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Serotype diversity of foot and mouth disease virus and molecular characterization of serotype O strains from 2019 and 2020 outbreaks in Kenya

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ABSTRACT

Foot and mouth disease (FMD) is a viral infection affecting ruminants and leads to great economic losses. Control and prevention have been a challenge despite the availability of vaccines. The causative agent exists in seven serotypes and is endemic in Kenya, with serotypes O, A, SAT (South African Territory) 1, and SAT 2 and having circulated in the recent past. This study was aimed at determining the current serotype diversity and serotype O variants during the study period. A cross-sectional study was conducted and a total of 267 epithelial samples were collected from animals during the disease outbreaks of 2019 and 2020. Antigen detection was performed using ELISA (Enzyme-Linked Immunosorbed Assay). The negative samples were inoculated on LFBK(Line of Fetal Bovine Kidney) monolayer cells followed by a repeat ELISA for CPE(Cytopathic Effect) positive samples. The partial VP1 gene for serotype O samples was amplified and directly sequenced. The generated sequences were analyzed and compared with the vaccine strain. The prevalence of FMDV was 65.9% (176/267) and serotypes SAT 1, O, SAT 2, and A in the order of decreasing prevalence were circulating. Serotype O viruses analyzed belonged to the EA 2 against the EA 1 vaccine strain in use. For better control of the disease, this study recommends close monitoring of the circulating serotypes and topotypes, and, regular vaccine matching to ensure vaccine effectiveness.

1. INTRODUCTION

Foot-and-mouth disease (FMD) is a contagious viral infection affecting all ruminants (WOAH, 2022). Although the mortality rate is low, the disease causes huge economic losses in reduced production and control costs (Knight-Jones & Rushton, 2013). Foot-and-Mouth Disease Virus is a non-enveloped positive sense RNA virus of the genus *Aphthovirus* in the family *Picornaviridae* and is phylogenetically divided into seven serotypes, O, A, C, SAT1, SAT 2, SAT 3, and Asia 1, with several subtypes that do not cross-protect (Mattion et al., 2004).

The viral capsid is made up of 60 copies each having four structural viral proteins (VP) 1 to VP4 with VP 1-VP3 forming the surface component of the capsid unlike VP 4 which is internal (Sáiz et al., 2002). Of these proteins, VP 1 is regarded as the major antigenic site of the virus harboring beta cell epitopes that induce antibody production (Strohmaier et al., 1982). Additionally, the VP 1 gene is highly variable among the various serotypes (longjam and Tayo, 2011). The Arginine (R), Glycine (G), and Aspartate (D) RGD motif, also located in this region plays an important role in receptor recognition.

Diagnosis of FMDV is done through virus isolation and identification of viral antigens or genome in samples. Disease prevalence may be determined through the detection of the viral agent or antibodies (WOAH, 2022).

The spread of the virus is influenced by animal movement, trade in animals and their products, and related wild reservoirs (Di Nardo et al., 2011), and the global distribution of serotypes results in seven endemic regional pools where serotype O is associated with the majority of the cases (Jamal and Belsham, 2013). Kenya lies in the Eastern Africa pool (pool 4) where serotypes O, A, SAT 1, and SAT 2 are endemic (Brito et al., 2017; King et al., 2020). Although serotype C was last isolated in Kenya in 2004, (Sangula et al., 2011), antibodies against this serotype were detected in samples collected in the country in 2010 (Kibore et al., 2014). Additionally, four of eleven topotypes of O serotype (EA 1, EA 2, EA 3, and EA 4) have occurred in the country before (Balinda et al., 2010; Wekesa et al., 2015 a).

Vaccination of animals is a key measure in disease control, saving farmers from massive losses (Hegde et al., 2009), although it requires repeated boosters and over 80% coverage (Kotecha et al., 2015). In Kenya, vaccination measures are practiced though challenged by the poor economic status of farmers (Nyaguthii et al., 2019). The vaccines used in Kenya are produced locally using ancient vaccine strains. These are OK77/78, AK5/80, and K52/84 for serotypes O, A, and SAT 2 all isolated in Kenya in the years 1978, 1980, and 1984 respectively. T 155/71, the vaccine strain for SAT 1 was isolated in Tanzania in 1771.

In the years 2019 and 2020, Kenya recorded an increased number of disease cases (King et al., 2020). The purpose of

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this study was to identify FMDV serotypes prevalence and serotype O topotypes among viruses that caused outbreaks in 2019 and 2020 in Kenya.

