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A first molecular epidemiological study of SAT-2 type foot-and-mouth disease viruses in West Africa

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SUMMARY

Thirty-one viruses causing SAT-2 outbreaks in seven West African countries between 1974 and 1991, and four viruses representative of East and Central Africa were genetically characterized in this study. Four major viral lineages (I–IV) were identified by phylogenetic analysis of an homologous 480 nucleotide region corresponding to the C-terminus end of VP1. Lineage I comprised two West African genotypes with viruses clustering according to year of isolation rather than geographical origin. Lineage II was represented by viruses isolated between 1979 and 1983 in two neighbouring West African countries, Senegal and The Gambia. Viruses from Nigeria and Eritrea, representative of West and East Africa respectively, constituted lineage III, whilst lineage IV, comprising viruses from Central and East Africa, was regionally and genetically distinct. This study revealed that unrestricted animal movement in West Africa is a major factor in disease dissemination and has also provided the first indication of trans-regional virus transmission.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals [1]. It is one of the most economically crippling afflictions of livestock due to the high cost of control and international trade restrictions imposed following an outbreak [2]. Estimates have put the cost of the recent outbreak in United Kingdom in excess of U$29 billion due to the ban on livestock exports, the cost of slaughter and disposal of the diseased and at-risk animals, as well as compensation to farmers [3].

FMD virus (FMDV) is a member of the *Aphthovirus* genus within the family Picornaviridae. There are seven serotypes worldwide, namely types A, O, C, SAT-1, SAT-2, SAT-3 and Asia-1. Four of these serotypes, O, A, SAT-1 and SAT-2 have been identified in West Africa. Of these, SAT-2 was the most prevalent FMDV type recovered between 1974 and 1991 from outbreaks in West Africa. Of the 12 West African countries, SAT-2 has been recorded in eight, namely Mali, Côte d'Ivoire, Ghana, Nigeria, Liberia, Senegal, The Gambia and Mauritania (Records of the OIE/WRL) but it is possible that some outbreaks have not been reported due to the endemicity of FMD to the region. Attention has focused on FMD because of a marked increase in outbreaks since 1991 in the West African region [4]. Currently, the growing population's high demand for animal protein and the need to compete in international markets have also raised the profile of the disease in West Africa.

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In southern Africa, the African buffalo (*Syncerus caffer*) plays an important role in virus maintenance and disease transmission to other cloven-hoofed animals [5, 6]. Unlike in southern Africa, the role of wildlife in the transmission of FMDV in West Africa is unclear. Wildlife populations in West African countries are comparatively small and their role in the epidemiology is therefore likely to be of minor importance due to the lower incidence of interaction between livestock and wildlife. A factor which may well be significant for virus spread in this region is the massive trans-boundary movement of livestock between neighbouring countries [7]. This is due to seasonal droughts which drive nomadic ranching and is exacerbated by a low standard of husbandry and a lack of fencing and defined grazing areas. Sheep and goats have been reported to play a role in the epidemiology of the disease elsewhere [8]. Although several million small ruminants occur in West Africa (Report of OIE/FAO, 1987), their role in the epidemiology is presently not known.

Molecular epidemiological studies of SAT-type viruses in southern Africa have revealed valuable insights into the origin and routes of transmission of the disease [6, 9, 10]. The molecular epidemiology of FMDV is based on nucelotide sequence determination of the immunogenic regions of the VP1 gene, as first described by Beck and Strohmaier [11]. It has been used successfully to trace the origin of FMD outbreaks in the case of illegal movements, interspecies transmissions and trans-continental introductions [4, 6, 10, 12–14].

Despite its importance and the regular involvement of SAT-2 type viruses in outbreaks in West Africa, little is known of the epidemiology of the disease. This study represents a first attempt to address this shortcoming by elucidating the regional genetic relationships of SAT-2 viruses recovered from outbreaks between 1974 and 1991.

MATERIALS AND METHODS

Viruses used in this study

Tissue culture isolates of all SAT-2 viruses causing outbreaks in West Africa were supplied by the World Reference Laboratory (WRL, UK). The geographical origin and isolation dates of the viruses used in this study are indicated in the Table. Viruses were grown in IBRS-2 (Insituto Biologico Rim Suino) cells in 25 cm^2 tissue culture flasks (Corning). Positive samples with 90% cytopathic effect were harvested and centrifuged at 3000 ***g*** for 10 min. The supernatant was stored in 20% glycerol at -80 °C.

