Sokoine University of Agriculture

PhD Thesis

Molecular Characterization and Antigenic Prediction of Foot-And-Mouth Disease Virus in relation to Vaccine Improvement in Endemic Settings in Africa

Mkama Mathias

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MOLECULAR CHARACTERIZATION AND ANTIGENIC PREDICTION OF FOOT-AND-MOUTH DISEASE VIRUS IN RELATION TO VACCINE IMPROVEMENT IN ENDEMIC SETTINGS IN AFRICA

By
MKAMA MATHIAS

Supervisors:
Prof. Christopher Kasanga
Prof. Philemon Wambura

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EXTENDED ABSTRACT

Foot-and-mouth disease (FMD) affects all cloven-hoofed livestock and wildlife, and it is caused by FMD virus (FMDV) that belongs to the genus *Aphthorvirus* and family *Picornaviridae*. The disease threatens food security and livelihoods across different parts of the world. There are seven FMDV serotypes (O, A, C, Asia1, SAT1-3), and studies in southern Africa describe buffalos to be persistently infected reservoirs of SATs and transmit the viruses to cattle. The FMD control is challenging and antigenic heterogeneity among field-circulating strains represents the most significant factor. The field's infinite variants pose a challenge to the effectiveness of vaccines and suggests for quick, reliable, and cost-effective methods for obtaining vaccine improvements relevant information timely. FMD is endemic in Africa; impacting the livelihoods of pastoral communities, and hinders the livestock sector by denying access of regional and global lucrative markets. Currently, the strategies deployed to control FMD in Africa, especially tropical Africa, are typically fragmented national-level focused activities with relatively poor outcomes, rather than regionally coordinated initiatives that have been used on other continents (South America, Europe) to reduce and even eliminate virus circulation. Studies have not demonstrated whether the buffalo can also act as carrier of the EurAsian serotypes O and A occurring in parts of East Africa. Also the current epidemiological status of the field circulating strains and their genetic-antigenic characteristics need to be understood. Also, it was unclear on the main challenges to FMD control under pastoral dominated and endemic settings in Africa that has hindered FMD control for decades.

This study investigated field reported outbreaks and clinically health buffalo of Tanzania to determine the molecular epidemiology of the circulating field strains between 2018 and 2021, their genetic-antigenic characteristics, and their evolutionary relationships using molecular techniques for FMDV detection, typing, and characterization. The seroprevalence of FMDV serotypes O and A in Tanzania were studied using NSP ELISA for natural infection screening and typed by Solid phase competitive ELISA (SPCE)

assay. Additionally; the B-cell antigenic epitopes of SAT1 types were mapped by combined publicly available immuno-informatics tools ranging from those employing propensity scales to the most recent machine learning and artificial intelligence models, to maximize the prediction authenticity, on the FMDV type SAT1 VP1 polypeptides from this study and those sourced from global rich databases.

The study identified three FMDV serotypes (A, O and, SAT1) circulating in the field as Africa Topotype G-I lineage, EA-2 Topotype, and Topotype I (NWZ) respectively. This study nucleotide sequences for serotypes O and SAT1 field strains were analysed categorically, and the shared percent identities of 92.0-100.0% and 96.9-98.8% were revealed respectively. The analysed 247 buffalo (37.6%, n =93) and cattle (58.3%, n =154) NSP ELISA positive serum samples from livestock-wildlife interface areas of Katavi, Mikumi, Mkomazi, and Ruaha ecosystems in Tanzania were further analyzed for FMDV serotype O and A by SPCE. A highest percentage seropositivity (PS) was revealed (67%, n= 103) and (55%, n= 51) in cattle and buffalo, whereas FMDV serotype A tested highest (54%, n= 83) and O (25%, n= 23) for cattle and buffalo sera, respectively. Also, some of the cattle (36%, n= 56) and buffalo (25%, n= 23) sera tested, showed evidence of multiple infection with FMDV serotypes O and A. Results showed that out of the 93 analyzed NSPE positive buffalo samples, 30.1% (28) of CI (19 – 39) had antibodies specific to FMDV Type A. Also 54.8% (51) of CI (39 – 64) and 24.7% (23) of CI (15 – 33) had antibodies specific to FMDV type O and those of mixed sero reaction respectively. Similarly, out of 154 NSPE positive cattle samples analyzed 53.9% (83) of CI (69 – 98), 66.9% (103) of CI (88 – 118) and 36.4% (56) of CI (44 – 70) had FMDV antibodies specific to type A, O, and mixed (O&A) seroreactions respectively. The results of compared serotype-specific seroreactions statuses revealed higher percentage for type O across all species, next type A, and the lowest score for mixed seroreactions. Similarly; at 95% CI, the analyzed cattle and buffalo NSPE positives expressed higher scores of (41.7%; 20.65%) by (33.6%; 11.34%) and (22.67%; 9.31%) on type O, type A and mixed seroreactions respectively. Results on Chi-square test of independence were significant, X^2 (df = 4, N = 330) = 31.0876, p =

.00001 at $p<.05$ criteria, indicating a relation between spp difference and variation in state of FMDV infection rates. The analyzed probang buffalo samples (n=89) from Serengeti national park using FMDV serotype O and A specific primers (one-step RT-PCR), 1.2% was revealed to be FMDV type O. Also, five consensus linear epitopes (PLE I – V) at positions 3-30, 44-59, 95-112, 134-149, 199-219 with 75%, 88%, 94%, 44 % and 24% of their aa reads conserved were observed respectively. Additionally; conformational epitopes (PCE I – VI) at positions 1-26, 82-87, 93-114, 131-149, 175-179, and 201-221 with 53 – 100% conserved were also identified. As the vaccines are challenged by the rapidly evolving FMDV in the field, the unveiling of conserved epitope domains is vital for enhancing future FMDV vaccines long-term effectiveness in the field.

This study has demonstrated FMDV type O infections in buffalo being the first molecular and serosurvey combined study to be undertaken on Eurasian FMDV types O and A in cattle and buffalos in Tanzania. The information generated from this study strengthens knowledge on FMDV epidemiology in Tanzania and Africa, and thereby contributing to the progressive control pathway-FMD program through tailored control initiatives. The unveiled challenges of FMD control amongst Africa's diverse pastoral communities' landscape for triggering strategical discussions on national and transboundary regional-based FMD control approaches in Africa.

