

## ORIGINAL ARTICLE

# Molecular Characterization of Foot-and-Mouth Disease Viruses Collected in Tanzania Between 1967 and 2009

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**Summary**

This paper describes the molecular characterization of foot-and-mouth disease viruses (FMDV) recovered from outbreaks in Tanzania that occurred between 1967 and 2009. A total of 44 FMDV isolates, containing representatives of serotypes O, A, SAT 1 and SAT 2 from 13 regions of Tanzania, were selected from the FAO World Reference Laboratory for FMD (WRLFMD) virus collection. VP1 nucleotide sequences were determined for RT-PCR amplicons, and phylogenetic reconstructions were determined by maximum likelihood and neighbour-joining methods. These analyses showed that Tanzanian type O viruses fell into the EAST AFRICA 2 (EA-2) topotype, type A viruses fell into the AFRICA topotype (genotype I), type SAT 1 viruses into topotype I and type SAT 2 viruses into topotype IV. Taken together, these findings reveal that serotypes O, A, SAT 1 and SAT 2 that caused FMD outbreaks in Tanzania were genetically related to lineages and topotypes occurring in the East African region. The close genetic relationship of viruses in Tanzania to those from other countries suggests that animal movements can contribute to virus dispersal in sub-Saharan Africa. This is the first molecular description of viruses circulating in Tanzania and highlights the need for further sampling of representative viruses from the region so as to elucidate the complex epidemiology of FMD in Tanzania and sub-Saharan Africa.

**Introduction**

Foot-and-mouth disease virus (FMDV; family *Picornaviridae*, genus: *Aphthovirus*) causes a highly contagious disease of ruminants and swine and exists as seven immunologically distinct serotypes, *viz.* O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3. FMD is endemic in most of the African countries where serotypes O, A, SAT 1 and SAT 2 predominate (Rweyemamu et al., 2000; Vosloo et al., 2002). Phylogenetic analyses of nucleotide sequence data of the VP1-coding region have been used to define genotypes, which occur in defined geographic areas (topotypes) for each of the FMDV serotypes (Samuel and Knowles, 2001; Knowles and Samuel, 2003; Vosloo et al., 2005).

Since the first documented outbreaks in Tanzania in 1927 and first isolation of the virus in 1954, many FMD outbreaks have occurred on an annual basis. Clinical manifestation of FMD in the country varies from mild in indigenous zebu cattle to more severe and overt clinical signs in exotic breeds. Unrestricted animal movements are an important mechanism by which FMD is spread (Rweyemamu et al., 2008; Di Nardo et al., 2011), and control measures implemented in Tanzania during outbreaks typically consist of quarantine and restriction of animal movements (Eisa and Rweyemamu, 1977; Kivaria, 2003).

Previous studies have provided evidence for the presence of four FMDV serotypes (O, A, SAT 1 and SAT 2) in Tanzania (Ferris and Donaldson, 1992; Swai et al., 2009; Kasanga et al., 2012). A serological survey of wildlife in

Tanzania suggested infection with O, A, SAT 1 and SAT 2 may be widespread in free-living African buffalo in many parts of the country, and FMDV infection of other wildlife species was also detected (Hamblin et al., 1990). Samples ( $n = 233$ ) from cases of suspected FMD in cattle occurring in Tanzania between 1967 and 2008 were submitted to the WRLFMD for virus isolation and serotyping (WRLFMD Records). A total of 35 (15%), 15 (6.4%), 47 (20.2%) and 27 (11.6%) were identified as FMDV types O, A, SAT 1 and SAT 2, respectively. FMDV genome was detected in a further 25 (10.7%) samples using real-time RT-PCR, and 84 (36.1%) samples were negative for FMDV in all tests. Thus, the occurrence of multiple FMDV serotypes in Tanzania complicates the control of the disease in the country through vaccination. The use of appropriate vaccines for control of FMD is dependent upon knowledge about the antigenic and genetic diversity of the circulating serotypes in the region. However, no detailed molecular epidemiological studies have been conducted on FMDV isolates from the country. The aim of this study was to characterize FMD viruses recovered from Tanzanian FMD outbreaks from 1967 to 2009 using VP1 sequence data, and the genetic relationships of these viruses were subsequently compared with other viruses collected from neighbouring countries.

## Materials and Methods

### Viruses

A total of 44 FMD viruses isolated from samples collected from FMD outbreaks in Tanzania (1967–2009) were selected for use in this study. Sampling locations and other sample details are shown in Table 1 and Fig. 1.

### RNA extraction, RT-PCR and DNA sequencing

Total RNA was extracted from cell culture passage viruses using RNeasy<sup>®</sup> kit (Qiagen Ltd., Crawley, West Sussex, UK). The VP1 region was amplified using a one-step RT-PCR kit (Qiagen) as previously described (Knowles et al., 2009). Oligonucleotide primers used for PCR amplification were selected based on serotype (Table 2). For type O, forward primers were either O-1C244F or O-1C272F and reverse primer EUR-2B52R. Primers used for serotype A were either A-1C562F or A-1C612F as a forward primer, with EUR-2B52R as a reverse primer. For serotype SAT 1, forward primers were either SAT1-P1-1228F, SAT1-1C559F or SAT1U-OS, each with SAT-2B208R as a reverse primer. SAT 2 RT-PCR used either SAT2-1C445F or SAT2-P1-1223F as a forward primer and SAT-2B208R as a reverse primer. For serotype A, amplification conditions were as previously described (Knowles et al., 2009), while the annealing temperatures of 50°C and 60°C were used for

