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Genetic relationships between foot-and-mouth disease type Asia 1 viruses

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SUMMARY

The sequence of 165 nucleotides at the 3′ end of the 1D (VP1) gene of foot-and-mouth disease (FMD) virus was determined for 44 type Asia 1 strains isolated from throughout Asia between 1954–92. Analysis of the relationships between the virus genomes showed epidemiological links not previously evident. The possible origin of the only outbreak of FMD Asia 1 to have occurred in Europe, in Greece in 1984, was identified because the nucleotide sequence of this virus was closely-related to the sequences of those present in the Middle East between 1983–5.

Variation in the region sequenced was not as great as that seen in the other FMDV serotypes and all viruses shared greater than 85% nucleotide identity. Thus all the virus isolates examined were considered to belong to a single genotype.

A database of Asia 1 virus sequences has been established which will facilitate the rapid analysis of new outbreaks strains.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious, economically devastating disease of cloven-hoofed animals affecting up to 70 domesticated and wild species [1]. The causative virus is a member of the genus aphthovirus of the family *Picornaviridae*. The FMDV virion has a positive-sense single-stranded RNA genome of approximately 8400 nucleotides. The icosahedral capsid consists of 60 copies of each of four structural polypeptides 1A (VP4), 1B (VP2), 1C (VP3) and 1D (VP1). Foot-and-mouth disease virus is divided into seven immunologically distinct types; O, A and C which occur in Europe, South America, Africa and Asia; SAT 1, SAT 2 and SAT 3 which are generally restricted to sub-Saharan Africa; and Asia 1 which only occurs in Asia.

Foot-and-mouth disease type Asia 1 was first identified in 1954 from samples submitted to Pirbright from Pakistan [2]. Retrospective testing of some atypical isolates from Izatnagar, India from 1951–2 were also found to belong to the Asia 1 serotype and are consequently the earliest documented Asia 1 virus isolates [3]. Asia 1 was also identified in Thailand from a 1954 sample [4]. The earliest record of Asia 1 in the Middle East is in 1957, where outbreaks were observed in Israel

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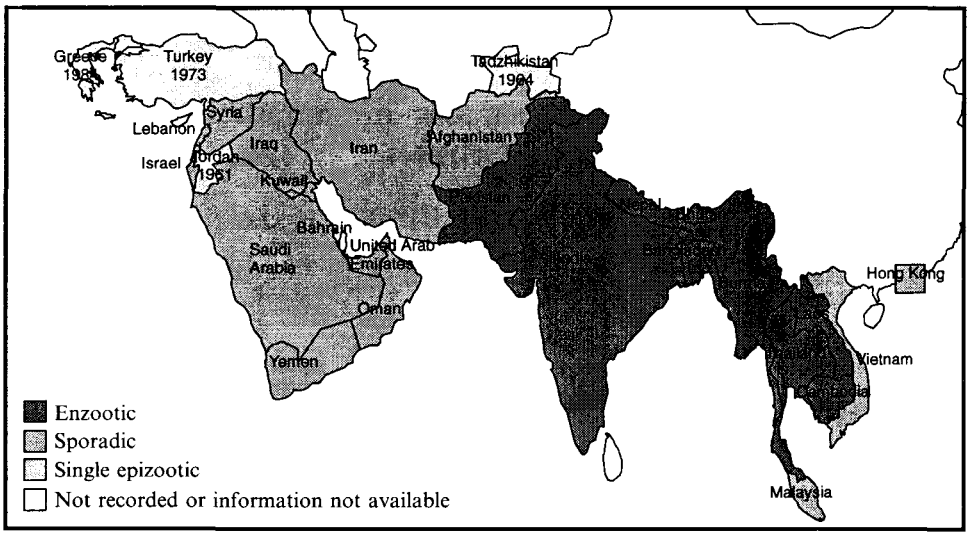

Fig. 1. Distribution of foot-and-mouth disease type Asia 1.