RNA extraction and cDNA synthesis

RNA was extracted directly from the cell culture suspension using a modified guanidinium-silica based method [15]. The cDNA was synthesized as previously described [16] using a universal oligonucleotide antisense primer P1 (5′-GAA GGG CCC AGG GTT GGA CTC-3′) targeting the highly conserved 2A/2B junction [11].

PCR amplification and DNA purification

A DNA band of 518 bp targeting the VP1 gene was amplified by RT–PCR following the methodology outlined by Bastos [16]. Briefly, the universal antisense primer, P1 was combined with one of two upstream primers, namely VP1Ub (5′-CCACGTACTACTTYTCTGACCTGGA-3′) [16] or VP3-AB (5′-CACTGCTACCACTCRGAGTG-3′). As the amplification success rate was low (<30%) a West African-specific primer termed SAT2U-OS was designed using sequences generated for West African SAT-2 viruses that amplified successfully with the VP3-AB and P1 primer combination. Subsequent amplification attempts with the SAT2U-OS primer (5′-CCA CNT TCG AGG TCA ACT TGA T-3′) which binds at nucleotide positions 139–160 of the VP1 gene ensured a high amplification success rate (>95%). PCR reactions were performed in a final volume of 50 μl using 0.25 μM of each primer (SAT2U-OS and P1) and 2U of *Taq* polymerase (DynaZyme, Espoo, Finland). After an initial denaturation step at 96 °C for 20 s, 39 cycles of denaturation at 96 °C for 12 s, annealing at 57 °C for 30 s and extension at 70 °C for 30 s were performed. The PCR product was run against a 100-bp DNA ladder (Promega, Madison, WI, USA) and the expected band of 518 bp was excised from the agarose gel and purified using Nucleopin Extract (Macherey Nagel, Düren, Germany), according to the manufacturer's specifications.

Nucleotide sequencing

Sequences were generated by either manual dideoxy-sequencing using a T7-DNA polymerase sequencing kit (Amersham, Life Science, Cleveland, OH,

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Table. *Summary of SAT-2 isolates used in this study*

Virus designation	Year of isolation	Country	Reference	Genbank accession no.
GHA/10/74	1974	Ghana	This study	AF426068
GHA/14/74	1974	Ghana	This study	AF426069
IVY/9/74	1974	Côte d'Ivoire	This study	AF426070
LBR/1/74B	1974	Liberia	This study	AF426071
NIG/1/75	1975	Nigeria	This study	AF426074
NIG/2/75	1975	Nigeria	This study	AF367139
SEN/1/75	1975	Senegal	This study	AF426076
SEN/5/75	1975	Senegal	This study	AF367140
GAM/9/79	1979	The Gambia	This study	AF426078
SEN/1/83	1983	Senegal	This study	AF426079
SEN/3/83	1983	Senegal	This study	AF426080
GHA/2/90	1990	Ghana	This study	AF426081
IVY/2/90	1990	Côte d'Ivoire	This study	AF426082
GHA/8/91	1991	Ghana	This study	AF426083
MAI/1/91	1991	Mali	This study	AF426084
MAI/2/91	1991	Mali	This study	AF426085
MAI/3/91	1991	Mali	This study	AF426086
MAI/5/91	1991	Mali	This study	AF426087
MAI/6/91	1991	Mali	This study	AF426088
MAI/7/91	1991	Mali	This study	AF426089
NIG/13/91	1991	Nigeria	This study	AF426090
NIG/1/74	1974	Nigeria	This study	AF426091
NIG/35/74	1974	Nigeria	This study	AF426092
GAM/8/79	1979	The Gambia	This study	AF426093
SEN/7/79	1979	Senegal	This study	AF426094
SEN/8/79	1979	Senegal	This study	AF426095
NIG/2/82	1982	Nigeria	This study	AF426096
SEN/5/83	1983	Senegal	This study	AF426097
SEN/7/83	1983	Senegal	This study	AF426098
IVY/7/90	1990	Côte d'Ivoire	This study	AF426099
SEN/2/75	1975	Senegal	This study	AF431732
ERI/12/98	1998	Eritrea	[30]	AF367126
KEN/3/57	1957	Kenya	Unpublished	AJ251473
ZAI/1/82	1982	DRC	[30]	AF367100
RWA/1/00	2000	Rwanda	[30]	AF367134

DRC, Democratic Republic of the Congo.

