The foot-and-mouth disease virus subtype variants in Kenya

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SUMMARY

The subtype variants found in Kenya in the past ten years have been studied. The type O and type SAT 2 subtypes have a distinct geographical distribution which appears to be associated with livestock movement patterns. The type A viruses have a greater tendency to antigenic variation and their geographical distribution is less distinct. In type C only minor differences exist between the three viruses studied.

INTRODUCTION

Within each of the immunologically distinct foot-and-mouth disease virus type groups are found subtype variants which are distinguished by the fact that immunization to one subtype variant does not confer as solid an immunity to another variant of the same type as to the homologous strain (Traub & Möhlmann, 1946; Galloway, Henderson & Brooksby, 1948). These antigenic differences can be measured by serological methods and the variants classified into groups according to the degree of immunological variation exhibited (Davie, 1962, 1964). These subtype variants probably arise in the field through genetic change followed by selection in the host population, which may exhibit a range of susceptibility as a result of earlier infection or immunization. Pringle (1964) has shown that genetically stable mutants of subtype variants can be produced *in vitro* and that genetic recombination does occur in crosses of mutants of the same strain (Pringle & Slade, 1968; Pringle, Slade, Elworthy & O'Sullivan, 1970) and in crosses of serologically related strains (Pringle, 1965). It has also been demonstrated experimentally (Hyslop & Fagg, 1965; Fagg & Hyslop, 1966) that serial passage of a strain in partially immune cattle produces antigenic variation in that strain. The work described here was undertaken to determine the ecological pattern of the foot-and-mouth disease viruses present in Kenya as a contribution to the present and future control programme against the disease.

METHODS

Isolation of field viruses

Samples of vesicular epithelium were collected from the tongues of clinically infected cattle during the course of field outbreaks. Viruses from suspensions of such material were isolated on primary bovine thyroid cells (Snowdon, 1966) and typed by the micro complement-fixation test.

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Production of specific antisera

Each field virus was adapted to grow in either a baby hamster kidney cell line (BHK 21 Cl 13, Macpherson & Stoker, 1962) or in primary bovine kidney cells. The virus was then passaged once more in monolayers of the same cells in three Thomson bottles, each containing 220 ml. of maintenance medium consisting of Eagle's minimum essential medium plus 10 % (v/v) tryptose phosphate broth. The virus was harvested 30–48 hr. later, when there was 100 % cytopathic effect (CPE). This suspension was then centrifuged at 1600 g for 30 min. and the supernatant inactivated with 0·05 % (v/v) acetylethyleneimine at 37° C. for 8 hr. and at 4° C. for a further 16 hr. The reaction was stopped with 2 % (w/v) sodium thiosulphate. The inactivated antigen was then concentrated in a two-phase liquid polymer system (Albertsson, 1958) and purified by rate zonal centrifugation through a 25–45 % sucrose gradient at 75000 g for $3\frac{1}{2}$ hr. The fraction containing the 140 S virion was identified by a micro complement-fixation test and emulsified in Freund's complete adjuvant. A 1 ml. dose of this vaccine was then given subcutaneously to an appropriate number of guinea-pigs of 700–800 g. body weight. One month later a second dose of freshly purified 140 S antigen, this time containing 0·25 mg. saponin as the adjuvant, was given. Ten days later the guinea-pigs were bled out and each serum assayed by a micro complement-fixation test. Antisera with antibody titres of 1/81 or greater were pooled and used in the subtyping test.

Identification of different subtypes

A micro complement-fixation test as described by Darbyshire, Hedger & Arrowsmith (1972) was used. Cross-fixation products were obtained for each group of viruses (Davie, 1964) and these are expressed as R values, where $R = 100\sqrt{(r_A r_B)}$ and

$$r_A = \frac{\text{complement fixed in reaction virus B} + \text{antiserum A}}{\text{complement fixed in reaction virus A} + \text{antiserum A}},$$

$$r_B = \frac{\text{complement fixed in reaction virus A} + \text{antiserum B}}{\text{complement fixed in reaction virus B} + \text{antiserum B}}.$$

In this study the following interpretation has been put on the R values obtained:

		R value
(i)	Different subtype	40 or lower
(ii)	Subtypes related but different	40–60
(iii)	Same subtype	60 or greater

The figures applied to the significance of R values have beeen arbitrarily chosen in all the work on subtype strains. The figure of 70 % was initially taken as it was believed that exposure following primary vaccination to a strain having an R value of less than this would result in significantly less protection than following exposure to the homologous strain. In the field work which is discussed in this paper we are dealing in general with a population of animals in which all but the youngest group have been revaccinated and, on the basis of observations on

vaccines in the field, we have chosen to classify only strains with an R value of 40 % or less as different. Those between 40 % and 60 % we have termed 'related' and over 60 % we have regarded as being within the limits of experimental error of the same subtype. It is to be expected that there will be a continuous series of variations and we therefore emphasize the arbitrary nature of the selection of values of R as having significance in cross-immunity studies.