MUHTASARI ULIOPANULIWA

Ugonjwa wa miguu na midomo (FMD) huathiri mifugo na wanyamapori wenye kwato zilizopasuka, unaosababishwa na kirusi cha ugonjwa wa miguu na midomo (FMDV) ambacho ni cha jenasi *Aphthorvirus* na familia ya *Picornaviridae*. Ugonjwa huo unatishia usalama wa chakula na maisha katika sehemu mbalimbali za dunia. Kuna serotypes saba za FMDV (O, A, C, Asia1, SAT1, SAT2, and SAT3), na tafiti kusini mwa Afrika zinaelezea nyati kuwa hifadhi aina ya virusi vya SAT na kuvisambaza kwa ng'ombe. Udhibiti wa FMD ni changamoto kutokana na tofauti za antijeni na zile za virus chanjo. Kubadirika kwa virusi mara kwa mara ni changamoto kwa ufanisi wa chanjo na kupendekeza kwa mbinu za haraka, za kuaminika na za gharama nafuu ili kuboresha chanjo kwa wakati. FMD ni janga katika Afrika; na ugonjwa huathiri maisha ya jamii za wafugaji, na huzuia sekta ya mifugo kwa kunyima upatikanaji wa masoko yenye faida ya kikanda na kimataifa. Hivi sasa, mikakati iliyowekwa kudhibiti FMD barani Afrika, haswa Afrika ya kitropiki ina matokeo duni, ukilinganisha na mipango ya kikanda ambayo imetumika katika mabara mengine (Amerika ya Kusini, Ulaya) kupunguza na hata kuondoa mzunguko wa virusi. Haijulikani kama nyati pia anaweza kuambukizwa na viruisi aina EurAsian O na A zilizopo katika sehemu za Afrika Mashariki. Pia haikuwa wazi juu ya changamoto kuu za udhibiti wa FMD chini ya mazingira yanayotawaliwa na wafugaji wa kuhamahama barani Afrika ambayo yamezuia udhibiti wa FMD kwa miongo kadhaa.

Utafiti huu ulichunguza milipuko ya FMD kwenye ng’ombe nchini Tanzania kati ya 2018 na 2021 ili kubaini aina za virusi sababishi, sifa zao za kijenetiki-antijeni, na uhusiano wao wa mabadiliko kwa kutumia mbinu za kimolekuli zilizotumia vinasaba-RNA. Maambukizi asilia ya FMDV aina ya O na A nchini Tanzania yalichunguzwa kwa kutumia kipimo cha serolojia (NSP ELISA) na baadae kubainisha uwepo wa aina ya O na A kwa kipimo cha SPCE. Pia nakala za antijeni za B-cell za virusi SAT1 zilibainishwa kwa kutumia zana zilizounganishwa za kinga-taarifa zinazopatikana hadharani kuanzia zile zinazotumia mizani ya uelekeo hadi mifumo ya hivi majuzi ya kujifunza kwa mashine na miundo ya akili bandia, ili kuongeza uhalisi

wa utabiri, kwenye virusi FMDV SAT1 kutoka polipeptidi za VP1 za utafiti huu na zile zilizotolewa kutoka hifadhidata duniani.

Utafiti ulibainisha aina tatu za FMDV (A, O na, SAT1) zinazozunguka Tanzania zenye nasaba ya Africa Topotype G-I, EA-2, na Topotype I (NWZ) mtawalia. Mifuatano ya nyukleotidi iliyozalishwa kutoka kwa aina hii ya utafiti O na SAT1 zilichanganuliwa, na zilionyesha utambulisho wa asilimia ufanano ya 92.0-100.0% na 96.9-98.8% mtawalia. Jumla ya sampuli za seramu chanya kwa NSP ELISA 247 (37.6%, n =93) na (58.3%, n =154) nyati na ng'ombe mtawalia, kutoka maeneo ya Katavi, Mikumi, Mkomazi na Ruaha nchini Tanzania zilizochambuliwa kwa FMDV serotype O na A kwa SPCE. Asilimia ya juu zaidi seropositivity (PS) ilifichuliwa (67%, n= 103) na (55%, n= 51) katika ng'ombe na nyati, ambapo FMDV serotype A ilifichuliwa (54%, n= 83) O na (25%), n= 23) kwa sera za ng'ombe na nyati, mtawalia. Pia baadhi ya ng'ombe (36%, n= 56) na nyati (25%, n=23) sera zilizojaribiwa, zilionyesha ushahidi wa maambukizi mchanganyiko (O na A). Matokeo yalionyesha kuwa kati ya sampuli 93 za nyati zilizochanganuliwa za NSPE, 30.1% (28) ya CI (19 - 39) walikuwa na kingamwili maalum kwa aina ya FMDV aina A. Pia 54.8% (51) ya CI (39 - 64) na 24.7% (23) ya CI (15 - 33) walikuwa na kingamwili maalum kwa FMDV aina O na zile za majibu mchanganyiko (FMDV O na A) mtawalia. Vile vile, kati ya sampuli 154 za ng'ombe chanya za NSPE zilichambuliwa 53.9% (83) ya CI (69 - 98), 66.9% (103) ya CI (88 - 118) na 36.4% (56) ya CI (44 - 70) walikuwa na FMDV kingamwili mahususi kwa aina ya A, O, na mchanganyiko (O na A) mtawalia. Matokeo ya kulinganisha hali za serotypes mahususi yalifichua asilimia kubwa zaidi ya aina O kwa spishi zote, aina inayofuata A, na alama ya chini zaidi kwa michanganyiko (O na A). Vile vile; katika 95% CI, matokeo chanya ya NSPE ya ng'ombe na nyati yalionyesha alama za juu zaidi za (41.7%; 20.65%) kwa (33.6%; 11.34%) na (22.67%; 9.31%) kwenye aina O, aina A na mchanganyiko(O na A) mtawalia. Matokeo ya t-test katika kiwango cha 0.05 cha kulinganisha umuhimu aina za O, A, na michanganyiko (O&A) kati ya ng'ombe na nyati yalionyesha kuwa, katika kiwango cha 0.05 cha umuhimu kulikuwa na tofauti ya takwimu katika alama za aina-A kwenye ng'ombe na nyati ambapo thamani ya p ilitimiza kigezo ($p<0.05$) na tofauti ya wastani hasi 0.238. Hakukuwa

na tofauti za umuhimu wa kitakwimu kati ya aina O na aina (O&A) michanganyiko kwa ng'ombe na nyati waliochanganuliwa kwa thamani zao za p [(0.059 na 0.063) na (0.058 na 0.052)] zilikidhi kigezo (p>0.05) matawalia. Sampuli za probang (n=89) kutoka kwa nyati wa mbuga ya Serengeti zilizochanganuliwa, 1.12% (n=1) zilikuwa na ushahidi wa vinasaba vya FMDV RNA, zilifichuliwa kuwa ni FMDV aina O kwa kutumia seti za vianzio mahususi O na A (hatua moja RT-PCR). Pia epitopes tano za mtiririko kwenye maeneo (3-30, 44-59, 95-112, 134-149, 199-219) na 75%, 88%, 94%, 44 % na 24 % ya aa hifadhiwa ulifichuliwa mtawalia. Na epitopes za kimuundo sita (1-26, 82-87, 93-114, 131-149, 175-179, and 201-221) zenye zilizohifadhiwa 53 - 100%. Chanjo zinakabiliwa na changamoto ya FMDV kubadirika kwa kasi, hivyo kubaini vikoa vilivyohifadhiwa ni muhimu kwa chanjo za FMDV kwa siku zijazo ili kuongeza matarajio ya ufanisi wa muda mrefu wa matumizi ya chanjo.