[5] and Iran [6]. FMD is enzootic in south-east Asia and in the Indian sub-continent, however, as far as is known only sporadic outbreaks occur in the Middle East and on the periphery of south-east Asia (Fig. 1). In the early 1970s FMD type Asia 1 appeared to spread from Pakistan through Afghanistan and Iran to Turkey and Iraq [7]. In 1973, the threat of Asia 1 to Europe led to the inclusion of vaccine against Asia 1 in the buffer zone in Thrace [8]. The furthest east Asia 1 has been recorded is in Hong Kong where it was first recorded in 1955 (OIE/FAO World Reference Laboratory for FMD records). Outbreaks of Asia 1 have occurred periodically in Hong Kong until 1980. Asia 1 eventually spread to Europe, where a single outbreak occurred in Greece near the Turkish border in 1984 [9].

Few comparative studies of FMDV type Asia 1 strains have been published. Most of these have been serological and concerned with the relationships between strains from discrete geographical regions [10–15, Samuel and Arrowsmith, unpublished data]. Monoclonal antibodies have been produced against an Indian Asia 1 strain, however, only a small number of isolates have been characterized [16].

Nucleotide sequencing was first used in the study of the epidemiology of FMD by Beck and Strohmaier [17] who investigated the origin of outbreaks of types O and A in Europe over a 20 year period. Since that time a number of other studies have attempted to use this technique for similar purposes; serotypes O [18–25], A [23, 26–29], C [30–34] and SAT 2 [35] have been studied.

Only two papers have been published reporting the nucleotide sequences of the 1D gene of FMDV Asia 1. One reported the sequence of a Russian lapinized vaccine strain originally isolated from Tadzhikistan (Tajikistan) SSR in 1964 [36] and the other the sequence of a vaccine strain (designated India/63/72) from the Indian Veterinary Research Institute (IVRI), Hebbal, Bangalore, originally isolated from Pune, Maharashtra in 1972 [37]. The latter sequence has not been included in this study since we could find no relationship with any other FMDV sequence.

This paper presents the results of a comparative study of FMD type Asia 1 virus isolates submitted to the WRL-Pirbright between 1954–92. Included are isolates from throughout Asia and from a single outbreak in Greece. The sequence of 165 nucleotides at the 3′ end of the 1D (VP1) gene of 44 virus isolates are compared.

MATERIALS AND METHODS

Viruses

The designation and origin of the FMD Asia 1 virus isolates used in this study are listed in Table 1. They were isolated in primary bovine thyroid (BTy) cells and adapted to either IB-RS-2 or BHK-21 cells for the sequencing studies.

Preparations of Virus RNA

Viruses were passaged once at a low multiplicity of infection (MOI) on either IB-RS-2 or BHK-21 cells grown in 175 cm^2 flasks. Following low speed centrifugation (2000 g for 10 min) the supernatant was mixed with 50% glycerol and stored at −20 °C. A further low MOI passage was performed which was then used to infect a confluent monolayer of the relevant cells at a high MOI. After complete CPE was observed cellular debris was removed by low speed centrifugation (2000 g for 10 min) and the virus pelleted from the supernatant by ultracentrifugation (140000 g for 3·5 h at 4 °C) through a 30% (w/v) sucrose cushion. Viral RNA was extracted directly from the pellet using phenol/chloroform in a procedure similar to that used for poliovirus [38].

Oligonucleotide primers

A universal FMDV oligonucleotide primer was synthesised on an Applied Biosystems (Foster City Ca.. USA) 381A machine and used following purification on a 20% polyacrylamide/8N urea gel. The sequence of the primer was 5′-GAAGGGCCCAGGGTTGGACTC and is complementary to codons 12–16 of the 2A gene and codons 1–2 of the 2B gene.

Nucleotide sequencing

Nucleotide sequencing was performed using the dideoxy-sequencing procedure for RNA templates [39, 40] with minor modifications [41].

Computer analysis

Nucleotide and amino acid sequences were analysed on an IBM compatible personal computer using programs written by one of the authors (NJK). All pairwise comparisons were performed by giving each base substitution equal statistical weight (ambiguities were ignored). A binary tree was constructed according to sequence relatedness across the interval of nucleotides 469 to 633 of the 1D gene using the Fitch-Margoliash and Least Squares methods as implemented in the computer program KITSCH (version 3·4) and a dendrogram plotted using the program PLOTGRAM (version 1·4) both from the PHYLIP phylogeny package [42].

