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Research article

Isolation and molecular characterization of Foot and Mouth Disease virus serotype O circulated in Kenya during the period 2013-2018

Eunice C. Chepkwony[1], **George C. Gitao**[2*], **Gerald M. Muchemi**[3] **and Abraham K. Sangula**[1]

[1] Foot and Mouth Disease National Reference Laboratory, Embakasi, Directorate of Veterinary Services, State Department for Livestock, Nairobi, Kenya
[2] Department of Veterinary Pathology, Microbiology, and Parasitology, University of Nairobi, Nairobi, Kenya
[3] Department of Public Health, Pharmacology, and Toxicology, University of Nairobi, Nairobi, Kenya

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***Corresponding author:**
George C. Gitao
cggitao@gmail.com

Abstract

The endemicity of Foot-and-mouth disease (FMD) in Kenya has been recognized for over a century, with the first recorded cases dating back to 1915. Production of effective vaccines against incursions of infection in endemic areas is achieved by evaluating the genetic and antigenic characteristics of the circulating viruses. The present study aimed to isolate, serotype, and molecularly characterize FMDV from Kenya from 2013-2018. Isolation was done from 58 field samples on BHK-21 cells, and serotyping of the isolated viruses was carried out using antigen ELISA. Isolated viruses were also analyzed using reverse transcription PCR, and the PCR products were subjected to sequencing. Based on the quality of obtained sequence spectra, only 51 isolates were aligned using MEGA v11.0.8, employing the ClustalW algorithm. SeaView version 5.0.4 was used to edit the alignment, and MEGA 11.0.8 was used to construct the phylogenetic tree and align it with the commercially used vaccinal strains (OK77/78 and OK82/98). With a few exceptions, isolates collected over the same period and those from the same regions consistently clustered in the same lineage or closer to each other. A total of 50/51 strains belong to the East African-2 (EA-2) topotype together with the vaccine strain OK82/98. However, only one strain (1/51) isolated from Tana River county belongs to the EA-1 topotype together with the current vaccine strain (OK77/78). None of these isolates was found to belong to the EA-and EA-4 topotypes. This study emphasizes the importance of regular surveillance and characterization of circulating virus strains for developing effective vaccines against FMD. It's proposed that future vaccine candidate strains selection could consider EA-2 topotype strains to control FMDV circulating in Kenya.

Keywords: Foot-and-mouth disease, FMDV-O serotype, Topotype, FMD outbreaks, Sequences, Vaccines

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Introduction

Foot and mouth disease (FMD) is a highly infective, debilitating viral disease with substantial economic implications in livestock production in many endemic developing countries (Brooksby, 1958; Arzt et al., 2011). It affects Artiodactyla wild and domestic species (Jamal and Belsham, 2013; Gilkerson and Hartley, 2017; McLachlan et al., 2019), leading to trade embargoes of livestock and their products with countries free from FMDV. The economic losses occur in terms of high morbidity in adults, particularly cattle and pigs, leading to reduced productivity and death in neonates (James and Rushton, 2002). The negative economic implications of trade restrictions at the national and farm level far outweigh the production losses from the illness due to sanitary control measures. In recent years, FMD has emerged in different regions of Africa and infected millions of livestock (Kardjadj, 2018; Mielke and Garabed, 2020; Zientara and Bakkali-Kassimi, 2021).

The disease causes pyrexia and blisters in the mouth, nose, teats, and feet, which turn into erosions. Clinically this manifests as excess salivation, lips smacking, teeth grinding (due to pain), nasal discharge, mastitis, lameness with lethargy, and anorexia. This leads to reduced milk production, infertility, weight loss, draught power, and death, which can be common in young animals due to heart muscle degen-

eration (Kitching, 2002). Secondary bacterial infection complicates the recovery process leading to protracted illness though many animals recover within two weeks. Lesions are not prominent in sheep and goats and are unapparent in wildlife species and buffaloes (Geering, 1967; Donaldson and Sellers, 2000). Transmission between animals can occur in various ways, including animal-to-animal contact and contaminated animal products. During the acute phase of the disease, the virus is excreted in all body excretions and secretions (Sumption et al., 2012). Peak transmission occurs when vesicles rupture, contaminating the environment, including pastures and water sources. The virus is also found in the lymph nodes and bone marrow of slaughtered infected animals with prolonged survival in frozen meat (Pizzi, 1998). Mechanical transmission can occur by animals, human fomites, and air. These diverse modes of transmission lead to easy virus movement across borders, often circumventing control measures in place.

The causative agent, Foot and mouth disease virus (FMDV), is a small non-enveloped RNA virus of the family *Picornaviridae* and genus *Aphthovirus* (Domingo et al., 1990; Thiry et al., 2001). Being non-enveloped, the virus is resistant and can survive for a long time in the environment, but it is susceptible to high and low pH. The capsid comprises four different structural proteins; VP1, VP2, VP3, externally, and VP4, which is internal. In addition, non-structural proteins (L, 2A, 2B, 3A, 3B, 3C, and 3D) contribute to viral amplification during infection (Belsham, 2005). The virus has high mutation frequencies leading to new lineages, which presents the importance of studying evolutionary changes that can occur in the viral strains (Rueckrt, 1996; Lauring and Andino, 2010).