USA) [16] or by automated DNA sequencing with a fluorescent dye deoxy-terminator (PerkinElmer, Wellesley, MA, USA) on an ABI Prism Model 377-18 (Applied Biosystems, Foster City, CA, USA). At least two separate amplifications and independent sequencing reactions were performed per sample with both primers.

Sequence analysis

Nucleotide sequences of the partial VP1 coding region were aligned and translated using the DAPSA program [17]. Phylogenetic reconstruction was performed using an homologous region of 480 nt corresponding to amino-acid positions 57–214 of the VP1 gene and the adjacent amino acids of 2A. Trees were inferred using both distance (neighbour-joining and UPGMA) and parsimony methods included in the MEGA program [18] with confidence levels being assessed by 10 000 bootstrap replications. Amino-acid variability was plotted for the 31 viruses of West African origin, using overlapping windows of 10 amino acids [18].

RESULTS

VP1 sequence analysis and comparison with existing sequence

In order to determine whether viruses from West Africa are distinct to this region, 35 viruses from

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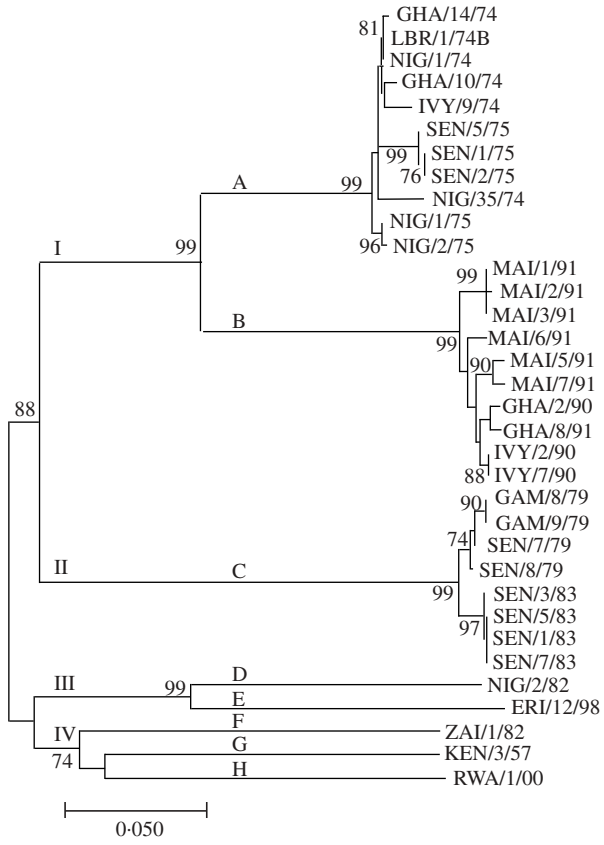

Fig. 1. Neighbour-joining tree depicting VP1 gene relationships of SAT-2 type FMD viruses from West Africa (1974–1991) and Central and East Africa (1957–2000). A distance of 5% is depicted by the scale. Bootstrap values >70 based on 10 000 replications are indicated as are the SAT-2 genotypes (labelled A–H) and the major viral lineages (labelled I–IV).

different geographical locations occurring north of the equator were selected for this study. Nucleotide sequences were determined for 31 SAT-2 viruses involved in outbreaks in West Africa between 1974 and 1991 and for four viruses from East and Central Africa recovered between 1957 and 2000. An homologous region of 480 nt was ultimately used for phylogenetic analysis which meets the general concept that the longer the sequence analysed the more accurate the FMD phylogeny that is inferred [19]. Eight major genotypes were identified by phylogenetic reconstruction (Fig. 1) based on high bootstrap support (>70%) [20] and the cut-off criterion that picornaviruses with more than 85% sequence identity belong to the same genotype [21]. These genotypes (labelled A–H) constituted four major evolutionary lineages (I–IV) that were associated with geographically distinct regions (Fig. 1). Lineages I and II were made up of viruses of West Africa origin, whilst lineages III and IV included viruses from West and East Africa, and Central and East Africa respectively.

