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## Analysis of Recent Serotype O FootandMouth Disease Viruses from Livestock in Kenya: Evidence of Four Independently Evolving Lineages

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**Analysis of Recent Serotype O Foot-and-Mouth Disease Viruses from Livestock in Kenya: Evidence of Four Independently Evolving Lineages**

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Subject Area:	Kenya, Foot-and-mouth disease, Serotype O FMDV, East Africa, Topotype, Outbreaks

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12 **of Four Independently Evolving Lineages**  
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### Summary

Foot-and-mouth-disease (FMD) is endemic in Kenya where four serotypes (O, A, SAT 1 and SAT 2) of the virus are currently in circulation. Within 2010 and 2011, the National Laboratory recorded an increase in the number of FMD outbreaks caused by serotype O virus. The characteristics of these viruses were determined to ascertain whether these were independent outbreaks or one single strain spreading throughout the country. The sequences of the complete VP1 coding region were analyzed from viruses sampled within different areas of Kenya during 2010 and 2011. The results indicated that the 2010 and 2011 outbreaks in Kenya were independent from each other. By comparison with earlier type O isolates from Eastern Africa, it was apparent that the outbreaks were caused by viruses from three different lineages of topotype EA-2 and a fourth virus strain belonging to topotype EA-4. The topotypes EA-1 and EA-3 were not detected from these outbreaks. Implications of these results for FMD control in Eastern Africa are discussed.

**Keywords:** Kenya; foot-and-mouth-disease; serotype O FMDV; East Africa; topotype; outbreaks

## Introduction

Foot-and-mouth disease (FMD) affects both domestic and wild cloven-hoofed animals such as cattle, pigs, sheep, and goats plus more than 70 species of wildlife (Alexandersen and Mowat, 2005; Thomson et al., 2003). The disease is clinically characterized by fever, lameness, and ~~vesicular lesions~~ vesicles on the tongue, feet, snout, and teats of susceptible species (Kitching, 2002). FMD ~~has been known since the sixteenth century as an important threat in the cattle industry and has continued to~~ is be one of the world's most economically important livestock diseases. ~~Losses due to FMD can be~~ with indirect losses due to trade ~~embargos~~ embargos on livestock and livestock products or and direct ~~one~~ effects such as reduced milk yields, weight loss, abortions, prenatal mortality and loss of cart power in draught animals (James and Rushton, 2002). Improved control of ~~the disease~~ the disease may contribute to poverty reduction and increased economic growth in developing countries (Perry and Rich, 2007) such as Kenya where the disease is endemic.

The causative agent ~~of the disease~~, foot-and-mouth disease virus (FMDV), occurs in seven immunologically and genetically distinct serotypes O, A, C, Asia 1, SAT 1, SAT 2, SAT 3 and multiple variants exist within each serotype (Grubman and Morgan, 1986; Knowles and Samuel, 2003). ~~These serotypes can be distinguished~~ able both serologically and by nucleotide sequencing. Vaccination against one serotype does not ~~cross-protect against other serotypes,~~ thus necessitating vaccination against all circulating each of the serotypes ~~circulating in an area or an outbreak. Furthermore~~ Additionally, FMDV has a high potential for genetic and antigenic variation, ~~this~~ hence which creates the need to constantly match vaccines to the circulating field viruses (Domingo et al., 2003).

The FMDV genome is a positive-sense single-stranded RNA molecule ~~with a length~~ of approximately 8400 nucleotides (nt) long that belongs to the genus *Aphthovirus* within the *Picornaviridae* family (Belsham, 2005). The genome encodes a polyprotein which is processed to the virus structural proteins (SP) designated VP1 (1D), VP2 (1B), VP3 (1C), and VP4 (1A) and multiple non-structural (NS) proteins (Rueckert, 1984). ~~Within the viral RNA, T~~ the VP1 coding region ~~has been found to be~~ is the most variable, hence many studies of evolutionary relationships between FMDV strains and serotypes are based on VP1 sequences have sequenced this part of the genome in order to determine and understand evolutionary relationships between FMDV strains and serotypes (Knowles and Samuel, 2003), and have. ~~Based on these~~

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3 ~~VP1 sequences, the serotypes have been~~ grouped the serotypes into ~~various~~ epidemiologically and  
4 genetically distinct regional clusters ~~in different regions of the world~~ defined as virus lineages and  
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VP1 sequences, the serotypes have been grouped the serotypes into ~~various~~ epidemiologically and genetically distinct regional clusters ~~in different regions of the world~~ defined as virus lineages and topotypes in accordance with intra-lineage and intra-topotype nucleotide identity  $\geq 92.5\%$  and  $\geq 85\%$ , respectively (Knowles and Samuel, 2003; Samuel and Knowles, 2001a).