Table 1. *Foot-and-mouth disease virus type Asia 1 isolates examined*

WRL ref. no.*	Geographical origin	Date collected‡	Animal
BAN/13/78	Laxipur, Bangladesh	30/11/78	Bovine
BAN/1/79	Pabna, Bangladesh	15/01/79	Bovine
BAN/30/79	Pabna, Bangladesh	11/06/79	Bovine
BAN/57/80	Tangail, Bangladesh	08/10/80	Bovine
BAN/1/87	Chittagong, Bangladesh	13/10/86	Bovine
BAR/1/85	Bahrain	25/04/85	Bovine
BHU/1/86	Bhutan	12/02/86	Bovine
BUR/12/77	Taungdwingyi, Burma	00/00/77	Bovine
BUR/4/78	Ye Kyi, Burma	00/00/78	Bovine
BUR/1/88	Kawhonu Township, Burma	06/06/88	Bovine
MYA/2/91	Monywa, Myanmar (Burma)	31/01/91	Bovine
CAM/9/80†	Tuk, Siem Reap, Cambodia	27/11/80	Bovine
CAM/1/88	Tuk, Siem Reap, Cambodia	08/09/88	Bovine
CAM/2/91	Kampong, Srok Sonrantang, Cambodia	21/06/91	Bovine
GRE/1/84	Evros, Thrace, Greece	20/06/84	Bovine
HKN/24/75	Hong Kong	12/02/75	Bovine
HKN/18/76	Lantau, Hong Kong	10/02/76	Bovine
HKN/20/80	Kowloon, Hong Kong	13/01/80	Bovine
IND/1/72	Jhansi, Uttar Pradesh, India	04/12/71	Bovine
IND/12/76	Madras, Tamil Nadu, India	00/00/76	Bovine
IND/8/79†	Ahmedabad, Gujarat, India	00/00/00	Not known
IND/5/89	Sambalpur, Orissa, India	18/01/88	Not known
IND/9/89	Khammam, Andhra Pradesh, India	07/05/88	Not known
ISR/1/57†	Israel	00/00/57	Not known
ISR/3/63	Yokneam, Israel	00/11/63	Not known
ISR/1/84	Tsefat, Northern Israel	00/06/84	Bovine
ISR/3/89†	Shamir, Northern Gallilee, Israel	18/06/89	Bovine
KUW/2/79	Farwania, Kuwait	21/01/79	Not known
KUW/2/81	Kuwait	00/00/81	Not known
LEB/3/83†	Kafer Kela, South Lebanon	01/11/83	Bovine
LEB/1/84	Addeissa, South Lebanon	00/00/84	Bovine
MAY/2/90	Padang Besar, Perlis, Malaysia	05/12/90	Bovine
NEP/28/90	Udaypur, Nepal	28/11/89	Bovine
OMN/8/80	Salalah, Oman	01/06/80	Bovine
OMN/2/82	Salalah, Oman	31/12/81	Bovine
PAK/1/54†	Okara, Punjab, Pakistan	05/03/54	Buffalo
SAU/2/80	Al Masanah, Riyadh, Saudi Arabia	07/06/80	Bovine
SAU/9/92	Riyadh, Saudi Arabia	11/04/92	Caprine
SAU/10/92	Tebrak, Riyadh, Saudi Arabia	21/04/92	Bovine
Tadzhikistan/64†	Tadzhikistan (Tajikistan) SSR	00/00/64	Not known
TAI/1/90	Nakhon Ratchasima Province, Thailand	30/10/90	Bovine
TUR/15/73†	Bayrampasa, Merkez, Kars, Turkey	12/08/73	Bovine
YEM/15/79†	Radar, Dhammar Highlands, Yemen	16/10/79	Bovine
YEM/2/80	Marbar, North Yemen	19/12/79	Bovine

* OIE/FAO World Reference Laboratory for Foot-and-Mouth Disease reference number.
† Vaccine strain.
‡ 00, date not known.