The VP1 is a pivotal capsid protein important in the replication of viral particles. It's highly polymorphic, having receptor-mediated attachment and humoral immune responses with major neutralization antigenic sites. In many previous studies, the VP1 nucleotide sequence has been used in the epidemiology of field outbreak investigations. It was also used in selecting and developing appropriate vaccines, improving diagnostic techniques, tracing outbreaks, and serotyping and sub-typing the viral strains. Other sites on the capsid involved in antigenic characteristics are VP2 and VP3 (Ludi and Rodriguez, 2013). Based on the capsid VP1 protein described previously, FMDV occurs in seven immunologically different serotypes: O, A, C, SAT1-3, and Asia-1. Each serotype also has topotypes, which are based on geographical locations, and within the topotypes, there are several subtypes (Bachrach, 1968; Wubshet et al., 2019). The most common serotypes found in four continents (Europe, South America, Asia, and Africa) are the O and A serotypes, while the SATs and Asia 1 serotypes are found in Africa and Asia, respectively. Serotype C originated in Europe and was detected in South America, East Africa, and India; however, it has not been encountered globally since 2004.

In Kenya, four serotypes, namely, O, SAT1, A, and SAT 2 are currently circulating, while serotype C was present up to 2004 when detected in Koibatek Subcounty of Baringo (Sangula et al., 2011). Frequent outbreaks occur mainly in cattle in the country with significant economic losses. Serotype O, like in the global distribution, was the most common serotype in the country, followed by SAT1, A, and SAT 2. Though widespread FMDV outbreaks in the country have been blamed on the presence of a wide wildlife specie/livestock interface. FMDV viruses circulated in buffalos and cattle in Kenya were found to be independently circulating strains in buffalos and cattle (Wekesa et al., 2015a). Globally, there are 11 known topotypes within the serotype O viruses, of which 4 were reported in East Africa, namely EA1-to EA4. Usually, the difference in topotypes is based on genetic and geographical distribution. The difference between one topotype and the next is a critical VP1 region nucleotide divergence of 15% (Samuel and Knowles, 2001). The EA-1 topotype has been detected in Kenya and Uganda since 1964 (Lloyd-Jones et al., 2017). The EA-2 topotype is found in East Africa, and both EA-3 and 4 are circulating in Ethiopia and Kenya.

Control of FMD in the country is implemented through vaccination and restriction of animal movement. Vaccination is carried out to control active outbreaks and build immunity using the locally produced inactivated vaccine. However, control strategies are impeded by the presence of multiple serotypes and strains in the country, together with rampant animal movement in search of pastures/water and also for trade. Vaccines are available from the local producer Kenya Veterinary Vaccines Production Institute (KEVEVAPI).

Confirmation of FMDV and serotyping is performed by submission of samples to the FMD National Reference Laboratory, Embakasi. Some counties have made efforts to institute subsidized regular vaccination programs. In addition, large-scale commercial farms routinely carry out vaccinations in their farms with the help of government or private veterinary service providers (Lyons et al., 2015). Currently, the country is developing an FMD control strategy in line with the Global Framework for control of Transboundary Animal Diseases (GF-TADS), in which vaccination is a crucial pillar for disease control. Vaccines of good quality are critical in controlling the disease. In the past, vaccine failures have been reported, probably due to the emergence of new strains in the field. To effectively control the disease, vaccines must contain strains related to those circulating in the area (González et al., 1992). Additionally, molecular characterization of field strains has been used to trace the sources/spread of viruses and to evaluate the mutational changes that have led to virus evolution and the development of lineages.

Despite FMD being endemic in Kenya, with serotype O causing the most outbreaks and several efforts to control using the locally available vaccine, a few studies have been conducted to study the genetic relationship between vaccine strains and circu-

Table 1: History of samples used for isolation and molecular characterization of FMDV.

