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THE SUITABILITY OF A ROLLED BHK_{21} MONOLAYER SYSTEM FOR THE PRODUCTION OF VACCINES AGAINST THE SAT TYPES OF FOOT-AND-MOUTH DISEASE VIRUS. I. ADAPTATION OF VIRUS ISOLATES TO THE SYSTEM, IMMUNOGEN YIELDS ACHIEVED AND ASSESSMENT OF SUBTYPE CROSS REACTIVITY

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ABSTRACT

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In an examination of 34 southern African SAT-type foot-and-mouth disease viruses, all but 1 attained satisfactory levels of infectivity within 6 passages in rolled BHK_{21} monolayer cell cultures. However, there were marked differences between adapted viruses with respect to the mass of immunogen (146S material) produced. Several isolates which consistently produced levels ≥ 2 $\mu g/m\ell$ were identified.

In cross neutralization tests using post-vaccinal sera, SAT–1 and SAT–2 isolates showed considerable diversity and none of the viruses tested would be expected to produce a broad-spectrum response if incorporated into a vaccine. On the other hand, when 2 of the SAT–2 isolates were incorporated into the same vaccine a distinctly broader response resulted.

INTRODUCTION

Currently, nearly all vaccines against foot-and-mouth disease (FMD) comprise concentrated and inactivated virus grown in cell or organ cultures which is adjuvanted with either aluminium hydroxide and saponin or oil emulsions. Criteria for the selection of successful vaccine strains have been detailed by Rweyemamu (1978).

In southern Africa, where the SAT types of FMD virus are almost exclusively responsible for disease outbreaks in domestic livestock, most vaccines used prior to 1980 were produced in BHK_{21} suspension cultures. Although this system is ideal for producing FMD vaccine on a large scale it is widely accepted that, in comparison with the A, O & C types, SAT type viruses adapt poorly to BHK_{21} cells grown in suspension and even where adequate adaptation is achieved, the immunogenicity of the vaccines produced is relatively poor (Pay & Schermbrucker, 1974; Mowat, Price, Owen & Taylor, 1975; Berger, Schermbrucker & Pay, 1975; Mowat, Garland & Spier, 1978; Preston, Owens & Mowat, 1982; Anderson, personal communication, 1985).

Conversely, the use of a Frenkel system (surviving bovine tongue epithelium in suspension culture) has been reported to produce SAT viral antigens whose immunogenicity is comparable with those of the A, O and C types although at least initially, there was some variability with respect to vaccine produced with SAT–2 viruses (Guinet, Falconer, Fargeaud, Dupasquier & Mannathoko, 1982). Unfortunately, however, the Frenkel system presents a logistical problem in the procurement of sufficient tongue epithelium for large-scale production.

This paper concerns the use of rolled monolayer cultures of BHK_{21} cells in a system developed in Italy (Nardelli & Panina, 1977) and which is at least adequate for the production of vaccines against A, O and C types (Panina, 1976). There have been few reports on the use of BHK_{21} monolayer cultures for the production of SAT type vaccine and none with reference to this particular system where the cells are grown up in suspension so as to obviate the necessity for trypsinization of cells required to seed monolayer cultures. Pay, Rweyemamu & O'Reilly (1978), however, have recorded that SAT type viruses are difficult to adapt to BHK_{21} monolayer cultures, and Preston *et al.* (1982) found that of 27 SAT–2 viruses tested only 1 produced titres $\geq 10^6$ pfu within 3 passages. On the other hand, there is a belief on the part of at least some vaccine manufacturers that vaccines produced from monolayer cultures are more immunogenic than those derived from BHK_{21} suspension cultures. It should be borne in mind though that both monolayer and suspension cultures of BHK_{21} cells vary between different laboratories in that they comprise mixed populations of cells with different susceptibilities to FMD virus (Clarke & Spier, 1980).

In addition to the difficulty of producing vaccines against SAT type viruses of adequate homologous immunogenicity (potency), the degree of antigenic diversity which exists within particularly the SAT–1 and SAT–2 types (Rweyemamu & Ouldridge, 1982) adds a further complication. As was pointed out by Pay (1983), it is essential to ensure that virus strains incorporated into FMD vaccine are appropriate for protecting against the subtypes prevalent in the field. However, in southern Africa, where challenge of domestic livestock is likely to be derived from contact with wild animal populations within which the degree and range of viral immunogenic variation has thus far been logistically too difficult to determine accurately, selection of appropriate vaccine strains is impossible other than to choose those strains or combinations thereof which induce a response with broad immunoligical cross reactivity.

This paper described the progress so far made towards the selection of SAT-type viruses which, when inactivated, are likely to be capable of inducing an adequate immune response with acceptable cross reactivity. Paper II will deal with aspects relating to large-scale production of vaccine virus, inactivation and adjuvanting of viral immunogens and the immune response of cattle to formulated vaccine.