RESULTS AND DISCUSSION

Nucleotide sequences are shown in Fig. 2 and deduced amino acid sequences are shown in Fig. 3. The sequence order was determined following the phylogenetic analysis which enabled clusters of nucleotide and amino acid substitutions to be

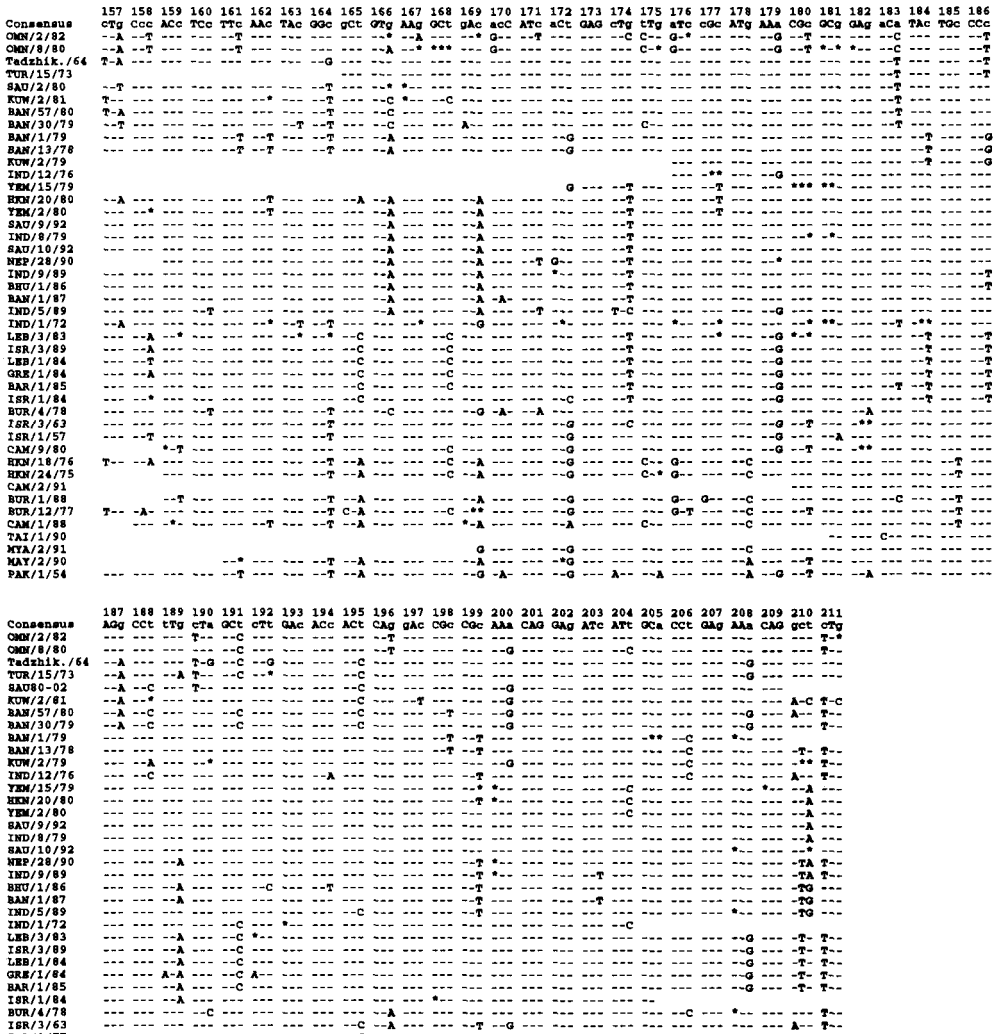

Fig. 2. Nucleotide sequences of the foot-and-mouth disease virus isolates studied. *, ambiguity; space, region not sequenced; —, same as consensus sequence.

easily visualized. Some of the sequences obtained were shorter than the region examined for most isolates, therefore two dendrograms were constructed one with all the sequences (Fig. 4) and one excluding IND/12/76, KUW/2/79, YEM/15/79, TAI/1/90 and CAM/2/91 (data not shown). No significant differences were observed in the branching order.

