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The sub-type classification of strains of foot-and-mouth disease virus

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SUMMARY

Sixteen foot-and-mouth disease virus (FMDV) strains of type SAT 1 were compared in complement-fixation tests. With the test used, the range of antigenic variation within a type appeared to be greater than previously described. The concept of a sub-type group within which all strains are more closely related to each other than to any strain outside the group was not supported. Considering the group of strains studied, it is suggested that the classification of strains is best achieved by nominating a *reference strain* for each sub-type. Others are classified as related strains in one or more sub-type groups according to their relationships with the reference strains.

INTRODUCTION

While foot-and-mouth disease virus (FMDV) strains can be classified into seven types which are immunologically quite distinct, within each type there is a range of antigenic variations between strains. Since Traub & Möhlmann (1946) first applied complement-fixation (CF) to the differentiation of intra-type strains, its use has become universally popular and such differentiation has been the main basis for the classification of strains into sub-types.

The World Reference Laboratory at Pirbright has classified strains into a large number of sub-types, from three sub-types within the Asia 1 type to 32 within type A. By classifying strains exclusively as one or another sub-type there is an implication that the antigenic variation within a type is such as to make discrete sub-type groups recognizable.

In this paper, the results are presented of a large number of cross-CF tests carried out between strains of one type to obtain an indication of the character and the range of antigenic variation within a type. On the basis of these results, new criteria are proposed for the classification of sub-types of FMDV.

MATERIALS AND METHODS

CF tests were carried out in microplates. The procedure and the reagents used have been previously described (Forman, 1974*a*, *b*) but several modifications were made to the method to enable a large number of tests to be carried out on a routine basis. Unless otherwise stated, the antigens used were harvests of infected BHK

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monolayers concentrated and partially purified by pelleting as described by Brown & Cartwright (1963) but without the final purification step of sucrose density gradient centrifugation. These are referred to as pelleted antigens. They were stored at −70° C. in small volumes, diluted in veronal-buffered saline. The results of many tests with antigens used before and after storage indicated that there was no significant alteration to the antigens on storage for up to three months.

Guinea-pig antisera were prepared by two methods. Live-virus antiserum was prepared by the hyper-immunization of guinea-pigs with live virus as described by Brooksby (1952). These antisera were obtained from the World Reference Laboratory. Anti-140S sera were prepared following the method of Cowan (1968). 140S antigen from the harvest of 20 BHK Roux flask monolayers was inactivated with acetylethyleneimine (0·05% for 30 hr. at 26° C.), purified by the procedure of Brown & Cartwright (1963) and double-emulsified with incomplete Freund's adjuvant (Difco Laboratories, Detroit, Michigan, U.S.A.) and Tween 80 detergent (Koch-Light Laboratories Ltd, Colnbrook, Bucks.). Five guinea-pigs were inoculated intramuscularly and hyperimmunized 4 weeks later with a similar amount of freshly prepared 140S antigen without adjuvant. They were exsanguinated after a further 10 days.

RESULTS

Cross-CF tests were carried out with 16 strains of type SAT 1 FMDV, to investigate the range and the pattern of variation between strains. The strains included one of each sub-type as classified by the World Reference Laboratory and a number of recent field isolates from West, East and Southern African countries. Unless otherwise specified, the antisera used were as follows:

RV 11/37 (Rhodesia, 1937)	SAT 1–2	140S
SWA 1/49 (South West Africa, 1949)	SAT 1–3	Live virus
SR 2/58 (Southern Rhodesia, 1958)	SAT 1–4	Live virus
SA 13/61 (South Africa, 1961)	SAT 1–5	Live virus
SWA 40/61 (South West Africa, 1961)	SAT 1–6	Live virus
TUR 323/62 (Turkey, 1962)	SAT 1–7	140S
GHA 14/68 (Ghana, 1968)		Live virus
BOT 1/68 (Botswana, 1968)		140S
MAL 3/70 (Malawi, 1970)		140S
UGA 47/71 (Uganda, 1971)		140S
KEN 3/72 (Kenya, 1972)		140S
RHO 4/72 (Rhodesia, 1972)		140S
ANG 9/72 (Angola, 1972)		140S
NIG 9/72 (Nigeria, 1972)		140S
GHA 7/73 (Ghana, 1973)		140S
ZAM 25/73 (Zambia, 1973)		140S

The first six strains above (SAT 1–2 to SAT 1–7) represent the six sub-types currently recognized by the World Reference Laboratory (there is no SAT 1–1 in the classification).