MATERIALS AND METHODS

Cell cultures

Adaptation for and growth of FMD viruses for vaccine production was conducted using BHK_{21} clone 13 cells[1] which are capable of growing either as rolled monolayer cultures or in suspension. Suspension culture was used for propagating the cells prior to seeding into 1 ℓ glass bottles which were rolled in an incubator at 36 °C. Virus

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suspensions were inoculated onto confluent monolayers which comprised approximately 400 × 10^6 cells per bottle.

Vac. 1 medium, which is a modification of Eagle's MEM, was used for both growth and maintenance of the BHK_{21} monolayers—in the former instance containing 5 % normal bovine serum (NBS) and in the latter with 2 % NBS. However, when used for growth of cell-adapted virus (i.e. vaccine virus production) Vac. 1 is used without the addition of serum.

Virus titrations were performed in tube cultures of IB-RS-2 cells maintained with Vac. 1 medium and 2 % NBS.

Viruses

The strains used in this study, their origin and initial passage history are shown in Table 1.

All stocks were stored in aliquots containing 20–45 % glycerol at −70 °C.

Adaptation of virus strains to BHK_{21} cultures

Confluent monolayers were washed with phosphate buffered saline (pH 7,2) and 10 mℓ of virus suspension inoculated into each of 2 roller cultures and the culture rolled for 1 h at 37 °C. The inoculum was then poured off and replaced with 80 mℓ of maintenance medium. The bottles plus an uninoculated control were incubated overnight (approximately 22 h) after which the presence or absence of cytopathic effect (CPE) was established, using an inverted microscope. The medium from the 2 inoculated cultures was then decanted and clarified by centrifugation for 15 min at 500 *g*. The process was then repeated for a further 5 passages or until complete CPE was observed. Infectivity titres were determined using both IB-RS-2 tube cultures and suckling mice as previously described (Gainaru, Thomson, Bengis, Esterhuysen, Bruce & Pini, 1986).

Sera

Convalescent sera were obtained from cattle 21 days after intradermolingual (idl) inoculation of virus. Post-vaccinal sera were likewise obtained from cattle 21 days or, as otherwise stated, after subcutaneous inoculation with formulated vaccine which had been checked for absence of residual live virus in BHK_{21} and IB-RS-2 cell cultures as well as by idl inoculation of susceptible cattle.

Sera were stored at −20 °C until required.

Subtyping

Virus subtyping was based on cross virus neutralization tests in microtitre cultures as described previously (Esterhuysen, Thomson, Flammand & Bengis, 1985), except that each serum was tested against 4 dilutions of each virus, the dilutions being 0,5 $\log_{10}$ apart and calculated to straddle the $\log_{10}^{2.0}$ $TCID_{50}$ dose. From the results obtained a regression line was calculated and the 50 % serum end-point titre at the $\log_{10}^{2.0}$ $TCID_{50}$ level established (Rweyemamu, Booth, Head & Pay, 1977).

Relationships between viruses were calculated as r values, i.e.,

$$r = \frac{\text{serum titre against the heterologous virus}}{\text{serum titre against the homologous virus}}$$

All neutralization titre determinations were repeated to identify spurious results and, where differences $\geq \log_{10}^{0.4}$ were found the test was redone.

146S quantification

Sucrose gradients (10–36 %) centrifuged in a SW 40 rotor of a Beckman L5-65B ultracentrifuge for 1 h at 40 000 rpm and scanned at 254 nm, were used to determine the 146S content of virus preparations (Barteling & Meloen, 1974; Doel, Fletton & Staple, 1982).

Results

Virus replication in BHK_{21} cells

Thirty-three of the 34 SAT viruses listed in Table 1 replicated satisfactorily (titres $\geq 10^{6,5}$ $TCID_{50}$/mℓ) in BHK_{21} monolayer cultures by the 6th passage. In all instances, the initial multiplicity of infection (moi) was ≥0,001. The only isolate which failed in this respect was ZAM 12/81 (SAT–2) which, despite an initial moi of 0,01, showed no demonstrable infectivity after the 2nd BHK_{21} culture passage.

Less than 50 % ($^{16}/_{33}$) of the isolates which replicated satisfactorily produced complete cytolysis by the 6th passage. A further 11 (33 %) induced 50–75 % CPE while the remaining 6 (18 %) produced 10–25 % CPE (excamples shown in Table 2).

Infectivity yields (determined in IB-RS-2 cell cultures and suckling mice) varied between 0,5 and 316 $TCID_{50}$ per cell at peak levels (i.e. up to $10^{9,3}$ $TCID_{50}$/mℓ). There was no obvious correlation between the extent of CPE induced and infectivity yield (results not shown).