It has been suggested that epidemiological relationships may be inferred by comparison of the nucleotide sequences of polioviruses [38] and FMD type SAT 2 viruses [35]. These authors used a difference of $< 5\%$ to indicate a close relationship and a difference of $> 15\%$ to distinguish genotypes. The genetic relationships between all the FMDV Asia 1 isolates studied are depicted in a

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                  160        170        180        190        200        210
Consensus    LpTSFNYGaVKAdtItElLirmKRAEtYCPRPlLAlDTTqdRRKQEIIAPEKQal
OMN/2/82     ------------*A-----V-------------------H--------------*
OMN/8/80     ----------**-A-----V----**-------------H---------------
Tadzhik./64  ----------------------------------------------------------
TUR/15/73          ----------------------------------------------------
SAU/2/80     ----------*-----------------------------------------------
KUW/2/81     -----*----*-----------------------------------------------TF
BAN/57/80    ----------------------------------------------------------T-
BAN/30/79    ------------N---------------------------------------------
BAN/1/79     ----------------------------------------------------*--*-
BAN/13/78    ----------------------------------------------------------V-
KUW/2/79                        ------------------------------------*-
IND/12/76                       -*----------------------------------T-
YEM/15/79                   *--------**-------------------*--------*--
HKN/20/80    ------------E-------------------------------*-----------
YEM/2/80     ------------E----------------------------------------------
SAU/9/92     ------------E----------------------------------------------
IND/8/79     ------------E-----------*----------------------------------
SAU/10/92    ------------E----------------------------------------*---
NEP/28/90      ----------E--A-------*-------------------*---------V-
IND/9/89     ------------E--*-------------------------*---------V-
BHU/1/86     ------------E----------------------------------------V-
BAN/1/87     ------------EN---------------------------------------V-
IND/5/89     ------------E----F-----------------------------------*-V-
IND/1/72     -----*----*-E--*---*----*--*---------*-----------
LEB/3/83     ------*-----------------*----------*----------------V-
ISR/3/89     ----------------------------------------------------V-
LEB/1/84     ----------------------------------------------------V-
GRE/1/84     ---------------------------------I--I----------------V-
BAR/1/85     ----------------------------------------------------V-
ISR/1/84     ---------------------------------------*-------
BUR/4/78     ------------EN--------------------------------------*---
ISR/3/63     ----------------------*-----------------------------T-
ISR/1/57     ----------------------------------------------------
CAM/9/80       *---------------------*-------------------------T-
HKN/18/76    ------------E------V-I-----------------------------*-S-
HKN/24/75      ----------E------V-I-------------------------------T-
CAM/2/91                          ------------------------------T-
BUR/1/88       ----------E------VGI-------------------------------
BUR/12/77    -H------P---*------V-I-------------------------------T-
CAM/1/88      -*---------*--------I-------------F-----------------T-
TAI/1/90                        *-P-------------N-------*-----*-
MYA/2/91               *--------I-------------------------------T-
MAY/2/90         *-------E--*-----I-------------------------*
PAK/1/54     ------------EN---M---I-------------------------------L-
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Fig. 3. Deduced amino acid sequences of the foot-and-mouth disease virus isolates studied. *, ambiguity; space, region not sequenced; —, same as consensus sequence.

