

Genome variation in the SAT types of foot-and-mouth disease viruses prevalent in buffalo (*Syncerus caffer*) in the Kruger National Park and other regions of southern Africa, 1986–93

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SUMMARY

Dideoxy nucleotide sequencing of a portion of the 1D gene of SAT-type foot-and-mouth disease viruses (FMDV) was used to derive phylogenetic relationships between viruses recovered from the oesophageo-pharyngeal secretions of buffalo in the Kruger National Park as well as several other wildlife areas in southern Africa. The three serotypes differed from one another by more than 40% while intratypic variation did not exceed 29%. Within each type, isolates from particular countries were more closely related to one another than to isolates from other countries lending credence to previous observations that FMDV evolve independently in different regions of the subcontinent.

INTRODUCTION

Foot-and-mouth disease (FMD) is an economically important disease of cloven-hoofed livestock in many parts of the world although it has been eradicated from North America and western Europe. In southern Africa its importance derives primarily from the constraints it places on the export of agricultural products of animal origin to the developed world (1).

Although there is no direct proof, it is generally accepted on circumstantial grounds that African buffaloes (*Syncerus caffer*) are the major source of FMD virus infection for domestic livestock in southern Africa (2,3). Not only are these infections established in buffalo populations throughout southern Africa (4) but a small isolated herd of animals maintained infection over a period of 24 years (5), showing that SAT (Southern African Territories) type viruses can be maintained by buffalo alone. In individual buffalo recovered from the acute stage of infection, which is usually asymptomatic, the SAT viruses may persist in the pharynx for periods of at least 5 years and probably longer (5). These animals are usually

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referred to as 'carriers' although their ability to transmit SAT viruses to either other buffalo or cattle is limited (6,7). Transmission to cattle has nevertheless been demonstrated (8,9). Furthermore, it is likely that these infections are maintained actively in breeding herds of buffalo and that the dynamics by which this occurs is determined to a large extent by fluctuations in the proportion of susceptible individuals in the herd (3). The proportion of susceptibles, however, is influenced by a variety of demographic variables as well as antigenic differences between the three virus types (SAT-1, 2 and 3) and also by intratypic antigenic variation.

It has been demonstrated that intratypic variation within SAT-2 virus isolates made from buffalo in the Kruger National Park (KNP) exists (3), but the extent of the variation within this, and the other two SAT types, is not known because of the difficulties of collecting a representative sample of buffalo viruses for testing.

Comparison of the sequence homology within a portion of the 1D gene of SAT-2 viruses obtained from epizootics of FMD involving species other than buffalo has shown that recent outbreaks of FMD in Zimbabwe, Namibia and South Africa were caused by dissimilar viruses (1). Similar geographic clustering has been demonstrated for type A FMDV from the Middle East (10), type C over a period of 60 years in Europe, South America and the Philippines (11) and also for wild isolates of poliovirus types 1 and 3 (12,13).

There is good evidence that the 1D gene codes for a major antigenic region of FMDV (14,15) as well as a protein that is important in the process of cell attachment (16,17). The regions of 1D responsible for its immunogenicity were identified between residues 141–160 (the so-called GH or FMDV loop) and the other at the C terminus (residues 200–213) (18–20).

The availability of a reasonable number of buffalo isolates from the KNP and other widely separated localities in southern Africa prompted us to compare the sequence homology of SAT type viruses over the region of the 1D gene in order to determine the extent of the variation in SAT viruses in different buffalo populations and to use this data to estimate antigenic variation between isolates within and between different localities.

MATERIALS AND METHODS

Geographic location of buffalo which were sampled

Most of the virus isolates were made from buffalo in the KNP which is situated in the north-eastern Transvaal (South Africa) (Fig. 1). These isolates were designated by the prefix KNP. A few isolates were also obtained from the Caprivi Strip of Namibia (designated NAM), the Kafue National Park in southern Zambia (ZAM) and the Hwange National Park in the north western Zimbabwe (ZIM). The precise locations are shown in Figure 1 and described in Table 1.

The Zimbabwean isolates were from young buffalo in the Hwange National Park caught to establish a FMDV-free herd. The first group was caught early in 1988 and kept under quarantine in pens in the Park. However, a few animals became infected, and SAT-2 virus was isolated from probangs (ZIM 1/88/2 and ZIM 7/88/2). The positive animals were removed, but the infection occurred again

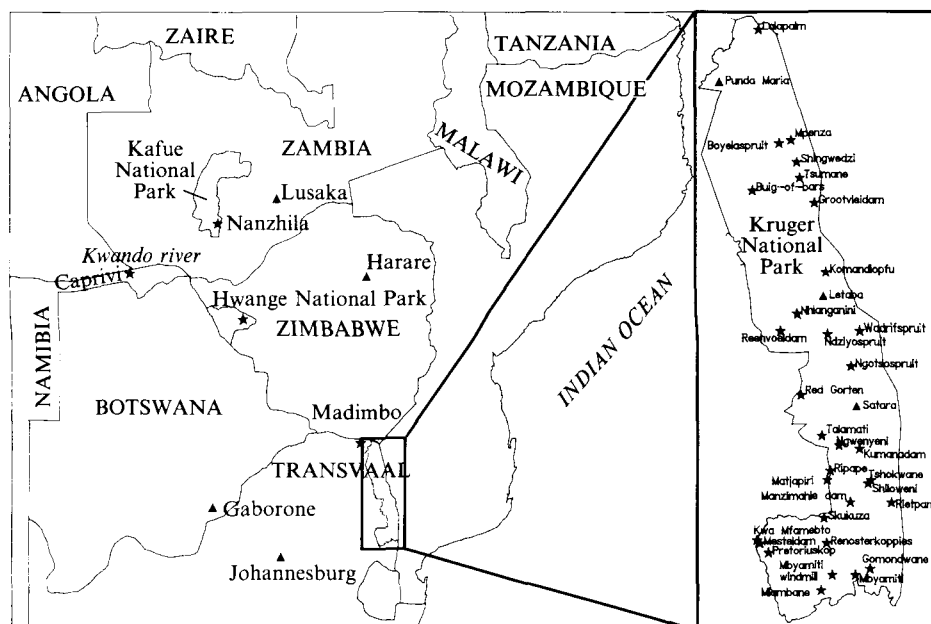


Fig. 1: Geographic location of the isolates sequenced in this study.

