CHAPTER IV

GENETIC HETEROGENEITY OF SAT-2 FOOT-AND-MOUTH DISEASE VIRUSES IN EAST AFRICA

4.1 Introduction

The SAT serotypes of FMD are prevalent in southern and eastern Africa and are endemic to most African buffalo populations in sub-Saharan Africa (Hedger *et al.*, 1973; Anderson, 1981; Vosloo *et al.*, 2002). The SAT-2 serotype has been recorded among East African countries *viz.,* Eritrea, Ethiopia, Sudan, Kenya, Uganda and Tanzania. However, there is no official record of this serotype in Somalia as very few samples from FMD outbreaks were submitted to the World Reference Laboratory (WRL) for FMD between 1960 and 1983.

The genetic diversity of SAT-2 FMD viruses has been investigated previously for eastern, western, central and southern African countries by Sangare *et al.* (2002) and Bastos *et al.* (2003). A total of V lineages of this serotype were identified in Africa where isolates from East Africa constituted two separate lineages. However, with the exception of a few isolates from Kenya and Eritrea previously described and included in the above studies, the molecular epidemiology of other SAT-2 FMD viruses from Ethiopia, Somalia, Sudan, Tanzania and Uganda has not been intensively studied.

The present study was carried out to determine the genetic variability of SAT-2 FMD viruses in East Africa and to compare them to previously reported VP1 gene sequences of SAT-2 viruses (Table 4.1).

4.2 Materials and Methods

4.2.1 Viruses studied

A total of 41 SAT-2 FMD viruses from Ethiopia, Sudan, Kenya, Uganda, Tanzania and Eritrea isolated between 1975 and 2000 were supplied by the WRL for FMD at the Institute for Animal Health, Pirbright (United Kingdom). These isolates were managed as described previously. To complement the study sequences of 26

isolates from 16 other countries in Africa and Saudi Arabia were selected from Genbank. The laboratory virus designations, country of origin, year of sampling and Genbank accession number of all isolates are indicated in Table 4.1.

Table 4.1 Summary of SAT-2 FMD viruses included in this study

Virus designations	Country of origin	Year of sampling	Reference	Genbank accession No.
ANG/4/74	Angola	1974	Bastos *et al.* (2003)	AF479417
BOT/1/98	Botswana	1998	Bastos *et al.* (2003)	AF367122
BOT/31/98	Botswana	1998	Bastos *et al.* (2003)	AF367125
BUN/1/91	Burundi	1991	Bastos *et al.* (2003)	AF367111
ERI/1/98	Eritrea	1998	This study	AY343933
ERI/12/98	Eritrea	1998	Bastos *et al.* (2003)	AF367126
ERI/4/98	Eritrea	1998	This study	AY343934
ETH/1/90	Ethiopia	1990	This study	AY343935
ETH/2/90	Ethiopia	1990	This study	AY343936
ETH/1/91	Ethiopia	1991	This study	AY343937
ETH/2/91	Ethiopia	1991	This study	AY343938
GAM/9/79	Gambia	1979	Sangare (2002)	AF426078
GAM/8/79	Gambia	1979	Sangare (2002)	AF426093
GHA/2/90	Ghana	1990	Sangare (2002)	AF426081
GHA/8/91	Ghana	1991	Sangare (2002)	AF426083
KEN/3/57	Kenya	1957	Unpublished	AJ251473
KEN/2/76	Kenya	1976	This study	AY343940
KEN/1/84	Kenya	1984	This study	AY344505
KEN/2/84	Kenya	1984	This study	AY343941
KEN/1/85	Kenya	1985	This study	AY343942
KEN/1/86	Kenya	1986	This study	AY343943
KEN/1/87	Kenya	1987	This study	AY343944
KEN/2/87	Kenya	1987	This study	AY343945
KEN/2/88	Kenya	1988	This study	AY343946
KEN/1/89	Kenya	1989	This study	AY343947
KEN/28/91	Kenya	1991	This study	AY343948
KEN/33/91	Kenya	1991	This study	AY343950
KEN/8/91	Kenya	1991	This study	AY343949
KEN/1/92	Kenya	1992	This study	AY343953
KEN/3/92	Kenya	1992	This study	AY343951
KEN/6/92	Kenya	1992	This study	AY343952
KEN/1/94	Kenya	1994	This study	AY343954
KEN/2/94	Kenya	1994	This study	AY343955
KEN/3/95	Kenya	1995	This study	AY343957
KEN/7/95	Kenya	1995	This study	AY343956
KEN/1/96	Kenya	1996	This study	AY343960
KEN/11/96	Kenya	1996	This study	AY343958
KEN/7/96	Kenya	1996	This study	AY343959
KEN/16/98	Kenya	1998	This study	AY343962
KEN/7A/98	Kenya	1998	This study	AY343961
KEN/5/99	Kenya	1999	Bastos *et al.* (2003)	AF367131
KEN/7/99	Kenya	1999	Bastos *et al.* (2003)	AF367132
KEN/9/99	Kenya	1999	Bastos *et al.* (2003)	AF367135

KNP/31/95	South Africa	1995	Bastos *et al.* (2003)	AF367119
MAL/3/75	Malawi	1975	Bastos *et al.* (2003)	AF367099
NIG/2/75	Nigeria	1975	Sangare *et al.* (2002)	AF367139
PAL/5/83	South Africa	1983	Bastos *et al.* (2003)	AF367102
RWA/1/00	Rwanda	2000	Bastos *et al.* (2003)	AF367134
SAU/6/00	Saudi Arabia	2000	Bastos *et al.* (2003)	AF367132
SEN/5/75	Sénégal	1975	Bastos *et al.* (2003)	AF367099
SEN/2/75	Sénégal	1975	Sangare (2002)	AF431732
SUD/6/77	Sudan	1977	This study	AY343939
SUD/9/77	Sudan	1977	This study	AY442014
TAN/1/75	Tanzania	1975	This study	AY343970
TAN/1/86	Tanzania	1986	This study	AY343971
UGA/51/75	Uganda	1975	This study	AY343963
UGA/3/76	Uganda	1976	This study	AY343964
UGA/8/76	Uganda	1976	This study	AY343965
UGA/3/91	Uganda	1991	This study	AY343966
UGA/9/95	Uganda	1995	This study	AY343967
UGA/19/98	Uganda	1998	This study	AY343969
UGA/28/98	Uganda	1998	This study	AY343968
ZAI/1/82	Zaire	1982	Bastos *et al.* (2003)	AF367100
ZAM/10/96	Zambia	1996	Bastos *et al.* (2003)	AF367121
ZIM/7/83	Zimbabwe	1983	van Rensburg & Nel (1999)	AF136607
ZIM/1/00	Zimbabwe	2000	Bastos *et al.* (2003)	AF367136
ZIM/267/98	Zimbabwe	1998	Bastos *et al.* (2003)	AF367130

4.2.2 Nucleic acid isolation and RT-PCR amplification

All procedures for RNA extraction, cDNA synthesis and DNA amplification were described previously sections 2.2.2, 2.2.3 and 2.2.4 with the exception of the downstream primer. The P1 primer and the upstream primer termed VP 3AB, binding to the VP3 encoding gene (5'-CACTGCTACCACTCRGAGTG-3') (Bastos *et al.*, 1998), were used to amplify an approximately 880 bp fragment.

4.2.3 DNA purification and Cycle Sequencing

The 880 bp PCR amplicon was excised from a 1.5% agarose gel and purified using the Qia Quick Gel Extraction Kit (Qiagen). Purified PCR products were sequenced using the Big Dye® version 3.0 Cycle Sequencing kit and the ABI Prism 310 Genetic Analyzer (Applied Biosystems) as previously described in section 2.2.6. Two independent sequencing reactions were performed per sample using the P1 and upstream primers as in the PCR. All data were submitted to Genbank under the accession numbers provided in Table 4.1.

4.2.4 Phylogenetic Analysis

The generated nucleotide sequences were aligned to published reference strains (Table 4.1) using the DAPSA program (Harley, 2001) and trimmed so that a homologous region of 648 bp corresponding to the complete VP1 encoding gene was used for phylogenetic analysis. Phylogenetic reconstruction was carried out using methods of analysis included in MEGA version 2.0 (Kumar *et al.*, 2001), with p-distance, pair-wise deletions of gaps and confidence levels assessed by 1000 boot-strap replications. Average pair-wise comparisons (distance, MEGA 2.0) were conducted to estimate divergence (Table 4.2) between lineages. Evolutionary lineages were distinguished on the basis of nucleotide sequence differences of $\geq$ 26% and boot-strap support > 80% while a divergence of $\geq$ 20% and < 26% distinguished genotypes. A variability plot of sequences of the 1D gene of 48 SAT-2 viruses from East Africa was represented using MEGA V1.02 (Kumar *et al.*, 1993) with the numbers of variable sites in overlapping windows of 10.

4.3 RESULTS

4.3.1 Phylogenetic analysis

The complete 1D gene sequences (648 bp) were used to compare phylogenetic differences between the SAT-2 viruses studied (Table 4.1). The Neighbour-joining (NJ) tree revealed 5 lineages and 15 genotypes from 67 virus isolates from Africa of which 41 East African isolates were characterized in this study. Identical genotypes were consistently obtained irrespective of the method of analysis used. Neighbour-joining (NJ), UPGMA and Parsimony methods produced trees with identical clusters (results not shown) indicating that the recovered phylogeny is a good estimate and reliable (Kim, 1993). It was observed that the NJ method gave rise to 3 distinct regionally associated grouping of lineages *viz.*, East African (lineages I & II), East-southern African (lineage V) and West African (III and IV) (indicated by bold arrow in Fig. 4.1). However, with all phylogenetic reconstruction methods used all the African isolates clustered into five lineages as this classification was further supported by the result of genetic distance comparison of the lineages (Table 4.2). The identified lineages (I – V) and genotypes (A – O) are illustrated in the phylogenetic tree (Fig. 4.1) and phylogeographic distribution of

genotypes (supported by 78% to 100% boot-strap support) can be summarized as follows:

East Africa

Lineage I: isolates from Eritrea, Saudi Arabia, Ethiopia, Sudan, Uganda, Zaire (DRC), Rwanda and Kenya.

- Genotype A: Kenya and Uganda
- Genotype B: Uganda
- Genotype C: Uganda and Zaire (DRC)
- Genotype D: Sudan
- Genotype E: Ethiopia
- Genotype F: Eritrea and Saudi Arabia
- Genotype G: Rwanda

Angola

Lineage II: isolate from Angola (Genotype H).

West Africa

Lineage III: isolates from Ghana, Niger and Senegal (Genotype I).

Lineage IV: isolates from Gambia and Senegal (Genotype J)

East-Southern Africa

Lineage V: isolates from Kenya, Malawi, Tanzania, Ethiopia, Burundi, South Africa, Zambia, Botswana and Zimbabwe.

- Genotype K: Zimbabwe and Botswana
- Genotype L: Zimbabwe
- Genotype M: Botswana and Zambia
- Genotype N: South Africa
- Genotype O: Kenya, Malawi, Tanzania, Ethiopia, Burundi

Most of the isolates within these lineages clustered into geographical localities in accordance with the FMD topotype concept of Knowles and Samuel (2003).

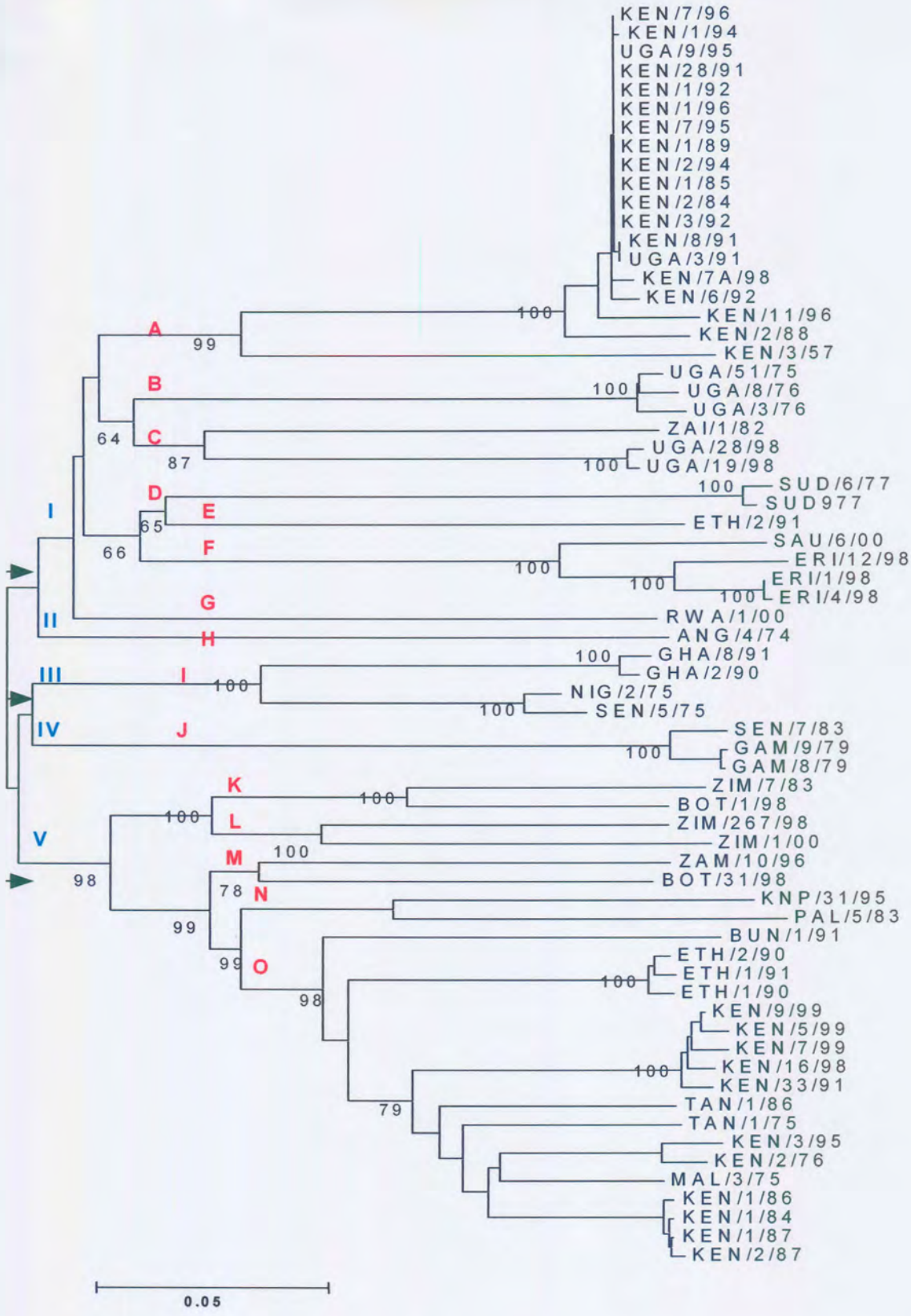

Fig. 4.1 Neighbour-joining tree depicting genetic relationships of serotype SAT-2 FMD viruses from East, West and southern Africa and Saudi Arabia. Boot-strap values were estimated based on 1000 replications. I - V and A – O correspond to lineages and genotypes, respectively. ► Indicates 3 distinct regionally associated grouping of lineages. The scale represents 5% differences at the nucleotide level.

Isolates from lineages I to IV were distributed into geographically defined areas, *viz*., East Africa, Angola and 2 in West Africa which corresponded to lineages I, II and III and IV, respectively. Isolates within lineage V were distributed throughout the southern African region and extended into central and East Africa, thus these isolates were not restricted to one geographically defined region. However, within this lineages 26 isolates from 9 countries clustered into 5 genotypes (K-O) and at the genotype level showed defined areas of distribution. Genotypes A to N appeared to cluster into geographically defined countries with common boundaries which can be termed topotypes. Eighteen isolates from 5 countries clustered in genotype O which showed a wider geographical distribution and this topotype should be further investigated by including more isolates from these countries.

The East African isolates in particular, constituted distinct regional associations where 7 topotypes (genotype A – F, O) were assigned. The only exception being genotype F where isolates from Eritrea and Saudi Arabia clustered together with sequence similarity of between 90 - 99%, thus providing evidence for links between outbreaks from these countries.

The five lineages demonstrated a higher level of genetic distance (divergence) ranging from 27 – 31% (Table 4.2). The sequence homology within each lineage varied between 80 - 99% and between lineages sequence similarities were found to be < 80%.

Table 4.2 Average pair-wise distance comparisons between lineages (I – V) of SAT-2 FMD viruses included in this study.

Lineages	**I**	**II**	**III**	**IV**
I East Africa				
II Angola	27.7			
III West Africa	27.1	28.0		
IV West Africa	29.3	30.4	27.3	
V East and Southern Africa	27.6	29.1	27.9	29.9

Description: No. of Taxa : 67, No. of Groups : 5, Gaps/Missing data : Pair-wise Deletion, Codon Positions : Noncoding, Distance method : Nucleotide: p-distance [Between group average], No. of Sites : 648, distance : estimate

4.3.2 Sequence variations and distribution of mutations

Nucleotide sequence similarities of isolates within genotypes were found to vary from genotype to genotype (Table 4.3). The lowest sequence variation (< 5%) was observed in genotype A between 16 Kenyan isolates (1984 – 1996) and 2 Ugandan isolates (1991, 1995) which revealed that the same virus was circulating over a period of 13 years between the two countries. Similarly, genotype O, spanning 23 years constituted of 18 isolates from Burundi (1), Ethiopia (3), Kenya (11), Tanzania (2) and Malawi (1) and demonstrated > 87% sequence identity.

Amino acid variability was plotted to determine whether mutations were randomly distributed or localized to specific regions of the VP1 gene. The nucleotide sequences of the full-length VP1 encoding gene of 48 isolates of East African origin showed 43.4% (171/648) of the sites were invariant across all isolates while at the amino acid level this translated into 52.7% (114/216) (underlined amino acids, Fig. 4.2). The hypervariable regions were located at amino acid positions 46 – 59, 76 – 90, 135 – 142, 148 – 160 (Fig. 4.2, 4.3). The latter 2 regions of variations were found within the immunogenic G-H loop of the gene (Pfaff *et al.*, 1988; Parry *et al.*, 1990) and amino acid positions 76-99 incorporates the D-E loop. The 'RGD' cell attachment site of the virus at amino acid positions 144-146 within the G-H loop is completely conserved across all isolates. Complete conservation was also observed at amino acids position between 175 – 191 in all 48 viruses while the amino acids position 192 – 210 exhibited variation between 20 - 40%. The VP1/2A cleavage site contained predominantly amino acid sequences KQ/LL, KQ/LC and RQTL.