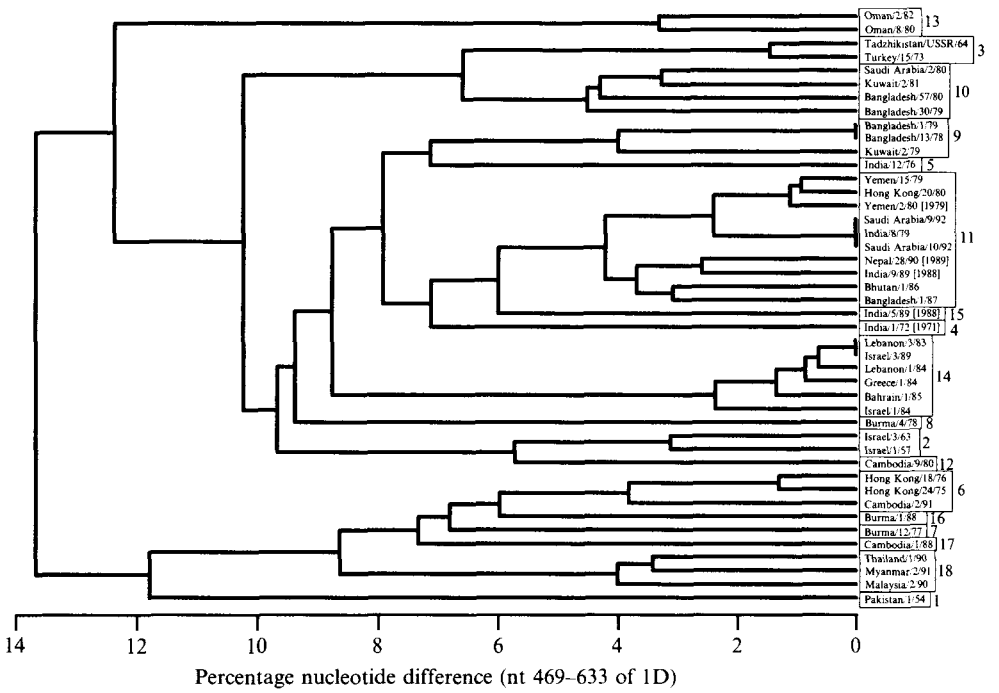

Fig. 4. Dendrogram depicting the genetic relationship between foot-and-mouth disease type Asia 1 viruses.

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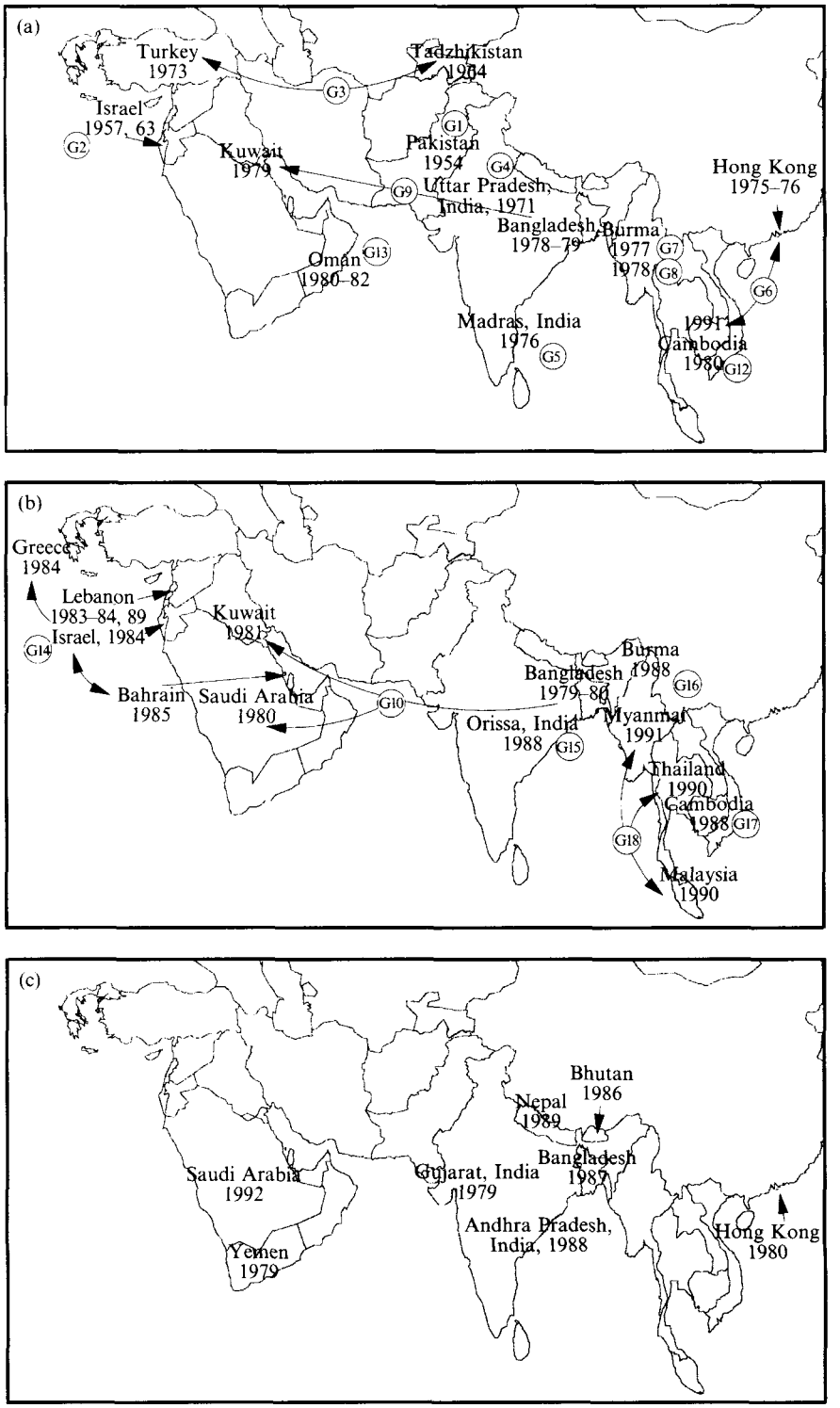