2. MATERIALS AND METHODS

The study areas for the 2019 and 2020 outbreaks
This study was conducted in 36 of the 47 counties (7 of the 8 provinces in Kenya) (Fig 1). Kenya lies in East Africa and borders five countries; Tanzania, Uganda, South Sudan, Ethiopia, and Somalia. The disease outbreak regions were mapped using QGIS version 3.30, GIS software (QGIS, 2019).

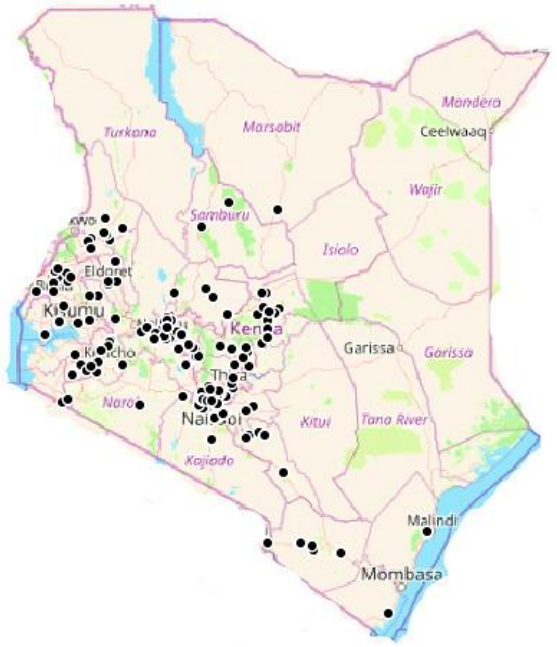

Fig. 1: Map of Kenya showing the county administrative regions and sampled areas of 2019 and 2020 (as dots)

Mouth and feet epithelial tissue samples were collected from bovines that presented with clinical symptoms of FMD. The samples were placed in screw-capped bottles containing transport media (50% glycerol in 0.04M phosphate-buffered saline PH 7.2-7.6) prepared and sterilized at the FMD laboratory Embakasi and transported in a cool box containing ice packs to the FMD laboratory for analysis.

Antigen detection *and serotyping ELISA*

A total of 267 (134 in 2019 and 133 in 2020) epithelial tissues were collected. One gram of epithelium samples was sliced and suspended into 10 ml of sterile 0.4M Phosphate Buffered Saline (PBS) prepared at the FMD Laboratory and ground with sterile sand in a mortar making a 10% suspension. This was centrifuged at 2000g for 10 minutes and the clear supernatant was used for viral antigen detection and serotyping using the IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna) ELISA -Italy. Optical densities were read at 450nm using 800 TS Microplate Reader –BioTek. Samples having direct OD readings of 0.1 and above were regarded as positive for the FMDV antigen.

Viral isolation and identification

Samples that were negative for FMDV antigen were inoculated on LFBK (line of Fetal Bovine Kidney) cells monolayer (Jackson et al., 2000) grown in Nunc™ EasYFlask™ Cell Culture Flasks 25 V/C-ThermoFisher and incubated at 37˚C for up to 48 hrs for CPE (Cytopathic Effect). The vaccine strain OK77/78 and an un-inoculated monolayer cell culture were used as positive and negative controls respectively. Positive cultures were harvested by freezing and thawing the material then centrifuged to collect the supernatant that was used for a repeat ELISA. The results were entered in a Microsoft Excel 2010 spreadsheet and then displayed in a table (Table 1). Chi-square was performed to determine significant differences in disease prevalence between the two sampling periods (2019 and 2020) and over the months (January to December).

Table 1: Geographical distribution of FMDV serotypes O, A, SAT 1, and SAT 2 in 2019 and 2020 in Kenya