Table 4.3. Pair-wise sequence divergence using the p-distance model. 0 indicate distance < 1%

Virus designation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1.KEN/1/85																																																										
2. KEN/1/94	0																																																										
3. KEN/3/92	0	0																																																							
4. KEN/7/96	0	0	0																																																					
5. KEN/7/95	0	0	0	0																																																				
6.KEN/28/91	0	0	0	0	0																																																				
7. KEN/2/84	0	0	0	0	0	0																																																			
8. KEN/1/89	0	0	0	0	0	0	0																																																	
9. UGA/9/95	0	0	0	0	0	0	0	0																																																
10. KEN/1/96	0	0	0	0	0	0	0	0	0																																															
11. KEN/2/94	0	0	0	0	0	0	0	0	0	0																																														
12. KEN/1/92	0	0	0	0	0	0	0	0	0	0	0																																													
13. KEN/8/91	0	0	0	0	0	0	0	0	0	0	0	0																																											
14. UGA/3/91	0	0	0	0	0	0	0	0	0	0	0	0	0																																									
15. KEN/7A/98	0	1	0	0	0	0	0	0	0	0	0	0	1	1																																							
16. KEN/6/92	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																																						
17. KEN/11/96	2	3	2	2	2	2	2	2	2	2	2	2	3	3	3	3																																						
18. KEN/2/88	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	6																																						
19. KEN/3/57	18	18	18	18	18	18	18	18	18	18	18	18	18	18	19	19	20	20																																						
20. UGA/28/98	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	25	24	27																																						
21. UGA/19/98	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	25	24	27	1																																				
22. UGA/3/76	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	26	26	25	22	22																																				
23. UGA/51/75	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	24	25	25	25	22	22	2																																				
24. UGA/8/76	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	26	26	26	22	21	2	1																																				
25. ETH/2/91	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	25	26	26	27	26	26	26	26	26																																				
26. SUD/6/77	26	27	26	26	26	26	26	26	26	26	26	27	27	27	27	28	27	30	26	26	26	26	26	24																																				
27. SAU/6/00	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	27	28	27	26	27	27	27	27	27	26	27																																		
28. ERI/12/98	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	29	27	28	29	28	27	27	27	25	26	9																																
29. ERI/1/98	26	27	26	26	26	26	26	26	26	26	26	26	27	27	27	27	28	27	27	28	27	27	27	27	26	26	9	4																																
30. ERI/4/98	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	28	27	28	28	28	27	27	27	26	27	9	4	0																																
31. GHA/8/91	27	27	27	27	27	27	27	27	27	27	27	27	27	27	28	28	29	28	29	29	28	25	25	25	28	29	30	30	31	31																																
32. GHA/2/90	27	27	27	27	27	27	27	27	27	27	27	27	27	27	28	28	29	27	29	29	29	25	25	25	28	28	30	30	30	30	1																																
33. ZIM/7/83	29	29	29	29	29	29	29	29	29	29	29	29	28	28	28	29	30	27	32	31	31	29	28	28	29	32	30	31	30	30	27	27																																
34. BOT/1/98	27	27	27	27	27	27	27	27	27	27	27	27	27	27	28	28	29	27	31	30	30	29	28	28	28	32	31	31	30	30	28	28	12																																
35. ZIM/267/98	27	27	27	27	27	27	27	27	27	27	27	27	28	28	29	27	30	27	27	28	27	27	29	30	30	30	29	29	27	27	21	19																																
36. ZIM/1/00	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	30	28	32	29	29	30	29	29	31	29	30	30	29	29	27	27	21	21	16																																
37. ZAM/10/96	27	27	27	27	27	27	27	27	27	27	27	27	27	27	28	29	26	31	31	31	29	28	29	28	29	32	30	31	30	31	27	27	24	24	26	24																																
38. BOT/31/98	27	27	27	27	27	27	27	27	27	27	27	27	27	27	28	28	29	25	30	30	30	29	28	29	28	32	30	30	30	30	28	27	25	24	26	25	17																																
39. KNP/31/95	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	32	30	32	29	29	29	28	28	25	32	28	29	28	29	28	27	26	26	27	26	21	21																																
40. PAL/5/83	31	32	31	31	31	31	31	31	31	31	31	31	32	32	32	32	33	31	31	29	29	29	29	29	27	31	28	27	27	27	28	28	26	26	27	27	22	22	16																																
41. ETH/2/90	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	26	31	29	29	29	28	29	28	30	29	29	28	29	29	29	25	24	24	25	20	21	20	23																																
42. ETH/1/91	27	27	27	27	27	27	27	27	27	27	27	27	27	28	28	29	25	31	29	29	29	28	29	27	30	28	28	28	28	29	29	25	24	24	26	20	22	20	23	1																																
43. ETH/1/90	27	28	27	27	27	27	27	27	27	27	27	28	28	28	28	29	26	31	29	29	29	28	28	31	29	29	29	29	28	28	25	24	24	25	20	22	20	23	1	1																																
44. TAN/1/86	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	26	30	28	28	29	28	29	27	30	28	29	28	29	29	28	23	24	24	25	20	22	21	23	14	15	15																																
45. KEN/9/99	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	30	27	31	30	30	28	27	28	27	30	30	30	29	29	29	28	25	25	26	27	21	22	20	23	15	15	15	13																																
46. KEN/5/99	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	30	31	27	32	30	30	28	27	28	28	31	30	31	30	30	29	29	26	26	26	28	21	22	21	24	15	16	15	13	1																																
47. KEN/7/99	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	30	31	27	32	30	30	28	28	28	29	27	31	30	30	30	30	29	29	26	25	26	28	21	22	20	24	15	16	15	13	1	2																																
48. KEN/16/98	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	30	27	32	30	30	28	28	28	27	30	30	30	29	29	29	29	26	25	26	27	21	22	20	23	15	15	15	13	1	1	2																																
49. KEN/4/99	28	29	28	28	28	28	28	28	28	28	28	28	29	29	29	29	30	27	31	30	30	28	27	28	28	30	29	30	29	29	29	28	26	25	26	27	21	22	21	23	14	15	14	13	1	2	2	1																																
50. KEN/33/91	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	30	26	31	30	30	28	28	28	27	31	29	30	29	29	29	29	26	26	26	27	21	23	21	24	15	15	15	14	1	2	2	1	2																														
51. TAN/1/75	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	30	26	30	31	31	29	28	29	26	30	28	29	28	28	28	28	25	24	24	25	21	22	19	22	13	13	13	10	10	11	11	10	10	11																														
52. KEN/3/95	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	31	27	32	29	29	29	29	29	29	31	29	30	30	30	27	27	25	24	24	24	20	22	21	24	14	14	14	12	11	12	12	12	12	12	10																														
53. KEN/2/76	29	30	29	29	29	29	29	29	29	29	29	29	30	30	30	30	31	27	31	29	29	29	29	29	28	30	29	30	29	29	27	26	24	23	24	23	19	21	20	23	13	14	14	10	11	12	11	12	12	12	8	2																														
54. KEN/1/86	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	25	29	29	29	29	28	29	28	30	29	29	28	28	27	27	23	24	23	25	19	22	21	22	12	13	12	11	11	12	11	11	11	12	10	8	9																														
55. KEN/1/84	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	24	29	29	29	29	29	29	28	30	29	29	28	28	27	27	23	25	24	25	19	22	22	22	13	13	13	11	11	12	11	11	11	12	10	8	9	0																														
56. KEN/1/87	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	25	29	29	29	29	28	29	28	30	29	29	28	28	27	27	23	24	23	25	19	21	21	22	12	13	13	11	11	11	11	11	11	12	10	8	9	0	0																														
57. KEN/2/87	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	29	30	25	29	29	29	29	29	29	28	31	30	29	28	28	27	27	24	25	24	25	19	22	22	22	13	13	13	11	11	12	12	12	12	12	10	9	9	1	0	0																														

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                          10        20        30        40        50        60        70        80
                 1. KEN/3/57  TTSAGEGAEV VTTDPTTHGG KVTTPRRVHT DVAFLLDRST HVHTNTTAFV VDLMDTKEKA LVGAILRSAT YYFCDLEVAC
                 2. ERI/1/98  ........D. .....S.... N.QEG..K.. E......... .....K.S.. .......K.. .......AS. .......I..
                 3. ERI/4/98  ........D. .....S.... N.QEG..K.. E......... .....K.S.. .......K.. .......AS. .......I..
                 4. ETH/2/90  ........D. .....S.... N.LEK.?M.. ....V...F. ....SK.T.N .......QH. ....L..AS. .......I..
                 5. ETH/1/91  ........D. .....S.... N.LEK..M.. ....V...F. ....SK.T.N .......QH. ....L..AS. .......I..
                 6. ETH/2/91  ........D. ..I.....  S..PA..I.. .......... .....K.T.N I......... .......... .......I..
                 7. SUD/6/77  ....S...D. I..G.A....  TEG.A..I.. .......... .....K...A .......R.. .......... ..........
                 8. SUD/9/77  ....S...D. I..G.A....  TEG.A..I.. .......... .....K...A .......R.. .......... ..........
                 9. ETH/1/90  ........D. .....S.... N.LEK..M.. ....V...F. ....SK.T.N .......QH. ....L..AS. .......IT.
                10. KEN/1/85  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                11. KEN/11/96 ........D. .......... T..AA..... .......... .....NL.D. KGK...N... .......... .......I..
                12. KEN/2/84  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                13. KEN/1/87  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.N .......QQ. ....L..AS. .......I..
                14. KEN/1/89  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                15. KEN/1/92  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                16. KEN/3/95  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.K ....N..QQ. ....L..AS. .......I..
                17. KEN/7/96  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                18. KEN/16/98 ........D. .....SS... S.VEK..M.. ....V...F. ....SK.T.N ...L...QH. ....L..AS. .......I..
                19. KEN/4/99  ........D. .....SS... S.VEK..M.. ....V...F. ....SK.T.N ...L...QH. ....L..AS. .......I..
                20. KEN/2/76  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.N .......QQ. ....L..AS. .......I..
                21. KEN/1/94  ........D. .......... T..AA..... .......... .....K.T.A .......... ...T...... .......I..
                22. KEN/1/84  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.N .......QQ. ....L..AS. .......I..
                23. KEN/1/96  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                24. KEN/1/86  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.N .......QQ. ....L..AS. .......I..
                25. KEN/28/91 ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                26. KEN/8/91  ........D. .......... T..AA..... .......... .....R.T.A .......... .......... .......I..
                27. KEN/2/87  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.N .......QQ. ....L..AS. .......I..
                28. KEN/2/94  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                29. KEN/7/95  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                30. KEN/6/92  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                31. KEN/2/88  ........D. .....S.... T.MAA..... ........F. .....K.T.A ......N.Q. ....L...T. .......I..
                32. KEN/33/91 ........D. .....SS... S.VEK..M.. ....V...F. ....SK.T.N ...L...QH. ....L..AS. .......I..
                33. KEN/7A/98 ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                34. KEN/3/92  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                35. UGA/9/95  ........D. .......... T..AA..... .......... .....K.T.A .......... .......... .......I..
                36. UGA/3/91  ........D. .......... T..AA..... .......... .....R.T.A .......... .......... .......I..
                37. UGA/51/75 .......... .......... S.GA...... .......... ....QK.S.A ...L...... .......... .....MDIT.
                38. TAN/1/75  ........D. .....S.... S.VEK..M.. ....V...F. .....K.T.S .......QQ. ....L..AS. .......I..
                39. TAN/1/86  ........D. .....S.... S.EEK..M.. ....V...F. .....KAT.N .......QQT ....L..AS. .......I..
                40. UGA/28/98 ........D. .......... S.RN...I.. ..T....... .....K.S.A .......... .......... H......I..
                41. UGA/3/76  .......... .......... S.GA...... .......... ....QK.S.A ...L...... .......... .....M.IT.
                42. UGA/8/76  .......... .......... S.G....... .......... ....QK.S.A ...L...... .......... .....M.IT.
                43. UGA/19/98 ........D. .......... S.RN...I.. ..T....... .....K.S.A .......... .......... H......I..
                44. ERI/12/98 ........D. .....S.... N.QEG..K.. E......... .....K.S.. .......G.. .......AS. .......I..
                45. SAU/6/00  ......S.D. .....S.... N.QEG..K.. E......... .....K.S.. .......... .......AS. .......I..
                46. KEN/9/99  ........D. .....SS... S.VEK..M.. ....V...F. ....SK.T.N ...L...QH. ....L..AS. .......I..
                47. KEN/7/99  ........D. .....SS... S.VEK..M.. ....V...F. ....SK.T.N ...L...QH. .I..L..AS. .......I..
                48. KEN/5/99  ........D. .....SS... S.VEK..M.. ....V...F. ....SK.T.N ...L...QH. ....L..AS. .......I..
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                          90        100       110       120       130       140       150       160
1.  KEN/3/57   ?GQTQARVLQ PNGAPRTTQL GDNPMVLSRN NVTRFAIPFT APHRLLSTVY NGECEYTKTV TAIRGDREVL AQKYSSAKHS
2.  ERI/1/98   V.DHTRVFW. .......... ......YAKG G......... .......... ....T....A .......AA. .A..ADNV.T
3.  ERI/4/98   V.DHTRVFW. .......... ......YAKG G......... .......... ....T....A .......AA. .A..ADNVYT
4.  ETH/2/90   V.EHTRVYW. .......... ......FAH. G.....V.Y. ....V.A.R. ....K..DR. SP.....A.. .A..ADSR..
5.  ETH/1/91   V.EHTRVYW. .......... ......FAH. G.....V.Y. ....V.A.R. ....K..DR. SP.....A.. .A..ADSR..
6.  ETH/2/91   V.EHKRVFW. ........T. ......Y.H. G.......Y. .......... ....D.K.AS .......A.. .A..ANT..T
7.  SUD/6/77   V.EHKRVFW. ........V. ......FAH. G.......Y. .......... ......N..S NP.....A.. .A.HKDVA..
8.  SUD/9/77   V.EHKRVFW. ........V. ......FAH. G.......Y. .......... ......N..S NP.....A.. .A..KDVA..
9.  ETH/1/90   V.EHSRVYW. .......... ......FAH. G.....V.Y. ....V.A.R. ....K..DR. SP.....A.. .A..ADSR..
10. KEN/1/85   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
11. KEN/11/96  V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
12. KEN/2/84   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
13. KEN/1/87   V.KHTRVFW. ........T. ......FAH. R.......Y. ......A.R. ....K..DR. S......A.. .A..ADSR.T
14. KEN/1/89   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
15. KEN/1/92   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
16. KEN/3/95   V.THTRVYW. ......A.A. ......FAH. G.....V.Y. ......A.R. ....N.KDR. S......A.. .A..A.SR.A
17. KEN/7/96   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
18. KEN/16/98  V.THKRVYWH ........T. ......FAH. G.......Y. ......A... ....K..DR. S......A.. .A..AESR.T
19. KEN/4/99   V.THKRVYW. ........T. ......FAH. G.......Y. ......A... ....K..DR. S......A.. .A..AESR.T
20. KEN/2/76   V.THTRVYW. ......A.A. ......FAH. G.....V.Y. ......A.R. ....K..DR. .......A.. .A..A.SR.A
21. KEN/1/94   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
22. KEN/1/84   V.KHTRVFW. ........T. ......FAH. R.......Y. ......A.R. ....K..DR. S......A.. .A..ADSR.T
23. KEN/1/96   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
24. KEN/1/86   V.KHTRVFW. ........T. ......FAH. R.......Y. ......A.R. ....K..DR. S......A.. .A..ADSR.T
25. KEN/28/91  V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
26. KEN/8/91   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
27. KEN/2/87   V.KHTRVFW. ........T. ......FAH. R.......Y. ......A.R. ....K..DR. S......A.. .T..ADSR.T
28. KEN/2/94   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
29. KEN/7/95   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
30. KEN/6/92   V.EHKRVFW. ....S.S... .V....F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
31. KEN/2/88   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
32. KEN/33/91  V.THKRVYW. .......MT. ......FAH. G.......Y. ......A... ....K..DR. S......A.. .A..AESR.T
33. KEN/7A/98  V.EHKRVFW. .......PKF ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
34. KEN/3/92   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
35. UGA/9/95   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
36. UGA/3/91   V.EHKRVFW. .......... ......F.HK K......... .......... ....K..EKT I......A.. ....A.T..A
37. UGA/51/75  V.EHARVFW. .......NE. .E....F.H. K.......Y. .......... ....A.S.P. S......QA. .A..A.GR.T
38. TAN/1/75   V.THTRVYW. ........P. ......FAH. G.......Y. ......A.M. ....K..DR. S......A.. .A..ADSR.T
39. TAN/1/86   V.THKRVYW. ........V. ......FAH. G.......Y. ......A... ....R..DK. .......A.. .A..ADSR.A
40. UGA/28/98  V.DHKRVFW. .......V.. ......F.H. G.P..... .......... ....D.NSK. .......Q.. Q...A.TR.A
41. UGA/3/76   V.EHARVFW. .......NE. .....IF.H. K.......Y. .......... .K.A.S.P. S......QA. .A..A.GG.T
42. UGA/8/76   V.EHARVFW. .......NE. ......F.H. K.....V.Y. .......... ....A.S.P. S......QA. .A..A.GR.T
43. UGA/19/98  V.DHKCVFW. .......V.. ......F.H. G......... .......... ....D.NSK. .......Q.. Q...A.TR.A
44. ERI/12/98  V.DHTRVFW. .......... ......YAKG G......... .......... ....T.A..A .......AA. .A..AASV.T
45. SAU/6/00   V.D?TRAFW. .......... ......FAKG G......... .......... ....V.K..P .......AA. .A..ADST.T
46. KEN/9/99   V.THKRVYW. ........T. ......FAH. G.......Y. ......A... ....K..DR. S......A.. .A..AESR.T
47. KEN/7/99   V.THKSVYW. ........T. ......FAH. G.......Y. ......A... ....K..DR. S......T.. .A..AESR.T
48. KEN/5/99   V.THKRVYW. ........T. ......FAH. G.......Y. ......A... ....K..DR. S......LL. .A..AESR.T
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                          170        180        190        200        214↓216
1.  KEN/3/57   LPSTFNFGFV TADKPVDVYY RMKRAELYCP RALLPAYTHA GGDRFDAPIGVAKQ LL
2.  ERI/1/98   ..Q.......  .V........ .......... .P.....D.. SR.........ER. T.
3.  ERI/4/98   ..Q.......  .V........ .......... .P.....D.. SR.........ER. T.
4.  ETH/2/90   ........H.  ...R...... .......... .P.....Q.N NR.........E.. .C
5.  ETH/1/91   ........H.  ...R...... .......... .P.....Q.N NR.........E.. ..
6.  ETH/2/91   ........H.  ...A...... .......... .......D.V .R.........ER. ..
7.  SUD/6/77   .......... .......... .......... .P.....S.N .R.........E.. .C
8.  SUD/9/77   .......... .......... .......... .P.....S.N .R.........E.. .C
9.  ETH/1/90   ........H.  ...R...... .......... .P.....Q.N NR.........E.. .C
10. KEN/1/85   .......... .......... .......... .........Q DR.........E.. ..
11. KEN/11/96  .......... .......... .......... .........Q DR.........E.. ..
12. KEN/2/84   .......... .......... .......... .........Q DR.........E.. ..
13. KEN/1/87   ........H.  ...Q...... .......... .P.....Q.G NR.........E.. .C
14. KEN/1/89   .......... .......... .......... .........Q DR.........E.. ..
15. KEN/1/92   .......... .......... .......... .........Q DR.........E.. ..
16. KEN/3/95   ........Y.  ...Q...... .......... .P.....Q.N NR.........K.. VC
17. KEN/7/96   .......... .......... .......... .........Q DR.........E.. ..
18. KEN/16/98  ........H.  ...Q...... .......... .P.....Q.G IR.........E.. .C
19. KEN/4/99   ........H.  ...Q...... .......... .P.....Q.G IR.........E.. .C
20. KEN/2/76   ........H.  ...Q...... .......... .P.....Q.N DR.........E.. .C
21. KEN/1/94   .......... .......... .......... .........Q DR.........E.. ..
22. KEN/1/84   ........H.  ...Q...... .......... .P.....Q.G NR.........E.. .C
23. KEN/1/96   .......... .......... .......... .........Q DR.........E.. ..
24. KEN/1/86   ........H.  ...N...... .......... .P.....Q.G NR.........E.. .C
25. KEN/28/91  .......... .......... .......... .........Q DR.........E.. ..
26. KEN/8/91   .......... .......... .......... .........Q DR.........E.. ..
27. KEN/2/87   ...R....H.  ...Q...... .......... .P.....Q.G NR.........E.. .C
28. KEN/2/94   .......... .......... .......... .........Q DR.........E.. ..
29. KEN/7/95   .......... .......... .......... .........Q DR.........E.. ..
30. KEN/6/92   .......... .......... .......... .........Q DR.........E.. ..
31. KEN/2/88   .......... .......... .......... .........Q DR.........E.. ..
32. KEN/33/91  ........H.  ...Q...... .......... .P.....Q.G IR.........E.. ..
33. KEN/7A/98  .......... .......... .......... .........Q DR.........E.. ..
34. KEN/3/92   .......... .......... .......... .........Q DR.........E.. ..
35. UGA/9/95   .......... .......... .......... .........Q DR.........E.. ..
36. UGA/3/91   .......... .......... .......... .........Q DR.........E.. ..
37. UGA/51/75  .......... .......... .......... .......D.. NR.........E.. .H
38. TAN/1/75   ........H.  ...Q...... .......... .P.....D.S DR.........E.. ..
39. TAN/1/86   ........H.  ...Q...... .......... .P.....Q.G DR.........E.. .C
40. UGA/28/98  .......... .......... .......... .P.....D.K NR.........ER. .S
41. UGA/3/76   .......... .......... .......... .......D.. NR.........E.. .H
42. UGA/8/76   .......... .......... .......... .......D.. NR.........E.. .H
43. UGA/19/98  .......... .......... .......... .P.....D.K NR.........ER. .Y
44. ERI/12/98  ..Q.......  .V........ .......... .P.....D.. SR.........ER. T.
45. SAU/6/00   ..........  .V........ .......... .P.....E.T ..........ER. T.
46. KEN/9/99   ........H.  ...Q...... .......... .P.....Q.G NR.........E.. .C
47. KEN/7/99   ........H.  ...Q...... .......... .P.....Q.G NR.........E.. .C
48. KEN/5/99   ........H.  ...Q...... .......... .P.....Q.G NR.........E.. .C
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Fig. 4.2 Amino acid sequence alignment of the 1D gene of 48 SAT-2 FMD viruses from East African countries. '.' Indicates an amino acid site identical to the sequence of KEN/3/57 and '?' indicates ambiguous sites. The cell attachment site the 'RGD' is in bold and the conserved amino acids are underlined.