Eight major SAT-2 genotypes were consistently recovered with neighbour joining, UPGMA and parsimony methods indicating that the trees were a robust estimate of the true viral relationships [22]. The geographical distribution of these genotypes is indicated in Figure 1 and constitutes the following major viral lineages.

Lineage I (*West Africa*). Genotype A (Ghana, Liberia, Côte d'Ivoire, Nigeria and Senegal, 1974–1975) and Genotype B (Ghana, Côte d'Ivoire, Nigeria and Mali, 1990–1991).

Lineage II (*West Africa*). Genotype C (The Gambia and Senegal, 1979–1983).

Lineage III (*West and East Africa*). Genotype D (Nigeria, 1982) and Genotype E (Eritrea, 1998).

Lineage IV (*East and Central Africa*). Genotype F (Democratic Republic of the Congo, 1982), Genotype G (Kenya, 1957) and Genotype H (Rwanda, 2000).

Viruses from West Africa constituted a distinctly West African assemblage of viruses (lineages I–II) and were grouped according to year of isolation rather than outbreak locality. In the case of genotypes A and B, viruses were recovered over a 1-year period respectively. Extensive circulation of a single virus-type in the field was, however, indicated by genotype C which included viruses recovered over a 4-year period (1979–1983). In all three genotypes (A–C), sequence identity values were consistently ⩾97% and bootstrap values were ⩾99%. The viruses representative of the remaining five genotypes displayed a geographical rather than temporal association. This was particularly true for lineage IV which comprised viruses from three neighbouring East and Central African countries despite being sampled over a 43-year period (1957–2000).

Amino-acid variation

In order to determine the distribution of mutations across the gene, amino-acid variation was plotted and regions of hypervariability were identified in which >40% of the sites were shown to vary. These hypervariable regions were located at amino-acid positions 131–149, 156–166, and 206–212 (Fig. 2). The former two hypervariable regions flank the RGD cell attachment site whilst the latter corresponds

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                66         76         86         96        106        116        126        136
KEN/3/57  KEKALVGAIL RSATYYFCDL EVACVGKHKR VFWQPNGAPR TTQLGDNPMV FSHNNVTRFA IPFTAPHRLL STVYNGECEY
GHA/10/74 .K......V. .A........ .I....E... .......... .......... ....K..... .......... ........N.
GHA/14/74 ........V. .A........ .I....E... .......... .......... ....K..... .......... ........N.
IVY/9/74  .K......V. .A........ .I....E... .......... .......... ....K..... .......... ........N.
LBR/1/74B ........V. .A........ .I....E... .......... .......... ....K..... .......... ........N.
NIG/1/74  ?.......V. .A........ .I....E... .......... .......... ....K..... .......... ........N.
NIG/35/74 ........V. .A........ .I....E... .......... A......... ....K..... .......... ........N.
NIG/1/75  ........V. .A........ .I....E... .......... .......... ....K..... .......... ........N.
NIG/2/75  ........V. .A........ .I....E... .......... .......... ...KK..... .......... ........N.
SEN/1/75  ........V. .A........ .I....D... .......... A......... ....K..... .......... ........N.
SEN/2/75  ........V. .A........ .I....D... .......... A......... ....K..... .......... ........N.
SEN/5/75  ........V. .A........ .I....D... .......... A......... ....K..... .......... ........N.
GAM/8/79  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
GAM/9/79  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
SEN/7/79  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
SEN/8/79  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
NIG/2/82  .......... .AS....... .I....E... .Y........ .......... .AK.G..... .......... ........S.
SEN/1/83  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
SEN/3/83  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
SEN/5/83  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
SEN/7/83  .D.T....L. .AT....A.. .I....D... .Y........ .......... ....R..... ..Y....... ........V.
GHA/2/90  ........V. .A........ .IT...E.A. .......... .......... .......... ..Y....... ........K.
IVY/2/90  ........V. .A........ .I....E.A. .......... .......... .......... ..Y....... ........K.
IVY/7/90  ........V. .A........ .I....E.A. .......... .......... .......... ..Y....... ........K.
GHA/8/91  ........V. .A........ .IT...E.A. .......... .......... .......... ..Y....... ........K.
MAI/1/91  ........V. .A........ .I....E.T. .......... .......... .......... ..Y....... ........K.
MAI/2/91  ........V. ?A........ .I....E.T. .......... .......... .......... ..Y....... ........K.
MAI/3/91  ........V. .A........ .I....E.T. .......... .......... .......... ..Y....... ........K.
MAI/5/91  ........V. .A........ .IT...E.A. .......... .......... ..R....... ..Y....... ........K.
MAI/6/91  ........V. .A........ .I....E.A. .......... .......... .......... ..Y....... ........K.
MAI/7/91  ........V. .A........ .IT...E.A. .......... .......... ..R....... ..Y....... ........K.
NIG/13/91 ........V. .A........ .IT...E.A. .......... .......... .......... ..Y....... ........K.
ZAI/1/82  .......... .......... .I....D.T. .......... ..K....... YA..H..... .......... ..?.......
ERI/12/98 .G........ .AS....... .I....D.T. .......... .......... YAKGG..... .......... ........T.
RWA/1/00  ........V. .......... ......D.A. .......... .......... .A..K..... ..Y....... ..........