Among the seven serotypes, FMDV O has been found to be dominant globally and has been responsible for some economically devastating outbreaks in countries which are normally free from FMD~~disease-free~~ such as the United Kingdom (Knowles et al., 2001; Samuel and Knowles, 2001b) and Japan (Hayama et al., 2012; Sakamoto and Yoshida, 2002). Similarly, in the region of East Africa (EA) where the disease is endemic, serotype O has been responsible for most outbreaks. Several recent studies have characterized these FMDV O isolates in EA and recommended inclusion of a broader range of virus isolates in future analyses for a better approach to FMD control within the region (Ayelet et al., 2009; Balinda et al., 2010a; Habiela et al., 2010; Kasambula et al., 2012). These studies found four topotypes of this serotype namely, East Africa 1 (EA-1) to EA-4 circulating in this region with no evidence of additional introductions except for transboundary exchange of viruses between neighbouring countries within the region. In Kenya, most FMDV O strains isolated within the country since 2000 belong to topotypes EA-2, EA-3 and EA-4, while the locally produced vaccine that is currently in use is based on strain O K77/78 which was isolated in 1978 and is classified within topotype EA-1 (Balinda et al., 2010a). The use of this vaccine may contribute to inadequate disease control, yet the FMD control policy in Kenya is to use locally produced vaccines and animal movement control. So far, the Directorate of Veterinary Services (DVS) has not received complaints or had records of FMDV O post-vaccination outbreaks although it lacks resources to undertake consistent post-vaccination monitoring (PVM). However, it relies on vaccine potency testing results (involving vaccination and challenge), generated by the state vaccine quality control laboratory that is independent from the producer.

Apart from serotype O, other serotypes including A, SAT 1 and SAT 2 have also been recently in circulation (Namatovu et al., 2013), while previously, serotype C has also been identified within the region (Vosloo et al., 2002). However, serotype C was last isolated from Kenyan cattle in 2004 (Roeder, 2009; Sangula et al., 2011).

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3 During 2010 and 2011, there was an increase in the recorded number of FMD outbreaks in Kenya. From  
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5 the use of virus isolation and antigen detection procedures ~~using enzyme linked immunosorbent assays~~  
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7 ~~(ELISAs)~~, the Kenyan National Laboratory found that 105 of the 265 districts in the country (40%) had  
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9 cases of FMD between January and December 2010 and this pattern continued into 2011. Among these, 74  
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11 districts (70%) had been affected by serotype O FMDV, the highest annual occurrence of this serotype  
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13 since ~~the~~ year 2000. It was not known whether these were independent outbreaks or if each case was part of  
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15 a single outbreak that quickly spread to the various districts of the country. In a comparable occurrence,  
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17 multiple FMD outbreaks ~~were reported to have taken place~~ within a short period of time during 2008-2009  
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19 in neighboring Uganda were shown to belong to a single outbreak that rapidly spread and affected large  
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21 areas (Kasambula et al., 2012).  
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23 Recently, Balinda et al., (2010a) sequenced the VP1 coding region of 46 viruses of serotype O collected in  
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25 Kenya between 1964 and 2008 and recommended a more widespread collection of viruses for appropriate  
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27 vaccine development and better regional FMD control strategies. Now, in this study, the VP1 coding region  
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29 sequences of recently (2010-2011) circulating serotype O FMDVs from various geographical areas of  
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31 Kenya have been determined and analysed. The study was aimed at determining the characteristics of these  
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33 VP1 sequences in order to understand the recent outbreak patterns.  
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## 36 **Materials and Methods**

### 37 *Source of viruses used in this study*

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39 Epithelial samples were selected from the national FMD laboratory (FMDL), Embakasi, Kenya virus  
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41 repository. These were field samples collected, from suspected clinical cases of FMD in cattle by  
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43 government District Veterinary Officers (DVOs), between February 2010 and September 2011 for the  
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45 purpose of serotype identification and mapping to guide FMD control strategies. The samples were  
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47 preserved in 50% glycerol-phosphate buffered saline (PBS) and transported on ice, in the shortest time  
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49 possible, to the FMDL, where they were subsequently aliquoted and preserved at -20°C. Virus isolation in  
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51 Baby Hamster Kidney (BHK) cells and FMDV antigen detection using enzyme linked immunosorbent  
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53 assays (ELISAs) were routinely performed according to procedures in the OIE manual (OIE, 2012). For the  
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55 purpose of this study, the samples were selected taking regional representation and quality of the material  
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57 into consideration. Thus samples were selected from different districts representing the eight administrative  
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3 regions (provinces) of the country, namely: Western, Nyanza, Rift-Valley, Central, Nairobi, Eastern, North-  
4 Eastern and Coast (Fig. 1). Viruses from a total of 35 samples collected between 1998 and 2011 have been  
5 isolated and sequenced in this study at the National Veterinary Institute, Technical University of Denmark,  
6 Lindholm (DTU Vet) and The Pirbright Institute, U.K. (Table 1). The sequences were analysed together  
7 with others from a previous study (Balinda et al., 2010a) and from the public sequence databases (Table 1).  
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### 13 ***Virus Isolation***

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16 At Lindholm and in Pirbright, for the purpose of sequencing, viruses were re-isolated on primary bovine  
17 thyroid (BTY) cells according to the OIE manual (OIE, 2012). Briefly, epithelia preserved in 50% glycerol-  
18 PBS at -20°C, were thawed at room temperature, weighed and ground using sterile sand, with a mortar and  
19 a pestle, in Eagles minimum essential media supplemented with protein hydrolysate, 2% fetal calf serum  
20 and antibiotics to make a 10% suspension. The suspensions were inoculated onto primary bovine thyroid  
21 (BTY) cells for 1 hour, followed by a change of media and then continued incubation. The cultures were  
22 observed for cytopathic effect (CPE) after 24 [and](#) 48 hours and harvested when CPE developed. Negative  
23 samples were further passaged onto fresh cells for another 48 hours.  
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### 33 ***Total RNA Extraction and cDNA Synthesis***

