MOLECULAR CHARACTERIZATION OF CIRCULATING FOOT AND MOUTH DISEASE VIRUSSEROTYPES A, SAT1 AND SAT2 IN KENYA FROM 2019 TO 2020 IN RELATION TO THE VACCINE.

BY

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UNIVERSITY OF NAIROBI

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DECLARATION

This thesis is my original work that has not been presented for award of a degree in this or any other university.

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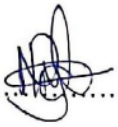
Signature.....  ..... **Date**.....27/05/2022.....

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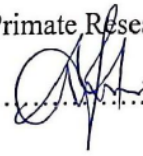
Signature.....  ..... **Date**.....27/05/2022.....

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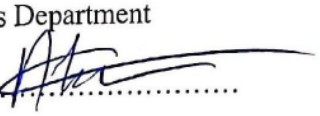
Signature .....  ..... **Date**.....30/5/2022.....

DEDICATION

This work is dedicated to my loving son Miles Njihia who has been my brightest star during my darkest nights. A special gratitude to my ever encouraging mother who prayed and kept supporting every step of my way.

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LIST OF ABBREVIATIONS

Ag	–	Antigen
ASALs	–	Arid and semi-arid lands
BHK	–	Baby hamster kidney
BTY	–	Bovine thyroid
cDNA	–	Complementary deoxyribonucleic acid
CFT	–	Complement fixation test
CPE	–	Cytopathic effect
DNA	–	Deoxyribonucleic acid
dNTP	–	deoxy nucleotide triphosphate
DTT	–	Dithiothreitol
ECL	–	Electro chemiluminescence
EDI	–	Elisa data information
EDTA	–	Ethylene diamine tetra acetic acid
ELISA	–	Enzyme Linked Immunosorbent Assay
EOC	–	Enzyme linked oligonucleotide capture
FMD	–	Foot and mouth disease
FMDV	–	Foot and mouth disease virus
IBRS	–	Instituto Biologico-Rim Suino
IRES	–	Internal ribosomal entry site
IU	–	International units
LFBK	–	Fetal porcine kidney
MAb	–	monoclonal antibody
MEM	–	Minimum essential media

MVPK	–	Mengeling-Vaughn Porcine Kidney
NASBA	–	Nucleic acid sequence based amplification
NSP	–	Non-structural proteins
NTR	–	Non-translated region
NWZ	–	North West Zimbabwe
OD	–	Optical density
OIE	–	Office International des Epizooties
ORF	–	Open reading frame
QAC	–	Quaternary ammonium compounds
RNA	–	Ribonucleic Acid
RT-PCR	–	Reverse Transcriptase Polymerase Chain Reaction
SAT1	–	South African Type 1
SAT2	–	South African Type 2
SP	–	Structural proteins
SSA	–	Sub Saharan Africa
TAE	–	Tris base, acetic acid and EDTA.
UV	–	Ultra violet
VNT	–	Virus Neutralization Test
VP1	–	Viral Protein 1

ABSTRACT

Foot and mouth disease (FMD) is a contagious disease of cloven-hooved domestic and susceptible wild animals. Repeated FMD cases are reported annually throughout the country despite vaccinations being carried out. This has hampered livestock industry development in Kenya due to production losses and that of trade. This study was conducted to investigate if there is a mismatch between the field strains and the vaccine. The study was conducted on 110 epithelial tissues submitted in the year 2019 and 2020 to Foot and Mouth disease laboratory, Embakasi for screening. The samples were collected from outbreaks and suspected clinical cases of FMD in cattle from different counties in Kenya for virus isolation, serotype identification and genetic characterization. All samples (n = 110) exhibited cytopathic effect on infected BHK-21 cell and the viruses were isolated. Serotype A (n=4), SAT 1 (n= 69) and SAT 2 (n= 37) were identified by antigen detection ELISA. Of the total 30 samples selected, 25 were confirmed positive by RT-PCR. Phylogenetic analysis of the isolates VP1 sequences were used to assess the genetic relatedness with the vaccine strain and other viruses retrieved from GenBank. The analysis showed that type A viruses belonged to the genotype VII (G-VII) within the AFRICA topo type. The viruses were closely related topo type A vaccine strain AK5/80 with 99.5%-99.8% nucleotide similarity. SAT 1 field isolates were closely related to the vaccine strain SAT1/T155/71 and had 99.7% - 99.9% identity with the vaccine. There was a similarity index of 99% - 100% between all the isolates and the reference vaccine strain in the country and all the isolates clustered together with their respective isolates on the tree. This shows there isn't much difference between the field strains in the current study and the vaccine strains. Therefore, there is a need to re-look at other factors which may influence vaccine efficacy including vaccine

stability, vaccination intervals and whether serotype O is similar to vaccine and or not to come up with effective vaccine strategies.

INTRODUCTION

1.1 Background information

FMD is endemic in Kenya and most parts of Africa and this creates a constant danger to regions free of the disease (Alexandersen & Mowat 2005). This endemic nature create waves of virus spread as different serotypes get incorporated and infect susceptible populations and spread to other regions. One serotype may persist in a region producing mild signs in young stock. Severe clinical disease may appear when a new serotype is introduced in the region and this has increasingly led to outbreaks being reported throughout the year (Kitching and Alexandersen, 2002).

FMD is caused by foot and mouth disease virus (FMDV) which exists as 7 immunologically distinct serotypes namely O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3. Within these serotypes, there exist a wide range of different strains (Knowles *et al*., 2003). This is caused by low fidelity of RNA polymerase and the ability of the genome to accommodate considerable amounts of mutations (Jamal *et al*., 2011).These serotypes are clinically indistinguishable with no cross-protection following infection or vaccination. These genetic and antigenic heterogeneity pose a major challenge for disease prevention by vaccination (Bai *et al*., 2011).

In Kenya, the most common control measures of FMD during outbreaks typically consist of imposition of quarantine in affected areas, restriction of animal movements and ring vaccinations (Asseged, 2005).During vaccinations, vaccine matching tests are not commonly done in the region due to scarcity of detection and characterization tests. Therefore, vaccination is usually done with 2-4 serotypes in attempt to provide protection (Mumford, 2007). The current vaccine

is made of historic strains and have a narrow antigenic spectrum. This has led to limited and short-term immune protection to animals and outbreaks have been reported even after vaccination (Kitching *et al*., 2007). Evidence of possible vaccine failure due to introduction of new strains was experienced in Kenya during the 2009 and 2010 outbreaks. The isolated field strain was noted to be 10% divergent from the vaccine strain (Chepkwony *et al*., 2012).This points to a possible mismatch between the vaccine strains and the circulating field strains.

In Kenya, the current vaccine in use is formulated as monovalent or polyvalent presented in 50, 100, 300ml vials which was developed in 1964.The vaccine contains chemically inactivated FMD viral strains derived from tissue culture of K 'O' 77/78, SAT 1 T 155/71, A'K 5/80 and SAT 2 K 52/84 adjuvant with saponin and aluminum hydroxide gel with recommended storage temperature at +4℃.Inactivated vaccines are recommended as they do not have the ability to multiply in vaccinated animals. In cattle subcutaneous injections are made at the rate of 3mls subcutaneously and revaccinations should be done every 6 months. For better protection, revaccinations should be done every 4 months (Chepkwony *et al*., 2012).

Occurrence of these outbreaks have been attributed to ineffective vaccine, uncontrolled livestock movement, low vaccination coverage and poor immune response upon vaccination. There is a major concern on development of new strains due to high potential for antigenic and genetic changes. (Sangula *et al*., 2010).

To facilitate new vaccine production and vaccine matching, molecular characterization of FMDV lineages has properly been utilized in developed countries and genome sequences have been used to trace back the outbreaks (Di Nardo *et al*., 2011). The VP1 region have been useful in analyzing molecular relationships and in determining the lineages and topo types. VP1 region information and has been utilized to assign genotypes which occur in a defined geographic

region for all the serotypes as topo types by showing genetic relatedness of the different strains (Jackson *et al*., 2007). This has been used as a tool for FMD surveillance and monitoring for development of new strains and introduction of new serotypes in an area it has never been reported (Knowles & Samuel 2003).

Insight into timely information on circulating serotypes/strains is important when considering an FMD control policy including vaccination programs (Asseged, 2005).The current study aimed at determining the antigenic and genetic characteristics of the field strains and correlating the isolates with the vaccine strains and assess if there was a mismatch.

1.2 Problem statement

Vaccination is the cheapest method of FMD control in endemic settings. This is however challenged by antigenic diversity between and within serotypes since vaccination with one serotype may fail to offer immune protection to another serotype or other genotypes of the same serotype (Paton *et al.*, 2005). New variants of the vaccine are periodically emerging creating antigenic mismatch and this is the main reason for vaccine failure. The virus antigenicity change due to high frequency of mutations especially on the VP1 region of the genome which can evade immunity though vaccinations. This results in emergence of immunologically distinct variants. This shows vaccine strains have to be carefully selected and matched and the need to continually monitor the field isolates and their relatedness to the vaccine (Parida, 2009). Vaccine matching is essential to assess if the vaccine will provide protection against the new isolate and for successful implementation of vaccination based FMD control policy. In Kenya, even though attempts to control the disease through vaccinations has been made, FMD still remain a threat as outbreaks are still happening in the country. This is alleged to be due to unavailability of vaccines of good quality and potency, and/or failure of vaccine matching with the circulating field strains and therefore do not offer protection to new circulating strains (Wekesa *et al.*, 2015).

1.3 Justification

Four FMDV serotypes are currently in circulation in Kenya and continue to cause spontaneous outbreaks despite routine vaccination programs (Sangula *et al.*, 2010). Recent studies have characterized serotype O and advocated for incorporation of a broader range of FMDV isolates for future studies to enhance FMD control in Kenya (Balinda *et al.*, 2010a). Also, detailed understanding of the circulating field strains with major emphasis on antigenic and genetic

characterization has not been exhaustively researched (Bari *et al*., 2014). This study will heighten the knowledge of FMDV antigenic and genetic characteristics in order to select appropriate vaccine strains.

Serotypes A, O, C, SAT1 and SAT2 have been reported in Kenya although type C has been considered nonexistent. Type O has previously been described as the serotype causing majority of the outbreaks in Kenya, followed by serotype SAT 2 and SAT 1. FMDV like other RNA viruses changes and mutates over time, thus one of the limitation in vaccination is the huge variation between and within serotypes. This highlights the need for continuous monitoring (Sangula *et al*., 2010).

Although vaccinations and restriction of animal movement have been applied in Kenya, the adherence to these regulations has not been thoroughly and consistently followed. Continuous monitoring of the antigenic relationships of FMDV isolates to vaccine strains is important in selecting appropriate vaccine strains. For the success of vaccination programs in Kenya, emerging strains need to be monitored (Sangula *et al*., 2010). This study aimed at isolating FMDV strains from field outbreaks, determined the antigenic and genetic characteristics and compared them with the FMDV strains in the current vaccine with the aim of identifying an appropriate vaccine candidate. This was done to aid in understanding why there have been frequent outbreaks despite routine vaccinations and enhance the development of a new vaccine in case there is a mismatch

1.4 Study objectives

1.4.1 General objective

The overall objective of this study was to determine the molecular characteristics of foot and mouth disease virus from field isolates to identify candidate vaccine strains.

1.4.2 Specific objectives

1. To determine the antigenic characteristics of FMDV field serotypes.
2. To determine the genetic characteristics of FMDV field serotypes.
3. To determine the correlation between current vaccine strains and the circulating foot and mouth disease field strains.

1.4.3 Research question

Is there any correlation between the current FMDV vaccine strain and the circulating FMDV field strains?

LITERATURE REVIEW

2.1 Disease history

The history of FMD is dates back in 1546 A.D in the era of Fracastorius Hieronymus in 1546.He reported of a disease that affected cattle near Verona, Italy (Fracastorius, 1546). The disease was later reported in Germany in 1754 by Adami and in Britain in 1839 and later became endemic in Europe and outbreaks were later recorded in Canada and United States for the first time (Henning, 1956). In 1894, Hutcheon recorded an outbreak in South Africa originating from Mashona land and the Northern parts of Transvaal in 1893 (Knowles, 1990). Later, Friedrich Loeffler and Paul Frosch reported that, FMD was caused by a filterable agent. This paved the way for the era of virology as this was the first time a filterable agent was shown to cause disease in animals (Loeffler *et al*., 1897).

In Africa, FMD was first described in 1780. Since then, the disease has remained endemic in most African countries due to evidence of circulating SAT serotypes which are reported to be distinctively adapted in African Buffalo in East, central and South Africa. Apart from Asia 1, all the other serotypes have been described as endemic in Africa (Knowles, 2009).

In Kenya, the disease was first characterized in 1932 and typing results availed since 1954 although the Maasai community was familiar with the disease. Since then, the disease has remained endemic in the country making it a challenge to export of meat and other animal products from Kenya to other countries like USA and European Union (Wariru, 1994).

2.2 Economic significance of FMD

Foot and mouth infections cause huge economic losses to farmers and this has a negative effect to the country's economy. FMD has a wide prevalence, with the virus circulating in approximately 77% of the world livestock population. As such it affects a large number of

animals when an outbreak occurs and infect a wide range of host species, this leads to a huge burden of the disease. Recent studies conducted estimated that, in Nakuru, 1 out of 20 smallholder dairy farms experience outbreak within 6 months period (Nyaguthii *et al.*, 2019). The cost of disease control has been approximated at 2.6 billion doses of FMD vaccine given yearly. The cost of vaccine purchase and distribution is approximated at $0.4 to $, this is dependent on the geographical location. (Sutmoller, 2002).

These losses can be direct or indirect. Direct losses comprise death of infected animals or reduced performance, loss of milk production by up to 80%, loss of draught power and lower weight gain. Hidden impacts of infertility, reduced fertility and abortions lead to need for replacement breeding herd and hence extra cost (Perry *et al*., 2007).

Indirect losses comprise of costs of treatment, control and management of the disease, revenue loss due to limitation of advanced technology, especially the use of improved breeds and improved production systems. Other indirect losses include use of suboptimal breeds after loss of productive animals. FMD has caused a shortfall in market opportunities both locally, regionally and internationally (Delgado *et al*., 2020).

2.3 Etiology of foot and mouth disease.

FMD is caused by FMDV which is an RNA virus of the genus *Aphthovirus,* of the family *Picornaviridae*. *Picornaviridae* family has 46 species categorized into 26 genera, which include genus *Enteroviruses* which encompasses *poliovirus* (PV), *rhinovirus, coxsackievirus and echovirus, Cardioviruses* like encephalomyocarditis and Theiler's viruses), *Hepatoviruses* like (hepatitis A virus (HEV) and *Aphthoviruses* which include foot-and-mouth virus. The word *Aphta* means vesicles in the mouth, which is consistent with the infection (ICTV, 2015).

The virus has seven serotypes reported to be in circulation in Africa namely O, A, C, SAT 1, SAT 2 and SAT 3. These serotypes are immunologically distinct and hence offer no cross protection. Some strains have however been reported to confer immunity to other strains of the same serotype while others do not (Woolhouse *et al*., 1996).

2.4 FMDV morphology and genome structure.

The virus is classified as an *Aphthovirus* of the family *Piconaviridae.* The viruses are small non-enveloped viruses with a diameter of 27 to 30 nm and has an almost spherical capsid assuming an icosahedral shape. The virion is made up of 70% protein, 30% is the nucleic acid and very small amount of lipids. On electron microscopy, FMDV appear smooth with a roundish outline. It has a molecular weight of 8.5*10^5 D and a sedimentation rate of 146S (Costa *et al*., 1984).

The nucleic acid is tightly packaged, non-segmented, single-stranded, positive-sense RNA, the genome size is 7.2-9.0 kilo bases and is made up of approximately 7,500 nucleotides (Cooper *et al*., 1978). Being a genomic RNA virus, replication is prone to errors and hence comprises of quasi-species rather than a defined genomic sequence creating antigenic and genetic diversity. This causes a major challenge in disease prevention, control and diagnosis (Domingo *et al*., 2002).

2.4.1 Foot and mouth disease virus genome

The FMDV Virions are constructed from 60 copies of each capsid coat proteins VP1, VP2, VP3, VP4 and a single copy of genome protein VPg. The genomic RNA is polyadenylated at its 3' end and has VPg protein covalently bonded to 50 end. The virus has 3 functional regions namely the 5' non-coding regulatory region), the protein-coding region categorized as L/Protein 1, Protein 2 and Protein 3, the 3' non-coding region (Belsham, 1993). The 50 non-coding regulatory region

has an S fragment of about 370 residues followed by polyC tract of 100-400 residues then a pseudoknot region which initiates protein synthesis. P1 encodes for the four capsid proteins while P2 and P3 encodes for the non-structural proteins which are responsible for viral multiplication and maturation of the virus (Domingo *et al*., 2002).