Sequencing code	FMD Lab. code	Month/Year collected	Source/ Sub-Location	Location	Division	Sub-county	County
O1	K9/18	Jan 2018	Kapiti	Mathatani	Mavoko	Kalama	MACHAKOS
O2	K12/18	Jan 2018	Kapiti	Mathatani	Mavoko	Kalama	MACHAKOS
O3	K14/18	Jan 2018	Salgaa	Salgaa	Rongai	Rongai	NAKURU
O4	K18/18	Jan 2018	Laikipia East	Sweetwaters	Thingithu	Laikipia East	LAIKIPIA
O5	K20/18	Jan 2018	Langas	Langas	Kapseret	Kapseret	UASIN-GISHU
O6	K21/18	Jan 2018	Ituka	Ituka	Kathonzweni	Kathonzweni	MAKUENI
O7	K22/18	Jan 2018	East Narasha	Olkinyei	Mara	Narok West	NAROK
O8	K42/17	Sep 2017	Bukengi	Bukhayo West	Matayos	Matayos	BUSIA
O9	K27/17	Jun 2017	Kasikeu	Kasikeu	Kilome	Mukaa	MAKUENI
O10	K28/17	Jun 2017	Township	Thika West	Thika	Thika	KIAMBU
O11	K29/17	Jul 2017	Township	Municipality	Municipality	Eldoret West	UASIN-GISHU
O12	K33/17	Aug 2017	Kapiyet	Kapiyet	Mosop	Mosop	NANDI
O13	K34/17	Aug 2017	Kebulonik	Sangalo	Mosop	Mosop	NANDI
O14	K37/17	Aug 2017	Kanyariri	Kanyariri	Kanyariri	Kabete	KIAMBU
O15	K42.17	Aug 2017	Westlands	Lower Kabete	Lower Kabete	Westlands	NAIROBI
O16	K53/17	Oct 2017	Endebes	Endebes	Kwanza	Kwanza	TRANS-NZOIA
O17	K59/17	Nov 2017	Township	Township	Thika	Thika	KIAMBU
O18	K63/17	Nov 2017	Makongi	Segero	Koisagat	Soy	UASIN-GISHU
O19	K79/17	Dec 2017	Nyathona	Kabatini	Kabatini	Nakuru North	NAKURU
O20	K81/17	Dec 2017	Aporodo	Ahero	Nyando	Nyando	KISUMU
O21	K67/16	Sep 2016	Juja	Juja	Juja	Thika	KIAMBU
022	K17/17	Mar 2017	Bruynsha	Bruynsha	Ruiru East	Ruiru	KIAMBU
O23	K39/17	Aug 2017	Gitaru	Gitaru	Kikuyu	Kikuyu	KIAMBU
O24	K41/17	Sep 2017	Merewet	Merewet	Mumetet	Moiben	UASIN-GISHU
O25	K56/17	Oct 2017	Kauti	Lower Kaewa	Kathiani	Kathiani	MACHAKOS
O26	K61/17	Nov 2017	Ruai	Ruai	Kasarani	Kasarani	NAIROBI
O27	K64/17	Nov 2017	Gichagi	Mountain View	Westlands	Westlands	NAIROBI
O28	K67/17	Nov 2017	Kipkenyo	Kipkenyo	Kapseret	Kapseret	UASIN-GISHU
O29	K11/18	Jan 2018	Kapiti	Mathatani	Mavoko	Kalama	MACHAKOS
O30	K25/18	Jan 2018	OlPajeta	Sweetwaters	Thingithu	Laikipia East	LAIKIPIA
O31	K26/18	Jan 2018	Withare	Withare	Ngubit	Laikipia East	LAIKIPIA
O32	K27/18	Jan 2018	Urudi	Urudi	North Nyakach	Nyakach	KISUMU
O33	K28/18	Jan 2018	Wasare	Wasare	North Nyakach	Nyakach	KISUMU
O34	K30/18	Jan 2018	Sukut	Kishaunet	Kapenguria	Kapenguria	WEST POKOT
O35	K33/18	Feb 2018	Kituluni	Kituluni	Kathonzweni	Kanthonzweni	MAKUENI
O36	K34/18	Feb 2018	South Kochongo	Kochongo	Ahero	Nyando	KISUMU
O37	K24/14	Feb 2014	Central	Kiwanjani	Kiwanjani	Kajiado Central	KAJIADO
O38	K22/14	Feb 2014	Yathui	Yathui	Kyamatula	Mwala	MACHAKOS
O39	K44/18	Feb 2018	Litein	Chesingoro	Litein	Bureti	KERICHO
O40	K25/14	Jan 2014	Muhoroni	Koru	Koru Central	Muhoroni	KISUMU
041	K39/14	Feb 2014	Rarieda	West Asembo	Mahaya	Rarieda	SIAYA
O42	K43/14	Feb 2014	Malili	Malili	Kautandini	Salama	MACHAKOS
O43	K46/14	Feb 2014	Kanduyi	East Sang'alo	Mwikhupo	Bungoma South	BUNGOMA
O44	K50/14	Feb 2014	Soy	Kabulgey	Kamukunji	Eldoret West	UASIN GISHU
O45	K55/14	Mar 2014	Assa	Assa	Tana Delta	Tana delta	TANA RIVER
O46	K61/14	Mar 2014	Ngata	Ngata	Ngata	Rongai	NAKURU
O47	K84/14	Mar 2014	Gilgil	Gilgil	Gilgil	Gilgil	NAKURU
O48	K86/14	Mar 2014	Wote	Wote	Wote	Makueni	MAKUENI
O49	K112/14	Jul 2014	Ruai	Shujaa	Ruai	Nairobi	NAIROBI
O50	K148/14	Oct 2014	Eldama Ravine	Eldama Ravine	Eldama Ravine	Koibatek	BARINGO
O51	K35/13	May 2013	Juja	Kiahuria	Komo	Thika West	KIAMBU
O52	K82/13	Oct 2013	Lanet	Lanet	Lanet	Nakuru East	NAKURU
O53	K61/15	Jul 2015	Soy	Kiplombe	Kabao	Eldoret West	UASIN GISHU
O54	K16/15	Jan 2015	Kikoe	Kikoe	Kikoe	Limuru	KIAMBU
O55	K44/15	Mar 2015	Kilibwoni	Kilibwoni	Kapnyoberai	Nandi	NANDI
O56	K35/16	June 2016	Kikobe	Gilgil	Gilgil	Gilgil	NAKURU
O57	K37/16	June 2016	Kikobe	Gilgil	Gilgil	Gilgil	NAKURU
O58	K45/18	Feb 2018	Mabasi	Kisiara	Bureti	Bureti	KERICHO
O59	OK77/78	May 1978	Kevevapi	Kevevapi	Kevevapi	Kevevapi	VACCINE STRAIN
O60	Ok77/78	May 1978	Kevevapi	Kevevapi	Kevevapi	Kevevapi	VACCINE STRAIN

