequencing reactions were also prepared according to the kit protocol, transferred to a thermal cycling block (OmniGene, Hybaid, UK) and subjected to the following thermal profile: 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1·5 min for 30 cycles. After addition of 3 μl of stopping solution the samples were heated at 70 °C for 2 min and 3 μl samples loaded onto a 6% polyacrylamide gel containing 7·5 M urea and electrophoresis was performed at 70 watts for 2 h. A further 3 μl of the sample was electrophoresed for 3 h.

Computer analysis

Nucleotide sequences were analysed on an IBM compatible personal computer using programs written by one of the authors (N.J.K.). All pairwise comparisons were performed by giving each base substitution equal statistical weight (ambiguities were ignored). A phylogenetic tree was constructed using the UPGMA method as implemented in the computer program NEIGHBOR and dendrograms plotted using the program DRAWGRAM both from the PHYLIP 3·5c phylogeny package [10]. The UPGMA method constructs a tree by successive (agglomerative) clustering using an average-linkage method of clustering.

RESULTS AND DISCUSSION

Viruses that have a difference of 5% or less in nucleotide sequence are considered closely related [6, 11]. Based on this criterion the results presented show that 12 distinct genetic sublineages (groups 1–12) can be identified in Saudi Arabia over the 13-year period studied (Fig. 1).

Group 1 contained the largest number of virus isolates, and consists of viruses isolated between 1988 and 1993. They are all related, by approximately 96·5% nucleotide identity, to a virus first seen in Israel in 1988 (O/ISR/1/88). The first outbreak caused by this genetic sublineage identified in Saudi Arabia was in the Al-Kharj region in September 1988 (O/SAU/8/88). The persistence of genetic group 1 in Saudi Arabia was possibly due to either carrier animals, which are known to occur on some of the dairy farms following outbreaks of disease, or was introduced from infected nomadic animals which are thought to be another source of outbreaks. The group 1 sublineage has been isolated from both nomadic and dairy animals and so possibly the original infection was introduced by nomadic animals and spread to the dairy farms where carrier animals subsequently became established (O/SAU/16/91 and O/SAU/7/91 were isolated from carrier animals). Viruses related to those in group 1 have caused extensive outbreaks of FMD in North Africa particularly in sheep and have caused a high incidence of mortality in lambs (A. R. Samuel, N. J. Knowles and D. K. J. Mackay, unpublished observations). There is no evidence of this genetic lineage in isolates studied before 1988 and so it appears that this group was a new introduction into the Kingdom during 1988. This corresponds temporally with other research which observed antigenic differences in viruses studied from the Middle East around that time [12].

Group 2 is the second largest group and consists of isolates only occurring in Saudi Arabia during 1994 and 1995. These isolates are closely related (< 5% nucleotide difference) to isolates from Bhutan, Nepal and India (A. R. Samuel and N. J. Knowles, unpublished observations) and this may be the consequence of the importation of sheep and goats into the Middle East from Asia during 1994. Group 2 viruses were responsible for all the outbreaks studied during 1994–5.

Group 3 is composed of three Saudi Arabian isolates: O/SAU/11/85, O/SAU/17/86 and O/SAU/44/91. The latter isolate may be a descendant of the two previous isolates and was possibly reintroduced from an outside source or persisted undetected in Saudi Arabia.

Group 4 includes strains that have been isolated in Saudi Arabia since 1990 and isolates from Syria in 1987, Turkey in 1988 and 1991 and Bulgaria in 1991