TABLE 1 SAT virus strains used in this study

Strain designation	SAT type	Species of origin	Passage history of starting material
SAR 1/73[1]	1	Impala	RS_2, BTY_1, RS_2
SAR 4/74[1]	1	Cattle	BHK_3, B_1
SAR 22/74[1]	1	Impala	RS_2
SAR 29/74[1]	1	Impala	RS_2
SAR 2/75[1]	1	Impala	BTY_4, RS_2
SAR 7/75[1]	1	Impala	BTY_1, RS_2
SAR 3/77[1]	1	Cattle	B_2
SAR 4/79[1]	1	Cattle	B_1
SAR 17/80[1]	1	Cattle	B_1
SAR 5/81[1]	1	Impala	CK_1, RS_2
SAR 9/81[1]	1	Cattle	BTY_3, BHK_3, RS_2
KNP 2/86/1	1	Buffalo	BTY_1
KNP 3/86/1	1	Buffalo	BTY_1
KNP 6/86/1	1	Buffalo	BTY_1
BOT 1/68[1]	1	Cattle	BHK_3, B_1
BOT 1/77[1]	1	Cattle	BHK_2, B_1
MOZ 3/77[1]	1	Cattle	BHK_1, B_1
MAL 1/85/1	1	Buffalo	CFK_1, BTY_1, CFK_1
SAR 3/77[1]	2	Cattle	B_2
SAR 3/79[1]	2	Cattle	B_1
SAR 2/81[1]	2	Cattle	B_1
PHALAB 1–6/83/2 (SAR 5/83)[2]	2	Cattle	B_1
SAR 16/83[1]	2	Impala	CK_1, BHK_2, RS_1
KNP 1/85/2	2	Impala	RS_2
KNP 7/86/2	2	Buffalo	BTY_1, RS_2
BOT 3/77[1]	2	Cattle	B_1
MOZ 4/78[1]	2	Cattle	B_1
MOZ 4/83[2]	2	Cattle	B_1
RHO 2/79[2]	2	Cattle	B_1
ZIM 7/83[2]	2	Cattle	B_1
ZAM 12/81[2]	2	Cattle	B_1
SAR 1/80[1]	3	Cattle	B_1
BEC 1/65[1]	3	Cattle	BHK_5, B_1
RHO 3/78[1]	3	Cattle	BHK_1, B_1

RS — IB–RS–2 cell cultures
BTY — Bovine foetal thyroid cell cultures
B_1 — Bovine tongue
CK — Calf kidney cell cultures
CFK — Calf foetal kidney cultures
[1] — Viruses supplied by The World Reference Laboratory, Pirbright, U.K.
[2] — Viruses supplied by The Botswana Vaccine Institute, Gaborone, Botswana

TABLE 2 Examples of the cytopathic effect induced as well as infectivity and 146S yields achieved during attempted adaptation of SAT isolates to rolled BHK_{21} cultures

Strain designation (type)	Passage level	Cytopathic effect[1]	IB-RS-2 titre[2] (moi)	Mouse titre[2] (moi)	146S (μg/mℓ)
SAR 2/75 (SAT–1)	B; BTY_4; RS_2	NA	6,7(0,01)	7,2(0,03)	ND
	B; BTY_4; RS_2 BHK_1	0	5,0	5,8	ND
	B; BTY_4; RS_2 BHK_2	0	5,2	5,1	ND
	B; BTY_4; RS_2 BHK_3	0	6,0	6,2	ND
	B; BTY_4; RS_2 BHK_4	0	6,7	6,6	ND
	B; BTY_4; RS_2 BHK_5	1	7,5	8,0	<0,3
	B; BTY_4; RS_2 BHK_6	2	7,5	8,1	<0,3
SAR 16/83 (SAT–2)	MALA; CFK; BHK_2; RS_2	NA	5,7(0,001)	6,8(0,013)	ND
	MALA; CFK; BHK_2; RS_2 BHK_1	0	4,2	6,1	ND
	MALA; CFK; BHK_2; RS_2 BHK_2	1	4,7	5,8	ND
	MALA; CFK; BHK_2; RS_2 BHK_3	2	5,5	ND	ND
	MALA; CFK; BHK_2; RS_2 BHK_4	2	6,2	5,2	ND
	MALA; CFK; BHK_2; RS_2 BHK_5	1	5,2	7,0	2,7
	MALA; CFK; BHK_2; RS_2 BHK_6	2	6,0	8,3	3,8
KNP 7/86/2 (SAT–2)	BUF; BTY_1; RS_2	NA	5,7(0,001)	5,8(0,001)	ND
	BUF; BTY_1; RS_2 BHK_1	0	6,0	5,7	<0,3
	BUF; BTY_1; RS_2 BHK_2	0	6,2	6,8	<0,3
	BUF; BTY_1; RS_2 BHK_3	1	7,2	7,5	<0,3
	BUF; BTY_1; RS_2 BHK_4	1	6,7	6,0	<0,3
	BUF; BTY_1; RS_2 BHK_5	2	6,7	6,4	<0,3
	BUF; BTY_1; RS_2 BHK_6	2	6,5	5,3	<0,3
SAR 1/73 (SAT–1)	RS_2; $BTY_1$1; RS_2	NA	6,2(0,04)	4,2(<0,001)	ND
	RS_2; $BTY_1$1; RS_2 BHK_1	1	6,7	4,3	0,3
	RS_2; $BTY_1$1; RS_2 BHK_2	2	6,2	<3,0	ND
	RS_2; $BTY_1$1; RS_2 BHK_3	2	8,2	<3,0	ND
	RS_2; $BTY_1$1; RS_2 BHK_4	2	6,7	<3,0	ND
	RS_2; $BTY_1$1; RS_2 BHK_5	4	5,5	<3,0	0,8
	RS_2; $BTY_1$1; RS_2 BHK_6	4	7,5	<3,0	0,4