SAT 2 and O viruses, respectively. RT-PCR products were sequenced using BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) using the following oligonucleotides (Table 2): NK72 for all serotypes; O-1C499F, O-1C583F, O-1C605eF, O-1D296F, O-1D296bF and O-1D628R for serotype O; A-1C612F for serotype A, SAT1-1C559F, SAT1U-OS, SAT1-1D394R and SAT1-1D200F for serotype SAT 1 and SAT2-1C445F, SAT2VP3-AB, SAT2-1C523F, SAT-1D209F and SAT2-D for SAT 2. DNA sequencing was performed on an ABI 3730 DNA Analyzer (Applied Biosystems). Nucleotide sequences covering the complete VP1-coding region were assembled using SeqMan Pro<sup>™</sup> part of the Lasergene 8.0 software package (DNASTar Inc., Madison, WI, USA).

### Phylogenetic analysis

Complete VP1 nucleotide sequences were aligned using BioEdit 7.0.5.3 (Hall, 1999) and Clustal W 1.83 (Thompson et al., 1994). These alignments were used to construct distance matrices using the Kimura-2-parameter nucleotide substitution model (Kimura, 1980) as implemented in the program MEGA 5.2 (Tamura et al., 2011). Midpoint-rooted neighbour-joining (NJ) trees were then constructed using MEGA 5.2. To construct maximum likelihood (ML) phylogenies (Nei and Kumar, 2000), each data set was tested for 24 common nucleotide substitution models using MEGA 5.2. The model with the lowest Bayesian information criterion (BIC) score was chosen to construct each tree. All positions with less than 95% site coverage were eliminated. The robustness of each tree topology (NJ and ML) was assessed with 1000 bootstrap replicates. Individual FMD viruses were classified into geographically restricted clusters, also known as topotypes, as previously described (Knowles and Samuel, 2003; Vosloo et al., 2004).

## Results

The geographic distribution of FMDV serotypes detected from 1967 to 2009 is shown in Fig. 1. Additional FMDV, which were available on the public sequence databases, were also included in the analyses. This included four VP1 sequences of Tanzanian viruses (two type O and two type SAT 2). The NJ trees showed essentially the same phylogenetic relationships between the viruses as the ML trees (data not shown).

### Phylogenetic analysis of serotype A

The evolutionary history was inferred using the maximum likelihood method based on the Hasegawa–Kishino–Yano (HKY) model (Nei and Kumar, 2000). The tree with the highest log likelihood (−7343.3387) is shown (Fig. 2). A

**Table 1.** Details of the Tanzanian foot-and-mouth disease virus isolates examined

Serotype	Topotype/ Lineage	WRLFMD Ref. No.	Geographic origin	Species	Collection date	Accession	Reference
A	AFRICA/G-I	TAN/2/68	Tanzania	Cattle	1968	KF561687	This study
A	AFRICA/G-I	TAN/3/68	Tanzania	Cattle	1967	KF561688	This study
A	AFRICA/G-I	TAN/4/80	Arusha Region, Tanzania	Not known	1980	KF561689	This study
A	AFRICA/G-I	TAN/11/2008	Iringa Region, Tanzania	Cattle	01/08/2008	KF561690	This study
A	AFRICA/G-I	TAN/12/2008	Iringa Region, Tanzania	Cattle	01/08/2008	KF561691	This study
A	AFRICA/G-I	TAN/4/2009	Morogoro Region, Tanzania	Cattle	01/05/2009	KF561692	This study
A	AFRICA/G-I	TAN/9/2009	Njombe District, Iringa Region, Tanzania	Cattle	13/06/2009	KF561693	This study
A	AFRICA/G-I	TAN/11/2009	Kibaha District, Pwani Region, Tanzania	Cattle	21/06/2009	KF561694	This study
A	AFRICA/G-I	TAN/42/2009	Mpwapwa, Dodoma Region, Tanzania	Cattle	07/09/2009	KF561695	This study
A	AFRICA/G-I	TAN/45/2009	Iringa Rural District, Iringa Region, Tanzania	Cattle	06/11/2009	KF561696	This study
A	AFRICA/G-I	TAN/47/2009	Bagamoyo, Pwani Region, Tanzania	Cattle	01/11/2009	KF561697	This study
A	AFRICA/G-VI	GHA/16/73	Ghana	Not known	1973	KF561698	This study
A	AFRICA/G-I	KEN/42/66 (K18/66)	Kenya	Not known	1966	KF561699	This study
A	AFRICA/G-I	KEN/7/2008 (K10/08)	Wei, Nakuru North, Rift Valley, Kenya	Cattle	09/02/2008	KF561700	This study
A	AFRICA/G-I	KEN/8/2008 (K13/08)	Gathanji, Kiambu West, Central, Kenya	Cattle	19/02/2008	KF561701	This study
A	AFRICA/G-I	KEN/28/2008 (K73/08)	Loitokitok, Rift Valley, Kenya	Cattle	01/08/2008	KF561702	This study
A	AFRICA/G-I	KEN/22/2009 (K63/09)	Olulunga, Narok South, Rift Valley, Kenya	Cattle	01/03/2009	KF561703	This study
A	AFRICA/G-V	NGR/2/73	Niamey, Niger	Cattle	1973	KF561704	This study
A	AFRICA/G-VII	UGA/13/66	Uganda	Not known	1966	KF561705	This study
O	EA-2	TAN/1/85	Iringa Region, Tanzania	Cattle	1985	KF561676	This study
O	EA-2	TAN/3/96	Kibaha District, Pwani Region, Tanzania	Cattle	25/07/1996	EU919241	M. Chitray, F. F. Maree and W. Vosloo, unpub.
O	EA-2	TAN/7/98	Kyela, Mbeya Region, Tanzania	Cattle	22/10/1998	AJ296320	Samuel and Knowles (2001)
O	EA-2	TAN/9/98	Kyela, Mbeya Region, Tanzania	Cattle	22/10/1998	KF561677	This study
O	EA-2	TAN/1/2004	Bagamoyo District, Pwani Region, Tanzania	Cattle	2004	KF561678	This study
O	EA-2	TAN/2/2004	Kibaha District, Pwani Region, Tanzania	Cattle	2004	KF561679	This study
O	EA-2	TAN/3/2004	Iringa Region, Tanzania	Cattle	2004	KF561680	This study
O	EA-2	TAN/12/2004	Chunya District, Mbeya Region, Tanzania	Cattle	2004	KF561681	This study
O	EA-2	TAN/14/2004	Songea, Ruvuma Region, Tanzania	Cattle	2004	KF561682	This study
O	EA-2	TAN/17/2004	Nkasi District, Rukwa Region, Tanzania	Cattle	2004	KF561683	This study
O	EA-2	TAN/16/2008	Morogoro Region, Tanzania	Cattle	25/09/2008	KF561684	This study
O	EA-2	TAN/5/2009	Morogoro Region, Tanzania	Cattle	01/05/2009	KF561685	This study
O	EA-2	TAN/44/2009	Makete District, Iringa Region, Tanzania	Cattle	27/10/2009	KF561686	This study
SAT1	I	T155/71	Tanzania	Cattle	1971	KF561706	This study
SAT1	I	TAN/2/71	Tanzania	Cattle	1971	KF561707	This study
SAT1	I	TAN/2/77	Tanzania	Cattle	1977	KF561708	This study