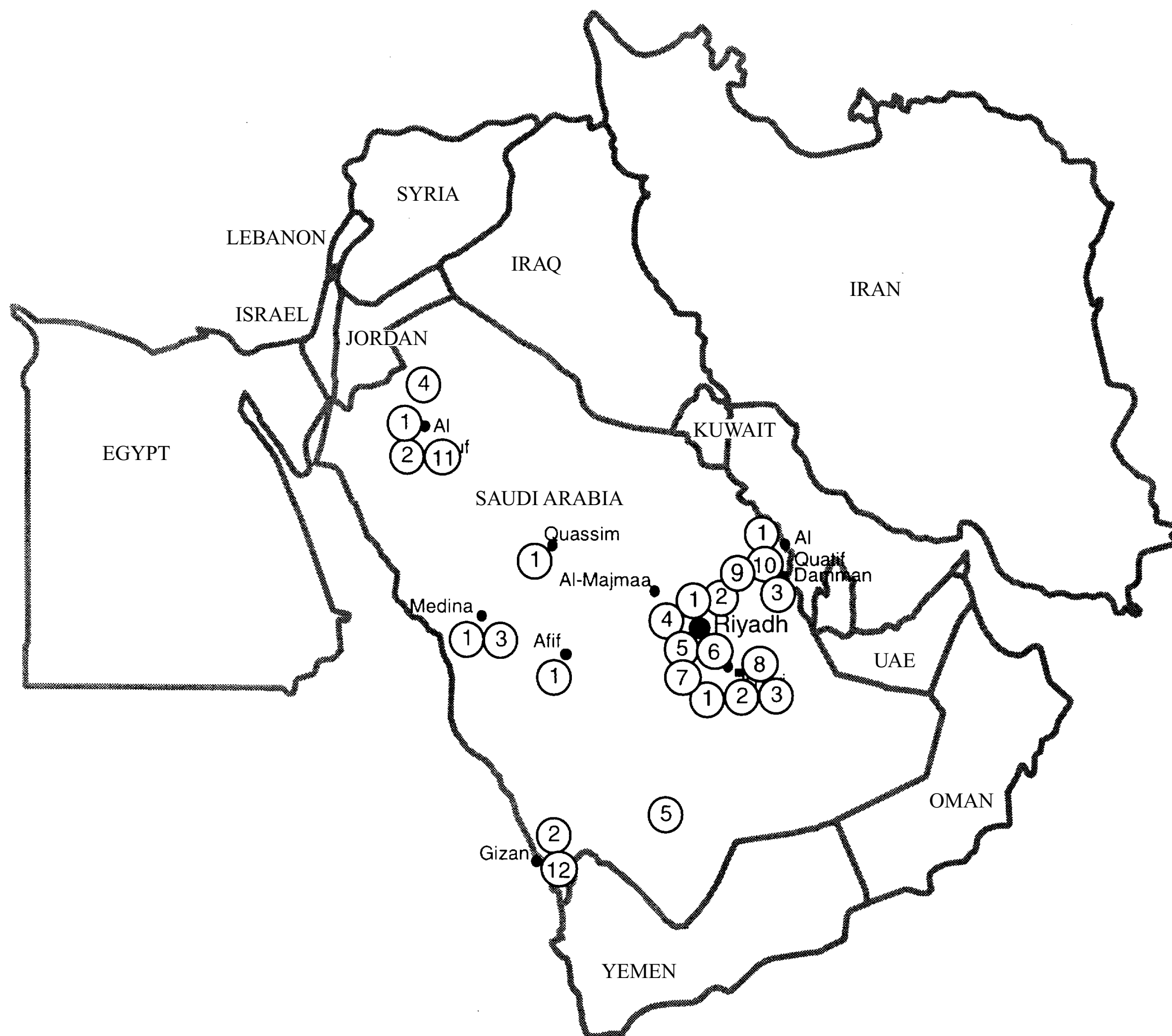

Fig. 2. Geographical location of the 12 genetic virus groups present in Saudi Arabia between 1983 and 1995.

that are closely related to these Saudi Arabian viruses. This group represents a genetic lineage present throughout the Middle East in the late eighties and early nineties [4] (A. R. Samuel and N. J. Knowles, unpublished observations). There is a close relationship (< 5% nucleotide difference) between some members of this group and isolates from other Gulf States (Bahrain and the United Arab Emirates) in 1992 and Indian isolates from 1988 and 1989. The isolate O/SAU/3/94, which is the sole member of group 9, is probably a virus which has evolved, either directly or indirectly, from this group.

The following genetic sublineages have occurred less frequently amongst the isolates that we have studied.

Group 5 (O/SAU/16/91 and O/SAU/49/91) is distinct from other Saudi Arabian groups and probably represents another strain introduced from outside the Kingdom. This group is most closely related to viruses from Bahrain (O/BAR/2/91) and Oman (O/OMN/58/91) although isolates belonging to this group have subsequently been found in Israel and Italy (A. R. Samuel and N. J. Knowles, unpublished observations).

Group 6 consists of the isolate O/SAU/95/93 and is genetically distinct. It was a sample which was submitted at the same time as samples of serotype A and this tends to suggest that this O strain was introduced into Saudi Arabia from another geographical area at the same time as the type A virus.