Amino acid in windows of 10	variable sites	Variablity plot
1-10	3	ÞÞÞ
2-11	4	ÞÞÞÞ
3-12	4	ÞÞÞÞ
4-13	5	ÞÞÞÞÞ
5-14	6	ÞÞÞÞÞÞ
6-15	5	ÞÞÞÞÞ
7-16	6	ÞÞÞÞÞÞ
8-17	6	ÞÞÞÞÞÞ
9-18	6	ÞÞÞÞÞÞ
10-19	5	ÞÞÞÞÞ
11-20	5	ÞÞÞÞÞ
12-21	5	ÞÞÞÞÞ
13-22	6	ÞÞÞÞÞÞ
14-23	6	ÞÞÞÞÞÞ
15-24	6	ÞÞÞÞÞÞ
16-25	7	ÞÞÞÞÞÞÞ
17-26	6	ÞÞÞÞÞÞ
18-27	5	ÞÞÞÞÞ
19-28	6	ÞÞÞÞÞÞ
20-29	6	ÞÞÞÞÞÞ
21-30	6	ÞÞÞÞÞÞ
22-31	6	ÞÞÞÞÞÞ
23-32	5	ÞÞÞÞÞ
24-33	5	ÞÞÞÞÞ
25-34	4	ÞÞÞÞ
26-35	4	ÞÞÞÞ
27-36	4	ÞÞÞÞ
28-37	4	ÞÞÞÞ
29-38	3	ÞÞÞ
30-39	4	ÞÞÞÞ
31-40	4	ÞÞÞÞ
32-41	3	ÞÞÞ
33-42	3	ÞÞÞ
34-43	2	ÞÞ
35-44	2	ÞÞ
36-45	2	ÞÞ
37-46	3	ÞÞÞ
38-47	4	ÞÞÞÞ
39-48	5	ÞÞÞÞÞ
40-49	5	ÞÞÞÞÞ
41-50	6	ÞÞÞÞÞÞ
42-51	7	ÞÞÞÞÞÞÞ
43-52	8	ÞÞÞÞÞÞÞÞ
44-53	9	ÞÞÞÞÞÞÞÞÞ
45-54	10	ÞÞÞÞÞÞÞÞÞÞ
46-55	10	ÞÞÞÞÞÞÞÞÞÞ
47-56	9	ÞÞÞÞÞÞÞÞÞ
48-57	9	ÞÞÞÞÞÞÞÞÞ
49-58	9	ÞÞÞÞÞÞÞÞÞ
50-59	9	ÞÞÞÞÞÞÞÞÞ
51-60	9	ÞÞÞÞÞÞÞÞÞ
52-61	8	ÞÞÞÞÞÞÞÞ
53-62	8	ÞÞÞÞÞÞÞÞ
54-63	7	ÞÞÞÞÞÞÞ
55-64	7	ÞÞÞÞÞÞÞ
56-65	7	ÞÞÞÞÞÞÞ
57-66	7	ÞÞÞÞÞÞÞ
58-67	6	ÞÞÞÞÞÞ
59-68	6	ÞÞÞÞÞÞ
60-69	6	ÞÞÞÞÞÞ
61-70	5	ÞÞÞÞÞ
62-71	6	ÞÞÞÞÞÞ
63-72	5	ÞÞÞÞÞ
64-73	5	ÞÞÞÞÞ
65-74	4	ÞÞÞÞ
66-75	3	ÞÞÞ
67-76	4	ÞÞÞÞ
68-77	5	ÞÞÞÞÞ
69-78	5	ÞÞÞÞÞ
70-79	5	ÞÞÞÞÞ
71-80	5	ÞÞÞÞÞ
72-81	4	ÞÞÞÞ
73-82	4	ÞÞÞÞ
74-83	5	ÞÞÞÞÞ
75-84	6	ÞÞÞÞÞÞ
76-85	7	ÞÞÞÞÞÞÞ
77-86	7	ÞÞÞÞÞÞÞ
78-87	7	ÞÞÞÞÞÞÞ
79-88	7	ÞÞÞÞÞÞÞ
80-89	7	ÞÞÞÞÞÞÞ
81-90	8	ÞÞÞÞÞÞÞÞ
82-91	8	ÞÞÞÞÞÞÞÞ
83-92	8	ÞÞÞÞÞÞÞÞ
84-93	7	ÞÞÞÞÞÞÞ
85-94	6	ÞÞÞÞÞÞ
86-95	6	ÞÞÞÞÞÞ
87-96	5	ÞÞÞÞÞ
88-97	5	ÞÞÞÞÞ
89-98	5	ÞÞÞÞÞ
90-99	5	ÞÞÞÞÞ
91-100	5	ÞÞÞÞÞ
92-101	5	ÞÞÞÞÞ
93-102	6	ÞÞÞÞÞÞ
94-103	6	ÞÞÞÞÞÞ
95-104	6	ÞÞÞÞÞÞ
96-105	5	ÞÞÞÞÞ
97-106	6	ÞÞÞÞÞÞ
98-107	6	ÞÞÞÞÞÞ
99-108	6	ÞÞÞÞÞÞ
100-109	6	ÞÞÞÞÞÞ
101-110	6	ÞÞÞÞÞÞ
102-111	7	ÞÞÞÞÞÞÞ
103-112	6	ÞÞÞÞÞÞ
104-113	7	ÞÞÞÞÞÞÞ
105-114	7	ÞÞÞÞÞÞÞ
106-115	7	ÞÞÞÞÞÞÞ
107-116	6	ÞÞÞÞÞÞ
108-117	6	ÞÞÞÞÞÞ
109-118	5	ÞÞÞÞÞ
110-119	5	ÞÞÞÞÞ
111-120	4	ÞÞÞÞ
112-121	3	ÞÞÞ
113-122	3	ÞÞÞ
114-123	2	ÞÞ
115-124	2	ÞÞ
116-125	3	ÞÞÞ
117-126	3	ÞÞÞ
118-127	3	ÞÞÞ
119-128	3	ÞÞÞ
120-129	3	ÞÞÞ
121-130	3	ÞÞÞ
122-131	3	ÞÞÞ
123-132	3	ÞÞÞ
124-133	4	ÞÞÞÞ
125-134	4	ÞÞÞÞ
126-135	4	ÞÞÞÞ

127-136	4	þþþþ
128-137	4	þþþþ
129-138	5	þþþþþ
130-139	5	þþþþþ
131-140	6	þþþþþþ
132-141	7	þþþþþþþ
133-142	8	þþþþþþþþ
134-143	7	þþþþþþþ
135-144	7	þþþþþþþ
136-145	6	þþþþþþ
137-146	6	þþþþþþ
138-147	5	þþþþþ
139-148	5	þþþþþ
140-149	5	þþþþþ
141-150	4	þþþþ
142-151	4	þþþþ
143-152	4	þþþþ
144-153	4	þþþþ
145-154	5	þþþþþ
146-155	6	þþþþþþ
147-156	7	þþþþþþþ
148-157	8	þþþþþþþþ
149-158	8	þþþþþþþþ
150-159	8	þþþþþþþþ
151-160	9	þþþþþþþþþ
152-161	8	þþþþþþþþ
153-162	7	þþþþþþþ
154-163	8	þþþþþþþþ
155-164	8	þþþþþþþþ
156-165	7	þþþþþþþ
157-166	6	þþþþþþ
158-167	5	þþþþþ
159-168	4	þþþþ
160-169	4	þþþþ
161-170	3	þþþ
162-171	3	þþþ
163-172	4	þþþþ
164-173	3	þþþ
165-174	3	þþþ
166-175	3	þþþ
167-176	3	þþþ
168-177	3	þþþ
169-178	3	þþþ
170-179	2	þþ
171-180	2	þþ
172-181	2	þþ
173-182	1	þ
174-183	1	þ
175-184	**0**	
176-185	**0**	
177-186	**0**	
178-187	**0**	
179-188	**0**	
180-189	**0**	
181-190	**0**	
182-191	**0**	
183-192	1	þ
184-193	1	þ
185-194	1	þ
186-195	1	þ
187-196	1	þ
188-197	1	þ
189-198	2	þþ
190-199	2	þþ
191-200	3	þþþ
192-201	4	þþþþ
193-202	4	þþþþ
194-203	4	þþþþ
195-204	4	þþþþ
196-205	4	þþþþ
197-206	4	þþþþ
198-207	4	þþþþ
199-208	3	þþþ
200-209	3	þþþ
201-210	2	þþ
202-211	1	þ
203-212	1	þ
204-213	2	þþ
205-214	2	þþ
206-215	3	þþþ
207-216	4	þþþþ

Fig. 4.3 Variability plot of sequences of 1D gene of SAT-2 viruses from East Africa. The numbers of variable sites in overlapping windows of size = 10 > 4 and < 7 = variable region 7-10 (Red)= Hyper variable region (>70%)
Black = conserved region

4.4 Discussion

Two lineages (I and V) with divergence of > 26% were identified for East Africa from isolates characterized in this study based on the phylogenetic analysis of the complete 1D gene sequences of SAT-2 viruses from 5 East African countries (Eritrea, Ethiopia, Kenya, Uganda and Tanzania). These lineages comprised of 12 genotypes including the 4 (4/7) new genotypes (A, B, D, E) identified for East Africa. The 3 other genotypes (C, F & G) which consisted of isolates from East Africa as identified by Bastos *et al.* (2003) clustered into two separate lineages whilst in this study the 3 genotypes clustered into one lineage (lineage I). The remaining genotypes within lineages I and V and the 3 other lineages (II – IV) consisted of isolates that have been characterized in earlier studies (Table 4.1; van Rensburg & Nel, 1999; Sangare *et al.*, 2002; Bastos *et al.*, 2003).

Twelve independently evolving genotypes were identified within lineages I and V. Genotype O within lineage V shared a genetic relationship with isolates from southern African countries and interestingly shared a common ancestor with buffalo viruses from South Africa (PAL/5/83, KNP/31/95; boot-strap support of 98%). This genotype (O) comprised of isolates from Ethiopia, Kenya, Malawi, Burundi and Tanzania collected over 20 years with a sequence similarity > 80% indicating a higher level of mutations compared to isolates in genotype A from lineage I (84 - 99%). Clustering of isolates from East Africa within lineage V with buffalo viruses from South Africa might indicate that the ancient migration of buffaloes caused the spread of the disease between East and southern African countries as this species is known to harbour viruses for up to 5 years (Condy *et al.*, 1985) and transmitting disease to other susceptible animals (Dawe *et al.*, 1994; Vosloo *et al.*, 1996; Bastos *et al.*, 2000).

The virus isolates comprising of genotypes A-G within lineage I were generally found to originate either from countries with common boundaries or from specific East African countries (Fig. 4.1) and were genetically heterogeneous. The Ugandan isolates of genotypes B and C were genetically sufficiently diverged to be assigned into two distinct genotypes and differed from other SAT-2 East African isolates. This indicated that similar strains of viruses can be confined to certain

localities and evolve within that locality over time while other strains can transcend country boundaries. This emphasizes the need to investigate the main factors which play a role in exchange of viruses between countries to minimize complication of the epidemiology of the disease in the regions. Restriction of animal movement in this region must be the first step in the control of the disease as well as development of polyvalent vaccines that contains a region specific cocktail of strains.

Genotype F within lineage I consisted of three isolates from Eritrea and Saudi Arabia, the latter being a country where SAT-2 does not normally occur. This result is consistent with the findings of Bastos *et al.* (2003) and the outbreak in Saudi Arabia was most probably caused by trade in livestock between the two countries.

Genotype A, within lineage I, which has a sequence identity between 96 – 99.5% (Table 4.3), is shared between 17 Kenyan and 2 Ugandan viruses and spans a 13 year period. This high level of sequence identity over such a long period is surprising and should be investigated further to determine whether the spread of these outbreaks were not due to incompletely inactivated vaccines. Of interest was that the isolate KEN/3/57 from the same genotype showed > 17% sequence dissimilarities with Kenyan isolates where this difference is very close to cut-off value for genotype, whilst high boot-strap support (99%) points to common ancestry.

Trans-boundary transmission of the disease due to animal movement is possible within lineages as the countries included in these lineages share common boundaries and animal trading across borders is a common practice (Ndiritu, 1984). The population of susceptible hosts for FMD in East African countries is high (the cattle and sheep population were estimated to be 57.6 and 98.9 million, respectively (McDermott and Arimi, 2002) and can easily maintain cycles of FMD epizootics. The livestock and the livestock production systems, illegal trading of animal and animal products as well as the presence of cloven-hoofed wild animals in the region favours the transmission of the disease between neighbouring countries and maintenance of the endemicity of FMD in the region.

It was also observed that the genetic diversity between topotypes (A-O) was more than 20% at nucleotide level. This genetic diversity could also be the reflection of the existence of extensive antigenic variation within SAT-2 viruses as was shown to be the case in SAT-1 and serotype A viruses (Vosloo *et al.*, 1996; Bastos *et al.*, 2003, the references therein). The result of the amino acid hypervariability plots of 48 isolates from Africa indicated that the GH-loop region had a continuous hypervariable region upstream and downstream of the 'RGD' which probably indicates the importance of this region for the virus to escape immune pressure. In contrast to other SAT type viruses the region within the C-terminus were not shown to be hypervariable (< 20%) whilst the amino acid positions 46-69 and 76-99 of the 1D gene accommodated hypervariable regions (> 70%).

This molecular epidemiological study of SAT-2 FMD viruses obtained from East Africa has provided valuable information with respect to the epidemiology of the disease in this region. These results demonstrated a distinct geographical grouping of SAT-2 in East Africa and stressed the genetic diversity within the serotype that reflects on the control of the disease by vaccination. Therefore, control of FMD will only be effective if the region co-operates to prevent transboundary spread of the disease which necessitates the need for regional or Pan-African management programme.

CHAPTER V

MOLECULAR EPIDEMIOLOGY OF SAT-1 FOOT-AND-MOUTH DISEASE VIRUSES IN EAST AFRICA ISOLATED BETWEEN 1971 AND 2000

5.1 Introduction

Over the past 30 years data from the WRL indicated that SAT-1 FMD viruses maintained endemicity in four East African countries, namely Uganda, Tanzania, Sudan and Kenya. This serotype has not been reported in Ethiopia, Eritrea and Somalia. Due to poor reporting from East African countries this figure is unlikely to provide a true distribution of SAT-1 in the region. Movement of animals across borders of countries is a common practice due to nomads and illegal animal trading. This favours the spread of the disease between countries as has been shown to occur in other African countries (Bastos and Sangare, 2001; Sangare *et al.*, 2001). The livestock population of 5 East African countries (Ethiopia, Kenya, Somalia, Tanzania and Uganda) is estimated to be over 57 million cattle and 98 million goats and sheep (McDermott and Arimi, 2002). There are also large numbers of African buffalos (*Syncerus caffer*) in East Africa, capable of maintaining and transmitting the SAT type FMD viruses (Thomson, 1996) and three independently evolving viral SAT-1 lineages were previously identified from persistently infected African buffalo populations within southern Africa (Bastos *et al.*, 2001).

There is little data available on the nucleotide sequence database of SAT-1 viruses of East African origin. With the exception of a number of isolates from Tanzania and Uganda (Bastos *et al.*, (2001) the phylogenetic relationships of SAT-1 viruses in East Africa has not been studied in any detail. In the present study, sequences from East African isolates and from previous studies were used to address the phylogeographical distribution of SAT-1 type viruses in Africa.

5.2 Materials and Methods

5.2.1 Viruses used in this study

This study included 23 new East African and 30 West, South and Central African virus isolates from previous studies. The WRL for FMD at the Institute for Animal Health, Pirbright (UK) supplied twenty (20/23) tissue culture virus isolates from East Africa and these viruses were propagated once on IB-RS-2 cells and stored at –70°C prior to further use. Sequences of all other isolates from Africa were obtained from Genbank. The laboratory designation, sampling date and geographical origin of all isolates are indicated in Table 5.1.

Table 5.1 Summary of SAT-1 FMD viruses used in this study.

Virus designations	Sampling year	Country of origin	Reference	Genbank Accession No
BOT/1/68	1968	Botswana	Knowles, 1998 (direct submission)	Z98203
BOT/24/77	1977	Botswana	Bastos *et al.* (2001)	AF301423
BOT/2/98	1998	Botswana	Bastos *et al.* (2001)	AF137402
BOT/37/98	1998	Botswana	Bastos *et al.* (2001)	AF137405
BOT/8/98	1998	Botswana	Bastos *et al.* (2001)	AF301432
KEN/11/91	1991	Kenya	This study	AY441994
KEN/9/91	1991	Kenya	This study	AY441995
KEN/4/98	1998	Kenya	This study	AY441993
KNP/96/81	1981	South Africa	This study	
SAR/9/81	1981	South Africa	Bastos *et al.* (2001)	AF056511
KNP/196/91	1991	South Africa	Van Rensburg *et al.* (2002)	AF283429
MAL/1/85	1985	Malawi	Bastos *et al.* (2001)	AF056509
MOZ/3/77	1977	Mozambique	Bastos *et al.* (2001)	AF056510
NAM/307/98	1998	Namibia	Van Rensburg (unpublished data)	
NGR/2/76	1976	Niger	Sangare *et al.* (2003)	AF431718
NGR/4/76	1976	Niger	Sangare *et al.* (2003)	AF431719
NIG/17/75	1975	Nigeria	Sangare *et al.* (2003)	AF431712
NIG/25/75	1975	Nigeria	Sangare *et al.* (2003)	AF431715
NIG/14/76	1976	Nigeria	Sangare *et al.* (2003)	AF431719
NIG/1/76	1976	Nigeria	Sangare *et al.* (2003)	AF431721
NIG/20/76	1976	Nigeria	Sangare *et al.* (2003)	AF431727
NIG/6/76	1976	Nigeria	Sangare *et al.* (2003)	AF431716
NIG/8/76	1976	Nigeria	Sangare *et al.* (2003)	AF431724
NIG/2/79	1979	Nigeria	Sangare *et al.* (2003)	AF431728
NIG/3/80	1980	Nigeria	Sangare *et al.* (2003)	AF431729
NIG/10/81	1981	Nigeria	Sangare *et al.* (2003)	AF431731
NIG/5/81	1981	Nigeria	Sangare *et al.* (2003)	AF431730
SUD/13/74	1974	Sudan	This study	AY442000
SUD/8/74	1974	Sudan	This study	AY441998
SUD/9/74	1974	Sudan	This study	AY441999
SUD/3/76	1976	Sudan	This study	AY441996
SUD/4/76	1976	Sudan	This study	AY441997
TAN/2/77	1977	Tanzania	This study	AY442008
TAN/3/80	1980	Tanzania	This study	AY442006
TAN/1/96	1996	Tanzania	This study	AY442003
TAN/19/96	1996	Tanzania	This study	AY442013
TAN/2/96	1996	Tanzania	This study	AY442001
TAN/5/96	1996	Tanzania	This study	AY442007
TAN/1/99	1999	Tanzania	Bastos *et al.* (2001)	AF301434

TAN/2/99	1999	Tanzania	Bastos *et al.* (2001)	AF301435
TAN/37/99	1999	Tanzania	This study	AY442005
TAN/51/99	1999	Tanzania	This study	AY442004
TAN/60/99	1999	Tanzania	This study	AY442002
UGA/13/74	1974	Uganda	This study	AY442010
UGA/1/97	1997	Uganda	Van Rensburg *et al.* (2002)	AF283439
UGA/3/99	1999	Uganda	This study	AY442009
UGA/7/99	1999	Uganda	This study	AY442011
ZAM/2/93	1993	Zambia	Bastos *et al.* (2001)	AF056514
ZAM/1/99	1999	Zambia	Bastos *et al.* (2001)	AF301436
ZIM/14/98	1998	Zimbabwe	Bastos *et al.* (2001)	AF137411
ZIM/3/88	1988	Zimbabwe	Bastos *et al.* (2001)	AF056520
ZIM/5/99	1999	Zimbabwe	Bastos *et al.* (2001)	AF301437
ZIM/7/99	1999	Zimbabwe	Bastos *et al.* (2001)	AF301439

5.2.2 Nucleic acid isolation and RT-PCR amplification

Viral RNA isolation, cDNA synthesis and RT-PCR amplification was carried out following the procedure described in section 2.2.2 - 2.2.4. The P1 primer and a primer VP 3AB binding to the VP3 encoding gene (5' CACTGCTACCACTCRGAGTG 3') (Bastos *et al.*, 2003) were used to amplify the complete 1D gene (880 bp) of SAT-1 viruses.