Sampling area	Isolated serotypes				
Province/County	A	O	SAT 1	SAT 2	Total
Central		24	20	1	45
KIAMBU		16	3	1	20
KIRINYAGA		2			2
MURANGA		3			3
NYANDARUA		3	9		12
NYERI			8		8
Coast		3		6	9
KILIFI		2			2
KWALE				1	1
TAITA TAVETA		1		5	6
Eastern	2	9	16	3	30
EMBU			2		2
ISIOLO			2		2
MACHAKOS	1	1	1		3
MAKUENI	1	8			9
MARSABIT				1	1
MERU			8	2	10
THARAKANITHI			3		3
Nairobi		4		1	5
NAIROBI		4		1	5
Nyanza		4	2	5	11
KISII			2	2	4
KISUMU		2		2	4
NYAMIRA		1			1
SIAYA		1		1	2
Rift Valley	3	20	40	9	72
BARINGO				1	1
BOMET		5	3	1	9
KAJIADO		2	1	1	4
KERICHO	1		1		2
LAIKIPIA		2	3		5
NAKURU	1	8	23	4	36
NANDI			2		2
NAROK			2		2
SAMBURU				2	2
TRANSNZOIA			2		2
UASINGISHU	1		2		3
WEST POKOT		3	1		4
Western		2	2		4
BUNGOMA			1		1
BUSIA		1			1
KAKAMEGA		1	1		2
Totals	5	66	80	25	176

Molecular Characterization of serotype O viruses

Viral RNA extraction was done for serotype O samples using PureLink® Viral RNA/DNA Mini Kit Invitrogen, and the VP 1 region was amplified using Invitrogen Platinum™ Taq DNA Polymerase-ThermoFisher US and the primers in Table 2 after cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit –Thermofisher US. Serotype O vaccine strain (OK77/78) and sterile nuclease-free water were used as positive and negative controls respectively. The cycling conditions were: 94˚C- 2min, 35 cycles of 94˚C-30 sec, 60˚C- 30 sec, and 72˚C-1 min, then infinite hold at 4˚C. The amplicons were confirmed by gel electrophoresis using 1.5% agarose and sent for sanger sequencing at Macrogen-Netherlands (Knowles et al., 2016).

Table 2: Serotype O primers used in the conventional PCR

Name	Sequence	Direction	Annealing region	Size
OC244F	GCAGCAAAACACATGTCAAACACCTT	+	VP3	1165
2B52R	GACATGTCCTCCTGCATCTGGTTGAT	-	2B	

Sequence analysis

The sequences were confirmed through the Basic Local Alignment Search Tool (BLAST) aligned in CLUSTAL in Bioedit version 7.2.5 and trimmed to 639 nucleotides of the entire VP 1 region guided by closely related sequences of FMDV serotype O obtained from BLAST. Phylogenetic analysis was done together with archived sequences obtained from the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena/browser/search) in MEGA X software version 10.2.6. The neighbor-joining tree was constructed and robustness was assessed by the 1000 replicates bootstrap implemented in the program. Nucleotide translation was done in Bioedit .

3. RESULTS

FMDV antigen prevalence

From the 267 samples collected, the prevalence of FMDV was 65.9% (176/267). The outbreaks occurred throughout the two years, with no significant difference in the overall disease prevalence between 2019 and 2020 ($p=0.932$, $df=1$), however, the difference in prevalence over the months in the two years was statistically significant ($p<0.001$, $df=11$) with a low number of cases reported in March and June 2019 (Fig 2) and April in 2020 (Fig 3). Comparatively, a high number of cases occurred in October 2019 and January 2020 (Fig 2 and Fig 3) .

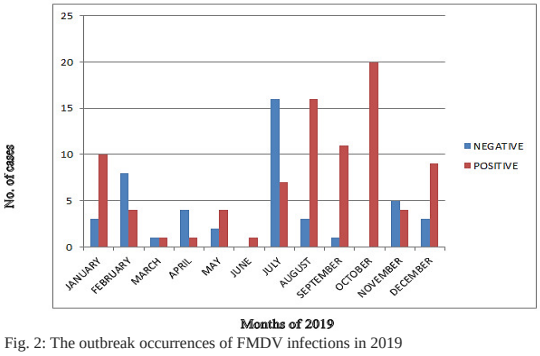

Fig. 2: The outbreak occurrences of FMDV infections in 2019

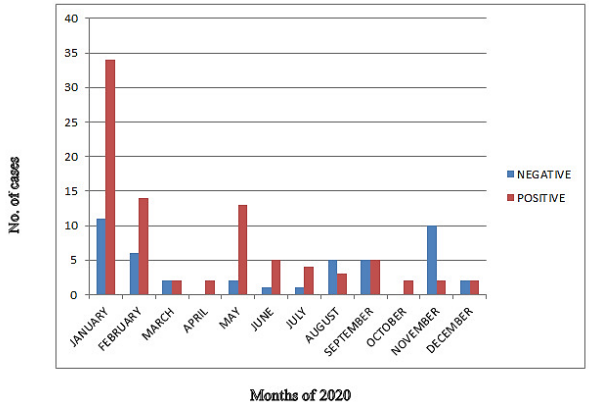

Fig. 3: The outbreak occurrences of FMDV infections in 2020

FMDV serotype diversity

Four of the seven serotypes of FMDV were identified and SAT 1 was the most predominant at 45.5% (80/176) followed by serotype O at 37.5% (66/176), serotype SAT 2 at 14.2% (25/176), and the least serotype A at 2.8% (5/176) (Table 1) .