Fig. 5. Geographical distribution of genetic groups of foot-and-mouth disease type Asia 1 virus studied. (a) Genetic groups 1–9, 12 and 13; (b) genetic groups 10, 14–18; (c) genetic group 11.

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dendrogram (Fig. 4). Based on the above criteria it can be seen that there are several distinct groups of viruses (difference > 5%), which have been arbitrarily numbered for convenience. Their geographic distribution is shown in Fig. 5.

Group 1 (PAK/1/54)

PAK/1/54 has been widely used as a vaccine strain, however, none of the isolates examined in this study was closely related to it.

Group 2 (ISR/1/57 & ISR/3/63)

A live attenuated vaccine prepared from chick embryo adapted ISR/1/57 [5] was used to control outbreaks of FMD type Asia 1 in Israel during the latter part of 1959 and 1960 [43]. These outbreaks were thought to be due to viruses coming from either Lebanon or Jordan, although Asia 1 was probably present in Israel as early as March 1958 [44]. The close relationship of ISR/3/63 to ISR/1/57 could be due either to reversion of the attenuated vaccine or to the viruses remaining unchanged in the field. Antigenically these two viruses have been shown to be distinguishable both from each other and from PAK/1/54. These virus strains constitute the three designated Asia 1 subtypes [10].

Group 3 (Tadzhikistan/64 & TUR/15/73)

The outbreak of FMD from which TUR/15/73 was derived was thought to have originated from the spread of a virus strain across Afghanistan and Iran during 1972–73 [7]. However, TUR/15/73 is almost indistinguishable from a Russian vaccine virus strain originally isolated in Tadzhikistan in 1964. Further analysis is in progress to determine the relationships between other viruses occurring in this area both in 1964 and during 1971–3.

Group 4 (IND/1/72 [1971]), group 5 (IND/12/76) and group 15 (IND/5/89 [1988])

Distinct virus isolates from India are not only represented in groups 4, 5 and 15 but also in group 11. This demonstrates the wide diversity of viruses from this country as suggested in previously reported antigenic studies [13].

Group 6 (HKN/24/75, HKN/18/76, CAM/2/91)

Asia 1 reappeared in Hong Kong in 1974 after an absence of 10 years. The two isolates examined were closely related, however, they were very different from a virus which was isolated in 1980 (group 11). A recent isolate from Cambodia appeared to be related to this group, whereas recent isolates from Thailand, Burma and Malaysia were not closely related (group 18).