[1] — Arbitrary scale, 0–4
[2] — Log_{10} $TCID_{50}$/mℓ
B — Bovine
BUF — Buffalo
BTY — Bovine foetal thyroid cells
RS_2 — IB–RS–S cells

TABLE 3 Correlation between virus titre and 146S values in vaccine production batches for 3 viruses

Run No.	BOT 1/77		ZIM 7/83		BOT 3/77	
	Virus titre[1]	146S[2]	Virus titre[1]	146S[2]	Virus titre[1]	146S[2]
1	7,4	1,8	7,8	3,0	8,4	4,0
2	7,4	1,6	7,2	3,8	8,4	3,8
3	6,4	1,2	6,9	3,3	8,4	4,6
4	8,2	2,8	7,8	3,3	8,8	2,0
5	9,2	2,4	7,8	4,1	8,0	5,4
6	8,6	3,3	7,4	3,5		
7	7,8	3,0	7,4	2,8		
8	8,6	3,4				
9	8,6	3,7				
10	7,8	2,4				
11	7,0	1,9				
12	8,0	2,0				
13	7,6	2,2				
14	7,2	1,9				
15	7,2	3,0				
Mean ± SD	7,8[3] ± 0,74	2,44[4] ±0,73	7,47 ± 0,35	3,40 ± 0,45	8,40 ± 0,25	3,96 ± 1,26
Cor. coeff.	0,69		0,05		−0,95	

[1] — Log_{10}/mℓ
[2] — μg/mℓ
[3] — Geometric mean
[4] — Arithmetic mean

Although there was generally close agreement between infectivity yield determined by titrations performed in IB-RS-2 cells and suckling mice, there were isolates which showed marked and repeated discrepancies in this regard (see SAR 1/73, Table 2).

Production of 146S particles

Since the 146S detection system was not operational at the time when adaptation attempts began, data are not available for all the strains used. However, as can be seen from Tables 2, 3 and 4, there was considerable variation with respect to the ability of different viruses, once adapted to growth in BHK_{21} cells, to produce 146S particles. Viruses, selected as potentially useful vaccine strains on the basis of growth characteristics, 146S production and virion stability (see Paper II), regularly produced 146S values >2 μg/mℓ (Table 3). At the other extreme, 2 isolates failed to provide any detectable 146S material in culture harvests which contained high levels of infectivity, viz. up to $10^{8.1}$ $TCID_{50}$/mℓ (Table 2). Other isolates provided intermediate values (Table 4). There was, overall, no correlation between infectivity and 146S yield (Table 3).

TABLE 4 146S values produced by some SAT isolates screened as possible vaccine strains

Isolate designation	SAR 17/80 (SAT–1)	SAR 3/77 (SAT–2)	SAR 2/81 (SAT–2)	PHALAB/ 1–6/83/2 (SAT–2)	MOZ 4/78 (SAT–2)	RHO 2/79 (SAT–2)	BEC 1/65 (SAT–3)	SAR 1/80 (SAT–3)	Test control[1] (ZIM 7/83)
No. of determinations	16	13	4	3	5	6	5	15	29
Mean ($\mu g/m\ell$)	1,40	2,60	0,78	0,70	0,76	0,95	1,52	1,18	3,15
Standard deviation	0,43	1,47	0,34	0,17	0,27	0,21	0,13	0,29	0,50

[1]Aliquots of this virus were stored at −70 °C and 146S values measured (usually in duplicate) twice weekly to provide an external standard

TABLE 5 "r" values of southern African SAT–1 viruses determined using convalescent[1] cattle sera