Of the 36 counties sampled, only Nakuru County reported all four serotypes. As per provinces, the Rift Valley and the Eastern regions recorded all four serotypes of FMDV while the Coast and Nairobi had only O and SAT 2, and the Western region had O and SAT 1. Serotype O was the only serotype that circulated in all seven provinces while SAT 1 was found in all the provinces except in Coast province. Only serotypes SAT 1 and O caused outbreaks in 2020 while serotype A circulated only in Eastern and Rift Valley provinces (Fig 4).

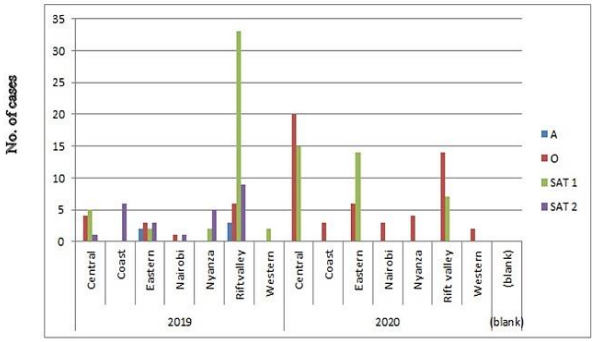

Fig 4. Occurrences of serotypes O, A, SAT 1 and SAT 2 in Kenya in 2019 and 2020

Serotype O topotypes

Fourteen VP 1 nucleotide sequences from six provinces (none from Nairobi and North Eastern) analyzed in this study belonged to EA 2 topotype with an average nucleotide divergence of 0.06 (6%) and 19.4% (124/639) variable sites. They were closely related to two other strains of EA 2, TAN/2/2004 and KEN/5/2002, and EA 4 strains. Two viruses collected in 2019 in RiftValley (K60/19 and K83/19) were closely related to K131 and K114 collected in 2020 from RiftValley and Nyanza provinces. The vaccine strain K77/78 belonged to EA 1. (Fig 5) .

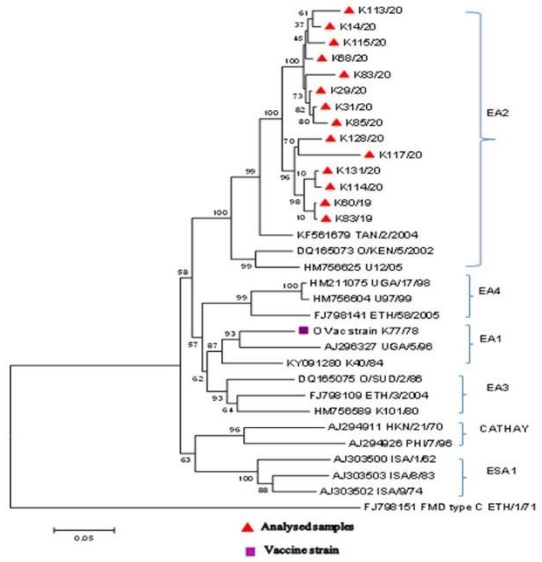

Fig. 5: Phylogenetic tree of FMDV VP 1 gene sequences from 2019/2020 outbreaks. The neighbor-joining method based on 1,000 bootstrap replicates was used. The sequences from this study are shown in a red triangle, the vaccine strain in a violet square, and other reference sequences are represented by accessory numbers.

Variation of VP 1 gene between circulating strains and the vaccine strain

All fourteen viruses analyzed in this study belonged to topotype EA 2 and were 23.3% and 9.8% different from the EA 1 vaccine strain in nucleotide and amino acid sequences respectively. At the highly variable region (G-H loop) located between residues 140 and 160 (Jeremy et al., 1990), some of the viruses showed some amino acid replacements majority at position 158 (K113/20, K115/20, K128/20, K117/20, K131/20, K114/20, K60/19 and K83/19). The RGD motif was conserved in all these sequences despite the replacements shown (Fig 6) .

