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Diversity and transboundary mobility of serotype O foot-and-mouth disease virus in East Africa: Implications for vaccination policies

Sheila N. Balinda [a,1], Abraham K. Sangula [a,d,1,*], Rasmus Heller [b], Vincent B. Muwanika [a], Graham J. Belsham [c], Charles Masembe [a], Hans R. Siegismund [b]

[a] Makerere University, Institute of Environment and Natural Resources, P.O. Box 7298, Kampala, Uganda
[b] Department of Biology, Ole Maaløes Vej 5, DK-2200, Copenhagen N, Denmark
[c] National Veterinary Institute, Technical University of Denmark, Lindholm, DK-4771 Kalvehave, Denmark
[d] Foot-and-Mouth Disease Laboratory, Embakasi, P.O. Box 18021, 00500 Nairobi, Kenya

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ABSTRACT

Foot-and-mouth disease (FMD) virus serotype O has been responsible for most reported outbreaks of the disease in East Africa. A sustained campaign for the past 40 years to control FMD mainly by vaccination, combined with quarantine and zoosanitary measures has been undertaken with limited success. We investigated the genetic relationships among serotype O strains in eastern Africa using complete VP1 coding region sequences obtained from 46 FMD virus isolates collected in Kenya in the years 1964–2008 and 8 Ugandan isolates collected between 1999 and 2006. In addition, 21 selected FMDV sequences from Genbank representing reference strains from eastern Africa and elsewhere were included in the Bayesian inference analyses and the detection of selection forces. The results confirmed previous observations that eastern Africa harbours four distinct topotypes (clades with >15% sequence divergence). All but one strain isolated post-2000 belonged to topotypes EA-2, EA-3 and EA-4, while all three vaccines have been based on strains in the EA-1 topotype. The estimated *dN*/*dS* ratios across the individual codons of the entire VP1 coding region revealed that purifying (negative) selection constituted the dominant evolutionary force. Cross-border disease transmission within the region has been suggested with probable incursions of topotypes EA-3 and EA-4 into Kenya and Uganda from neighboring Ethiopia and Sudan. We conclude that the vaccines have probably been effective in controlling EA-1, but less so for the other topotypes and propose a more comprehensive representation of topotypes in the development of new vaccines in recognition of the considerable diversity and transboundary nature of serotype O.

1. Introduction

Foot-and-mouth disease (FMD) is a vesicular disease affecting cloven hoofed domestic and wild animals (Coetzer et al., 1994). It is mainly transmitted through direct contact involving infected aerosol deposition in the respiratory tract and mechanical transfer of the virus to susceptible animals. Disease dissemination may occur indirectly through contaminated surfaces and products (Alexandersen and Mowat, 2005). The causative agent, foot-and-mouth disease virus (FMDV), comprises a positive sense RNA within a protein capsid. The RNA genome has a single large open reading frame (ORF) encoding a polyprotein that is eventually processed into 12 mature proteins. The virus capsid contains sixty copies of each of the four structural proteins with VP1-3 exposed on the outside while VP4 is located internally (Acharya et al., 1989; Belsham, 2005). FMD viruses, like other members of *Picornaviridae*, are very diverse and exist in seven serotypes: O, A, C, Asia 1 and the Southern African Territories (SAT) 1, SAT 2 and SAT 3 as determined using virus neutralization assays. The VP1 coding region has been used extensively in molecular characterization and to determine evolutionary relationships (Bastos et al., 2003; Knowles and Samuel, 2003; Sangare et al., 2003), resulting in a comprehensive nucleotide data library of this part of the virus coding region.

In East Africa, serotype O has been responsible for most of the reported outbreaks (Vosloo et al., 2002). A recent study has found that type O viruses in this region belong to four topotypes, EA-1 to EA-4 (Ayelet et al., 2009), based on the concept of topotype classification (Samuel and Knowles, 2001; Knowles and Samuel, 2003). Over the last 40 years, a sustained campaign to control FMD

* Corresponding author at: Makerere University Institute of Environment and Natural Resources, Molecular Biology Laboratory, P.O. Box 7298, Kampala, Uganda. Tel.: +256 775633133; fax: +256 41 530134.
E-mail addresses: aksangula@muienr.mak.ac.ug, aksangula@gmail.com, sbalinda@gmail.com (A.K. Sangula).
[1] Equal-Joint first authorship.

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has been undertaken in East Africa with limited apparent success. This has mainly been through the use of vaccination to control outbreaks and for routine prophylaxis applied together with quarantine measures adopted during FMD outbreaks which are usually coupled to zoosanitary measures to prevent indirect disease dissemination. A single vaccine strain for serotype O FMDV (K77/78) has been in use in Kenya and Uganda for over 25 years but other vaccine strains used in the past include K120/64 and K83/79. The limited efficiency of vaccination in controlling FMD can be attributed, in part, to the existence of six of the seven known serotypes in this region (Vosloo et al., 2002) since immunity to one serotype does not confer protection against another. Different strains within a single serotype also exhibit significant diversity, as was shown through the distinction between two type A strains using cross neutralization tests (Brown, 2003). Consequently, antigenic diversity may limit the efficiency of vaccines to combat different strains of the same serotype (Mumford, 2007).

