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Neutralization kinetics studies with type SAT 2 foot-and-mouth disease virus strains

II. Antigenic differentiation of vaccine strains

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SUMMARY

A comparison of homologous and heterologous rates of neutralization demonstrated that antigenic relationships of foot-and-mouth disease virus strains could be differentiated quantitatively by the kinetics of neutralization method described previously (Rweyemamu, Booth & Pay, 1977). Conventional formulae for r, R and D values could be applied to results obtained by this method. It was demonstrated that results analysed this way gave R values which were similar to those obtained with other neutralization test methods but which were generally smaller than those obtained with complement fixation test results. It was demonstrated that there were wide differences between the vaccine strains tested as demonstrated by R value relationships. An examination of r values, however, demonstrated that antisera to the Moz 1/70 strain were highly reactive with most of the virus strains from Central and Southern Africa. The selection of FMD virus strains with a wide serological range for vaccine production is discussed.

INTRODUCTION

In a previous communication we reported an investigation of some factors influencing the kinetics of neutralization of Type SAT 2 foot-and-mouth disease (FMD) virus strains (Rweyemamu *et al.* 1977). In the present paper we describe the application of the technique to an evaluation of antigenic relations between several SAT 2 foot-and-mouth disease vaccine strains. Results obtained with this method are compared with those by other serological techniques in common use in our laboratory.

MATERIALS AND METHODS

Virus strains and antisera

The type SAT 2 foot-and-mouth disease virus strains tested were Ken 3/57, Uga 6/70, Tan 5/68, Tan 1/72, Rho 2/72, Swa 1/68, Swa 4/69 and Zam 8/75. Methods for their cultivation, storage and preparation of corresponding antisera have been described elsewhere (Rweyemamu *et al.* 1977).

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Neutralization kinetics test

This was carried out as described in detail in the accompanying paper (Rweyemamu *et al.* 1977). The neutralizing activity in the convalescent rabbit sera was generally too low to permit accurate determination of inter-strain differences unless the test was performed in the presence of anti-rabbit IgG goat serum* (Rweyemamu *et al.* 1977). In such cases, therefore, virus and a predetermined dilution of antiserum, both of which had been prewarmed at 37 °C, were mixed together at 37 °C in a water bath. Samples of 0·5 ml serum-virus mixtures were recovered at 1·25 and 2·5 min, and thereafter at 2·5 min intervals up to 15 min. Each sample was immediately mixed with 0·5 ml of the anti-IgG serum (diluted 1/20) then placed on ice for 1 min before being diluted 100-fold in chilled medium and subsequently assayed for surviving plaque forming activity on monolayers of BHK 21 cells.

The neutralization rate constant (K) was calculated as described previously (Rweyemamu *et al.* 1977). When pairs of virus strains were compared using antiserum to one of them the degree of serological relatedness was expressed as the ratio

$$r = \frac{\text{Heterologous } K \text{ value}}{\text{Homologous } K \text{ value}}$$

or as the normalized rate constant $NK = 100 \times r$. The extent to which two strains (e.g. a and b) were cross-related was denoted by the value

$$R = 100\sqrt{(r_a \times r_b)}.$$

In many cases the values of r_a and r_b were found to be very different. Thus the extent to which one strain was serologically dominant over the other was calculated by the formula of Stellman, Moreau & Roumiantzeff (1972) $D = \sqrt{r_a/r_b}$.

Complement fixation test

The complement fixing antibody activity of antisera was titrated in a 'chess-board' type test in microtitre plates as described by Forman (1974) using three 50% haemolytic doses of complement. Fixation was at 37 °C for 60 min. Serum titres were determined as the highest dilution of antiserum which fixed 2 units of complement in the presence of an optimal quantity of antigen. The serological relatedness of two strains compared by antiserum to one of them was given by the expression

$$r = \frac{\text{Antibody titre against heterologous virus}}{\text{Antibody titre against homologous virus}}.$$

Other neutralization tests

Plaque reduction test. Serial twofold dilutions of antisera were incubated for 1 h at room temperature with a constant dose of virus containing about 100 p.f.u./

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0·1 ml. The neutralizing antibody titre was defined as the highest dilution of the antiserum which reduced the plaque count by at least 50 % in BHK 21 monolayers, inoculated with 0·2 ml of serum-virus mixture.