The notation of viruses and subtype groups used

In this study three different notations are used to describe the virus strains. These are:

(a) The laboratory typing reference number of the strain, e.g. O–K120/64 is type O virus isolated in 1964.

(b) For reasons of clarity only, each isolate has been given a number, e.g. O-1, A-6, C-2, etc., and these are used in Fig. 1 to show the origin of these viruses.

(c) Where different subtype groups in Kenya have been established, these groups are referred to as O/K_1, O/K_2, O/K_3, etc. This should not be confused with the World Reference Laboratory (WRL), Pirbright, notations for different subtype groups which are O_1, O_2, O_3, etc. Although in this study no attempt was made to compare the different subtypes found with known subtype groups from other countries, some of the strains used have previously been classified by the WRL (J. B. Brooksby, personal communication), as follows:

$$\text{subtype group } O/K_1 = \text{WRL group } O_9,$$
$$\text{subtype group } A/K_2 = \text{WRL group } A_{23},$$
$$\text{subtype group SAT } 2/K_2 = \text{WRL group SAT } 2_3.$$

Virus strain A-K140/69, which is related to subtype groups A/K_3, A/K_4 and A/K_6, has also been shown to be related to WRL group A_{22}.

RESULTS

Field outbreaks are mainly caused by types O and A viruses. Type SAT 2 outbreaks do occur but are less common. In 1970 51 % of all outbreaks were type O, 30 % were type A and 17 % were type SAT 2. In 1971 66 % were type O, 19 % were type A and 14 % were type SAT 2. Type C outbreaks occur, but very infrequently. In this study, virus isolates of types O, A, C and SAT 2 from field outbreaks in different parts of the country were compared (Fig. 1). The antigenic interrelations of each type group of viruses, expressed as R values, are given in Tables 1–4.

In order to try to relate the R values obtained with possible significance to vaccination, it was first necessary to show that the complement-fixation test used was not influenced to any degree by the presence of non-specific antibodies in the antisera to the 12 S protein subunits present in most unpurified virus suspensions. These were shown by Bradish & Brooksby (1960) to fix complement with hetero-typic antisera and could therefore be responsible for increased values of R. Real differences between the entire virion which is the immunogenic particle (Randrup, 1954; Brown & Crick, 1959) would then be less apparent. Consequently, a series of complement-fixation tests was carried out with three type O subtype variants
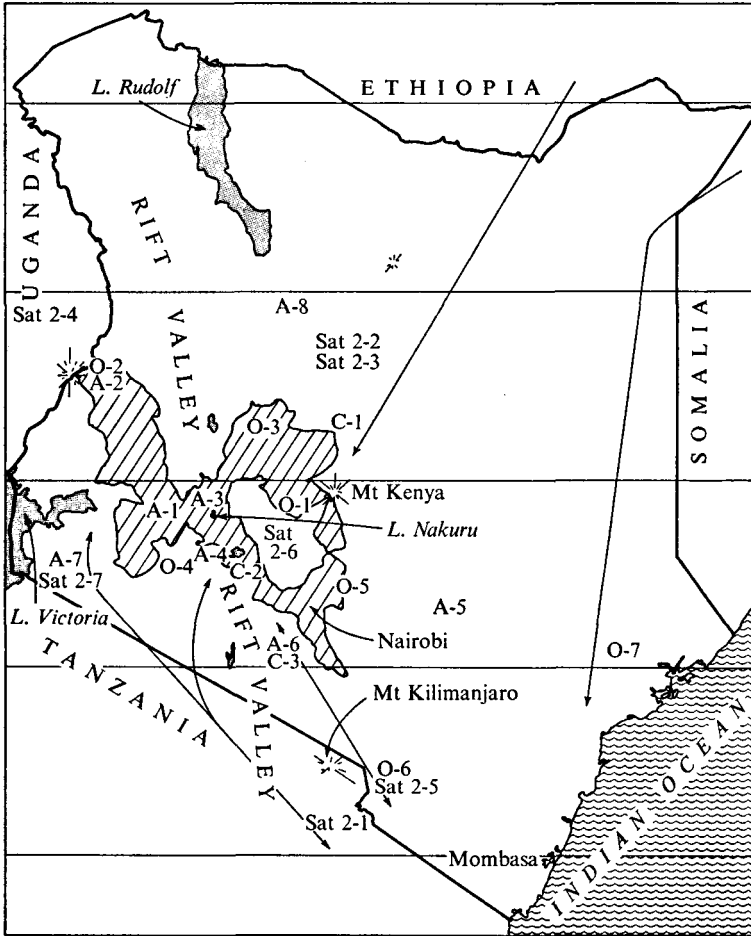
Fig. 1. Location of field outbreaks from which the various subtypes were originally recovered. ▨, Vaccinated area. →, Livestock movements.