Utafiti huu ulibaini maambukizo ya FMDV aina ya O katika nyati kuwa utafiti wa kwanza kufanyika kwa njia ya serolojia na molekuli katika ng'ombe na nyati nchini Tanzania. Utafiti ulizalisha na kukusanya taarifa zinazoweza kuimarisha ujuzi wa kukabiri FMDV Tanzania na katika nchi zingine barani Afrika. Changamoto zilizofichuliwa za udhibiti wa FMD miongoni mwa jumuiya mbalimbali za wafugaji barani Afrika ni msingi wa kuanzisha na kuendeleza mijadala ya kimkakati juu ya udhibiti wa FMD kitaifa, barani Afrika, dunia nzima.

DECLARATION

I, MKAMA MATHIAS MASHINAGU, do hereby declare to the Senate of Sokoine University of Agriculture that, this dissertation is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Mkama Mathias **Date**
(PhD Candidate)

The above declaration is confirmed by,

_____ _____

Prof. Christopher J. Kasanga **Date**
(Supervisor)

_____ _____

Prof. Philemon N. Wambura **Date**
(Supervisor)

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DEDICATION

I would like to dedicate this work to Almighty God, who gave me good health, energy, strength, ability, and perseverance in doing this work; my beloved wife Nyanzobe Ndaki; my precious parents Naomi Lutemela and Mathias Mashinagu, for their endless love, passion, prayers, and support in their own unique ways during the entire duration of my study.

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Status: Manuscript published in the *Journal of Transboundary and emerging diseases.*

Paper II: Mkama Mathias, Philemon Wambura, Sharadhuli Kimera, Donald King, David Paton, Francois Maree, Herbertha Mpete, Sengiyumva Kandusi, Mark Rweyemamu, and Christopher Kasanga. **Molecular epidemiology of Foot-and-mouth disease virus in Tanzania during 2020 to 2021 outbreaks.**

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Status: Manuscript submitted to the *Journal of Scientific Research and Reports.*

LIST OF ABBREVIATIONS AND SYMBOL

%	Percent
µl	microliter
^{0}C	Degree Centigrade
3ABC	Nonstructural Protein 3ABC.
3C^{pro}	Nonstructural Protein 3C
3D^{pol}	Nonstructural Protein 3D
aa	Amino Acid
ABI	Applied Biosystems
ADB	Africa Development Bank
Ag-ELISA	Antigen ELISA
AU-FMD	Africa Union-FMD
BEC	Bechuanaland
BEI	Binary Ethylene-Imine
BHK-21	Baby Hamster Kidney-21
bp	Base pair
BVI	Botswana Vaccine Institute
cDNA	Complementary deoxyribonucleic acid
CEO	Chief Executive Officer
CFT	Compliment Fixation Test
CGIAR	The Consortium of International Agricultural Research Centers
COLSAFA	la Comisión Sudamericana de Lucha contra la Fiebre Aftosa (COSALFA) South American Commission for the Fight against FMD
CPE	Cytopathic Effects
Cre	Cis-acting replicative element
DIVA	Differentiate between infected and vaccinated animals
dNTP	Deoxy nucleotide triphosphate
DVS	Director of veterinary services
EA	East Africa
EAF	East Africa Federation
ECOWAS	Economic Community of West African States
ECTAD	Emergency Center for Transboundary Animal Diseases
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
EU-FMD	European Union-FMD
EURO-SA	Europe –South America
FAO	Food and Agriculture Organization
FMD	Foot and mouth disease

FMDV	Foot and mouth disease virus
g	gram
GF-TADS	Global Framework-TADS
GRZ	Government of the Republic of Zambia
HACCP	Hazard Analysis Critical Control Point
IB-RS-2	Instituto Biologica Rim Suino-2
IFPHTM	Intermediate Fellowship in Public Health and Tropical Medicine
ILRI	International Livestock Research Institute
IRES	Inter ribosomal entry sites
ISH	Insitu hybridization
IZSLER	Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna
kb	Kilobase pair
KEVEVAPI	Kenya Veterinary Vaccines Production Institute
Km	Kilometer
KNP	Kruger National Park
LFBK	Cell line of Fetal Porcine Kidney
LPBE	Liquid Phase Blocking ELISA
L^{pro}	Protein-L
M2525	Diprenorphine hydrochloride
M99	Etorphine hydrochloride
MEGAX	Molecular Evolutionary Genomic Analysis version X
MgCl2	Magnesium Chloride
ml	milliliter
mM	millimole
mPCR	Multiplex polymerase chain reaction
MVPK-1	Mengeling-Vaughn Porcine Kidney-1 (Fetal porcine kidney cell lines)
NCBI	National Center for Biotechnology Information
ng	nanogram
NK72	Nick Knowles 72
nm	nanometer
NP	National Park
NSP	Nonstructural protein
NSPE	Nonstructural protein-enzyme linked immunosorbent assay
OIE	Office Internationale des Epizooties
OP	Oesophageo-pharyngeal
ORF	Open Reading Frame
P1-3	Viral protein of FMDV Genome Segment P
PANVAC	Pan African Veterinary Vaccine Center
PBS	Phosphate Buffer Saline
PCE	Predicted conformational epitope