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35 At The Pirbright Institute, total RNA extraction and cDNA synthesis were done as described by Ayelet et  
36 al. (2009) and Habiela et al. (2010), while at DTU Vet these were done as follows:

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38 Viral RNA was extracted from CPE positive cell culture harvests using the QIAmp<sup>®</sup> viral RNA mini kit  
39 (Qiagen) following the viral RNA mini spin protocol as described by the manufacturer, [using](#) 60 µl of AVE  
40 buffer [for elution](#) (as supplied) and the RNA was stored at -80°C.  
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45 To synthesize cDNA, the RT master mix was made using PE Biosystems TaqMan RT reagents (part N808-  
46 1234) in accordance with the manufacturer's instructions. [Briefly](#), for each reaction, 1.5 µl of 10x TaqMan  
47 RT buffer (500 mM KCl, 100 mM TrisHCl, pH 8.3), 3.3 µl of 25 mM MgCl<sub>2</sub>, 3.0 µl of dNTP (2.5 mM  
48 each), 0.5 µl of 50 µM random hexamers, 0.3 µl of RNase inhibitor (20U/µl) and 0.375 µl of MultiScribe  
49 reverse transcriptase (100 U/µl, Moloney RT) were used making a total of 9 µl per reaction. RNA samples  
50 (6 µl) were added to the master mix to make 15 µl per reaction and incubated at 48°C for 45 min, and 95°C  
51 for 5 min. The cDNA products were stored at -20°C.  
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### ***Real time RT-PCR to detect FMDV RNA***

Quantitative RT-PCR was performed using TaqMan<sup>®</sup> Universal 2X PCR Master Mix (PE Biosystems). The TaqMan PCR was run as described previously (Reid et al., 2003) using the FMDV MultiII IRES primers (FMDV Multi II forward primer and FMDV Multi II reverse primer) and FMDV Multi II-288 probe (FAM-labelled). The reaction mixtures (25 µl) contained 2.25 µl of 10 pmol/ µl forward primer, 2.25 µl of 10 pmol/ µl reverse primer, 1 µl of 5 pmol/ µl probe and 12.5 µl of the universal master mix. This 18 µl ~~of this~~ master mix was mixed with 7 µl cDNA template and ~~t~~. The tubes were sealed with optical caps and, ~~then~~ incubated in a Mx3005P cycler (Stratagene) for 50 cycles; 50°C 2min (UNG digest), 95°C 10min (heat activation of TaqGold) and 50 x ( 95°C 15s and 60°C 60s), then held at 4°C.

### ***RT-PCR to amplify and sequence FMDV VP1 coding sequence***

At The Pirbright Institute, these methods were done as described by Ayelet et al. (2009) and Habiela et al. (2010), while at (DTU Vet) as follows:

Samples which were shown to contain significant amounts of FMDV RNA (Ct <32) (Shaw et al., 2007) were selected for VP1 coding region amplification. The cDNA sequence was amplified using reverse primer 11-FPN 28 (5' GGGCCCAGGGTTGGACTC 3') and forward primer 11-FPN 29 (5' GCIGCIGACTACGCITACACYGC 3') (note, I = inosine and Y = C and T). These primer sequences were flanked the region between found between positions nt 3936 and 3169 in of the virus genome respectively and includesing the entire VP1 coding region. The primers were and designed with reference to strain O UKG 35/2001, GenBank accession number AJ53914 (Mason et al., 2003).

The reaction mixture for each amplification (50 µl) comprised RNAase free water (25µl), 5.0 µl 10X PCR buffer, 4.0 µl of 25 mM MgCl<sub>2</sub>, 0.4 µl of 10 mM dNTPs, 5.0 µl of each primer (10 pmol/µl), ~~and~~ 5.0 µl cDNA template and plus 20 U of AmpliTaq Gold<sup>®</sup> DNA polymerase (Applied Biosystems). The PCR was run in a thermocycler (Gene Amp<sup>®</sup> PCR system 3700 version 3.0 - Applied Biosystems) using the touchdown method for 1 cycle; 95°C 5min, 1 cycle; 95°C 15s, 5 cycles; 55°C 30s, 1 cycle; 72°C 1 min 20s, 40 cycles; (95°C 15s, 50°C 30s, 72°C 1 min.20s), 1 cycle; 72°C 7 min and 4°C holding.

The PCR products (ca. 885 bp) were viewed by electrophoresis on 1.5 % agarose gels (Seakem GTG agarose in 1 X TAE - low EDTA buffer) at 120 volts for 1 hour in parallel with a molecular weight marker

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ΦX 174-RF DNA (Amersham, Biosciences). Purification of the PCR products was achieved using the QIAquick PCR purification kit (Qiagen). Products for cycle sequencing were quantified using a NanoDrop® 1000 Spectrophotometer (Thermo Scientific) and for each reaction, 10 - 13 ng of amplicon was used. Cycle sequencing, in both directions, was performed using the Big dye Terminator V 3.1 kit (Applied Biosystems) and run on an automated DNA Sequencer (ABI PRISM 3700; Applied Biosystems).

### Sequence Analysis

The nucleotide sequences in this study were edited using SeqMan Pro software (Lasergene package, DNASTar, Inc.). Serotypes of the generated sequences were identified and compared with previous data using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov). The sequences were aligned by MUSCLE incorporated in MEGA software version 5.05 (Tamura et al., 2011) and trimmed to 639 nucleotides comprising the complete VP1 coding region.