Table 1. R *values – type O viruses*

Virus	Laboratory typing reference no.	Antiserum						
		O-1	O-2	O-3	O-4	O-5	O-6	O-7
O-1	K120/64	100	—	—	—	—	—	—
O-2	K248/71	47	100	—	—	—	—	—
O-3	K1/72	65	45	100	—	—	—	—
O-4	K171/72	36	35	25	100	—	—	—
O-5	K61/71	38	16	—	23	100	—	—
O-6	K4/71	21	14	—	39	87	100	—
O-7	K217/72	78	40	—	34	37	21	100

and three type A variants, using both unpurified virus harvests and preparations of the purified 140 S particle. The R values obtained are compared in Tables 5 and 6 and are essentially the same for both antigens, indicating that in routine tests using antisera raised by the method described the presence of 12 S antigen is relatively unimportant.

Table 2. R *values – type A viruses*

Virus	Laboratory typing reference no.	Antiserum							
		A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8
A-1	K18/66	100	—	—	—	—	—	—	—
A-2	K46/65	27	100	—	—	—	—	—	—
A-3	K140/69	36	43	100	—	—	—	—	—
A-4	K180/71	15	36	60	100	—	—	—	—
A-5	K104/72	15	34	40	32	100	—	—	—
A-6	K179/71	12	31	63	100	44	100	—	—
A-7	K27/72	17	25	39	—	24	24	100	—
A-8	K82/72	21	30	68	—	23	25	37	100

Table 3. R *values – type C viruses*

Virus	Laboratory typing reference no.	Antiserum		
		C-1	C-2	C-3
C-1	K267/67	100	—	—
C-2	K182/69	68	100	—
C-3	K42/73	52	—	100

Table 4. R *values – type SAT 2 viruses*

Virus	Laboratory typing reference no.	Antiserum						
		SAT 2-1	SAT 2-2	SAT 2-3	SAT 2-4	SAT 2-5	SAT 2-6	SAT 2-7
SAT 2-1	T5/68	100	—	—	—	—	—	—
SAT 2-2	K3/57	18	100	—	—	—	—	—
SAT 2-3	K159/70	46	26	100	—	—	—	—
SAT 2-4	U6/70	8	14	—	100	—	—	—
SAT 2-5	K83/72	61	22	23	13	100	—	—
SAT 2-6	K147/72	83	20	32	18	43	100	—
SAT 2-7	K162/72	49	21	32	—	32	50	100

Table 5. *A comparison of* R *values obtained using purified and unpurified antigen – type O viruses*

Serum	O-1		O-4		O-6	
	Purified antigen	Unpurified antigen	Purified antigen	Unpurified antigen	Purified antigen	Unpurified antigen
O-1	100	100	—	—	—	—
O-4	44	36	100	100	—	—
O-6	32	21	39	39	100	100

Type O viruses

The R values of the type O viruses included in this study are given in Table 1. These may be placed in three subtype groups, as shown in Table 7.

