JVMR. pISSN: 2357-0512 - eISSN: 2357-0520 Mashinagu et al., 2024. 10.21608/jvmr.2024.276435.1099

Original Article

Molecular Epidemiology of Foot-and-Mouth Disease Virus in Tanzania during 2020 to 2021 Outbreaks

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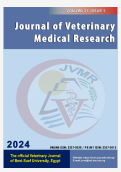

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Abstract

Background: Food and mouth disease (FMD) is an endemic disease of cattle and other cloven-hoofed animals. The objective of this study was to investigate the genetic characteristics and evolutionary relationships for the 2020 to 2021 field circulating Food and mouth disease virus (FMDV) obtained from reported outbreaks in different parts of Tanzania. Methods: The epithelial tissues were collected from lesions (oral, nasal, interdigital) of FMD suspect cases, stored, and shipped to the laboratory for analysis. In the laboratory, the samples were prepared for nucleic acid extraction, FMDV detection, typing, sequencing, and phylogeny analysis. The construction of the phylogenetic trees was done by aligning current field strains nucleotide sequences with those from past studies stored in GenBank database. Results: The study identified three FMDV serotypes (A, O, and SAT-1) to be circulating in the field as Africa Topotype G-I lineage, EA-2 Topotype, and Topotype I (NWZ) respectively. The identified field strains showed diverse scores of shared identity among current and past study strains. The generated nucleotide sequences from this study type O and SAT-1 field strains were analyzed categorically and showed shared percent identities of 92.0-100.0% and 96.9-98.8% respectively. Conclusion: The sequencing and analysis of the VP1 coding region enhance FMDV knowledge on the genetic and evolutionary relationships existing among field strains, and commend for improved future strategies for effective national, regional, and global FMD control measures .

Keywords: FMDV, Serotype A, Serotype O, Serotype SAT-1, Tanzania

Citation. Mashinagu MM, Wambura PN, Kimera S, King DP, Paton DJ, Maree F, Mpete H, Kandusi S, Rweyemamu MM, Kasanga CJ. Molecular Epidemiology of Foot-and-Mouth Disease Virus in Tanzania during 2020 to 2021 Outbreaks. J Vet Med Res., 2024; 31(1): 15–22. https://doi.org/10.21608/jvmr.2024.276435.1099.

Article History: **Received**: 13-Mar-2024 **Accepted**: 04-Jun-2024

1. Introduction

Foot-and-mouth disease (FMD) is a disease of all cloven-hoofed (livestock and wildlife) animals, with severe agricultural and socio-economic implications at national, regional, and global community levels (Belsham and Bøtner, 2015; Knight-Jones et al., 2016). FMD undermines the livestock sector by causing production and productivity losses enhanced by trade embargoes and enormous progressive control costs across countries in the world (Knight-Jones et al., 2016).

The disease is caused by a highly infectious Foot-and-mouth disease virus (FMDV) that belongs to the genus *Aphthorvirus* of the family *Picornaviridae* (Zell et al., 2017). FMD has been reported worldwide and countries are classified into 6 stages ranging from 0 to 5 depending on the progressive control pathway-FMD (PCP-FMD) achievements acquired (Sumption et al., 2012). It is a highly contagious disease as its primary spread is through direct contact between infected and naïve animals, aerosols, contaminated environments, and fomites (Belsham and Bøtner, 2015). There are 7 worldwide reported FMDV antigenically distinct serotypes O, A, C, Asian1, Southern African Territories (SAT)-1, SAT-2, and SAT-3 circulating as 7 pools of FMDV strains under identified geographic regions globally (Brito et al., 2017). The African continent has the largest number of FMD virus serotypes reported (O, A, C, SAT-1, SAT-2, and SAT-3) than any other continent in the world (Vosloo et al., 2002).

FMDV is a single-stranded, positive sense RNA genome virus with approximately 8500 nucleotides in size (Lloyd-Jones et al., 2017; Mahapatra et al., 2015) with a single linear open reading frame (ORF) of approximately 7000 nucleotides that differs in size or length between different serotypes (Carrillo et al., 2005; Orsel et al., 2007). The FMD virus genome is enclosed by a 30 nm diameter size icosahedral capsid that is composed of 60 copies of structural proteins (SP) named VP1, VP2, VP3, and VP4 that are derived from the P1 region of its genome (Mason et al., 2003). The latter are capsid building blocks, where the VP1-3 are located on the surface and VP4 internally located (Bari et al., 2014). VP1 expresses the highest variability followed by VP3 then VP2 being the least (Carrillo, 2012). The VP1 region is the main segment of the genomic whole capsid segment (P1) utilized as the landmark for FMDV molecular serotyping, determining the antigenic epitopes, and the evolutionary relationships existing among isolates. The genetic variability expressed at the VP1 region is intensified by FMDV broad host range, high replication (>10^5 new virus particles per 5 hours), and high mutation rates (10^{-5} to 10^{-3} per nucleotide per FMDV genome) situation (Belsham and Bøtner, 2015; Singh et al., 2019). The latter facilitates the frequent emergence of antigenically distinct variant strains of FMDVs in the field that need to be studied properly as a benchmark for future effective control strategies.