Protein 3C (protease) cleaves reactions for polyprotein processing while 2C is a mutation site and catalyzes RNA synthesis processes leading to resistance. 3B encodes for three copies of VPg protein which is bound to 50 terminal and 3D which act as the virus RNA-dependent RNA polymerase (Lopez *et al*., 2002). The 30 non-coding regulatory region has 90 residues and is an interaction site of virus and host cell proteins for RNA replication. Despite the distinctive regulatory and coding regions, it is thought that the coding regions of the genome may also be involved in regulatory functions (Domingo *et al*., 2002).

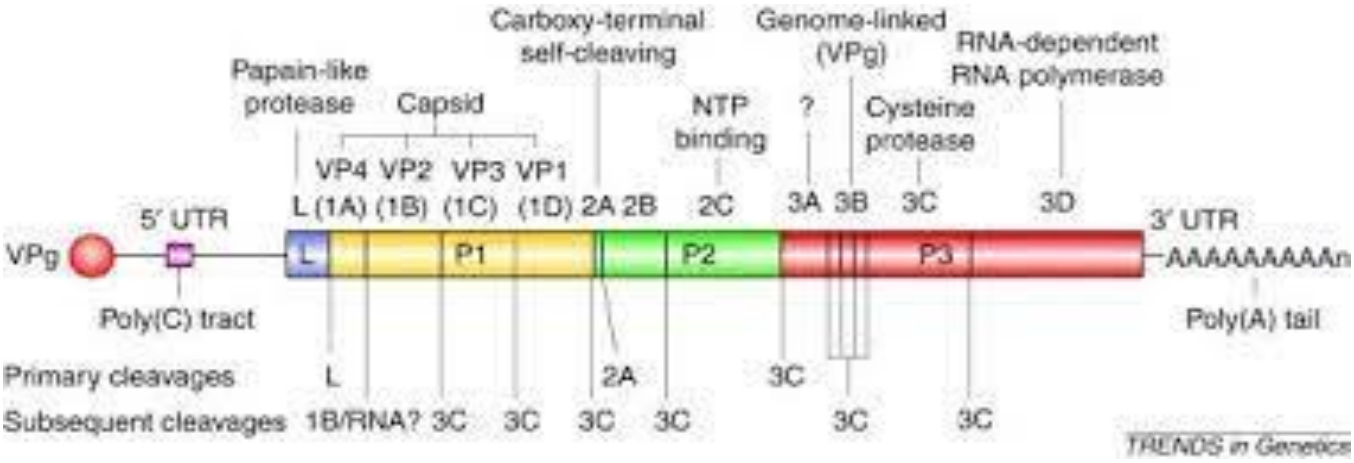

Figure 1.1: Genome layout of FMD virus.

Source: TRENDS in Genetics.

2.4.2 Foot and mouth disease virus structural proteins

All 7 serotypes have distinct strains that exhibits both antigenic and genetic variation, which translates to varying degree of virulence and infectivity (Cooper *et al*., 1978). The outermost capsid protein coat constitutes 60 capsomers comprising of 4 viral proteins (VP) namely VP1, VP2, VP3, and VP4 (John *et al*., 2007).

The capsid is composed of VP1, VP2 and VP3, VP4 is located within the capsid. Viral Protein 1 is the most infective and alteration of VP1 can cause development of new strains of the same serotypes, creating more complex antigenic and genetic variations leading to vaccination failure (Kitching *et al*., 1988). Viral Protein 1, VP2 and VP3 present as wedge-like structures on the surface and has antigen neutralizing sites (N-Ags) which are the sites for binding neutralizing antibodies (Hayon *et al*., 2001). The small VP4 molecules are found inside the virion. The non-enveloped virus capsid harbors the RNA genomes that measures approximately 7.2 to 8.4 kilo bases. It is covalently bonded at the 5′ terminal by phosphodiester linkage to the small virus proteins (VPg) which is made of 22 to 25 amino acids (Acheson, 2011).

Different genetic, immunologic and biochemical tests have identified 4 antigenic sites of the virus (Laporte and Lenoir 1973). Particularly interesting is a major, immunodominant site in the G–H loop of viral protein 1 (VP1) (Cavanagh *et al*., 1977). A remarkable feature of the G–H loop is that it is protrudes to the surface forming up to 54% of the virus surface (Strohmaier *et al*., 1982). G-H loop act as cell receptors for the virus and in antibody binding (Domingo *et al*., 2002). The VP1 sequence analysis is utilized as a tool in assessing the genetic similarity with other FMD viruses and therefore, estimate the possibility of a vaccine to offer immune protection after vaccination as most of the vaccines target the VP1 region (v) (Cavanagh *et al*., 1977).

2.4.3 Foot and mouth disease virus accessory proteins

The VP-g is followed by a long 5′ non translated region (NTR) made up of 500 to 1200 nucleotides containing replication signals and the internal ribosomal entry site (IRES). The IRES is followed by one large open reading frame (ORF) encoding for the polyprotein and finally a short 3′ NTR made up of 30 to 650 nucleotides with a poly A tail. The genome is arranged in 1ABCD-2ABC-3ABCD units. (Acheson, 2011).

There are 4 cleavage products formed after translation. This include the L protease amino terminal (Lpro), 2BC and P3 which are the capsid protein precursors. They make the non-structural proteins after cleavage (Belsham, 1993). Leader protein (Lpro) is a protease which is bound to the N end (Ryan *et al*., 1991).

The function of 3C protease is to break P1 into 1AB (VP0), 1C (VP3), and 1D (VP1). Cleavage of 1A/1B (VP4/VP2) happens at a later stage of virus formation process and is involved in virus capsid maturation. 2C/3A cleavage is facilitated by 3Cpro and this leads to production of intermediate proteins and mature proteins (Newman *et al*., 1994), (Capozzo *et al*., 2002). 2A/2B junction cleavage is facilitated by 2A peptide by separating P1 and itself from 2BC/P3 (Belsham, 1993). This change does not dependent on L and 3C and the resulting 2A region is short (approximately 18 amino acids). This region has the ability to mediate cleavage of its C terminal end (Donnelly *et al*., 1997).

2.5 Foot and mouth disease virus serotypes in Kenya.

Six serotypes have been reported to be in circulation in Africa namely except Asia type 1. The serotypes are genetically classified according to individual VP1 sequence (Knowles *et al*., 2003). Different subtypes have evolved within these serotypes (Pereira, 1977). The serotypes are

immunologically distinct and hence offer no cross protection. Out the seven, five serotypes has been reported in Kenya, this include O, A, SAT1, SAT2 and C (Kitching *et al*., 1988) and multiple topotypes of the serotypes are in circulation. These are O/EA-2, O/EA-3, O/EA-4, A/AFRICA/G-I, A/AFRICA/G-IV, SAT1/I, SAT2/IV, and SAT2/VII. Recovery from FMD infection and vaccination with one of the serotypes does not offer immune protection to other serotypes and sometimes this protection may not give protection within the same serotype. Some strains have been reported to confer immunity to other strains of the same serotype while others do not, this is caused by antigenic and genetic variation with differing virulence. This creates a complex situation in FMD control (Woolhouse *et al*., 1996).

In a recent study in Kenya, The VP1 coding regions of the current viruses isolated between 2014 and 2016 were identical to those of previously published VP1 sequences. The SAT1 strain nucleotide sequences were 88.3%, 94.5%, 92.1% and 92.3% identical while SAT2 were 92.3% and 94.1% identical. Considering the widespread endemicity and rapid mutation rate of FMDV, maintaining recent near-complete references is critical for understanding the regional molecular epidemiology (Palinski *et al*., 2019).

Serotype A was first recorded in Kenya in 1952. In Kenya, the genetic diversity of serotype A has not been extensively studied as compared to other circulating serotypes in the country. In a study, serotype A in Kenya was reported to be the most diverse of all the other serotypes. This diversity was attributed to the use of different type A vaccines namely; K35/1980, K5/1980, K179/66 and K18/71 (Wekesa *et al*., 2015). The study reported four previously genotypes (G-I, G-III, G-VII and G-VIII), within the Africa topo type and a sub lineage from G-I. Genotypes G-III and G-VIII that were first isolated in 1964 are now considered nonexistent. G-VII was last

reported in 2005, G-I is currently in circulation together with the new lineage (Wekesa *et al*., 2015).

2.6 Physical and chemical properties of FMDV

FMDV is fairly stable in the environment and endures longer at cold temperatures and hence can persist for months in frozen, chilled or refrigerated state. The virus is however inactivated by temperature above 50 ℃ for at least 30 minutes. It can survive and remain infective for a significant period, days to weeks in organic matter and up to a month in contaminated environment and fodder depending on environmental temperatures. The infectivity of FMDV is not significantly affected by sunlight and hence the virus from carcass organs can remain infective for some time as the pH does not sufficiently decline after death (Alexandersen *et al*., 2003). The virus is vulnerable in both low and high pH but is viable between pH 6.0 to 9.0. The virus survives for a long period in frozen raw meat and for 15 seconds at pH 4.6 in milk and milk products. In dry areas, the virus can survive for long in proteins of epithelial fragments. (OIE, 2016).

The virus is susceptible and rendered inactive in 2% sodium hydroxide, 4% sodium carbonate, 0.2% citric acid, 2% acetic acid, 3% sodium hypochlorite, 1% potassium peroxymonosulfate, 1% sodium chloride and chlorine dioxide. However, it is resistant to common disinfectants including alcohol, quaternary ammonium compounds (QAC) and phenolic compounds more so in organic material (Sahle, 2004).

2.7 Distribution of FMDV in the world

Foot and mouth disease has a worldwide distribution excluding North America, Australia, New Zealand, Greenland and Western Europe. Any region has an FMD status either as epidemic,

endemic (outbreaks), sporadic or free. Through imposition of stringent actions especially on trade restrictions of animals and animal products from endemic regions, most developed nations has managed to successfully eradicate and control the disease (Kitching, 1999). According to (OIE 2012), 36 countries has acquired FMD free status but remains endemic in Africa, Asia, Middle and Far East and some parts of South America despite routine and continuous use of FMDV vaccine in vast areas. Effects of FMD are felt regionally and globally due to its transboundary nature (Asseged, 2005).

2.8 Epidemiology and distribution of FMDV in Africa

The occurrence of foot and mouth disease has been reported in single occurrence or outbreaks. It is considered endemic in Kenya and in most of the developing nations. The disease is characterized by high morbidity rate of up to 100% in susceptible herds and low mortality rates especially in adult livestock. Young stocks have been reported to have a high mortality (Musser, 2004). FMD sero prevalence in cattle is 54% at herd level in Ethiopia (Bayissa *et al*., 2011) and 52.5% in Kenya (Kibore *et al*., 2013).

2.9 Distribution and occurrence FMDV in Kenya.

In Kenya and other East Africa countries, five serotypes have been reported. Serotype O has been reported to be the dominant serotype causing outbreaks in the country followed by SAT 2, SAT1 and A respectively. Majority of the Kenyan outbreaks has been demonstrated to be caused by serotypes O and SAT2. Serotypes A and C outbreaks has been reported to occur at a lesser frequency. Actually, only 1 outbreak has been reported involving serotype C since 2004 (OIE, 2009).

According to (Sangula, 2006), an increment of SAT1 and SAT2 outbreaks were reported. The rise was directed to presence of a new strain in Transmara district and had spread to other areas causing major outbreaks in vaccinated animals in central Kenya. Phylogenic reconstruction analysis of the strain revealed a 10% antigenic variation from the vaccine strain, although the strains were not matched with the vaccine to confirm (Chepkwony *et al.*, 2012).

According to 2007 FMD laboratory annual report, all the serotypes are present in the country with Rift valley province experiencing high serotype prevalence rate between (2001-2007) followed by Central, Eastern, Nyanza, Nairobi and Coast. Continuous transmission due to constant movement of animals especially the pastoralist livestock in East African region is common. These form an FMD maintained ecosystem is due to wildlife-livestock interactions, constant movement of animals, and existence of robust livestock market creating stock routes from outside into the country. This include the Kenya-Tanzania Maasai border and the Kenya-Somali boarder (FAO/AU-IBAR workshop, 2006).

Sero-prevalence of FMD is significantly higher in adult cattle > 2 years compared to young calves < 1 year. This is attributed to high exposure risk and animal movement in search of water and pasture in the pastoral communities. This increases chances of domestic and wildlife interaction particularly the African buffalo. In the highland areas, adult dairy cattle are kept in the farms for a longer period for milk. This increases chances of FMD and especially if the farm had had the disease previously, this make them chronic carriers and keep spreading the virus. Calves are reared around the homesteads and this minimizes chances of contracting the disease (Kibore *et al*., 2013).

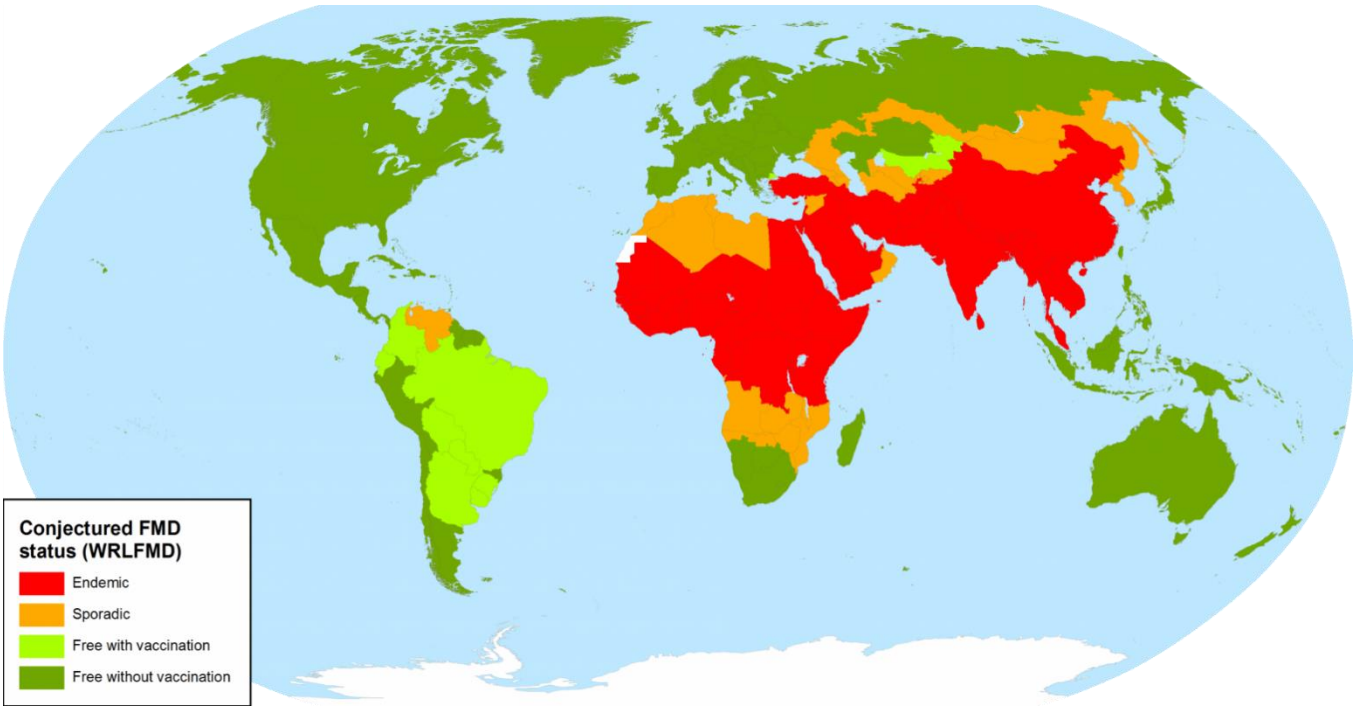

Figure 2.2: World map showing global conjectured Foot and Mouth disease status.

Source: https://www.wrlfmd.org/foot-and-mouth-disease/occurrence(2020).

2.10 Transmission and spread of FMD

FMD is extremely transmissible, contagious and consequently only a small number of the viral particles is required to cause infection in susceptible hosts (Sellers *et al*., 1971). Transmission is through direct contact of naive animal with infected animal, secretions and excretions. FMD virus has been demonstrated in semen, saliva, urine, milk, fecal material, vesicular fluid, fomites and virus contaminated animal products (Sellers *et al*., 1971).

Agricultural tools, human beings, vehicles and air transmission increases chances of mechanical transmission and spread of FMDV (Donaldson *et al*., 1987). Movement of equipment and materials from infected herds/ flocks also accounts for virus spread between contaminated and

uncontaminated environment. Insects and birds have been identified as mechanical vectors of the virus although no biological vectors have been identified (Donaldson, 1997).

Wind/airborne transmission can occur due to huge numbers of infected pigs which results to aerosolized virus in the air. Since cattle inspire more air than shoats and pigs, they are easily infected through breathing. Infected swine aerosolized virus can spread a considerable distance of 20 to 300 km away infecting susceptible cattle and sheep 10 – 100 km away respectively (Alexandersen *et al*. 2003). Pigs become infected by direct contact with vesicular lesions and vesicular fluid of infected animals or consuming food contaminated with the virus. (Thomson, 1996).