**Table 1.** (continued)

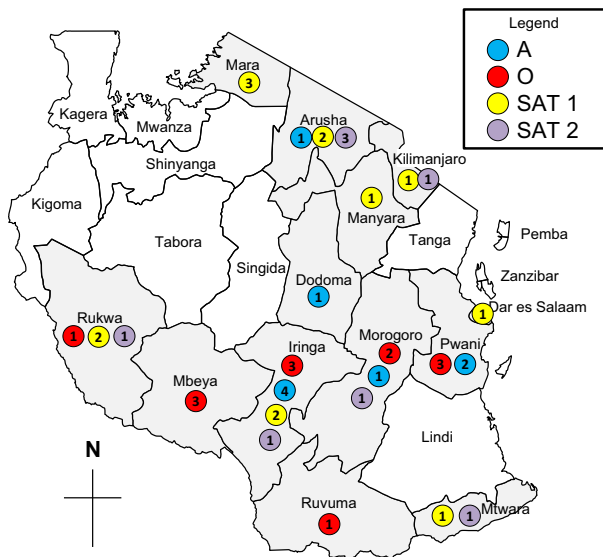
Serotype	Topotype/ Lineage	WRLFMD Ref. No.	Geographic origin	Species	Collection date	Accession	Reference
SAT1	I	TAN/3/80	Sumbawanga District, Rukwa Region, Tanzania	Cattle	1980	KF561709	This study
SAT1	I	TAN/1/96	Arusha, Arusha Region, Tanzania	Cattle	17/05/1996	KF561710	This study
SAT1	I	TAN/5/96	Koibate, Arusha Region, Tanzania	Cattle	1996	KF561711	This study
SAT1	I	TAN/6/99	Mtwara Urban, Mtwara Region, Tanzania	Cattle	01/06/1999	KF561712	This study
SAT1	I	TAN/18/99	Sumbawanga District, Rukwa Region, Tanzania	Cattle	1999	KF561713	This study
SAT1	I	TAN/19/99	Musoma, Mara Region, Tanzania	Cattle	1999	KF561714	This study
SAT1	I	TAN/21/99	Simanjiro, Manyara Region, Tanzania	Cattle	1999	KF561715	This study
SAT1	I	TAN/25/99	Musoma District, Mara Region, Tanzania	Cattle	1999	KF561716	This study
SAT1	I	TAN/26/99	Hai District, Kilimanjaro Region, Tanzania	Cattle	1999	KF561717	This study
SAT1	I	TAN/37/99	Iringa, Iringa Region, Tanzania	Cattle	1999	KF561718	This study
SAT1	I	TAN/43/99	Iringa, Iringa Region, Tanzania	Cattle	1999	KF561719	This study
SAT1	I	TAN/51/99	Kinondoni District, Dar es Salaam Region, Tanzania	Cattle	04/08/1999	KF561720	This study
SAT1	I	TAN/60/99	Serengeti National Park, Mara Region, Tanzania	Wildebeest	1999	KF561721	This study
SAT2	IV	TAN/1/75	Sanya Juu, Moshi, Kilimanjaro Region, Tanzania	Cattle	1975	AY343970	Sahle et al. (2007)
SAT2	IV	TAN/1/86	Mtawanya, Mtwara Region, Tanzania	Cattle	07/05/1986	AY343971	Sahle et al. (2007)
SAT2	IV	TAN/4/2004	Arusha Region, Tanzania	Cattle	2004	KF561722	This study
SAT2	IV	TAN/6/2004	Arusha Region, Tanzania	Cattle	2004	KF561723	This study
SAT2	IV	TAN/9/2004	Nkoanua, Arumeru District, Arusha Region, Tanzania	Cattle	2004	KF561724	This study
SAT2	IV	TAN/18/2004	Nkasi District, Rukwa Region, Tanzania	Cattle	2004	KF561725	This study
SAT2	IV	TAN/21/2004	Kihonda, Morogoro Urban District, Morogoro Region, Tanzania	Cattle	2004	KF561726	This study
SAT2	IV	TAN/43/2009	Makete District, Njombe District, Iringa Region, Tanzania	Cattle	27/10/2009	KF561727	This study