                                                                                                ⇓
               146        156        166        176        186        196        206        214  216
KEN/3/57  TKTVTAIRGD REVLAQKYSS AKHSLPSTFN FGFVTADKPV DVYYRMKRAE LYCPRALLPA YTHAGGDRFD APIGVAKQ   LL
GHA/10/74 STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
GHA/14/74 STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
IVY/9/74  STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSRH.V. .....E..   .?
LBR/1/74B STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
NIG/1/74  STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
NIG/35/74 STS..PV... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
NIG/1/75  STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
NIG/2/75  STS..P.... .A...A..AN T..T...... ..Y....A.. ........S. .....P.... .D.QSR.... .....E..   .C
SEN/1/75  STS..P.... MA...A..A. T..T...... ..Y....A.. ........S. .....P.... .D.QSH.... .....E..   .C
SEN/2/75  STS..P.... MA...A..A. T..T...... ..Y....A.. ........S. .....P.... .D.QSH.... .....E..   .C
SEN/5/75  STS..P.... .A...A..A. T..T...... ..Y....A.. ........S. .....P.... .D.QSH.... .....E..   .C
GAM/8/79  AD...P.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
GAM/9/79  AD...P.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
SEN/7/79  AD...P.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
SEN/8/79  AD...P.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
NIG/2/82  K.ET...... .A...A..A. T..T...... .......A.. ........T. .....P.... .D...R.... .....ER.   T.
SEN/1/83  AD..AP.... .Q...A...N R..Q...... Y.Y....E.. .......... ?......... .D.HSR.... S....E..   .C
SEN/3/83  AD..AP.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
SEN/5/83  AD..AP.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
SEN/7/83  AD..AP.... .Q...A...N R..Q...... Y.Y....E.. .......... F......... .D.HSR.... S....E..   .C
GHA/2/90  NT..QP.... .A...N..AN R..T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
IVY/2/90  NT..QP.... .A...N..AN R..T...... ..Y....... .......... .....P.... .D.QSH.... .....E..   .C
IVY/7/90  NT..QP.... .A...N..AN R..T...... ..Y....... .......... .....P.... .D.QSH.... .....E..   .C
GHA/8/91  DT..QP.... .A...N..AN R..T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
MAI/1/91  NT..QP.... .A...N..AH RE.T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
MAI/2/91  NT..QP.... .A...N..AH RE.T...... ..Y....... .......... ....?P.... .D.QSR..V. .....E..   .C
MAI/3/91  NT..QP.... .A...N..AH RE.T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
MAI/5/91  NT..QP.... .A...S..AN R..T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
MAI/6/91  DT..QP.... .A...N..AN R..T...... ..Y....R.. .......... .....P.... .D.QSR.... .....E..   .C
MAI/7/91  NT..QP.... .A...N..AN R..T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
NIG/13/91 NT..QP.... .A...N..AN R..T...... ..Y....... .......... .....P.... .D.QSR.... .....E..   .C
ZAI/1/82  RTN....... .Q......A. T..A...... .....T.... .......... .....P.... .D.QNR.... .....E..   .C
ERI/12/98 A..A...... .AA..A..AA SV.T..Q... .....V.... .......... .....P.... .D..SR.... .....ER.   T.