2.11 Risk factors for FMD transmission and spread

The virus affects more than 70 domestic species and wild *Artiodactyla* and therefore faster spread of the disease. The epidemiology of FMD is not very well understood. This is affected by different factors (Nishiura *et al*., 2010).

2.11.1 Virus factors

These factors include variation in virulence which affect (disease severity, amount of virus released and time taken for viral release), stability of the virus in different environments and chances of virus to persist for a long time. FMD problem in East Africa is intensified by the existence of multiple strains within and between serotypes (Smith *et al*., 2014).

The genomic RNA of FMDV is small and extremely susceptible to genetic change and hence high rates of mutations and continuous emergence of new variants. This occurs as a result of RNA replication errors, recombination, host selection and constant evolutionary pressure (OIE,

2013). Even within the serotypes, antigenic drift occurs leading to emergence of new strains. These strains spread from one region to another due to uncontrolled movement via porous national borders and this make control through vaccination a constant challenge (Wekesa *et al*., 2015a).

The RNA-dependent RNA polymerase, VP1 and 3D affect the virulence of the infective strain. VP1 choice for receptors and multiplication on host cell were key factors causing variation in virulence and severity on the host (Nishi *et al*., 2019). Changes in VP1 are highly responsible for genetic and antigenic variation of the virus and has led to development of new subtypes. As such, development of new sub types has been considered a major challenge in FMD control by vaccination. Due to this antigenic drift, it is crucial to match with the vaccine strains (Haydon *et al*., 2001).

2.11.2 Host factors

FMDV replication and spread also depends on host species susceptibility, hosts physiological status, immunological and nutritional status, number of hosts per unit area, livestock contact and movements with other susceptible animals or contact with other animals with ability for mechanical transmission (Nishiura *et al*., 2010).

Severe infection has been reported in exotic cattle, sheep and goat breeds as compared to the indigenous breeds. Cattle FMD outbreaks are among the most critical and highly reported. This has caused delay in the progression of the livestock industry by minimizing livestock outputs and hampering trade of animal and their products (Thomson, 1996).

The species infected affects how the virus aerosolizes and distance the virus can move e.g. pigs release more infective virus through exhalation than cattle and sheep (Thomson, 1996). Infected

swine excrete enormous amounts of the virus through exhalation up to 3000 times more compared to sheep, goats and cattle, encouraging spread of aerosolized virus to susceptible animals, environment contamination, equipment and formites. Therefore, pigs are deemed as main amplifiers of the virus. Other factors that affect the amount of virus shed in the environment include host disease stage and the number and concentration of infected animals (Alexandersen *et al*., 2003).

2.11.3 Environmental factors

Well maintained and decontaminated environment can act as a barrier for virus spread, but when right atmospheric condition for the virus prevail, the environment can act as a ground for virus dissemination and spread (Ferguson *et al*., 2001). Humidity of <55% with low winds encourages virus spread through aerosolization. The virus substantially move over water than over land. Due to this multiple factors, there is high potential for variation and adaptation leading to development of complex evolution patterns (Sobrino & Domingo 2001).

2.11.4 Role of wildlife in FMD spread

There exists a complex epidemiology involving multiple FMD serotypes and topo types in circulation between domestic and wild animals in Africa. These serotypes circulate naturally in a wide range of species (Thomson *et al*., 1996). Presence of a large number of wildlife and lack of implementation of regulations for livestock and wildlife movement in the country and across borders has become a major dynamic in dissemination and transmission of FMD (Bronsvoort *et al*., 2004).

Extensive livestock farming, repeated contact at feeding, watering and collection points, has become risks factors for contact with the wildlife. It is also worth noting that, most of the parks are not properly fenced and this created a channel for immediate disease spread to domestic animals (Wekesa *et al*., 2015b). The African buffalo has been documented to be the only carrier for FMDV serotype SAT and can transmit the virus to other susceptible African buffalos and cattle. Serotypes O, A, C and Asia-1 are not spread to naive animals from carriers (Alexandersen *et al*., 2002).

2.12 FMD pathogenesis, gross and histopathological lesions

Primary replication occurs in the epithelium of the pharynx. Upon infection, FMDV multiplies at the initial entry site, be it the mucus membrane, lymphoid tissues of the upper respiratory tract or in dermis or sub cutis upon of a skin abrasion. This lead to production of primary vesicles, or "aphthae". The vesicles form on epithelial stratum spinosum of cells. Disease outcome vary with the host species infected and the strain involved (Burrows *et al*., 1981).

The virus is spread all over the body as free virus or in conjunction with mononucleated cells, creating a state of viremia 24-48 hours after infecting the epithelium in pigs and cattle. This leads to virus spread to other tissues and organs which causes formation of vesicles in the feet and mouth. High concentration of virus is found in the glandular tissue and the stratum spinosum and secondary replication occurs (Burrows *et al.,* 1981). This acute phase lasts about 7 days, this is consistent with development of strong humoral immunity (Salt, 1993).

Stratum spinosum layer swells up, degenerates and ruptures. Edema fluid fills up forming vesicles which merge together to create a bullae which are typical of the disease. In some instances, the rumen, reticulum and omasum epithelial lining may develop visible lesions

(Kitching, 1992). Infected young animals develop macroscopic greyish areas of degeneration in the myocardium, more so in the left ventricle and appear stripped commonly (tiger heart). Skeletal muscle cells may undergo hyaline degeneration (Kitching, 1992).

2.13 Immune response to FMD

Host response to FMDV infection is through production of antibodies. Usually, the levels of neutralizing antibodies directly correlate with immune protection observed inside the host. This lead to a complex interaction between the immune system and the virus (Jullef *et al*., 2009). Genetically unrelated viruses can be close antigenically where some field strain show high antibody response than the homologous virus. Viral mutations have been shown to affect antigenicity of the virus (Barnett *et al*., 2001).

2.14 Clinical manifestations of FMD

FMD produces an acute, systemic, vesicular disease after primary infection through the upper respiratory system. Clinical signs sometimes differ depending on the serotype and strain involved (Sutmoller, 2002). The incubation period for FMD is usually 2 to 14 days although it can be as short as 24 hours. Manifestation of initial signs depends on amount of virus, host animal species and infection route. Infected animals start shedding the virus before manifestation of any signs (OIE, 2013).

Animals present with anorexia, fever (up to 40℃) lasting one or two days and shivering and reduced milk production (by 80%) in 2–3 days. This is followed by development of vesicular eruptions in the mouth, tongue, palate, dental pads, lips, gums, muzzle, teats, interdigital spaces and coronary band (Kitching, 1992). In a day, the vesicles rupture and recovery occurs in 8–15 days. Hypersalivation and nasal discharge are consistent with development of oral and nasal

lesions respectively. Initially, the discharge is mucoid then changes to mucopurulent due to secondary bacterial involvement (Knight-Jones and Rushton 2013).

Oral lesions rupture quickly leaving shallow erosions surrounded with raw epithelium and may coalesce forming large wounds. Teat lesions pose challenge milking and mastitis might follow as a secondary bacterial infection once the vesicles rupture. Feet lesions may remain intact for 2 to 3 days before rupture depending on floor type. These render animals lame, recumbent and sometimes results in under-run sole and chronic lameness (Musser, 2004).

2.15 Diagnosis of FMD

Definitive diagnosis of FMD infection is paramount in effective control and in the implementation of eradication measures in Kenya and other endemic settings as a way to stamping out policy in FMD free areas. Diagnosis has be done through various serological and molecular techniques. These tests are able to either detect viral antigen, antibody response as a result of an active infection or the viral nucleic acid material (Remond *et al*., 2002).

2.15.1 Virus isolation

Development of susceptible cells has led to a way of laboratory isolation of the virus and a way to study viral characteristics in cells invitro. FMDV has been shown to infect primary cell lines of porcine, bovine and ovine. Other cell lines have been shown to be susceptible to FMDV and are the most commonly used for detecting low amount of infectivity. These include like IBRS-2, MVPK-1 clone 7, LFBK cell line and 5 BHK-21 (House *et al.*, 1988). In a study, FMDV field isolates were passaged in BHK21 monolayer cell cultures and showed characteristic cytopathic effects (CPE) and had considerably adapted in the 3rd and 5th passage. Cytopathic effect

developed within 48 hours and the cells showing no CPE were inoculated on fresh cultures (Goel & Rai 1985).

Epithelial samples collected from outbreaks were confirmed positive for FMDV by RT-PCR test by showing CPE upon passage on BHK21cell culture. The virus has also shown a positive result when RT-PCR was done from tissue culture fluid from primary calf thyroid (BTY) cells monolayers. Inoculated BTY tubes are incubated for 4 days and examined daily for evidence of CPE. Confirmation of specificity is done using antigen detection ELISA (Roeder and Blanc, 1987).

Cell culture is nevertheless time consuming, requires a lot of labor is less sensitive. It is conducted in a biosafety laboratory, other lab cell culture facilities and employs a lot of caution handling the samples and the equipment. Sometimes this can be expensive to acquire and difficult to maintain as it requires technical knowhow (Rweyemamu, 1982).

2.15.2 Enzyme linked immuno-sorbent assay (ELISA).

Techniques for FMDV serotyping techniques have been developed as a result of multiple existing serotypes been reported. Diagnosis is generally grounded on demonstration of the viral antigen and hence used as an important tool for antigenic characterization. Some serological procedures have been used in diagnosis whereby, illustration of high specific antibody titer in unvaccinated animals predicts a diagnosis. Antibody response towards nonstructural proteins 3A, 3D, 3ABC, 2C and Lb predicts an infection as they follow a disease experience. Vaccinated animals demonstrate antibody response towards nonstructural proteins 2B, 2C, 3AB, 3ABC and 3C. ELISA provides a model of detecting specific nonstructural proteins response for all susceptible species (King *et al*., 2012).

The Ag-ELISA kit involves type-specific MAbs, together with FMDV antigen reaction. It is the most user-friendly method for serotyping FMD viruses (Grazioli *et al*., 2020). ELISA is highly specific, sensitive and quantitative and has the advantage of being rapid, less variable, fewer false-positive results, not dependent on tissue culture systems and is able to distinguish between immune antibody response due to infection or vaccination. This is because superior FMDV vaccines are composed of structural protein (SP) viral capsid protein with little or no non-structural capsid proteins (Alexerndersen *et al*., 2000).

During natural FMDV infection, NSP of the virus are expressed eliciting immune response that is detected using diagnostic approaches. During replication, 8 different NSPs are generated which are potential serological targets for diagnostic assays. Antibody response towards recombinant Lb, 2C, 3A, 3D, and 3ABC FMDV NSPs follows exposure to infection while vaccinated animals show an antibody response to NSP especially 3AB, 3ABC, 2B and/or 3C, 2C. ELISA format provide a generic approach to detect NSP-specific responses for all species that are susceptible to FMD (King *et al*., 2012).

2.15.3 Virus neutralization test (VNT).

VNT is specific, sensitive and quantitative and takes two to three days to provide a result. Low titer false-positive results are sometimes anticipated in a small ratio of sera. VN tests are generally conducted in tissue culture grade microtiter plates, employing susceptible cells such BHK-21 or lamb or pig kidney monolayers. Wells showing CPE are considered positive for the virus and neutralization titers are represented as the reciprocal of the final dilution of serum present in the serum-virus mixtures at the 50% endpoint (OIE, 2009).

2.15.4 Molecular techniques

Introduction of molecular methods of diagnosis has made FMD diagnosis easier. These techniques are based on demonstration of a whole or a part of viral gene in suspected samples (Meyer *et al*., 1991).

2.15.5 Polymerase chain reaction

Polymerase chain reaction (PCR) is widely employed technique since its intervention and is based on demonstration of nucleic-acid material. The technique have led to advancement as a number of reverse transcription PCR (RT-PCR) methods for detecting specific FMDV RNA in the recent past (Rodriguez *et al*., 1994).

Due to the high sensitivity and specificity of PCR, this molecular technique have been evaluated and employed as a major diagnostic tool for FMD and as a method of differentiating FMD from other vesicular diseases (Reid *et al*., 1998). Diagnosis by detection of nucleic acid by real time fluorogenic PCR amplification has become a precise way of quantifying the amount of nucleic acid material in a sample. This technique is fast, quantitative and can run more samples compared to conventional PCR (Reid *et al*., 2002).

2.16 Genetic characterization of FMDV

Although basic antigenic characterization for epidemiological studies has been conducted, information on genetic and immunogenic characteristics of these strains is inadequate *(Maradei et al*., 2011). To characterize the strain, FMDV RNA is extracted from epithelial samples and reverse transcription carried out using random primers and reverse transcriptase enzyme. After PCR, the amplified products are purified from 1% agarose gels and the recovered DNA material

is quantified and sequenced. VP1 sequence data comparisons has increasingly become the main method to determine genetic relatedness of viruses (Knowles *et al*., 2016).

2.17 Vaccine matching tests

There are different factors that come into play in immune protection against FMD, the major factor being the matching of field strain to the one present in the vaccine and such, the type of strain used. Currently matching is done through cross reactivity of the vaccinated bovine serum against the vaccine and the field strain (Paton *et al.*, 2005).

Field outbreaks despite routine vaccines is an evidence of inadequately or unmatched vaccine or vaccines of poor quality. Genetic characterization and profiling have been used to show emergence or reemergence of a serotype or subtype in an area which the vaccine might not effectively protect. Once the virus has been serotyped and selected for vaccine matching, *in-vitro* molecular methods can be used to compare genetic relatedness of the field strain and the homologous vaccine strain (OIE, 2006).

2.18 Prevention and control of FMD.

The FMD progressive control pathway (PCP–FMD) tool lay major emphasis on regular monitoring of the circulating serotypes, regular vaccination and enhancing bio-security (Namatovu *et al*., 2013). In Kenya and other developing nations, the control of FMD revolves around vaccinations and control of animal movement since eradication programs are expensive (Nderitu, 1984). To effectively control the disease by the latter method, the vaccines used must contain the representative strains of the serotype in circulation as FMD control has potential to enhance food security, poverty alleviation and national development (Gonzalez *et al.*, 1992).

2.18.1 Foot and Mouth Disease control by vaccination

Due to the contagious nature and high frequency of spread of the virus, no country is considered safe from the disease. Control through regular vaccination with effective vaccine has been employed in different countries even those declared FMD free. There is need for timely reporting of outbreaks for efficient actions to be taken. Control through vaccination can only be realized when the vaccines are effective and administered regularly (Bruner *et al*., 1973).

FMD control in endemic and FMD free countries can be achieved by use of the present conventional FMD vaccines although protection is short lived (~6 months) and need revaccinations for effective control. It is also good to note that vaccination does not prevent development of carrier state. For the vaccine to be effective, there is need for adopting new methods for vaccine strain selection, use alternative methods of vaccine testing and use of new generation efficacious vaccine (Parida, 2009).

2.18.2 Foot and mouth disease vaccine in Kenya

East Africa, has 2 major FMD vaccine producing plants with limited information on the protective value of the produced vaccines. Kenya uses historic viruses for vaccine production and vaccine matching tests are rarely carried out. In Kenya, the current vaccine in use is formulated as monovalent or polyvalent presented in 50, 100, 300ml vials. It contains chemically inactivated FMD viral strains derived from tissue culture of K 'O' 77/78, SAT 1 T 155/71, A'K 5/80 and SAT 2 K 52/84 adjuvant with saponin and aluminum hydroxide gel with recommended storage temperature at +4℃. Potency of the vaccine is tested in cattle to guarantee at least 6.6 PD 50 (50% protective dose) (Chepkwony *et al*., 2012).

2.18.3 Implications of FMD vaccine in Kenya

New variants within and between serotypes continue to arise increasing the antigenic and genetic diversity of subtype. This limits the vaccines ability to cross protect within and between serotypes. This is caused by the virus mutations which happens due to inability of enzyme RNA polymerase to proof read the strands. This has confounded efforts to develop vaccines that can cross protect (Richard *et al*., 2016).To effectively control the disease by vaccination, the vaccines used must contain the representative strains of the serotype in circulation as FMD control has the ability to improve food security, alleviating poverty and encourage development (Gonzalez *et al.,* 1992). Another major factor is the potency/strength of the vaccine. This factor is influenced by antigenicity of the strain used, antigen quantity and quality and other formulations added to make up the vaccine (OIE, 2006).

2.19 Livestock migratory corridors and stock routes in Kenya.

Existence of stock routes all over the country ensures constant maintenance of FMD. This is because animals sourced from the region are introduced without proper documentation and screening. These animals some of who are diseases are trekked long distances and this ensures dissemination of the virus through saliva and feces. This form of migration happens in search of livestock market where there is huge demand for beef. The chain is organized in a way that, animals are trekked from pastoral areas to primary or secondary markets. From these markets, animals are trucked or trekked to terminal markets most of which terminate in Nairobi and Mombasa (Kibore *et al*., 2013).