discrete gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0.6032)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+I), 42.5518% sites]. Eleven serotype A virus isolates detected in Tanzania between 1967 and 2009 all fell into genotype I (G–I) within the AFRICA topotype. In this genotype, the Tanzanian viruses were grouped in two major subclusters, one with older viruses detected between 1967 and 1980, while the other comprised of viruses detected between 2008 and 2009. Tanzanian viruses from 2009 fell into two groups differing by 84.8–85.3% nt identity. One group (A/TAN/9/2009) was closely related to two Tanzanian viruses from the previous year (94.5% nt id); all three viruses were from the Iringa Region. The second group, consisting of A/TAN/4/

2009, A/TAN/11/2009, A/TAN/42/2009, A/TAN/45/2009 and A/TAN/47/2009, from four contiguous regions of Tanzania (which included Iringa), had an intragroup variability of 97.5–99.2% nt id and were closely related to two Kenyan viruses (A/KEN/28/2008 and A/KEN/22/2009) with nt id values of 97.5–99.2%. Intermediate between the two groups were two Kenyan viruses (A/KEN/7/2008 and A/KEN/8/2008) with 84.5–85.0% nt id and 91.7–93.3% nt id to the first and second groups, respectively.

#### Phylogenetic analysis of serotype O

The evolutionary history was inferred using the maximum likelihood method based on the Tamura–Nei (TN93) model (Nei and Kumar, 2000). The tree with the highest



**Fig. 1.** Map of Tanzania showing geographic location of the 48 virus isolates (1967–2009) analysed in this study.

log likelihood (−11962.9132) is shown (Fig. 3). A discrete gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0.6762)]. Thirteen serotype O viruses detected in various geographic locations of Tanzania between 1985 and 2009 were clustered in eight lineages within the East

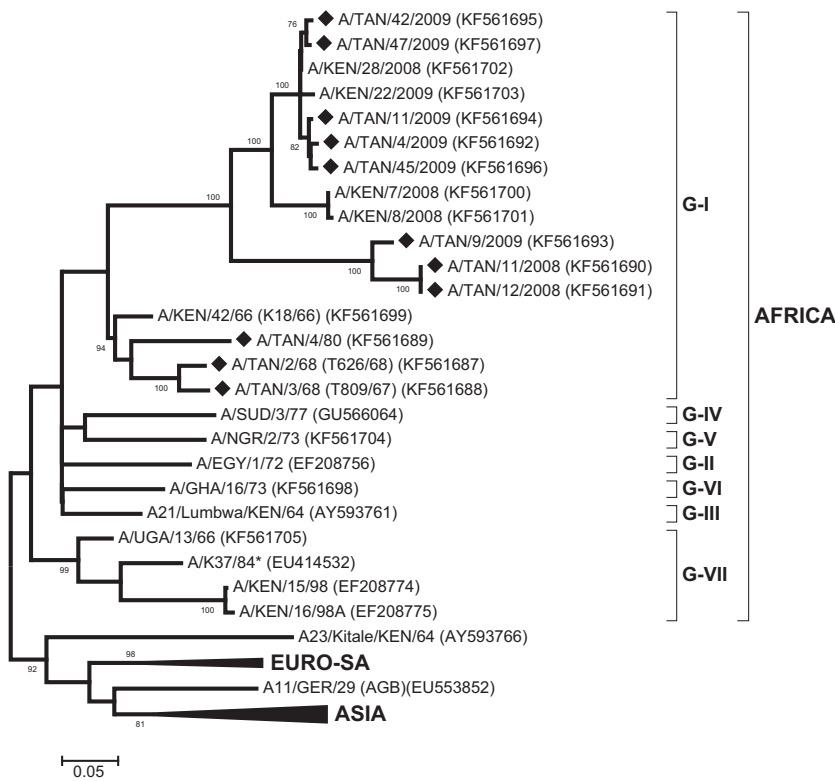
Africa 2 (EA-2) toptotype, (i) O/TAN/1/85; (ii) O/TAN/3/96; (iii) O/TAN/7/98; O/TAN/9/98 (closely related to a virus from Malawi (O/MAL/1/98; 99.2–99.5% nt id); (iv) O/TAN/1/2004; (v) O/TAN/2/2004, O/TAN/3/2004 and O/TAN/12/2004 [closely related to a virus from Kenya (O/K48/05; 98.1–98.4% nt id)]; (vi) O/TAN/14/2004 and O/TAN/17/2004; (vii) O/TAN/16/2008; and (viii) O/TAN/5/2009 and O/TAN/44/2009 [most closely related to viruses from Uganda in 2005–2006; 95.8–96.4% nt id (Fig. 3)].