PLE	Predicted linear epitope
PCP	Progressive Control Pathway
PCR	Polymerase Chain Reaction
pH	Measure of alkalinity or acidity
PI	Percentage Inhibition
PKs	Pseudoknots of RNA
pmol	picomole
PS	Percentage Seropositivity
PVS	Protein variability Server
qRT-PCR	Quantitative Real time-polymerase chain reaction
RGD	Arginine-glycine-aspartic acid
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT-PCR	Reverse-Transcription - Polymerase Chain Reaction
SACIDS	Southern African Centre for Infectious Diseases Surveillance
SADC	Southern African Development Community
SADC/TADS	SADC-Transboundary animal diseases
SAT	Southern Africa territory
SP	Structural Protein
SPCE	Solid Phase Competition/ competitive ELISA
SUA	Sokoine University of Agriculture
TAD	Transboundary Animal Disease
TAHC	Terrestrial Animal Health Code
TANAPA	Tanzania national parks authority
TAWIRI	Tanzania wildlife research institute
TBE	Tris base, Boric acid and EDTA containing buffer
$TCID_{50}$	Median tissue culture infective dose (amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated)
TVLA	Tanzania Veterinary Laboratory Agency
U.S $	United States Dollar
UK	United Kingdom
UNOWAS	United Nations Office for West Africa and the Sahel
USA	United States of America
UTR	Untranslated Region
VNT	Virus neutralization test
VP	Viral proteins
w/v	Weight/volume
WB-ACE	World Bank-Africa Center of Excellence
WOAH	World Organization for Animal Health
WRL	World Reference Laboratory
ZVC	Zonal Veterinary Center

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Foot-and-mouth disease virus (FMDV) causes a highly contagious Foot-and-mouth disease (FMD) to all cloven-hoofed animals and the disease is potentially accounted for food security and socio-economic devastating impacts (Mason & Grubman, 2009). This is cited to be the first animal infectious pathogen identified as a virus by Loeffler and Frosch in 1898 (Brown, 2003). The virus belongs to the genus *Aphthorvirus* of the family *Picornaviridae* (Lloyd-Jones *et al.,* 2017). FMD is endemic in Tanzania since its first documentation in 1927 and first virus typed isolation was made in 1954 (Rweyemamu & Loretu, 1972). There are seven antigenically distinct FMDV serotypes O, A, C, Asia1, and the Southern African Territories (SAT) 1, SAT2 and SAT3 that have been reported worldwide (Lloyd-Jones *et al.,* 2017). The infection or vaccination with any of the serotype cannot confer protective immunity against other serotypes, and some antigenically distinct topotypes exist even within same serotype (*OIE/FAO*, 2015). FMDV is a single-stranded, positive-sense RNA genome virus with approximately 8.5kb nucleotides in size (Lloyd-Jones *et al.,* 2017; Mahapatra *et al.,* 2015), whereby 7000 nucleotides of the genome size is occupied by its open reading frame (ORF) (Gao *et al.,* 2016; Mason *et al.,* 2003). The FMDV genome is enclosed by a 30nm diameter size icosahedral capsid that is composed of 60 copies of each of the four structural proteins (SPs) (VP1, VP2, VP3, and VP4) derived from the P1 region of its open reading frame (Mason *et al.,* 2003). The FMDV SPs play a potential role in capsid assembly and stability, virus binding, and antigenicity (Wright *et al.,* 2011). It's the three-dimensional arrangements of the viral SPs VP1, VP2, VP3, and the innermost VP4 that offers surface-exposed antigenic sites that elicit responses to vaccination or infection (Jackson *et al.,* 2003). The high replication and mutation rates as well as its broad host range, impart significant genetic deviations that influence the viral antigenic features subject to time and geographic location variabilities (Belsham & Bøtner, 2015). This situation facilitates frequent emergence of antigenically distinct variant strains of FMDVs in the field that need to be studied properly as benchmark for future

effective control strategies. The frequent studies need to provide knowledge of the viruses' spatio-temporal distribution patterns, the genetic-antigenic characteristics of serotypes circulating strains, antigenic correlation of circulating strains and available vaccine strains, and explicit knowledge of the role of every susceptible host in the epidemiology of the disease. Despite of other animal diseases reported in Africa, FMD plays a great role in restricting animals and their products access global lucrative markets, the situation that undermine livestock sector contribution to livelihoods improvement in African animal keeping communities. Currently the African continent contains the largest number of FMDV serotypes in the world history of the disease, and it is only FMDV Asia1 type that has never been reported (Vosloo *et al.,* 2002). Of the six known serotype of FMDV in Africa, four serotypes (A, O, SAT 1 and SAT 2) have been isolated and identified in Tanzania (Rweyemamu & Loretu, 1972; Rweyemamu & Loretu, 1973; Rweyemamu *et al.,* 2008). However, serotype C appears to have undergone extinction, and its latest published sequence data goes back to a 1996 strain from Kenya, that was phylogenetically analyzed with type C virus multiple nucleotides sequences archived in world-wide databases, and was inferred as an African/ Middle-east specific topotype (Paton *et al.,* 2021; Sangula *et al.,* 2011).

FMD control measures depend on the country disease status (EU-FMD, 2011;Paton *et al.,* 2009), where by vaccination, culling (infected and in contact animals) and movement control (fencing and quarantine) techniques are applied to effect prevention and control measures (Bergmann *et al.,* 2005). The FMD control has been challenging and difficult to achieve due to the presence of vital hindrances. Some of the challenge involves its broad host spectrum as FMDV is potentially infective to over 70 species of livestock and wildlife origin (Alexandersen & Mowat, 2005). Cattle and buffalo have been identified most as the main species involved in the transmission and as reservoirs of the virus in the livestock and wildlife animal populations respectively (Omondi *et al.,* 2020). The lack of compensatory policy particularly in cases of diseases epidemics and the involvement of wildlife in the epidemiology of the disease, then culling of the infected and all in contact animals cannot be applicable