This movement has created three major corridors (Southern, Northern and North Eastern) of migration all over the country and extend to the neighboring countries. The North Eastern corridor comprise of six routes where three routes serve animals from Ethiopia border to Nairobi and Mombasa. The other three routes serve from Mandera, Somalia and Wajir through different routes and terminate in Nairobi and Mombasa. The Northern corridor has two routes that serve from Samburu and Turkana through different primary and secondary markets and finally terminate in Nairobi. The southern corridor has two routes that serve from Migori and Narok and terminate in Nairobi and Mombasa respectively through several primary and secondary markets (Kibore *et al*., 2013).

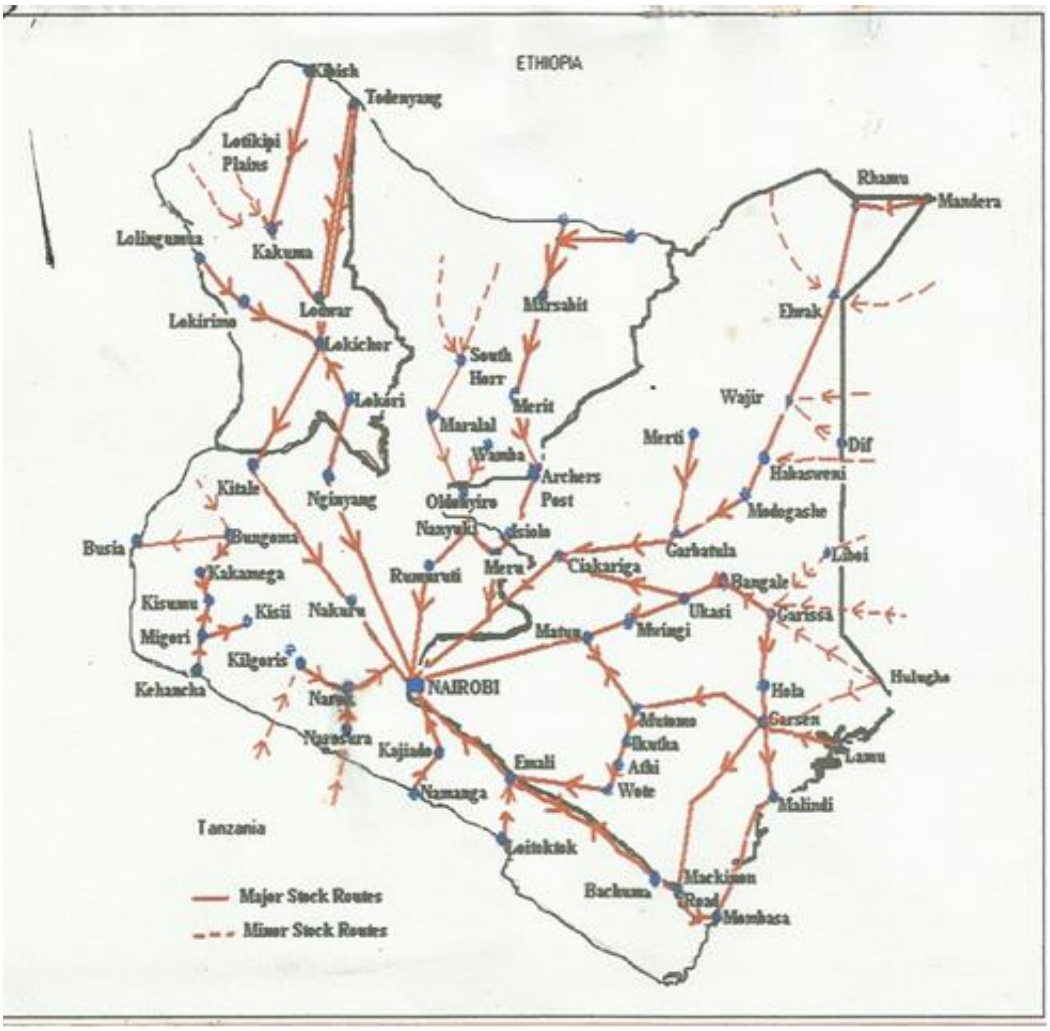

Figure 2.3: Map of Kenya showing livestock routes into the country.

Source: Source: AU-IBAR & NEPDP (Kenya livestock sector study, 2006)

2.20 Factors limiting FMD control in Kenya.

Foot and mouth control is surrounded by complex dynamics which should be considered for effective control. The virus contagiousness is very high and is endemic in most African countries hence it is widely distributed. These makes the virus spread fast and maintain itself in the environment. The virus has a wide host range and has the ability to establish carrier state in susceptible animals. The virus has a wide antigenic diversity leading to no or poor cross

protection. Immunity acquired through infection or vaccination is also short lived. There is poor or no surveillance and diagnostic facilities in the country and control measures are poorly implemented (Tamilselvan *et al*., 2009).

Kenya border lines are inhabited mostly by pastoralists creating porous borders between Eastern African countries. The pastoralists have close linkages with their counterparts from the neighboring countries and this facilitates cross border movement of people and livestock in search of water and feed. This creates a vibrant and flourishing livestock market at the borders (Kibore *et al*., 2013).

Presence of these robust animal market creates demand and hence cross border animal movement in search of market. The animals trek for long distances creating stock routes. Existence of these stock routes act as dissemination routes for FMDV through excretions and saliva. These routes include those from North Eastern, Southern and Northern corridors and mostly terminate in Nairobi or Mombasa. This ensures constant maintenance of transboundary diseases including FMD and spread of new serotypes and /or strains in the region (Kibore *et al*., 2013).

Social norms especially in the pastoral areas complicate restriction of animal movements. These practices include pastoralism, communal grazing and use of livestock as a mode of dowry payment, dowry and animal movements across borders. Wildlife especially the African buffalo have been shown to act as maintenance host the virus and therefore, lack of fences around vaccination zones and national parks between Eastern Africa countries ensures constant transmission of the disease (Namatovu *et al.*, 2013).

Low availability and affordability of vaccines especially in remote rural areas and existence of different circulating virus serotypes and strains that do not match the vaccine strains complicate

the control of the disease. Vaccine impurity as presence of NSP traces in vaccine may cause false positive reaction to animals. Climatic disasters like drought, floods and wars that lead to displacement of people and their livestock make vaccination a challenge. Poor legislation and law enforcement, misdiagnosis and poor participation and cooperation from communities in disease control. In addition, poor infrastructure in the remote rural areas, limited disease reporting and surveillance and lack of local, regional and international collaborations in disease control, prevention and eradication. Also, delivery of the vaccines and veterinary services in the horn of Africa is hampered by civil wars from time to time. Poor vaccine potency as a result of poor cooling systems for the vaccines in the field (Chepkwony *et al*., 2012).

MATERIALS AND METHODS

3.1 Study area

This study was conducted in 27 Counties in Kenya. Kenya has 7 agro ecological zones categorized as I, II, III, IV, V, VI, and VII. Zone I to IV are non- pastoral areas while zones V to VII are the pastoral zones and form the arid and semi-arid areas of the country where most of the samples were collected. The ASALs constitute 80% of the Kenyan landmass and houses approximately 20% of its human population. The economy of the ASALs is supported by livestock production and supports 90% of the human population. Kenya borders Tanzania to the South, Uganda to the West, Somalia to the East, Ethiopia to the North and South Sudan to the North West.

3.2 Sample size determination

This study utilized samples submitted in the year 2019 and 2020 to Foot and Mouth disease laboratory, Embakasi for foot and mouth disease screening. The samples were collected from outbreaks and suspected clinical cases of FMD from different counties in Kenya. 110 samples had been sent to the lab for analysis during the study period.

The counties which formed the focus of this study were Nakuru, Meru, Laikipia, Nyandarua, Isiolo, Tharaka Nithi, Machakos, Samburu, Siaya, Marsabit, Baringo, Taita Taveta, Makueni, Kajiado, Nairobi, Kwale, Uasin Gishu, Narok, Bungoma, Kakamega, Nandi, West Pokot, Bomet, Transzoia, Kericho, Embu, Nyeri. These counties were purposively chosen based on different criteria which included presence of livestock markets, presence of wildlife-livestock interfaces, presence of national parks, existence of livestock movements, presence of stock routes and the level of disease abundance determined by disease prevalence as shown in table 3.1.

Table 3.1: Criteria for County selection and the disease prevalence.

County	% Prevalence	Livestock Markets	National Parks	Wildlife Livestock Interfaces	Stock Routes	A	SAT 1	SAT 2
Nakuru	22.7	-	✓	✓	✓	✓	-	✓
Meru	35	-	✓	✓	✓	-	✓	-
Laikipia	49.2	-	✓	✓	✓	-	✓	-
Nyandarua	37.5	-	✓	✓	✓	-	✓	-
Isiolo	33.9	-	-	✓	✓	-	✓	-
Tharaka	40	-	-	✓	-	-	✓	-
Machakos	43.2	-	-	✓	✓	✓	-	-
Samburu	40	-	✓	✓	✓	-	-	✓
Siaya	62.1	-	-	-	-	-	✓	✓
Marsabit	18	-	✓	✓	✓	-	-	✓
Baringo	100	-	-	✓	-	-	-	✓
Taita Taveta	40.2	-	✓	✓	-	-	-	✓
Makueni	26.8	-	-	✓-	✓	✓	-	-
Kajiado	67.6	✓	✓	✓	-	-	-	✓
Nairobi	-	✓	✓	-	✓	-	-	✓
Kwale	42.2	-	-	✓	✓	-	✓	-
Narok	90.4	✓	✓	✓	✓	-	✓	-
Bungoma	100	-	-	✓	-	-	-	✓
Kakamega	100	-	✓	✓	-	-	✓	-
Nandi	100	-	-	-	-	-	✓	-
West Pokot	100	-	-	✓	✓	-	✓	-
Bomet	70	-	-	-	-	-	-	✓
Transzoia	100	-	✓	✓	✓	-	✓	-
Embu	82.9	-	-	-	✓	-	✓	-
Nyeri	5.3	-	-	-	✓	-	✓	-

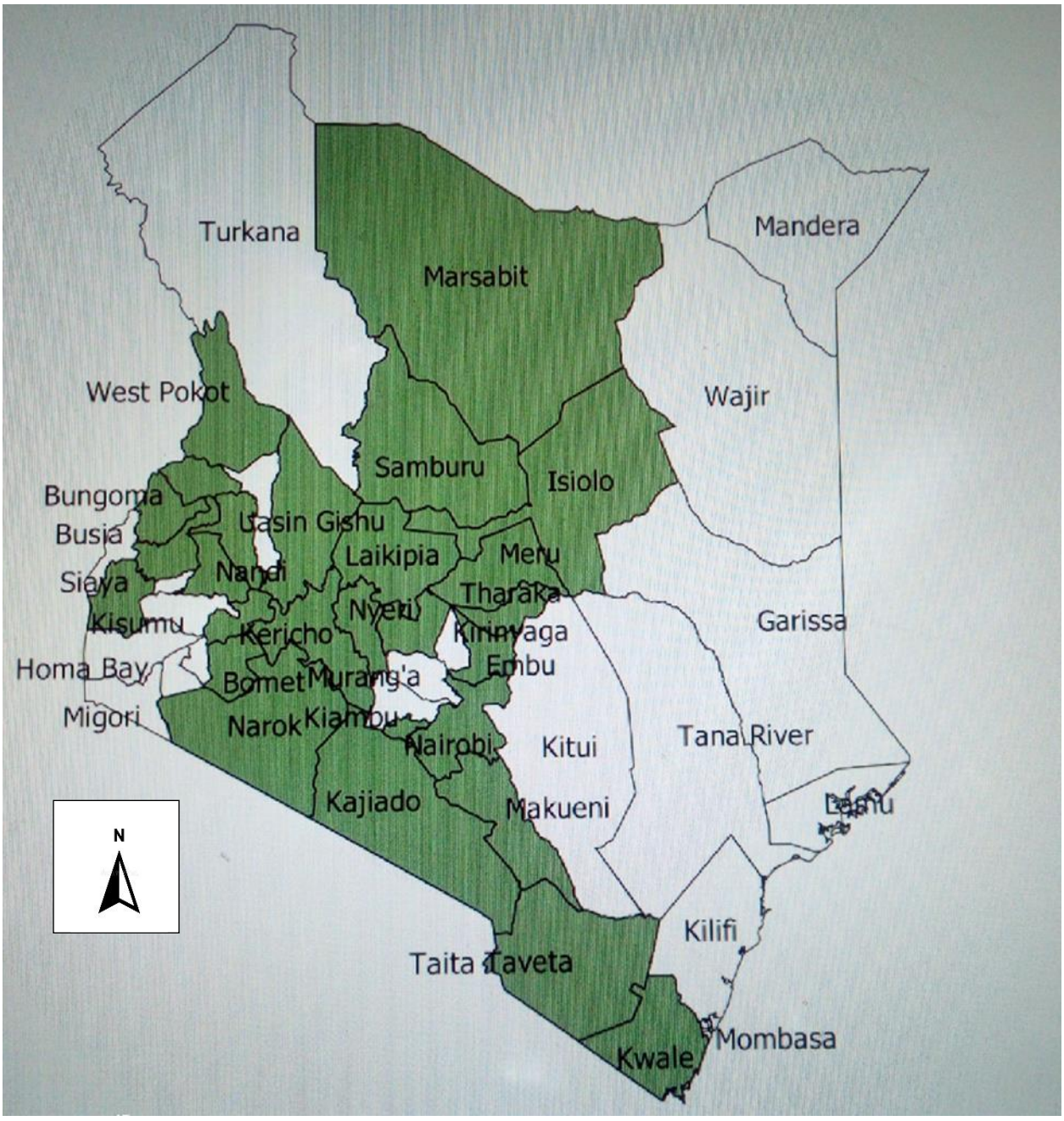

Figure 3.1: Map of Kenya showing Counties (shaded) forming the focus of the study.

3.3 Sample collection and preservation

The study utilized 110 samples submitted from the field upon FMD disease recognition by clinical signs. For laboratory diagnosis, epithelial tissue were the samples of choice. Routine clinical cases of foot and mouth disease are sampled and samples put in transport media composed of 50% glyceral, 0.04M phosphate buffer, antibiotics (penicillin 1000 I.U, neomycin sulphate 100 I.U, mycostatin 100 I.U and polymixin B 50 I.U). pH is maintained at 7.2-7.6 by adding adding phenol red indicator dye. The samples are kept at low temperature and transported on ice to FMD laboratory, Embakasi. This is done following OIE protocol.

3.4 Virus isolation and determination of FMDV antigenic characteristics of the field serotypes.

110 epithelial samples were received at the laboratory and recorded on submission book and kept refrigerated at 4℃ until they were processed.

3.4.1 Laboratory preparation of epithelial tissues

The epithelial samples were removed from the media and dried on a blotting paper to remove glycerol which could otherwise be toxic to cell cultures. The samples were weighed and the weight recorded. A suspension was prepared by thoroughly but carefully grinding the sample in sterile sand using pestle and mortar. A small volume of antibiotics, tissue culture media were added to make a 10% suspension. The suspension was mixed thoroughly and clarified through centrifugation at 2000g for 10 minutes. The tissue suspension was collected in sterile universal bottles and the sediment discarded. This procedure was repeated for all the samples.

3.4.2 Virus inoculation in tissue culture

The virus was cultured and isolated from BHK-21 cell line. This cell line has been shown to increase susceptibility of the virus enhancing the rate of virus multiplication (LaRocco *et al.*, 2013). This procedure was carried out as previously described (OIE, 2018).

In brief, 500 µL of the suspension was inoculated with 500 µL of MEM (added with L-glutamine 2 mM, penicillin 75 µg/mL, and streptomycin 100 µg/mL) on BHK-21 monolayer and incubated at 37 ◦C for 1 h. The inoculum was aspirated and the flasks without the inoculum were incubated with 5% carbon dioxide, 10 mL of fresh MEM (added with L-glutamine 2 mM, penicillin 75 µg/mL, and streptomycin 100 µg/mL) and 3% fetal bovine serum.

The cell cultures were monitored every 12 hours under an inverted microscope for cytopathic effects (CPE). Where CPE was not detected, the cells were inoculated on fresh cultures and incubated for 48 hours and monitored every 12 hours for CPE. Cells showing 70-100% CPE were harvested and serotyping was done using antigen detection ELISA.

3.4.3 Antigen detection Enzyme- Linked Immunosorbent Assay (ELISA) and serotyping

Isolates that had CPE positive were forwarded for FMDV serotyping. Detection and serotyping of FMDV by Ag-ELISA was carried out using FMDV antigen detection and Serotyping ELISA Kit (IZSLER, Brescia, Italy and TPI, Pirbright, UK). This included detection and typing of serotypes O, A, SAT1 and SAT2 following manufacturers' instructions. A pan FMD test, detecting any isolates of serotypes O, A, SAT 1 and SAT 2 was included in the kit to complement the specific typing and to detect FMD viruses which might have escaped binding to selected serotype-specific MAb.