lating strains. Therefore, the aim of this study is the isolation and molecular characterization of field strains circulating during the period 2013 and 2018, compared with documented sequences obtained from the GenBank and the current Kenyan serotype O vaccine strain (OK77/78).

Material and methods

Selection of field isolates for the study

Tissue samples and original suspected samples serotyped as FMDV "O" were obtained from the archived samples at FMD National Laboratory sample storage. Samples were collected mainly by the Sub-county Veterinary Officers (SCVOs) from cattle suffering from FMD outbreaks from 2013 to 2018. The main clinical signs were lethargy and salivation, typical vesicular lesions on the mouth, and ulcers in the interdigital space (Figure 1). A total of 58 field samples and two vaccinal strains were obtained from 19 regions with a history of multiple outbreaks during this period. In addition, the vaccinal strain OK77/78, obtained from our virus bank, was also included in this study. Further data about the history of used samples, including the date of sample collection and location, are shown in Table 1.

Virus isolation

The frozen epithelial tissues were thawed at room temperature and washed three times using sterile phosphate-buffered saline (PBS) at a pH of 7.2-7.6. The tissue suspension was clarified at 3,000rpm for 10 min, and the supernatant was collected and filtered by a Millipore filter of 0.45-μM. Isolation was done on Baby Hamster Kidney cells (BHK-21, FMD Laboratory, Kenya). All procedures were conducted under the Biosafety cabinet level 2. Briefly, 1 mL of the suspension was inoculated on confluent BHK-21 monolayer cells grown on 25 cm^2 tissue culture flasks and incubated at 37°C for 1 hr for adsorption of the virus, followed by the addition of 9 mL Eagle's Minimal Essential Medium supplemented with 2% fetal calf serum (GE Life Science, Carlsbad, USA) and incubated at 37°C and 5% CO2 in a humidified incubator. Cytopathic effects (CPE) were observed daily under the inverted microscope, and cells were harvested when 85–100% of CPE was observed. In case of no CPE within 72 hr PI, three passages were done to consider the sample negative. Then, the virus was clarified and kept at -70°C until required for ELISA and PCR

Serotyping of isolated FMDV

The isolated viruses were serotyped using an Izsler FMDV antigen detection ELISA kit (Istituto Zooprofilatico Sperimentale, Brescia, Italy), according to the manufacturer, to confirm that all viruses included in this study are serotype "O". Briefly, 50 μL/well from each diluted samples in diluent buffer (1:2) were added to the ELISA plate and incubated for 1 hr at room temperature (18-30°C). After three washes, the conjugate was added by 4× washing, followed by adding 50 μL/well Chromogen/substrate solution to all wells. The plates were covered and left at room temperature in the dark for 20 mins. The reaction was stopped by adding 50 μL/well of the stop solution, following the same order used to add the substrate solution. The well content was mixed well before reading using an ELISA reader at 450 nm wavelength. The interpretation was made by considering OD450 $\geq$0.1 positive for FMD while OD450 <0.1 negative for FMD.

Amplification of FMDV VP1

According to the manufacturer's instructions, RNA was extracted using Qiagen QIAamp viral RNA Minikit (Qiagen, Hilden, Germany). QIAGEN One-Step RT-PCR kit (Qiagen, Hilden, Germany) was used. PCR was performed using O-1C244F (5'-GCA GCA AAA CAC ATG TCA AAC ACC TT-3') EUR–2B52R (5'-GAC ATG TCC TCC TGC ATC TGG TTG AT-3'), as forward and reverse primers, respectively, according to Knowles et al. (2005). Briefly, 5 μL of the extracted RNA was added to a master mix containing 13 μL of nuclease-free water, 4 μL of each primer, 10 μL of 5× PCR Buffer (12.5 mM of MgCl2), 2.0 μL of deoxynucleotide Triphosphate, 2.0 μL Enzyme Mix and 10 μL of 5× Q- solution. The PCR cycling program was chosen according to the primer sets as shown in Table 2. The PCR was run in a thermocycler (Gene Amp® PCR system 3700 version 3.0 - Applied Biosystems). PCR products (885 bp) were viewed by electrophoresis on 1.5% agarose gels.

Sequencing and sequence analysis

Fragments of appropriate recovered from the gel were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions before sequencing PCR products. The DNA was quantified using a NanoDrop®1000 Spectrophotometer (Thermo Scientific). Purified DNA was sent to Macrogen Inc. (Amsterdam, Netherlands) for sequencing. The obtained sequences (n=51) and the additional sequences obtained from NCBI were aligned using MEGA v11.0.8, employing the ClustalW algorithm. The SeaView v5.0.4 software was used to edit the alignment. MEGA v11.0.8 was used to construct a phylogenetic tree.