RWA/1/00  ....S..... .A...A..A. G..T...... .......... N......... .....PF... .D..SR.... .....E..   .A
```

Fig. 2. Deduced amino-acid sequences of the C-terminal half of the VP1 genes of SAT-2 strains from West Africa including viruses from east and central Africa. Dots indicate sequence identity with the master sequence, KEN/3/57. ? Indicates undefined amino acids due to sequence ambiguities. The RGD is embolded and the cleavage site of VP1/2A (KQ/LC or RQ/TL) is indicated by a vertical arrow above the sequence.

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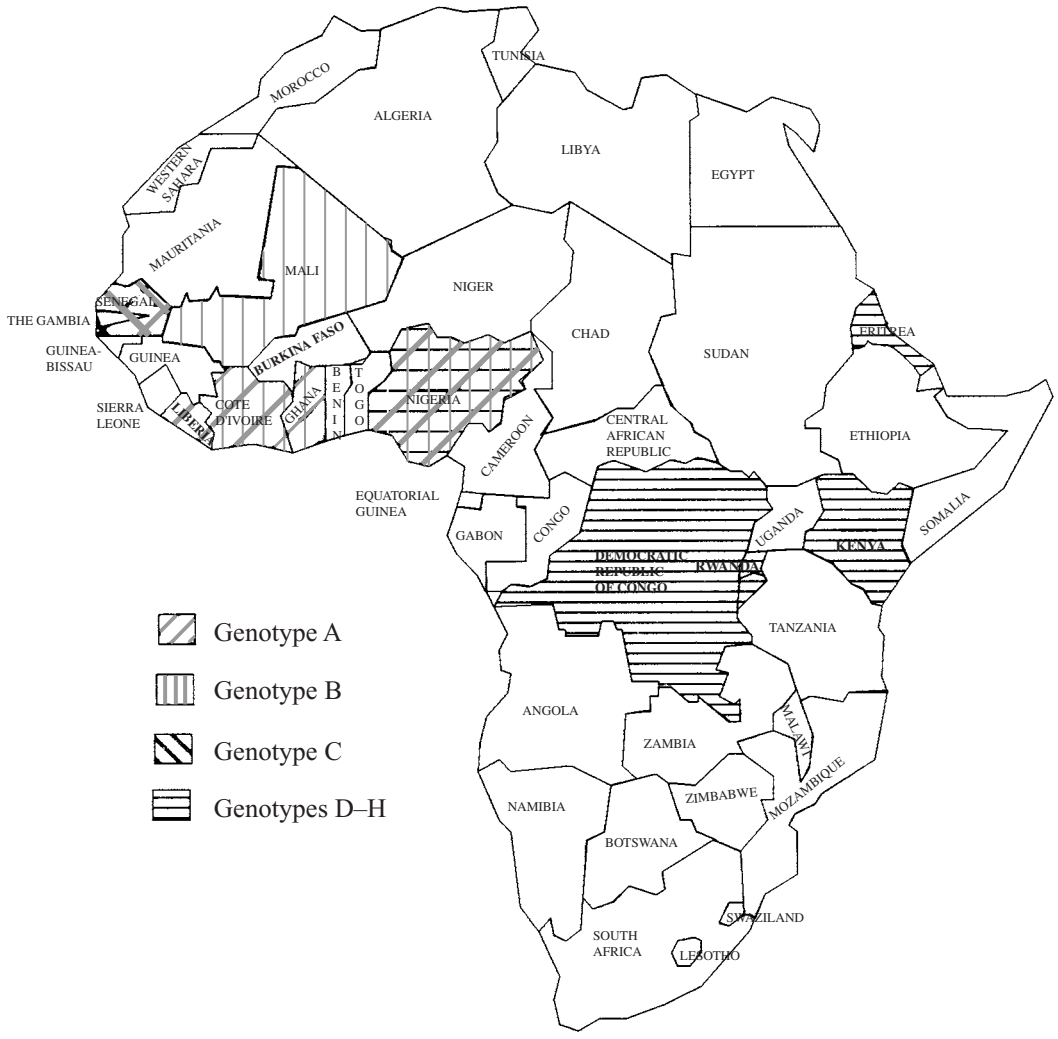

Fig. 3. Map of Africa indicating genotype distribution of SAT-2 type FMDV in West, East and Central Africa.

to another highly immunogenic portion of the gene, namely the C-terminal region [23–25]. The cell attachment site, Arg-Gly-Asp (RGD) at amino-acid residues 145–147, within the G–H loop was completely conserved across all viruses included in this study. In contrast to other serotypes where a leucine predominates at amino-acid position 148 where it enhances and stabilizes α-helix formation [26], all SAT-2 viruses in this study had an arginine at this position, with the exception of two viruses from Senegal (SEN/1/75 and SEN/2/75) which had a methionine instead. A cysteine residue at the base of the G–H loop (position 135) was conserved among all the isolates (Fig. 2) and is associated with disulphide-bond formation in serotype O viruses which is believed to promote conformational epitope formation [25, 27]. Two distinct cleavage-site sequences were observed in SAT-2 viruses of West African origin, namely, KQ/LC and RQ/TC (Fig. 2). The KQ variant was always associated with an LC in 2A, whilst a change from KQ to RQ in VP1 was always coupled with a change from LC to TC in 2A.

DISCUSSION

Four distinct evolutionary lineages (Figs. 1, 3) were identified by sequencing the VP1 gene of 35 SAT-2 viruses consisting of 31 isolates from West Africa and four from Central and East Africa. Lineages I and II consisted of viruses from West Africa exclusively. Unique West African lineages have similarly been reported for serotype A, where all West African viruses were shown to constitute a single, large evolutionary lineage covering a period of 26 years [28]. Thus two distinct West African topotypes are distinguishable, however, genotypes within these

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topotypes did not display the same locality-specific grouping of viruses found within southern African topotypes [10, 29].

The sequencing results indicate that three unrelated epizootics involving viruses from three different genotypes (A–C) occurred in West Africa between 1974 and 1991. The first epizootic affected numerous neighbouring West African countries, including Ghana, Côte d'Ivoire, Liberia, Nigeria and Senegal, between 1974 and 1975. The second involving The Gambia and Senegal was caused by a virus with an extended field presence (1979–1983). The third occurred over a 2-year period (1990–1991) and affected Mali, Côte d'Ivoire, Ghana and Nigeria. For each of these epizootics it was shown that