in Africa. This has been applicable mostly in Europe and in other developed countries, like during UK FMDV outbreak 2001 (Leforban, 2002). The movement control has been facing difficulties as the vast majority of the cattle herds are under pastoral communities, and are kept in the proximity of porous border-conserved lands strategically for pastures access during seasons of the year with deprived pastures and water (Bronsvoort *et al.,* 2004; Fè Vre *et al.,* 2006). This state facilitates increased frequency of interactions between livestock and wildlife, sustain virus circulations and complicate the epidemiology of the disease in the country and the region at large (Vosloo et al, 2005). Studies carried out in southern Africa have shown that persistently infected African buffalo have so far been potentially proved that, buffalo (*Syncerus Caffer*) successfully transmit FMDV serotypes SAT1, SAT 2 and SAT 3 to cattle (SADC, 2018). Persistently infected cattle and buffalo (*Syncerus Caffer*) are cited to be the potential sources of new FMD outbreaks in endemic countries (Grubman & Baxt, 2004), and they can maintain the virus for 6 months up to 3 years and 5 to 24 years respectively (WOAH/OIE, 2009b). In the persistently infected cattle and buffalo FMDV is maintained in Oesophageo-pharyngeal epithelial cells (Longjam *et al.,* 2011; Thomson, 1996). And the major means of viral transmission at interface is through animals interactions that occur either within conserved lands or at close vicinity communal grazing lands (Mkama *et al.,* 2014). It therefore important to understand the role of African buffalo in the epidemiology of Eurasian FMDV serotypes O and A to establish FMDV infection spectrum and enhance FMD future tailored mitigation measures. Vaccination stands to be the main FMD control option and can be achieved by administering purified or un-purified vaccines (Asokan, 2009; Hostnik, 1997; Keeling *et al.,* 2003; Meeusen *et al.,* 2004; Nicholls, 1983; Parida, 2009b;Ferrari *et al.,* 2016). The FMD vaccines are recommended as prophylactics, or during quarantine after suspecting or confirmed FMD cases to prevent the virus spread and where appropriate the vaccinated animals may be subsequently slaughtered to reduce the delay in re-establishing trading status. Despite of being the main control option, effective vaccination faces challenges mainly caused by the distinct antigenic variabilities prevailing within and between FMDV serotypes (Carrillo *et al.,* 2005) squealed by its

complicated epidemiology. This state have persisted in various geographic areas for decades, enhancing evolution of new subtypes or topotypes within each serotype that portray significant genetic and antigenic distinct characteristics (Martínez *et al.,* 1992). This state result into FMD persistent outbreaks reports in Tanzania and other African countries, and mark Africa as global potential reservoir for FMDV and impedes the global progress on strategic initiatives for FMD control and eradication (Paton *et al.,* 2009). Tanzania is potential on cattle heads, and it possesses about 33,928,391 (Ministry of Agriculture, 2021) being ranked fourth in Africa after Ethiopia, Chad, and Sudan (Statista, 2022). Then any effort related to strategic FMD control is expected to unleash magnificent contribution in the national economy and improving lives of stakeholders under the livestock production (ILRI & CGIAR, 2017) as well as conservation value chains, as currently we don't export live animals and animal products to lucrative international markets.

This study was intended to generate updated knowledge of current circulating FMD field strains (O, A, SAT1, SAT2 and SAT3) distribution, and characterizing them to respective serotypes and topotypes. To unveil the epidemiological state of the cosmopolitan Eurasian FMDV serotypes O and A at select livestock-wildlife interface areas in order to understand the threat spectrum posed by wildlife in the future FMD control strategies. To conduct a genetic and antigenic identity analysis between current study African type (SAT1) strain, vaccine strains and prototype strains to understand the essential antigenic epitope domains on the VP1 regions that could be considered in selecting suitable vaccine for novel tailored vaccine development to achieve adequate protection of animals against FMDV field challenges (Parida, 2009). This study molecular, serological, bioinformatics and immune-informatics analytical tools to achieve the FMDV molecular epidemiology, its antigenic characterization and the establishment of preliminary serological related evidence of infection in animals. The study also utilized publicly available data from global rich databases relevant to this work to enrich/ enhance the outcome/output of this project.

1.2 Problem Statement and Study Justification

FMD is endemic in most of African countries and of the seven globally identified distinct serotypes, six serotypes (A, O, C, SAT1, SAT2 and SAT3) have been reported in Africa with marked regional differences in their distribution (Vosloo *et al.,* 2002). With exception to Serotype C, the 5 prevalent serotypes exhibit inter and intra serotype genomic and antigenic variability. The high rate of mutation and multiple circulating distinct strains confer diversified antigenicity (Mahapatra *et al.,* 2015). This situation present the major challenge for FMD control in Africa endemic countries where vaccination is marked to be the priority approach when compared to other control methods (movement control, slaughter of infected and in contact animals). The updated information of circulating field strains at different geographic location and antigenic characterization knowledge of prevalent FMDV is highly needed on this situation for strengthened suitable vaccine strains selection. Failure to address this knowledge gap in endemic areas of Africa on appropriate time basis has rendered futile eradication efforts for decades. Even though, the sustainable global FMD control and eradication cannot be achieved without addressing and supporting the control of the disease in endemic countries (Leforban, 2002) and emphasis on regional program(s). Studies done in Africa on FMDV are vastly on molecular characterization of FMDV VP1 genome region and no study has been done to unveil how suitably the VP1 genomic-antigenic characteristics of FMDV relate can be harnessed for future tailored vaccine development. The VP1 coding region exhibit high genetic diversity that reflect on expressed viral antigenic properties (Maree *et al.,* 2011) and vital for protective immunity if explicitly addressed. The combination of molecular, bioinformatical, and immunological/serological tools was adopted for this FMD study in in Tanzania and Africa at large. The study was expected to improve knowledge for future FMD vaccines, enhance understanding of currently circulating strains, and provide the insights of buffalo epidemiological role in improving future FMD control approaches and outcomes. Also, the results of this study contribute to the FMD-progressive control pathway (PCP-FMD) in African countries as guided by WOAH.

1.3 Research Questions

1.3.1 Overall research question

What are the genetic and antigenic characteristics of FMDV strains attributable to vaccine efficacy for FMD control in endemic settings in Africa?

1.3.2 Specific research questions

i) What are the current FMDV strains circulating in different geographic locations in Tanzania?
ii) What are the infection statuses of FMDV serotypes O and A in cattle and buffalo at selected livestock-wildlife interface areas of Tanzania?
iii) What are the predictable antigenic epitopes available on VP1 polypeptides of FMDV type SAT1 strains in Africa?

1.4 Objectives

1.4.1 General objective

To determine the antigenic characteristics attributable to vaccine efficacy for improvement of FMD control in endemic settings in Africa.

1.4.2 Specific objective

i) To determine the molecular epidemiology of FMDV circulating strains in Tanzania from FMD outbreaks.
ii) To determine the epidemiological evidence of Eurasian strains O and A circulating at selected livestock-wildlife interface.
iii) To examine the characteristics of predictable antigenic epitopes of select FMDV strains in Africa.

1.5 Literature Review

1.5.1 Foot-and-mouth disease virus

The FMDV causes a highly contagious foot-and-mouth disease (FMD) in all cloven-hoofed animals (livestock and wildlife), with devastating consequences on food security and livelihoods (Lee *et al.,* 2012; Mason *et al.,* 2003). In all vulnerable cloven-hoofed hosts, blister- like vesicles develops on the tongue, mouth, teats, and coronary bands of the feet (Donaldson, 2019). The virus belongs to the genus *Aphthorvirus* and family *Picornaviridae* (King *et al.,* 2000).