Briefly, 250 µL of original tissue homogenates or cell culture supernatants were diluted at a ratio of 1:2 in the specific dilution buffer and incubated for 1 h at room temperature. Into one row of the ELISA plate, 50 µL per well, pre-coated with selected type-specific capture MAbs and one additional pan-FMDV MAb was added. The same pan-FMDV MAb conjugated to peroxidase (Conjugate A) was used to complete the detection and typing of serotype A. A mix (Conjugate B) comprising one SAT1, one SAT 2, and one cross-reactive MAbs was used for the detection and typing of SAT 1 and SAT 2 serotypes. The two Conjugates were incubated for 1 h at room temperature, after which the reaction was developed using TMB (3,30,5,and 50 - tetrametilbenzidina) as substrate. After the addition of a stop buffer (0.6N H_2SO_4), optical density was measured at 450 nm using an ELISA reader. Washes between each step were performed.

The results were determined by the reading of the optical density (OD) and interpreted as follows.

- Optical density (OD) < 0.1 was interpreted as negative for FMDV.
- OD ≥ 0.1 with the pan-FMDV catching MAb and < 0.1 with the type-specific MAbs was interpreted as positive for FMDV untyped.
- OD ≥ 0.1 with at least one of the two type A MAbs and with the pan-FMDV MAb denoted a positive result for serotype A.
- OD ≥ 0.1 with the type SAT 1 catching MAb; some samples could be positive also with the pan-FMDV MAb. This denoted a positive result for SAT 1.
- OD ≥ 0.1 with the type SAT 2 catching MAb; some samples could be positive also with the pan-FMDV MAb. This denoted a positive result for SAT 2;

OD ≥ 0.1 with the type O MAb and with the pan-FMDV MAb denoted a positive result for serotype O.

3.4.3.1 Sample preparation

The plate was properly numbered, well aligned and 50ul of each sample distributed into 8 wells of a column. Another 50ul of the diluent buffer was put in wells of column 11 and 12 of the plate and incubated for 1 hour at 18-30℃.

3.4.3.2 Washing

The wells were emptied and tapped to remove any residual fluid. Each well was filled with 200ul washing solution and incubated for 3 minutes at room temperature. This washing cycle was repeated 3 times and residual fluid removed from the plate from tapping firmly onto a clean absorbent paper.

3.4.3.3 Conjugation

A volume of 50ul of diluted conjugates was appropriately added to each well. Conjugate A was added into rows from A to F and conjugate B was added into rows G and H. The plate was covered and incubated for 1 hour at room temperature. The plate was washed as above leaving the last one for 5 minutes.

3.4.3.4 Substrate/chromogen solution

A volume of 50ul of substrate/chromogen solution was added into each well, equilibrated at room temperature. The plate was covered and left at room temperature for 20 minutes in a dark drawer. Blocking was done to stop the reaction by adding 50ul of stop solution in each well. The plate content was mixed thoroughly before reading.

3.4.3.5 Reading

After blocking, the plate was placed in a photometer to initiate the reading sequence. Reading was done at 450nm wavelength on a spectrophotometer ELISA microplate reader. The software was used to automate the reading of optical density (OD) values.

3.5 Determining the genetic characteristics of FMDV field isolates.

3.5.1 RNA extraction

The genomic viral RNA was extracted from 110 inoculants showing CPE in cell cultures using PureLink™ Viral RNA/DNA Mini Kit following manufacturer's instructions. In brief, the lysate was prepared by adding the lysis buffer, carrier RNA to proteinase K. 96-100% ethanol was added and loaded onto the spin column. This was followed by washing the column twice with wash buffer and eluted with RNase free water into a recovery tube.

3.5.1.1 Preparation of the lysate

To prepare the lysate, the previously frozen samples were thawed at room temperature and 200ul of cell free sample was added into a sterile micro centrifuge tube with 25ul proteinase K to make up to 225ul starting material. 200ul of lysis buffer (containing 5.6ug carrier RNA) was added and mixed by vortexing for 15 seconds and incubated at 56℃ for 15 minutes in a water bath. This was followed by brief centrifugation at 6800g for 1 minute to remove any droplets that could be inside the lid.

3.5.1.2 Binding and washing

A volume of 250ul of 96% ethanol was added, mixed and incubated at room temperature for 5 minutes. This facilitated binding of RNA to the membrane. The tube was centrifuged at 6800g for 1 minute to remove any drops from inside the lid. The lysate with ethanol was then

transferred into the viral spin column, centrifuged at 6800g for 1 minute and the flow though discarded with the collection tube.

The first wash was done by adding 500ul wash buffer (WII) with ethanol to the spin column placed in a 2ml clean wash tube and centrifuged at 6800g for 1 minute. The flow through was discarded and the spin column placed on the same wash tube. This wash procedure was repeated again but this time, the wash tube holding the flow through was discarded.

The spin column was placed in a 2ml clean wash tube and centrifuged at 10000g in a micro centrifuge for 1 minute to completely dry the membrane. The wash tube was removed from the centrifuge and discarded together with the flow through. The spin column was placed in a 1.5ml recovery tube.

3.5.1.3 Elution

A volume of 50ul of sterile RNase free water (E3) was added to the center of the column, closed the lid and incubated for 1 minute. The column was centrifuged at 10000g for 1 minute and the purified viral RNA recovered in the recovery tube. The spin column was discarded.

3.5.1.4 RNA quantification

The concentration and purity of viral RNA was quantified in (ng/ul) using the NanoDrop Resolution Life Science PC Software. To calibrate the Bio-Drop machine, 1.5 ul of the RNAse free water was placed on the machines inbuilt sample port to read at zero (0ug/ul). In this case, RNAse free water was used as the calibrating media since it was used as the RNA eluting solution during RNA extraction. This was repeated for all samples, results read and recorded. RNA was aliquoted into 25ul volume and stored at -80℃ awaiting cDNA synthesis.

3.5.2 cDNA Synthesis

The first strand complementary DNA was done on 30 purposively selected samples. This was done using the first strand cDNA synthesis Invitrogen™ superscript ™ II reverse transcriptase kit in following manufacturer's instructions. The kit has increased thermal stability and reduced Rnase H activity. The superscript™ II reverse transcriptase enzyme can be used to synthesize first strand cDNA at higher temperatures providing increased specificity, high yields of cDNA and more full length product and generated cDNA of up to 12.3kb.

Briefly, for each reaction, 4ul of superscript 5x cDNA synthesis buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15mM MgCl2), 1ul of dNTP mix (1omM each), 1ul of random hexamer primers (250ng), 8ul of total RNA, 2ul of sterile distilled water, 1ul (200 units) reverse transcriptase, 1ul of RNase inhibitor (40 units/ul), 2 ul 0.1M DTT in 2 mixes to make a total of 20 ul per reaction were prepared.

3.5.2.1 Annealing

On ice, mix 1 was prepared by adding the random hexamers, total RNA, dNTP mix and distilled water were mixed in a nuclease free tube, mixed and drops collected by brief centrifugation. The tubes were then incubated at 65℃ for 5 minutes then quickly chilled on ice to stop the annealing.

3.5.2.2 Reverse transcription

Mix 2 was prepared by adding the buffer, DTT and Rnase OUT into the tube. The content was mixed gently and incubated at 25℃ for 2 minutes. The reverse transcriptase enzyme was added and mixed gently by pipetting and incubated at 25℃ for 10minutes, followed by another incubation at 42℃ for 50 minutes.

3.5.2.3 Inactivation

The reaction was inactivated by heating at 70℃ for 15minutes then left for 5 minutes at 4℃ before removing it out of the machine and chilled on ice. This was repeated for all the samples and the cDNA products were quantified before proceeding to polymerase chain reaction.

3.5.2.4 Product quantification

The concentration and purity of cDNA were quantified in (ng/ul) using the Nano-Drop Resolution Life Science PC Software. To calibrate the Bio-Drop machine, 1.5 ul of the elution media (RNAse free water) was placed on the machines inbuilt sample port to read at zero (0ug/ul). In this case, RNAse free water was used as the calibrating media since it was used as the RNA eluting solution during RNA extraction. This was repeated for all samples and results read and recorded. The cDNA products were then stored at -20℃ awaiting polymerase chain reaction (PCR) for FMDV screening.

3.5.3 RT-Polymerase Chain Reaction to detect FMDV RNA

RT-PCR was performed using Platinum® TaqMan Universal 2X PCR Master Mix (PE Biosystems). The TaqMan PCR was run following manufacturer's instructions using the FMDV designed respective primer sets specific to serotype A, SAT1 and SAT2 (forward primers and reverse primers).

3.5.3.1 Preparation of the Super mix

A 0.5ml sterile micro centrifuge tube was placed on a rack and the following components were added per reaction. The mixture contained 18.15ul ddH20, 2.5ul 10*PCR buffer, 0.75ul 50mM Magnesium chloride and 0.5ul 10mM dNTPs. Another 90 sterile tubes were placed on a rack and each 30 marked A, B and C to represent the 3 serotypes A, SAT 1 and SAT 2 respectively. In each tube 1ul of the respective cDNA template was added followed by 21.9ul of the universal

mix. 1ul of respective forward and reverse primers were added followed by 0.1ul platinum® *Taq* DNA polymerase.

The tubes were capped and thoroughly mixed by vortexing and incubated in a thermocycler (SimpliAmp Thermal Cycler) using the following thermal cycling profiles. Initial denaturation at 94℃ for 2 minutes to completely denature the template and activate the enzyme. 40 cycles with denaturing at 94℃ for 15 seconds, annealing at 60℃ for 30 seconds and extension at 68℃ for 1 minute and final extension at 68℃ for 5 minutes. The reaction was held at 4℃ after cycling and samples stored at -20℃ awaiting further analysis.

3.5.3.2 Amplification

Twenty three (23) samples showing bands of interest were considered positive and were amplified through a second PCR run. Specific primers set for FMDVA A-1C612F 5′-TAGCGCCGGCAAAGACTTTGA-3′ and FMDVA EURO-2B52R 5′-GACATGTCCTCCTGCATCTGGTTGAT-3′ were used to amplify the VP1 region of serotype A. FMDV SAT1-1C559F 5′-GTGTATCAGATCACAGACACACA-3′ and FMDV SAT-2B208R 5′-ACAGCGGCCATGCACGACAG-3′ were used to amplify the VP3 region of SAT1 and FMDV SAT2-1C445F 5′-TGGGACACMGGIYTGAACTC-3′ and FMDV SAT-2B208R 5′-ACAGCGGCCATGCACGACAG-3′ were used to amplify VP1 region of SAT2.

The RT-PCR was conducted following the above previously described protocol. The thermal cycling profiles used for amplification were initial denaturation at 94°C for 2minutes, 40 cycles with denaturing at 94°C for 15 seconds, annealing at 62°C for 30 seconds and extension at 68°C for 1 minute and a final extension at 68°C for 5 minutes. The annealing temperatures for all the serotypes was increased to facilitate separation of the bands.

The PCR products were again analyzed on 1.0 % agarose gel electrophoresis stained with ethidium bromide at 100 V for 2hours. This time, only one comb was placed on the tray to create a single row of wells on the gel. This was meant to give space to allow proper fragment separation and allowed more time to run to enhance cutting of the fragments. Fragments of 814bp, 1043bp and 1145bp for serotype A, SAT1 and SAT2 respectively were visualized and cut from the gel. The DNA fragments were sliced using a clean scalpel and was cut as close to the band as possible to minimize the gel volume, put in sterile pre-weighed labeled tubes and stored at -4℃ awaiting DNA purification.

3.5.4 Agarose gel electrophoresis

The RT-PCR products of estimated band size of 814bp, 1043bp and 1145bp for serotype A, SAT 1 and SAT2 respectively were analyzed through 1.0 % agarose gel electrophoresis stained with ethidium bromide for 1 h at 100 V.

3.5.4.1 Preparation of the buffer

In a cylindrical flask, 100mls of 10* TAE buffer stock solution (Tris Base, EDTA 0.5M Ph 8.0 and glacial acetic acid) was measured and diluted with 900mls of distilled water to make 1000mls solution.

3.5.4.2 Gel preparation

A gram of agarose powder was weighed and put in a conical flask. 100mls of reconstituted TAE was added to the flask to make 1% gel. The precipitate was boiled at 100℃ for 1 minute 45 seconds to dissolve the powder and the solution cooled through running cold tap water. 5ul of intercalating dye (Ethidium bromide) was added to the solution and mixed thoroughly to stain the gel. The gel was carefully poured into a casting mould making sure there were no bubbles and allowed to settle for 30 minutes.

To load the samples into the wells, 10uls of samples were mixed with the loading dye on a Para film then carefully into the wells. 5ul of the DNA ladder was loaded onto the first and last wells of every row and the gel was allowed to run at 100v for 1 hour. The gel was visualized under ultra-violent light for visible bands.

3.5.6 DNA purification

Purification of the DNA was done following the Thermo scientific GeneJET extraction kit #K0691 protocol. Briefly, the excised DNA fragment was dissolved in a binding buffer to dissolve the gel, denature proteins and promote DNA binding to the silica membrane in the column. The binding buffer had a yellow color indicator which allowed monitoring of the solution pH to enhance DNA binding. Single step wash was done to remove the impurities and the purified DNA was eluted from the column with an elution buffer.

3.5.6.1 DNA extraction from Agarose gel

The concentrated wash buffer was first diluted with 96% ethanol in a ratio of 1:5 before use. After adding the ethanol, the check box on the bottle was marked to indicate the wash buffer had been diluted. To get the exact weight of the gel, the tube with the gel was weighed and recorded and then subtracted the weight of an empty tube as follows.

Weight of tube with the gel – Weight of the empty tube = Weight of the gel

In every sample, 1:1 weight by volume of the binding buffer was added to the agarose gel. The gel mixture was incubated for 10 minutes at 50-60℃ in a shaker until the gel slice was completely dissolved. To facilitate melting, the tubes were inverted gently until all the gel was dissolved to form a yellow mixture which indicated that the pH was optimal for DNA binding.

Before loading on the spin column, the gel mixture was vortexed briefly. The solubilized gel was transferred to the GeneJET purification column and centrifuged at 10000 revolutions per minute

for 1 minute. The flow through was discarded and the spin column placed back into the same collection tube.

3.5.6.2 Binding and washing

Binding was recommended since the purified DNA was to be used for sequencing. 100ml of binding buffer was added in the purification column and centrifuged at 10000 rev/min for 1 minute. The flow through was discarded and the purification column placed back on the collection tube. This binding step was repeated a second time to enhance binding of the DNA to the membrane. This was followed by washing whereby, 700mls of wash buffer diluted with ethanol was added to the purification column and centrifuged at 10000 rev/min for 1 minute. The flow through was discarded and the column placed back on the same collection tube. The empty GeneJET purification column was centrifuged at 10000 rev/min for 1 minute to completely remove any residual wash buffer.

3.5.6.3 Elution

The dried GeneJET purification column was transferred into a sterile 1.5 ml micro centrifuge tube and 30ul of elution buffer added to the center of the purification column membrane. This was followed by centrifugation at 10000 rev/min for 1 minute. The column was discarded and the purified DNA collected in the micro centrifuge tube. This procedure was repeated for all the samples and the purified DNA was kept at -20℃.

3.5.7 DNA sequencing

Twenty three (23) purified amplicons were sent for sequencing for both forward and reverse strands, sequencing of the amplified and purified fragments was done by Humanizing genomics Macrogen in Netherlands. The amplicons were sequenced with Applied Biosystems 3500 XL Genetic Analyzer by an automated fluorescence-based technique, following the manufacturer's

instructions. Cycle sequencing was done and ran on Sanger sequencer as described below. Obtained fragments were assembled in a unique sequence for each sample using the SeqMan software.

3.5.7.1 Preparation of the templates

Cycle Sequencing was performed on PCR products after purification. The contents of the BigDye ™ Terminator v3.1 Cycle Sequencing Kit, samples and the primers were thawed and stored on ice. Vortexing of the sample tubes was done for 3 seconds, then briefly centrifuged for another 3 seconds with a benchtop micro centrifuge to collect contents at the bottom of the tubes. In a MicroAmp™ Optical 96-Well Reaction Plate, 4 ul of the ready reaction mix, 1ul forward primer, 4ul RNase free water and 1ul of the template were added to make up to 10 ul total volume. This reaction was repeated using 1ul reverse primer for the reverse reaction. The plate was sealed with a MicroAmp ™ clear adhesive film, vortex for 3 seconds and briefly centrifuged at 1000g for 10 seconds in a swinging bucket centrifuge to collect all the contents at the bottom of the wells.