**Phylogenetic analysis of serotype SAT 1**

The evolutionary history was inferred using the maximum likelihood method based on the TN93 model (Nei and Kumar, 2000). The tree with the highest log likelihood (−8127.7373) is shown (Fig. 4). A discrete gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0.7745)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+I), 32.2379% sites]. The 16 serotype Tanzanian SAT 1 virus isolates from samples collected between 1971 and 1999 all fell into toptotype I (aka NWZ) (Fig. 4). One sublineage (with 99.1–100% nt id), represented by SAT1/TAN/19/99 (Mara), SAT1/TAN/25/99 (Mara), SAT1/TAN/21/99 (Manyara), SAT1/TAN/26/99 (Kilimanjaro) and SAT1/TAN/51/99 (Dar es Salaam), was identified in the north of Tanzania and clustered closely

**Table 2.** Oligonucleotide primers used for RT-PCR and sequencing

Serotype	Primer name	Primer sequence (5'–3')	Use
O and A	EUR-2B52R	GAC ATG TCC TCC TGC ATC TGG TTG AT	RT & PCR
O	O-1C244F	GCA GCA AAA CAC ATG TCA AAC ACC TT	PCR
O	O-1C272F	TBG CRG GNC TYG CCC AGT ACT AC	PCR
A	A-1C562F	TAC CAA ATT ACA CAC GGG AA	PCR
A	A-1C612F	TAG CGC CGG CAA AGA CTT TGA	PCR & SEQ
SAT 1 and SAT 2	SAT-2B208R	ACA GCG GCC ATG CAC GAC AG	RT & PCR
SAT 1	SAT1-P1-1228F	AAC CTG CAC TTC ATG TAC AC	PCR
SAT 1	SAT1-1C559F	GTG TAT CAG ATC ACA GAC ACA CA	PCR & SEQ
SAT 1	SAT1U-OS	GTG TAC CAG ATC ACT GAC AC	PCR & SEQ
SAT 2	SAT2-1C445F	TGG GAC ACM GGI YTG AAC TC	PCR & SEQ
SAT 2	SAT2-P1-1223F	TGA ACT ACC ACT TCA TGT ACA CAG	PCR
All	NK72	GAA GGG CCC AGG GTT GGA CTC	SEQ
O	O-1C499F	TAC GCG TAC ACC GCG TC	SEQ
O	O-1C583F	GAC GGY GAY GCI CTG GTC GT	SEQ
O	O-1C605eF	TAG CTA GCG CCG GCA AGG ACT TCG AG	SEQ
O	O-1D296F	ACA ACA CCA CCA ACC CAA C	SEQ
O	O-1D296bF	ACA ACA CCA CCA ATC CAA C	SEQ
O	O-1D628R	GTT GGG TTG GTG GTG TTG T	SEQ
SAT 1	SAT1-1D394R	GGY TTG TAC TTR CAR TCA CCG TTG TA	SEQ
SAT 1	SAT1-1D200F	TGC GYG CIG CCA CGT ACT AYT TCT C	SEQ
SAT 2	SAT2VP3-AB	CAC TGC TAC CAC TCR GAG TG	SEQ
SAT 2	SAT2-1C523F	GAC ACN CCM GCM ATG GC	SEQ
SAT 2	SAT-1D209F	CCA CAT ACT ACT TTT GTG ACC TGG A	SEQ
SAT 2	SAT2-D	GGT GCG CCG TTG GGT TGC CA	SEQ



**Fig. 2.** Midpoint-rooted maximum likelihood tree showing the relationships between the serotype A viruses collected from Tanzania. The three serotype A topotypes are labelled AFRICA, ASIA and EURO-SA. Seven proposed African genotypes, G-I to G-VII, are also shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. \*reference number not assigned by the WRLFMD.

with Kenyan viruses (SAT1/K96/99 and SAT1/K114/99; 98.5–99.4% nt id) isolated in the same year. A single virus (SAT1/TAN/60/99) isolated from a wildebeest in the Serengeti National Park also belonged to this sublineage (98.9–99.4% nt id). However, a genetically distinct second sublineage (87.2–88.1% nt id to the first sublineage and with intravariability of 99.1–99.7% nt id), represented by SAT1/TAN/6/99 (Mtwara), SAT1/TAN/18/99 (Ruwka), SAT1/TAN/37/99 (Iringa) and SAT1/TAN/43/99 (Iringa), was found in southern Tanzania. The most closely related viruses to this group were SAT1/TAN/2/77 and SAT1/TAN/3/80, which shared 86.9–87.3% and 89.6–90.0% nt id, respectively (Fig. 4).