Group 7 (BUR/12/77), group 8 (BUR/4/78) and group 16 (BUR/1/88)

As found with virus isolates from India, Burma also has a very diverse population of viruses being represented additionally in group 18.

Group 9 (BAN/13/78, BAN/1/79, KUW/2/79) and group 10 (BAN/30/79, BAN/57/80, SAU/2/80, KUW/2/81)

The Asia 1 serotype appeared for the first time in Kuwait in 1978 [45] and in Saudi Arabia in 1980 [46]. Ata [45] suggested the possible origin of the Asia 1 outbreak in Kuwait was buffalo imported from India, however, the grouping of

isolates from Bangladesh and Kuwait suggests a possible link with the trade between these countries. The finding of two different groups within this 3-year period emphasises the diversity of viruses in the Far East.

Group 11 (*IND/8/79, YEM/15/79, YEM/2/80* [*1979*], *HKN/20/80, BHU/1/86, BAN/1/87, IND/9/89* [*1988*], *NEP/28/90* [*1989*], *SAU/9/92 & SAU/10/92*)

Viruses in this group appear to be fairly widely distributed occurring throughout Asia except for south-east Asia.

Group 12 (*CAM*/9/80) *and group 17* (*CAM*/1/88)

These two groups show the wide diversity of virus strains in this country additionally reported in group 6.

Group 13 (*OMN/8/80, OMN/2/82*)

An isolated group, closely related to each other, but distinct from the other groups (> 12% nucleotide difference).

Group 14 (*LEB/3/83, LEB/1/84, ISR/1/84, GRE/1/84, BAR/1/85, ISR/3/89*)

Viruses belonging to this group all differ from each other by less than 2% and are therefore very closely related. This is not surprising since most of the viruses were isolated from the Middle East during 1983–5. However, the reason for the reappearance of this virus in Israel in 1989 is unknown. It appears that the outbreak in Greece in 1984 originated from virus circulating at that time in the Middle East and not from the vaccine (Iran/73 strain) which was being used in the Thrace buffer zone. LEB/3/83 has been used as a vaccine strain.

Group 18 (*TAI/1/90, MAY/2/90, MYA/2/91*)

Malaysia is normally free from FMD and the 1990 isolate, MAY/2/90, is closely related to recent isolates from both Thailand and Myanmar (Burma), but not to a 1991 isolate from Cambodia (see group 6).

If approximately 10% or less similarity is used to cluster viruses then groups 6, 7, 16, 17 and 18 form a super-group consisting of viruses isolated only from Far Eastern countries (Cambodia, Hong Kong, Malaysia, Myanmar (Burma) and Thailand). All the other groups, except for groups 1 (Pakistan) and 13 (Oman), cluster to form another super-group which includes viruses isolated in the Far East (Burma, Cambodia and Hong Kong), the Indian sub-continent (Bangladesh, Bhutan, India and Nepal), the Middle East (Bahrain, Israel, Kuwait, Lebanon, Saudi Arabia, Turkey and Yemen) and the USSR (Tadzihikistan). Such groupings could be explained by the spread of FMD Asia 1 viruses from the Indian sub-continent to both the east and the west. However, Asia 1 does not appear to spread from south-east Asia in a westerly direction. Future studies which will include more virus isolates from throughout Asia may help to clarify the epidemiological situation.

The high degree of similarity between such a geographically and chronologically diverse group of isolates indicates that they are more conserved genetically than other FMDV serotypes (N. J. Knowles, A. R. Samuel, unpublished data). None of the sequences showed a greater divergence than 14% and if similar criteria to

those used by Rico-Hesse and colleagues [38] for polioviruses and Vosloo and colleagues [35] for FMD type SAT 2 viruses are applied then all type Asia 1 viruses studied so far could be regarded as members of a single genotype.

Although FMD Asia 1 was thought to only occur sporadically in the Middle East, being introduced from enzootic regions, recent studies of FMD type O in Saudi Arabia have revealed the presence of Asia 1 viruses. These viruses have only been found in association with type O FMDV as they could only be isolated as a mixed infection [47].

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