Substitution models were determined in the MEGA software. Codon positions included were 1st+2nd+3rd. Maximum Likelihood fits of 24 different nucleotide substitution models were estimated and Akaike Information Criterion (AIC) estimated. Non-uniformity evolution rates were modeled using discrete gamma distribution (G). Thus, Tamura Nei substitution model with gamma distribution and invariable rates (I) (TN93+G+I) was chosen as the best that describes the substitution pattern (Tamura et al., 2011). The evolutionary history was inferred using the Neighbor-joining method (Saitou and Nei, 1987) and a bootstrap consensus tree estimated from 1000 replicates (Felsenstein, 1985) taken to represent the evolutionary history of the taxa analyzed.

The sequences in this study were compared to selected serotype O Kenyan isolates from 1978 to 2009 and to isolates obtained from neighbouring countries during 1986-2009, using two 1983 isolates from Hong Kong and Indonesia as outgroups. The taxa were grouped into topotypes defined by an intra-topotype nucleotide identity within the VP1 coding sequence of >85% (Knowles and Samuel, 2003; Samuel and Knowles, 2001a).

### Results

Thirty five FMD viruses were all having been successfully isolated in cell tissue culture and tested positive for serotype O FMDV using antigen ELISAs. Virus harvests were also shown to contain significant

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3 amounts of FMDV RNA by real time RT-PCR assays (Ct <32), hence were selected for The VP1 coding  
4 region from each of these samples was amplified by RT-PCR and subsequently sequenced. BLAST  
5 searches using of the VP1 coding region of these sequences confirmed that these were of serotype O FMDV  
6 in each case. Generally, a Apart from one, all the other 2010-2011 viruses sequenced in this study were  
7 distributed among three distinct lineages (with each lineage having  $\geq 7.5\%$  differences in nucleotide  
8 sequence within the VP1 coding region) and were classified within the EA-2 topotype (labeled as lineages  
9 A, B and C in Fig. 2). In a Neighbor-joining tree, ~~These lineages grouped, in a Neighbor-joining tree,~~  
10 together with viruses from Uganda that had been collected in 2004-2006 (Fig. 2). The one other virus  
11 (K72/2010) belonged to the EA-4 topotype (labeled as lineage D in Fig. 2), originated from district 14 (Fig.  
12 1) and grouped together with some earlier Ethiopian and Ugandan viruses. This virus made up an  
13 independently evolving sub-lineage within this topotype with nucleotide differences of about 11% and 9%  
14 compared to the Ethiopian and Ugandan viruses, respectively.

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Lineage A, within the EA-2 topotype (Fig. 2), incorporated nine viruses from this study, including K31/2011 and K249/2010. These viruses had about 97% intra-topotype nucleotide identity within the VP1 coding sequence. They were collected between December 2010 and April 2011 (Table 1) from six districts (Siaya, Nyandarua, Thika, Baringo, Rongai and Koibatek) located in three different provinces (Central, Rift Valley and Nyanza) (Fig. 1). This group differed in their nucleotide sequence from lineages B and C by about 12 % and 10 - 11% respectively.

Lineage C, in the EA-2 topotype, included nine isolates from both 2010 and 2011, including K224/2011 from Kinango in Coast province and K98/2011 that grouped together with several others obtained from several districts around the southern and central parts of the Rift Valley province of the country (Fig. 1). They clustered together with others collected from Coast province in 2010, Central in 2005 and Nyanza in 2009. This lineage also contained three independently evolving sub-lineages (Fig. 2) with about 6% nucleotide differences among them.

The third lineage (B) within the EA-2 topotype included only one isolate ~~from~~ this study (K251/2010) (Fig. 2). This virus was collected from district 3 in the Western province of the country, near the border with Uganda (Fig. 1). It had 93% nucleotide identity to viruses collected in 2002 from the same province in Kenya and 91-92% identity with those from Eastern Uganda between 2002 and 2009 and about 99%

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3 nucleotide identity with the 2011 viruses from the same region in Uganda (data not shown). However, this  
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5 lineage had about 12% nucleotide difference from viruses in lineage A and about 12% from those in  
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7 lineage C (within the VP1 coding region).  
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## 10 Discussion