*. Virus isolate (see Table 1).

in the remaining animals (ZIM 1/89/2, ZIM 3/89/2 and ZIM 6/89/2) during later months. All the animals were released and the whole exercise was repeated during 1989. Again, some animals had lost their maternal immunity sufficiently to be infected and ZIM 32/89/2 was isolated on that occasion.

Four SAT-3 isolates from the Madimbo Strip on the border between SA and Zimbabwe were obtained from buffaloes which are separated from the KNP buffalo by a game-proof fence. These buffaloes were sampled after an outbreak of SAT-3 occurred in cattle at Chikwarakwara (Zimbabwe) i.e. an area adjacent to the Madimbo Strip. These viruses were designated NTV (northern Transvaal-1/91/3, 2/91/3, 3/91/3 and 4/91/3).

Viruses

Oesophageo-pharyngeal (O/P) specimens (probangs) were collected using 'probang cups' (21). The buffalo were mostly between 8 months and 2.5 years (22). Isolates were obtained during routine culling in the KNP and from animals anaesthetized in the Caprivi Strip, Zambia and Zimbabwe. The viruses were isolated on primary bovine thyroid or pig kidney cells and then stored at -70°C (stock virus).

Partial purification of FMDV isolates, RNA extraction and sequence analysis

The methods for passaging of stock viruses, the partial purification of the viruses, RNA extraction and the dideoxy-sequencing procedure for RNA templates have been described (1) as has the sequence of the primer and its location on the genome (23). The dendrograms of the nucleotide sequences were

Table 1. *Virus isolates included in this study*

Virus Designation	Date of isolation	Geographical origin		Extent of area sequenced
SAT-1				
KNP 2/86/1	14/03/86	31° 51' E-24° 47' S	Tshokwane	469-679
KNP 8/86/1	14/03/86	31° 51' E-24° 47' S	Tshokwane	475-673
KNP 6/86/1	14/03/86	31° 51' E-24° 47' S	Tshokwane	475-679
KNP 10/86/1			KNP	475-684
KNP 11/88/1	26/09/88	31° 50' E-24° 48' S	Shiloweni	426-683
KNP 13/88/1	19/10/88	31° 50' E-24° 48' S	Shiloweni	469-685
KNP 3/89/1	29/06/89	31° 58' E-24° 54' S	Rietpan	426-685
KNP 8/89/1	06/12/89	31° 13' E-25° 07' S	Mesteldam	430-687
KNP 15/89/1	20/10/89	31° 13' E-25° 07' S	Mesteldam/Mbyaniti	427-687
KNP 20/89/1	17/10/89	31° 12' E-25° 06' S	Kwa Mfamebto	478-684
KNP 26/89/1	17/10/89	31° 12' E-25° 06' S	Kwa Mfamebto	457-684
KNP 27/89/1	18/10/89	31° 34' E-25° 22' S	Mlambane	442-684
KNP 29/89/1	20/10/89	31° 40' E-24° 36' S	Ngwenyeni	442-684
KNP 32/89/1	14/11/89	31° 35' E-24° 59' S	Skukuza	460-684
KNP 39/89/1	20/10/89	31° 40' E-24° 36' S	Ngwenyeni	469-683
KNP 1/90/1	27/06/90	31° 20' E-24° 00' S	Reënvoëldam	457-684
KNP 27/91/1	14/06/91	31° 44' E-24° 54' S	2 km south of Manzimahle dam	462-690
KNP 39/91/1	10/06/91	31° 51' E-25° 15' S	Gomondwane windmill	463-684
KNP 139/91/1	09/07/91	31° 27' E-24° 20' S	Red Gorten windmill	431-693
KNP 148/91/1	25/07/91	31° 36' E-25° 07' S	Renosterkoppies	460-690
KNP 172/91/1	16/07/91	31° 26' E-23° 55' S	Upper reaches - Nhlanganini	465-684
KNP 196/91/1	04/06/91	31° 38' E-25° 17' S	Mbyamiti windmill	478-689
KNP 27/92/1	12/06/92	31° 27' E-25° 12' S	Tsumane, Shingwedzi	466-687
KNP 29/92/1	18/06/92	31° 13' E-22° 25' S	Lalapalm, Pafuri	469-684
KNP 36/92/1	23/06/92	31° 24' E-23° 00' S	Mpenza windmill, Shingwedzi	463-689
KNP 45/92/1	25/06/92	31° 32' E-23° 20' S	Grootvleidam, Shingwedzi	431-691
KNP 26/93/1	10/08/92	31° 11' E-23° 16' S	Buig-of-bars windmill	485-686
NAM 2/89/1	30/08/89	23° 20' E-27° 50' S	West of Kwando river, Caprivi	404-683
ZAM 2/93/1	21/05/93	26° 00' E-16° 30' S	Nanzhila, Kafue National Park, Zambia	472-688
ZAM 4/93/1	21/05/93	26° 00' E-16° 30' S	Nanzhila, Kafue National Park, Zambia	496-689
SAT-2				
KNP 7/86/2	10/11/86	31° 26' E-23° 07' S	10 km east of Shingwedzi	447-661
KNP 8/86/2	10/11/86	31° 26' E-23° 07' S	10 km east of Shingwedzi	427-661
KNP 7/88/2	08/07/88	31° 58' E-24° 54' S	Rietpan	486-652
KNP 8/88/2	08/07/88	31° 58' E-24° 54' S	Rietpan	444-667
KNP 9/88/2	27/05/88	31° 50' E-24° 48' S	Shiloweni	379-662
KNP 10/88/2	27/05/88	31° 50' E-24° 48' S	Shiloweni	437-659
KNP 14/88/2	19/10/88	31° 37' E-24° 44' S	Ripape	445-661
KNP 13/89/2	16/10/89	31° 13' E-25° 07' S	Mesteldam	441-668
KNP 19/89/2	25/10/89	31° 37' E-24° 44' S	Ripape	372-673
KNP 33/89/2	24/10/89	31° 36' E-24° 47' S	Matjapiri	502-666
KNP 34/89/2	25/10/89	31° 37' E-24° 44' S	Ripape	502-666
KNP 2/90/2	27/06/90	31° 20' E-24° 00' S	Reënvoëldam	490-677
KNP 3/90/2	27/06/90	31° 20' E-24° 00' S	Reënvoëldam	487-675
KNP 93/91/2	26/06/91	31° 34' E-24° 33' S	Talamati	428-671
KNP 105/91/2	26/06/91	31° 47' E-24° 37' S	Kumanadam	486-669
KNP 107/91/2	25/06/91	31° 44' E-24° 11' S	Ngotsospruit	473-675

Table 1. (cont.)