Table 1. *Values for* R (%) *determined by cross-CF between strains of foot-and-mouth disease virus type SAT* 1

SWA 1/49	SR 2/58	SA 13/61	SWA 40/61	TUR 323/62	GHA 14/68	BOT 1/68	MAL 3/70	UGA 47/71	KEN 3/72	RHO 4/72	ANG 9/72	NIG 9/72	GHA 7/73	ZAM 25/73	
30	27	16	23	9	3	14	4	10	—	15	⩽3	—	9	19	RV 11/37
	23	35	32	21	7	—	—	—	—	—	—	—	—	—	SWA 1/49
		27	55	32	—	14	—	—	—	—	—	—	—	—	SR 2/58
			42	24	10	—	21	—	—	23	10	—	18	25	SA 13/61
				32	< 6	—	—	—	—	—	—	—	—	—	SWA 40/61
					22	33	21	30	43	23	11	—	10	10	TUR 323/62
						—	4	—	—	5	⩽ 3	—	5	⩽ 3	GHA 14/68
							28	31	30	16	7	—	7	15	BOT 1/68
								18	96	16	13	—	4	25	MAL 3/70
									14	—	—	—	3	—	UGA 47/71
										—	—	—	—	—	KEN 3/72
											11	11	12	23	RHO 4/72
												—	2	4	ANG 9/72
													65	—	NIG 9/72
														3	GHA 7/73

The results of the tests are summarized in Table 1. The values for R represent geometric mean values of at least two tests. If individual values for R between two strains differed by twofold or more, further tests were carried out.

The range of values of R shown in the Table is large and suggests a continuous gradation from virtual identity (96%) to 2% or less. There is no clear pattern of variation, in that strains which are furthest apart geographically or in time of isolation are not necessarily those showing the greatest differences. Also there is no indication that the strains can be readily classified exclusively into groups of more closely related strains.

To obtain an indication of the importance of antiserum quality in the determination of quantitative relationships between strains, tests were carried out using antisera prepared by the two different methods described. Live-virus and 140S antisera of the strains RV 11/37 and BOT 1/68 were tested against pelleted antigens, purified 140S antigens and 12S antigens (the last prepared by heating purified 140S antigen at 56° C. for 30 min.). Table 2 shows the results of cross-CF tests and in Table 3, antiserum titres to homologous 140S and 12S antigens are shown.

It is evident from Table 2 that the values determined for r and R were similar with pelleted or purified 140S antigens but different with 12S antigens. This suggests that the pelleted antigens were of good quality, i.e. that they consisted mainly of 140S antigen. However, the values for r determined with either pelleted or 140S antigens were lower for each strain when 140S antisera rather than live-virus antisera were used. The homologous titres (Table 3) of three of the four antisera were greater with 140S than with 12S antigens. But for the BOT 1/68 live-virus antiserum the anti-12S titre was greater than the anti-140S titre. This indicates only one of the possible reasons for the variation in specificity of antisera

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Table 2. *Cross-CF tests using live virus and 140S antisera, with pelleted, purified 140S and 12S antigens*

Antigens	Antisera	r_1 (RV 11/37)	r_2 (BOT 1/68)	R (%)
Pelleted	Live-virus	0·30	0·71	46 %
Pelleted	140S	0·12	0·25	18 %
Purified 140S	Live-virus	0·25	0·85	47 %
Purified 140S	140S	0·06	0·25	12 %
12S	Live-virus	0·30	0·35	33 %
12S	140S	0·30	0·25	30 %

Table 3. *Antiserum titres against homologous purified 140S and 12S antigens*

Antigen	Antiserum	Antiserum titre
140S	RV 11/37 live-virus	204
12S	RV 11/37 live-virus	128
140S	RV 11/37 140S	204
12S	RV 11/37 140S	181
140S	BOT 1/68 live-virus	115
12S	BOT 1/68 live-virus	181
140S	BOT 1/68 140S	724
12S	BOT 1/68 140S	181

and this aspect was not investigated further. However, results obtained with other strains (A. J. Forman, to be published) suggest that anti-140S sera do not invariably possess greater sub-type specificity than live-virus antisera.

DISCUSSION

The results presented in this paper demonstrate that the serological differences between FMDV strains within a type can be very large (a value for R of 2 % represents a difference between homologous and heterologous antiserum titres of 50-fold). The CF test used by a number of previous workers for FMDV strain differentiation has been shown to be invalid in principle (Forman, 1974*a*) and it appears likely that this earlier method was less discriminating of the differences between strains.

