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Variation among strains of type A foot-and-mouth disease virus in the Eastern Mediterranean region 1964–1972

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SUMMARY

Variants of type A FMD virus from the Eastern Mediterranean region over the years 1964–72 have been shown to belong to a group distinct from the Western European strains as represented by A_5 Westerwald. This group appears to derive from the A_{22} strain first recognized in 1964 and indicates the possibility of new strains supplanting old in the field.

INTRODUCTION

Among the strains of foot-and-mouth disease virus which have been particularly important in the last twenty years, A_{22} has a special place. The extent of its difference from earlier A strains, the wide area which was involved in its spread, the changes in antigenic character of the strain as it established itself in new areas and, finally, its emergence as the main endemic strain of type A in much of the area involved are all characteristics which single out A_{22} from other new variant strains which have been encountered.

The earliest date of receipt at Pirbright of a sample of virus of the strain subsequently known as A_{22} was 19 October 1964. Samples then arrived from Mosul and Kirkul in Iraq. During November of the same year further samples from Abu Ghraib, Mosul, Amara and Romah, all in Iraq, were found to be identical with the original. The strain also appeared during the same month in Van Province in Turkey. The number of cases of disease associated with the strain was alarmingly high and the countries in the East rapidly became aware that a new epizootiological situation confronted them. During December samples came from Syria, Lebanon and Jordan and the provinces of Turkey as far west as Istanbul. In Israel the disease appeared early in January. During this period, too, information became available that a strain of type A of unusual character was present in Iran as early as September 1964. Samples received in March 1965 were identified as related to the strain prevalent in the other countries (see Fig. 1).

The precise date of entry of the strain into the USSR is not known by the author but during the spring of 1965 it spread widely in many provinces. The strain also succeeded in passing through the barrier of vaccinated animals at the borders of Greece and Turkey and there was a limited outbreak in Greece in June 1965.

The spread of A_{22} through the countries of the Near East bears a striking resemblance to that of the SAT 1 strain which, coming from Africa, set up the disease in the Persian Gulf in the winter of 1961–2. SAT 1 spread during the ensuing months along lines very similar to those of the movement of A_{22}. In view of

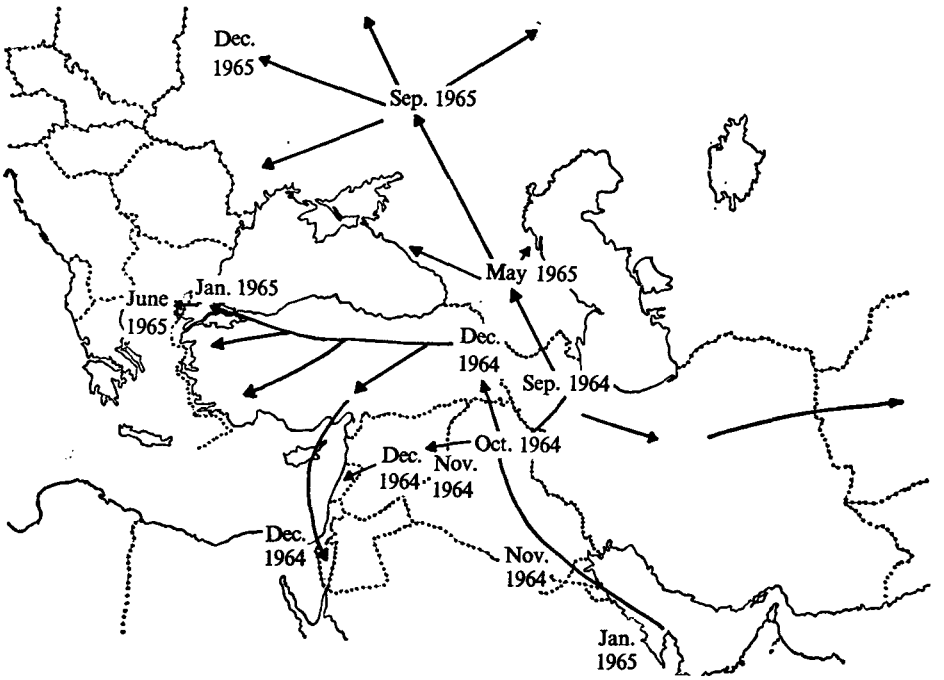

Fig. 1. The progress of A_{22} foot-and-mouth disease during 1964–5. Originating in Iran and Iraq in September and October 1964 it spread towards the west and south through the remainder of 1964 and early 1965, northwards throughout 1965, and later eastwards into USSR.