In recent years, there has been an increased government interest in controlling FMD in Tanzania to enhance livestock sector contribution to the national economy by enabling international lucrative markets access to live animals and their products exports that are currently hindered by the FMD endemic situation (James and Rushton, 2002; ILRI and CGIAR, 2017). Tanzania ranks third in the number of cattle in Africa with a total estimate of over 30 million cattle herds 1.4% of the global and 11% of Africa cattle population (FAO, 2014), therefore FMD control bears feasible socio-economic impacts in the country. FMD is endemic in Tanzania, and four serotypes (O, A, SAT-1, and SAT-2) have been reported to cause outbreaks in different geographical locations (Kivaria, 2003; Kasanga et al., 2012). The FMD control in endemic countries continues to be challenging due to its prevailing complicated epidemiology instigated by the presence of multiple serotypes, subtypes, and even topotypes that are widely distributed, higher numbers of livestock herds, biodiversity richness of susceptible host animal species in numerous conservation areas across the country, and uncontrolled animal movements (Tekleghiorghis et al., 2016). This situation implicates the persistent FMD endemicity in the country and similarly in other sub-Saharan region countries (Vosloo et al., 2002; Tekleghiorghis et al., 2016). The latter could be the reason for the current FMD field situation presenting an increased frequency of FMD outbreaks to the extent of even reporting the disease throughout all seasons of the year.

According to FAO and OIE stipulations on FMD control based on a long-term progressive risk reduction approach (Rweyemamu et al., 2008; Paton et al., 2009), updated knowledge of FMDV circulating field strains is a requirement. Tanzania is at stage one of the PCP-FMD and its advancement to stage 2 requires monitoring of circulating strains to understand the epidemiology of FMD in the country to enhance tailored mitigation options whereby vaccination remains as main FMD intervention of choice under this state. The vast of studies done on FMDV in Africa and other parts of the world deploy VP1 genomic region to infer the molecular characteristics of the virus (Knowles et al., 2016; Dyirakumunda et al., 2017).

Thus, this study was undertaken to investigate the occurrence and genetic characteristics of FMDV serotypes from 2020 to 2021 field-reported FMD outbreaks by deploying molecular analytical techniques for the VP1 genomic region of the FMDV as a target. This study strengthens knowledge of FMD's current status in Tanzania based on circulating viral strains at studied geographical areas, their genetic diversity, and existing evolutionary relationships amongst current and previously identified strains. The gathered information is also vital for implementing tailored mitigation measures on FMD and contributes to the PCP-FMD advancement in Tanzania.

2. Materials and Methods

2.1. Samples and Study Area

This study aimed to collect Cattle tissue samples from districts that reported FMD outbreaks in the duration between 2020 and 2021 across Tanzania. A total of 11 districts (Chalinze, Bagamoyo, Moshi rural, Babati, Mbogwe, Bukombe, Biharamulo, Ngara, Mvomero, Morogoro rural, and Kibaha) reported FMD outbreaks in the stipulated duration (Figure 1). The samples were collected by following World Organisation of Animal Health (OIE) guideline (WOAH, 2013). Epithelia tissue samples were obtained from well-restraint clinically sick animals, stored in cryovials with Viral transport media, and stored in a liquid nitrogen tank. The obtained samples were shipped to the Department of Veterinary Microbiology, Parasitology, and Biotechnology laboratory at the Sokoine University of Agriculture, Morogoro, and stored at -80°C till analyzed.

2.2. Sample Preparation and RNA Extraction

The field obtained epithelial tissue samples under -80°C storage conditions were allowed to equilibrate at room temperature, and ground using mortar and pestle in TBE buffer solution. The suspension products were centrifuged at 12000 rpm and the supernatant was collected for RNA extraction. Then RNA extraction was done using Qiagen RNeasy® Mini Kit, Qiagen GmbH Strasse1, Hilden Germany by following the manufacturers' instructions manual. The extraction products were quantified spectrophotometrically using Nanodrop and all products below the ratio of 2.0 were rejected for further analysis.

2.3. Detection and Typing of FMDV Genome

The extraction products obtained were screened to infer the presence of FMD virus genomes in every field sample under study. The screening was done by a one-step RT-PCR using PAN primers.