5.2.3 DNA purification and Cycle Sequencing

The 880 bp PCR amplicon was purified and sequenced as described in sections 2.2.6 and 4.3.4 respectively. Two independent sequencing reactions were performed per sample using the P1 and upstream primers VP 3AB (Bastos *et al.*, 2003). All data were submitted to Genbank under the accession numbers provided in Table 5.1.

5.2.4 Phylogenetic analysis

Two sets of sequences were used in this study and sequence alignments and phylogenetic analysis were carried out as described in previous chapters. The 880 bp sequence fragment was trimmed resulting in homologous regions of 396 and 663 base pairs corresponding to the partial and complete 1D encoding gene respectively and these were used as two sets of data for phylogenetic analysis. Lineages were distinguished on the basis of nucleotide sequence differences of > 23% and boot-strap support $\geq$ 80% while a divergence of $\geq$16% distinguished genotypes.

5.3 Results

5.3.1 Phylogenetic analysis

In order to assess the phylogenetic relationships of SAT-1 type FMD viruses from East Africa and other African countries, two sets of sequence data were analyzed based on the availability of sequences of viruses from Genbank. An homologous region of 396 nt corresponding to amino acid positions 90 – 221 C-terminus of the 1D gene and the complete sequence (663 nt) of the 1D/2A protein were used for phylogenetic analysis.

In order to place our results within the context of earlier molecular epidemiological studies and to have a better representation of viruses from different regions of the continent, a total of 53 viruses with sequence length of 396 nt were analysed. The NJ method using the p-distance model revealed 8 major lineages labelled I - VIII and 11 distinct genotypes labelled A – K (Fig. 5.1) from the partial sequences of the 1D gene of 39 isolates.

Figure 5.2 represents analysis of the complete 1D gene sequences of 21 isolates from East Africa and 18 isolates from other African countries. The NJ method of analysis recovered the same clustering of isolates *ie.*, 8 lineages as revealed with the partial sequence analysis of the 1D gene. The recovered genotypes in Fig. 5.2 were also found to coincide with genotypes as in the partial sequence analysis (Fig. 5.1).

Analysis of the two sets of data with the UPGMA and Maximum Parsimony methods recovered the same clustering of viruses as distinguished by NJ tree. The average pair-wise distance comparisons between lineages (I – VIII) revealed uncorrected sequence divergence in excess of 23% (Table 5.2).

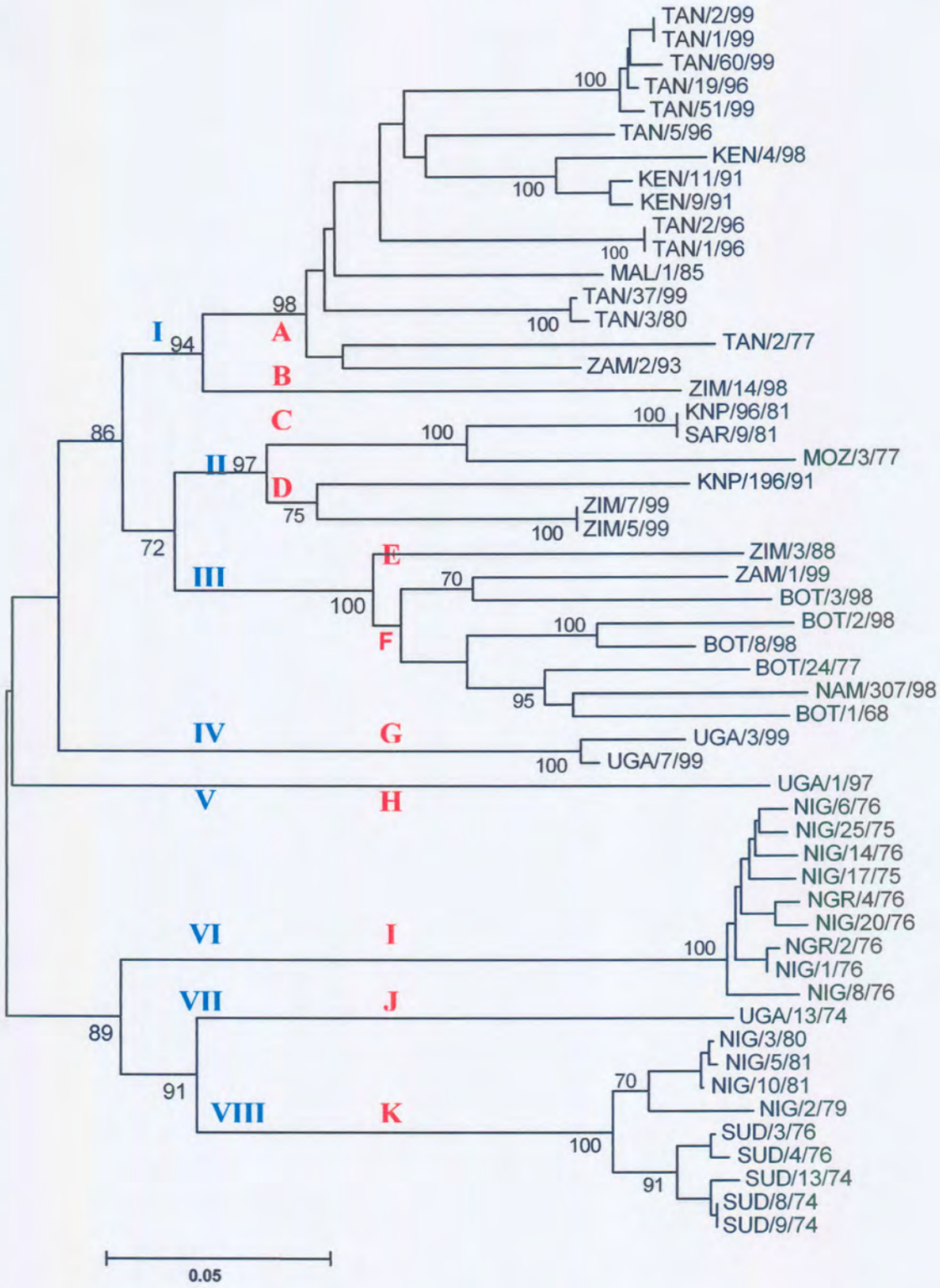


Fig. 5.1 Neighbour-joining tree based on 396 nt of the 1D gene (amino acid position 90 - 221) depicting genetic relationships of 53 SAT-1 FMD viruses from Africa. lineages (I-VIII) and genotypes (A-K) were identified. P-distance was used to estimate genetic distances and boot-strap values of ≥ 70% are indicated and are based on 1000 replications. The scale represents 5% differences at the nucleotide level.

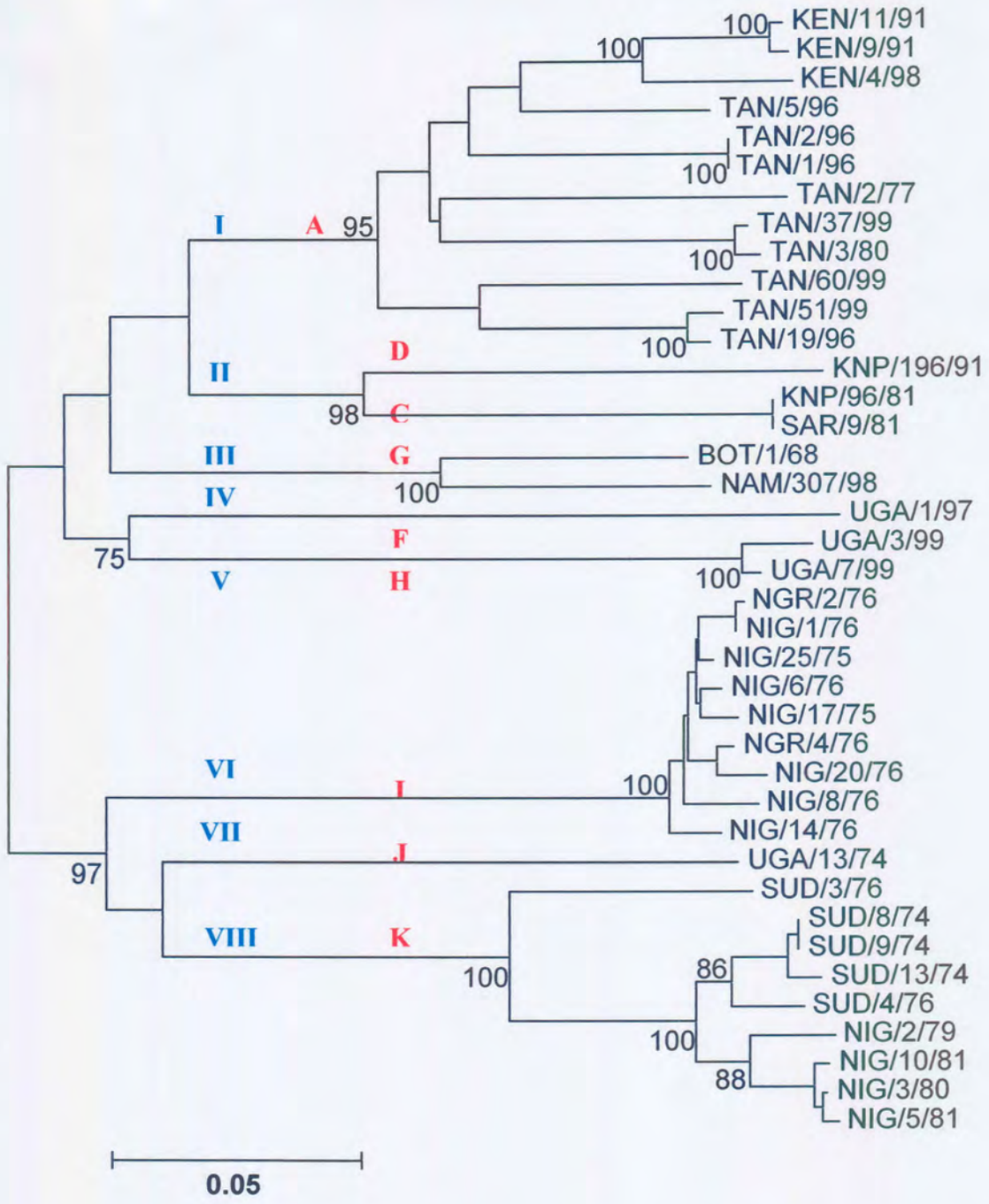


Fig. 5.2 Neighbour-joining tree based on 663 nt of 1D/2A gene (6 nt from 2A) depicting phylogenetic relationships of 39 SAT-1 FMD virus isolates from Africa. Eight major lineages labelled I -VIII were identified based on uncorrected sequence divergence in excess of 23% and genotypes labelled A – K coincided with Fig. 5.1 (genotype B & E in Fig.5.1 were not presented here as their complete 1D gene sequence were not available). The scale represents 5% differences at the nucleotide level.

Lineage I (East-southern Africa)

Genotype A: Tanzania, Kenya, Zambia and Malawi

Genotype B: Zimbabwe

Lineage II (Southern Africa)

Genotype C: South Africa and Mozambique

Genotype D: Zimbabwe

Lineage III (Southern Africa)

Genotype E: Zimbabwe

Genotype F: Zambia, Botswana and Namibia

Lineage IV (East Africa)

Genotype G: Uganda

Lineage V (East Africa)

Genotype H: Uganda

Lineage VI (West Africa)

Genotype I: Nigeria and Niger

Lineage VII (East Africa)

Genotype J: Uganda

Lineage VIII (East-west Africa)

Genotype K: Nigeria and Sudan

Isolates from East Africa were distributed into 5 lineages (Fig. 5.1) where the 3 lineages IV, V and VII were found to be distinct to the Uganda. The other 2 lineages (I and VIII) comprised of viruses from East Africa clustering with viruses from other regions in Africa. Viruses from Zambia, Malawi, Zimbabwe and East Africa (Tanzania and Kenya) clustered in lineage I (Fig. 5.1) while lineage VIII showed historical relationships between Sudan and West African (Nigerian) isolates. The latter lineage represented one of the two lineages identified by Sangare *et al.* (2003).

The complete 1D gene sequences of 3 isolates KNP/196/91, UGA/1/97 (van Rensburg *et al.*, 2002) and NAM/307/98 (Van Rensburg, unpublished) included in this study were found to be heterogeneous and clustered in lineages II, IV and III, respectively (Fig. 5.2). These three lineages showed high divergent values (> 30%) (Table 5.2) and represented previously identified lineages by Bastos and co-workers (2001).

Table 5.2 Average pair-wise distance comparisons between lineages (I – VIII) of SAT-1 FMD viruses included in this study.

Lineages	I	II	III	IV	V	VI	VII
I (East Africa & Zimbabwe)							
II (Southern Africa)	23.5						
III (Southern Africa)	25.8	23.8					
IV (Uganda)	26.1	28.0	27.5				
V (Uganda)	31.2	30.8	30.8	32.3			
VI (West Africa)	32.3	31.9	31.2	31.1	32.5		
VII (Uganda)	29.9	35.8	31.7	31.4	34.8	29.3	
VIII (West and East Africa)	29.2	34.4	32.8	33.8	35.0	27.6	23.7

Description: No. of Taxa : 53, No. of Groups: 8, Gaps/Missing data : Pair-wise Deletion, Codon Positions : Noncoding, Distance method : Nucleotide: p-distance [Between group average], No. of Sites : 396, Distance (d) : estimate .

5.3.2 Sequence variations

Sequencing results showed that 53% of the amino acids across the complete 1D gene of the 36 SAT-1 viruses compared in this study were conserved (Fig. 5.3). In the remaining part of the 1D gene dispersed variability (for overlapping windows of 10 amino acids) was observed and hypervariable regions were located at amino acid positions 135 – 163 (135 – 147, 152 –163) and 196 – 220 in which 70% or more of the sites varied. These regions coincided with the immunogenic G-H loop and the C-terminus, respectively. The RGD motive at positions 149 – 151 were conserved in all 36 isolates and two amino acids at the +1 (L) or –1 (I) positions of the motive were also conserved in 32 isolates. Conserved sites (amino acids) were also found at amino acid positions 131-133 (YNG), 137 (Y), 156 (A) and 158 (R) within the immunogenic G-H loop of the 1D gene of all isolates which possibly plays a crucial role in virus cell interaction.

The variability plot revealed that distinct amino acids were only shared between the Sudanese and the Nigerian isolates at deduced amino acid positions 141-144 (NEDT), 160 (R), 161 – 163 (RQS) and 220 (M). Variable regions were also observed at amino acid positions 13 – 35, 44 –56, 91 – 112 and 172 - 179 (Fig. 5.4) in which 40 - 70% of the sites varied. The average proportion of nucleotide differences across the complete 1D sequence between all isolates was estimated to be 24%.

	10	20	30	40	50	60	70	80
BOT/1/68	TTSAGEGADP	VTTDASAHGG	DTRTTRRAHT	DVTFLLDRFT	LVGKTNDNKL	VLDLLSTKEK	SLVGALLRAA	TYYFSDLEVA
KEN/4/98	....	.....TQ...	GR..A..H..	..S.......	.....QN.R.	T....Q....	A....I....	....
KEN/11/91	........E.	.....TQ...	GR..AY.H..	..S.I.....	...R.QN.R.	T....Q....	A....I....	....
KEN/9/91	........E.	.....TQ...	GR..AY.H..	..S.I.....	...R.QN.R.	T....Q....	A....I....	....
SUD/3/76	........E.	......Q...	GR.AA..Q..	..S.......	.....VN...	T....K....	A....I..S.	....A.....
SUD/4/76	V	..V..A....	NQ.R...V..	..A.......	.....Q...M	...M.K....	A....I..S.	....A.P...
SUD/8/74	V	..V..A....	NQ.R...V..	..A.......	.....QN.RM	...M.K....	A....I..S.	....A.....
SUD/9/74	V	..V..A....	NQ.R...V..	..A.......	.....QN.RM	...M.K....	A....I..S.	....A.....
SUD/13/74	V	..V.TA....	NQ.R...V..	..A.......	.....QN.RM	...M.K....	A....I..S.	....A.....
TAN/2/96	........E.	......Q...	GR.A.H.H..	..S.......	.....VA...	T....Q....	A....I....	....
TAN/60/99	V	..V..A....	NQ.R...V..	..A.......	.....Q...M	...M.K....	A....I....	....
TAN/1/96	........E.	......Q...	GR.A.H.H..	..S.......	.....VA...	T....Q....	A....I....	....
TAN/51/99	........E.	......Q...	GR.AA..Q..	..S.......	.....VN...	T....Q....	A....I....	....
TAN/37/99	........E.	......Q...	GR.VA..H..	..S.I.....	.....VN.R.	T....Q....	A....I....	....
TAN/3/80	........E.	......Q...	GR.V...H..	..S.I.....	.....VK.R.	T....Q....	A....I....	....
TAN/5/96	........E.	......Q...	GR..A..H..	..S.I.....	.....V....	T....Q....	A....I....	....
TAN/2/77	........E.	......Q...	GR..A..H..	..S.I.....	.....V....	T....Q....	A....I..V.	....
UGA/3/99	........E.	......Q...	TS.RV..Q..	..S.......	.I...H....	.....Q....	A....I..S.	....
UGA/13/74	V	..V..T....	TQ.A...V..	..A.......	.....V...M	...M.Q..K.	A....I..S.	....A.....
UGA/7/99	........E.	......Q...	TS.RV..Q..	..S.......	.I...HN...	.....Q....	A....I..S.	....
UGA/1/97	........E.	......Q...	NA.P...Y..	N.E.......	.I...HN..M	...M.R.EK.	A....I..S.	....
TAN/19/96	........E.	......Q...	GR.AA..Q..	..S.......	.....VN...	T....Q....	A....I....	....
KNP/96/81	........E.	..V...Q...	NS.GVH.Q..	..S.......	.....QN..M	T....Q....	A....I....	....
NIG/10/81	V	..V.TA....	NQ.R...V..	..A.......	.....R...M	...M.K....	A....I..S.	....A.....
NIG/2/79	V	..V.TA....	NQ.R...V..	..A.......	.....Q...M	...M.K....	A....I..S.	....A.....
NIG/3/80	V	..V.TA....	NQ.R...V..	..A.......	.....R...M	...M.K....	A....I..S.	....A.....
NIG/5/81	V	..V.TD?...	??.R...V..	..A.......	.....R...M	...M.K....	A....I?.S.	.?..A.....
NGR/2/76	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....I..S.	....
NGR/4/76	V	?.........	N..P...V..	..A.......	.....V...M	.....K....	A....V..S.	....
NIG/1/76	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....I..S.	....
NIG/6/76	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....V..S.	....
NIG/8/76	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....V..S.	....
NIG/17/75	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....I..S.	....
NIG/25/75	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....I..S.	....
NIG/14/76	V	....	N..P...V..	..A.......	.....V...M	.....K....	A....I..S.	....
NIG/20/76	V	..?.......	N..P...V..	..A.......	.....V...M	.....K....	A....V..S.	....