FMD viruses are considered as the world's smallest category of viruses with a positive sense, Single-stranded RNA genome (Pico~) (Alexandersen *et al.,* 2002). FMD has a global distribution, with some countries being endemic and others experiencing sporadic or epidemic outbreaks (Jamal & Belsham, 2013; Jean-Francois *et al.,* 2009; Paton *et al.,* 2018). The World Organization for Animal Health (WOAH/OIE) and the Food and Agriculture Organization (FAO) recognize FMD as one of the most transboundary diseases in the world, with only (Greenland, Iceland, New Zealand, and the Oceania Islands) that have never reported any outbreak (Bronsvoort *et al.,* 2004). FMD prevents countries from benefitting on lucrative animal and animal products markets (Alexandersen *et al.,* 2002; Paton *et al.,* 2009; EU-FMD, 2011).

1.5.2 The history of foot-and-mouth disease virus

The brief history of FMDV is based on Knowles's 1990 report. In his report, Knowles began by outlining how the documentation from Hieronymi Fracastorii (1546) might be suggestive of FMD. In Northern Italy in the year 1514, Fracastorii described a condition as unusual and affecting exclusively cattle. In 1780, in Southern Africa, Le Vaillant (1795) reported a disease in cattle that "attacked the feet of oxen, causing them to swell enormously, causing suppuration, and the hooves could occasionally slough off." Gordon Cumming (1850) and General S.J.P. Kruger (1858) both recorded a probable outbreak of FMD in Southern Africa. FMD was present in Rhodesia and Swaziland in 1892 (Sinclair, 1922) and then in Rhodesia in 1894 to 1895 (Edmonds, 1922). Adami documented the occurrence of the disease for the first time in Germany in 1754 (Henning, 1956), but records from Great Britain indicate the very first report was made in August 1839. In 1895, the disease infiltrated Brazil, and in 1910, it apparently impacted Uruguay, Peru, and Chile. In 1898, Loeffler and Frosch revealed that the agent of foot-and-mouth disease could be filtered, and in 1922, Vallée and Carré demonstrated the presence of two immunological types of FMDV by cross-immunity experiments in cattle. They were designated O (Oise, a department in northern France) and A according to their areas of origin (Allemagne - Germany). Waldmann and Trautwein (1926) identified three immunologically distinct types, A, B, and C. The three types became

designated by international agreement, as Vallée O, Vallée A and Waldmann C and later simply as O, A and C. After cross-protection experiments in cattle and guinea pigs, samples originating from Bechuanaland (BEC/1/48) were determined to be different from O, A, and C at the WRL in 1948. These new types were designated as Southern African Territories (SAT) types 1, 2, and 3. In 1951 to 1952, viruses were isolated across India (Dhanda *et al.,* 1957) and 1954 in Pakistan then designated as the Asia 1 serotype (Brooksby and Rogers, 1957). The discovery of FMDV by Loeffler and Frosch in 1898 makes it the first animal disease pathogen to be recognized as a virus (Brown, 2003), and its worldwide control strategies were the driving force nucleus for the founding of the World Organization for Animal Health WOAH (founded as OIE) in 1928.

1.5.3 Foot-and-mouth disease food security and economic consequences

FMD normally causes negligible mortalities in mature animals, except in immature animals (calves and piglets) whereby case fatality may approach 100 percent (James & Rushton, 2002; Knight-Jones & Rushton, 2013; WOAH, 2016). The disease causes catastrophic consequences, such as weight loss, decreased milk yield, jeopardize draught power in agriculture, and ultimately results in a protracted period of productivity loss (Grubman & Baxt, 2004). According to the World Organization for Animal Health (WOAH), FMD is on the A list of infectious animal diseases and has been recognized as the greatest obstacle to the worldwide trade of animals and related products (Aggarwal *et al.,* 2002; Grubman & Baxt, 2004). Then, in order to feed the world's rapidly expanding population, the freedom of food product transportation is an essential component but compromised by FMD existing epidemiological situation. Following an outbreak of FMD, the process of returning to FMDV-free status has always been expensive economically and difficult or challenging to achieve. A practical example was the 2001 FMD outbreak across the United Kingdom, almost 4 million animals were culled and 2.5 million additional animals were destroyed for welfare reasons (Scudamore & Harris, 2002). It was estimated that the epidemic caused overall losses of around $12 billion, and the tourism industry contributed around 36% of the overall losses encountered. The

described commitment described by United Kingdom government by executing serious and strategic FMD mitigation measures enabled to end the virus circulation, with the final case record in the United Kingdom was on September 30, 2001, and the country regained its FMD-free status without vaccination on January 22, 2002 (Scudamore & Harris, 2002). The government had to compensate for the damaged agriculture and food chain industries about $4.2 billion due to FMD impacts (Thompson *et al.,* 2002).

1.5.4 Foot-and-mouth disease virus host range

The foot-and-mouth disease virus is characterized to exhibit a broad host spectrum of both livestock and wild animals, and can infect more than 70 species within 20 families (Alexandersen *et al.,*2002). Besides sheep, goats, pigs, and water buffalo, in the livestock side, cattle are the most susceptible livestock species (WOAH/OIE, 2018). Sheep and goats often present milder clinical signs of the disease compared to pigs and cattle (Alexandersen *et al.,* 2002; WOAH/OIE, 2018). FMDV may also infect camelidae, bactrian, camels, new world camelids, deer, antelopes, elephants, and giraffe (Larska *et al.,* 2009). Experimental infection of rats, mice, and guinea pigs is possible; and additional species susceptible to FMD viruses include llamas and alpacas, although they play no part in the epidemiology of FMD (Alexandersen & Mowat, 2005)**.**

1.5.5 Conditions for foot-and-mouth disease virus survival

The FMDV is pH-sensitive, with an optimum pH range between 7.2 and 7.6, and is rendered inactive at pH values between 6.0 and 9.0. Within the pH range of 6.0-7.2 to 7.6-9.0, FMDV remains in a condition of partial inactivation. The FMDV is somewhat stable at lower temperatures, lasting for one year at 4^0C, though its survival period decreases as the temperature raises (Gibbs, 2003). FMDV may persist in hay for up to 15 weeks and may be protected from high ambient temperatures by feces and other organic debris (Cottral, 1969). Upon slaughter, the virus is inactivated within 48 hours provided the pH decreases below 6.0, as it usually occurs in the skeletal muscles during the rigor mortis phase. However, in non-replicative form, the virus may persist in lymph nodes and bone marrow (Cottral, 1969), and the virus can survive for years

when tissues containing FMDV are frozen at -75^{0}C. FMDV are sensitive to disinfectants; hence, cleaning combined with disinfection is effective in removing the agent from contaminated facilities (Davies, 2002).