(Forward Primer: GCCTGGTCTTTCCAGGTCT; Reverse Primer: CCAGTCCCCTTCTCAGATC) that targets the 5'UTR region of the FMD virus genome. The protocol involved 50°C (30 min.) for reverse transcription, 95°C (15 min.) for (transcriptase enzyme denaturation, polymerase activation, and cDNA unwinding, denaturation 95°C (1 min.), annealing 55°C (1 min.), elongation 72°C (2 min.) and final elongation 72°C (5 min.) for 35 cycles. The PCR products were visualized in a transilluminator after being electrophoresed in 1.5% agarose gel along with SafeView™ Classic ladder of 100 bp size. The samples that tested positive (328 bp band size) for FMDV PAN- Primers were further analyzed by using FMDV serotype-specific primers. The serotype-specific primers were for FMDV serotypes (A, O, SAT-1, -2, and -3) and the analysis was done as previously described (Knowles et al., 2016).

The PCR amplification protocol was 50°C (30 min.) for reverse transcription, 95°C (15 min.) for (transcriptase enzyme denaturation, polymerase activation, and cDNA unwinding), denaturation 95°C (1 min.), annealing 60°C (1 min.), elongation

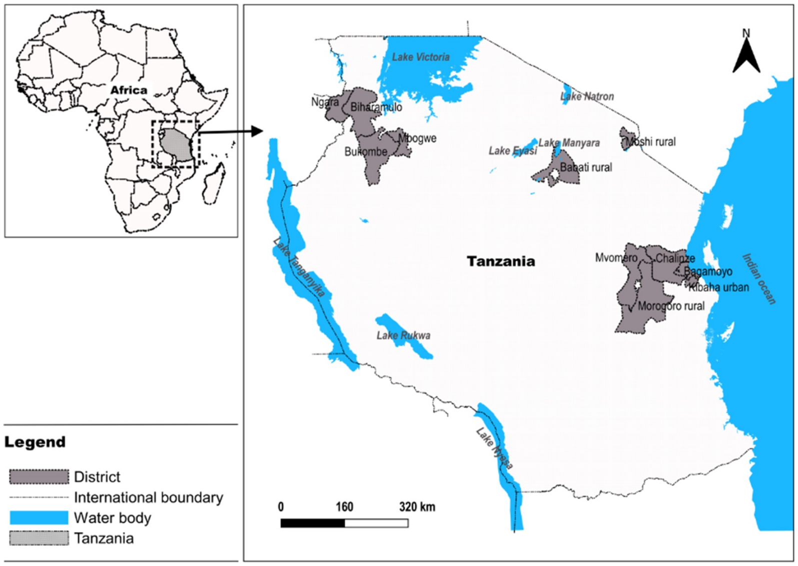

Figure 1: Map of Africa and Tanzania showing areas where samples and previous study nucleotide sequences used in this study were obtained and analyzed.

Table 1: Antimicrobial treatments applied to control *L. monocytogenes* in Domiati cheese.

S/N	District	Total samples	Positive PAN-PCR results	Serotype	Topotype
1	**Chalinze**	3	-	-	
2	**Bagamoyo**	11	3	SAT-1	I(NWZ)
3	**Kibaha**	5	3	A	G-I
4	**Moshi rural**	16	10	SAT-1	I(NWZ)
5	**Babati**	1	-	-	-
6	**Mbogwe**	43	29	O	EA-2
7	**Bukombe**	2	2	-	-
8	**Biharamulo**	4	-	-	-
9	**Ngara**	8	6	O	EA-2
10	**Mvomero**	15	-	-	-
11	**Morogoro rural**	5	2	-	-
12	**Total**	113	55	3	3

72°C (2 min.) and final elongation 72°C (5 min.) for 35 cycles. The PCR amplicons generated with diverse band sizes depending on each primer set deployed were observed in a 1.5% Agarose gel electrophoresis with SafeView™ Classic ladder of 100 bp size. The properly typed samples were identified and qualified for the VP1 amplification process using respective serotype-specific primer sets.

2.4. FMDV VP1 Amplification

The reaction master mix was prepared in a separate PCR clean room by adding 8 μl of nuclease-free water, 2.5 μl of FMDV serotype-specific forward primer (4 pmol/ μl), 5 μl of FMDV serotype-specific reverse primer (4 pmol/ μl), 5 μl of 5× buffer (containing 2.5 mM $MgCl_2$), 1 μl of dNTPs mix and 1 μl of Qiagen OneStep RT-PCR enzyme mix (QIAGEN OneStep RT-PCR kit (Qiagen, Germany), 2.5 μl of the viral RNA was lastly added to the RT-PCR tube. Template-free amplification controls (RT-PCR tubes with nuclease-free water only instead of RNA sample) for each primer set were included and amplified parallel to the RNA samples to monitor any chances of cross-contamination in the process. The RT-PCR tubes with reaction mixtures and the control tubes were placed in a thermocycler (Applied Biosystems, ABI 9700; USA) and the appropriate PCR cycling program was set based on the serotype and respective primers as described by (Knowles et al., 2016). When the amplification process was done, the tubes were held at 12°C waiting for cycle sequencing processes.