Microneutralization test. The test was carried out as a two-dimensional checker-board in microtitre plates. Serial twofold dilutions of antisera were incubated for 1 h at room temperature mixed with a range of 0·5 log step dilutions of the virus under test in equal volumes of serum and virus (0·05 ml each). Cells were added in 0·05 ml volumes at a seeding rate of $7{\cdot}5 \times 10^4$ cells/cup. The plates were incubated at 37 °C for 2 days; then the medium was tipped off into a bowl of 2 % citric acid and the monolayers were stained with 0·1 % crystal violet in 10 % formol-saline. The cytopathic effect had resulted in total lysis of infected cells and therefore only uninfected monolayers could fix and take up stain. Virus neutralization was marked by the presence of residual intact stained monolayers.

At each virus dose the corresponding degree of neutralization was thus determined. A dose–response curve was drawn and from such a curve neutralizing antibody titre was determined as the dilution of antiserum which neutralized exactly 100 TCID 50 of the virus.

Cell metabolic inhibition test. This was based on the method described by Martin & Chapman (1961) except for using IB-RS-2 pig kidney cells (de Castro, 1964).

Data obtained using the three tests were converted to r and R values as described for the complement fixation and neutralization kinetics tests.

RESULTS

Neutralization kinetics tests

In Figs. 1 and 2 it will be observed that suitably diluted antisera demonstrated clear homologous and heterologous neutralization rates. In the case of low titre rabbit convalescent sera, however, effective comparison of homologous and heterologous neutralization rates could only be made by enhancing the reactions with anti-IgG serum (Fig. 2). As previously demonstrated (Rweyemamu *et al.* 1977) such enhancement did not significantly alter the relation between heterologous and homologous reactions.

The results obtained on the rate of neutralization for several strains are summarized in Table 1. The K and NK values presented are the means of at least two separate determinations. For ease of comparison the r and R values calculated for the various combinations of pairs of strains are shown in Table 2. From this table it will be observed that the two strains from Namibia (Swa 1/68 and Swa 4/69) were closely related, if not identical ($R = 80\,\%$) and the two viruses that were isolated in Tanzania 4 years apart (Tan 5/68 and Tan 1/72) were found to be similar to each other as well as to the Namibian strain, Swa 1/68. The Tanzanian strain Tan 5/68 ,was also related to the strains isolated from Mozambique in 1970 (Moz 1/70). Differences between all the other strains were found to be wide ($R < 15\,\%$).

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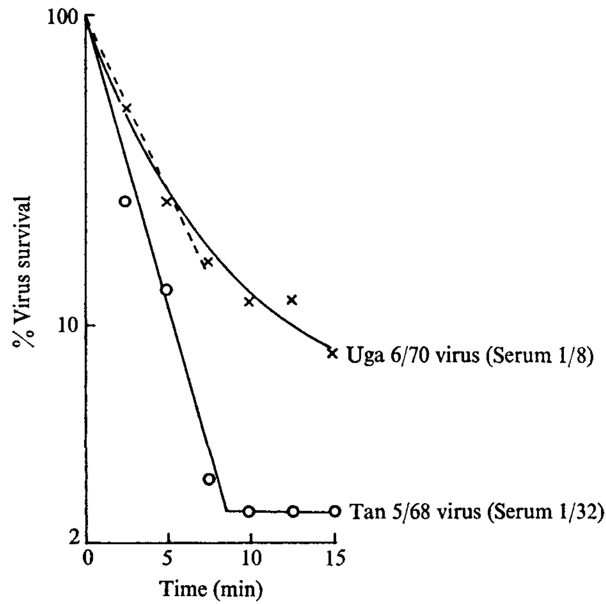

Fig. 1. Homologous and heterologous neutralization kinetics of Uga 6/70 and Tan 5/68 FMD type SAT 2 strains by Tan 5/68 rabbit antiserum.