1.5.6 Scientific classification of foot-and-mouth disease virus

FMDV belongs to genus *Aphthorvirus* and family *Picornaviridae* (King *et al.,* 2000). It possesses a single-stranded, positive-sense RNA genome enclosed in the smallest (Pico) size capsid (Gao *et al.,* 2016; Mason *et al.,* 2003).

1.5.7 Global distribution of foot-and-mouth disease virus serotypes

There are seven antigenically distinctive serotypes of FMDV (O, A, C, SAT1, SAT2, SAT3, and Asia1) that have been identified globally (Fig. 1.1) (Jean-Francois *et al.,* 2009; Kitching *et al.,* 2007; WOAH/OIE, 2018; Rweyemamu *et al.,* 2008). The serotypes O, A, and C are designated as Europe and America serotypes, the Southern African Territories (SAT) types 1, 2, and 3 that were first discovered in 1948 by WRL (Knowles, 1990) from samples originating in Africa are designated as African serotypes. The SATs have caused occasional outbreaks in the Middle East, but have failed to establish themselves outside of Africa (Vosloo *et al.,* 2002). In the early 1950s, viruses isolated from India in 1951 and 1952 (Dhanda *et al.,* 1957) and Pakistan in 1954 were identified as the Asia 1 serotype (Brooksby & Rogers, 1957). In contrast to serotypes A, O, and C, Asia1 has never spread to other continents (Fig. 1.1). The FMDV evolution processes to multiple serotypes ad topotypes is enhanced by its rapid replication rate and lack of a proofreading mechanism, broad host range in varying geographic regions. The mutation rate of FMDV has been estimated to be $1.46x10^{-3}$ substitutions/site/year, which is comparable to that of other RNA viruses (Belsham & Bøtner, 2015). The most recent common ancestor appears to have evolved around 481 years ago (early 16th century). In Africa, serotype C was recorded and documented in Kenya, Uganda, Zambia, Angola, Tunisia, and Ethiopia, however it has now become extinct since there is no evidence of ongoing FMD epidemics caused by type C viruses (Vosloo *et al.,* 2002).

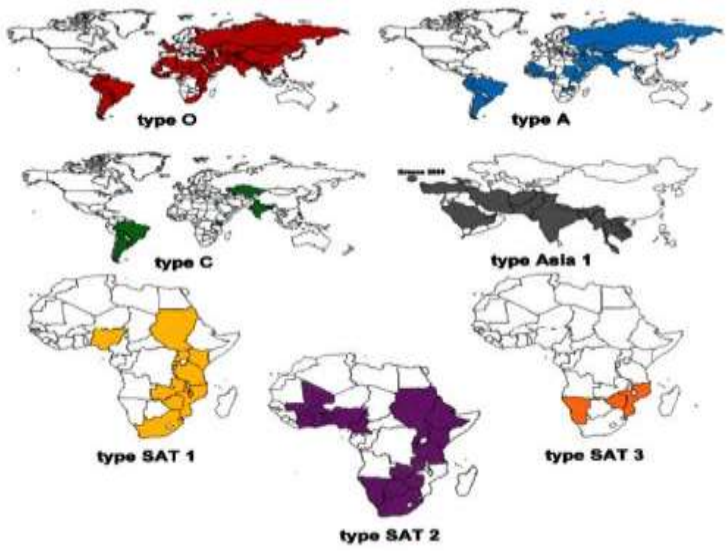

Figure 1. 1: Global and Africa continent map showing foot- and –mouth disease virus serotypes O, A, C, SAT1, SAT2, SAT3, and Asia1 distribution patterns (Shahriari & Habibi-Pirkoohi, 2018).

1.5.8 Foot-and-mouth disease virus serotypes reported in Tanzania

The first occurrence of FMD in Tanzania was recorded in 1927; isolation and typing were achieved in 1954 (Rweyemamu & Loretu, 1973). FMD is endemic in Tanzania and by the year 1958, serotypes A, O, and SAT 2 had already been identified; in 1971, SAT 1 was further recorded for the first time (Rweyemamu & Loretu, 1973; Vosloo *et al.,* 2002). The FMDV serological survey conducted between 1994 and 2004 in East African wildlife (African buffalo) revealed the presence of FMDV type SAT3 in Tanzanian African buffalo (Bronsvoort *et al.,* 2008). With this report, a total of five FMDV serotypes have been identified (Serotypes A, O, SAT1, SAT2, and SAT3), although isolation and genomic inferences need to be accomplished particularly on SAT3 type.

1.5.9 Structure of the foot-and-mouth disease virus capsid

The FMDV icosahedral capsid is made from 60 copies of the SPs VP1, VP2, VP3 and VP4 (Carrillo *et al.,* 2005), with an estimated diameter of 30nm size. VP1-3 are the surface-exposed proteins (Fig. 1.2), and their 3D structure serves as the main antigenic determinants, and VP1 harboring the virus's key antigenic epitopes (Dyirakumunda *et al.,* 2017). The VP4 protein is positioned closest to the viral genome and presents the lowest proportion of the antigenic epitopes (Carrillo, 2012).

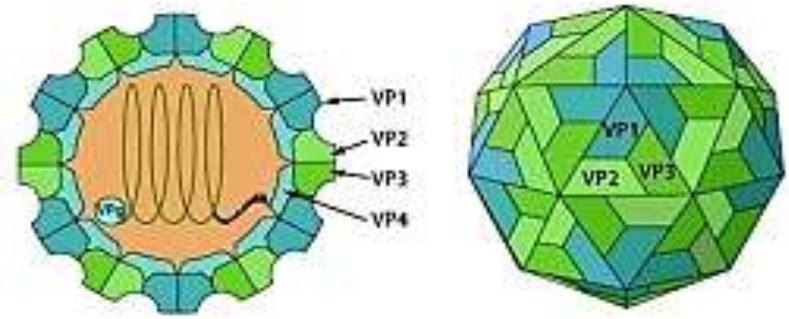

Figure 1.2: Foot-and-mouth disease virus icosahedral capsid structure schematic representation and the capsid building blocks schematic orientation of structural proteins VP1, VP2, VP3 and VP4 (Shahriari & Habibi-Pirkoohi, 2018).