this and of the antigenic relation which has later been shown between A_{22} and certain A strains from Africa, it must be recorded that samples received for typing from Kuwait had been typed as A as early as February 1964 and through to December 1965. Since type A had earlier been found in this area and there was no immediate prospect of vaccination, no subtyping work was carried out on these samples and this applies also to samples from Bahrain examined in January 1965. It is possible that a type A infection was prevalent in the countries of the Persian Gulf during the earlier months of 1964 and that spread had taken place in the summer and autumn of that year – some months, in fact, before it was detected as an epizootic strain. Unfortunately, it has not proved possible to revive the material from Kuwait and Bahrain and the initial stages of the A_{22} epizootic must therefore remain a matter for conjecture.

At an early stage in the series of outbreaks reported, it became apparent that differences existed between some of the strains which were being examined. These interrelationships are discussed later but in general it could be said that the major A_{22} subtype strains were an A-Iraq which spread into Turkey and the other countries of the Eastern Mediterranean, the A-Iran which was the prevalent strain in that country, and the A-USSR. These strains were different from each other but formed a group showing a very clear difference from the Western European A, in particular A_5.

The epizootic of type A occurring in 1972 involved Turkey, Greece and Lebanon,

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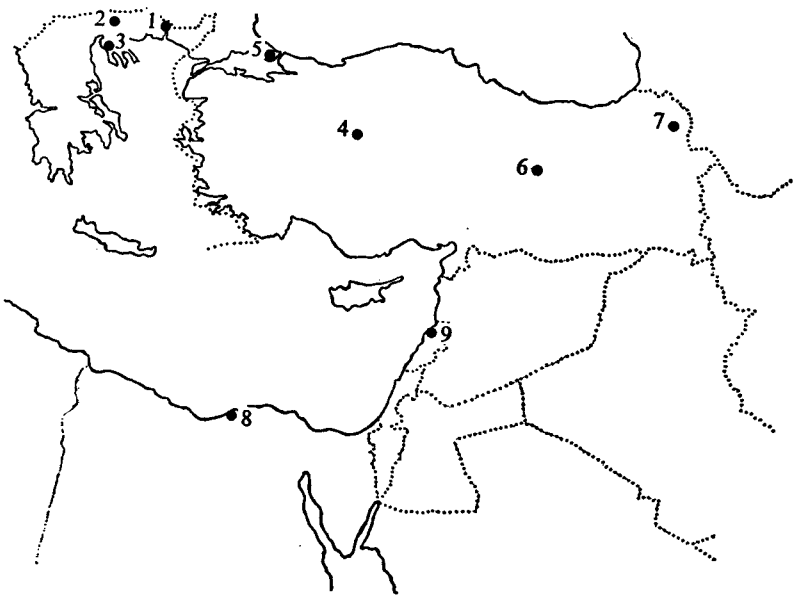

Fig. 2. A type FMD samples tested during the epizootic of summer, 1972.

Sample no.	Location	Country
1	Xanthi province	Greece
2	Serrai province	
3	Thessaloniki province	
4	Ankara province	Turkey
5	Istanbul province	
6	Erzincan province	
7	Kars province	
8	Alexandria	Egypt
9	Bsarma	Lebanon

with an isolated outbreak in Egypt (see Fig. 2). Outbreaks were recorded in Greek Thrace, appearing first in the province of Evros in April, affecting a small number of cattle, and seemed to be an isolated focus. However, the infection was detected a few days later in Xanthi province and then spread to neighbouring Drama and Serrai, with an isolated outbreak due to movement of animals in Attica in Southern Greece. Cattle, sheep and pigs were infected, in small groups of animals in which easy spread of the disease would have been expected; but the number of diseased animals was relatively small compared with the total animal populations in the provinces and in many of the villages affected there was not complete spread.