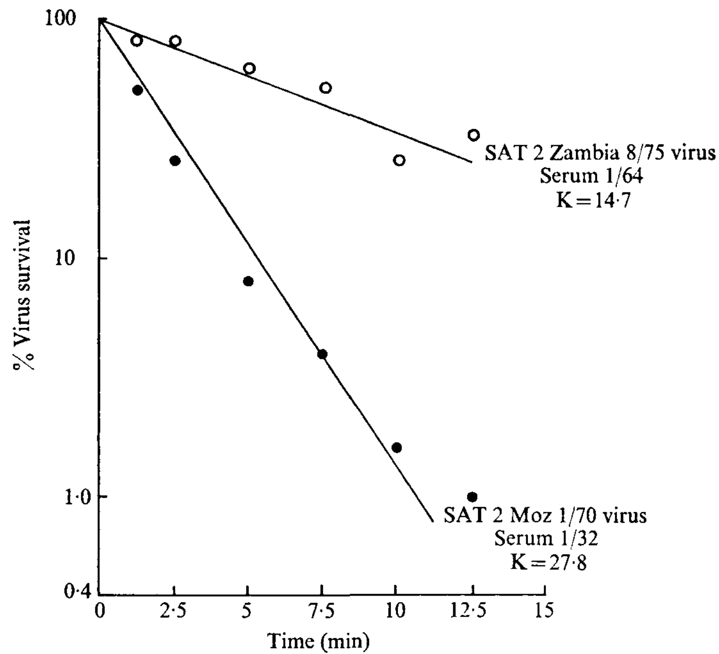

Fig. 2. Homologous (SAT 2 Moz 1/70) and heterologous (SAT 2 Zambia 8/75) neutralization kinetics enhanced by anti-IgG serum.

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Table 1. *Comparison of SAT 2 FMD viruses by rabbit antisera*

Neutralization rate constants (K); normalized constants (NK).

erum ...	Ken 3/57		Uga 6/70		Tan 5/68		Tan 1/72		Rho 2/72*		Swa 1/68		Swa 4/69	
irus	K	NK	K	NK	K	NK	K	NK	K*	NK	K	NK	K	NK
en 3/57	165·4	100	2·8	4·9	4·7	20·6	ND	ND	4·5	12·2	1·8	9·8	1·2	6·4
ga 6/70	9·4	5·7	57·7	100	4·0	17·5	ND	ND	5·8	15·6	0·4	2·3	2·4	12·8
an 5/68	4·1	2·5	2·2	3·9	22·8	100	38·6	35·1	6·5	17·5	5·2	28·0	2·0	10·6
an 1/72	ND	ND	ND	ND	12·2	53·5	109·9	100	ND	ND	5·9	31·3	3·0	16·0
oz 1/70	3·5	2·1	4·0	7·0	6·4	28·1	ND	ND	5·2	14·0	3·0	16·0	3·9	20·7
ho 2/72	6·1	3·7	3·5	6·0	3·0	13·2	ND	ND	37·1	100	4·8	25·7	0·85	4·5
wa 1/68	5·9	3·6	1·5	2·6	15·8	69·3	42·8	38·9	ND	ND	18·7	100	21·4	113·8
wa 4/69	ND	ND	ND	ND	ND	ND	ND	ND	1·2	3·2	10·4	55·7	18·8	100

ND = Not done.
* These K values were enhanced by the use of anti-rabbit IgG serum.

Table 2. *Neutralization kinetics of SAT 2 FMD viruses by rabbit antisera: r and R (in parentheses) values*

Virus ... / Serum	Ken 3/57	Uga 6/70	Tan 5/68	Tan 1/72	Moz 1/70	Rho 2/72	Swa 1/68	Swa 4/69
Ken 3/57	1·0 (100)	0·06	0·02	ND	0·02	0·04	0·04	ND
Uga 6/70	0·05 (6)	1·00 (100)	0·04	ND	0·07	0·06	0·03	ND
Tan 5/68	0·21 (7)	0·18 (9)	1·00 (100)	0·54	0·28	0·13	0·69	ND
Tan 1/72	ND	ND	0·35 (44)	1·0 (100)	ND	ND	0·34	ND
Moz 1/70	0·41 (9)		0·94 (43)	ND	1·0 (100)	0·38		
Rho 2/72	0·12 (7)	0·16 (10)	0·18 (15)	ND	0·14 (23)	1·00 (100)	ND	0·03
Swa 1/68	0·10 (6)	0·02 (3)	0·28 (44)	0·31 (33)	0·16	0·26	1·00	0·56
Swa 4/69	0·06	0·13	0·11	0·16	0·21	0·05 (4)	1·14 (80)	1·0 (100)

Variation in the neutralization kinetics results

In order to assess the order of repeatability of neutralization kinetics, data obtained from two types of experiments are presented in Tables 3 and 4. In the first experiment the variation in the homologous K values was assessed by results of 14 replicate tests with strain Uga 6/70. The resultant K values are presented in Table 3 and demonstrate a range of 40·48–69·70, mean K value of $55·49 \pm 8·13$ and the standard error of the mean was 4%.