Brooksby (1968) suggested the following values for R for type and sub-type differentiation by CF.

(1) type difference R = 10 % or lower,
(2) sub-types widely different R = 10–32 %,
(3) sub-types different R = 32–70 %,
(4) difference within sub-type R = 70–100 %.

It has been shown previously (A. J. Forman, to be published) that there is no heterotypic fixation with 140S antigen provided it remains intact, so it is unnecessary to define a maximum value for R for type differentiation. The results shown in Table 1 indicate that, if R = 70 % was the smallest difference separating sub-

Table 4. *Sub-type classification of SAT 1 strains*

Sub-type	Reference strain	Related strains
SAT 1/1	RV 11/37	SWA 1/49, SR 2/58
SAT 1/2	SA 13/61	SWA 1/49, SR 2/58, SWA 40/62
SAT 1/3	TUR 323/62	SR 2/58, SWA 40/61, BOT 1/68, UGA 47/71, KEN 3/72
SAT 1/4	GHA 14/68	—
SAT 1/5	MAL 3/70	BOT 1/68, KEN 3/72
SAT 1/6	RHO 4/72	—
SAT 1/7	ANG 9/72	—
SAT 1/8	GHA 7/73	NIG 9/72
SAT 1/9	ZAM 25/73	—

types, then almost all of the strains examined in this group would be classified as different sub-types, which would provide little information regarding the inter-relationships between the strains. It is suggested that if methods similar to those described in this paper are used, then a more realistic level for sub-type differentiation would be $R = 25\%$. This represents a fourfold difference between the homologous and heterologous antiserum titres of the two strains being compared. It is only around this level of differentiation that differences detected in microplate tests become meaningful, the detection of smaller differences requiring more accurate techniques (such as the tube test previously described; Forman, 1974*a*, *b*).

The term 'sub-type' has never been precisely defined. Brooksby (1968) proposed it as follows: 'A sub-type can then be defined as a group of strains which can be differentiated from other groups of strains within a type by serological methods or by cross immunity experiments at a level of immunity lower than that possessed by recently recovered animals – for example, in vaccinated animals.' The implication is that strains within a group are more closely related to each other than to strains in other groups. However, examination of the relationships obtained for the SAT 1 strains (Table 1) reveals that they cannot be placed into such groups based on a level of differentiation of $R = 25\%$.

A different approach is proposed in which only *reference strains* would be classified exclusively, one for each sub-type, such that the relationship between any two reference strains is $R \leqslant 25\%$. The selection of reference strains with incomplete historical data would be somewhat arbitrary but has a chronological basis. Once selected, reference strains would be fixed. All others would be classified as *related strains* in all sub-type groups with whose reference strain they have a relationship of $R > 25\%$. From the results obtained with the SAT 1 strains (Table 1), a sub-type classification could be made as shown in Table 4.

The exhaustive characterization of related strains is not essential as the division into sub-types depends only on the identification of reference strains. The six strains presently classified in the World Reference Laboratory as separate sub-types are all contained in the first three sub-type groups in Table 4. The identification of a further six groups demonstrates the antigenic diversity of SAT 1 strains which have not been previously classified. Recognizing the fact that sera were used that possibly had different powers of discrimination, this Table should be regarded

only as an indication of the principle involved and not as a proposed revision of the present classification.

In accordance with the proposed new classification, a sub-type can be redefined as follows: 'An FMDV sub-type is an antigenic grouping of closely related strains within a type. All strains within a sub-type are related to one particular reference strain, such that $R > 25\%$. Reference strains of each sub-type differ from each other to the extent that $R \leqslant 25\%$. A strain can be classified into more than one sub-type group according to its relationship to the reference strains.'

Stellman, Moreau & Favre (1972) and Stellman, Moreau & Roumiantzeff (1972) introduced the concept of dominance into the classification of FMDV strains. They suggested that the antisera of dominant strains are more cross-reactive and proposed a mathematical system for the determination of dominance on the basis of the inequality in values for r. No attempt has been made in this paper to incorporate the dominance characteristic into strain differentiation. It is apparent from earlier work (Forman, 1974*b*) that if the 140S antigens of two strains are compared in cross-CF tests, then the values for r of the two antisera are not necessarily equal. However, it was shown in this paper (Table 2) that the value for r can differ between two antisera of the same strain. This suggests that considerable care must be taken before attributing unequal values of r between strains to the effect of dominance.

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