Virus Designation	Date of isolation	Geographical origin		Extent of area sequenced
KNP 155/91/2	18/07/91	31° 36' E–24° 01' S	Ndziyospruit	486–662
KNP 9/93/2	17/08/92	31° 20' E–23° 01' S	Boyelaspruit	469–669
NAM 1/89/2	30/08/89	23° 25' E–16° 41' S	West of Kwando river, Caprivi	437–652
NAM 3/89/2	30/08/89	23° 25' E–16° 41' S	West of Kwando river, Caprivi	433–669
ZIM 1/88/2*	15/07/88	27° E–19° S	Hwange National Park	552–666
ZIM 7/88/2*	15/07/88	27° E–19° S	Hwange National Park	549–666
ZIM 1/89/2	15/11/88	27° E–19° S	Hwange National Park	384–666
ZIM 3/89/2	15/11/88	27° E–19° S	Hwange National Park	450–661
ZIM 6/89/2	15/11/88	27° E–19° S	Hwange National Park	372–665
ZIM 32/89/2	30/05/89	27° E–19° S	Hwange National Park	430–666
ZAM 9/93/2	21/05/93	26° 00' E–16° 30' S	Nanzhila, Kafue National Park, Zambia	457–675
ZAM 10/93/2	21/05/93	26° 00' E–16° 30' S	Nanzhila, Kafue National Park, Zambia	457–670
SAT-3				
KNP 1/86/3	14/03/86	31° 51' E–24° 47' S	Tshokwane	379–670
KNP 9/86/3	11/04/86		KNP	382–669
KNP 5/88/3	20/02/88	31° 16' E–25° 10' S	Pretoriuskop	455–676
KNP 6/88/3		31° 16' E–25° 10' S	Pretoriuskop	455–680
KNP 41/89/3	14/11/89	31° 35' E–24° 59' S	Skukuza	451–676
KNP 5/90/3	27/06/90	31° 20' E–24° 00' S	Rënvoëldam	453–674
KNP 10/90/3	25/06/90	31° 20' E–24° 00' S	Reënvoëldam	454–660
KNP 75/91/3	26/06/91	31° 46' E–25° 17' S	Mbyamiti mouth	379–668
KNP 92/91/3	26/06/91	31° 33' E–24° 34' S	Talamati	452–665
KNP 176/91/3	04/07/91	31° 47' E–24° 00' S	Wadrifspruit	459–669
KNP 13/92/3	07/08/92	31° 36' E–23° 42' S	Komandlopfu	460–669
NTV 1/91/3	12/09/91	30° 54' E–22° 19' S	Pafuri-Madimbo Strip	473–654
NTV 2/91/3	12/09/91	30° 54' E–22° 19' S	Pafuri-Madimbo Strip	439–661
NTV 3/91/3	12/09/91	30° 54' E–22° 19' S	Pafuri-Madimbo Strip	481–664
NTV 4/91/3	12/09/91	30° 54' E–22° 19' S	Pafuri-Madimbo Strip	566–663
ZAM 1/93/3	21/05/93	26° 00' E–16° 30' S	Nanzhila, Kafue National Park, Zambia	375–675
ZAM 5/93/3	21/05/93	26° 00' E–16° 30' S	Nanzhila, Kafue National Park, Zambia	370–679
ZAM 7/93/3	21/05/93	26° 00' S–16° 30' S	Nanzhila, Kafue National Park, Zambia	369–677

* Sequencing performed at AFRC Institute for Animal Health, Pirbright Laboratory, England

drawn using the neighbour-joining (NJ) method of Saitou and Nei (24) employing the CLUSTALV package (25) and rooted using an outgroup. To estimate the accuracy of the tree topology, the bootstrap method was used (26). The random number generator seed of 111 and 10^3 bootstrap replicates were used. The significant nodes ($p \leq 0.05$) are indicated in the relevant figures by an *.