It is interesting to note that a sample received in 1973 from Kavala province in the north of Greece showed on one-way tests a similarity to those strains received in 1972. This particular province, bordering Serrai, Drama and Xanthi, showed no evidence of infection the previous year.

As A_{22} is endemic in Turkey, it is not possible to know precisely when this new strain emerged to become responsible for the 1972 epizootic. Samples submitted in June 1972 came from areas in which the disease was first observed in the latter half

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of May. These areas ranged from Istanbul in the west to Kars province, on the Russian border, in the east. At the present time it is uncertain whether this strain crossed the border into Russia.

The A type outbreak was also widespread in North Lebanon and an isolated focus was detected in Egypt in local cattle in Alexandria.

Initial unidirectional tests indicated the strains from the various countries to be related to the A_{22} group and the subsequent development of the strain set out in this paper shows the large number of substrains which have developed; but it must be emphasized throughout that the differences within the A_{22} group are always much less than the difference between this group and that of the classical European A strains, for example A_5. As work on these strains has progressed, there have been improvements in the methods and it is believed that the picture which is presented of continuing change of antigenic characteristics is the real one. Cross-vaccination experiments in cattle both at Pirbright and in France have shown the very clear difference between the A_{22} group and the classical European A strains.

MATERIALS AND METHODS

Strains of A type FMD used in investigation

A_5 Westerwald/50:	Received in 1950s from Tubingen, Germany, and used as reference subtype strain for A_5 European subtype group.
A_{22} Iraq 24/64:	Received from Dr Berzanji of Iraq on 12 November 1964 as a sample of epithelium from a cow affected with FMD in Mosul. Assigned subtype number 22 (WRL Sheet 13).
A_{26} Argentine/66:	Received from Dr Palacios of the Pan American Centre, Brazil, in November 1967. The sample was bovine epithelium from an outbreak near Buenos Aires (WRL Sheet 13).
A_{28} (TUR 1/69):	Received from Dr Karagozoglu on 23 January 1969 and isolated from cattle affected with FMD at Polatli in the district of Ankara, Turkey. This strain was assigned subtype number A_{28} (WRL Sheet 13).
USSR 2/66:	Received on 2 February 1966 from the USSR. No further history known.
TUR 1/70:	Received from Dr Boz on 27 August 1970 as first passage material in cattle. The original epithelium was obtained from cattle affected with FMD in Civril, Denizli district, Turkey.
KEN 140–/69:	Received from Nairobi on 23 July 1969 as a sample from an outbreak at Nakuru Ranch, Kenya (WRL Sheet 17).
GRE 1/72:	Received on 18 April 1972 from Dr Cardassis and isolated from material obtained from cattle in the department of Xanthi, Northern Greece. The outbreaks involved cattle, sheep and goats (WRL Sheet 13).

TUR 6/72: Received on 25 May 1972. Sample of epithelium from an outbreak in Macun village, district of Polatli, province of Ankara. Animals affected were cattle, sheep, goats and buffalo (WRL sheet 15).

LEB 2/72: Received from Dr Rizkallah. Sample of original material from Bsarma, North Lebanon.

EGY 1/72: Received from Dr Böhm on 7 June 1972. Sample of epithelium from one local Baladi bovine, one of 51 animals affected at Alexandria. Buffalo and imported cattle from Somalia in the same herd were not affected at that time.

A_{24} Cruzeiro/55: Received in March 1965 from the Pan American FMD Centre, Rio de Janeiro. Sample of original material from cattle involved in an outbreak at Granja São Crispim, Municipio Cruzeiro, State of São Paulo, in May 1955.

Antigens

The antigens for each strain were produced either in IB-RS-2 (clone 60) or in BHK 21 (clone 13) cell lines.

IB-RS-2 (*clone* 60). Cell line of porcine kidney (de Castro, 1964). Monolayer roller cultures were produced in 20-oz. medical bottles, using a growth medium consisting of Hanks' saline, 10% lactalbumin hydrolysate, 10% ox serum and antibiotics (100 i.u./ml. neomycin, 400 i.u./ml. penicillin). The cultures were washed with medium and overlaid with maintenance medium of Earle's saline, amino acids and antibiotics as above, followed by inoculation with seed virus, and incubated at 37° C. overnight. Cultures were harvested between 18 and 24 hr. when 100% CPE is normally showing. The tissue culture fluid was clarified by low-speed centrifugation and used in complement-fixation tests.