For effective disease control, an understanding of the mechanisms responsible for the maintenance of FMDV is required. Key to this process is improved insight into the evolutionary forces such as selection and genetic drift operating on populations of the virus. In addition, as previously suggested by Heath et al. (2006), the evolution of aphthoviruses and enteroviruses (other members of the family *Picornaviridae*) includes recombination and thus analyses towards its identification in FMD viral sequence data sets may be desirable as it is known to bias estimates of phylogenies (Schierup and Hein, 2000).

In this study, the complete VP1 coding region sequences were used to determine the evolutionary relationships and processes shaping this most prevalent FMD virus serotype in eastern Africa.

2. Materials and methods

2.1. Virus isolates

A total of 54 type O virus isolates obtained between the years 1964 and 2008 from Kenya and Uganda were included in this study (Table 1). Fig. 1 indicates their respective districts of origin. The Kenyan isolates were part of the collection of outbreak samples submitted to the Embakasi FMD laboratory, Nairobi, and were mainly generated from epithelium samples from which viruses were isolated and passaged in BHK cells. All the Ugandan samples were from oropharyngeal fluid and swab samples (from buccal and foot lesions) collected during field outbreak investigations.

2.2. RNA extraction, cDNA synthesis, PCR and cycle sequencing

RNA was extracted from cell culture supernatants and directly from clarified samples of oropharyngeal fluid or swab samples using the QIAamp® Viral RNA kit (Qiagen, Germany) according to the manufacturer's instructions. The cDNA synthesis was carried out using Ready-To-Go™ You-Prime First – Strand Beads (GE Healthcare Life Sciences, Sweden) with random hexamer (pdN_6) primers.

PCR of two overlapping fragments (to cover the VP1 region) using 0.2 pmol primers 8-A PN 64 with 8-A PN 98 and 8-A PN 84 with 8-A PN 85; SNB-84 with SNB-85 (designed specifically for Eastern African viruses) as well as primers previously used to target the entire VP1 region O-$1C_{564}$ and FMD-$2A_{34}$ (NK-72) (Table 2) were performed in a final volume of 50 μl using 2–5 ng of