2.5. Sequencing of FMDV VP1 Fragment

The VP1 PCR amplicons were purified using Illustra kit and cycle sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies). A total 10 μl reaction mixture was prepared, each with 2 μl 5× sequencing buffer mixed with 0.5 μl BigDye Terminator v3.1 (both reagents are supplied with the kit), 3 μl of FMDV universal reverse sequencing primer (NK72) or serotype/ topotype specific sequencing forward some reverse primers at (1.6 pmol) (Knowles et al., 2016), and 5–20 ng of target DNA. The cycle-sequencing PCR reactions were carried out in each of the primers in 0.2 ml thin-walled tubes by deploying a protocol of 96°C for 1 min and 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min (Applied Biosystems, ABI 9700; USA). After the cycling was done the thermocycler was set to hold the tubes at 4°C while waiting for further procedures.

The obtained cycle-sequencing PCR products were cleaned up by ethanol precipitation. The latter used 5 μl containing 125 mM EDTA and 60 μl of 100% ethanol added to each reaction tube containing the sequencing PCR products, then vortexed and incubated for at least 15 min at room temperature

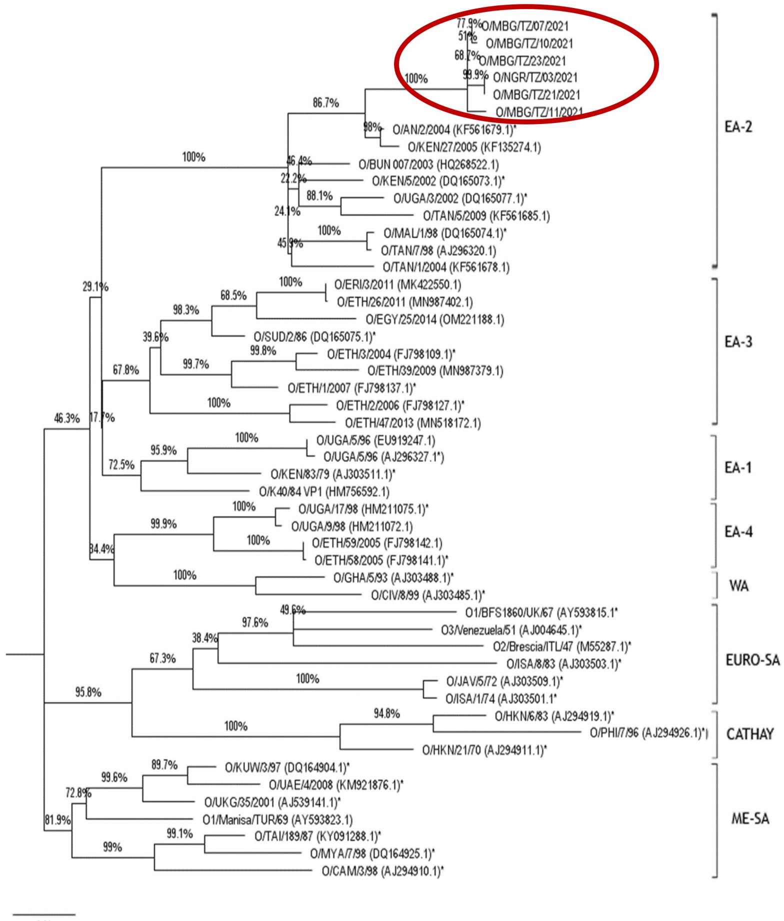

Figure 2: Midpoint-rooted maximum likelihood phylogenetic tree showing the genetic relationship between 2020/2021 fields identified FMDV type-O strains and GenBank database archived nucleotide sequences from previous outbreaks in African countries.

to allow precipitation to occur. The precipitation was carried out in the dark enclosure as the BigDye Terminator reagent is light-sensitive. After precipitation was done, the tubes were centrifuged at 13,000 rpm for 30 min at 4°C and the supernatant was pipetted and discarded without disturbing the pellet. Thereafter; the pellets were washed with 60 μl of 70% ethanol and centrifuged at 13,000 rpm for 30min at 4°C, the supernatant was removed and the pellets were shaded from direct light and dried in an oven drier for 60 min to ensure no ethanol remnants. The samples were finally re-suspended in 20 μl of Hi-Di Formamide (Life Technologies) and loaded onto the ABI 3500 DNA Analyzer where the sequencing reactions were allowed to run according to the manufacturer's instructions.