The Neighbor-Joining method was used to infer evolutionary changes between strains, applying 1000 bootstrap replications. Computations of the distance between evolutionary changes were done using the Kimura 2-parameter method, showing the total base substitutions in each site. The tree shows evolutionary distances denoted by the length of the branches as the tree is drawn to scale, and pairwise deletion was used to rid ambiguous positions. All the sequences used to construct this phylogenetic tree were collected from different counties in Kenya and Uganda. Details of additional sequences used for constructing the phylogenetic tree are shown in Table 3.

Figure 1: Clinical picture of FMD outbreaks investigated in Kenya. A) Lethargy and salivation, B) Typical vesicular lesions on the mouth, C) Lesion in the inter-digital space.

Table 2: Standard thermocycling protocol used for RT-PCR amplification of FMDV serotype O VP1 region.

Cycling parameter	Temperature	Duration	Number of cycles
Reverse transcription	50°C	30 min	1
Inactivation	95°C	15 min	1
Denaturation	95°C	60 sec	35
Primer annealing	60°C	60 sec	
Extension	72°C	120 sec	
Final extension	72°C	5 min	1

Results

Virus isolation

In this study, 58 samples collected during 2013 -2018 were used for the isolation and molecular identification of FMD viruses. Isolation of the FMD virus on BHK-21 revealed that all samples showed a CPE by the 3rd passage. The main CPEs were cell monolayer sloughing, and these sloughed cells were roughly round, swelling within 72 hrs after inoculation, and cell death (Figure 2).

Molecular characterization

Amplification of VP1

All samples were positive using PCR and showed a band of 885 bp, viewed by electrophoresis on 1.5% agarose gels (Figure 3).

Sequence analysis

Initially, all the sequences were automatically trimmed, excluding 9 samples due to the high average error rate (1%), indicating low quality that may produce erroneous analysis results. The number of samples considered for further analysis was 51, compared with 54 NCBI sequences (Table 3). The forward and reverse reads were paired for each sample, and each sample's consensus sequence was edited manually by trimming low-quality reads from the 3‘ and 5‘ ends.

Figure 4 demonstrates the five different FMDV clades, and Figure 5 shows the four FMDV topotype groupings and subtypes. The phylogenetic tree analysis showed that all the FMDV isolates were grouped into five clades. Fourteen isolates getting grouped into clade 1, four isolates were grouped into clade 2, 27 isolates into clade 3, 22 isolates were into clade 4, and 36 isolates into clade 5. The sequences from the current study clustered in clades 3, 4, and 5 represent different lineages. In clade 3, samples collected in 2017 and 2018 clustered together, while those collected in 2016 and 2017 are more closely related to those reported earlier in 2005 and 2007. In clade 4, four samples collected in 2014, two in 2016, and one collected in 2013 clustered together. In contrast, viruses reported in the early 2000s were grouped separately in a sub-cluster together with those collected in the same period in Uganda.

In clade 4, it's important to note that one of the vaccinal strains from NCBI, K82/022/98, which was recruited as a vaccine strain in 2000, is in this clade though not commonly incorporated in recent vaccines. The vaccine strain OK77/78 lies in clade 1 and is more closely related to the samples collected in the 1990s, with only one sample from the current study isolated from Tana River county. Samples collected over the same period and those collected in the same county or neighboring counties consistently clustered in the same clade or closer to each other.

Identifying where the study isolates belong in terms of topotype, prototype, and strains

FMDV sequences were organized by serotype and, within each serotype, topotype. For the FMDV "O" serotype isolates in this study, analysis was conducted to determine its specific topotype and prototype in terms of lineage and sub-lineage. Serotype O in East Africa exists in four topotypes: East Africa -1(EA-1), EA-2, EA-3, and EA-4. Samples analyzed in the current study were mainly grouped in the EA-2 topotype.

Based on the phylogenetic analysis shown in Fig-

Table 3: Sequences used in phylogenetic analysis.