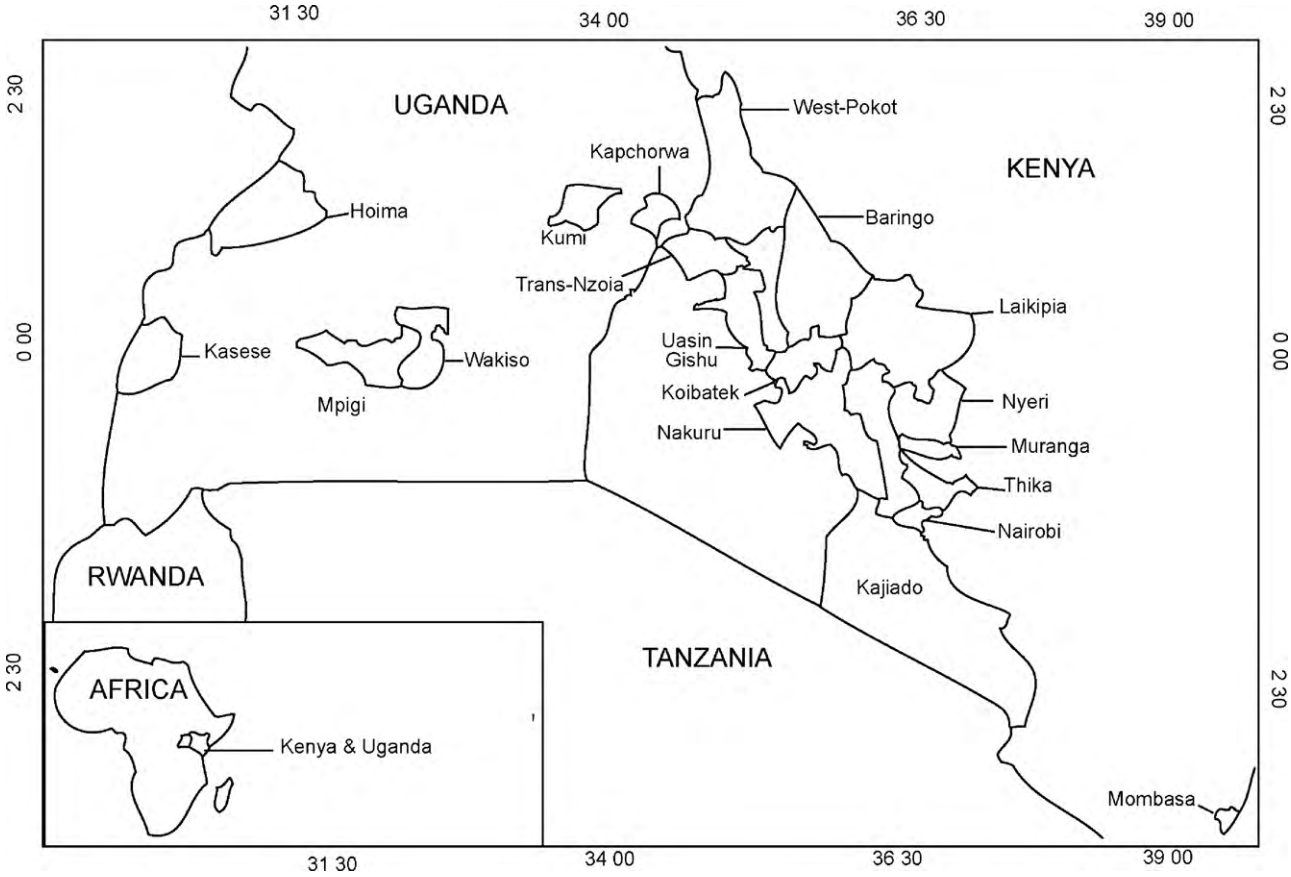

Fig. 1. The districts of origin of the FMD virus samples used in the study from both Kenya and Uganda are shown.

Table 1
Foot-and-mouth disease virus strains and sequences used in the study.

Isolate designation	District/country	Accession No.	Topotype
K120/64	Laikipia, Kenya	This study	
BFS/UK/67	United Kingdom	AY593815	EURO-SA
K77/78	Nakuru, Kenya	This study	
K83/79	Nyeri, Kenya	AJ303511	EA-1
K101/80	Laikipia, Kenya	This study	
K103/82	Thika, Kenya	This study	
HKN/6/83	Hong Kong	AJ294919	CATHAY
ISA/8/83	Indonesia	AJ303503	ISA-1
K11/84	Kiambu, Kenya	This study	
K131/85	Kiambu, Kenya	This study	
K114/87	Kiambu, Kenya	This study	
K121/91	Kiambu, Kenya	This study	
K51/92	Nakuru, Kenya	This study	
K52/92	Kiambu, Kenya	This study	
K11/93	Kiambu, Kenya	This study	
K34/93	Laikipia, Kenya	This study	
GHA/5/93	Ghana	AJ303488	WA
K29/95	Kiambu, Kenya	This study	
K56/95	Kiambu, Kenya	This study	
UGA/5/96	Uganda	AJ296327	EA-1
K82/98	Kiambu, Kenya	This study	
MAL/1/98	Malawi	DQ165074	EA-2
UGA/17/98	Uganda	WRL	EA-4
K117/99	Nakuru, Kenya	This study	
SUD/1/99	Sudan	WRL	
U/97/99	Uganda	This study	
K63/00	Trans Nzoia, Kenya	This study	
K109/00	Uasin Gishu, Kenya	This study	
K117/00	Nyeri, Kenya	This study	
K130/00	Trans Nzoia, Kenya	This study	
K131/00	Nairobi, Kenya	This study	
K141/00	West Pokot, Kenya	This study	
K145/00	Laikipia, Kenya	This study	
K147/00	Trans Nzoia, Kenya	This study	
K150/00	Uasin Gishu, Kenya	This study	
K45/01	Nakuru, Kenya	This study	
K61/01	Mombasa, Kenya	This study	
UKG/35/01	United Kingdom	AJ294910	ME-SA
KEN/5/02	Nakuru, Kenya	DQ165073	EA-2
K79/02	Nakuru, Kenya	This study	
UGA/Kumi/02	Kumi, Uganda	FJ461344	
UGA/Kap/02	Kapchorwa, Uganda	FJ461345	
UGA/3/02	Uganda	DQ165077	EA-2
K55/03	Nakuru, Kenya	This study	
ETH/3/04	Ethiopia	FJ798109	EA-3
U/13B/04	Hoima, Uganda	This study	
U/14B/04	Hoima, Uganda	This study	
U/17B/04	Hoima, Uganda	This study	
U/20B/04	Hoima, Uganda	This study	
TAN/2/04	Tanzania	WRL	EA-2
K5/05	Laikipia, Kenya	This study	
K31/05	Laikipia, Kenya	This study	
K48/05	Kiambu, Kenya	This study	
ETH/58/05	Ethiopia	FJ798141	EA-4
U/12/05	Wakiso, Uganda	This study	
K50/06	Uasin Gishu, Kenya	This study	
ETH/2/06	Ethiopia	FJ798127	EA-3
U/18/06	Mpigi, Uganda	This study	
U/25/06	Mpigi, Uganda	This study	
UGA/KSE/06	Kasese, Uganda	EF611987	
K2/07	Kiambu, Kenya	This study	
K6/07	Koibatek, Kenya	This study	
K28/07	Laikipia, Kenya	This study	
K30/07	Laikipia, Kenya	This study	
K31/07	Kiambu, Kenya	This study	
K82/07	Muranga, Kenya	This study	
ETH/1/07	Ethiopia	FJ798137	EA-3
K1/08	Nairobi, Kenya	This study	
K4/08	Thika, Kenya	This study	
K11/08	Kiambu, Kenya	This study	
K14/08	Baringo, Kenya	This study	
K31/08	Kajiado, Kenya	This study	
K32/08	Thika, Kenya	This study	

GenBank accession numbers for sequences generated in the study range from HM756587 to HM756640.