	90	100	110	120	130	140	150	160
BOT/1/68	CVGTNAWVGW	TPNGSPVLTE	VGDNQVVFSR	RGTTRFALPY	TAPHRVLATV	YNGDCKYKPT	GTAPRENIRG	DLATLAARIA
KEN/4/98	...E.K....	....A.E.S.	....P....H	N.........	.....C...A	.........N	AE...TH...	...V..E...
KEN/11/91	...V.K..R.	....A.E.S.	....P....H	N.........	.....C...A	.........N	TE...TH...	......E...
KEN/9/91	...V.K..R.	....A.E.S.	....P....H	N.........	.....C...A	.........N	TE...TH...	......E...
SUD/3/76	.....R....	L...A..PQ.	....P....H	N........F	....	I	NEDT.T....	.........VR
SUD/4/76	.....R....	L...A..PQ.	....P....H	N........F	....	I	NEDT.T....	.........VR
SUD/8/74	.....K....	L...A..PQ.	....P....H	N........F	....	I	NEDT.T....	.........R
SUD/9/74	.....K....	L...A..PQ.	....P....H	N........F	....	I	NEDT.T....	.........R
SUD/13/74	.....K....	L...A..PQ.	....P....H	N.A......F	....	I	NEDT.T....	.........R
TAN/2/96	.....K....	....A.E.A.	....P....H	N.........	.....C...A	....S..RSH	NEE..AH...	....
TAN/60/99	.....K....	....A.E.N.	....P....H	NE........	.....C...A	.........N	SE...TH...	.FT...E...
TAN/1/96	.....K....	....A.E.A.	....P....H	N.........	.....C...A	....S..RSH	NEE..AH...	....
TAN/51/99	.....K....	....A.E.N.	....P....H	NE........	.....C...A	.........N	SE...THF..	......E...
TAN/37/99	...V.G....	....A.E.S.	....P....H	N.........	.....C...T	....	.E...TH...	......E...
TAN/3/80	...V.G....	....A.E.S.	....P....H	N.........	.....C...T	....	.E...TH...	......E...
TAN/5/96	.....K....	....A.E.S.	....P.....	N.........	.....C...A	.........N	HET..TH...	......T...
TAN/2/77	...E.K....	....A.E.S.	....P....H	N.........	.....C....	.....R....	TES..TH...	......E...
UGA/3/99	.....K....	....A.Q...	....PSH..H	N.........	.....L....	....	EQP..T....	...V..Q...
UGA/13/74	.....K....	L...A..PS.	....P....H	N.V.......	....	...N.R...N	TE...PT...	....
UGA/7/99	.....K....	....A.Q...	....P.I..H	N.........	.....L....	....	EQP..T....	...V..Q...
UGA/1/97	.....E....	V...T.DVS.	....P.....	D.A......F	......V..S	...N...QDQ	S.T.LTHV..	......E...
TAN/19/96	.....K....	....A.E.N.	....P....H	NE........	.....C...A	.........N	SE...TH...	.F....E...
KNP/96/81	.L.E.K....	....A.E.E.	....P....N	..A......F	.....C...T	.........A	.....D....	...V..Q...
NIG/10/81	.....K....	L...A..PR.	....P....H	N........F	....	....	NEDT.T....	.........R
NIG/2/79	.....K....	L...A..PR.	....P.....	N........F	....	....Y....I	NEDT.T....	.........VR
NIG/3/80	.....K....	L...A..PR.	....P....H	N........F	....	....	NEDT.T....	.........VR
NIG/5/81	.....K....	L...A..PR.	....P....H	N........F	....	....	NEDT.T....	.........VR
NGR/2/76	.....K....	V...A..PK.	....P....H	N.........	.........T	....Y...AQ	SVES..-...	...V....V.
NGR/4/76	.....K....	V...A..PK.	....P....H	N.........	.........T	.........AQ	PVEN..-...	...V..V.V.
NIG/1/76	.....K....	V...A..PK.	....P....H	N.........	.........T	.........AQ	SVES..-...	...V....V.
NIG/6/76	.....K....	V...A..PK.	....P....H	N.........	.........T	.........AQ	PVEN..-...	...V....V.
NIG/8/76	.....K....	V...A..PKG	....P....H	N.........	.........T	.........AR	PVEN..-...	...V....V.
NIG/17/75	.....K....	V...A..PK.	....P....H	N.........	.........T	.........AQ	PVEN..-...	...V....V.
NIG/25/75	.....K....	V...A..PK.	....P....H	N.........	.........T	.........AQ	PVEN..-...	...V....V.
NIG/14/76	.....K....	V...A..PK.	....P....H	N.A.......	.........T	.........AQ	PVEN..-...	...VV...VV
NIG/20/76	.....K....	V...A..PK.	....P.I..H	N.........	.........T	.........AQ	PVEN..-...	...V..V.V.

						VP1 ↓ 2A
	170	180	190	200	210	221
BOT/1/68	SETHIPTTFN	YGMIYTQAEV	DVYLRMKRAE	LYCPRPVLTH	YDHNGRDRYK	TTLVKPAKQ LS
KEN/4/98	....	..R...E.D.	...V......	....	...K......	VA.T..... .C
KEN/11/91	....	..RL..E.D.	...V......	....	...K.N....	VA.T..... .C
KEN/9/91	....	..RL..E.D.	...V......	....	...K.N....	VA.T..... .C
SUD/3/76	EQS.....	..I.L.E...	...V......	.........T	...ASA....	VP..A.E.. MA
SUD/4/76	EQS.....	..I.L.E...	...V......	.........T	...ASA....	VP..A.E.. MA
SUD/8/74	EQS.....	..I.L.E...	...V......	.........T	...ASA....	VS..A.E.. MA
SUD/9/74	EQS.....	..I.L.E...	...V......	.........T	...ASA....	VS..A.E.. MA
SUD/13/74	EQS.....	..I.L.E...	...V......	.........T	...ASA....	VS..A.E.. MA
TAN/2/96	....	..R...E...	...V......	....	...Q.K....	VA.T..... .C
TAN/60/99	....	..R...E...	...V......	....	...Q......	VA.T..... .C
TAN/1/96	....	..R...E...	...V......	....	...Q.K....	VA.T..... .C
TAN/51/99	....	..R...E...	...V......	....	...Q.K....	VA.T..... .C
TAN/37/99	....	..R.......	...V......	....	...Q......	VA.T..... .C
TAN/3/80	....	..R.......	...V......	....	...Q......	VA.T..... .C
TAN/5/96	....	..R...E...	...V......	....	.E.Q.K....	VA.T..... .C
TAN/2/77	....	..R...E...	...V......	V.........	.N.Q.K....	.A.T..... .C
UGA/3/99	.K.....S..	..Q...ES..	...V......	....	...GDK....	IE..... .E
UGA/13/74	EQA.....	..I.L.EG..	...V......	.....F...M	...A.Q..R.	IR..A.... VC
UGA/7/99	.......S..	..Q...ES..	...V......	....	...GDK....	IE..... .C
UGA/1/97	QNA....S..	..Q...E...	...V......	T.....L...	...A.KN...	.K..A.... .C
TAN/19/96	....	..R...E...	...V......	....	...Q.K....	VA.T..... .C
KNP/96/81	G.........	..R...E...	...V......	......L...	.....K....	.AIT..... .G
NIG/10/81	EQS.....	..I.L.E...	...V......	.........T	...ALA....	VS.IA.E.. MA
NIG/2/79	EQS.....	..I.L.E...	...V......	.........T	...ASA....	VS.IA.E.. MA
NIG/3/80	EQS.....	..I.L.E...	...V......	.........T	...ALA....	VS.IA.E.. MA
NIG/5/81	EQS.....	..I.L.E...	...V......	.........T	...ALA....	VS.IA.E.. MA
NGR/2/76	E.........	....L.ES..	...V......	.....FL..T	.....A....	....A.E.. .A
NGR/4/76	E.........	....L.ES..	...V......	.....FL..T	...D.A....	....A.E.. .A
NIG/1/76	E.........	....L.ES..	...V......	.....FL..T	.....A....	....A.E.. .A
NIG/6/76	E.........	....L.ES..	...V......	.....FL..T	.....A....	....A.E.. .A
NIG/8/76	E.........	....L.ES..	...V......	.....FL..T	.....A....	....A.E.. .A
NIG/17/75	E.........	....L.ES..	...V......	.....FL..T	.....A..H.	....A.E.. .A
NIG/25/75	E.........	....L.ES..	...V......	.....FL..T	.....A....	....A.E.. .A
NIG/14/76	E.........	....L.ES..	...V......	.....FL..T	.....A....	....A.E.. .A
NIG/20/76	E.........	....L.ES..	...V......	.....FL..T	...D.A....	....A.E.. .A

Fig. 5.3 Sequence alignment of 221 amino acids of the 1D gene of 36 SAT-1 FMD viruses. The cell attachment site of the viruses (RGD) in the GH-loop is highlighted at positions 149-51. Dots (.) indicate amino acids identical to BOT/1/68. '?' indicates amino acids that could not be determined. '-' indicates viruses that have a codon deletion corresponding to amino acid position 147.

A	B	C
1-10	2	þþ
2-11	2	þþ
3-12	2	þþ
4-13	3	þþþ
5-14	3	þþþ
6-15	4	þþþþ
7-16	5	þþþþþ
8-17	6	þþþþþþ
9-18	6	þþþþþþ
10-19	5	þþþþþ
11-20	4	þþþþ
12-21	5	þþþþþ
13-22	6	þþþþþþ
14-23	5	þþþþþ
15-24	6	þþþþþþ
16-25	6	þþþþþþ
17-26	6	þþþþþþ
18-27	5	þþþþþ
19-28	6	þþþþþþ
20-29	6	þþþþþþ
21-30	6	þþþþþþ
22-31	6	þþþþþþ
23-32	5	þþþþþ
24-33	6	þþþþþþ
25-34	5	þþþþþ
26-35	5	þþþþþ
27-36	4	þþþþ
28-37	4	þþþþ
29-38	3	þþþ
30-39	3	þþþ
31-40	3	þþþ
32-41	2	þþ
33-42	3	þþþ
34-43	2	þþ
35-44	3	þþþ
36-45	2	þþ
37-46	3	þþþ
38-47	4	þþþþ
39-48	4	þþþþ
40-49	5	þþþþþ
41-50	6	þþþþþþ
42-51	7	þþþþþþþ
43-52	6	þþþþþþ
44-53	6	þþþþþþ
45-54	6	þþþþþþ
46-55	6	þþþþþþ
47-56	6	þþþþþþ
48-57	5	þþþþþ
49-58	6	þþþþþþ
50-59	6	þþþþþþ
51-60	5	þþþþþ
52-61	5	þþþþþ
53-62	5	þþþþþ
54-63	5	þþþþþ
55-64	4	þþþþ
56-65	4	þþþþ
57-66	4	þþþþ
58-67	4	þþþþ
59-68	3	þþþ
60-69	3	þþþ
61-70	3	þþþ
62-71	2	þþ
63-72	2	þþ
64-73	2	þþ
65-74	2	þþ
66-75	3	þþþ
67-76	2	þþ
68-77	3	þþþ
69-78	3	þþþ
70-79	2	þþ
71-80	2	þþ
72-81	2	þþ
73-82	3	þþþ
74-83	3	þþþ
75-84	4	þþþþ
76-85	3	þþþ
77-86	4	þþþþ
78-87	3	þþþ
79-88	3	þþþ
80-89	4	þþþþ
81-90	4	þþþþ
82-91	5	þþþþþ
83-92	4	þþþþ
84-93	4	þþþþ
85-94	3	þþþ
86-95	4	þþþþ
87-96	3	þþþ
88-97	4	þþþþ
89-98	5	þþþþþ
90-99	5	þþþþþ
91-100	6	þþþþþþ
92-101	5	þþþþþ
93-102	5	þþþþþ
94-103	5	þþþþþ
95-104	5	þþþþþ
96-105	5	þþþþþ
97-106	6	þþþþþþ
98-107	6	þþþþþþ
99-108	5	þþþþþ
100-109	4	þþþþ
101-110	4	þþþþ
102-111	5	þþþþþ
103-112	6	þþþþþþ
104-113	7	þþþþþþþ
105-114	7	þþþþþþþ
106-115	6	þþþþþþ
107-116	5	þþþþþ
108-117	4	þþþþ
109-118	4	þþþþ
110-119	4	þþþþ
111-120	4	þþþþ
112-121	3	þþþ
113-122	2	þþ
114-123	1	þ
115-124	1	þ
116-125	1	þ
117-126	2	þþ
118-127	3	þþþ
119-128	3	þþþ
120-129	3	þþþ
121-130	3	þþþ
122-131	3	þþþ
123-132	3	þþþ
124-133	3	þþþ
125-134	4	þþþþ
126-135	5	þþþþþ
127-136	5	þþþþþ
128-137	4	þþþþ
129-138	5	þþþþþ

130-139	6	þþþþþþ
131-140	6	þþþþþþ
132-141	**7**	**þþþþþþþ**
133-142	**8**	**þþþþþþþþ**
134-143	**9**	**þþþþþþþþþ**
135-144	**9**	**þþþþþþþþþ**
136-145	**9**	**þþþþþþþþþ**
137-146	**9**	**þþþþþþþþþ**
138-147	**10**	**þþþþþþþþþþ**
139-148	**10**	**þþþþþþþþþþ**
140-149	**9**	**þþþþþþþþþ**
141-150	**8**	**þþþþþþþþ**
142-151	**7**	**þþþþþþþ**
143-152	**7**	**þþþþþþþ**
144-153	**7**	**þþþþþþþ**
145-154	**7**	**þþþþþþþ**
146-155	**7**	**þþþþþþþ**
147-156	6	þþþþþþ
148-157	6	þþþþþþ
149-158	5	þþþþþ
150-159	6	þþþþþþ
151-160	**7**	**þþþþþþþ**
152-161	**8**	**þþþþþþþþ**
153-162	**8**	**þþþþþþþþ**
154-163	**8**	**þþþþþþþþ**
155-164	**7**	**þþþþþþþ**
156-165	6	þþþþþþ
157-166	6	þþþþþþ
158-167	5	þþþþþ
159-168	6	þþþþþþ
160-169	5	þþþþþ
161-170	4	þþþþ
162-171	3	þþþ
163-172	2	þþ
164-173	2	þþ
165-174	3	þþþ
166-175	4	þþþþ
167-176	4	þþþþ
168-177	5	þþþþþ
169-178	5	þþþþþ
170-179	6	þþþþþþ
171-180	6	þþþþþþ
172-181	6	þþþþþþ
82	6	þþþþþþ
174-183	5	þþþþþ
175-184	5	þþþþþ
176-185	4	þþþþ
177-186	4	þþþþ
178-187	3	þþþ
179-188	2	þþ
180-189	1	þ
181-190	1	þ
182-191	2	þþ
183-192	2	þþ
184-193	2	þþ
185-194	1	þ
186-195	1	þ
187-196	2	þþ
188-197	3	þþþ
189-198	3	þþþ
190-199	3	þþþ
191-200	4	þþþþ
192-201	3	þþþ
193-202	4	þþþþ
194-203	4	þþþþ
195-204	5	þþþþþ
196-205	6	þþþþþþ
197-206	6	þþþþþþ
198-207	6	þþþþþþ
199-208	6	þþþþþþ
200-209	**7**	**þþþþþþþ**
201-210	**7**	**þþþþþþþ**
202-211	**8**	**þþþþþþþþ**
203-212	**8**	**þþþþþþþþ**
204-213	**9**	**þþþþþþþþþ**
205-214	**9**	**þþþþþþþþþ**
206-215	**9**	**þþþþþþþþþ**
207-216	**8**	**þþþþþþþþ**
208-217	**8**	**þþþþþþþþ**
209-218	**8**	**þþþþþþþþ**
210-219	**7**	**þþþþþþþ**
211-220	**7**	**þþþþþþþ**
212-221	7	þþþþþþþ

Fig. 5.4 Variability plot of complete sequences of the 1D gene of SAT-1 viruses from Africa. Under **A** amino acids in a window of 10 were indicated while **B** and **C** indicated the number of variable sites and variability per amino acid, respectively.

Key: Between 4-7 = variable region. 7-10 = Hyper variable region (≥7)

5.4 Discussion

Genetic characterization of SAT type viruses is well documented for the southern African region with the emphasis on buffalo isolates (Vosloo *et al.*, 1992; Esterhysen, 1994, Vosloo *et al.*, 1995; Van Rensburg and Nei, 1999; Bastos, 2001; Bastos *et al.*, 2001; Van Rensburg *et al.*, 2002; Bastos *et al.*, 2003) as well as for west Africa (Sangare, 2002; Sangare *et al.*, 2003). A total of 23 (23/53) virus isolates from East Africa as well as 30 geographically representative isolates from previous studies were included in this study to determine the genetic relationships of African SAT-1 isolates. In the present analysis of nucleotide sequences of the 1D gene, divergence between lineages and genotypes varied from 23.5 to 35% and 16.9 to 23.5%, respectively. Thus, the SAT-1 virus types are genetically heterogeneous and distantly related at both lineage and genotype level. This shows an independent evolution of lineages and genotypes and a high rate of mutation of FMD viruses (Sobrino *et al.*, 1983) and a higher level of intratypic variation for SAT type viruses (Vosloo *et al.*, 1995; Bastos *et al.*, 2001; Bastos *et al.*, 2003).

A divergence of > 20% in nucleotide sequences distinguishes topotypes for the SAT type FMD viruses (Bastos *et al.*, 2003; Knowles and Samuel, 2003). The present divergence in nucleotide sequence was > 23% for lineages and > 16% for genotypes. Since viruses within genotypes were distributed into distinct geographical localities, topotypes could be assigned. The three Ugandan lineages (IV, V and VII) represented isolates from 1974, 1997 and 1999 and are distinct for Uganda (Fig. 5.2).

The historical evolutionary relationships of SAT-1 type viruses in Africa can clearly be seen in Fig. 5.1 and Fig. 5.2. These phylogenetic trees revealed three major genetically related combinations of lineages. The first major cluster composed of lineages I – III which included virus isolates from East and southern Africa while the second major cluster (lineages IV and V) consisted of isolates from Uganda and is distinct for East Africa. The last major cluster (lineage VI - VIII) constituted virus isolates from West Africa (Nigeria and Niger) and East Africa (Uganda and Sudan). This showed that East African isolates are historically related with both

West and southern African isolates. The phylogenetic link between the West and the East African isolates was further supported by the observation that distinct amino acids located within the GH-loop (NEDT, R, RQS) and C-terminus (M) which were not found in the other African isolates (Fig. 5.3).