The viruses of group O/K_1 all originated in the north and east of the country, while those of groups O/K_2 and O/K_3 were isolated in the south. The livestock movement patterns that traditionally occur within these two regions are shown

Table 6. *A comparison of* R *values obtained using purified and unpurified antigen – type A viruses*

	A-4		A-1		A-5	
Serum	Purified antigen	Unpurified antigen	Purified antigen	Unpurified antigen	Purified antigen	Unpurified antigen
A-4	100	100	—	—	—	—
A-1	6	15	100	100	—	—
A-5	36	32	12	22	100	100

Table 7. *Subtype groups – type O viruses*

Group	Virus	Related virus
O/K_1	O-1, O-3, O-7	O-2
O/K_2	O-4	—
O/K_3	O-5, O-6	—

Table 8. *Subtype groups – type A viruses*

Group	Virus	Related virus
A/K_1	A-1	—
A/K_2	A-2	—
A/K_3	A-4, A-6	A-3
A/K_4	A-5	A-3, A-6
A/K_5	A-7	—
A/K_6	A-8	A-3

Table 9. *Subtype groups – type SAT 2 viruses*

Group	Virus	Related viruses
SAT 2/K_1	SAT 2-1	SAT 2-3
	SAT 2-5	SAT 2-7
	SAT 2-6	—
SAT 2/K_2	SAT 2-2	—

in Fig. 1. From the north cattle move south into the Rift Valley area and coastal belt. In the south-west livestock movement is extensive but mainly confined to this region, although there is some movement into the Rift Valley and the area bordering Lake Victoria. Of the two strains isolated in the Rift Valley region, strain O-2 from the north-west is antigenically related to the northern subtype while strain O-5 is the same subtype as one of the southern strains (O-6).

Type A viruses

Amongst the type A viruses included in this study (Table 2), six subtype groups have emerged as shown in Table 8. The geographical distribution of the different type A subtypes does not show such a distinct regionalization as the type O subtypes, although each subtype group does originate in a different part of the country. The larger proportion of distinct groups found indicates a greater tendency to antigenic variation in the type A viruses. Strain A-3 is antigenically related to groups A/K_3, A/K_4 and A/K_6.

Type C viruses

Few outbreaks of type C infection have occurred and only three strains have been examined. These were all closely related (Table 3) but from the few data available it cannot be concluded that C strains are more antigenically stable.

Type SAT 2 viruses

The SAT 2 viruses examined (Table 4) fell into two subtype groups as shown in Table 9. One subtype group contains those viruses isolated in the south of the country, while the other contains the northern strain SAT 2-2. Strain SAT 2-3 isolated in 1970 has some antigenic relation to one of the southern strains, SAT 2-1, but not to the others. It is, however, a different subtype from strain SAT 2-2 isolated at the same place in 1957. The geographical distribution of the type SAT 2 subtypes follows the same pattern as the type O subtypes.

DISCUSSION

In an enzootic area subtype variants may arise by mutation of the parent strain or by recombination between subtype variants where a dual infection occurs. Any mutation of the genome affecting the antigenic composition of the surface proteins of the virion will be immediately expressed and such a variant will then be subject to natural selection in the population of immune and partially immune animals (Pringle, 1969). Although such selection pressure itself may act towards the appearance of subtype variants, these variants will not easily establish themselves in this population. New subtypes are only likely to arise by gradual transition from the parent strain to establish themselves as stable mutants (Pringle, 1964; Hyslop & Fagg, 1965). The results of this study indicate that this is probably what happens in an enzootic area. Relatively few different subtypes were found, while the number of serologically related but still antigenically dissimilar strains found, particularly amongst the type A and type O viruses, supports the view that there is a gradual transition away from the parent strains. Where different subtypes were found, they were usually from different geographical areas. There appeared to be some relation between the distribution of the different subtypes and livestock movement patterns (Fig. 1). In general, the strains found in the north of the country are different from those found in the south and west. There is little direct movement of livestock between these areas; rather, livestock movement takes place from these areas towards the Rift Valley and Central Highlands.

The significance of the presence of different subtypes in relation to disease control by regular vaccination is not clear. In Kenya, since 1968 a vaccination programme requiring routine vaccination of all cattle over three months of age against types O and A every 6 months has been carried out in the Central Highlands (Fig. 1). This programme was started in a small area and gradually extended so that by 1973 the area shown in Fig. 1 was being covered. An inactivated vaccine containing strains of subtype groups A/K_1 and O/K_1 was used. Many of the field strains, particularly those of type A, show quite marked antigenic differences from the vaccine strains yet the number of type O and A outbreaks of disease in the vaccinated areas has fallen from 40 in 1968 to nine in 1969, five in 1970, two in

1971, three in 1972 and four in 1973. These results are largely due to a good level of herd immunity being maintained and, at the same time, minimizing the challenge through rigid quarantine procedures following a disease outbreak. Whether the same results would have been achieved if, following the appearance of a new subtype, no quarantine measures were enforced and the weight of challenge allowed to build up is uncertain.

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