#### Phylogenetic analysis of serotype SAT 2

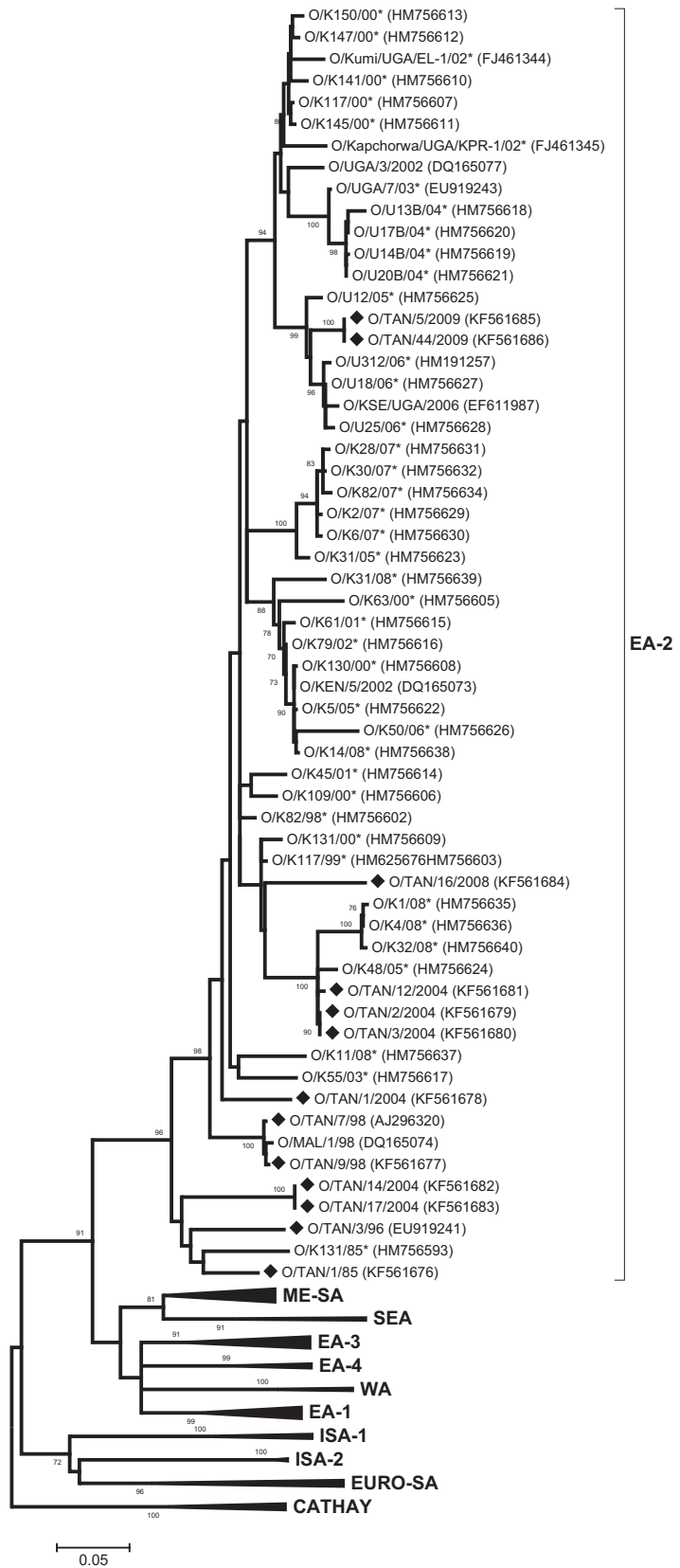
The evolutionary history was inferred using the maximum likelihood method based on the HGY model (Nei and Kumar, 2000). The tree with the highest log likelihood (−11571.3763) is shown (Fig. 5). A discrete gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0.8124)]. The rate variation model allowed for some sites to be evolutionarily invariable [(+I), 33.2835% sites]. The serotype SAT 2 viruses found in Tanzania between 1975 and 2009 all belonged to topotype IV. Within this topotype, five viruses

collected in 2004 (intra-isolate 98.5–100% nt) were closely related to a Kenya isolate from the same year (SAT2/K120/04; 97.8–98.3% nt id), whereas an isolate from 2009 was not closely related to any of the other viruses (maximum nt id 89.2% with SAT2/KEN/9/99). Older Tanzanian viruses from 1975 (SAT2/TAN/1/75) and 1986 (SAT2/TAN/1/86) were not closely related to any other viruses, but grouped with Kenyan viruses from 1974 (SAT2/K183/74; 91.5% nt id) and 2006 (SAT2/K6/06; 90.0% nt id), respectively (Fig. 5).