Despite this progress there are still some very basic questions that need to be addressed. It is interesting to note that Uganda has 3 distinct lineages within its border. There is also very little movement control within Uganda and between bordering countries. Why should one country then have such different viruses. As mentioned above the question of the heterogeneity of the Ugandan isolates which clustered into three distinct lineages needs to be addressed. Analysis of the complete 1D gene sequence indicated that these lineages differed by more than 30% to each other and > 26% to other lineages found in Africa (Table 5.2). The high divergence between lineages (> 23%) can be explained by multiple cycles of epizootics and independent evolution of variants. The overlapping distribution of lineages of different serotypes can also lead to intra- and intertypic recombination of viruses in endemic countries like Uganda. Study based on the complete genome sequences of viruses may be very useful in determining the prevalence and the frequency of genetic recombination in the field (Knowles and Samuel, 2003).

Different isolates of SAT-1 viruses from Tanzania included in this study clustered into genotype A. The 10 Tanzanian isolates obtained from 1977 to 1999 (Fig. 5.1 and Fig. 5.2) constituted of 5 groups of unrelated viruses (base on < 90% sequence similarities). Within each of the first (TAN/2/99, 1/99, 60/99, 19/96, 51/99) and second groups (TAN/1/96 and TAN2/96) a high percentage of sequence similarity (> 97%) were found which on the other hand indicated that closely related viruses in each group were responsible for the outbreaks. However, the third (TAN/5/96), fourth (TAN/3799, TAN/3/80) and fifth groups (TAN/2/77) were found to differ from each other and from the first 2 groups by > 10%. Isolates of the same year *ie*., from 1996 were distributed into more than 3 groups which showed a minimum of 3 variant of viruses that were responsible for outbreaks in Tanzania. Phylogenetic analysis also showed that the Kenyan (1991 and 1998), Malawian (1985), Zambian (1993) and the Tanzanian isolates

clustered as genotype A whereas one Tanzanian isolate from 1977 clustered with a Zambian isolate of 1993 within genotype B (Fig. 5.1).

In the phylogenetic analysis by Bastos and co-workers (2001) two 1999 isolates from Tanzania clustered with Zambian isolates while one Ugandan isolate (UGA/1/97) differed from southern African isolates and appeared as a distinct lineage. The other isolates from southern Africa included in the same study showed a geographical clustering and 3 topotypes were assigned. Sangare *et al.* (2003) have also identified two independently evolving viral lineages of this serotype in West Africa. The present study showed that the SAT-1 type FMD virus populations circulating in East Africa are genetically heterogeneous and phylogenetically clustered into 3 distinct Ugandan lineages and 2 shared lineages with West and southern Africa. These results also complemented the previous studies by Bastos *et al.* (2001), Sangare *et al.* (2003) and Knowles and Samuel, (2003) and assisted in complying a comprehensive SAT-1 sequence database for East African countries.

CHAPTER VI

SEROLOGICAL SURVEY TO DETERMINE THE ROLE OF SMALL RUMINANTS AND WILDLIFE IN THE EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE IN ETHIOPIA AND EVALUATION OF SEROLOGICAL TESTS

6.1 INTRODUCTION

Ethiopia is characterized by its central highlands, rising up to 4600 metres above sea level (masl) and peripheral lowlands descending down to 100 metres below sea level (mbsl). Three major agro-climatic zones occur in Ethiopia *viz.*, dry land (north-east and the rest of low-lying areas in the peripheries), tropical rainy climate (west mainly, also eastern regions) and moderate climate (central plateau) (Anonymous, 1998b).

Animal diseases are widespread in all agro-ecological zones of Ethiopia. At present the three major diseases of socio-economic importance are FMD, contagious bovine pleuropneumonia (CBPP) and trypanosomosis. However, diseases such as contagious caprine pleuropneumonia (CCPP), peste des petit ruminants, lumpy skin disease, African horse sickness, Newcastle disease, cowdriosis, fasciolasis, haemonchosis, etc. are still rampant (Anonymous 1998b; Anonymous, 2000). The conventional methods of controlling these diseases either through immunization or therapy are in most instances not completely effective. Almost all vaccines for the major animal diseases are produced in the country while the vaccines for CCPP, rabies and FMD are imported on occasion. In Ethiopia, rinderpest is the only animal disease investigated by sero-surveillance at national level and the country has been declared free of the disease (Abraham *et al.*, 1998).

The current situation of FMD in Ethiopia is alarming. There is no national control strategy, no legislation exists for making FMD notifiable to the veterinary authorities nor for animal movement restrictions to be imposed. Therefore, livestock are at risk from endemic strains as well as from antigenic variants prevailing in neighbouring countries. The official data may not exhibit the reality of the disease, due to the

insidious nature of the disease, the unreported cases by farmers, as well as the few samples submitted to Pirbright for identification. However, records from the National Animal Health Research Center and the National Veterinary Institute of Ethiopia indicated that serotypes O, A, C and SAT-2 were responsible for FMD outbreaks during 1974 – 2003. However, a national survey on the incidence and distribution of the disease has not yet been conducted. The role of small and wild ruminants in the epidemiology of FMD is therefore not known.

Good quality data on the impact of FMD on animal production in Ethiopia are lacking. The prevalence of the disease, the existing circulating viral isolates, variation in the severity of outbreaks and the role of small ruminants and cloven-hoofed wild animals in the country need to be investigated. This chapter describes an initial attempt of using sero-surveillance of FMD through the detection of antibodies in serum samples collected from cattle, small ruminants and cloven-hoofed wild animals to address this need.

6.2 Materials and Methods

6.2.1 Study area

Ethiopia is divided into 8 administrative regions (Fig. 6.1). Within each region there are zones which are further divided into districts. There are 3 major agro-ecological zones in Ethiopia *viz*., the dry climate, the tropical rainy climate and the temperate climate. Classification of the country into agro-ecological zones or administrative regions is of no value to study FMD. However, classification according to farming systems is more beneficial as the type of farming system may influence the transmission and spread of the disease. The two major farming systems in Ethiopia are indicated in Fig. 6.2:

1. The communal (pastoral) farming system is occupied mainly by nomads and it represents the dry climate and low land (green-shaded).
2. The semi-intensive and intensive farming system covers the tropical rainy climate and temperate climate which are mostly high land and mid altitude areas (yellow-shaded).

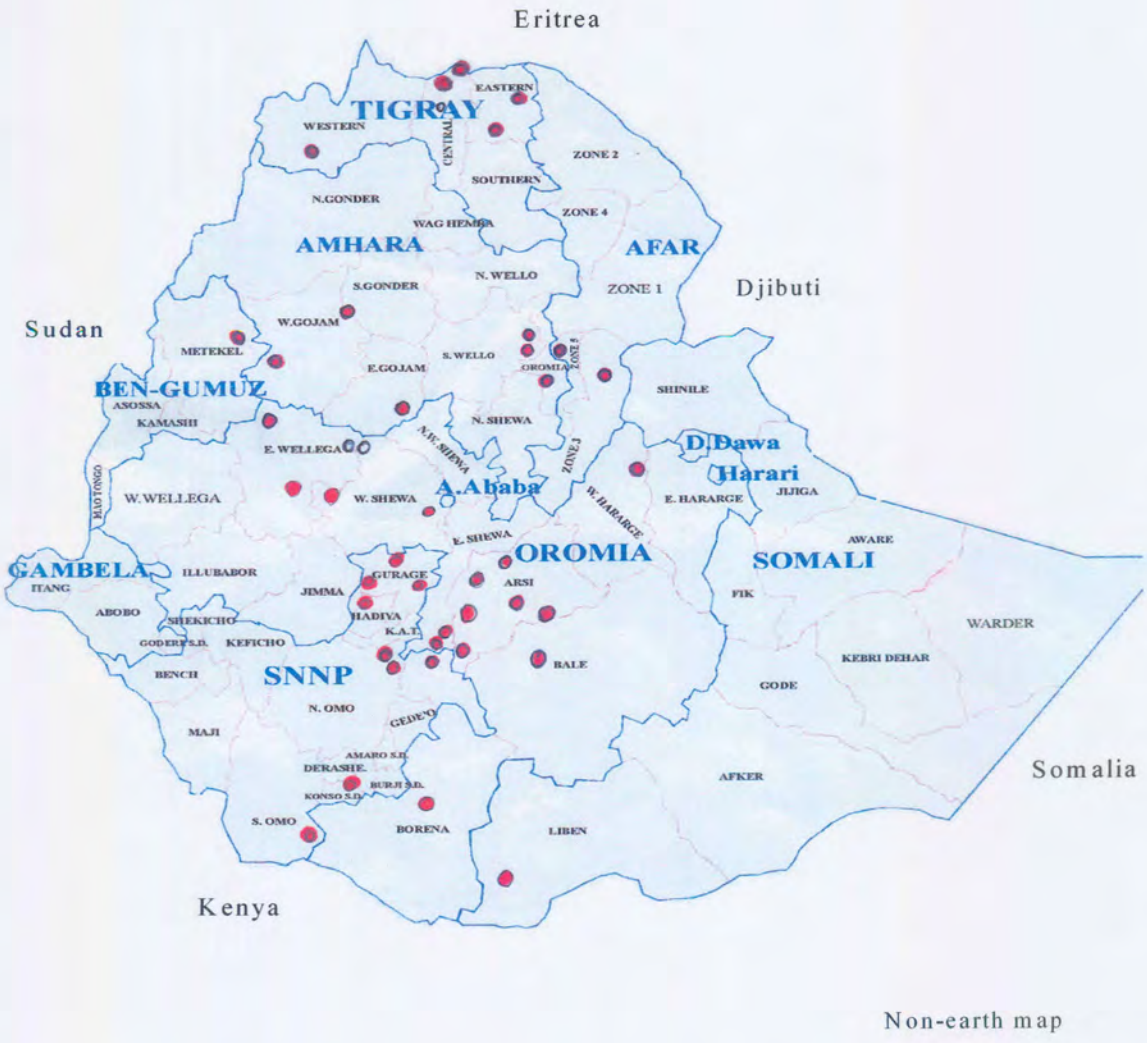


Fig. 6.1 Administrative map of the Federal Republic of Ethiopia, depicting regions (blue print) and zones (black). ● indicates distribution of reported foot-and-mouth disease outbreaks during serum sampling in 2001 (January to March)

6.2.2 Serum samples

Serum samples from cattle collected over a 5 year period for sero-surveillance of rinderpest and sera from sheep, goats and wild ruminants were collected. Field sera were sampled from sheep and goats above one year of age and cattle ranging between 1 and 3 years of age. All serum samples collected represent sera from non-vaccinated animals. The species of wildlife sampled are indicated in Table 6.3a.

Fig. 6.2 Map of Ethiopia showing the different farming systems (source: Ministry of Agriculture of Ethiopia, 1999).

Serum samples collected from each farming system (Fig. 6.2) and stored in the serum bank were selected by applying simple random or systematic random sampling. The same protocol was designed for the control of rinderpest to detect a sero-prevalence of 5%, with a precision level of 5% of the true level 95% of the time. This allowed the detection of one antibody positive case to rinderpest in the sampling unit (herd).

Serum sample size determination: The districts within each farming system as well as the size of the livestock population were considered in the preparation of

the sample framework so that all the study populations had an equal chance of being selected.

It was assumed that 50% of the cattle and small ruminants (sheep and goats) had antibodies to FMDV. This estimate was assumed to be within 5% (allowable error) for cattle and small ruminants of the true level, 95% of the time. It was also estimated that 20% of wildlife (buffalo, warthog, hart beest, lesser kudu, eland, menlik's bush buck and mountain nyala) sampled would be sero-positive with a desired absolute precision of 10% of the true level, 95% of the time.

$n = 1.96^2 P\hat{O} / L^2$ (where $\hat{O} = 1 - P$)

n = required sample size

P = estimate of prevalence (%)

L = desired absolute precision (%)

Cattle (P = 50%, L = 5%)

$n = 1.96^2 \times 0.5(1-0.5) / (0.05)^2 = 384.16$ ... (400)

Small ruminants (P = 50%, L = 5%)

$n = 1.96^2 \times 0.5(1-0.5) / (0.05)^2 = 384.16$ ... (400)

Wild animals (P=20%, L = 10%)

$n = 1.96^2 \times 0.2(1-0.2) / (0.1)^2 = 61.46$ ... (62)

To compensate for the error due to multistage sampling the number of samples was doubled. Therefore, the total number of serum samples for both cattle and small ruminants (sheep and goats) were 800 each (Table 6.1). Since the population of wild animals is very small compared to cattle and small ruminants, the sample size remained the same.

Criteria of sampling percentage: It has been established that 40% of the animals are farmed in the communal farming system, while 60% of them are farmed in the intensive and semi-intensive farming system (Anonymous 1998b). Thus, the number of serum samples collected is a reflection of the representative percentage livestock population per farming system (Table 6.1) and the sample size within each category is summarized in Table 6.2. Sampling units within administrative regions were randomly selected representing the 2 different farming

systems. The number of serum samples tested per administrative region is a reflection of the percentages of livestock in relation to the total livestock population in the 2 farming systems.

Table 6.1 Total number of serum samples collected per farming system.

Farming system	**Serum samples**		
	Cattle	Sheep & goats	Wild ruminants
Communal farming	320 (40%)	320 (40%)	77
Intensive & semi-intensive	480 (60%)	480 (60%)	
Total	800	800	77

Table 6.2 Number of serum samples collected from randomly selected districts within the administrative regions, representing the 40 and 60% animal distribution in the (A) communal and (B) intensive/semi-intensive farming systems, respectively.

Adminstrative region	**Cattle**		**Small ruminants**		**Cattle**	**Small ruminant**
	A	B	A	B	Total	Total
Tigray	7	54	50	46	61	96
Afar	101		80		101	80
Amhara		124		139	124	139
Oromia	42	214	54	219	256	273
Somali	100		80		100	80
Benshangule	30		24		30	24
S.N.N.P.R.	20	88	14	76	108	90
Gambela	20		18		20	18
Total	320	480	320	480	800	800

6.2.3 Antibody detection tests

6.2.3.1 Liquid phase blocking ELISA

An adaptation of the method described by Hamblin *et al.* (1986a & 1986b) and applied by Esterhuysen *et al.* (1994) was used.

Serum samples: - Serum samples were stored at -20°C. Once thawed, samples were kept at 4°C until used. Phosphate buffered saline (PBS) containing 0.05% casein (PBS-C) was used as diluent for sera and conjugate while PBS containing Tween 20 (PBS-T) was used as washing buffer to remove unbound reagents. Sera were initially diluted (1:10) in PBS-C and two fold dilutions of these sera (1:20 – 1:80) in duplicates were tested for serotyping against 6 serotypes of FMD virus where the antigen (inactivated virus from cell culture supernatant) and antisera used in the liquid phase blocking ELISA (Lpb ELISA) was prepared from the following isolates (Esterhuysen, personal communication).

SAT-1: SAR/9/81 and BOT/1/68	A: A_5 Allier
SAT-2: ZIM/7/83 and KNP/19/89/2	O: SAR/11/00
SAT-3: KNP/10/90/3	C: C_1 Noville

Antibody blocking titres were expressed at the 50% level of inhibition compared with the virus control. Any sera dilution where 50% of the maximum optical density (MOD) reading was inhibited were considered reactive (positive) where the MOD for each plate was determined by subtracting the OD value of the negative antigen from the OD value of the positive antigen. Sera with titres $\geq$ 1/40 were considered positive.

6.2.3.2 The UBI FMDV non-structural enzyme-linked immunosorbent assay (3ABC ELISA)

The United Biomedical Inc. FMD virus enzyme-linked immunosorbent assay (UBI FMDV NS EIA) is a useful test for identifying infected animals (Mackay, 1998 and references therein). All the reagents, buffers, microplates, control reactive and non-reactive sera were supplied by the manufacturer (UBI, USA). They were stored at +4°C and always pre-warmed to room temperature before used. Briefly, the test sera as well as a set of negative and positive references were added to ELISA plates pre-coated with 3ABC antigen and incubated for 60 min at 37°C. After incubation, the plates were washed (at least 300 µl/well/wash) with diluted wash buffer (PBS) using an automatic microplate washer. Excess liquid was removed from the reaction microplate by inverting and tapping on absorbent paper until no further moisture appeared on the paper. An enzyme conjugated to anti-species antibody was added to the plate and incubated for another 30 min. After incubation the plates were washed as described above. One hundred µl of TMB substrate solution was added to each well. After incubation of the microplate in

the dark for 15 min at 37°C, 100 µl of stop solution (1M H_2SO_4) was added to each well. The absorbance of each well was read at 450 nm. The positive and negative results of test sera for each plate were established using the formula provided by the manufacturer where the results were expressed as an index derived by dividing the absorbance value of the serum tested by that of the cut-off control.

6.2.4 Data analysis

The sample size was determined in accordance with the estimation of the prevalence of FMD in Ethiopia (Section 6.2.2). All data collected were edited and evaluated from the sample sheet. Descriptive statistical methods were applied to estimate the percentage of agreement between the two different ELISAs in detecting antibody to FMD virus. The data comparing the Lpb ELISA and the 3ABC ELISA were assessed by cross tabulating and the percentage of agreement was estimated. The results were analysed in three groups representing serum samples from three species of animals (wildlife, small ruminants and cattle).

6.3 Results

The Lpb ELISA and 3ABC ELISA were used to test sera for the presence of antibodies mounted against structural and non-structural (3ABC) proteins of FMDV respectively. Heterogeneity of FMDV due to changes in the structural proteins of the virus may have an effect on the sensitivity of the Lpb ELISA and the antigen used in this test may not be recognized by some of the antibodies in the test sera. Therefore, the 3ABC ELISA was used since it employs synthetic peptides to the more conserved non-structural proteins compared with the solid-phase immunosorbent assay for detection of antibodies to infectious FMDV and may be more sensitive to score positive sera (Mackay, 1998).

In this study the origin of the reactive and non-reactive sera was used to evaluate the potential role of the farming systems on the incidence of FMD as well as the role of wildlife and small ruminants in the epidemiology FMD. The serotype identification by Lpb ELISA and the reactive and non-reactive sera detected by the 3ABC ELISA (per row) in each table represents the same animals i.e. if the serum scored positive in Lpb ELISA and positive in 3ABC ELISA in the same row, it

represents the same animal. All reactive (positive) sera are denoted by R (+) and all non-reactive sera by NR (-).

6.3.1 Analysis of sera from wildlife

Cross tabulation representing the number of positive and negative serum samples from wildlife are presented in Table 6.3a. The different serotypes identified are presented in Table 6.3b. Sera reactive to both tests were detected in buffalo, eland and mountain nyala while sera from other wild species included in this study were found to be non-reactive (Table 6.3a). One serum from eland and mountain nyala each tested with the Lpb ELISA, was positive for serotype C of FMD (Table 6.3b) but had low titres (1.7, 1/52) and was probably due to false positive reactions. These reactions were not confirmed on serum neutralization test and tested negative on the 3ABC ELISA (Table 6.3a). Serotype A, SAT-1 and SAT-2 were identified in 21 (n = 25) sera from buffaloes where only 16/21 (76.19%) of the same sera were found to be positive with the 3ABC ELISA (Table 6.3b). There were therefore 7 sera positive with the Lpb ELISA that didn't react with 3ABC ELISA. In contrast, 2 sera were detected as reactors in the 3ABC ELISA and not in the Lpb ELISA. Five doubtful reactive sera to SAT-3 by the Lpb ELISA were considered as negative as low values for the same sera were detected by the 3ABC ELISA (Appendix II). A total of 52/77 sera were detected as non-reactors in both tests (Table 6.6).

Table 6.3a Summary of reactivity of serum samples tested by the Lpb ELISA and 3ABC ELISA. The numbers in each row represent the same serum samples.

Origin of serum	Lpb ELISA		3ABC ELISA	
	R (+)	NR (-)	R	N R
Buffalo	21		14	7
		4	2	2
Eland (Tautotragus oryx)	1	4		5
Mountain Nyala (Tragelaphus buxtfoni)	1	6		7
Tiang (Taurotragus oryx)		11		11
Lelwel H. beest (Alcelaphus buselaphus lelwel)		5		5
Swayne's H. beest (A. budelaphus swaynei)		11		11
White earned kob (Kobus kob cencotis)		4		4
Lesser kudu (Tragelaphus imberbis)		3		3
Menlik's bush buck (T. scriptus meniliki)		2		2
Warthog (Phacocherus aethiopicus)		4		4
Total	23	54	16	61

R(+) = Reactive serum NR(–) = Non-reactive serum

Table 6.3b: Foot-and-mouth disease virus serotypes identified from reactive sera of wildlife.