3.5.7.2 Cycle sequencing

The tubes were placed in a thermal cycler and the correct volume set. The reaction was incubated for 1 minute at a temperature of 96℃ followed by denaturation at 96℃ for 10 seconds, annealing at 50℃ for 5 seconds and extension at 60℃ for 4 minutes. Sequencing was ran for 25 cycles after which the reaction was held at 4℃ until purification was done.

3.5.7.3 Purification

The bottle of BigDye X Terminator™ beads was vortexed for 8 to 10 seconds before mixing with the SAM solution. 45ul of SAM solution was added to 10ul bead solution to make 55ul total solution. The solution was transferred to each sample and plate sealed with a clear adhesive film.

The tubes were vortexed for 20 minutes at 1,800 rpm and centrifuged at 1000g for 2 minutes in a swinging bucket centrifuge.

125 mM EDTA solution was dispensed directly into each sample tube then 70% ethanol was added and the tubes capped. This was followed by vortexing for 3 seconds and brief centrifugation for 10 seconds at 1000g. The tubes were then incubated at room temperature for 15 minutes.

3.6 Phylogenetic analysis

Using BioEdit software, 14 generated sequences and reference homologous vaccine sequences were aligned through multiple sequence alignment. Analysis was done of generated VP1 nucleotide sequences by assembling and verifying using Clustal W algorithm in the MEGA software. The nucleotide sequences were aligned and reconstructed using Neighbor- joining method in MEGA version 11.0.8 software and was used to characterize the isolates and to construct phylogenetic tree.

Data generated from FMDV VP1 gene sequences data were retrieved from the GenBank BLAST database for performing comparative multiple sequences. A phylogenetic tree of FMDV was constructed using the nucleotide sequences based on VP1 sequences data of the viruses sequenced and reference isolates from Kenya and other Africa countries. Individual FMD viruses were classified into geographically restricted clusters (topotypes) as previously described (Knowles and Samuel, 2003). Analysis was made of divergent and closely related strains and a matrix relationship created.

RESULTS

4.1 Serological test results

FMD virus was isolated from 110 epithelial samples on BHK 21 cells by showing cytopathic effect and confirmed by antigen detection ELISA.

4.1.1 Tissue culture results

There was successful isolation of FMDV on BHK-21 cell line from all the samples. In this cell line, all 110 samples (100%) induced effective infection on the cells after 24–48 hours. The cytopathic effects of the virus was demonstrated by detachment from the culture vessels, aggregate formation, cell lysis which released the virus in the culture suspension.

4.1.2 ELISA results

The virus isolates were subsequently typed by Ag-ELISA to determine the serotype involved. Out of 110 samples, 4% were type A (4/110), 63% were SAT 1 (69/110), and 33% were SAT 2 (37/110).

4.1.3 Polymerase chain reaction results

Out of the 110, 30 samples were selected for PCR, 13% were type A (4/30), 43% were SAT 1 (13/30), and 43% were SAT 2 (13/30). Out of the 30, 23 DNA samples produced visible bands of interest. 4 for type A, 2 for SAT 2 and 17 for SAT 1.

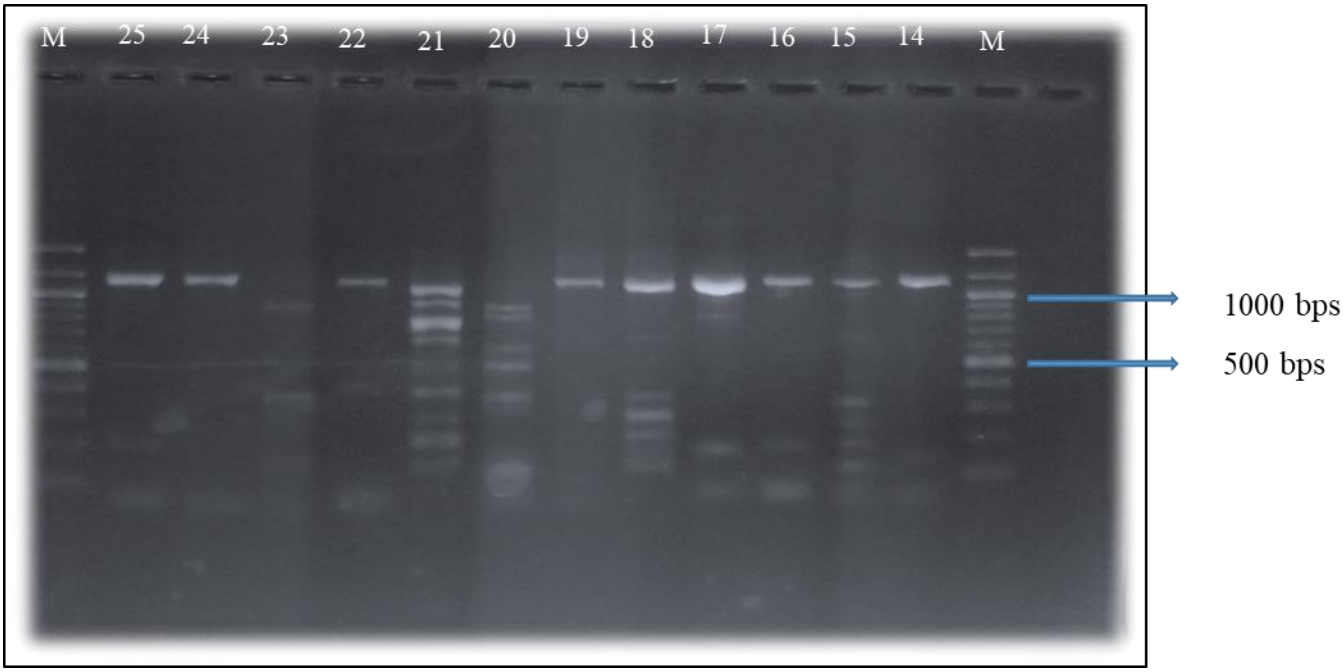

Figure 4.1: Gel electrophoresis DNA image (1.0%, 100 volts, 0.5X TBE,). PCR products visualized under UV light showing the DNA fragments of interest for SAT1 (Samples 14-25). (M is the DNA marker).

Only 2 out of 13 samples of SAT 2 produced visible bands and the other 10 samples were visualized as SAT 1 amplicons.

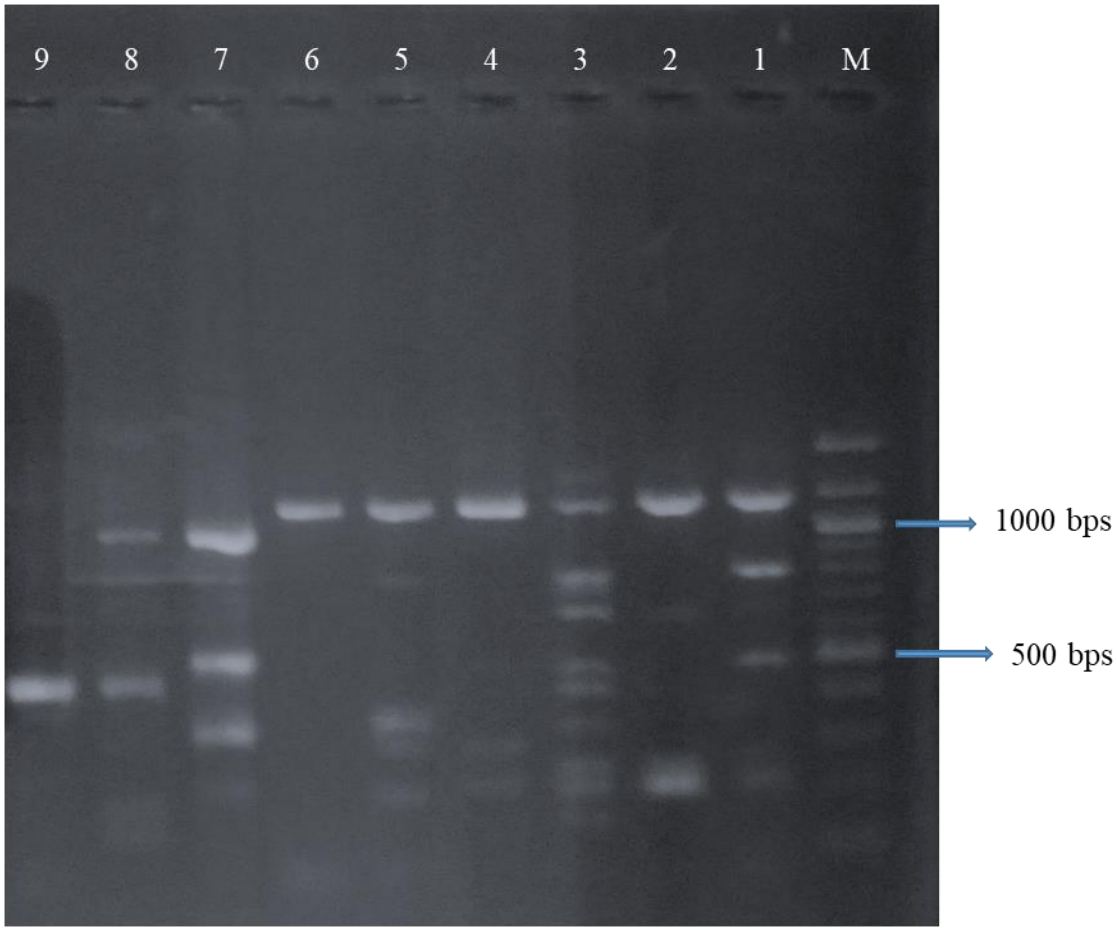

Figure 4.2: Gel electrophoresis DNA image (1.0%, 100volts, 0.5X TBE,). PCR products visualized under UV light showing the DNA fragments of interest for SAT2 (Samples 1-3) and SAT1 (Samples 4-9).

FMDV DNA amplicons of 814 base pairs were amplified for serotype A. All the 4 samples of type A produced visible bands.

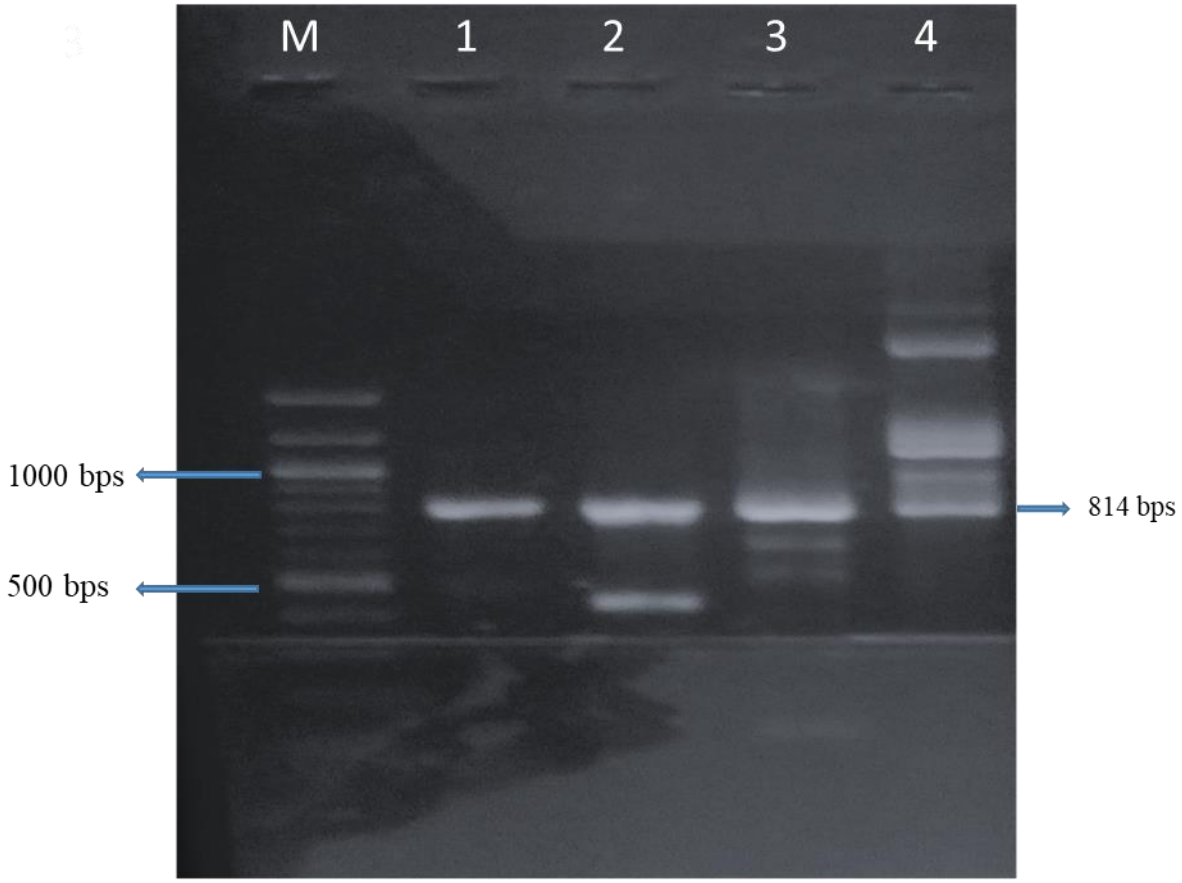

Figure 4.3: Gel electrophoresis DNA image (1.0%, 100 volts, 0.5X TBE,). PCR products visualized under UV light showing the DNA fragments of interest for serotype A (Samples 1-4), (M is the DNA marker).

4.1.4 DNA sequencing results

A total of 23 DNA samples were submitted for DNA sequencing for both forward and reverse strands, 4 samples for type A, 17 for SAT 1 and 2 for SAT 2 including the vaccine strains. Sequencing yielded a total of 2 VP1 sequences of serotype A FMDV, 11 sequences of SAT1 FMDV and no sequence was generated for SAT2 FMDV for both forward and reverse strands except for the vaccine strain.

Table 4.2: Serological typing and molecular results.

Sample ID	Elisa results	PCR Results	DNA Sequencing
k39/20	sat 1	sat 1	sat 1
k70/19	sat 1	sat 1	sat 1
k48/20	sat 1	sat 1	sat 1
k26/20	sat 1	sat 1	sat 1
k77/19	sat 1	no results	no results
k85/19	sat 1	sat 1	sat 1
k96/19	sat 1	sat 1	sat 1
k92/19	sat 1	sat 1	sat 1
k102/19	sat 1	sat 1	no results
k125/19	sat 1	sat 1	sat 1
T155/71	sat 1	sat 1	sat 1
k77/19	sat 2	sat 1	no results
k42/19	sat 2	sat 1	no results
k36/19	sat 2	sat 1	A
k33/19	sat 2	sat 2	no results
k75/19	sat 2	no results	no results
k12/19	sat 2	sat 1	no results
k10/19	sat 2	sat 1	no results
k9/19	sat 2	no results	no results
k2/19	sat 2	no results	no results
k34/19	sat 2	no results	no results
k20/20	sat 1	sat 1	no results
Ak5/80	A	A	A
k59/19	A	A	no results
k121/19	sat 1	sat 1	sat 1
k26/19	A	A	A
k43/19	A	A	no results
k39/19	sat 2	no results	no results
K52/84	sat 2	sat 2	sat 2
k100/19	sat 2	no results	no results

4.1.5 FMDV DNA sequence analysis

FMDV VP1 sequences were trimmed and assembled (both forward and reverse). The forward and reverse complement nucleotide sequences delimited by forward and reverse primers of FMDV serotypes A, SAT1 and SAT2 were aligned using BioEdit software to obtain a consensus nucleotide sequence. One consensus sequence from each of the three serotypes was used to search for other highly similar sequences (mega blast) available in the GenBank (NCBI) using the BLAST online tool for performing comparative multiple sequence analysis. Highly similar sequences (from 97-100% identity and 99-100% query cover with high score and low E-value ≤ 0) from different African and other countries were downloaded and saved in FASTA format with their access numbers which were later included in the phylogenetic tree.

A phylogenetic tree was constructed using the nucleotide sequences of the present and reference isolates. The neighbor-joining method with the maximum composite likelihood nucleotide substitution model and the pairwise deletion was performed using MEGA version 11.0.8 software. These downloaded sequences were then selected based on the criteria of the information available regarding the location and the year of isolation. Using Clustal-W program incorporated in MEGA software, the retrieved data were aligned separately and phylogenetic analyses were carried out using the neighbor joining method following nucleotide substitution model and confidence level assessed by 1000 bootstrap replications

4.2 Phylogenetic analysis of FMDV serotypes A, SAT 1 and SAT 2

The partial VP1 gene sequence information was used to construct a phylogenetic tree and study the genetic relationships between the current isolates with the isolates retrieved from the data

base. For the analysis 25 nucleotide sequences were included, 14 from the current study and 11 nucleotide sequences retrieved from the gene bank.