Table 4 gives homologous and heterologous K values for two antisera. Homologous and heterologous values were obtained in the same set of tests. Although

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Table 3. *Variation between homologous* K *values in replicate neutralization kinetics of FMD type SAT* 2 *Uga* 6/70 *virus*

Test no.	K value
1	58·80
2	53·00
3	58·80
4	51·60
5	63·45
6	67·56
7	55·20
8	56·60
9	54·00
10	69·70
11	47·10
12	45·30
13	55·20
14	40·48
Mean K	55·49 ± 8.13

Table 4. *Between test variations in homologous and heterologous* K *values and the corresponding r values*

	SAT 2 virus strain					
Test serum ...	Tan 5/68	Uga 6/70	Tan 1/72		Swa 1/68	
	K	K	K	r	K	r
Tan 5/68 rabbit antiserum	25·76	—	11·78	0·46	12·88	0·50
	16·68	—	9·40	0·56	14·10	0·85
	28·85	—	13·40	0·46	19·60	0·68
	22·20	—	14·30	0·64	15·70	0·71
Mean	23·37	—	12·22	0·53	15·57	0·69
r based on mean K				0·52		0·67
Uga 6/70 rabbit antiserum		58·80			1·72	0·03
		53·0			1·60	0·03
		58·8			1·25	0·02
Mean		56·87			1·52	0·03

there was variation from test to test in individual K values r values varied less dramatically, indicating that in general variations in homologous K values corresponded with those in the heterologous K values. Therefore, provided both sets of values are obtained as pairs in the same test, the effect of variation in K value can be minimized by comparison of either mean K or mean r values but homologous and heterologous K values obtained in separate tests could easily result in misleading r values unless many replicates are used.

It will be observed that the data in Table 4 indicate that r values obtained for both Tan 1/72 and Swa 1/68 viruses using Tan 5/68 antiserum do not differ significantly from 1·00 at $P = 0·05$. In contrast the data with antiserum to Uga 6/70 virus demonstrate a wide significant difference between Uga 6/70 and Swa 1/68 strains.

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Table 5. *Comparison of R values obtained with the neutralization kinetics, micro-neutralization, plaque reduction and complement fixation tests using the same rabbit antisera and complement fixation tests using guinea-pig sera to purified* 146S *antigens*

Virus	Ken 3/57	Uga 6/70	Tan 5/68	Rho 2/72	Swa 1/68
Serum and test method					
Ken 3/57					
Kinetics					
Plaque					
Micro N.					
CFT-Rabbit	100				
CFT-Gp					
Uga 6/70					
Kinetics	6				
Plaque	6				
Micro N.	4	100			
CFT-Rabbit	35				
CFT-Gp	27				
Tan 5/68					
Kinetics	7	9			
Plaque	1	4			
Micro N.	9	5	100		
CFT-Rabbit	29	59			
CFT-Gp	35	30			
Rho 2/72					
Kinetics	7	10	15		
Plaque					
Micro N.				100	
CFT-Rabbit					
CFT-Gp	38	42	47		
Swa 1/68					
Kinetics	6	3	44		
Plaque	17	10			100
Micro N.	9	11	50		
CFT-Rabbit	32	46	54		
CFT-Gp	22	29	42	30	

Table 6. *Probable antigenic dominance among SAT* 2 *vaccine strains*

			D = values*			
Virus ...	Ken 3/57	Uga 6/70	Tan 5/68	Moz 1/70	Rho 2/72	Swa 1/68
Serum						
Ken 3/57	1·00	1·10	0·31	0·22	0·58	0·63
Uga 6/70	0·91	1·0	0·47	ND	0·61	1·22
Tan 5/68	3·24	2·12	1·0	0·55	0·85	1·57
Moz 1/70	4·53	ND	1·83	1·00	1·65	ND
Rho 2/72	1·73	1·63	1·18	0·61	1·0	0·77
Swa 1/68	1·58	0·82	0·64	ND	ND	1·00

* For each antiserum if $D > 1$ strain dominant and $D < 1$ strain dominated.