Antisera: virus against which the antiserum was produced	Viruses								
	BOT 1/77	SAR 4/74	SAR 4/79	SAR 17/80	SAR 9/81	KNP 2/86/1	BOT 1/68	MOZ 3/77	MAL 1/85/1
BOT 1/77 (3,37)[2]	1,00	0,46	0,98	0,96	0,64	0,69	0,24	0,19	0,15
BOT 1/77 (3,28)	1,00	0,77	0,72	1,03	0,53	1,06	0,51	0,24	**0,45**
SAR 4/74 (3,39)	0,03	1,00	0,03	0,18	0,08	0,05	0,05	0,01	0,02
SAR 4/74 (3,36)	0,03	1,00	0,05	0,33	0,06	0,16	0,03	0,06	0,10
SAR 4/79 (3,22)	0,13	0,30	1,00	0,59	0,72	0,27	0,35	0,15	0,02
SAR 4/79 (3,35)	0,24	0,31	1,00	0,60	0,27	0,45	**0,06**	0,12	0,01
SAR 17/80 (3,35)	0,30	0,41	0,25	1,00	0,52	0,57	0,42	0,13	0,17
SAR 17/80 (3,24)	0,17	0,86	0,22	1,00	0,74	0,55	0,08	0,10	0,12
SAR 17/80 (3,32)	0,31	0,69	0,62	1,00	ND	ND	**0,27**	0,09	**0,04**
BOT 1/68 (3,16)	0,20	0,40	0,19	0,33	0,54	0,38	1,00	0,25	0,07
BOT 1/68 (3,26)	0,14	0,21	0,15	0,18	ND	ND	1,00	0,16	0,06
MOZ 3/77 (2,92)	0,51	1,36	0,81	1,67	ND	ND	0,88	1,00	0,09
MOZ 3/77 (2,81)	0,46	1,91	0,31	1,99	1,88	1,07	0,47	1,00	0,11
MAL 1/85/1 (3,16)	0,17	0,44	0,19	0,38	ND	ND	0,12	0,02	1,00
MAL 1/85/1 (3,11)	0,44	**1,90**	**1,50**	**1,80**	0,19	1,06	**0,93**	**0,21**	1,00

[1] — Obtained 3 weeks after infection
[2] — Log_{10} VN_{50} titre against the homologous virus
0,45 — Denotes reactions where the 2 antisera to a specific virus provided results with ≥3-fold difference

TABLE 6 "r" values of southern African SAT–1 viruses determined using sera from cattle vaccinated once with BOT 1/77 vaccine

Serum No.	Viruses				
	BOT 1/77	SAR 4/74	SAR 4/79	SAR 17/80	MAL 1/85/1
1 (2,3)[1]	1,00	0,09	0,37	0,13	0,09
2 (2,4)	1,00	0,03	0,22	0,06	0,08
3 (2,4)	1,00	0,06	0,24	0,08	0,08
4 (2,3)	1,00	0,08	0,27	0,14	0,12
5 (2,2)	1,00	0,13	0,33	0,18	0,17
6 (2,3)	1,00	0,04	0,35	0,22	0,11
Mean ± SD	1,00	0,07	0,30	0,14	0,11

[1] — Log_{10} VN_{50} titre against BOT 1/77 (homologous reaction)

Immunogenic variation within SAT–1 and SAT–2 types

For both SAT–1 (Table 5) and SAT–2 (Table 7) viruses, cross neutralization tests performed using convalescent cattle sera demonstrated good cross reactivity (r ≥0,25) against most of the viruses tested, with 1 exception in each case, viz., SAR 4/74 for SAT–1 and BOT 3/77 for SAT–2. Convalescent antisera to SAR 4/74 failed to neutralize the heterologous SAT–1 strains satisfactorily (r ≤0,25) with the exception of SAR 17/80 (mean r = 0,25) (Table 5), while BOT 3/77 antiserum had r values against heterologous SAT–2 strains ≤0,1 (Table 7). Conversely, amongst the SAT–1 viruses tested, MOZ 3/77 and MAL 1/85/1 were not effectively neutralized by antisera to any of the other SAT–1 viruses (Table 5).

In some instances the r values provided by the 2–4 antisera to an individual virus were appreciably (≥3-fold) different. In 2 cases (MAL 1/85/2 in Table 5 and ZIM 7/83 in Table 7) there were consistent differences between 2 or more antisera.

In contrast to the above results, antisera derived from cattle which had been inoculated with inactivated BOT 1/77 (Table 8) showed much narrower cross reactivities than corresponding convalescent antisera (Tables 5 & 7). For example, while convalescent antisera to BOT 1/77 neutralized SAR 17/80 virus effectively (r ≃ 1,0 Table 5), post-vaccinal serum to BOT 1/77 was much less effective against SAR 17/80 (mean r = 0,14, Table 6), i.e. there was >sevenfold difference between the 2 types of antisera. Likewise, the mean difference between the r values of ZIM 7/83 antisera against PHALAB 1–6/83/2, using the 2 different types of antiserum was sixfold (deduced from values shown in Tables 7 & 8).