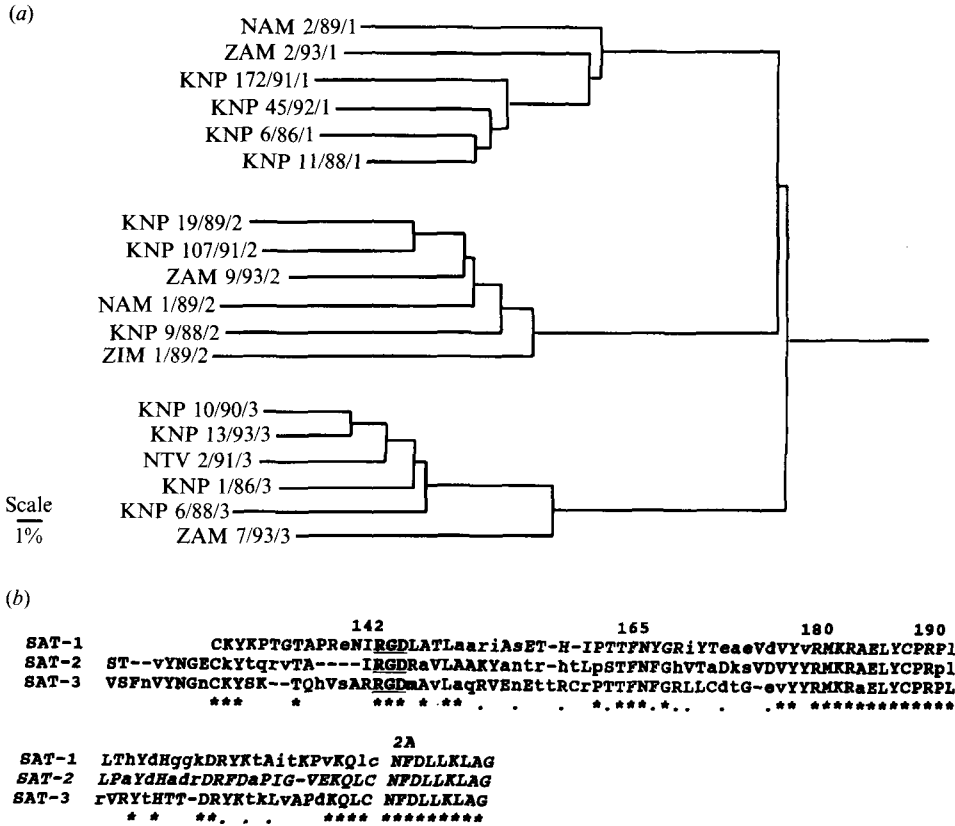


Fig. 2a. Dendrogram depicting the relationships between selected SAT-1, 2 and 3 buffalo isolates from southern Africa. The percentage nucleotide sequence divergence between any two strains is the sum of the distances along the abscissas to the connecting node. The last number in the virus designation indicates the SAT type. Fig. 2b. Alignment of the consensus amino acid sequences of SAT-1, 2 and 3. Upper case indicates consensus between all the sequences of a serotype, while lower case indicates that some variation occurred. *, Homology between all three consensus sequences; ., conservative change; —, gaps to accommodate alignment; *RGD*, cell attachment motif.

RESULTS

Comparison of the amino sequences of the three serotypes

The nucleotide sequences derived in this study have been submitted to GenBank. Representatives of the three virus types were used to construct a dendrogram of the nucleotide differences between the three SAT types. Large nucleotide sequence variation (> 40%) occurred between SAT-1, 2 and 3 isolates (Fig. 2a) although some amino acid sequences were highly conserved in the isolates of the three types. An alignment of the consensus amino acid sequences obtained using CLUSTALV is shown in Fig. 2b. Lower case letters indicate positions where the amino acids were not conserved between the isolates of the same serotype but show the amino acid occurring most often. The asterisks indicate amino acids conserved between the three virus types and dots indicate conserved changes. The *RGD* sequence is underlined in Fig. 2b. This sequence has been implicated as the

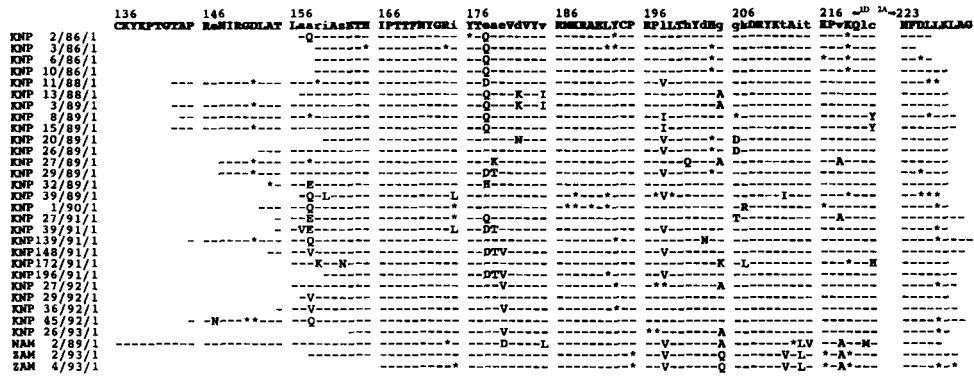


Fig. 3. Deduced amino acid sequences of the SAT-1 buffalo isolates included in this study. The consensus sequence is shown in the upper case where all the amino acid residues were identical and in the lower case where the sequences differed. *, Sequence ambiguity: —, same as consensus sequence. Regions not sequenced are blank.

cell attachment site (16). The SAT-1 and 2 isolates had isoleucine before the RGD and the SAT-3 had arginine. The alignment showed that the lengths of the main antigenic regions (residues 142–165 as described for SAT-3 by Brown and colleagues (27)) contain deletions and therefore varied between the three virus types (Fig. 2b). Several regions outside the main antigenic region seemed to be conserved *viz.* PTTFNFR (163–170; SAT-3 sequence) and the most conserved region being VYYRMKRAELYCPRPLL (178–194) (Fig. 2b). The amino acids KQLC at the amino terminal of 1D (at the junction between P1 and 2A) were also conserved between the three types (Fig. 2b). The position of the primer is such (23) that it was possible to obtain a few amino acids of the N-terminus of the 2A protein. All the isolates had the same amino acid sequence (NFDLLKLAG).

Deduced amino acid sequences of SAT-1 isolates obtained from buffalo and the inferred phylogeny

Some of the KNP isolates were obtained from the same herd on the same day, others from herds situated in areas close to one another over short periods of time, and the rest from widely separated areas. The deduced amino acid sequences are shown in Figure 3. Several regions of conservation in the deduced amino acid sequences were identified although only eight of the KNP sequences included parts of the main antigenic region as well as the RGD sequence. However, a number of nucleotide sequence ambiguities occurred in the RGD region, so that it was not possible to determine whether this motif was conserved between the isolates. Some of the regions outside the main antigenic area also seemed to be conserved *viz.* positions 158–177 with single variations at 159, 160, 162 and two isolates demonstrating variation at position 175, 186–197 and in the 2A region (223–231).

From Fig. 4, the dendrogram obtained utilizing the nucleotide sequences, it was found that in some instances, where isolates were obtained from the same herd on the same day, they were closely related, eg. KNP 3/86/1, KNP 6/86/1 which were isolated at Tshokwane on 14/03/86 (Table 1; Fig. 1) showed only approximately