Table 2
Primers used in the study.

Primer ID	Primers
8-A PN 64	GGTTGGACTCCACATCTCC
8-A PN 84	TACTACACCCAGTACAGCG
8-A PN 85	TGCAGCTTCGGGTGCTCC
8-A PN 98	GCATCCACTTACTACTTTGC
O-1C_{564}[a]	AATTACACATGGCAAGGCCGACGG
FMD-2A_{34}(NK72)[a]	GAAGGGCCCAGGGTTGGACTC
SNB-85	TGC(AG)(GC)CTTC(AG)GGTGC(CT)CCGTTCGGG
SNB-84	GCCCAATACTACACCCAGTACAGCGGC

[a] Knowles and Samuel (1994).

cDNA, and 2.5 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems), 200 μM of each dNTP (dATP, dCTP, dGTP, dTTP) and 1.5 mM $MgCl_2$. Following the activation of AmpliTaq Gold® DNA polymerase at 95 °C for 5 min, reaction mixtures were denatured at 95 °C for 15 s followed by 60 °C for 2 min to allow for primer annealing. For each cycle, a chain elongation step at 72 °C for 1 min 20 s was allowed. This process was repeated 30 times and final extension continued at 72 °C for 5 min. The resultant PCR products were analysed by electrophoresis on 2% agarose gels using as a molecular weight marker ΦX 174-RF DNA (Amersham, Biosciences). Purification of the PCR products to remove oligonucleotide primers, dNTPs and enzyme was achieved with a QIAquick® PCR purification kit (Qiagen). Cycle sequencing was performed in both directions using Big dye Terminator V 3.1 kit (Applied Biosystems) and ran on an automated DNA Sequencer (ABI PRISM® 3700) by Macrogen in Korea.

2.3. Sequence analysis

Sequencher software 4.8 (Gene Code Corporation) was used to assemble the sequences and multiple alignments by log-expectation comparison were carried out using MUSCLE incorporated within Geneious 4.7.6 software (Drummond et al., 2009). Determination of the most appropriate model of evolution was performed using a hierarchical likelihood-ratio test of 24 models implemented in PAUP*(v. 4.0 beta 10) (Swofford, 2001) and MrModeltest (v 2.2) (Nylander et al., 2004). The GTR+I+G model was used with rate variation following a gamma distribution across sites and a proportion of invariable sites was identified as the best model of evolution and was used in subsequent analyses. Most previous studies concerned with topotype designation within FMDV serotypes have used distance-based phylogenetic approaches such as neighbour-joining or UPGMA trees without taking the temporal scale of sampling into account. Instead, we applied the Markov Chain Monte Carlo (MCMC) method available in BEAST 1.4.7 (Drummond et al., 2005), incorporating the sampling dates in a Bayesian framework that co-estimates divergence times as well as tree topology. This approach also allows several different demographic scenarios to be tested together with the phylogenetic relationship between the samples. First, we tested the fit of the data to two different demographic models, the constant population size and the Bayesian skyline plot (BSP) and to two types of molecular clock, a strict and a relaxed (uncorrelated exponential) molecular clock (Drummond and Rambaut, 2007). This was done by calculating approximate Bayes factors comparing the marginal likelihoods of each model in Tracer v1.4 (http://beast.bio.ed.ac.uk/). The 10,000 trees saved during the MCMC chain were summarized using TreeAnnotator v1.4.7 (part of the BEAST package) to produce a maximum clade credibility (MCC) tree, discarding the first 1000 trees as burn-in. We used DnaSP v. 4.10.9 (Rozas et al., 2003) to estimate the average number of nucleotide substitution per site (Nei, 1987, eq. 10.20) between putative topotypes.