Overall, therefore, convalescent cattle sera were consistently more cross reactive than sera derived from immunized cattle. However, as shown in Table 9,

TABLE 7 "r" values of southern African SAT–2 viruses determined using convalescent [1] cattle sera

Antisera: virus against which the antiserum was produced	Viruses								
	ZIM 7/83	BOT 3/77	SAR 3/77	SAR 3/79	SAR 2/81	SAR 5/83	KNP 1/85/2	MOZ 4/83	RHO 2/79
ZIM 7/83 (3,20)[2]	1,0	0,03	0,51	0,49	0,56	1,03	1,03	0,60	1,03
ZIM 7/83 (3,09)	1,0	0,02	0,30	0,30	0,11	0,55	0,11	0,47	0,60
ZIM 7/83 (3,20)	1,0	0,01	0,06	0,07	ND	0,30	0,73	0,33	0,42
ZIM 7/83 (3,18)	1,0	0,03	**0,04**	**0,10**	ND	**0,30**	**0,76**	1,02	**0,30**
BOT 3/77 (3,31)	0,03	1,0	0,03	0,03	0,03	0,06	ND	0,01	0,03
BOT 3/77 (3,04)	0,05	1,0	0,04	0,06	0,04	0,10	ND	0,02	0,06
SAR 3/77 (2,74)	0,15	0,16	1,0	0,35	0,24	0,44	ND	0,52	0,81
SAR 3/77 (2,60)	**0,67**	0,25	1,0	0,39	0,29	0,86	ND	0,28	1,22
SAR 3/79 (3,03)	0,22	0,15	0,16	1,0	0,23	0,83	ND	0,26	0,79
SAR 3/79 (2,84)	0,30	**0,62**	**0,66**	1,0	0,29	2,0	ND	0,21	1,03
SAR 2/81 (2,54)	0,39	0,30	0,35	0,42	1,0	0,63	ND	0,66	2,39
SAR 2/81 (2,52)	0,31	0,84	0,32	0,81	1,0	1,33	ND	0,44	2,33
SAR 5/83 (3,53)[3]	0,25	0,38	0,51	0,55	0,22	1,0	ND	0,67	0,63
MOZ 4/83 (2,86)	0,50	0,32	0,29	0,89	0,28	0,40	ND	1,0	2,05
MOZ 4/83 (2,86)	0,64	0,66	0,29	0,73	0,48	0,72	ND	1,0	0,94
RHO 2/79 (2,39)	0,18	0,12	0,12	0,14	0,28	0,63	ND	0,83	1,0
RHO 2/79 (2,44)	0,11	0,11	0,18	0,19	0,25	0,27	ND	**0,16**	1,0

[1] — Obtained 3 weeks after infection

[2] — Log_{10} VN_{50} titre against the homologous virus

[3] — Pool of 4 sera

0,04 — Denotes reactions where the 2–4 antisera to a specific virus provided results with ≥3-fold difference

TABLE 8 "r" values of southern African SAT–2 viruses determined using sera of cattle immunized twice with monovalent ZIM 7/83 vaccine

Serum No.	Viruses							
	ZIM 7/83	BOT 3/77	SAR 3/77	SAR 3/79	SAR 5/83	MOZ 4/83	RHO 2/79	KNP 1/85/2
1 (2,84)[1]	1,0	0,02	0,02	0,02	0,07	0,06	0,06	0,04
2 (2,62)	1,0	0,01	0,03	0,02	0,11	0,04	0,15	0,03
3 (2,39)	1,0	0,03	0,03	0,03	0,18	0,31	0,36	0,09
4 (2,49)	1,0	0,05	0,09	0,08	0,06	0,31	0,13	0,15
5 (2,74)	1,0	0,01	0,03	0,05	0,03	0,07	0,05	0,06
6 (2,54)	1,0	0,05	0,02	0,04	0,10	0,17	0,15	0,42
7 (2,48)	1,0	0,06	0,08	0,03	0,09	0,08	0,13	0,06
Mean ± SD	1,0±0	0,03±0,02	0,04±0,03	0,04±0,02	0,09±0,05	0,15±0,12	0,15±0,10	0,12±0,14

[1] — Log_{10} VN_{50} titre against ZIM 7/83

administering vaccine containing 2 SAT–2 viruses (BOT 3/77 and ZIM 7/83) resulted in greater r values. Taking the examples used above, a mean r value of 0,09 was obtained by ZIM 7/83 post-vaccination sera against PHALAB 1–6/83/2 (Table 8). When BOT 3/77, which had the narrowest cross reactivity of all the SAT–2 viruses (Tables 7 & 8), was incorporated into the vaccine with ZIM 7/83, the resulting mean r values against PHALAB 1–6/83/2 were 0,92 and 1,76 (Table 9). The sera obtained from cattle which had been vaccinated twice with the bivalent vaccine were not appreciably more cross reactive than those of cattle inoculated once only (Table 9).