11 This study has analyzed FMDVs isolated from [serotype O FMD outbreaks in](#) all the eight provinces of  
12 Kenya from February 2010 until September 2011 ~~that were responsible for the serotype O FMD outbreaks~~  
13 ~~during this period.~~ Three independently evolving lineages belonging to toptype EA-2 were the most  
14 commonly occurring and found to be responsible for the majority of these recent outbreaks in Kenya. In  
15 addition, this study showed that there was another virus strain belonging to toptype EA-4 circulating in  
16 2010 and that none of the recent viruses belonged to toptypes EA-1 and EA-3. Unlike two studies recently  
17 undertaken in neighboring Uganda (Kasambula et al., 2012; A. Namatovu, G. J. Belsham, C.  
18 Ayebazibwe, M.T. Dhikusooka, S. N. Wekesa, H. R. Siegismund, V. B. Muwanika, K. Tjørnehøj,  
19 unpublished results) which found only closely related serotype O FMDVs causing outbreaks, this study  
20 found different lineages even between neighbouring districts, indicative of different recent common  
21 introductions of the viruses. In addition, the different lineages were spread in several provinces of the  
22 country, suggestive of widespread transmission of the viruses within the country.  
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25 Viruses in lineage A consisted of several isolates that occurred late in 2010 and early 2011 that were  
26 distributed across 3 different regions of the country (Nyanza, Rift valley and Central). This group seems to  
27 have evolved from one recent common ancestor and within five months (December 2010 to April 2011), it  
28 had rapidly spread across a wide area within these three ~~different~~ regions of Kenya. In particular, virus  
29 K24/2011 from Thika and K31/2011 from Kisumu were collected [almost concurrently](#) about 380 km ~~road~~  
30 ~~distance~~ apart. ~~yet they occurred almost concurrently. Furthermore, the period of~~ this occurrence  
31 coincided with two major national holidays and festive seasons (Christmas and Easter), during which  
32 animal movements for sale and slaughter are usually increased, which may explain the rapid spread.  
33  
34 Considering that FMD control measures in Kenya mainly rely on animal movement control and  
35 vaccination, and that the virus had spread widely within a short period, it is likely that [the control systems](#)  
36 [in the country were breached during this period. there were loopholes in the control systems in the country](#)  
37 [at that time.](#) Similarly, lineage C viruses appear to have circulated across three different regions of Kenya  
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3 that are also widely separated. Viruses within this lineage were obtained from different districts within the  
4 southern and central parts of the Rift Valley and the coastal region. In addition, it is noteworthy that this  
5 lineage contained three independently evolving sub-lineages, two of them in the Rift Valley and one in the  
6 Coast region. These observations are suggestive of inadequate countrywide disease control measures that  
7 ought to be investigated and addressed by the relevant authorities and stakeholders.  
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12 The Western regions of Kenya and Eastern parts of Uganda have traditionally been associated with  
13 movement of animals across the borders for ~~various reasons including~~ trade, grazing and cattle raiding.  
14 This probably is the reason why lineage B, including K251/2010 from this study collected in December  
15 2010 from Kwanza district in western Kenya was ~~confined to~~ observed in these two countries. This virus is  
16 closely related to an earlier virus from the same region in Kenya near the border with Uganda and other  
17 earlier viruses from Eastern Uganda. Furthermore, two other viruses recently isolated from Kumi and  
18 Bukedea districts in Eastern Uganda in March 2011 have been found to be closely related to this virus (A.  
19 Namatovu, G. J. Belsham, C. Ayebazibwe, M.T. Dhikusooka, S. N. Wekesa, H. R. Siegismund, V. B.  
20 Muwanika and K. Tjørnehøj, unpublished results). This is not surprising because, even though Kenya and  
21 Uganda are both East African countries, they have varying levels of disease control and biosecurity  
22 measures. Nevertheless, both countries use animal movement control as one of the methods of disease  
23 control. These findings ~~have therefore~~ confirmed the transboundary mobility of serotype O as observed in a  
24 previous studies (Balinda et al., 2010a), hence necessitating enhancement of border surveillance and  
25 harmonization of livestock trade and animal movement regulations between the two countries if the much  
26 desired regional control of FMD is to be achieved.  
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43 Viruses belonging to topotype EA-4 have not been reported in Kenya previously but this topotype has been  
44 found in Ethiopia in 2005 (Ayelet et al., 2009) and earlier in Uganda in 1998-1999 (Ayelet et al., 2009;  
45 Balinda et al., 2010b). The identification of this topotype in Kenya in this study, unlike in previous studies,  
46 is probably due to the inclusion of a wider geographical representation of isolates in the present analysis  
47 that has additionally included the North-Eastern, Nyanza and Coastal provinces of Kenya. This finding is  
48 suggestive of a probable recent incursion of EA-4 into Kenya as an emerging topotype that needs to be  
49 thoroughly investigated. Consequently, this reveals that border disease control measures should be  
50 scrutinized and possible problems addressed. On the other hand, this virus may have been circulating  
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3 earlier within the country undetected, therefore raising the need for regular surveillances and virus  
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5 characterization to ascertain the strains of viruses in circulation.  
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7  
8 By 2008, the EA-2 toptype had been found to be mainly confined to Uganda and Kenya for about ten  
9  
10 years (Balinda et al., 2010b). This study has found that this toptype was the most commonly occurring in  
11  
12 Kenya. This observation is largely consistent with previous studies in the East African region including  
13  
14 Ethiopia, Kenya and Uganda (Ayelet et al., 2009; Balinda et al., 2010a) which indicated that recent FMDV  
15  
16 O viruses in Kenya and Uganda are all grouped in toptype EA-2 and that there are hardly any incursions  
17  
18 from outside the region. Apart from Kenya and Uganda, the EA-2 toptype has also been found in Burundi  
19  
20 (1980, 1999 and 2003), the Democratic Republic of the Congo (2006 and 2010), Rwanda (1998 and 2004),  
21  
22 Tanzania (1980, 1984, 1985, 1996, 1998, 2004, 2009 and 2012), northern Malawi (1998) and northern  
23  
24 Zambia (2000 and 2010) (N.J. Knowles, unpublished data; Balinda et al., 2010a; Di Nardo et al., 2011)  
25  
26 suggesting that EA-2 has been widespread in this region at least since the early 1980's. This is not  
27  
28 unexpected as earlier studies have found these countries do rely strongly on one another for livestock trade  
29  
30 (Rweyemamu et al., 2008) and pastures especially for the nomadic communities around the national  
31  
32 borders. However, studies on the distribution of toptype EA-2 in the region as well as the other toptypes  
33  
34 ought to be extended to other countries such as Burundi, Rwanda, Democratic Republic of Congo (DRC),  
35  
36 Somalia and South Sudan where the FMD epidemiological situation is unclear