S.No.	Sequence name	Collection year	County	Country	County code	Serotype	Accession no.
52	K32/08	2008	Thika	Kenya	22	O	HM756640.1
53	K31/08	2008	Kajiado	Kenya	34	O	HM756639.1
54	K14/08	2008	Baringo	Kenya	30	O	HM756638.1
55	K11/08	2008	Kiambu	Kenya	22	O	HM756637.1
56	K4/08	2008	Thika	Kenya	22	O	HM756636.1
57	K1/08	2008	Nairobi	Kenya	47	O	HM756635.1
58	K82/07	2007	Murang'a	Kenya	21	O	HM756634.1
59	K31/07	2007	Kiambu	Kenya	22	O	HM756633.1
60	K30/07	2007	Laikipia	Kenya	31	O	HM756632.1
61	K28/07	2007	Laikipia	Kenya	31	O	HM756631.1
62	K6/07	2007	Koibatek	Kenya	30	O	HM756630.1
63	K2/07	2007	Kiambu	Kenya	22	O	HM756629.1
64	U25/06	2006	Mpigi	Uganda	-	O	HM756628.1
65	U18/06	2006	Mpigi	Uganda	-	O	HM756627.1
66	K50/06	2006	Uasin Gishu	Kenya	26	O	HM756626.1
67	U12/05	2005	Wakiso	Uganda	-	O	HM756625.1
58	K48/05	2005	Kiambu	Kenya	22	O	HM756624.1
59	K31/05	2005	Laikipia	Kenya	31	O	HM756623.1
70	K5/05	2005	Laikipia	Kenya	31	O	HM756622.1
71	U20B/04	2004	Hoima	Uganda	-	O	HM756621.1
72	U17B/04	2004	Hoima	Uganda	-	O	HM756620.1
73	U14B/04	2004	Hoima	Uganda	-	O	HM756619.1
74	U13B/04	2004	Hoima	Uganda	-	O	HM756618.1
75	K55/03	2003	Nakuru	Kenya	32	O	HM756617.1
76	K79/02	2002	Nakuru	Kenya	32	O	HM756616.1
77	K61/01	2001	Mombasa	Kenya	1	O	HM756615.1
78	K45/01	2001	Nakuru	Kenya	32	O	HM756614.1
79	K150/00	2000	Uasin Gishu	Kenya	26	O	HM756613.1
80	K147/00	2000	Trans Nzoia	Kenya	27	O	HM756612.1
81	K145/00	2000	Laikipia	Kenya	31	O	HM756611.1
82	K141/00	2000	West pokot	Kenya	24	O	HM756610.1
83	K131/00	2000	Nairobi	Kenya	47	O	HM756609.1
84	K130/00	2000	Trans Nzoia	Kenya	27	O	HM756608.1
85	K117/00	2000	Nyeri	Kenya	19	O	HM756607.1
86	K109/00	2000	Uasin Gishu	Kenya	26	O	HM756606.1
87	K63/00	2000	Trans Nzoia	Kenya	27	O	HM756605.1
88	U97/99	1999	N/A	Uganda	-	O	HM756604.1
89	K117/99	1999	Nakuru	Kenya	32	O	HM756603.1
90	K82/98	1998	Kiambu	Kenya	22	O	HM756602.1
91	K56/95	1995	Kiambu	Kenya	22	O	HM756601.1
92	K29/95	1995	Kiambu	Kenya	22	O	HM756600.1
93	K34/93	1993	Laikipia	Kenya	31	O	HM756599.1
94	K11/93	1993	Kiambu	Kenya	22	O	HM756598.1
95	K52/92	1992	Kiambu	Kenya	22	O	HM756597.1
96	K51/92	1992	Nakuru	Kenya	32	O	HM756596.1
97	K121/91	1991	Kiambu	Kenya	22	O	HM756595.1
98	K114/87	1987	Kiambu	Kenya	22	O	HM756594.1
99	K131/85	1985	Kiambu	Kenya	22	O	HM756593.1
100	K40/84	1984	Kiambu	Kenya	22	O	HM756592.1
101	K11/84	1984	Kiambu	Kenya	22	O	HM756591.1
102	K103/82	1982	Thika	Kenya	22	O	HM756590.1
103	K101/80	1980	Laikipia	Kenya	31	O	HM756589.1
104	K77/78	1978	Nakuru	Kenya	32	O	HM756588.1
105	K120/64	1964	Laikipia	Kenya	31	O	HM756587.1

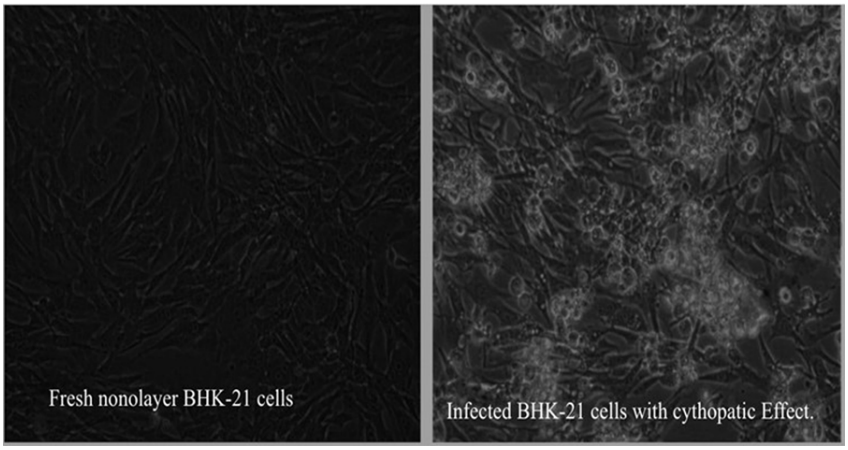

Figure 2: BHK-21 cells at 48 hrs post-infection with FMDV showing cytopathic effect including cell rounding.