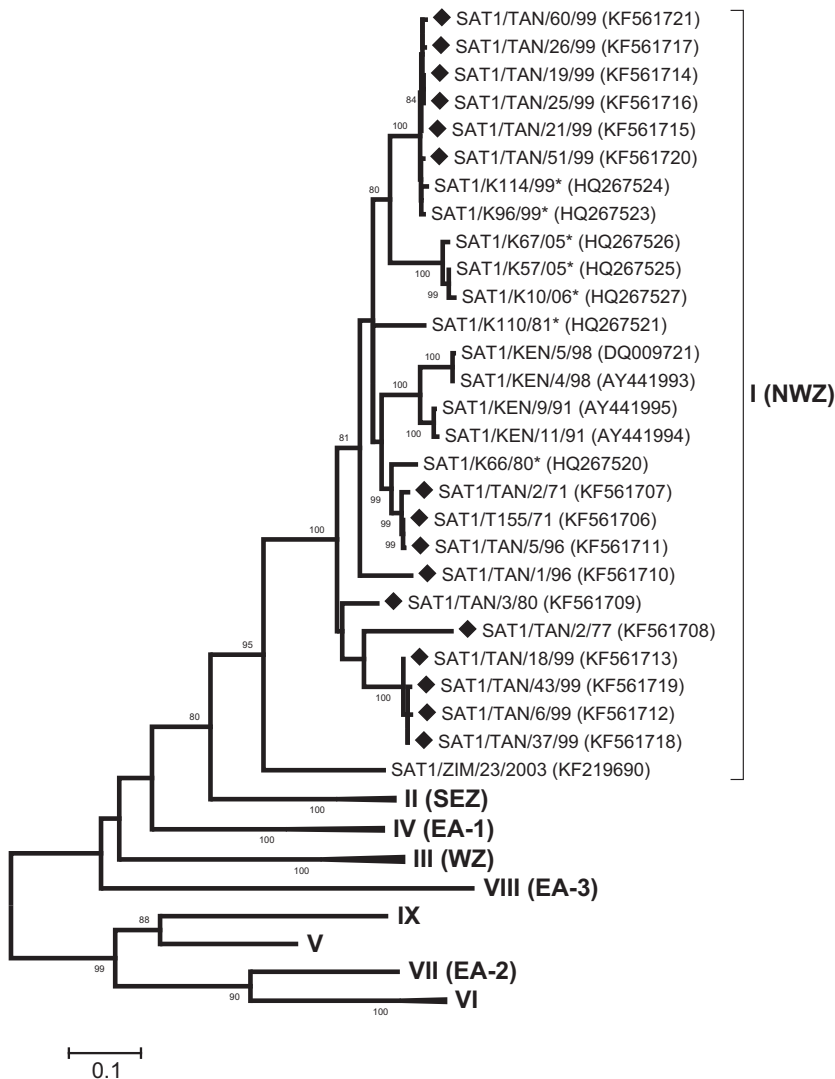
#### Discussion

This study describes the first molecular analysis of FMDV virus isolates collected from Tanzania. Since 1954, only four of the seven FMDV serotypes (O, A, SAT 1 and SAT 2) have been detected. This observation is consistent with previous recent serotyping studies that reported the presence of serotypes O, A, SAT 1 and SAT 2 in various locations in Tanzania (Swai et al., 2009; Kasanga et al., 2012) and indicate that the epidemiology of FMD in the country is complicated by the presence of multiple serotypes.

This study used phylogenetic analysis to define the genetic relationships between Tanzanian FMD viruses and those that have been collected from neighbouring countries (Figs 2–5). From the phylogenetic trees constructed, it is possible to infer the genetic relationship of isolates, and



**Fig. 3.** Midpoint-rooted maximum likelihood tree showing the relationships between the serotype O viruses collected from Tanzania. The 11 serotype O topotypes are labelled EURO-SA, CATHAY, ME-SA, SEA, ISA-1, ISA-2, WA and EA-1, EA-2, EA-3 and EA-4. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. \*reference number not assigned by the WRLFMD.



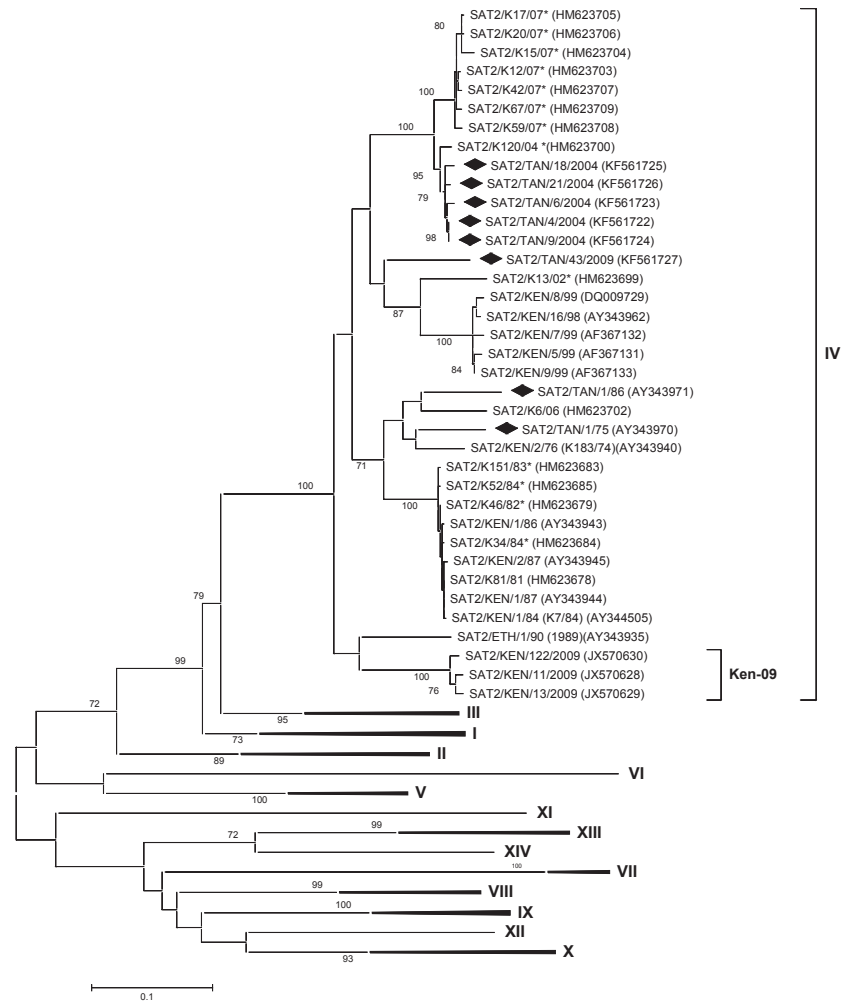
**Fig. 4.** Midpoint-rooted maximum likelihood tree showing the relationships between the serotype SAT 1 viruses collected from Tanzania. The nine serotype SAT 1 topotypes are labelled from I to IX. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. \*reference number not assigned by the WRLFMD.