Origin of serum	Serotype identified				3ABC ELISA
	SAT-1	SAT-2	A	C	
Buffalo (Omo National Park)	+13				+8, –5
	+2				+1, –1
	+3				+3
		+1			+1
		+2			+1, –1
	– 4				(+2, -2)
Eland (Omo National Park)				+1	–1
Mountain Nyala (Bale Mountain Park)				+1	–1

6.3.2 Analysis of sera from small ruminants

Eight hundred serum samples from sheep and goats (400 + 400) were tested using the Lpb ELISA and the 3ABC ELISA (Table 6.4). Antibodies to serotypes A (25), O (43) and to both A & O (19) were identified. There were no serum samples reactive to serotype C and the 3 SAT types of FMD virus. Both tests detected the same 692 sera as non-reactive and the 87 sera mentioned above as reactive. An additional 21 sera were positive on the 3ABC ELISA while not reacting on the Lpb ELISA (692 + 21 = 713). This indicated that 14.9% (21/108) of the sera were not recognized as reactors by the Lpb ELISA. Of interest is that all the reactive sera to 3ABC ELISA were collected from the areas where there were FMD outbreaks (Fig. 6.2) due to serotypes A and O. All the serum samples taken from the low-land (Somalia, Gambela and Benshanule) were found to be non-reactive in both assays (Table 6.4).

Table 6.4 Summary of results of Lpb ELISA and 3ABC ELISA using sera from small ruminants.

Regions	Lpb. ELISA			3ABC ELISA		Total
	RS and serotype identified		NR			
	A	O		R	NR	
Tigray		4	92	4	92	96
Afar	5 (A+O)	11 (O)	64	20	60	80
Amhara	4 (A+O) 2 (A)	20 (O)	113	29	110	139
Oromia	10 (A+O) 12 (A)	8 (O)	243	33	240	273
Somali			80		80	80
Benshangule			24	4	20	24
S.N.N.P.R.	11 (A)		79	16	74	90
Gambela			18	2	16	18
Total	87		713	108	692	800

6.3.3 Analysis of sera from cattle

The number of reactive and non-reactive sera as well as the serotypes identified in sera from cattle in each administrative region are presented in Table 6.5. Serotypes O, A, SAT-1 and SAT-2 were identified in bovine serum samples but there were no sera reactive to serotype C and SAT-3. Serotype O (150/210) was most prevalent and had a wide distribution throughout Ethiopia while serotype A was limited to 3 administrative regions with common boundaries (Table 6.5). The positive sera due to serotypes A and O were detected in both farming systems in the administrative regions of Afar, Amhara and Oromia where more than 80% of the livestock population of Ethiopia occur. Serotype O was also detected from serum collected from the low-land area of Tigray. The distribution of serotypes A and O was not confined to a particular farming system as it covered the low-land area in the south (Oromia), central high lands and low land areas of the North East and Western part of Ethiopia. It seems that the size of susceptible cattle population plays a role in the maintenance of FMD rather than the farming system.

Table 6.5 Summary of test results of Lpb ELISA and 3ABC ELISA using sera from cattle.

Regions	Lpb ELISA					3ABC-ELISA		Total
	RS & serotype of FMDV identified				NR	R	NR	
	A	O	SAT-1	SAT-2				
Tigray		12 (O)			49	14	47	61
Afar	12(A+O) 11(A)	20 (O)			58	40	61	101
Amhara	7 (A)	23 (O)			94	34	90	124
Oromia	12(A+O) 8 (A)	39 (O)	7 (SAT-1&2) 11 (SAT-1)	16 (SAT-2)	163	95	161	256
Somali					100		100	100
Benshangule					30		30	30
S.N.N.P.R.		32 (O)			76	31	77	108
Gambela					20		20	20
Total	210 (26.5%)				590	214	586	800

The distribution of all reactive sera to SAT types (34) was limited to the southern part of the country (Borena and Southern Shoe zones). Antibodies to serotype SAT-1 and SAT-2 (24) were detected from cattle in the low land area of Borena zone adjacent to the Kenyan border and Omo National Park which is a rich habitat for wild ruminants. Nearly equal numbers of non-reactive sera (307) (52.1%) were collected from the low-land areas in the region of Tigray, Oromia, Somali, Benshangule and Gambela while 283 (47.9%) non reactive sera were from intensive and semi-intensive farming systems (Fig. 6.2).

Summary of cross tabulation comparisons of test results of serum samples from the three groups of animals detected by blocking ELISA and 3ABC-ELISA is presented in Table 6.6. The results indicated that both assays identified the same 306/1677 and 1327/1677 sera as reactive and non-reactive, respectively. Disagreement between the two tests was found on test results of 44/1677 sera. The 3ABC ELISA identified 13/44 (29.5%) as reactive (negative on Lpb ELISA) and the remaining 31/44 (70.4%) as non-reactive (positive on Lpb ELISA).

Table 6.6 Cross tabulation comparisons of reactive and non-reactive sera from bovine, small ruminants and buffaloes detected by blocking ELISA and 3ABC ELISA.

Wildlife		3ABC ELISA		Total
		R	NR	
Lpb ELISA	R	14	2	16
	NR	9	52	61
Total		23	54	77
Small ruminants		3ABC ELISA		Total
		R	NR	
Lpb ELISA	R	87	21	108
	NR	0	692	692
Total		87	713	800
Cattle		3ABC ELISA		Total
		R	NR	
Lpb ELISA	R	205	8	213
	NR	4	583	587
Total		209	591	800

6.4 Discussion

In a country such as Ethiopia where FMD is endemic, and where large numbers of susceptible domestic and wild ruminants exist with limited vaccination on some dairy farms, serological surveys are a pre-requisite to understand the epidemiology of FMD. To delineate the epidemiological profiles of the endemic occurrence of FMD in Ethiopia, 1677 sera from different species were investigated using serological tests. Of the animal species included in this study, cattle, sheep, goats and buffalo are more susceptible to FMD (FAO, 1984). In addition the few species of cloven-hoofed wildlife from Ethiopia investigated in this study may become infected with FMD but their role in the epidemiology of FMD is unclear. In Ethiopia the highest sero-prevalence was detected in buffalo 84% (21/25) where antibodies to serotypes A, SAT-1 and SAT-2 were identified for the first time in this species. Antibodies to the same serotypes of FMD viruses were also identified in cattle serum collected in the vicinity of the buffalo populations (Omo National Park). Both tests confirmed the presence of antibodies to FMDV in clinically normal buffalos, indicating a possible carrier state as has been recognized previously (Hedger, 1968,1970; Burrows *et al.,* 1971; Rossi *et al.,* 1988). These animals could represent a potential risk of FMD maintenance and a potential

source of infection for other susceptible cloven-hoofed animals in Ethiopia. This is likely to be the same scenario in the neighbouring country Kenya, as the presence of antibodies against 5 serotypes of FMD in buffalo was previously reported in Kenya by Anderson (1981, 1985).

Serological results indicated that the two ELISAs were able to detected 306 (18%) and 1327 (79%) sera as seropositive and seronegative, respectively. However, thirty one sera were reactive with the Lpb ELISA while they were non-reactive in the 3ABC ELISA (Table 6.6). This may be explained by differences in the life span of antibodies to structural and non-structural (NS) proteins (3ABC) in infected animals or false positive reaction on the Lpb ELISA, but also false negative reaction on the 3ABC ELISA. Antibodies to the non-structural proteins can be detected in infected cattle and pigs for up to one year after infection (reviewed by Mackay, 1998) while antibodies to the structural proteins can last 1-2 years (McCullough *et al.*, 1992) and even longer. On the other hand another 13 sera that were positive with the 3ABC ELISA appeared as negative in the Lpb ELISA which could be possible due to heterogeneity of the viruses included in the latter assay and prevalent viruses in Ethiopia. For example, the ZIM/7/83 (SAT-2) that was used for Lpb ELISA reagent preparation and the ETH/2/91 Ethiopian field isolate clustered into 2 different lineages (divergence 27.6%) (Fig. 4.1). Thus the Lpb ELISA used in this study may lack sensitivity to some genetic variants of FMDV (previous chapters) as the immuno-reagents for this test were developed against southern African isolates (Section 6.2.3.1). The results indicate that the Lpb ELISA should either be customized for each region in Africa to ensure sensitivity or that viruses from different regions should be included as a mixture in the assay. It would seem that ELISA kits will provide better results if designed for specific geographic locations, incorporating immuno-reagents that can recognize homologous and heterologous population of circulating variants.

Sample size was determined on the basis of the assumption that 50% of cattle and small ruminants and 20% of wild ruminants had antibodies to FMD. In this study from the results indicated that 26.25% (210/800) cattle, 10.8% (87/800) small ruminants and 29.8% (23/77) of wild ruminants have been exposed. This showed that more serum samples must be tested to estimate the actual sero-prevalence of the disease. In conclusion therefore, the detection of antibody to the NS protein

3ABC in conjunction with specific antibody detection by the Lpb ELISA provided conclusive evidence of previous infection with FMD. In addition, the distribution of reactive sera (Table 6.4, 6.5) and non-reactive sera did not show the importance of farming systems in the occurrence of the disease. Although the number of sera analysed in this study is small, the serology results indicated that buffaloes are the only wildlife species that play an important role in the epidemiology of FMD in Ethiopia. Although, small ruminants become infected, the low percentage of positive animals indicated that they are probably not important in the epidemiology of the disease in Ethiopia. It has also been reported that clinically healthy sheep was shown to be seropositive to infection with FMDV in Kenya and Morocco (Ndarathi, 1991; Blanco *et al.*, 2002). Therefore more epidemiological studies including serosurveillance and virus isolation is required to confirm the role of small ruminants and wildlife in Ethiopia.

CHAPTER VII

GENERAL DISCUSSION AND RECOMMENDATIONS

Complete and partial nucleotide sequence data of the 1D gene were used to study the genetic variation and relationships of isolates within serotype O, SAT-1 and SAT-2 FMD from East Africa. These three serotypes as well as serotype A have been reported to be most prevalent in sub-Saharan African countries (Vosloo *et al.*, in 2002). Previous studies on the molecular epidemiology of FMD virus focused mainly on southern and West African isolates (section 5.4, the references therein). Knowles *et al.* (1998) studied serotype A in some detail and reported the presence of six regionally and genetically distinct virus lineages. Serotype C is rare and the last outbreak was recorded in Kenya in 2000 (Kitching, 2002a). Serotype SAT-3 rarely occurred in East Africa and has been reported only in Uganda (Vosloo *et al.*, 2002). Therefore, serotypes A, C and SAT-3 were not included in this study.

A summary of lineages and genotypes (topotypes) identified in this and other studies are presented in Table 7.1 and Fig 7.1A-C. In this study distinct geographical localization of virus isolates were observed at genotype level. The distribution of isolates to defined localities can also be seen at lineage level (Table 7.1). In some cases 2 to 3 genotypes were found to be overlapping (*e.g.* Ugandan isolates).

The interpretation of the genetic relationships of FMD virus isolates in terms of lineage and genotype was based on uncorrected sequence divergence values between clusters in the phylogenetic tree with significant boot-strap support and agreements between different methods of phylogenetic reconstruction applied. The optimum cut-off values for clearer definition of genotypes and lineages are summarized in Table 7.2. Differences of approximately > 15% in nucleotide sequence to distinguish genotype (Rico-Hesse *et al.*, 1987) and cut-off values in excess of 15 - 20% nucleotide differences (VP1 gene) to distinguish between subgroups/topotypes have previously been used (Vosloo *et al.*, 1992, Bastos *et al.*, 2001, 2003: Sangare *et al.*, 2001, 2003; Samuel and Knowles 2001a,b; Knowles and Samuel 2003).

Table 7.1 Summary of phylogenetic analysis of FMD virus isolates from East Africa (EA) and elsewhere. References are indicated for previously established topotypes.

Lineages	Topotypes	Country
O		
I	A (North-Eastern EA)	Ethiopia, Eritrea, Kenya, Somalia, Sudan
	B (Sudan-West Africa)	Sudan, Algeria, Ghana, Burkina Faso
	C (Central EA)	Kenya, Uganda
	D (Sudanese)	Sudan
	E (Uganda I)	Uganda
	F (Uganda II)	Uganda
	G (South-Eastern EA)	Tanzania, Uganda
	H (The Middle East-South Asia)	India, Turkey, Saudi Arabia, Bangladesh, Iraq, South Africa (Sangare *et al.*, 2001; Samuel & Knowles, 2001b)
II	I (Cathay)	Taiwan, Russia (Samuel & Knowles, 2001b)
III	J (Angolan)	Angola (Sangare *et al.*, 2001)
	K (Euro-S. America)	United Kingdom, Argentina (Samuel & Knowles, 2001b)
SAT-1		
I	A	Tanzania, Kenya, Zambia, Malawi
	B	Zimbabwe (Bastos *et al.*, 2001)
II	C	South Africa, Mozambique (Bastos *et al.*, 2001)
	D	Zimbabwe (Bastos *et al.*, 2001)
III	E	Zimbabwe (Bastos *et al.*, 2001)
	F	Zambia, Botswana, Namibia (Bastos *et al.*, 2001)
IV	G	Uganda
V	H	Uganda
VI	I	Nigeria, Niger (Sangare *et al.*, 2003)
VII	J	Uganda
VIII	K	Nigeria, Sudan (Sangare *et al.*, 2003 and this study)
SAT-2		
I (East Africa)	A	Kenya, Uganda
	B	Uganda
	C	Uganda, Zaire (DRC)
	D	Sudan
	E	Ethiopia
	F	Eritrea, Saudi Arabia (Bastos *et al.*, 2003 and this study)
	G	Rwanda (Bastos *et al.*, 2003)
II (Angola)	H	Angola (Bastos *et al.*, 2003)
III (West Africa)	I	Ghana, Niger, Senegal (Sangare *et al.*, 2003)
IV (WA)	J	Gambia, Senegal (Sangare *et al.*, 2003)
V (East-Southern Africa)	K	Zimbabwe and Botswana (Bastos *et al.*, 2003)
	L	Zimbabwe (Bastos *et al.*, 2003)
	M	Botswana and Zambia (Bastos *et al.*, 2003)
	N	South Africa (Bastos *et al.*, 2003)
	O	Kenya, Malawi, Tanzania, Ethiopia, Burundi

In this study, the cut-off values determined for genotypes varied from 13 to 20% and lineages from 20 to 26% (Table 7.2). The differences in cut-off values for each serotype are most probably associated with the differences in the level of intratypic

variation between serotypes. Higher cut-off values were observed for SAT type virus isolates than for serotype O. Similarly, higher levels of intratypic variations were observed for SAT type virus isolates than for serotypes O, A, C (Vosloo *et al.*, 1995; Bastos, 1998, Bastos *et al.*, 2001, 2003; Van Rensburg and Nel 1999). The nucleotide sequence divergence between genotypes within each serotype was found to be higher in the SAT-1 and SAT-2 virus isolates compared to O viruses (Table 7.2). The variation level between some lineages within SAT-1 (30-35%) is almost borderline for variation between serotypes, as was indicated between serotypes O and C (Domingo *et al.*, 1990).

Table 7.2 Summary of nucleotide sequence differences used as cut-off values to define genotypes and lineages as well as divergence between genotypes within each serotype.

Serotypes	Genotypes (%)	Lineages (%)	Divergence between genotypes (%)
O	> 13	> 20	13 – 20
SAT-1	> 16	> 23	16 - 24
SAT-2	> 20	> 26	20 - 27

For serotype O all 66 isolates from Africa as well as 6 isolates from the Middle East/Asia and South Africa clustered into one evolutionary lineage containing 8 distinct genotypes (Table 7.1). Isolates from East Africa clustered into 7 distinct topotypes (A - G), except one topotype (B) which included isolates from Sudan and West Africa and this study has revealed the historical relationship between East and North-western African isolates. Furthermore, the phylogenetic tree linked all 7 topotypes as well as one topotype (H) which included isolates from the Middle East and South-East Asia to the same node with significant boot-strap support of 92%, suggesting the possibility of the same ancestor. This lineage also diverged (20-24%) from the previously determined 2 lineages included in this study (Samuel and Knowles, 2001b; Knowles and Samuel, 2003; Sangare *et al.*, 2001) where one lineage included the Cathay topotype and the other the Angolan, and Euro-South American topotypes (Fig. 3.1).

The phylogeographical distribution of serotype O topotypes in East Africa can be divided into 2 major areas. Three topotypes (Table 7.1, A, B & D) were found to

be distributed in the northern part of East Africa (Kenya and north of Kenya) while the other 4 topotypes (C, E, F & G) were distributed in Kenya and countries to the south. The North Eastern African topotype (A) has a wide distribution across 5 countries with common boundaries namely Ethiopia, Eritrea, Somalia, Kenya and Sudan. One of these countries, Sudan harboured another 2 topotypes, the Sudanese (D) as well as the Sudan-West Africa topotypes (B). From countries south of Kenya, Uganda was extremely diverse in its topotype distribution with one topotype being shared with Kenya (C) and the other (G) with Tanzania as well as having two distinct topotypes (D & E) exclusive to Uganda. The overlapping topotype distribution in East Africa provides the basis for a proposal of FMD control. Countries north and south of Kenya have to consider the genetic diversity of isolates within topotypes of their respective sub-regions for the implementation of effective control strategies. Kenya, being centrally situated within East Africa has to consider all topotype distributions since the country harboured both groups of topotypes. Kenya and Ethiopia are producing vaccines against this serotype but detailed studies on the level of protection against all circulating field strains within these countries should be determined.

Comparison of amino acids at the immunodominant sites between the vaccine strain (ETH19/77) and other Ethiopian outbreak isolates revealed genetic variation. In all 36 compared isolates (Fig. 2.2) the hypervariable region was located at the highly immunogenic site of the 1D gene, the G-H loop. These results also indicated that further research is needed to assess whether the vaccine strain will provide adequate protection against field variants of type O FMD viruses currently circulating in Ethiopia. The distribution of East African topotypes revealed in this study, is of major importance as each country in this region is under constant threat of importing new variants. Therefore, it is strategically important to consider a regional approach for effective disease control.

In Ethiopia, FMD is a very common disease in cattle. The clinical signs of the disease in this species of animals are well recognized by herds men. The majority of the farmers interviewed during sample collection expressed the exact clinical signs of the disease in cattle. However, the majority of the same farmers didn't know the occurrence of the disease in small ruminants. The pastoral community in the southern part of the country (Borena) observed 2-3 outbreaks of FMD per year

and this was also confirmed by animal health assistance personnel in the region. In this study, serological survey results also indicated the involvement of 2 to 3 serotypes in outbreaks within the same herds of cattle and small ruminants (Table 6.4, 6.5). Alternatively, serotypes may persist in a region and rarely appear clinically, producing only mild infection where it can be only detected serologically. Generally the disease is clinically less obvious in indigenous breeds to the country where FMD is endemic (Kitching, 2002a).