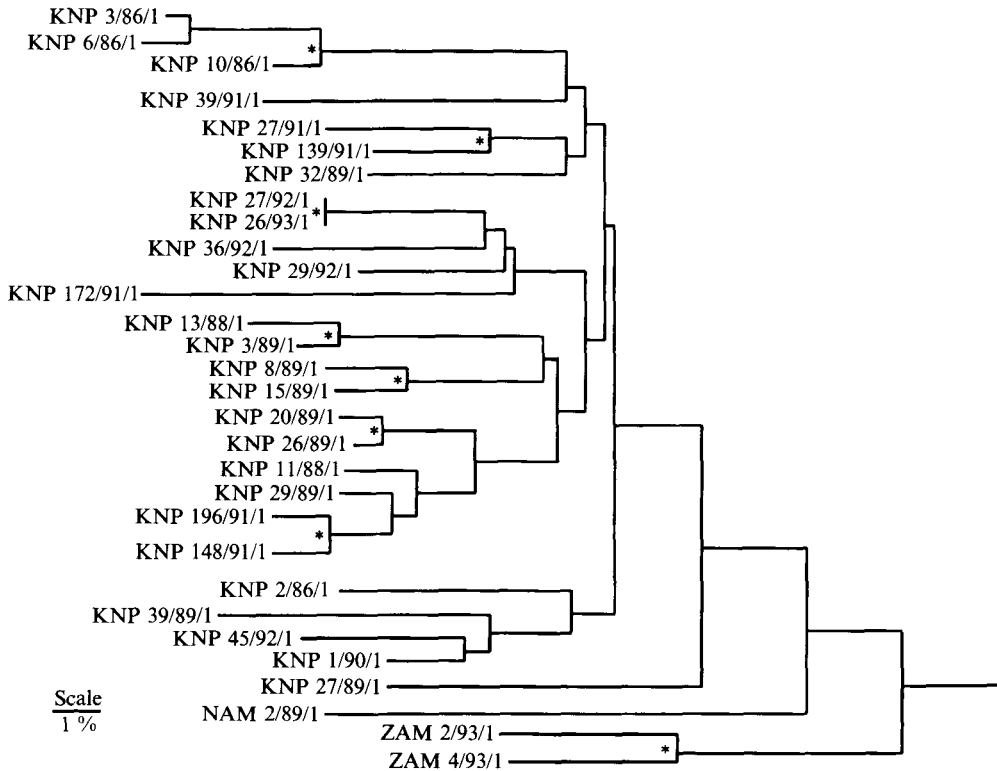


Fig. 4. Dendrogram depicting the relationships between the SAT-1 buffalo isolates from southern Africa between 1986 and 1993. The percentage sequence divergence between any two strains is the sum of the distances along the abscissas to the connecting node. *, Significant nodes.

1.5% nucleotide difference. KNP 20/89/1 and KNP 26/89/1 isolated at Kwa Mfamebto, likewise had less than 2% nucleotide difference. It was also found that viruses isolated from areas situated close to one another, but on different occasions, were also closely related. This is demonstrated by KNP 13/88/1, isolated from Shiloweni (Fig. 1) during 1988 and KNP 3/89/1, isolated from Rietpan (only a few kilometres away) but 8 months later, which showed approximately 3% nucleotide difference (both these nodes are significant).

Conversely, in some instances SAT-1 viruses isolated from the same herd on the same day had significant differences (Fig. 4): KNP 2/86/1 was isolated on the same occasion as KNP 3/86/1, KNP 6/86/1 and KNP 10/86/1 but differed by approximately 16% from these three. KNP 29/89/1 and KNP 39/89/1 likewise differed by 14% from each other although they were isolated from the same herd at Ngwenyeni on the same day.

KNP 27/92/1, KNP 26/93/1, KNP 36/92/1 and KNP 29/92/1 were isolated from northern areas of the KNP (Fig. 1; Table 1) and were more closely related to each other than to the rest of the KNP isolates which originated in southern areas, indicating that genetic differences occur between herds more isolated from each other (Fig. 4). However, KNP 45/92/1, was also obtained from the north of

	127	137	147	157	167	177	187	197	207	217
	STVYNGECKY	LqrVTAIRGD	KavLAARVaa	trhtLpSTFF	PGVYTaDksV	DVYIEMERAS	LYCFRqILPa	YKsd-DKDFD	qFIGVRRGLC	WFFLAKLAG
KNP 7/86/2	---	---	---	---	---	---	---	---	---	---
KNP 8/86/2	---	---	---	---	---	---	---	---	---	---
KNP 7/88/2	---	---	---	---	---	---	---	---	---	---
KNP 8/88/2	---	---	---	---	---	---	---	---	---	---
KNP 9/88/2	---	---	---	---	---	---	---	---	---	---
KNP 10/88/2	---	---	---	---	---	---	---	---	---	---
KNP 14/88/2	---	---	---	---	---	---	---	---	---	---
KNP 13/89/2	---	---	---	---	---	---	---	---	---	---
KNP 19/89/2	---	---	---	---	---	---	---	---	---	---
KNP 33/89/2	---	---	---	---	---	---	---	---	---	---
KNP 34/89/2	---	---	---	---	---	---	---	---	---	---
KNP 2/90/2	---	---	---	---	---	---	---	---	---	---
KNP 3/90/2	---	---	---	---	---	---	---	---	---	---
KNP 93/91/2	---	---	---	---	---	---	---	---	---	---
KNP 105/91/2	---	---	---	---	---	---	---	---	---	---
KNP 107/91/2	---	---	---	---	---	---	---	---	---	---
KNP 155/91/2	---	---	---	---	---	---	---	---	---	---
KNP 9/93/2	---	---	---	---	---	---	---	---	---	---
SIM 1/88/2	---	---	---	---	---	---	---	---	---	---
SIM 7/88/2	---	---	---	---	---	---	---	---	---	---
SIM 1/89/2	---	---	---	---	---	---	---	---	---	---
SIM 3/89/2	---	---	---	---	---	---	---	---	---	---
SIM 6/89/2	---	---	---	---	---	---	---	---	---	---
SIM 32/89/2	---	---	---	---	---	---	---	---	---	---
NAM 1/89/2	---	---	---	---	---	---	---	---	---	---
NAM 3/89/2	---	---	---	---	---	---	---	---	---	---
ZAM 9/93/2	---	---	---	---	---	---	---	---	---	---
ZAM 10/93/2	---	---	---	---	---	---	---	---	---	---

Fig. 5. Deduced amino acid sequences of the SAT-2 buffalo isolates included in this study. The consensus sequence is shown in the upper case where all the amino acid residues were identical and in the lower case where the sequences differed. *, Sequence ambiguity; —, same as consensus sequence. Regions not sequenced are blank.

the KNP at Grootvleidam, but was more closely related to KNP 1/90/1 and KNP 39/89/1, isolated from the south of the Park than to the other northern isolates (Fig. 4).