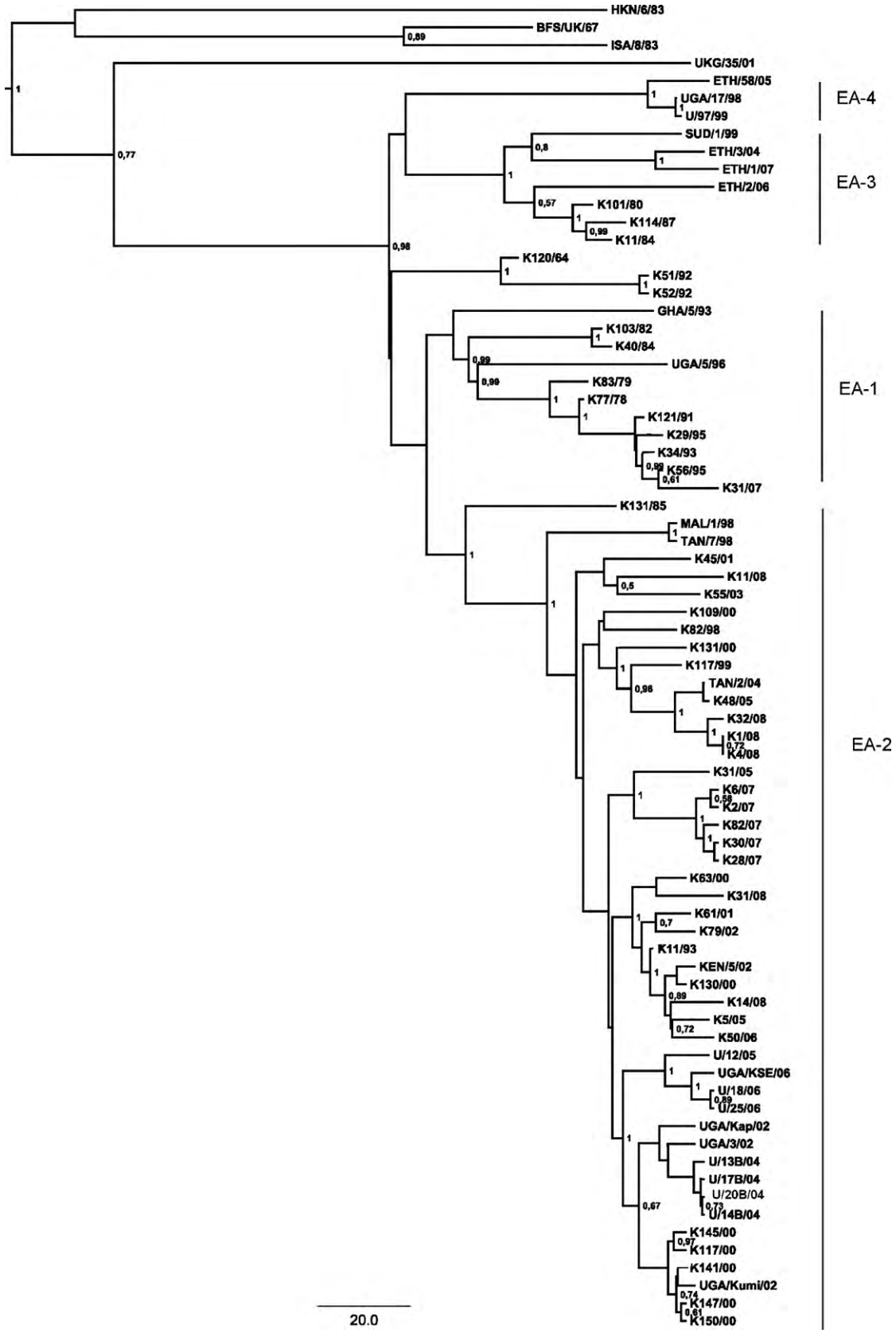

Fig. 2. Phylogenetic relationships between East African strains of serotype O FMD Viruses. The tree is based on the nucleotide sequence of the VP1 (1D) coding region with selected reference strains from East Africa and elsewhere. Other sequences deposited in Genbank from East African strains were also included as indicated. Trees were estimated using Bayesian inference analysis (BEAST) with a relaxed uncorrelated exponential molecular clock (see Section 2). The scale bar shows the estimated number of amino acid substitutions per site per year.