Discussion

Recommended characteristics for FMD virus vaccine strains (Rweyemamu, 1978) include: rapid and regular growth in the cell system with high virus yields, high content of immunizing antigen in virus harvests and appropriate serological (subtype) specificity.

Ideally, peak titres should be reached within 24 h and be at least $10^{6.5}$ pfu/mℓ with cell survival less than 50 % at that time (Rweyemamu, 1978). These recommendations, however, give no minimum acceptable value for the 146S content for suitable vaccine strains, presumably because different virus types vary with regard to 146S production potential. For example, type C viruses, which are generally good immunogens, are also relatively poor 146S producers (Panina, G. F., personal communication, 1981).

The selection system employed in this investigation (i.e. rapid passage of the original inoculum) was designed to provide vaccine stocks which replicate rapidly and resulted in 33 of the 34 virus isolates tested (Table 1) reaching titres $\geq 10^{6.5}$ $TCID_{50}$/mℓ of culture harvest by the 6th passage within 22 h. Thus in this respect the success rate was 97 %. The number of culture passages was deliberately limited to 6 in order to minimize changes in growth characteristics and antigenic structure which has been shown to occur during limited passage in BHK cells (Van Bekkum, 1978; Pay, 1983; Brown, 1985).

Although the basis for the recommendation that cell survival at 24 h should be less than 50 % is not clear, since there is no obvious correlation between CPE and virus titre or 146S production (for examples see Table 2), all but 6 (i.e. 81 %) of the isolates which replicated satisfactorily fulfilled this requirement.

The above results differ from those reported by Pay *et al.* (1978) and Preston *et al.* (1982), where only 3/52 (6 %) and 21/58 (36 %) of the SAT–1, 2 and 3 isolates investigated adapted adequately to growth in BHK monolayer cell cultures. The reason for these discrepancies is not clear, although they may reflect differences in BHK_{21} cultures between different laboratories as

TABLE 9 "r" values of southern African SAT-2 virus isolates determined using sera from cattle immunized with vaccine containing both ZIM 7/83 and BOT 3/77 viruses

No.	Antiserum to.	Homologous titre	Viruses							
			ZIM 7/83	BOT 3/77	SAR 3/77	SAR 3/79	SAR 5/83	MOZ 4/83	RHO 2/79	KNP 1/85/2
1	ZIM 7/83	2,50[1]	1,00	0,64	0,09	0,09	1,29	0,08	0,14	0,06
2	ZIM 7/83	2,10	1,00	0,20	0,11	0,36	1,03	0,23	0,26	0,17
3	ZIM 7/83	1,62	1,00	3,19	0,43	0,33	0,76	0,86	0,33	0,24
4	ZIM 7/83	2,33	1,00	1,50	0,05	0,11	0,53	0,12	0,11	0,05
5	ZIM 7/83	1,73	1,00	1,00	0,30	0,67	1,00	0,30	0,31	0,28
	Mean ± SD		1,00±0	1,31±1,16	0,20±0,16	0,31±0,23	0,92±0,29	0,32±0,32	0,23±0,10	0,16±0,10
6	ZIM 7/83	2,51	1,00	3,81	0,59	1,07	2,22	0,89	1,48	1,07
7	ZIM 7/83	3,07	1,00	1,23	0,09	0,62	1,23	0,35	0,81	0,25
8	ZIM 7/83	2,98	1,00	1,09	0,10	0,41	0,68	0,24	0,36	0,16
9	ZIM 7/83	3,10	1,00	0,80	0,09	0,51	0,53	0,30	0,78	0,15
10	ZIM 7/83	2,88	1,00	1,56	0,10	0,40	1,92	0,35	0,37	0,29
	Mean ± SD		1,00±0	1,70±1,21	0,19±0,22	0,60±0,28	1,32±0,74	0,43±0,26	0,76±0,46	0,38±0,39
1	BOT 3/77	2,30	1,57	1,00	0,14	0,14	2,03	0,12	0,22	0,10
2	BOT 3/77	1,40	5,04	1,00	0,86	1,80	5,20	1,16	1,32	0,84
3	BOT 3/77	2,13	0,31	1,00	0,13	0,10	0,24	0,27	0,13	0,07
4	BOT 3/77	2,50	0,66	1,00	0,03	0,07	0,35	0,08	0,11	0,03
5	BOT 3/77	1,73	1,00	1,00	0,30	0,67	1,00	0,30	0,31	0,28
	Mean ± SD		1,72±1,92	1,00±0	0,29±0,33	0,56±0,74	1,76±2,05	0,39±0,44	0,42±0,51	0,26±0,36
6	BOT 3/77	3,09	0,26	1,00	0,16	0,28	0,58	0,24	0,39	0,28
7	BOT 3/77	3,16	0,81	1,00	0,07	0,05	1,00	0,29	0,66	0,21
8	BOT 3/77	3,02	0,91	1,00	0,09	0,37	0,62	0,22	0,33	0,15
9	BOT 3/77	3,02	1,24	1,00	1,00	0,64	0,66	0,37	0,98	0,19
10	BOT 3/77	3,07	0,64	1,00	0,09	0,26	1,23	0,22	0,22	0,18
	Mean ± SD		0,77±0,36	1,00±0	0,28±0,40	0,32±0,21	0,82±0,28	0,27±0,06	0,52±0,31	0,20±0,05