2.6. Phylogenetic Analysis

The cDNA nucleotide sequences of VP1 origin obtained from different FMDV field isolates that had already been typed into their respective serotypes were assembled using SeqMan Pro (Lasergene package DNAstar Inc., Madison, Wisconsin, USA). The nucleotide sequences from each FMDV serotype were aligned with multiple similar VP1 nucleotide sequences sourced from GenBank (NCBI) database using CLUSTAL W (Thompson et al., 1994). The evolutionary history was inferred by using the Maximum-Likelihood method, and the selection of the best model for the construction of the phylogenetic tree was achieved by the use of aligned multiple cDNA nucleotide sequences in MEGAX (Nei and Kumar, 2000). The General time reversal (GTR) model combined with gamma distribution and proportion of invariant sites (GTR+G+I) algorithms was applied. The tree was constructed and visualized in MEGAX (Kumar et al., 2018) and the phylogenetic tree obtained was additionally manipulated in FigTree program v1.4.4 for enhanced visualization.

3. Results and Discussion

In this study, a total of 113 FMD tissue samples were collected (Table 1), and 48.67% (n= 55) of the FMD tissue samples collected had nucleic acid materials for FMDV when screened by one-step RT-PCR using PAN primers. These results provided evidence of FMDV strains circulating and responsible for the outbreaks in the field. Also, the screening results confirmed that the profile of vesicular lesions manifested in cattle herds in the field was due to FMD outbreaks. This study identified three FMDV serotypes (O, A, and SAT-1), circulating and causing FMD outbreaks in different geographical locations during the stipulated study period in Tanzania.

Unlike in other studies on FMD outbreak investigation in Tanzania where type O, A, SAT-1, and SAT-2 were reported (Kasanga et al., 2012; Sallu et al., 2014). This study did not

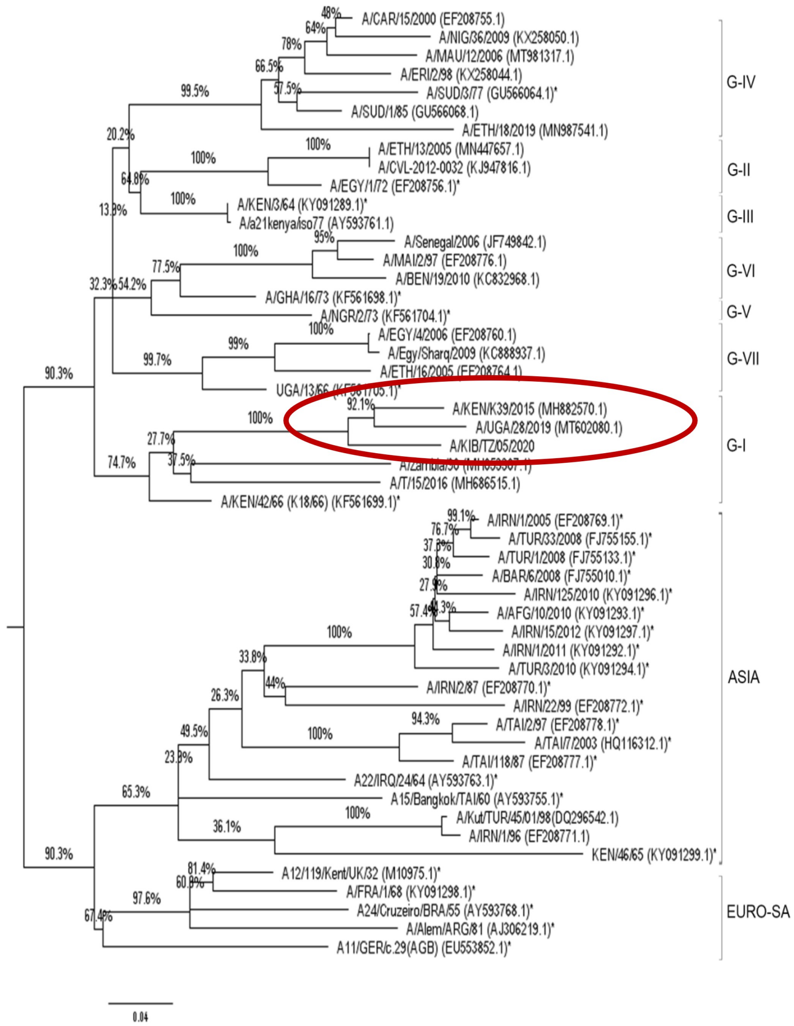

Figure 3: Midpoint-rooted maximum likelihood phylogenetic tree showing the genetic relationship between 2020/2021 fields identified FMDV type-A strains and GenBank database archived nucleotide sequences from previous outbreaks in African countries.