BHK 21 (*clone* 13). Monolayer cultures of baby hamster kidney cells were prepared and the cultures used as above.

Antisera

Hyperimmune guinea-pig antisera were used. Guinea-pigs weighing 600–800 g. were inoculated intradermally with vesicular plantar pad material of high titre. Animals not showing secondary lesions were killed and the remainder kept for 28 days. These were given three injections of fresh infected guinea-pig pad and lymph suspension together with saponin intradermally at intervals of 2 days. On the 10th day the animals were bled and the collected sera pooled after individual titration. The pooled sera were tested for type specificity, then filtered through Seitz EK pads, after which they were inactivated at 56° C. for 30 min., and stored in 10 ml. amounts at −20° C.

Complement

The complement used was normal guinea-pig serum. Adult male guinea-pigs were starved for 24 hr., bled from the throat and the pooled serum stored with Richardson's preservative at 4° C. (Richardson, 1941).

Haemolytic indicator system

This was prepared and used as previously described (Darbyshire, Hedger & Arrowsmith, 1972).

Complement-fixation test

A chessboard complement-fixation technique was used in this investigation with 1·5-fold dilutions of sera and antigens and a constant 5 haemolytic unit dose of complement, with controls of 5, 2 and 1 haemolytic units, in disposable plastic micro-plates supplied by Linbro and obtained from Biocult Laboratories Ltd, Glasgow. The sera, antigens and complement were incubated at 37° C. for 60 min. The volumes used and the additions of sensitized sheep red cells were as described elsewhere (Darbyshire *et al.* 1972). The end-point was taken as the reciprocal of the highest dilution of serum giving 50% lysis of red blood cells by the optimal dilution of antigen.

Estimation of antigenic relationships

Bradish, Brooksby & Tsubahara (1960) and Bradish & Brooksby (1960) devised a method for the comparison of minor antigenic differences between strains of foot-and-mouth disease virus. They introduced a quantity called the cross-fixation relationship r, which is now defined as the comparison of the reactions of one serum with two viruses, i.e. with viruses A and B and homologous antisera a and b respectively, the ratio ra for serum a would be:

$$ra = \frac{Ba \text{ (heterologous reaction)}}{Aa \text{ (homologous reaction)}}$$

For antiserum b the relationship rb would be obtained by:

$$rb = \frac{Ab}{Bb}.$$

These values are also written r_1 and r_2.

The cross-fixation relationship was then used to calculate the cross-fixation product (CFP), i.e. $r_a \times r_b$ (or $r_1 \times r_2$).

Davie (1964) classified strains of CFP $> 0{\cdot}5$–$1{\cdot}0$ into the same subtype, while those of CFP $< 0{\cdot}5$ were considered to be different.

This method of distinguishing strains has been slightly altered to the present one of expressing the antigenic relationship R as a percentage (Archetti & Horsfall, 1950; Chu, Andrewes & Gledhill, 1950; Jordan & Gaylin, 1953; Wenner, Kamitsuka & Lenahan, 1956; Ubertini *et al.* 1964). The R value is derived from

$$R = \sqrt{(r_1 r_2)} \times 100.$$

A CFP of 0·5 corresponds to $R = 70\%$ so that, by this method, strains having values of 70–100% would be considered to belong to the same subtype group, while those with $R = < 70\%$ should be regarded as different (Brooksby, 1967).