The Namibian isolate NAM 2/89/1 was clearly unrelated to any of the KNP buffalo isolates, differing by more than 17% from the KNP isolates. The two isolates obtained from Zambia, ZAM 2/93/1 and ZAM 4/93/1 grouped together with approximately 8% nucleotide differences but differed from the Namibian and KNP isolates by 20 and 25% respectively (Fig. 4). NAM 2/89/1 differed from the KNP and Zambian buffalo isolates by unique amino acids at position 180 (D instead of E/V), 185 (L → V/I), 215 (V → T) and 221 (M → L). The Zambian isolates likewise differed from the rest with unique amino acids at positions 205 (Q → A/K/G), 212 (V → I/T) and 214 (L → I) (Fig. 3).

Deduced amino acid sequences of SAT-2 isolates obtained from buffalo and the inferred phylogeny

As was the case with the SAT-1 viruses, the 18 SAT-2 KNP isolates were obtained from regions throughout the KNP (Fig. 1; Table 1). The deduced amino acid sequences of the SAT-2 buffalo isolates are shown in Fig. 5. Several regions of conservation were identified within the amino acid sequences *viz.* positions 162–173 with single changes at 162 and 172, 176–199 with some changes at 198, 202–216 and the 2A protein (217–225). KNP 7/86/2 and KNP 8/86/2 differed from the rest of the KNP as well as the other isolates at positions 155–158 with TGKQANTR. As would be expected from such a change in the major antigenic region, this caused antigenic variation in relation to the other KNP isolates (results not shown). Only four of the sequences were long enough to contain the conserved RGD motif.

The SAT-2 buffalo isolates from the KNP showed sequence variation as high as 20% (Fig. 6). As in the case of the SAT-1 isolates, it was found that isolates from the same herd obtained on the same day were sometimes closely related. For example, KNP 7/86/2 and KNP 8/86/2, isolated at Shingwedzi differed by 3% from each other and KNP 19/89/2 and KNP 34/89/2 from Ripape differed by approximately 1%, while KNP 33/89/2, isolated the next day from a

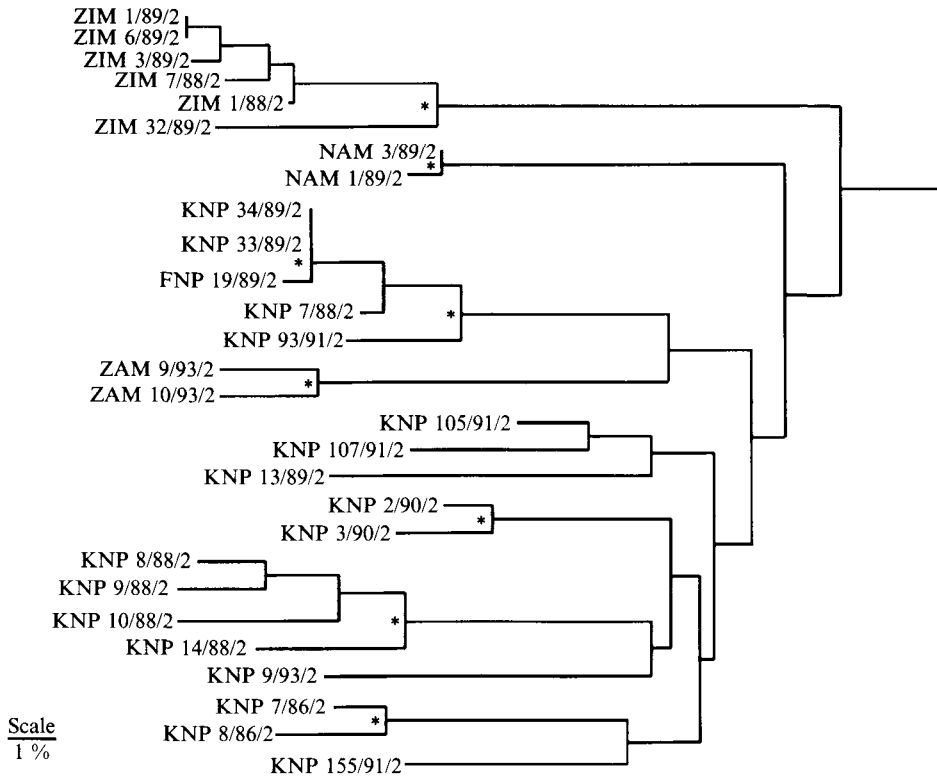


Fig. 6. Dendrogram depicting the relationships between the SAT-2 buffalo isolates from southern Africa between 1986 and 1993. The percentage sequence divergence between any two strains is the sum of the distances along the abscissas to the connecting node. *, Significant nodes.

neighbouring area, was 100% homologous to KNP 34/89/2. KNP 2/90/2 and KNP 3/90/3 were isolated at Reënvoëldam and differed by 3% from each other. The converse was also found; KNP 7/88/2 and KNP 8/88/2 were isolated at Rietpan on the same day and differed by 20% from each other (Fig. 6). Similarly, KNP107/91/2 and KNP155/91/2, which differed by more than 14%, were isolated within a month of each other from buffalo in localities which are close to each other (Fig.1; Table 1). KNP 9/93/2, which was isolated in the same vicinity as KNP 7/86/2 and KNP 8/86/2 in the north of the KNP, but 6 years later, differed by 19% from the latter two.

Two buffalo isolates from a herd in the Caprivi were included in the comparison, as well as two from Zambia and six isolates from Zimbabwe. The Zimbabwean isolates were clearly distinguishable from the KNP and Namibian isolates by several unique amino acid substitutions at positions 160 (E → T/A/S), 196 (G → A) and 207 (S → A). The Namibian isolates could likewise be distinguished with unique substitutions at positions 148 (E → A) and 198 (E → D/T/N) as could the Zambian isolates at positions 157, 160, 192–193 and 200 (Fig. 5).