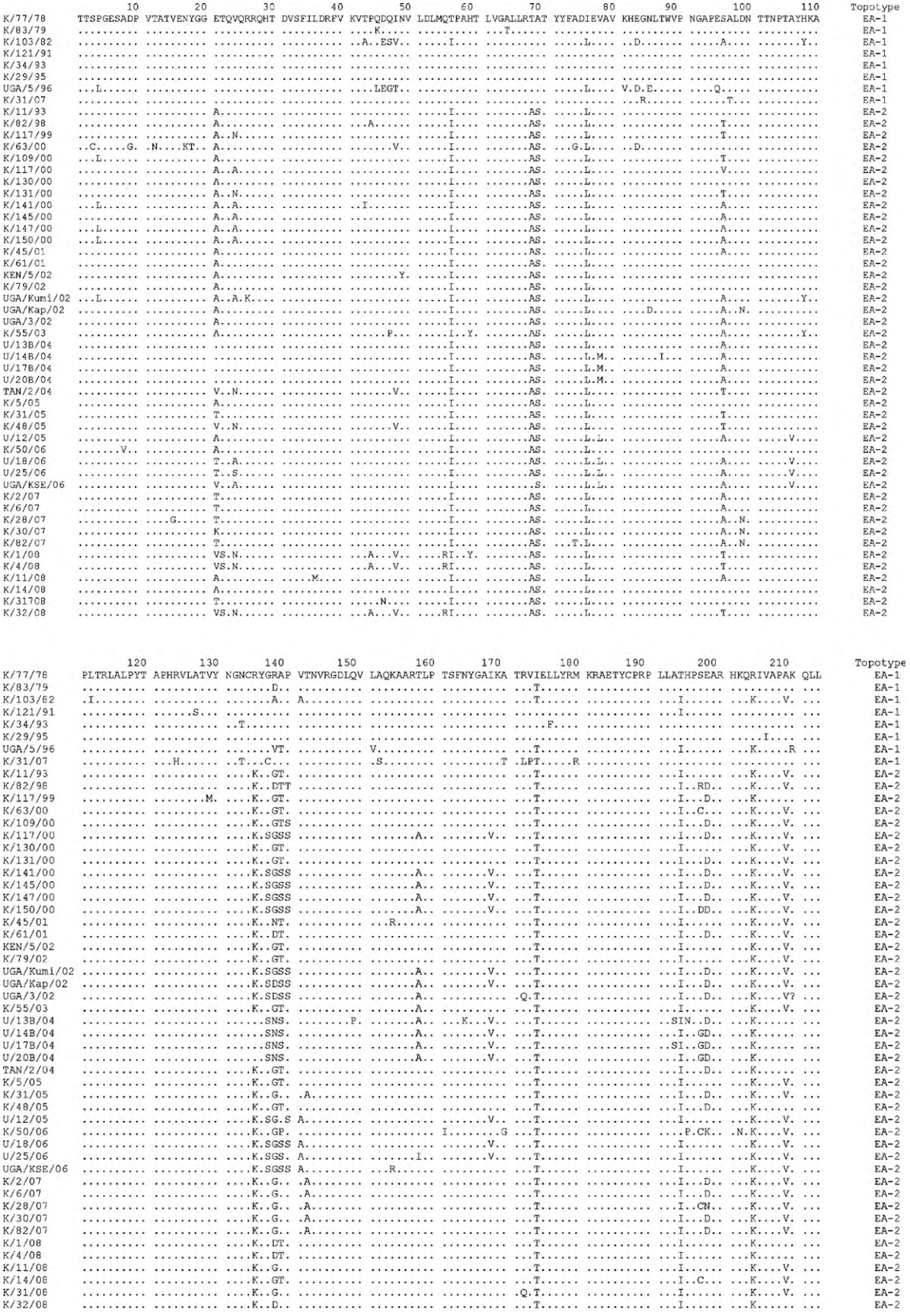

Fig. 3. Amino acid alignments of 54 serotype O FMD viruses representative of topotypes EA-1 and EA-2. A '.' indicates an amino acid site identical to that of the sequence KEN/77/78 and '?' denotes an ambiguous (undetermined) site; UGA/3/02 with an "N" at position 631 probably translates into either lysine (K), glutamic acid (E) or glutamine (Q).

The translation, alignment and identification of variable sites within amino acids were carried out using MEGA 4 (Tamura et al., 2007). Overall, site-specific selection pressures acting within this region were determined by estimating the *dN*/*dS* using two likelihood procedures available in the HyPhy package and accessed through the Datamonkey web-server. These methods are the single-likelihood ancestor counting (SLAC) method and the random-effects (REL) method (Kosakovsky Pond and Frost, 2005).

Recombination was investigated using RDP (Martin and Rybicki, 2000), GENECONV (Padidam et al., 1999), Chimaera (Posada and Crendall, 2001) and Maxichi (Posada and Crendall, 2001) within the RDP3 beta 22 software (Martin et al., 2005). The analyses using each of the mentioned methods were conducted twice for repeatability.

3. Results

3.1. Phylogenetic relationships

The complete VP1 coding region of 54 FMDV samples have been amplified by RT-PCR and then the sequences determined. The 46 samples with origins from Kenya were obtained between the years 1964 and 2008 while the 8 Ugandan samples were obtained during the years 1999–2006 within the districts of origin summarized in Fig. 1. The sequences used here were selected to include representation of serotype O strains from West Africa and elsewhere in addition to the East African strains. Analyses involving the detection of recombination revealed no evidence for this evolutionary process within the dataset (of VP1 coding sequences), hence phylogenetic analyses were performed under the assumption that the sequences in this study were not generated by recombination. The Bayes factor test showed no significant support for the more complex BSP demographic model, but strongly supported a relaxed (uncorrelated exponential) rather than a strict clock. Branch rate heterogeneity was severe with a coefficient of variation of 0.90 (95% HPD: 0.79–1.01) and inferred rates on individual branches ranging from 9.81×10^{-5} to 1.02×10^{-2}. Hence, results from the constant population size model evolving under a relaxed clock are reported throughout.