With the strains from 1969–72 the situation appears complicated since, if there were rigid adherence to the classification outlined above, most of these strains would have to be regarded as new subtypes but inspection of Table 1 shows

Table 1. *r values calculated from the optimum serum titres*

	Virus									
Serum	A_{22} USSR 2/66	A_{22} IRAQ 24/64	A_{22} LEB 2/72	A_{22} TUR 1/70	A_{28} TUR 1/69	A_{22} GRE 1/72	A_{22} KEN 140/69	A_{26} ARG 66	A5 West	EGY 1/72
A_{22} USSR 2/66	*1·0*	0·83	0·65	0·40	0·34	0·45	0·34	0·14	0·11	0·44
A_{22} Iraq 24/64	0·59	*1·0*	0·43	0·60	0·44	0·48	0·39	0·21	0·15	0·48
A_{22} LEB 2/72	0·41	0·59	*1·0*	0·65	0·49	0·39	0·32	0·07	0·07	0·18
A_{22} TUR 1/70	0·44	0·43	0·41	*1·0*	0·54	0·56	0·25	0·10	0·17	0·17
A_{28} TUR 1/69	0·31	0·50	0·37	0·66	*1·0*	0·58	0·55	0·20	0·25	0·33
A_{22} GRE 1/72	0·35	0·31	0·34	0·63	0·58	*1·0*	0·59	0·24	0·24	0·15
A_{22} KEN 140/69	0·12	0·26	0·35	0·54	0·67	0·51	*1·0*	0·18	0·43	0·30
A_{26} ARG 66	0·13	0·18	0·08	0·18	0·19	0·26	0·24	*1·0*	0·26	0·24
A_5 West	0·14	0·14	0·16	0·26	0·27	0·23	0·25	0·28	*1·0*	0·16
EGY 1/72	0·13	0·19	0·10	0·11	0·14	0·18	0·24	0·11	0·12	*1·0*

asymmetry of reaction, i.e. the new viruses react well with the older A_{22} Iraq 24/64 serum but the A_{22} virus does not react so well with the new sera, so that when greater emphasis is placed on the *r* values it is more feasible to class them as strains belonging to the A_{22} subgroup.

RESULTS

The results shown in Tables 1 and 2 were calculated from optimum serum titres. The *r* values are the mean values from several tests for each virus and serum and from these were derived the *R* values listed in Table 2.

A study of Tables 1 and 2 suggests:

(1) EGY 1/72 is greatly divergent from the others, though possibly linked to A_{22} rather than to European strains.

(2) LEB 2/72 is closely linked to the two earlier strains.

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Table 2. *R* % *derived from r values of Table* 1

A_{22} USSR 2/66	100									
A_{22} Iraq 24/64	70	100								
A_{22} LEB 2/72	52	50	100							
A_{22} TUR 1/70	42	51	52	100						
A_{28} TUR 1/69	33	47	43	60	100					
A_{22} GRE 1/72	40	39	43	59	58	100				
A_{22} KEN 140/69	20	32	34	37	61	59	100			
A_{26} ARG 66	14	19	8	13	20	25	27	100		
A_5 WEST	12	15	11	21	26	24	33	27	100	
EGY 1/72	24	30	13	14	22	16	27	16	14	100
	A_{22} USSR 2/66	A_{22} Iraq 24/64	A_{22} LEB 2/72	A_{22} TUR 1/70	A_{28} TUR 1/69	A_{22} GRE 1/72	A_{22} KEN 140/69	A_{26} ARG 66	A_5 West	EGY 1/72

(3) GRE 1/72 is more closely linked to A_{28} TUR 1/69 and TUR 1/70 but less related to Iraq/64 and USSR/66.

(4) KEN 140/69 is related to the newer A_{22} strains rather than to the older ones.

DISCUSSION

The history of A_{22} and its derivative strains provides a good opportunity for detailed observation of the changes which a strain of virus can undergo during the phases of initial epizootic spread in a hitherto unaffected region and the later establishment of the strain as enzootic.

The 1972 outbreak in Greece led to an increased interest in the strains comprising the A_{22} subgroup, raising the following questions:

(1) Whether the more recent strains should be regarded as one group.

(2) Whether subdivision should be attempted within the group.

(3) Whether the possibility exists of the newer strains supplanting the older ones in these areas.