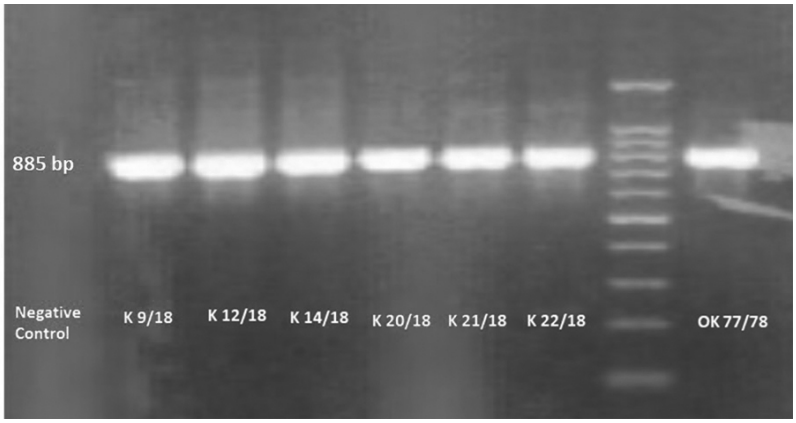

Figure 3: Electrophoresis of selected PCR products on 1.5% agarose gels.

ure 5, all the serotype O viruses isolated in this study, except the strain currently used for serotype O vaccines OK77/78 and field isolate K55/004/14, clustered within the EA-2 topotype. The OK77/78 and K55/004/14 vaccine strain and one isolate from Tana River collected in an outbreak in 2014 belong to the EA-1 topotype. The vaccine strain OK82/98 represented in the analysis by K82/022/98 belongs to the EA-2 topotype. The field strains of the EA-2 topotype circulated in Kiambu in 2013 and caused outbreaks in Nairobi and many parts of the Rift Valley area: Nakuru, Baringo, Kajiado, and Trans Nzoia and moving into the Western region in Bungoma in 2014. They continued to cause outbreaks in Kiambu in 2015 and 2016 and moved to the neighboring area (e.g., Nakuru). Several outbreaks of EA-2 topotype were recorded in subsequent years of 2017-2018 in Nairobi, Kiambu, Machakos, Makueni, and in the Rift Valley in Trans Nzoia, Nandi, Nakuru and affected Kisumu, and Busia.

Discussion

Serotype "O" of the FMDV continues to cause outbreaks in many counties all over the country. This study shows that outbreaks in Kenya and neighboring countries occurring in the same period are closely related. Some strains cross national boundaries, like Uganda, clustered with circulating strains in Kenya mainly due to uncontrolled animal movements across porous borders and illegal trading activities. Animal movement is thought to play a key role in the onward transmission of the strains around the country and across country borders (Kerfua et al., 2018).

In this study, 51 FMDV isolates obtained between 2013 to 2018 were characterized. The serotype "O" EA-2 topotype continues to be the most prevalent serotype/topotype causing outbreaks in Kenya, resulting in serious economic losses in the livestock industry. Similarly, Lloyd-Jones et al. (2017) reported similar dominance of EA-2 from 80 isolates of FMD type O collected between 1993-2012 in Kenya. More recently, a study found EA-2 to be the most commonly detected topotype together with EA-4, with no detection of EA-1 and EA-3 (Wekesa et al., 2015b). These data show the dynamic changes in FMDV circulating strains at different periods in the country.

The EA-1 topotype strains still occur in the region, despite the lower prevalence and homologous vaccination using the strain OK77/78 belonging to the same topotype. Future vaccine candidates can be selected from the EA-2 group of viruses banked in the FMD National Laboratory repository, consisting of several isolates successfully sequenced in this study, and reactivation of OK 82/98 belonging to this EA-2 topotype could be considered.