detect FMDV type-SAT-2 however, it was lastly detected in 2016 study samples collected in Kilimanjaro, Arusha, Iringa, Morogoro, and Coast Region areas (Mfuru et al., 2018). The study done in 2008 – 2013 showed that of all outbreaks reported in that study duration SAT-2 accounted for 2.85% (Sallu et al., 2014). FMDV type-SAT-2 could have probably been circulating in areas where no outbreaks were reported to enable samples to be taken for analysis during the study period.

3.1. Foot-and-Mouth Disease Virus Serotype O

The FMD viruses serotype O detected in this study were from outbreak samples obtained from the Ngara and Mbogwe districts. The FMD virus type O exhibits a historical cosmopolitan occurrence, and past studies in Tanzania have reported type O to be circulating in the sampled areas for decades (Kasanga et al., 2012). The phylogenetic analysis conducted inferred the existing genetic and evolutionary relationships amongst GenBank data, and this study nucleotide sequences through expressed clustering patterns (Figure 2). In the phylogenetic tree the field identified FMDV serotype O nucleotide sequences with 633 nt size clustered together with reference sequences derived from prototype strains (O/TAN/2/2004 [KF561679.1], O/MAL/1/98 [DQ165074.1], O/UGA/3/2002 [DQ165077.1] and O/KEN/5/2002 [DQ165073]) Tanzania, Malawi, Uganda and Kenya origin isolates, respectively with 100% bootstrap value. The phylogenetic tree topology, the published articles cited in this current study, and the WRLFMD (Pirbright, UK) reports stipulate that, all 2020 to 2021 FMDV serotype O Tanzania field isolates belonged to topotype EA-2.

The prototype (O/TAN/2/2004 [KF561679.1]), a 2004 Tanzania origin isolate described the closest relatedness as compared to the other prototypes included in the analysis with shared identities of 95.46 – 96.24% with current study strains. The shared identity of 92.0-100.0% revealed in this study field isolates, whereas the far distant (O/NGR/TZ/03/2021) and (O/MBG/TZ/ 21/2021) isolates showed the highest identity of 100.0% compared to close distance isolates within Mbogwe district (Figure 2). The identity disparities amongst analyzed sequences portrayed in the phylogenetic tree describe their existing genetic and evolutionary relationships influenced by their

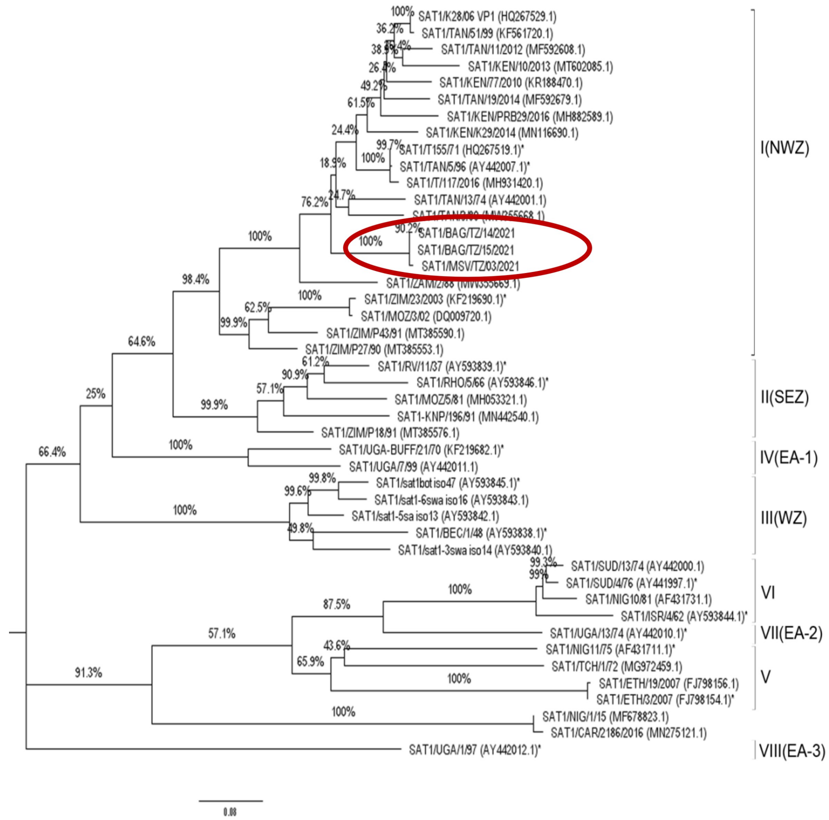

Figure 4: Midpoint-rooted maximum likelihood phylogenetic tree showing the genetic relationship between 2020/2021 fields identified FMDV type-SAT-1 strains and GenBank database archived nucleotide sequences from previous outbreaks in Africa countries.