The Zambian isolates differed from the rest of the isolates by more than 17% (Fig. 6). The two Namibian viruses, isolated on the same day from the same herd

	123	133	143	153	163	173	183	193	203	213 ^{ZIM}	217
	VETPaVYTHQc	KYTKIQhVcA	KRGDmAvLaq	KVEmSttRCr	PFTTFPQMLL	CdtGevYTHN	ENaKLYCPNP	LcVHYtETTD	RYRtLlVcPd	EQGLC	NPDLKIKAG
KNP 1/86/3	T-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 9/86/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 5/88/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 6/88/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 41/89/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 5/90/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 10/90/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 75/91/3	T-----	-----T*	-----A*R	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 92/91/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 176/91/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
KNP 13/93/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
NTV 1/91/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
NTV 2/91/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
NTV 3/91/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
NTV 4/91/3	-----	-----X-	-----A-T-	-----T	-----T	-----E-	-----T	-----*	-----E	-----E	-----*
ZAM 1/93/3	-----T-	-----C-P	-----L-SS	-----T-QE--L	-----S-D	-----V--*	-----VA--K-E	-----VA--K-E	-----VA--K-E	-----VA--K-E	-----VA--K-E
ZAM 5/93/3	-----T-	-----C-P	-----L-SS	-----T-QE--L	-----S-D	-----V--*	-----VA--K-E	-----VA--K-E	-----VA--K-E	-----VA--K-E	-----VA--K-E
ZAM 7/93/3	-----T-	-----C-P	-----L-SS	-----T-QE--L	-----S-D	-----V--*	-----VA--K-E	-----VA--K-E	-----VA--K-E	-----VA--K-E	-----VA--K-E

Fig. 7. Deduced amino acid sequences of the SAT-3 buffalo isolates included in this study. The consensus sequence is shown in the upper case where all the amino acid residues were identical and in the lower case where the sequences differed. *, Sequence ambiguity; —, same as consensus sequence. Regions not sequenced are blank.

were closely related to each other (less than 0.5% nucleotide difference; $p \leq 0.05$), but differed by more than 20% from the other SAT-2 isolates. The Zimbabwean isolates likewise formed a related group (0–11% nucleotide difference). The five viruses obtained from the first captive group, but on two different occasions, differed by 0–2.5%, indicating that disease was introduced by one or a few individuals and that the same virus infected the group on both occasions. The nodes separating these isolates were not significant, as these isolates were so closely related. ZIM 32/89/2, which was isolated from a different group of captive animals differed by approximately 11% from the previous isolates and was obviously a different virus (Fig. 6). The Zambian isolates differed by 4% from each other, but by between 18 and 29% from the rest of the SAT-2 viruses.

Deduced amino acid sequences of SAT-3 isolates obtained from buffalo and the inferred phylogeny

The deduced amino acid sequences of eleven SAT-3 buffalo isolates from the KNP are shown in Fig. 7. Several regions of conservation were identified from these sequences. The conserved regions were from positions 163–173, 179–204 with variations at 185, 194 and 198, and 213–216 and 217–225 (the 2A region). Although the precise origin of KNP 9/86/3 was unknown, evidence existed that it was isolated at Tshokwane at the same time as KNP 1/86/3. These two isolates have unique amino acids at positions 152 and 175. KNP 5/88/3 and KNP 6/88/3 were both isolated at Pretoriuskop, probably on the same occasion and also have unique changes at positions 156, 162 and 177–178 (Fig. 7).

Figure 8, the dendrogram obtained from the nucleotide sequences, indicates instances of close relationship between isolates obtained from single herds on the same day. These were KNP 1/86/3 and KNP 9/86/3 isolated at Tshokwane, KNP 5/88/3 and KNP 6/88/3 isolated at Pretoriuskop and KNP 5/90/3 and KNP 10/90/3 isolated at Reënvoëldam (Fig. 8; nodes $p \leq 0.05$). Between 0.5 and 16% variation occurred between the KNP isolates.

The NTV isolates had unique codons at positions 206–207 while Zambian isolates could clearly be distinguished from the KNP and NTV isolates by unique amino acids at positions 131 (T → N), 139 (C → H), 147, 151–152, 158–159, 175, 207 and 210 (Fig. 7). From Figure 8 it was clear that the KNP, the NTV and the Zambian viruses (Fig. 1; Table 1) formed different branches. The Zambian isolates differed by between 22 and 27% from the NTV and KNP isolates respectively, but

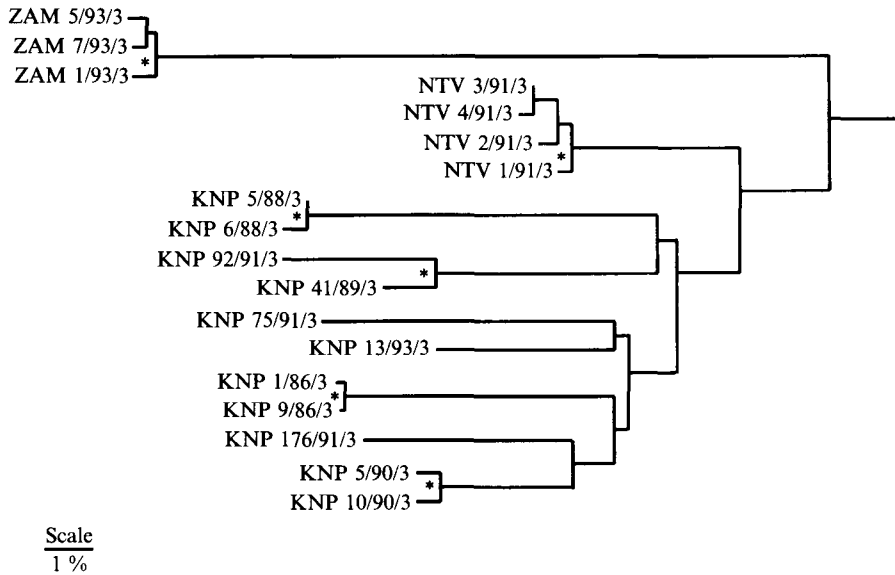


Fig. 8. Dendrogram depicting the relationships between the SAT-3 buffalo isolates from southern Africa between 1986 and 1993. The percentage sequence divergence between any two strains is the sum of the distances along the abscissas to the connecting node. *, significant nodes.

were closely related to each other with less than 1% nucleotide differences (Fig. 8). The NTV isolates were likewise closely related (less than 2% nucleotide difference) indicating that the animals were probably originally infected by only one virus.