Groups 7 (O/SAU/2/84 and O/SAU/34/84) and 8 (O/SAU/2/83 and O/SAU/4/83) consisted of isolates from the early 1980s that are distinct both from each other and from subsequently isolated viruses.

Group 10 is represented by a single virus isolate, O/SAU/1/88, which is from the east of the Kingdom (Fig. 2) and is distinct from other Saudi Arabian groups.

Group 11 consists of O/SAU/1/87, which is most closely related to O/ISR/2/85, which has been used as a vaccine strain in parts of the Middle East, and is probably representative of a strain circulating in the Middle East at that time.

Group 12 is represented by a single isolate, O/SAU/31/92, collected from an outbreak which occurred in Gizan in the south west of the Kingdom (Fig. 2) that is not closely related to other genetic sublineages found in Saudi Arabia; the most closely related is a virus (6·5% nucleotide difference) from North Yemen in 1987 (O/NYE/10/87). This may be indicative of a link between Yemen and Saudi Arabia which would not be surprising considering the proximity of Gizan to the Yemen border. The current disease status of Yemen is uncertain but samples of FMDV type O isolated and sequenced by the World Reference Laboratory for FMD (WRL-FMD) in 1991 were not closely related to Saudi Arabian isolates (A. R. Samuel and N. J. Knowles, unpublished observations).

The results of this study show that over the 13-year period in which viruses from Saudi Arabia have been studied 12 apparently distinct genetic sublineages are apparent. None of the isolates examined was identical or very closely related to vaccine virus strains that have been used in the Middle East, namely O_1/Sharquia/EGY/72, O_1/Manisa/TUR/69, O/ISR/2/85 and O_1/BFS 1860/UK/67, which would tend to exclude the possibility that improperly inactivated vaccines have been responsible for any of the outbreaks.

Despite the occurrence of distinct genetic groups all the Saudi Arabian FMD type O viruses examined could be considered as belonging to a single genotype if the same criteria that other workers have used with poliovirus genetic relationships are applied, i.e. viruses related by more than 85% nucleotide identity belong to the same genotype [6]. Antigenically, isolates from the Middle East have remained closely related (R. P. Kitching, unpublished observations) although some minor antigenic variation has been measurable using monoclonal antibodies [12].

An interesting feature of the outbreaks of FMD type O in Saudi Arabia is that it was possible to isolate FMD virus of serotype Asia 1 from some of the samples collected. In one instance serotypes O, A and Asia 1 were recovered from a single sample (SAU/42/91) from Gizen in the south-west of the Kingdom [13]. This phenomenon was not restricted to any of the serotype O genetic groups.

Although isolates of FMDV are composed of a mixture of different RNA species, so-called 'quasi-species' [14], we believe that the genetic sublineages found in Saudi Arabia are distinct, and are not due to the selection of different components of a single Saudi Arabian genetic sublineage. This is supported by other studies of virus isolates from outbreaks of FMD type O that occurred in North Africa during 1989–91 (A. R. Samuel, N. J. Knowles and D. K. J. Mackay, (unpublished observations). The viruses isolated were related to the Saudi Arabian genetic group 1 and, throughout the 3-year course of an extensive epidemic which spread from east to west across North Africa, the viruses recovered and sequenced belonged to a single genetic sublineage and did not exhibit the genetic diversity seen in Saudi Arabian isolates. The rate of fixation of mutations for FMDV types A and C has been measured at 10^{-2} to 10^{-3} substitutions/nucleotide/year [16–18] (N. J. Knowles and A. R. Samuel, unpublished observations) and is consistent with the data presented here.

Another feature this study highlights is that the main genetic groups do not remain confined to particular regions of the Kingdom (Fig. 2), but occur in many different areas, which is not unexpected considering the extensive movement of the animals belonging to the nomadic people across the country. It can also be seen that group 2 is the only genetic lineage apparent during 1994 and 1995. It appears that, after its introduction into Saudi Arabia, it quickly became established as the dominant strain.

Although samples representing various farms from different governates in the Kingdom have been examined in this study, there have been outbreaks which have either not been reported to the relevant authorities or, if reported, samples have not been collected and submitted for laboratory analysis [15]. It is therefore not possible to be certain that all genetic groups of serotype O that may be circulating in Saudi Arabia have been identified. However, whilst acknowledging this non-random selection, this is the first genetic analysis of type O FMD viruses from outbreaks within a geographically restricted FMD-enzootic region.

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