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38 Topotype EA-3 was last documented in Kenya in 1999 (KEN/1/1999) and in the same year in Uganda  
39  
40 (Balinda et al., 2010a), while in the recent past it has been mainly confined to Eritrea, Ethiopia and Sudan  
41  
42 (Ayelet et al., 2009; Habiela et al., 2010). However, Di Nardo et al., (2011) have recently determined  
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44 livestock movement patterns in the region indicating movements from Ethiopia, Sudan and Somalia into  
45  
46 Kenya that may pose a risk of disease transmission in that direction. As a result the current confinement of  
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48 EA-3 to these countries may not remain constant since this toptype could easily move back into Kenya if  
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50 control measures at the borders are inefficient. Moreover, there is need for more surveillance in the  
51  
52 Northern and North-Eastern parts of Kenya neighbouring these countries to ascertain the absence of this  
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54 toptype within the country.

55  
56 The majority of East African countries, including Kenya, rely on vaccination and animal movement control  
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58 for FMD control measures. However, the current vaccine strain K77/1978 [belongs to toptype EA-1 and](#)  
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3 has been used since 1981, not only in Kenya but also by several countries in the region such as Uganda,  
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5 Ethiopia, Sudan, Burundi and Somalia. This study and other recent studies in Kenya (Balinda et al.,  
6  
7 2010a), in Uganda (Kasambula et al., 2012; A. Namatovu, G. J. Belsham, C. Ayebazibwe, M.T.  
8  
9 Dhikusooka, S. N. Wekesa, H. R. Siegismund, V. B. Muwanika and K. Tjørnehøj, unpublished results), in  
10  
11 Ethiopia (Ayelet et al., 2009) and in Sudan (Habiela et al., 2010) have found that the [VP1 coding region of](#)  
12  
13 [this vaccine which belongs to topotype \(EA-1\) strain](#) has more than 15% nucleotide divergence, ~~within the~~  
14  
15 [VP1 coding region](#), from the majority of the currently circulating strains. The antigenic relationship  
16  
17 between this vaccine strain and FMDV field strains has not been established, yet, as described by Paton et  
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19 al., (2005), appropriate vaccine strain selection is essential for FMD control programs that depend on  
20  
21 vaccination. Therefore, although vaccination failures have not been recently recorded, there is a need to  
22  
23 undertake consistent PVM and vaccine matching.

24  
25 In conclusion, the existing systems to control the spread of FMDV within Kenya and the entire region of  
26  
27 [Eastern Africa](#) seem inadequate and require re-evaluation. Therefore, there is need for increased  
28  
29 surveillance, better disease control measures at source and efficient animal movement control both within  
30  
31 the country and across the borders. Additionally, it is necessary to undertake studies to assess the  
32  
33 effectiveness of the current serotype O vaccine strains using virus neutralization tests or ELISAs as  
34  
35 recommended by OIE (OIE, 2012).

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2  
3 **Declaration of Conflict of Interest**  
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5 All authors declare that they have no conflict of interest.  
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### List of figures and tables

**Fig. 1.** Map of Kenya showing geographical districts and administrative regions (underlined) from which 2010-2011 samples analyzed in this study were collected. The encircled numbers correspond to the listing in Table 1.

**Fig. 2.** A Neighbor-joining tree based on nucleotide sequences of the VP1 coding region of serotype O viruses from Kenya in comparison with other East African topotypes. (●) indicates the 2010-2011 viruses in this study and (▲) the current vaccine strain. The values on the nodes show posterior probabilities. The encircled letters A-D show the lineages in which the recent Kenyan isolates group. EA -1 to 4 are the East Africa topotypes that have been found in Kenya.

**Table 1.** List of serotype O FMD viruses analyzed in this study, including viruses collected in Kenya during 2010-2011 and sequenced in this study, the current vaccine strain and other older viruses from Kenya and rest of the Eastern Africa region.

<sup>a</sup> K and KEN - prefixes used in FMDL Embakasi and at the Pirbright Institute, respectively for Kenyan isolates. <sup>b</sup> Current vaccine strain.

**Table 1.** List of serotype O FMDV viruses analyzed in this study, including viruses collected in Kenya during 2010-2011 and sequenced in this study, the current vaccine strain and other older viruses from Kenya and rest of the Eastern Africa region.