DISCUSSION

Although only the carboxy terminal part of 1D was examined in this study, the results from Figure 2a, indicating that the SAT types differed by more than 40% from each other, corresponded well with the findings of Palmenberg (28) who used the whole of 1D in her comparisons. It was also demonstrated that the A, C, O and Asia 1 serotypes are more similar to each other than to the SAT types, though A/C and Asia 1/O cluster into smaller, separate branches. The SAT types have approximately 10% more nucleotide heterogeneity than the European types (28).

The lengths of the main antigenic regions of SAT-1, 2 and 3 around the RGD motif varied (Fig. 2b). SAT-1 and 3 have the same number of amino acids, but three more than SAT-2. It was also demonstrated that the lengths of the main antigenic regions of representatives of serotypes A, O, C and SAT-3 varied (27). SAT-3 possessed five additional amino acids relative to the O₁ and nine amino acids more than the C₁ serotypes (27) suggesting that this part of the protein has been subject to strong selective pressure, presumably immunological. Furthermore, the fact that this part of the protein is a protrusion on the surface of the virus, and not essential for the integrity of the virus particle (29,30), explains why these regions can differ in length.

Areas of amino acid conservation outside the major antigenic region were also identified (Fig. 2b). The sequence VYYRMKRAELYCPRPLL (178–194) was very similar in all the FMDV types included in this study (Fig. 2b), while the sequence CPRP is conserved in all picornaviruses (28). Comparative studies of the deduced primary amino acid sequences of various European FMDV 1Ds indicate that conservation of amino acid sequences is localized at positions 1–42, 62–80, 85–129, and 171–191 (31). The 171–191 region was included in the sequences obtained in this study. Conserved regions are probably part of the skeletal framework of 1D and therefore essential for the integrity of the virion. Consequently, dramatic changes within these regions are less likely to occur (31).

2A of FMDV has been shown to be an oligopeptide of only 16 amino acids (NFDLLKLAGDVESNPG). The sequence results available of all seven serotypes, including the part of the sequences of SAT-1, 2 and 3 determined in this study (NFDLLKLAG), show that 2A is the most highly conserved protein in the genome (32). This implies that the sequence may have a crucial role in replication. Ryan and colleagues (33) demonstrated that 2A may have proteolytic activity and may mediate polyprotein cleavage at the carboxy-terminus of 2A.

It is apparent that genetic variation occurs within the FMDV genome of all three SAT types that infect buffalo in the KNP being up to 19%, 20% and up to 20% for SAT-1, 2 and 3 respectively. For all three types variation occurred within isolates obtained from individual herds although most isolates obtained from specific herds were closely related (Table 1; Figs. 4, 6 and 8). In contrast close relationships were detected between isolates acquired from areas separated by distances greater than 30km and over long time periods. Although isolates obtained from the north of the KNP seemed in some instances to group separately from those obtained from more southerly areas of the Park, this was not always the situation. Possibly because fewer SAT-3 isolates were sequenced, it appeared that there is slightly less intratypic variation within SAT-3 viruses in the KNP than is the case for SAT-1 and 2. When more information becomes available the validity of this observation should be confirmed.

Studies on breeding herds of buffalo in the KNP indicate that they are not permanent entities, but split and coalesce periodically. It is nevertheless possible to associate herds with certain geographic areas over long periods of time. Herds often fragment after disturbance, and detached animals may remain either as small separate groups or join other herds (34). This may explain the relationship between KNP 33/89/2 and KNP 34/89/2 which were isolated from buffalo culled on consecutive days in the same area (Matjapiri and Ripape respectively) and which are 100% homologous over the region sequenced. The same applies to KNP 29/89/1, which was isolated from a different herd than KNP 20/89/1 and KNP 26/89/1 but is more closely related to them than to an isolate made from the same herd (KNP 39/89/1). This observation may be due to herd fragmenting after the disturbance of the culling operation, and the subsequent joining with another herd in the vicinity which was then culled the next day. The possibility of co-circulating, related viruses spread by natural mixing of herds is also possible. Lone bulls are known to be more mobile than herds, and may be an explanation for finding related sequences from seemingly separate herds (KNP 8/89/1, KNP 15/89/1 and KNP 11/88/1, KNP 29/89/1; KNP 33/89/2, KNP 7/88/2). It is

therefore extremely difficult to determine whether certain sequences occur only in certain areas or herds within the KNP.

A clear distinction between buffalo isolates from the different southern African countries is observed from the sequences of all three types. Isolates from Namibia seem to form a group, as do those obtained from Zimbabwe, Zambia and the KNP. This was also evident when isolates from cattle and impala obtained from these countries were sequenced and compared (1,35). This suggests that FMDV have evolved separately in the buffalo populations in the different countries. This is not unexpected since movement of animals between countries does not occur under normal circumstances.

This geographic distribution of genomes was also observed for outbreaks involving type A in cattle in the Middle East where the Saudi Arabian isolates were closely related to each other and the Iranian viruses likewise presented a distinct genetic group (10). Isolates of type C over a period of six decades showed six major evolutionary lineages corresponding with the geographical area from which they were isolated (11). This distribution is probably more marked for FMDV where the movement of animals and their products is more restricted than is the case with human infections. For instance, Rico-Hesse and colleagues (12) demonstrated that numerous geographic foci of endemic circulation exist but that links sometimes occur between cases of wild poliovirus type 1 isolated from distant localities. This was also shown for hepatitis A (36).

The six SAT-2 isolates from Zimbabwe were from young captive buffaloes kept in close contact with each other and therefore the sequence variation does not represent that found among SAT-2 isolates in natural buffalo populations in Zimbabwe. The close homology (less than 2% nucleotide sequence variation) between the various isolates made from the first group of buffaloes suggests that initial infection originated from one animal. The second group of buffaloes became infected with a related, but different strain of the virus (9% nucleotide sequence difference from the other isolates).

The seven isolates obtained from the Kafue Park in Zambia were probably from a single herd and are not representative of the overall situation in Zambia. However, it was possible to determine that the isolates of the same serotype were related and differed from the isolates obtained in other countries.

Although it is difficult to relate differences in nucleotide sequence to antigenic change, the genetic divergence between the various buffalo isolates suggests antigenic differences between SAT type isolates (3) and this has serious implications for immunization programmes since most vaccines have relatively narrow antigenic spectra (37).

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