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Table 7. *Probable antigenic dominance among SAT 2 vaccine strains*

Virus ... ... Serum and test method	Ken 3/57	Tan 5/68	Uga 6/70	Moz 1/70	Swa 1/68	Swa 1/69	Rho 2/72	Zambia 8/75
			r values					
SAT 2 MIT(RCS) Ken 3/57	—	0·04	0·06	0·09	0·05	0·07	0·04	0·1
Micro N. (GPAS)	1·0	0·28	0·19	0·30	0·12	0·14	0·14	0·31
Kinetics (RCS)	—	0·02	0·06	0·02	—	—	0·04	—
SAT 2 MIT(RCS) Moz 1/70	1·0	0·53	1·0	1·0	—	0·89	1·0	0·76
Micro N. (GPAS)	0·32	1·0	0·50	1·0	0·20	0·62	0·22	0·46
Kinetics (RCS)	0·41	0·94	ND	1·0	—	—	0·38	0·64
			Average *r* values					
Ken 3/57	1·0	0·11	0·10	0·14	0·09	0·11	0·07	0·21
Moz 1/70	0·58	0·82	0·75	1·0	0·2	0·76	0·53	0·62

MIT = metabolic inhibition test; Micro N. = microneutralization text; RCS = convalescent rabbit serum; GPAS = guinea-pig antiserum.

Relation between neutralization kinetics results and those obtained in other serological tests

Five SAT 2 vaccine strains were compared by two-way cross-reactions in the neutralization kinetics, plaque reduction, microneutralization and complement fixation tests. The intrinsic errors of the microneutralization and complement fixation tests have been reported elsewhere (Rweyemamu, Pay & Parker, 1976). The *R* values obtained in the various tests are summarized in Table 5.

The strains from Tanzania (Tan 5/68) and Namibia (Swa 1/68) were similar and the *R* values obtained for this pair did not vary in all the test systems. In all the other combinations the three neutralization systems demonstrated similar degrees of differentiation, indicating wide differences between the vaccine strains tested. In contrast the complement fixation test, especially with rabbit antisera, did not demonstrate such divergencies between strains. On average *R* values obtained in the complement fixation test were approximately four times greater than those obtained with the neutralization test systems.

Probable antigenic dominance among vaccine strains

Table 6 has been derived from the data given in Table 2 and gives an expression of dominance (Stellmann *et al.* 1972) between relationships detected among the type SAT 2 vaccine strains based on the results of neutralization kinetics tests. It is evident that the Ken 3/57 strain is dominated by almost all the other strains. In contrast the Moz 1/70 and, to a lesser extent, Tan 5/68 strains appear to be dominant over the other strains. This phenomenon was investigated further by testing the cross-neutralization activity of antisera to Ken 3/57 and Moz 1/70 strains against viruses previously isolated from Eastern and Central Africa in a metabolic inhibition test, a microneutralization cytopathic endpoint test and the neutralization kinetics test. The tests were carried out with rabbit and guinea-

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pig antisera and the resultant r values are summarized in Table 7. It will be observed that in the three neutralization test systems antisera to the Ken 3/57 strain had a low cross-neutralization activity (i.e. low r values) against the other strains. In contrast the Moz 1/70 antisera had a high cross-reactivity with other strains from Eastern and Central Africa.

DISCUSSION

The results reported in this paper have demonstrated that neutralization kinetics techniques may be used quantitatively in antigenic analysis of FMD virus strains provided steps are taken to minimize variations in the kinetics slope as previously outlined (Rweyemamu *et al.* 1977). Our main objective in these and other serological studies has been to attempt to rationalize the selection of suitable vaccine strains for use mainly in Eastern and Central Africa. Because of inadequate antigenic analysis of the SAT 2 viruses that have been isolated from this region we have tended to produce vaccines from many strains from this region as far as possible using vaccine derived from each strain in the country of original isolation. As Pay & Schermbrucker (1974) demonstrated, the majority of these strains give vaccines of only marginal potency with the exception of SAT 2 Ken 3/57 which consistently produces vaccine of high potency. Our results have shown this strain to have a narrow serological range. Anderson, Anderson & Doughty (1974) demonstrated that in Kenya SAT 2 virus strains from southern Kenya were more related to the Tan 5/68 strain than the Ken 3/57 strain but those from central and northern Kenya were related to Ken 3/57 strain. There is also indication of similarity between strains from Tanzania, Mozambique and Namibia. This has raised a possibility of rationalizing the selection of strains that are suitable for a relatively wide region. To this effect a comparison of interaction of sera to Ken 3/57 and Moz 1/70 strains with other SAT 2 strains has been of interest. The results summarized in Table 7 have shown that the Moz 1/70 antisera have a high reactivity with all strains derived from Eastern and Central Africa and that the Ken 3/57 has a low reactivity with other strains. On this basis it seems that vaccines derived from the Moz 1/70 strain would be effective over a wide region of Eastern, Central and Southern Africa.