No.	Virus Ref. number <sup>a</sup>	Date collected	Country/Region/ District	Accession No.	Topotype	Reference
1	K182/2010	26/08/2010	Kenya/Rift Valley/Laikipia	KF207885	EA-2	This Study
2	K241/2010	17/12/2010	Kenya/Central/Thika-West	KF207886	EA-2	This Study
3	K251/2010	29/12/2010	Kenya/Western/Kwanza	KF207887	EA-2	This Study
4	K24/2011	04/02/2011	Kenya/Central/Thika	KF207881	EA-2	This Study
5	K31/2011	12/02/2011	Kenya/Nyanza/Siaya	KF207882	EA-2	This Study
6	K38/2011	02/03/2011	Kenya/Rift Valley/Rongai	KF207883	EA-2	This Study
7	K47/2011	08/04/2011	Kenya/Rift Valley/Baringo	KF207884	EA-2	This Study
8	KEN/1/2011 (K10/11)	12/01/2011	Kenya/Central/Thika-West	KF135289	EA-2	This Study
9	KEN/5/2011 (K32/11)	15/02/2011	Kenya/Central/Nyandarua	KF135290	EA-2	This Study
10	KEN/6/2011 (K33/11)	19/02/2011	Kenya/Rift Valley/Rongai	KF135291	EA-2	This Study
11	KEN/15/2011 (K91/11)	18/09/2011	Kenya/Rift Valley/Gilgil	KF135292	EA-2	This Study
12	KEN/16/2011 (K98/11)	22/09/2011	Kenya/Rift Valley/ Masaba	KF135293	EA-2	This Study
13	UGA/APAC/2009 KEN/100/2010	2009	Uganda/North/Apac	JN974309	EA-2	Kasambula et al., 2012
14	(K72/10) KEN/137/2010	28/04/2010	Kenya/Rift Valley/ Eldoret	KF135281	EA-4	This Study
15	(K152/10) KEN/145/2010	06/07/2010	Kenya/Rift Valley/Transmara	KF135282	EA-2	This Study
16	(K211/10) KEN/146/2010	19/10/2010	Kenya/Rift Valley/Kericho	KF13528	EA-2	This Study
17	(K220/10) KEN/148/2010	16/11/2010	Kenya/Rift Valley/Nakuru	KF135284	EA-2	This Study
18	(K222/10) KEN/150/2010	17/11/2010	Kenya/Rift Valley/Rongai	KF135285	EA-2	This Study
19	(K224/10) KEN/151/2010	22/11/2010	Kenya/Coast/Kinango	KF135286	EA-2	This Study
20	(K233/10) KEN/152/2010	01/12/2010	Kenya/Rift Valley/Kajiado	KF135287	EA-2	This Study
21	(K249/10)	23/12/2010	Kenya/Rift Valley/Koibatek	KF135288	EA-2	This Study
22	KEN/6/2009 (K16/09)	01/02/2009	Kenya/Rift Valley/Kipkelion	KF135279	EA-2	This Study
23	KEN/10/2009 (K29/09)	01/02/2009	Kenya/Nyanza/Bondo	KF135280	EA-2	This Study
24	HKN/6/83	1983	Hong Kong	AJ294919	CATHAY	Knowles et al., 2001
25	ISA/8/83	1983	Indonesia	AJ303503	ISA-1	Samuel and Knowles, 2001
26	K1/2008	08/01/2008	Kenya/Nairobi/Nairobi-East	HM756635	EA-2	Balinda et al., 2010
27	KEN/3/2008 (K4/08)	23/01/2008	Kenya/Central/Thika	KF135276	EA-2	This Study
28	KEN/4/2008 (K6/08)	06/02/2008	Kenya/Rift valley/Nakuru	KF135277	EA-2	This Study
29	K11/2008	20/02/2008	Kenya/Central/Kiambu-West	HM756637	EA-2	Balinda et al., 2010
30	K14/2008	22/02/2008	Kenya/Rift Valley/Baringo	HM756638	EA-2	Balinda et al., 2011
31	K31/2008	05/05/2008	Kenya/Rift Valley/Kajiado	HM756639	EA-2	Balinda et al., 2012
32	K32/2008	05/05/2008	Kenya/Central/Thika	HM756640	EA-2	Balinda et al., 2013
33	KEN/36/2008 (K104/08)	01/12/2008	Kenya/Rift Valley/Molo	KF135278	EA-2	This Study
34	K82/2007	01/10/2007	Kenya/Central/Murang'a	HM756634	EA-2	Balinda et al., 2010
35	K30/2007	18/06/2007	Kenya/Rift Valley/Laikipia	HM756632	EA-2	Balinda et al., 2010
36	K2/2007	11/01/2007	Kenya/Central/Kiambu	HM756629	EA-2	Balinda et al., 2010
37	KEN/12/2007 (K57/07)	23/07/2007	Kenya/Eastern/Imenti-North	KF135275	EA-2	This Study
38	KEN/4/2005 (K7/05)	26/01/2005	Kenya/Central/Nyeri	KF135272	EA-2	This Study
39	KEN/14/2005 (K48/05)	21/07/2005	Kenya/Central/Kiambu	KF135273	EA-2	This Study
40	KEN/27/2005 (K46/05)	15/07/2005	Kenya/Central/Kiambu	KF135274	EA-2	This study
41	KEN/24/2004 (K139/04)	30/07/2004	Kenya/Central/Murang'a	KF135269	EA-2	This study
42	KEN/29/2004 (K170/04)	21/10/2004	Kenya/Eastern/Machakos	KF135270	EA-2	This study
43	KEN/30/2004 (K176/04)	24/12/2004	Kenya/North- Eastern/Marsabit	KF135271	EA-2	This Study
44	KEN/3/2002 (K56/02)	29/07/2002	Kenya/Western/Busia	KF135268	EA-2	This Study