how FMD viruses might be dispersed between countries in sub-Saharan Africa at different times and places. These findings indicate that serotype A viruses (Fig. 2) are constantly evolving with time and geographic location and give rise to variant viruses that are genetically unrelated. Figure 2 shows that the group containing the Tanzanian strains isolated between 2008 and 2009 also contained viruses detected from Kenya in 2008 (A/KEN/8/2008) suggesting viruses from Tanzania and Kenya share common epidemiological links. Furthermore, a group of viruses from Tanzania (A/TAN/3/68, A/TAN/2/68 and A/TAN/4/80) also was most closely related to an older virus from Kenya (A/KEN/42/66), implying that the ancestral history of this lineage is shared in those East African countries.

The phylogenetic tree for serotype O isolates (Fig. 3) features the close genetic relationship of Tanzanian viruses and FMD viruses collected elsewhere in the region (Kenya and Uganda), suggesting that long-distance animal

movements through trade and pastoralism could contribute to the spread of FMD across East Africa. It is also clear that FMD viruses occasionally spread into the northern parts of some Southern African countries (as evidenced by the close relationship between O/MAL/1/98 and Tanzanian viruses from the same year). Studies conducted elsewhere (Kivaria, 2003; Habiela et al., 2010; Di Nardo et al., 2011) described further that the spread of FMDV can also be associated by animal movement, especially in the pastoral livestock systems in Africa and Asia.

SAT strains are known to be endemic in most Southern African countries with African buffalo being incriminated as reservoirs for these viruses (Vosloo et al., 2004, 2007). Phylogenetic analysis of the SAT viruses (Figs 4 and 5) shows high degree of nucleotide heterogeneity in SAT 1 viruses that were isolated from different geographic areas and times in Tanzania. Interestingly, in 1999, SAT 1 viruses belonging to topotype I were clustered in two dif-



**Fig. 5.** Midpoint-rooted maximum likelihood tree showing the relationships between the serotype SAT 2 viruses collected from Tanzania. The 14 serotype SAT 2 topotypes are labelled from I to XIV. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. \*reference number not assigned by the WRLFMD.

ferent lineages implying a possibility of independent evolutionary events among viruses from a particular geographic location. These observations raise the possibility that epidemiological factors and evolutionary characteristics of viruses in a defined geographic location are related and may contribute significantly to FMD events and emergence of FMDV variants.

The general trend for the occurrence of FMDV serotypes in Tanzania shows that serotype A virus has been present for long time in southern and central parts of the country. The type A viruses were incriminated to cause FMD outbreaks up to early 1980s, and there has been no evidence of serotype A detection in Tanzania until very recently (Swai et al., 2009; Kasanga et al., 2012). In part, the apparent disappearance of FMDV type A for almost 30 years (from the 1980s to the 2008) could reflect the low level of livestock sampling in the country. However, it is also possible that this could be ascribed to several aspects, including epidemiological factors related to maintenance and transmission of the virus, vaccination pressure, climatic and ecological

changes in specific geographic areas of disease occurrence. Further studies including in-depth molecular characterization are required to clarify the reoccurrence phenomenon of serotype A in similar environments after long-time disappearance of the virus.

In recent years, there appears to be a north–south division of FMD viruses in East Africa with topotypes/genotypes occurring in Ethiopia, Eritrea, Somalia and Sudan (O/EA3, A/G-IV, A/G-VII, SAT1/IX, SAT2/VII, SAT2/XIII) being different to those occurring to the south in the Democratic Republic of the Congo, Kenya, Tanzania and Uganda (O/EA-2, O/EA-4, A/G-I, SAT1/I and SAT2/IV) (Ayelet et al., 2009; Balinda et al., 2010; Habiela et al., 2010; Sangula et al., 2010; Kasambula et al., 2012; Wekesa et al., 2013), although occasionally viruses from one region may appear in the other, for example, O/EA-4 in Ethiopia (Ayelet et al., 2009). Occasionally, viruses normally present in East Africa may spread to other regions such as the recent multiple incursions of SAT 2 into Egypt and the Middle East (Ahmed et al., 2012).

We have sequenced the VP1-coding regions of four FMDV serotypes O, A, SAT 1 and SAT 2, which have been cocirculating in Tanzania between 1967 and 2009. The genetic characteristics of FMDV within each serotype revealed variations in nucleotide sequences; however, the overall genotypes/topotypes to which these viruses belonged were stable and consistent with those present in other countries in East Africa. The presence of multiple serotypes and genotypes/topotypes as well as the complex epidemiology of FMD complicates the control of the disease through vaccination and establishment of FMD-free zones. Therefore, further studies in both domesticated and wild animals are required to determine the phylogeography of viruses, investigate the genetic and antigenic characteristics of the circulating strains so that a rational control method for FMD in Tanzania and neighbouring countries can be recommended. These data provide historical context for a number of ongoing studies that aim to describe the epidemiology of FMD in Tanzania and the factors that influence the maintenance of the disease in the country.

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