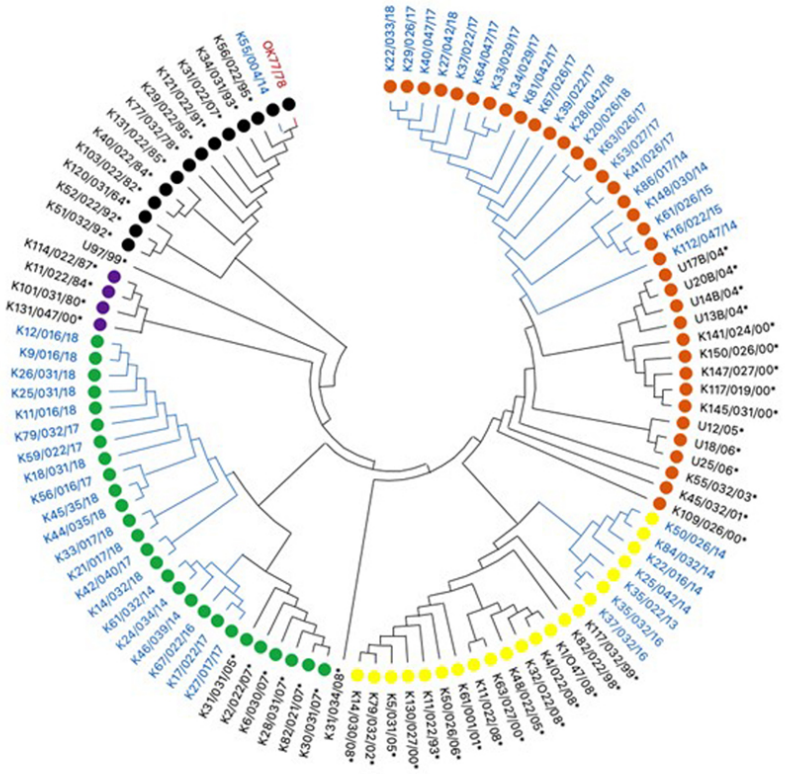

Figure 4: Phylogenetic tree representing the relationship between the 105 FMDV isolates, serotype O. All the FMDV isolates were organized into five separate clades: clade 1 (black), clade 2 (purple), clade 3 (green), clade 4 (yellow) and clade 5 (orange). The samples colored blue are those generated in this study, the ones colored red are serotype O Kenya vaccine strain, and those colored black with an asterisk at the end are the samples obtained from NCBI.

The particular new vaccine candidate strain to be selected will depend on vaccine matching tests and the adaptability of the strain to tissue culture cells. Lloyd-Jones et al. (2017) reported that the vaccine strain O/KEN/77/78 showed low *in-vitro* antigenic matches in neutralization experiments with recently circulating EA-2 and EA-3 viruses compared to the ME-SA topotype vaccine strains, including O/PanAsia-2 and O/Manisa. Hence, developing new vaccine candidates from these isolates after testing for antigenic matches is important to enhance the effective control of FMD in the East African region. However, it is recommended that the original vaccine strain OK77/78 shall be retained as one of the vaccine antigen reserves whenever increased cases of EA-1 topotype strains are encountered, as seen in this study. The OK 82/98 vaccine strain can also be reactivated to protect animals against new circulating strains.

In the current study, five FMDV clades were recognized between 2013 and 2018, of which the serotype "O" EA-2 topotype is the most commonly circulating. Viruses circulating in the same period, neighboring counties, and/or countries tend to belong to one lineage. Previously reported strains, however, appear to cluster with recent viruses in one lineage in some instances. Only one isolate in this study belongs to the EA-1 topotype, which also has the vaccine strain OK77/78.

This study emphasizes the importance of regular surveillance and characterization of circulating FMDV virus strains for selecting effective vaccine strains and other FMD control strategies. Choosing a suitable strain of the EA-2 topotype as a vaccine candidate for future use and/or reactivating vaccine strain OK82/98 in FMD control is recommended based on extensive antigenic matching studies to ascertain their ability to protect against the circulating strains. It is, however, prudent to maintain the current vaccine strain as a reserve antigen, as EA-1 strains may be detected in future surveillance.

Article Information

Conflict of Interest. The authors have no conflict of interest to declare.

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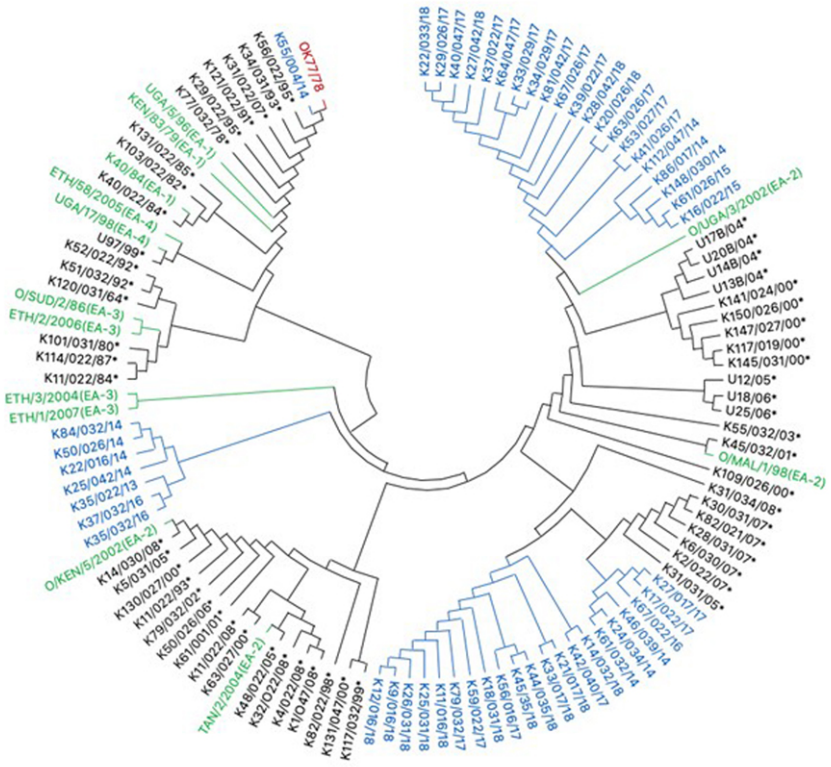

Figure 5: Phylogenetic tree representing the relationship between the 105 FMDV isolates, and serotype O. Parameters included: pairwise deletion, 1000 replicates for bootstrap analysis, and neighbor-joining method for tree construction. The isolates colored blue are those generated in this study, the ones colored red are the vaccine strains, isolates colored green are those with a known topotype, and those colored black with an asterisk at the end are the samples obtained from NCBI.

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