geographic locations variabilities (Figure 2). In the phylogenetic tree, the 1998 FMD outbreaks in Tanzania and Malawi had isolates (O/TAN/7/98 [AJ296320.1] and O/MAL/1/98 [DQ165074.1] sharing 97.3% identity, the 2004 and 2005 Kenya and Tanzania isolates (O/KEN/27/2005 [KF135274.1] and O/TAN/2/2004 [KF561679.1]) shared 98.6% identity whilst 2011 Eritrea and Ethiopia had isolates (O/ERI/3/2011 [MK422550.1] and O/ETH/6/2011 [MN987402.1]) sharing 99.8% identity.

Also, the field strains identified during this study clustered closely to the 2005 Kenyan isolate (O/KEN/27/2005 [KF135274.1]) with 98% bootstrap support (Wekesa et al., 2015). These findings inference for the possibility of cross-border virus incursions, and agree with Di Nardo et al. (2011) study that described border areas to be experiencing the burden of transboundary livestock diseases including FMD fuelled by cross-border legal and illegal socio-economic activities. The FMDV strains expressing less than 15% variation in the sequenced VP1 segment are considered to be of the same genotype, and the ones with less than 5% variation are considered to be closely related (Samuel et al., 1999; Knowles et al., 2016). The virus strains under the EA-2 cluster have expressed a shared identity of 85.2-100%. This degree of relatedness in the topotype EA-2 viruses signifies that, if FMD vaccines developed from strains belonging to topotype EA-2 identified in this study they are likely to confer suitable protection against viral incursions of the EA-2 topotype category.

3.2. Foot-and-Mouth Disease Virus Serotype A

The FMDV serotype A was identified in the analyzed field obtained samples. The FMDV type-A is also widely distributed, having been reported worldwide in the history of the disease (Brito et al., 2017). The virus was detected in outbreak samples obtained from the Kibaha district of Tanzania. The current geographic occurrence of type A is consistent with the past studies that described type A to be circulating in the mentioned areas (Kasanga et al., 2012; Sallu et al., 2014). Also, studies have described the Eastern areas to be FMD higher risk areas due to frequent reports of multiple types of FMD outbreaks of (O, A, SAT-1, and SAT-2) origin (Kasanga et al., 2012). In this study, SAT-1 and A types were identified in samples from close distance districts of Bagamoyo and Kibaha districts respectively. The nucleotide sequences for the FMDV field identified as serotype A had a 621 bp size. Based on the phylogenetic tree constructed from FMD virus type A nucleotide sequences, this study nucleotide sequence clustered with a reference nucleotides sequence with (A/KEN/42/66 [KF561699.1]) a Kenyan 1966 isolate (Kasanga et al., 2015). The observed clustering pattern in the phylogenetic tree, the published articles cited in this work, as well as the WRLFMD (Pirbright, UK), reports infer that the FMDV type A Tanzania 2020 field isolates belonged to Africa topotype G-I Lineage. The phylogenetic tree described type A to have a closer clustering with (A/KEN/K39/2015 [MH882570.1]) and (A/UGA/28/2019 [MT602080.1]) Kenyan and Uganda previous studies identified strains respectively, than any other nucleotide sequences from sub Saharan countries (Figure 3).

The 2015 Kenyan (A/KEN/K39/2015 [MH882570.1]) nucleotide sequence shared a 90.8% highest identity followed by the 2019 Uganda (A/UGA/28/2019 [MT602080.1]) nucleotide sequence that expressed 89.7% shared identity with A/KIB/TZ/05/2020 strain. These are the highest percentage identity levels expressed in the cluster list of the Africa topotype G-I Lineage that ranged from 82.3-90.8% identity. The phylogenetic tree indicates FMDV type A to exhibit multiple topotypes and lineages (G-I to G-VII) circulating in the sub-Saharan region, where G-I to G-III lineages are vastly reported in the East and Central parts of Africa. The phylogenetic tree also depicts the genetic evolutionary relationships existing between the EURO-SA, Asia, and African topotypes as they are distinct and have been evolving and circulating in different geographic areas (Brito et al., 2017). The multiple topotypes state reveals the significant antigenic richness existing within type FMDV type-A, this state complicates suitable vaccine strain(s) selection among local isolates capable of controlling incursions of self-lineage and other lineages effectively.