The overall mean rate of substitutions per site per year was estimated to be 2.76×10^{-3} (95% HPD: 1.84×10^{-3} to 3.63×10^{-3}). This resulted in an estimated root of the tree at 164 years (95% HPD: 80–299 years). Phylogenetic relationships inferred from the maximum clade credibility tree confirmed that the type O samples from eastern Africa belonged to four distinct viral topotypes (with a minimum sequence divergence of 15% among them) termed EA-1 to EA-4 (Fig. 2). Four samples did not cluster into the topotype designations (K/120/64, K/51/92, K/52/92 and K/131/85) but should probably be attributed to topotypes EA-1 (first three samples) and EA-2 (latter sample), respectively. They were not sufficiently divergent from these topotypes to be designated as separate topotypes. Interestingly, the West African sample included in this analysis grouped as a sister taxon to EA-1, but with sufficient sequence divergence to be designated in a separate topotype, WA (following the nomenclature in Ayelet et al., 2009). Hence, WA and EA-1 collectively formed a sister clade to EA-2. The posterior probability of some of the nodes separating topotypes were not very high: EA-1/WA: 0.29; EA-1, WA/EA-2: 0.46; EA-3/EA-4: 0.47.

The earliest topotype identified (EA-1) was found in Kenya and Uganda. The majority of the viruses in this topotype are from the period 1964–1996. However, K/31/07 is an exception being the only recent strain from this topotype. The topotype EA-2 was represented by Kenyan, Ugandan, Tanzanian and Malawian isolates. It was the predominant topotype within the East African samples, and all but one (K/31/07) of the recently collected (2000–2008) samples from Kenya and Uganda belonged to this topotype. EA-3 and EA-4 are mainly restricted to Ethiopia, Eritrea and Sudan in recent times, although they did previously circulate in Kenya and Uganda. The most recent representative of topotype EA-4 in Uganda occurred about 10 years ago (strain U/97/99 in Fig. 2), and EA-3 was last observed in Kenya about 20 years ago (K/114/87).

3.2. Amino acids

The alignment of the deduced amino acid sequences for the entire VP1 proteins of 54 Kenya and Uganda viruses belonging to topotypes EA-1 and EA-2 is shown in Fig. 3. Two hundred and eighty three (44%) variable sites have been determined across the 642 nucleotides of these viruses which encode substitutions to 80 (37.4%) of the 214 amino acids (Fig. 3). The majority of the amino acid variations were clustered within antigenic site 1(A) corresponding to the G-H loop (residues 135–161) and the car boxy terminus region (residues 191–212).

In both topotypes, some level of amino acid conservation was observed within the G-H loop, with the cysteine amino acid residue (135) at the base of the loop conserved within all the strains. Similarly, the RGD motif within this loop of VP1, which binds to the cellular integrin receptor located at residues 146–148 was completely conserved. Residues flanking the RGD motif were also conserved as far as the −2 position. Conservation was also observed as far as +4 (RGDLQVL), in most of the viruses with the exception of U/13B/04 (EA-2) at +2 with P (Proline) instead of Q (glutamine) and UGA/5/96 (EA-1) with V (Valine) instead of L (Leucine) at position +4. A variation between topotypes EA-1 and EA-2 at position 69–70 was observed, with the former comprising T (Threonine), A (Alanine) and the latter A (Alanine), S (Serine) respectively.

3.3. Selection

To identify the forces shaping evolution, the ratio of non-synonymous to synonymous substitutions (*dN*/*dS*) was determined. An overall mean of *dN*/*dS* of 0.12 for the entire VP1 coding region was estimated. However, the per site *dN*/*dS* analyses revealed 130 of the sites to be under negative selection and no positively selected sites as determined using the SLAC method at $p = 0.1$. On the other hand, REL, at a specified significance of Bayes Factor = 50 identified three positively selected sites at positions 45, 47 and 109, and 156 negatively selected sites.

4. Discussion

The East African dataset generated in this study is the largest and the most geographically representative of FMDV serotype O assembled so far for Kenya and Uganda in a single analysis. Comparing our findings with those of Tully and Fares (2008), who used the same Bayesian phylogenetic approach to infer divergence dates within the whole FMDV tree, some interesting differences are apparent. The mean rate of evolution inferred in the present study was slightly lower than that obtained by Tully and Fares (2008), but 95% HPD intervals overlapped considerably between the studies. However, the inferred age of the root of the tree presented in this study was much older (164 years) than that derived for all serotype O viruses by Tully and Fares (2008) (92 years), even though a broader diversity of topotypes was included there. This is probably attributable to the fact that they applied a demographic model of exponential growth, which was not supported by our data. The appropriateness of inferring any dynamic demographic model from a broad variety of FMDV topotypes is questionable, given that they cannot be considered to constitute a single population, hence violating the assumptions of

demographic inferences based on the coalescent (Halloran and Holmes, 2009). It is also possible that the sample composition plays an important role in dating divergences in this manner, as we have shown that rates of evolution differ substantially among branches in the type O phylogeny. Given the variability and inconsistencies in the estimated evolutionary rates and the divergence times between studies, the possibility of a radically changed time scale of historical divergence within FMDV should be addressed in future studies.