The progress of A_{22} and its derivative strains is shown briefly in Fig. 1. In tracing the development of A_{22} it is natural to begin with the three strains first received from representative regions, i.e. Iraq 1964, Iran 1965 and USSR 1966. These three strains, although separated in time of receipt at Pirbright, probably represent the first wave of infection through the Near Eastern countries and into Russia; they already showed some slight difference, although this was probably only just significant, and it was agreed at that time that no useful purpose would b served by attaching new subscript numbers to different strains. Unfortunately, in the present series A_{22} Iran is not included but, since later strains appear to be derived more from A_{22} Iraq on its westward spread, this may not be too important. Again, an initial test on a strain recovered from Turkey in 1965 and labelled in Turkey as 'Mahmatli' showed little or no difference from A_{22} Iraq.

The first indication of a greater change in the A_{22} strain came when the isolate later named 'Polatli' in Turkey and known as A_{28} TUR 1/69 broke through the 'Mahmatli' vaccine, but since that time no further strains closely related to 'Polatli' have been recovered. Although 'Polatli' had been assigned subtype number A_{28}, it will be seen from the Tables that there is a greater justification for regarding it as a branch of A_{22} rather than as a new subtype.

The next main event in the history of A_{22} appears to be the occurrence of the Greek 1972 strain which was recovered from Xanthi in Northern Greece and formed a pattern of westward spread of disease which was subsequently traced back to Turkish Thrace. Samples were received from Turkey from areas reaching from Istanbul in the west to the province of Kars in the east.

It seems reasonable to point out here that there have been two other type A outbreaks in Greece – one in 1969 in Serrai and the other in 1971 in Crete. As work on these indicates that they are very different from the early A_{22} strains (i.e. Iraq 24/64 and USSR 2/66) and further comparisons are necessary to establish their relation with the other subgroups, and as no suggestion has been made that they were linked in any way with the spread of A_{22}, it was thought unnecessary to deal with them in any detail here since this is a paper primarily concerned with the A_{22} family of strains.

The 1972 epizootic consists of three separate geographical foci, viz. Greece and Turkey in the north and Lebanon and Egypt to the south. The strain from Egypt appears the most outlying strain, though it shows more identity with the original A_{22} strains, Iraq 24/64 and USSR 2/66, than with any other strain. The other separate focus at this time, Lebanon, was produced by a virus which is most certainly a member of the A_{22} family. The GRE 1/72 strain appears equally related to A_{28} TUR 1/69 and TUR 1/70, with less relation to the classic strains USSR 2/66 and Iraq 24/64. The other strain of interest, KEN 140/69, shows closest relation with A_{28} TUR 1/69 and GRE 1/72 and also has a unidirectional relation with TUR 1/70 and A_5 Westerwald. There is, therefore, the suggestion that if EGY 1/72 is included it has diverged most from the classical A_{22}, that the Kenya strain is reasonably close to the newer A_{22} strains and that, earlier than this, divergences have been relatively slight though perhaps they are sufficient to be of taxonomic value.

As it is difficult to draw conclusions about attempted groupings of the strains which have been provisionally discussed as A_{22} derivatives, it is suggested that:

(*a*) these strains are more distinct from Western European strains (as represented by A_5 Westerwald) than they are from each other, and

(*b*) on such evidence as there is from the field, vaccines against some of the earlier strains appear to have been reasonably effective against the new ones.

An important aspect of a survey of this nature lies in the possible detection not only of emergent strains but of new strains supplanting older ones in the field. This is illustrated by a strain received from the province of Kavala in Northern Greece in 1973 which when examined in unidirectional tests was shown to be similar to the GRE 1/72 strain. As previously remarked in this paper, Kavala province was not involved in the original 1972 epizootic, so that isolation of the virus a year after the major outbreak may have some significance, suggesting that the divergent A_{22} strain is supplanting the older one in this area.

On the basis of these observations it is suggested that the principles of subtype grouping should be revised to take account of the relation between new strains and established vaccine strains rather than to attempt to include all new strains within an arbitrary group. The antigenic characterization of new strains would then be indicated by their '*r*' and '*R*' values in relation to the established strains rather than by describing them as being, for example, A_1, A_2, ..., A_x. In characterizing the strains, particular reference should be made to *r* values to allow for the asymmetric relations between strains. The collection of data on vaccine performance against variant strains with known *r* and *R* values in comparison with vaccine strains will lead to a better basis for decisions on the need to replace existing vaccine strains with strains appearing in the field.

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