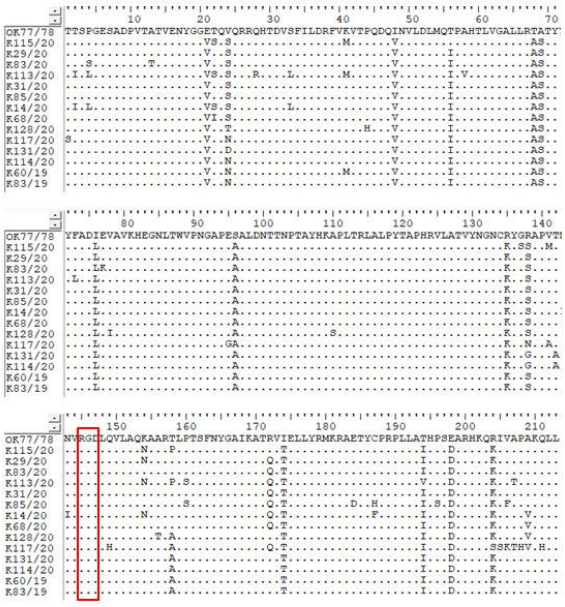

Fig. 6: Amino acid sequence alignment for the VP1 region for serotype O viruses analyzed in this study together with the vaccine strain OK/77/78. The dots indicate the regions identical to the vaccine strain while the RGD motif is shown in the red box.

4. DISCUSSION

Foot and mouth disease is endemic in the Eastern Africa region and serotypes O, A, SAT1, and SAT2 are circulating (Brito et al., 2017). From this study, the prevalence of FMDV was 65.9% and confirmed previous findings that serotypes O, A SAT 1, and SAT 2 circulate in the country (Kibore et al.,2014; King et al., 2020) .

During the study period, outbreaks occurred continually, and the disease prevalence between the two years (2019 and 2020) was statistically insignificant but different over the months where January 2020 and October 2019 recorded the highest number of cases while low numbers were reported around March, April and June in the two years. Reduced interactions between animals in search of water and pasture may have played a role as March and April coincide with one of the rainy seasons in Kenya.

Serotype SAT 1 was the most prevalent serotype (45.5%), despite leading in infections in endemic regions of the world including Eastern Africa (Ayelet et al., 2009; Brito et al., 2017). Serotype O was at 37.5%. while other serotypes, SAT 2 (14.2%) and A (2.8%) were also present. Geographically, all four serotypes were circulating in the Eastern and Rift Valley regions of the country, areas with higher cattle populations compared to other regions in Kenya coupled with uncontrolled extensive grazing (Rendel, 2018) while Coast and Nairobi recorded only serotypes O and SAT 2. The western province recorded serotypes O and SAT 1 in the two years studied. Serotype A circulated in Eastern and Rift Valley in 2019 and was the least prevalent serotype. Serotype O was the only serotype observed to circulate in all the provinces while SAT 1 was not found in Coast province. Since SAT 1 serotype has been least prevalent in the region in previous years, livestock and wildlife interactions (Wekesa et al., 2015b; Omondi et al., 2020) and a decline in population immunity (Casey-Bryars et al., 2018) may have contributed to the serotypes prevalence shift in Kenya witnessed in the study period.

Serotype O viruses analyzed from this study belonged to the EA 2 topotype similar to another study conducted in the Uganda-Tanzania border area (Kerfua et al., 2019). Some viruses had amino acid replacements at the major antigenic site (G-H loop). Viruses from the current study however had an average nucleotide divergence of 6% compared to 4.9% of the previous study. Similarly, all serotype O viruses collected between 2013 and 2018 from Kenya belonged to EA 2 except one virus isolated in 2014 (Chepkwony et al., 2022). The current study confirms previous findings that EA 2 is the predominant topotype in East Africa (Balinda et al., 2010; Wekesa et al., 2015a). The amino acid divergence of 9.8% between the analyzed viruses from the vaccine strain (OK77/78) may suggest poor broad neutralization of the circulating strains.

5 .CONCLUSIONS

The outbreaks of FMD in Kenya in 2019 and 2020 were caused by serotypes O, A, SAT 1, and SAT 2 where SAT 1 caused the majority of the infections. All the serotype O viruses analysed belonged to EA 2 topotype. This study demonstrates the necessity of continuous FMDV surveillance and monitoring of viral antigenic and genetic variants to match the vaccines used with the most recent epidemiologic situation.

CONFLICTS OF INTEREST

All the authors declare that there is no conflict of interest regarding the publication of this paper.

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