The phylogenetic relationships found here are consistent in some respects with those previously determined (Samuel and Knowles, 2001; Knowles and Samuel, 2003; Ayelet et al., 2009) in that four topotypes within serotype O exist within the Eastern African region. However, our phylogeny differed from those previous published by grouping the only West African sample within the clade comprising the four East African topotypes, making the East African topotypes paraphyletic. Of the known topotypes within East Africa, only EA-1 and EA-2 were observed in Kenya and Uganda within the last 10 years. Of these, EA-2 was by far the most dominant. On the other hand, topotypes EA-3 and EA-4 were mainly found in Ethiopia, Eritrea and Sudan, although both topotypes appeared to have previously circulated in Kenya and Uganda; EA-3 was found in Kenya in the 1980s while an incursion of EA-4 into Uganda occurred in 1999.

Vaccines against type O in East Africa have all been based on virus isolates from the topotype EA-1. Only one virus sample from all the post-2000 samples included in this study was attributable to this topotype, whereas the remaining 31 were from the other three topotypes. This could suggest that vaccine programs have been successful, but that type O vaccines generally do not cross-protect well against other topotypes, confirming what has been observed for type A (Araujo et al., 2002). Based on the dated phylogeny inferred in this study, we conclude that all four East African topotypes probably already existed in 1964, the time of the collection of the first strain on which a vaccine was based. Furthermore, there was no indication of positive selection in the amino acid positions that were variable among the vaccine strains and the other topotypes. This leads us to conclude that the diversification of type O FMDV was most likely not an evolutionary response to the selection pressure afforded by vaccine programs. The current dominance of topotypes EA-2 and EA-3 in East Africa probably just reflects the lack of protection induced by the previous vaccine programs against these topotypes. This instills some confidence that vaccine programs can be used to control the disease as long as the full diversity of the serotype is taken into account and vaccines are not exclusively based on single topotypes. However, as EA-1 has continued to circulate, in Kenya at least, in the recent past (2007), the vaccines have not been able to eliminate this topotype completely. This could be attributable to lack of comprehensive vaccine programs and to the survival of virus strains in untreated livestock reservoirs or potentially type O is being maintained in wildlife populations. Anti-O antibodies have been detected among buffalo populations in Uganda indicating previous exposure to serotype O (Ayebazibwe et al., 2010).

Despite the limited representation of virus sequences from some countries in the region, the phylogenetic relationships provided indications of transboundary events within the East African region. The Sudanese (1999) isolate, within topotype EA-3 appeared closely related to Ethiopian isolates obtained in 2004 and 2007. Similarly, the close evolutionary relationship between the Malawian (MAL/1/98), Tanzanian (TAN/7/98), Ugandan (UG/kumi/2002) and the Kenyan K/141/00 strains suggests that FMD virus strains can transgress borders relatively quickly. This is perhaps not very surprising, as uncontrolled animal movement across the border points is known to occur within the East African region but our evolutionary analysis underlines the transboundary nature of type O FMDV. More surprising was the observation that the only West African sample included in this study grouped as a sister taxon to EA-1 and hence within the East African topotype diversity. If true, this further stresses the lack of geographical sorting in type O genetic diversity but it should be examined in more detail by including more West African samples along with dense East African sampling.

Taken together, the considerable natural diversity and the transboundary nature of FMDV serotype O leads us to propose comprehensive control programs that use frequently administered vaccines based on strains from all four topotypes in East Africa, and possibly even also on WA strains. This is necessary to prevent "exotic" varieties of type O originating from introduced livestock from starting new epidemics. Of course it cannot be excluded that FMDV has the capability to escape vaccine programs by acquiring adaptive resistance to the selection pressure, given its rapid rate of evolution. However, on the basis of our evolutionary analyses we conclude that vaccines have been reasonably successful in controlling the topotype they were based upon.

An overall $dN/dS < 1$ suggests that negative selection (purifying selective pressure) within the VP1 coding region is dominant resulting in amino acid conservation as observed previously (Tully and Fares, 2006). Nevertheless, positive selection on a few codon sites has also been revealed in this study. In other studies, several sites were identified as undergoing positive selection within this widely geographically distributed serotype. Interestingly, even the entire RGD motif, a region known for extensive conservation in all the serotypes was suggested as undergoing positive selection in this serotype (Tully and Fares, 2006). The differences in observed sites undergoing positive selection in these two studies may be attributed to the nature of sampling with the current study mainly focusing on the East African strains while the analysis of Tully and Fares (2006) included samples with a wide geographic distribution coupled to various sampling times for an attempted random representation of the world wide situation (Tully and Fares, 2006).

This work has analyzed the evolutionary relationships within serotype O of FMDV in Eastern Africa. The occurrence of transboundary disease transmission was evident, and effective control strategies to combat this should be adopted by applying vaccines that more broadly reflect the diversity of East African topotypes. This should be implemented by the respective governments in a joint regional effort.

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