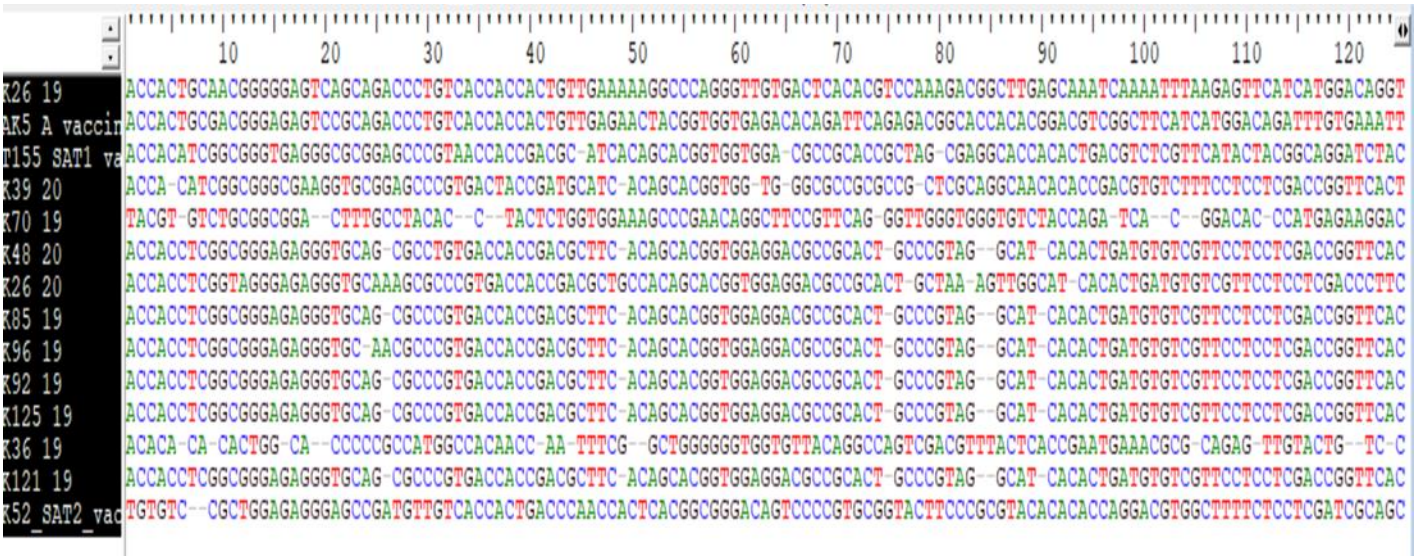

Figure 4.4: Multiple alignment of sequences for the field strains and vaccine strains for serotype A, SAT 1 and SAT 2

```
Alignment: C:\BioEdit\Protein sequences multiple allignment.gb

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50         60
AK5_A_vacc      ---------- ---------- ---------- ---------- ---------- ----------
K70_19          YVSAADFAYT YSGGKPEQAS VQGWVGVYQI TDTHEKDGAV VVTVSAGPDF EFRMPIKDGL
K26_19          ---------- ---------- ---------- ---------- ---------- ----------
AK5_80          ---------- ---------- ---------- ---------- ---------- ----------
K48_20          ---------- ---------- ---------- ---------- ---------- ----------
K39_20          ---------- ---------- ---------- ---------- ---------- ----------
T155_SAT1_      ---------- ---------- ---------- ---------- ---------- ----------
K26_20          ---------- ---------- ---------- ---------- ---------- ----------
K36_19          ---------- ---------- ---------- -HTHWHPRHG HNQFRLGGGV TGQSTFTHRM
K125_19         ---------- ---------- ---------- ---------- ---------- ----------
K26_19          ---------- ---------- ---------- ---------- ---------- -------KPS
K121_19         ---------- ---------- ---------- ---------- ---------- ----------
K52_SAT2_v      ---------- ---------- ---------- ---------- ---------- ----------
K85_19          ---------- ---------- ---------- ---------- ---------- ----------
K96_19          ---------- ---------- ---------- ---------- ---------- ----------
K92_19          ---------- ---------- ---------- ---------- ---------- ----------


                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         70         80         90        100        110        120
AK5_A_vacc      --TTATGESA DPVTTTVENY GGETQIQRRH HTDVGFIMDR FVKLNSLS-P THVIDLMQTP
K70_19          DETTSAGEGA APVTTDASQH GGGRRTARRH HTDVSFLLDR FTLVGKTVDN KLTLDLLQTK
K26_19          ---------- ---------- ---------- ----EFIMDR CYDLLEVSSP THVIDLMQTH
AK5_80          ---------- ---------- ---------- ---------- ---------- ----------
K48_20          ---------- ---------- ---------- ---------P PRRE-RVQRL ----------
K39_20          ---------- ---------- ---------- ---------P HRRA-KVRSP ----------
T155_SAT1_      ----GLASSR PSLIALTPWV SSSNADTRRL KSGPCLVDMS CCMLLMLSTS FVKFSLTSAK
K26_20          ---------- ---------- ---------- ---------H LGRE-RVQSA R---DHRRCH
K36_19          KRAELYCPRA LLPAYTHQDR DRFDAPIGVE KQLFNFDLLK LAGD-VESNP GPNHSRRDSH
K125_19         --TTSAGEGA APVTTDASQH GGGRRTARRH HTDVSFLLDR FTLVGKTVDN KLTLDLLQTK
K26_19          EGCIMVGRGV TYVFKGGHRG AVGHPSQIVL MPHHHLQVGE VVRGRAQQRA DQAVLVSLHE
K121_19         ---------- ---------- ---------- ---------P PRRE-RVQRP ----------
K52_SAT2_v      ------CP-- -------LER EPMLSPL--- TQPLTAGQSP CGTS-RVHTP GRGFSPRSQH
K85_19          --TTSAGEGA APVTTDASQH GGGRRTARRH HTDVSFLLDR FTLVGKTVDN KLTLDLLQTK
K96_19          --TTSAGEGA TPVTTDASQH GGGRRTARRH HTDVSFLLDR FTLVGKTVDN KLTLDLLQTK
K92_19          --TTSAGEGA APVTTDASQH GGGRRTARRH HTDVSFLLDR FTLVGKTVDN KLTLDLLQTK


                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        130        140        150        160        170        180
AK5_A_vacc      ERGLVGALLR AATYYFSDLE IVVRHDGNLT -WVPNGAPEV ALQNESNPTA YHKAPFTRLA
K70_19          EKPLVGAILR AAACY----- ---------- ---------- ---------- ----------
K26_19          QHGLVGALLR AATYYC---- --AREARGWS -WP------- ---------- ----------
AK5_80          ---FQKGLFT ---------- ---------- ---------- ---------- ----------
K48_20          ---------- ---------- ---------- ---------- ---------- ----------
K39_20          ---------- ---------- ---------- ---------- ---------- ----------
T155_SAT1_      KKGPGSPTSL SLRRNFNGVQ PTHLFVPTHV RATL------ ---------- ----------
K26_20          STVEDAALLK LASH------ ---------- ---------- ---------- ----------
K36_19          SGPPRSHRLV DIFSSIAKRH VHQARSRLQP VGDRV----- ---------- ----------
K125_19         EKALVGAILR AATYYFSDLE VACVGTNKWV GWTPNGAPEL SEVGDNPVVF SHNG-TTRFA
K26_19          FIDMSGDSLH SSFTNLSMMN SDTCVVPSLD VCFTTVVLKQ WW-------- ----------
K121_19         ---------- ---------- ---------- ---------- ---------- ----------
K52_SAT2_v      TRAYEQDHFC GGL------- ---------- ---------- ---------- ----------
K85_19          EKALVGAILR AATYYFSDLE VACVGTNKWV GWTPNGAPEL SEVGDNPVVF SHNG-TTRFA
K96_19          GKALVGAILR AATYYFSDLE VACVGTNKWV GWTPNGAPEL SEVGDNPVVF SHNG-TTRFA
K92_19          EKALVGAILR AATYYFSDLE VACVGTNKWV GWTPNGAPEL SEVGDNPVVF SHNG-TTRFA
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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       190        200        210        220        230        240
AK5_A_vacc      LPYTAPHRVL ATVYNGTSKY STG----ASG GRGDMAALAA RVA--AQLPA SFNYGALRAT
K70_19          ---------- ---------- ---------- ---------- ---------- ----------
K26_19          ---------- ---------- ---------- ---------- ---------- ----------
AK5_80          ---------- ---------- ---------- ---------- ---------- ----------
K48_20          ---------- ---------- ---------- ---------- ---------- ----------
K39_20          ---------- ---------- ---------- ---------- ---------- ----------
T155_SAT1_      ---------- ---------- ---------- ---------- ---------- ----------
K26_20          ---------- ---------- ---------- ---------- ---------- ----------
K36_19          ---------- ---------- ---------- ---------- ---------- ----------
K125_19         LPYTAPHRCL ATAYNGDCKY KPMSEAPRTN IRGDLATLAA RIASETHIPT TFNYGRIYTE
K26_19          ---------- ---------- ---------- ---------- ---------- ----------
K121_19         ---------- ---------- ---------- ---------- ---------- ----------
K52_SAT2_v      ---------- ---------- ---------- ---------- ---------- ----------
K85_19          LPYTAPHRCL ATAYNGDCKY KPMSEAPRTN IRGDLATLAA RIASETHIPT TFNYGRIYTE
K96_19          LPYTAPHRCL ATAYNGDCKY KPMSEAPRTS IRGDLATLAA RIASETHIPT TFNYGRIYTE
K92_19          LPYTAPHRCL ATAYNGDCKY KPMSEAPRTN IRGDLATLAA RIASETHIPT TFNYGRIYTE


                ....|....| ....|....| ....|....| ....|....| ....
                       250        260        270        280
AK5_A_vacc      TIHELLVRMK RAELYCPRPL LAT-EVTGAD RHKQKIIAPA KQLM
K70_19          ---------- ---------- ---------- ---------- ----
K26_19          ---------- ---------- ---------- ---------- ----
AK5_80          ---------- ---------- ---------- ---------- ----
K48_20          ---------- ---------- ---------- ---------- ----
K39_20          ---------- ---------- ---------- ---------- ----
T155_SAT1_      ---------- ---------- ---------- ---------- ----
K26_20          ---------- ---------- ---------- ---------- ----
K36_19          ---------- ---------- ---------- ---------- ----
K125_19         AEVDVYVRMK RAELYCPRPV LTHYDHQGRD RYKVALTKPA KQLC
K26_19          ---------- ---------- ---------- ---------- ----
K121_19         ---------- ---------- ---------- ---------- ----
K52_SAT2_v      ---------- ---------- ---------- ---------- ----
K85_19          AEVDVYVRMK RAELYCPRPV LTHYDHQGRD RYKVALTKPA KQLC
K96_19          AEVDVYVRMK RAELYCPRPV LTHYDHQGRD RYKVALTKPA KQLC
K92_19          AEVDVYVRMK RAELYCPRPV LTHYDHQGRD RYKVALTKPA KQLC
```

Figure 4.5: Multiple alignment of amino acid sequences for type A, SAT1 and SAT2.

Phylogenetic tree of FMDV A, SAT1 and SAT2 DNA sequences.

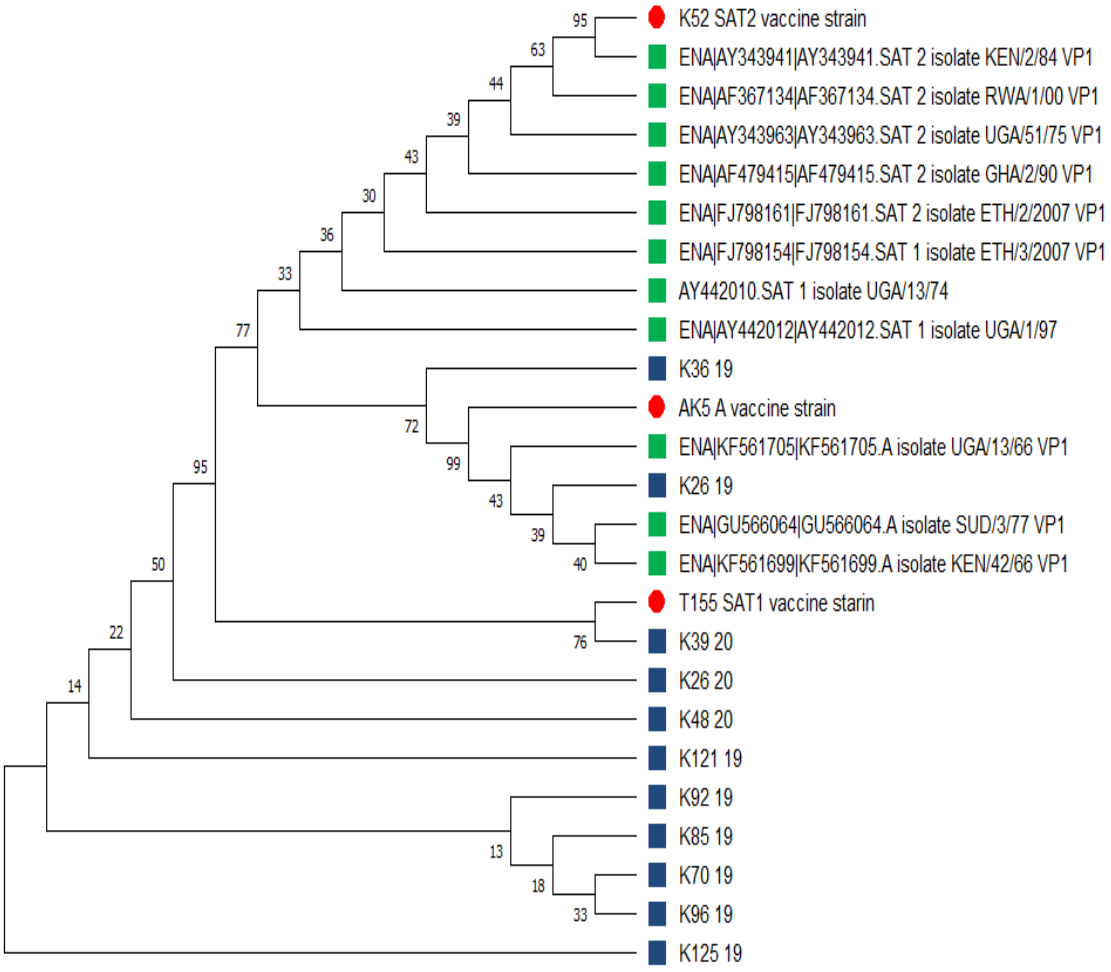

Figure 4.6: DNA Phylogenetic analysis for serotype A, SAT1 and SAT2 of foot-and-mouth disease virus. The blue, red and green color represent the sample, vaccine and gene bank data respectively.

Phylogenetic tree of FMDV A, SAT1 and SAT2 Amino acid sequences.

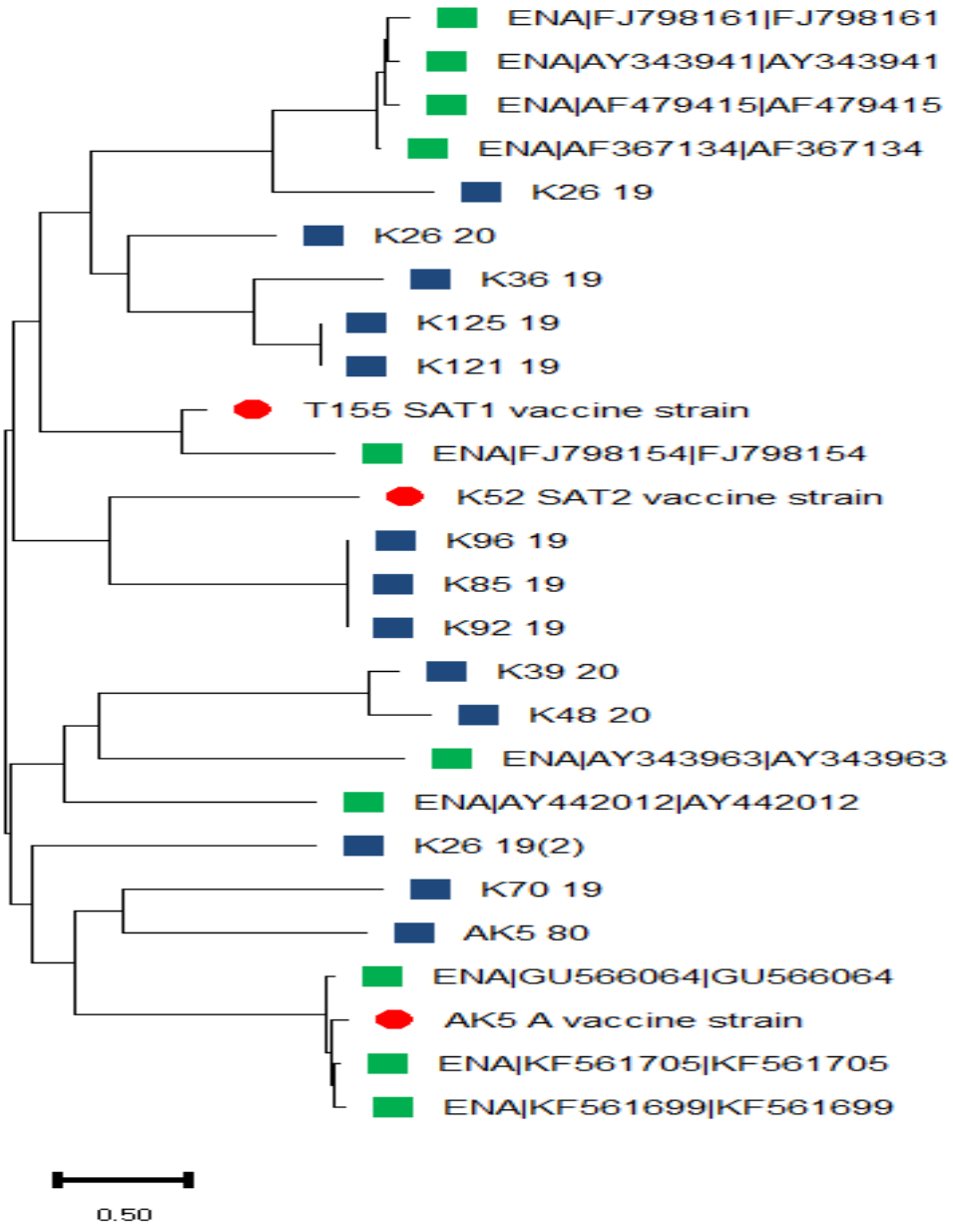

Figure 4.7: Amino acids Phylogenetic analysis for serotype A, SAT1 and SAT2 of foot-and-mouth disease virus. The blue, red and green color represent the sample, vaccine and gene bank data respectively.