A comparison of results obtained with other tests showed that differences detected by the neutralization kinetics were of the same order as those obtained in the plaque reduction and the microneutralization c.p.e. test. The neutralization tests generally showed wider differences between strains than those shown by the complement fixation test. The specificity of the complement fixation test was further decreased when rabbit sera prepared by inoculation of animals with crude tissue culture live virus were used. This is not surprising since the CF test detects both those antigens which are involved in neutralization and those that are not (Cowan & Trautman, 1967; Forman, 1975). This has also emphasized that criteria set out for differentiating FMD strains based on the complement fixation test (Brooksby, 1968; Forman, 1975; Pereira, 1976) may not strictly apply to results obtained in neutralization test systems (Rweyemamu *et al.* 1976).

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REFERENCES

Anderson, E. C., Anderson, J. & Doughty, W. J. (1974). The foot-and-mouth disease virus subtype variants in Kenya. *Journal of Hygiene* **73**, 237–44.

Brooksby, J. B. (1968). Variants and Immunity: definitions for serological investigations. *International Symposium on Foot-and-Mouth Disease. Variants and Immunity.* Lyon 1967. *Symposium Series. Immunobiological Standardization* **8**, 1–10.

Cowan, K. M. & Trautman, R. (1967). Immunochemical studies of foot-and-mouth disease. 1. Complement fixation reactions with isolated antigenic components. *Journal of Immunology* **99**, 729–36.

De Castro, M. P. (1964). Behaviour of the foot-and-mouth disease virus in cell cultures: susceptibility of the IB-RS-2 cell line. *Archivos do Instituto Biologico, São Paulo* **31**, 63.

Forman, A. J. (1974). A study of foot-and-mouth disease virus strains by complement fixation. 1. A model for the fixation of complement by antigen/antibody mixtures. *Journal of Hygiene* **72**, 397–405.

Forman, A. J. (1975). The subtype classification of strains of foot-and-mouth disease virus. *Journal of Hygiene* **74**, 227–32.

Martin, W. B. & Chapman, W. G. (1961). The tissue culture colour test for assaying the virus and neutralizing antibody of foot-and-mouth disease and its application to the measurements of immunity in cattle. *Research in Veterinary Science* **2**, 53–61.

Pay, T. W. F. & Schermbrucker, C. G. (1974). Production of foot-and-mouth disease vaccines against various virus types and subtypes. *Bulletin. Office international des Épizooties* **81**, 1261–70.

Pereira, H. G. P. (1976). Subtyping of foot-and-mouth disease virus. *A paper presented at the International Symposium on Foot-and-Mouth Disease. International Association of Biological Standardization*, Lyon, 1976.

Rweyemamu, M. M., Booth, J. C. & Pay, T. W. F. (1977). Neutralization kinetics studies with type SAT 2 foot-and-mouth disease virus strains. I. Factors that influence the rate and pattern of neutralization. *Journal of Hygiene* **78**, 99–112.

Rweyemamu, M. M., Pay, T. W. F. & Parker, M. J. (1976). Serological differentiation of foot-and-mouth disease virus strains in relation to selection of suitable vaccine viruses. *A paper presented at the International Symposium on Foot and-Mouth Disease. International Association of Biological Standardization.* Lyon, 1976.

Stellman, C., Moreau, Y. & Roumiantzeff, M. (1972). Biomathematical system of relationship and dominance for the classification of foot-and-mouth disease strains. *Archiv für die gesamte Virusforschung* **37**, 357–64.

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