the viruses differed from each other by no more than 3% across the nucleotide region characterized in this study. Furthermore, bootstrap values, based on 10000 replications, were $\geqslant$99% for each of the epizootics/genotypes identified here. These three genotypes constitute two distinct evolutionary lineages (I–II), discerned on the basis of high levels of bootstrap support and nucleotide sequence differences in excess of 20%, as indicated in similar molecular epidemiological studies of FMD viruses [4, 10].

Whilst lineages I and II are distinctly West African, the inclusion of other viruses from African localities north of the equator indicate that at least two additional lineages are present in this continental region. Lineages III and IV comprised viruses primarily from Central and Eastern Africa. One exception was NIG/2/82, which was the only virus from a West African country to occur within these viral lineages. The absence of this virus from West African-specific genotypes and its grouping with viruses from East and Central Africa is indicative of trans-regional transmission between East and West Africa. Thus not only is there extensive movement of viruses within the West African region, but trans-regional transmission is possible, leading to extensive heterogeneity in the field. This heterogeneity is likely to be reflected antigenically [30] and should be addressed by the selection of vaccine strains representative of the major regional genotypes in circulation in West Africa, in order to ensure food safety through immunization.

In contrast to southern Africa where the geographical origin of an outbreak can be traced with great accuracy [6, 10], the outbreaks in West Africa appear to have multiple foci in different countries, making it difficult to determine the original source of the infection. This grouping of viruses from West Africa according to year of isolation rather than sampling locality indicates that unrestricted animal movement occurs. In order to adequately control the disease it is therefore clear that restriction of animal movement will be a key factor in achieving this, together with the development and administration of custom-made vaccines.

In summary, this first molecular epidemiology of FMD virus SAT-2 in West Africa has provided valuable insights into the epidemiology of the disease in West Africa. Unrestricted animal movement is a major factor in disease transmission, resulting in the rapid spread of infection which in turn leads to the inability to discern the source of the outbreak. Of importance was the identification of the threat of introduction from neighbouring countries and possible virus movement between different continental regions. The latter significantly affects the antigenic and genetic diversity of viruses in the West African region and has implications for control of the disease through vaccination. In addition to the intra-typic complexities as illustrated here for the SAT-2 serotype, the epidemiology of FMD in West Africa is further exacerbated by the presence of four of the seven known serotypes.

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