No. 1–5 — Cattle vaccinated once only
No. 6–10 — Cattle vaccinated twice with a 3-week interval
For ZIM 7/83 — There is sig. diff. ($P \leq 0,05$) between 1 and 2 inoculations
For BOT 3/77 — No sig. diff. between 1 and 2 inoculations (two way analysis of variance)
[1] — $Log_{10} VN_{50}$
SD — Standard deviation

described by Clarke & Spier (1980). Additionally, in our investigation, 6 passages were undertaken, whereas in the 2 quoted above only 3 were conducted.

The production of 146S particles by different strains varied independently of infectivity titre between ≤0,3 and 6,5 $\mu g/m\ell$ (Tables 2, 3 & 4). Furthermore, although statistically significant differences between different viruses could not be demonstrated with respect to 146S production because of large variances, the fact that with 2 strains (SAR 2/75 and KNP 7/86/2, Table 2) no detectable 146S material was present in the culture fluid while others (e.g. ZIM 7/83 and BOT 3/77, Table 3) consistently produced levels ≥2 $\mu g/m\ell$, suggests that 146S production capacity is inherent in individual strains in this system.

Why high titre virus peparations should contain no detectable 146S particles is difficult to explain. A possibility is that such strains are unusually labile and the virus disintegrated during density gradient centrifugation.

Taking the ability of strains to achieve 146S levels ≥2 $\mu g/m\ell$ as an arbitrary dividing line between good and poor 146S producers resulted in 6/15 (40 %) of the isolates being designated "good" (Tables 2, 3 & 4). Since 146S particle content is the only accepted *in vitro* measure of vaccine virus immunogenic potential and because there is a clear dose response association between 146S values and serum neutralizing antibody (Rweyemamu, Black, Boge, Thorne & Terry, 1984), only viruses whose 146S potential was ≥2 $\mu g/m\ell$ were considered as worthy of further investigation as potential vaccine strains.

The practical implications of subtype variation in relation to FMD vaccines have been described by Pay (1983). In southern Africa, this problem is particularly acute because the extent and rate of change in the antigenic make-up of virus populations circulating in wild animals, the (presumed) usual source of infection for domestic livestock in this region, has not been investigated in any detail.

The most striking aspect of the serological relationships investigated in this study was the remarkable discrepancy in results obtained using convalescent sera as opposed to those obtained from cattle immunized once or twice, i.e. in general convalescent sera showed broader cross reactivity than did sera from immunized cattle (compare Tables 5 & 7 with Tables 6 & 8). Furthermore, convalescent sera obtained from different cattle inoculated with the same virus preparation sometimes differed considerably in their subtype cross-reactivity (Tables 5 & 7). Thus isolated BOT 1/77 (SAT–1) and ZIM 7/83 (SAT–2), which were selected as potential vaccine strains on the basis of growth characteristics, infectivity titres and 146S production levels, as well as the broad cross reactivity of convalescent antisera against them, failed in the latter respect when inactivated virus (vaccine) was used to produce the antisera (Tables 6 & 8). This was despite the fact that these 2 viruses, when inactivated, induced good homologous antibody responses (Tables 6 & 8). On the basis of these results vaccines produced using these 2 viruses could not be expected to induce a broad immune response to either SAT–1 or SAT–2 viruses. Table 9, however, demonstrates that when inactivated BOT 3/77 was incorporated into SAT–2 vaccine together with ZIM 7/83 there was a broadening of serological cross reactivity against other SAT–2 strains (Table 8), i.e. in this respect the 2 viruses behaved synergistically. This is interesting in view of the fact that BOT 3/77 has a notoriously narrow antigenic spectrum (Table 7; Pay *et al.*, 1978).

Although there are no recent published results with which to compare ours, Guillemin, Mannathoko & Mosienyane (1986) reported remarkably different intratypic cross reactivity of antisera obtained from cattle immunized with ZIM 7/83. Thus they obtained r values against RHO 2/79 and KNP/1/85/2 (their nomenclature RSA 11/85) of 0,96 and 0,37 respectively as opposed to our figures of 0,15 and 0,12 (Table 8). On the basis of the results of Guillemin *et al.* (1986) one would reach a different conclusion from that suggested by ours.

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