Serological evaluation was used to determine the FMD incidence in Ethiopia using a liquid phase blocking ELISA and 3ABC ELISA from sera of non-vaccinated cattle, small ruminants and wild ruminants. The results obtained from these tests identified SAT-1 and SAT-2 FMD viruses circulating in buffalo and cattle populations in the southern part of the country. Since 1992, SAT-2 viruses have been isolated from FMD outbreaks while to date no SAT-1 virus has been isolated in the country. However, it is clear that SAT-1 viruses are present, but remain unrecorded as the surveillance system is inadequate. Furthermore, the highest sero-prevalence was detected in buffalo (84%) where antibodies to SAT-1 and SAT-2 were identified for the first time in these species in Ethiopia while both serotypes were also identified in cattle serum collected in the vicinity of buffalo populations (Omo National Park). Although antibodies to the 2 SAT types as well as serotype A and O have been identified from cattle and small ruminants sera, outbreaks due to O and A are predominant, with SAT-2 having the lowest incidence in Ethiopia and SAT-1 being the least frequently recovered serotype from outbreaks in East Africa. Antibodies to serotype A and O were also identified from the sera of small ruminants but for all of these reactive sera the distribution was limited to the areas where FMD outbreaks were reported during the period of sample collection. The present study highlighted the importance of buffalo in the epidemiology of FMD in Ethiopia and showed that other wild and domestic small ruminants are insignificant in the epidemiology of the disease in Ethiopia. The susceptibility of small ruminants to FMD can vary with strains of virus and the clinical signs may be indistinguishable from those produced by a variety of other causes and for this reason most cases of FMD in sheep and goats are misdiagnosed. However, this species have played a significant role in the transmission of the disease to susceptible hosts in other parts of the world

(Kitching and Hughes, 2002). Furthermore, small ruminants (sheep and goats) may become carriers of FMDV for variable periods of time (Callis, 1996).

Research addressing the role of small ruminants and other wildlife in the epidemiology of FMD in Ethiopia and other East African countries has been very limited in comparison to southern African countries. In order to further clarify the possible role of small ruminants and wildlife in virus maintenance and virus transmission during inter-epizootic periods and to gather more information on epidemiological data which can assist in the control the disease, extensive epidemiological studies involving both serological surveys and virus isolations need to be conducted.

The molecular epidemiology of SAT-2 was based on the phylogenetic analysis of the complete VP1 nucleotide sequences of 41 East African and 26 isolates from other African countries. Phylogenetic analysis identified 5 lineages and 15 genotypes from 13 African countries and Saudi Arabia (Table 7.1). Lineage I comprised 7 genotypes which consisted of viruses from East Africa, Zaire (DRC), Rwanda and Saudi Arabia while lineage V consisted of viruses occurring throughout southern African countries and extending into East Africa. Previously identified lineages (III and IV) from West Africa and one lineage (II) from Angola (Sangare *et al.*, 2003) included in this study were found to represent distinct isolates for their respective regions. These results indicated that the SAT-2 viruses were extremely diverse, but according to genotype distribution could be divided into 15 topotypes. Some of the East African topotypes showed links between countries within the region as well as with the southern and central African countries.

The East African isolates were distributed within lineages I and V (Table 7.1). Lineage I consisted of the majority of East African isolates as well as individual isolates from Saudi Arabia and Rwanda and 7 topotypes were assigned. The distribution of each topotype was limited to one or 2 countries. These genetically independent entities corresponded to geographically defined areas. Alternatively, the other topotype (O) within lineage V showed wide distribution which covered 5 countries (Burundi, Malawi, Tanzania, Kenya and Ethiopia). The geographic distribution of topotype O stretches from south to north across these countries.

Interestingly this topotype shared a common ancestor with buffalo virus isolates from South Africa in topotype N within the same lineage which indicated historical relationships between East African and South African isolates. It is possible that isolates within this topotype have a wider range of susceptible hosts.

The presence of 3 topotypes within Uganda (Table 7.1) showed the higher level of intratypic variation of SAT-2 virus isolates from this country. In contrast, the majority of virus isolates in genotype A, from Kenya and Uganda, were less diverged (< 6%) even though these isolates span a period of 13 years (Table 4.3). This genetic conservation over 13 years needs further investigation. Similarly, a close genetic relationship was observed between the vaccine strain ETH/19/77 and the field strain ETH/5/95 in Ethiopia. One speculation can be the re-introduction of the same virus from a common source such as a poorly inactivated vaccine.

SAT-1 maintains endemicity in many sub-Saharan African countries with marked regional differences in distribution. The Neighbour-joining method was used to construct phylogenetic trees based on partial nucleotide sequencing of the 1D gene (396 bp) from 52 SAT-1 virus isolates from Africa. This analysis identified 8 lineages and 11 genotypes (Table 7.1) when nucleotide differences of > 23% for lineages and > 16% for genotypes were used as cut-off values. The same pattern of phylogenetic relationships was confirmed using the complete 1D gene sequence of 39 selected isolates of SAT-1 viruses from Africa.

High intratypic variation (divergence 23.5 – 35%) was observed across lineages, except for the lineages distinct to Uganda, the other lineages were distributed between two or more countries. It seems reasonable to assume that viruses in each genotype evolved independently over years. A historical relationship between Sudan and West African (Nigerian) isolates were also observed in one of the genotypes. The genetic relationships of this serotype as well as serotype O was indicative of some exchange of viruses taking place in the past which can be related to the nomadic movement of animals and links between East and West Africa. These preliminary results showed the importance of molecular studies to assist in a better understanding of the genetic relationships of FMD viruses in Africa.

Three distinct lineages for Uganda were identified with high divergent values between each other (> 30%) and are by far the most heterogeneous. The Ugandan isolates characterized in this study including the above two serotypes revealed the presence of 8 genotype in this country. As this study highlighted, there is no explanation why are some strains are restricted to certain areas, while others are not. The precise mechanism that enable the virus to achieve this in not clear. It can however be predicted that within each serotype there is a spectrum of strains with their own antigenic and epidemiological characteristics (Kitching, 2002) which are likely to be important in survival mechanism of the viruses.

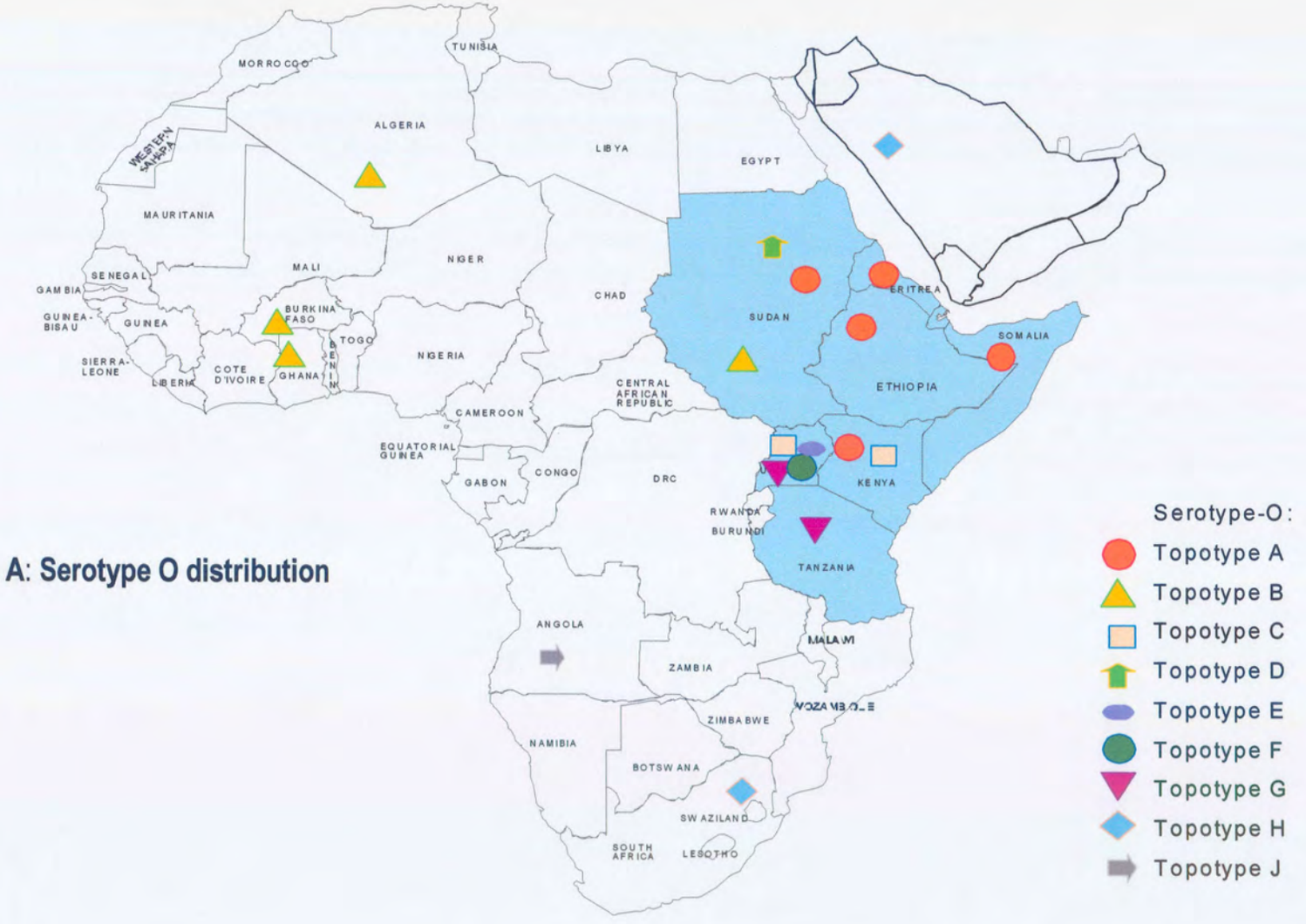
A variability plot based on sequence alignment of 221 amino acids of the 1D gene of 52 SAT-1 viruses correspond with immunogenic sites the G-H loop and the C-terminus regions (Fig. 5.4) which coincided mostly with the previous finding from southern and western African isolates (Bastos *et al.*, 2001; Sangare *et al.*, 2001). SAT 2 in contrast with SAT-1, were not hypervariable at amino acid positions 192 – 208 (the C-terminus) of the 1D gene. This was found with 48 SAT-2 viruses (Fig. 4.2) which exhibited a variation between 20 – 40% in this region. In addition within SAT-2 viruses two regions at amino acid positions 46 – 59 and 76 – 90 were shown to be hypervariable which were not observed in SAT-1 viruses.

The identification of numerous lineages and genotypes of FMD virus in East Africa having distinct or overlapping distribution and the links between East Africa and West Africa as well as southern Africa indicate that exchange of viruses between these regions has occurred in the past and the highest interatypic variation was observed in SAT-1 and SAT-2 viruses. The intratypic genetic variation in excess of 20% on the nucleotide sequence level were shown to be antigenically poorly related. This may lead to difficulties in the effective control of FMD by vaccination (Bastos *et al.*, 2003). Therefore, it is vital that the correct vaccine strains are selected that induce antibodies capable of full protection against field variants circulating within a country or region.

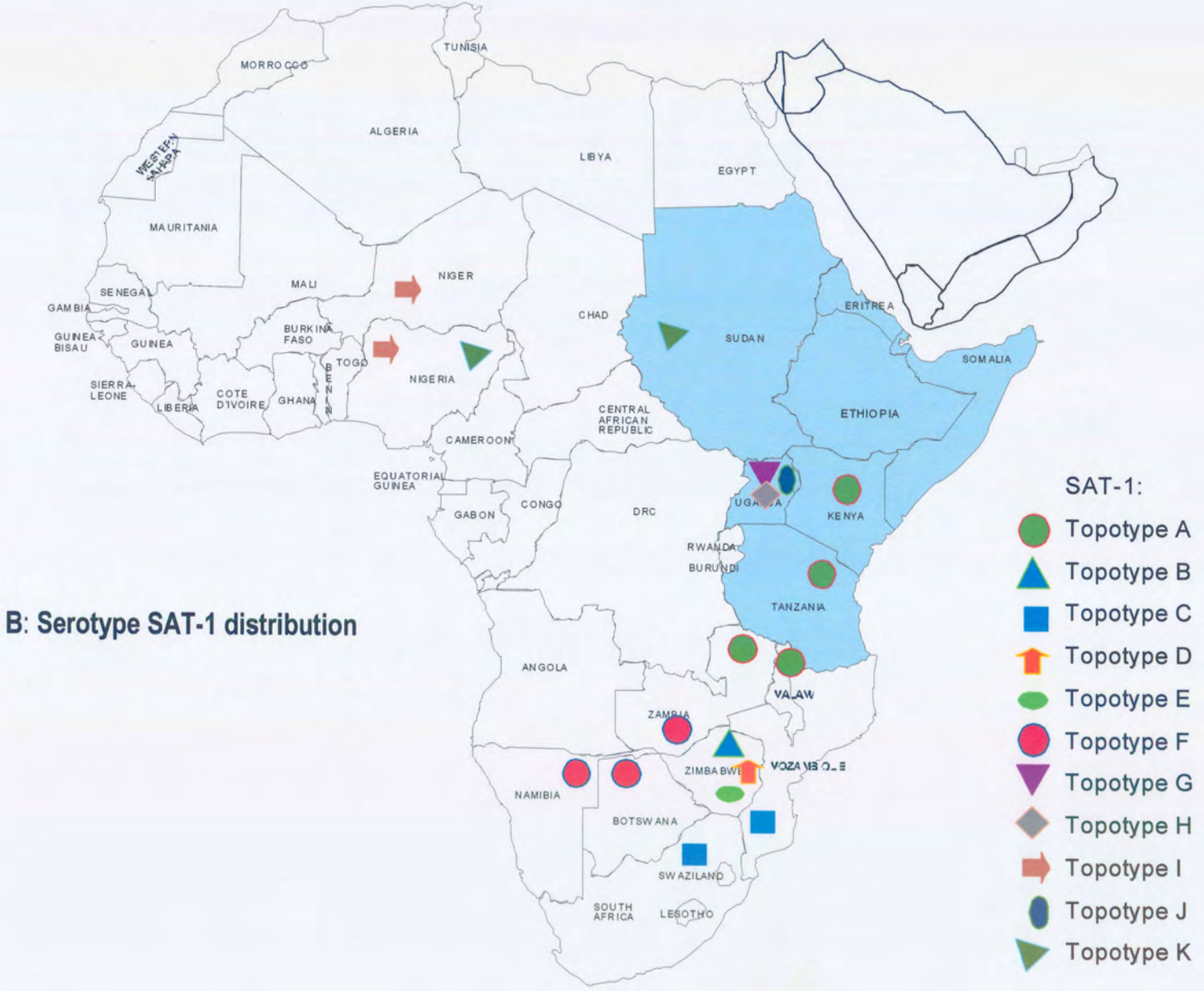
Understanding of the distribution of genotypes and lineages in Africa is vital and important in the development of future disease control strategies. It is likely that the genetic diversity on the nucleotide sequence level of the VP1 gene observed in this study is reflected antigenically. It can be seen that the one strategy by

vaccination only is complex partly because of the complex epidemiology involved. Our result indicated that some topotypes have a wider geographical distribution when others restricted within a country. This might be explained by the differences in survival mechanism of FMD viruses which might be linked to host rang and their ability to transmit rapidly and cause infection. It was reported that the PanAsian strain has been isolated from cattle, sheep, goats, pigs, water buffalo, deer, gazelles, antelopes and camels (Samuel and Knowles, 2001a) while other virus of serotype O was found to be a pig-specific strain in South-East Asia (Kitching, 2002b).

This study as well as previous studies based on the major immunogenic regions of the VP1 gene of SAT types (Bastos *et al.*, 2001, 2003; Vosloo *et al.*, 2002; Sangare *et al.*, 2003; Knowles and Samuel 2003) illustrated the distribution of distinct topotypes in Africa and a higher level of intratypic variation within each SAT type. Thus, a further detailed study on the evaluation of available vaccines and the development of multivalent vaccines for most developing countries of Africa on the basis of strict geographical clustering of independently evolving virus populations should be encouraged. Furthermore, restrictions on animal movement must be enforced to minimize further transboundary transmission of the disease. The virus characterization approach also provides a base-line database for tracing the origin of outbreaks. Effective control of FMD also requires a regional approach where the governments must be accountable for the planning and co-ordination of disease control programmes.



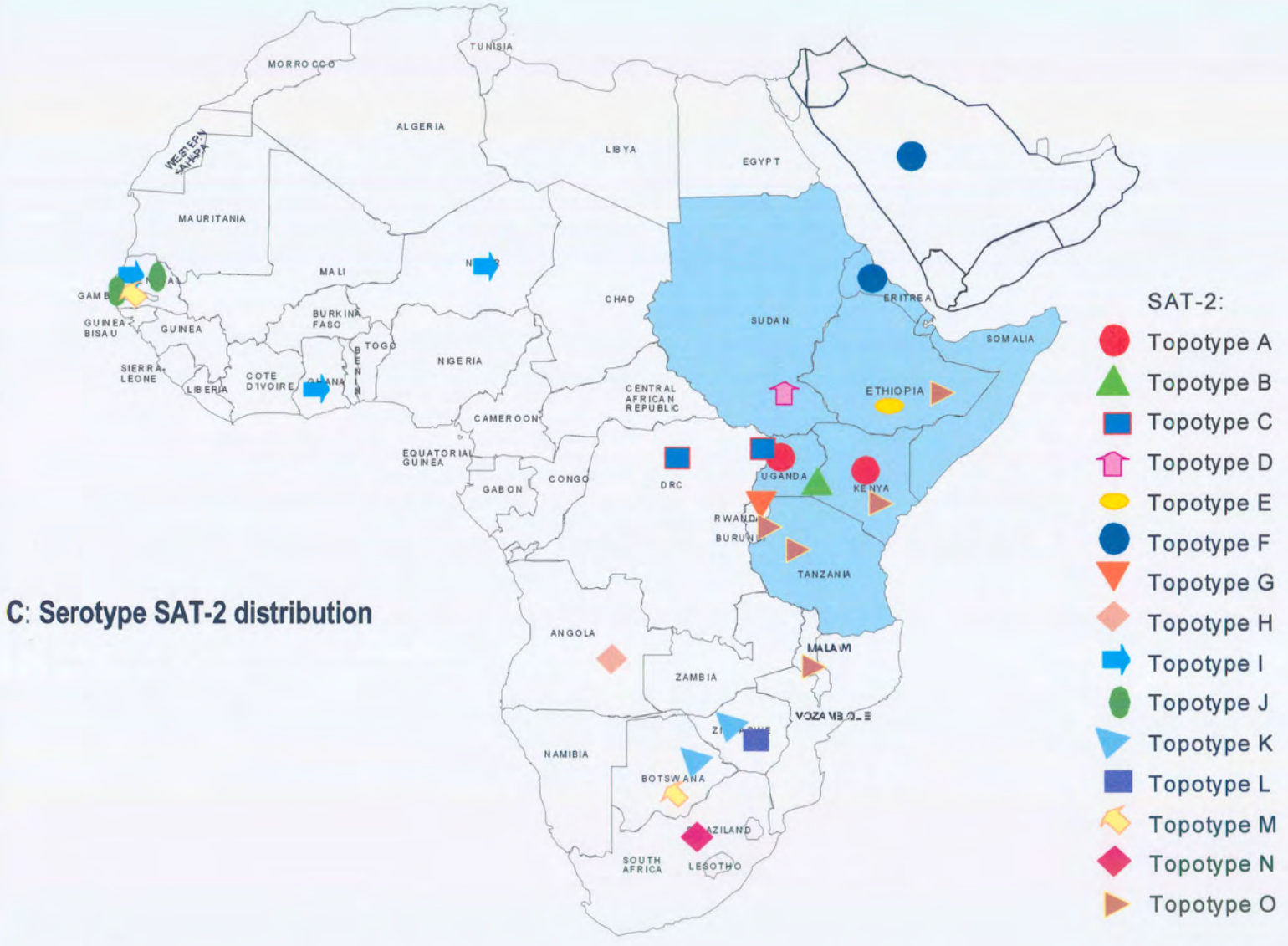
A: Serotype O distribution



B: Serotype SAT-1 distribution

SAT-1:

- Topotype A
- Topotype B
- Topotype C
- Topotype D
- Topotype E
- Topotype F
- Topotype G
- Topotype H
- Topotype I
- Topotype J
- Topotype K



C: Serotype SAT-2 distribution

Fig. 7.1 Distribution of various Topotypes of FMD virus isolates identified in Africa (A: type O, B:SAT-1, C: SAT-2).