Table 4.3: Showing percentage of nucleotide identity and divergence among field isolates and vaccine strains of A, SAT1 and SAT2.

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1		99.8	99.6	99.6	99.6	99.6	99.5	99.6	99.6	99.6	99.6	99.5	99.6	99.5	99.8	99.8	99.8	99.6	99.5	99.6	99.5	99.5	99.5	99.5	99.5	1
2	0.2		99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.5	99.6	99.5	99.9	99.9	99.9	99.5	99.6	99.6	99.5	99.5	99.5	99.6	99.6	2
3	0.4	0.4		99.9	99.8	99.8	99.7	99.8	99.8	99.8	99.8	99.5	99.8	99.6	99.6	99.6	99.6	99.6	99.7	99.7	99.6	99.7	99.6	99.7	99.6	3
4	0.4	0.4	0.1		99.9	99.9	99.8	99.9	99.9	99.9	99.9	99.5	99.9	99.7	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	4
5	0.4	0.4	0.2	0.1		100.0	99.9	100.0	100.0	100.0	100.0	99.5	100.0	99.6	99.6	99.4	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	5
6	0.4	0.4	0.2	0.1	0.0		99.9	100.0	100.0	100.0	100.0	99.5	100.0	99.7	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	6
7	0.5	0.4	0.3	0.2	0.1	0.1		99.9	99.9	99.9	99.9	99.5	99.9	99.6	99.5	99.6	99.6	99.6	99.7	99.6	99.6	99.6	99.5	99.6	99.6	7
8	0.4	0.4	0.2	0.1	0.0	0.0	0.1		100.0	100.0	100.0	99.5	100.0	99.6	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	8
9	0.4	0.4	0.2	0.1	0.0	0.0	0.1	0.0		100.0	100.0	99.5	100.0	99.6	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	9
10	0.4	0.4	0.2	0.1	0.0	0.0	0.1	0.0	0.0		100.0	99.5	100.0	99.6	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	10
11	0.4	0.4	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0		99.5	100.0	99.6	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	11
12	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		99.5	99.4	99.5	99.5	99.5	99.5	99.4	99.5	99.5	99.4	99.4	99.5	99.5	12
13	0.4	0.4	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.5		99.6	99.6	99.6	99.6	99.7	99.7	99.7	99.7	99.7	99.6	99.7	99.7	13
14	0.5	0.5	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.6	0.4		99.5	99.6	99.5	99.6	99.7	99.7	99.8	99.9	99.7	99.7	99.7	14
15	0.2	0.1	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.4	0.5		99.9	99.9	99.6	99.5	99.6	99.5	99.5	99.5	99.6	99.6	15
16	0.2	0.1	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.1		99.9	99.6	99.5	99.6	99.5	99.6	99.5	99.5	99.6	16
17	0.2	0.1	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.5	0.1	0.1		99.6	99.5	99.6	99.5	99.5	99.5	99.6	99.6	17
18	0.4	0.5	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.4	0.4	0.4	0.4		99.6	99.7	99.7	99.6	99.6	99.6	99.7	18
19	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.3	0.3	0.5	0.5	0.5	0.4		99.7	99.7	99.7	99.7	99.7	99.7	19
20	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.3	0.3		99.7	99.7	99.7	99.7	99.7	20
21	0.5	0.5	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.2	0.5	0.5	0.5	0.3	0.3	0.3		99.8	99.7	99.7	99.7	21
22	0.5	0.5	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.6	0.3	0.1	0.5	0.4	0.5	0.4	0.3	0.3	0.2		99.8	99.7	99.7	22
23	0.5	0.5	0.4	0.3	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.6	0.4	0.3	0.5	0.5	0.5	0.4	0.3	0.3	0.3	0.2		99.7	99.6	23
24	0.5	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.4	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.3		99.6	24
25	0.5	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.4		25
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	

(1.) K26_19 **(2.)** AK5_A_vaccine_strain **(3.)** T155_SAT1_vaccine_strain **(4.)** K39_20 **(5.)** K70_19 **(6.)** K48_20 **(7.)** K26_20 **(8.)** K85_19 **(9.)** K96_19 **(10.)** K92_19 **(11.)** K125_19 **(12.)** K36_19 **(13.)** K121_19 **(14.)** K52_SAT2_vaccine_strain **(15.)** ENA|GU566064|GU566064.A isolate SUD/3/77 VP1 **(16.)** ENA|KF561705|KF561705.A isolate UGA/13/66 VP1 **(17.)** ENA|KF561699|KF561699.A isolate KEN/42/66 VP1 **(18.)** ENA|AY442012|AY442012.SAT 1 isolate UGA/1/97 **(19.)** ENA|FJ798154|FJ798154.SAT 1 isolate ETH/3/2007 VP1 **(20.)** ENA|FJ798161|FJ798161.SAT 2 isolate ETH/2/2007 VP1 **(21.)** ENA|AY343963|AY343963.SAT 2 isolate UGA/51/75 VP1 **(22.)** ENA|AY343941|AY343941.SAT 2 isolate KEN/2/84 VP1 **(23.)**

ENA|AF367134|AF367134.SAT 2 isolate RWA/1/00 VP1 **(24.)** ENA|AF479415|AF479415.SAT 2 isolate GHA/2/90 VP1 **(25.)** AY442010.SAT 1 isolate UGA/13/74.

4.2.1 Phylogenetic analysis of FMDV serotype A

Two VP1 sequences of serotype A were compared to the vaccine strain sequence and other reference isolates from African origin in the GenBank. The analysis showed that the field isolates K26/19 and K36/19 were closely related to serotype A vaccine strain AK5/80. The 2 isolates clustered together with the reference isolates A/UGA/13/66 (accession number KF561705), A/SUD/3/77 (accession number GU566064) and A/KEN/42/66 (accession number KF561699) on the tree. K26/19 showed 99.8% nucleotide similarity and K36/19 showed 99.5% nucleotide similarity with the vaccine strain. The two serotype A isolates showed 99.5% sequence identity with each other and 99.5% to 99.9% identity with the reference isolates. The isolates fell in genotype VII (G-VII) within the AFRICA topotype and were closely related to one Ugandan virus A/UGA/13/66 with a nucleotide identity value of 99.8% and 99.5% for K26/19 and K36/19 respectively.

From the amino acid phylogenetic tree, 4 samples (K70/19, K26/19, K48/20 and K39/20) grouped together with the vaccine strain AK5/80 for serotype A. This shows that the amino acid sequences were of type A. There was variation in (K70/19, K48/20 and K39/20) as their DNA sequences clustered together with SAT1 vaccine strain SAT1/T155/71. Only K26/19 DNA and amino acid sequences clustered as type A strain. This phylogenetic incongruence could be as a result of stochastic errors or due to heterotachy causing substitutions in the nucleotide and amino acid sequences, which led to sequence evolution.

4.2.2 Phylogenetic analysis of FMDV serotype SAT 1

Nine VP1 sequences of serotype SAT 1 were compared to the vaccine strain sequence and other reference isolates from African origin in the GenBank. The analysis showed that the field isolates were closely related to the vaccine strain SAT1/T155/71 (accession number KF561706). The isolates showed 99.7% to 99.9% nucleotide identity with the vaccine strain and 99.9% to 100% similarity with each other. The isolates clustered together on the tree. All the SAT1 isolates fell in Africa topotype I (North West Zimbambwe) with one sub lineage represented by K92/19, K85/19, K70/19 and K96/19. The 4 were isolated the same year.

From the amino acid phylogenetic tree, 4 samples (K26/20, K36/19, K125/19 and K121/19) clustered together with the SAT1 vaccine strain SAT1/T155/71. This show that, the amino acid sequences belonged to SAT 1 serotype. The 3 DNA sequences (K26/20, K125/19 and K121/19) had also clustered together as SAT1 strains. This shows there were no variations between their DNA and amino acid sequences. DNA sequences of K36/19 had previously clustered together with serotype A vaccine whereas its amino acid sequences clustered as SAT1. This shows there was variation between K36/19 DNA and amino acid sequences.

4.2.3 Phylogenetic analysis of FMDV serotype SAT 2

Since there was no SAT 2 sequence generated, the vaccine strain was compared to the reference isolates. The vaccine strain SAT2/K52/84 was closely related to a Kenya isolate SAT2/KEN/2/84 (accession number AY343941) which is a topo type IX and had a 99.9% nucleotide similarity. All the SAT 2 reference isolates clustered together with SAT 1 isolates on the tree. These include SAT 2 isolates ETH/2/2007 (accession number FJ798161), UGA/51/75 (accession number AY343963), KEN/2/84 (accession number AY343941), RWA/1/00 (accession number AF367134) and GHA/2/90 (accession number AF479415). The SAT 1

included UGA/1/97 (accession number AY442012), ETH/3/2007 (accession number FJ798154) and UGA/13/74 (accession number AY442010).

From the amino acid phylogeny, 3 samples (K96/19, K85/19, and K92/19) clustered together with the SAT2 vaccine strain SAT2/K52/84. This shows that the amino acid sequences belonged to SAT 2 serotype. DNA sequences of the 3 strains had previously clustered together with SAT1 vaccine SAT1/T155/71 on the phylogenetic tree and there was no DNA sequence clustering around SAT 2 vaccine.

5.0 DISCUSSION

This study reports on serological and molecular characterization of FMD cases reported in cattle between 2019 and 2020 in Kenya. The serological results indicate that FMDV is endemic in the country and multiple FMDV serotypes are in circulation as it was shown by (Sangula *et al*., 2010).

5.1 Serological findings

BHK-21 cell line was used to isolate the virus from clinical samples. FMDV positive samples detected by RT-PCR, all the 110 (100%) samples showed CPE, which appeared as rounding in monolayer cell cultures, complete cell destruction and detachment from the surface of the flask. This observation in CPE of FMDV agrees with previous reports (Sulayeman *et al*., 2018). Previously, Paixao *et al*., 2008 and Attia *et al*., 2017 reported that FMD virus isolation on BHK-21 cell is the most reliable diagnostic method. From the total, samples serotyped by antigen detection ELISA, Out of 110 samples, 4% were identified as type A (4/110), 63% were SAT 1 (69/110), and 33% were SAT 2 (37/110). Out of the 110, 30 samples were selected for PCR, 13% were type A (4/30), 43% were SAT 1 (13/30), and 43% were SAT 2 (13/30). SAT 1 is the second most reported serotype after serotype O in Kenya, causing most of the SAT outbreaks.

5.2 Molecular findings

Out of the total analyzed DNA samples (n = 23), only 14 VP1 sequences were obtained for phylogenetic analysis including the vaccine strains. 3 VP1 sequences for serotype A, 10 for SAT1 and 1 for SAT 2. This low rate (14/23) of sequence recovery could be explained by low concentration of the template, failed sequencing reactions, chromatograms background noise, mixed template, insertions or deletions on the chromatograms and early termination of read

lengths. The 3 VP1 sequence analysis of serotype A isolate identified in the study and other sequences data available in the GenBank database indicated that, the sequence of serotype A clustered to genotype VII (G-VII) of the AFRICA topotype. This included the vaccine strain. In Kenya, genotype (G-VII) was last recorded in 2005. They showed 99.5% to 99.9% nucleotide similarity with the Ugandan reference sequence A/UGA/13/66. This indicated that the serotypes were closely related and could be genetically related with one another. This agrees with (Knowles and Samuel 2003) who reported that serotype A with 95 % nucleotide similarity in the VP1 region are closely related. The two isolate were also closely related with viruses from Kenya and Sudan which belong to genotype (G-I) and (G-IV) respectively with nucleotide identity of 99.5%. This close relation could be due to uncontrolled trans-border animal movement between East African countries. This agrees with (Wekesa *et al*., 2013), who reported that there is high genetic diversity and wide distribution of serotype A in Eastern Africa region due to trans-border movement. This highlights the importance of continuous surveillance and genetic and antigenic characterization.

The isolates showed a total of 0.2% – 0.5% nucleotide differences with the VP1 sequence of the vaccine strain AK5/80. This indicated that the vaccine strain and field isolates were closely related. Knowles and Samuel reported that FMD viruses with >15% of nucleotide sequence differences in the VP1 sequence are considered unrelated (Knowles and Samuel, 2003). Serotype A vaccine matching was last done in 2001 and it was shown that serotype A vaccine (AK5/80) was protective against all serotype A outbreaks from all the provinces except Eastern province. AK35/1980 was protective against Eastern province FMDV outbreaks (Wekesa *et al*., 2013).

The 9 isolates of SAT1 had a 99.9 - 100% nucleotide similarity with each other and 99.7% - 99.9% identical to the vaccine SAT1/T155/71 (accession number KF561706). This shows that

they are closely related to the vaccine. The isolates clustered together in the phylogenetic tree suggesting that they form a single clade for SAT 1 of Africa topotype 1 North West Zimbambwe (NWZ). The VP1 coding region of these isolates were identical to those of previously reported samples. This corresponds to a study by (Kasanga *et al.*, 2015), who reported that all 16 SAT 1 isolated from Tanzania between 1971 and 1999 fell into topotype I. One sub lineage clustered together with Kenya isolates of the same year (SAT1/K96/99 and SAT1/K114/99) with nucleotide identity of 98.5 to 99.4%). This close genetic relatedness of lineages suggest that cross border movement between East African countries is a contributor of virus dispersal which has significant implications for FMDV surveillance and control. The same results were also arrived by (Sahle *et al*., 2007), who reported that the Kenyan isolates clustered together with Tanzanian isolates within topotype I and had a >10% nucleotide difference from the Tanzanian isolates. The genetic characteristics of the serotypes in this study revealed high similarity in nucleotide sequence and the field strains were consistent with those present in East African countries.

6.0 CONCLUSION

1. It is evident that multiple lineages and sub-lineages of FMDV serotypes A, SAT1 and SAT2 are currently circulating in Kenya. This highlights the need to continuously monitor their occurrence and assessing possibility of a foreign serotype. Therefore, continuous surveillance, genetic and antigenic characterization including vaccine matching of field isolates both on a local and regional scale are of importance.
2. The serological and molecular observations of this study urge for continuous surveillance of FMD enabling to monitor the infection status and the spread of FMDV serotypes in livestock as well as in wildlife populations in Kenya and Eastern African region.
3. Other factors that can lead to vaccine failure like cold chain maintenance, vaccine efficacy and efficiency should be regularly checked and evaluated.

7.0 RECOMMENDATIONS

- It is anticipated that the results of this study will motivate further work to characterize FMDV from field outbreaks in the country where antigenic and genetic status of virus is poorly understood.
- A strategic trans-border animal movement control is required in order to curb the spread from infected zones to non-infected areas. Animal screening is required to ascertain their FMD status before they are moved from one area to another. Emphasis should be on animal movement and trade based on sanitary measures including availability of inspectorate and certification systems for animals and animal products in Eastern Africa region.
- Regular FMD outbreak investigation should be conducted to have more detailed information about the serotypes and topo types circulating in the country.
- Vaccine matching studies should be conducted for field strains circulating in the country against the vaccine strains.
- Regular vaccination program should be started to control the outbreak of the disease.

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