3.3. Foot-and-Mouth Disease Virus Serotype SAT-1

The field identified FMDV type-SAT-1 were from outbreak samples obtained from the Bagamoyo and Moshi rural districts of Tanzania. The past studies (Kasanga et al., 2012) reported the circulation of FMDV type-SAT-1 in areas under investigation This study identified SAT-1 possessed nucleotide sequences with 390 and 655 nucleotide sizes for strains sourced from Moshi Rural and Bagamoyo districts, respectively. The topology of the phylogenetic tree generated from a list of FMD virus type-SAT-1 nucleotide sequences of this study and GenBank origin described characteristic clustering pattern (Figure 4). A close relatedness was observed between type SAT-1 current study and reference prototypes (SAT-1/ZIM/23/2003 [KF219690.1], SAT-1/T155/71 [HQ267519.1] and, SAT-1/TAN/5/96 [AY442007.1]) 2003 Zimbabwe, 1971 and 1996 Tanzania FMD outbreak strains.

The inference made through the phylogenetic tree generated, the published articles cited in this study and the WRLFMD (Pirbright, UK) reports indicated the type-SAT-1 2021 Tanzanian field strains belonged to topotype I (NWZ). The SAT-1 strains of this study shared had 96.9-98.8% shared identity and expressed closer clustering than other sequences in the SAT-1 type list of sequences analyzed (Figure 4). These findings are in agreement with Sallu et al. (2014) study that identified FMDV of topotype I (NWZ) to be circulating and causing outbreaks in different areas in Tanzania. The shared identity of 77.4-99.0% was expressed for nucleotide sequences within the same topotype I(NWZ) and at least 67.2-70.3% across topotypes I(NWZ) and V. The strains with close geographic relationships had exceptionally higher shared percentage identity (99.0% for SAT-1/MOZ/3/02 versus SAT-1/ZIM/23/2003) and (93.6% for SAT-1/K28/06 versus SAT-1/TAN/11/2012). This situation emphasizes enhancing geographical areas/regions based on FMD control strategies that execute tailored vaccines based on identified topotypes or lineages rather than generalized vaccines that have failed to confer effective field performances under the current African context.

In this study, the nucleotide sequences of the current circulating wild-type virus strains have been analyzed, and found no evident new variants circulating in the field. However, the distantly obtained SAT-1 and O identified in this study had similar genetic characteristics and these findings provide prospects for feasible FMD control if strategic FMD control mitigations are to be implemented across endemic African countries.

4. Conclusion

The findings of this study provide evidence of FMD presence in Tanzania with multiple outbreaks that implicate food security and the livelihoods of communities. The multiple FMDV types (A, O, and SAT-1) identified from samples obtained from diverse geographic locations reveal the epidemiological complexity of FMD in the country and call for strategized mitigation measures featured on frequently updated field data.

The genetic and evolutionary relationship revealed amongst strains across countries examined during this study, infer the persistence and significance of FMD transboundary consequences. The aspect of uncontrolled animal movements is regarded as the main contributing factor to the viruses spread across districts and even crossing country borders. This state needs to be translated as the landscape for the concerned countries to accord on improving coordinated national, regional, and global FMD control initiatives. The degree of percentage shared identity expressed within and between FMDV types (A, O, and SAT-1) topotypes in this study enhances knowledge for tailored vaccines and vaccination to improve FMD control outcomes.

Tanzania has also been involved in progressive control pathway for FMD (PCP-FMD) strategic initiatives and is estimated to be at PCP level 1, the updated knowledge on circulating field strains is vital as it comprehends control strategies (suitable vaccine selection). Therefore, the information of this study significantly advances knowledge on FMDV currently circulating in cattle herds and the underlying molecular and spatial epidemiology of the FMDV in Tanzania and Africa. However, future studies need to be on redefining the FMDV susceptible hosts spectrum due to the richness of livestock and wildlife diversity in the country or region, The knowledge of FMDV whole capsid antigenic characteristics of the circulating strains versus the available vaccine strains, and the community level of awareness and attitudes on FMD consequences for unleashing future participatory control approaches. Though the 5' UTR FMDV genome target region for PAN primers is highly conserved, but need for revised performance of the primers is a requirement

Article Information
Ethical Approval. Not Applicable.
Funding. This study was funded by the World Bank and Tanzania Government (WB-ACE II Grant PAD1436) through the SACIDS Foundation for One Health Africa Centre of Excellence for Infectious Diseases of Humans and Animals Project for PhD fellowships and the laboratory analysis work was funded by the Intermediate Fellowship in Public Health and Tropical Medicine Project that is under Welcome Trust Funding.
Conflict of Interest. The authors declare no conflict of interest.

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