45	KEN/7/98 (K30/98)	1998	Kenya	KF135266	EA-3	This Study
46	KEN/1/99 (K1/99)	1999	Kenya	KF135267	EA-3	This Study
47	K50/2006	21/07/2006	Kenya/Rift Valley/Uasin Gishu	HM756626	EA-2	Balinda et al., 2010
48	K31/2005	27/05/2005	Kenya/Rift Valley/Laikipia	HM756623	EA-2	Balinda et al., 2010
49	K79/2002	01/10/2002	Kenya/Rift Valley/Nakuru	HM756616	EA-2	Balinda et al., 2010
50	K114/1987	1987	Kenya	HM756594	EA-2	Balinda et al., 2010
51	K101/1980	1980	Kenya	HM756589	EA-2	Balinda et al., 2010
52	K83/1979	1979	Kenya/Central/Nyeri	AJ303511	EA-1	Samuel and Knowles, 2001
53	K77/1978 <sup>b</sup>	1978	Kenya/Rift Valley/Nakuru	HM756588	EA-1	Balinda et al., 2010
54	U97/1999	1999	Uganda	HM756604	EA-2	Balinda et al., 2010
55	UGA/5/96	1996	Uganda	AJ296327	EA-1	Samuel and Knowles, 2001
56	ETH/27/2007	01/12/2007	Ethiopia	FJ798139	EA-3	Ayelet et al., 2009
57	ETH/58/2005	20/04/2005	Ethiopia	FJ798141	EA-4	Ayelet et al., 2009
58	ETH/60/2005	20/04/2005	Ethiopia	FJ798143	EA-4	Ayelet et al., 2009
59	ETH/1/79	1979	Ethiopia	HM211077	EA-2	Balinda et al., 2010
60	K56/1995	1995	Kenya	HM756601	EA-2	Balinda et al., 2010
61	UGA/17/98	1998	Uganda	HM211075	EA-2	Balinda et al., 2010
62	SUD/26/2004	2004	Sudan	GU566054	EA-3	Habiela et al., 2010
63	SUD/1/99	1999	Sudan	DQ165076	EA-3	Habiela et al., 2010
64	SUD/2/86	1986	Sudan	DQ165075	EA-3	Habiela et al., 2010
65	U18/2006	2006	Uganda/Mpigi	HM756627	EA-2	Balinda et al., 2010
66	U12/2005	2005	Uganda/Wakiso	HM756625	EA-2	Balinda et al., 2010
67	U17B/2004	2004	Uganda/Hoima	HM756620	EA-2	Balinda et al., 2010

<sup>a</sup> K and KEN are prefixes used in FMDL Embakasi and at the Pirbright Institute, respectively for Kenyan isolates; <sup>b</sup> Current vaccine strain.

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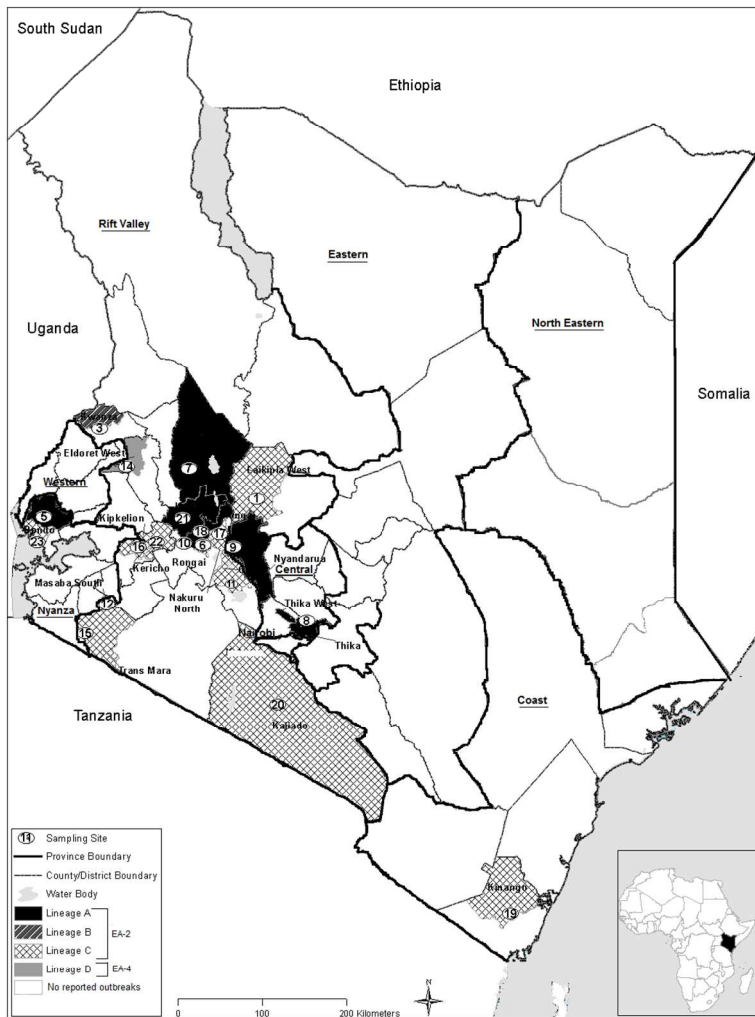


Fig. 1. Map of Kenya showing geographical districts and administrative regions (underlined) from which 2010-2011 samples analyzed in this study were collected. The encircled numbers correspond to the listing in Table 1.

323x437mm (96 x 96 DPI)