1.5.10 Foot-and-mouth disease virus genome Organization

The FMDV is comprised of a positive sense, single-stranded RNA genome enclosed in an icosahedral symmetry capsid made of 60 copies each of four structural proteins (VP1 [1D], VP2 [1B], VP3 [1C], and VP4 [1A]) (Gao *et al.,* 2016; Grubman & Baxt, 2004). The organization of FMDV genome partakes similar basics as those of other members of the *Picornaviridae*, and the nomenclature of its proteins was established by (Rueckert & Wimmer, 1984). The FMDV genome is approximately 8200 nucleotides length size excluding the poly(C) and poly(A) tracts (Jackson *et al.,* 2007). Its open reading frame (ORF) is of approximately 7000 nucleotides length size, and based on the presence of cleavage sites is divided into four regions named as L^{pro}, structural protein (P1) and non-structural proteins (P2

and P3) (Rueckert & Wimmer, 1984) (Fig. 1.3). The FMDV genome ORF is flanked by 5' untranslated region (5' UTR) upstream and 3' UTR downstream. The FMDV 5' UTR and 3' UTR contains about 1300 and 161 nucleotides by size respectively (Forss *et al.,* 1984; Gao *et al.,* 2016). The 5' UTR is comprised of five functional elements named sequentially from 5' end is the S fragment, poly(C) tract, RNA pseudoknot, the cis-acting replicative element (cre-structure) (Mason *et al.,* 2002) and internal ribosome entry site (IRES) (Forss *et al.,* 1984; Kanda *et al.,* 2016), vital for virus RNA replication and translation processes. At the 3' end of the FMDV genome is the 3'UTR bearing a poly-A tract that thought to be necessary for genome replication (Carrillo, 2012; Gao *et al.,* 2016; Jackson *et al.,* 2007; Mason *et al.,* 2003). After the 5' UTR, the L^{pro} region follows and contains two in-frame AUG initiation codons that encode two L proteins, Lab and Lb (Robertson *et al.,* 1985; Sangar *et al.,* 1987). Thereafter is the P1 region that encodes the four structural proteins, 1A (VP4), 1B (VP2), 1C (VP3) and 1D (VP1) for viral capsid assembly. The 1A (VP4), 1B (VP2), 1C (VP3) and 1D (VP1) assemble to form a protomer where five protomers assemble together to form a pentamer. Twelve pentamers reorganize to enclose the viral RNA to create a virus particle (provirion). The VP1-3 are surface exposed while the VP4 is internally located contacting the RNA. The P2 region encodes three non-structural (NS) proteins named as 2A, 2B and 2C and where the last P3 region codes for other three NS proteins termed as 3A, 3B, $3C^{pro}$ and $3D^{pol}$ protein (Fig. 1.3) (Mason *et al.,* 2003; Rueckert & Wimmer, 1984).

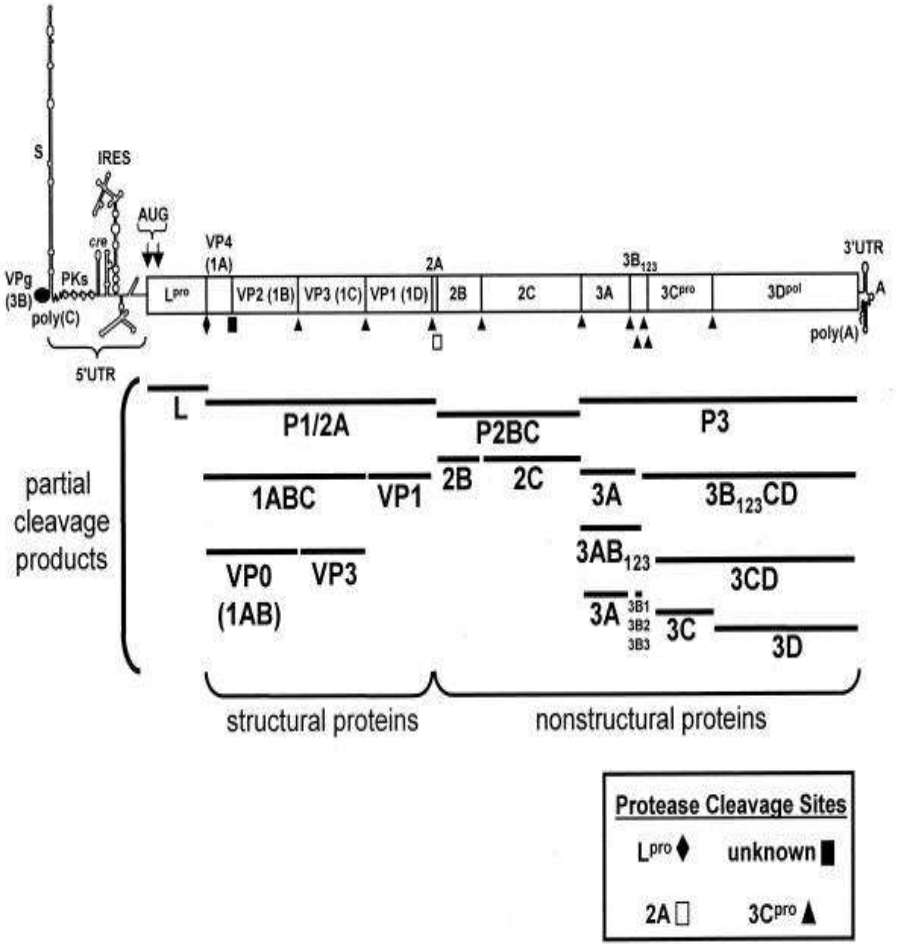

Figure 1.3: **Schematic representation of the FMDV genome, the ORF is presented within the boxed area, and the viral proteins labeled based on the nomenclature of (Rueckert & Wimmer, 1984). Also indicated are the functional portions of the genome, the positions of the principal cleavages and the proteases involved, as well as the partial protein cleavage products, figure adapted from Mason *et al.,* (2003).**

1.5.11 Foot-and-mouth infection virus source(s) of infection

The FMDV particles are mostly found in the secretions, excretions, and aerosols from clinically manifesting or recovered animals. Four

days before the onset of obvious clinical signs, the milk and semen from infected cattle may contain virus particles, with titers of approximately $\log_{10}$ 6.7 $TCID_{50}$ per ml in milk and $\log_{10}$ 6.2 $TCID_{50}$ per ml in semen, $\log_{10}$ 4.9 $TCID_{50}$ per milliliter of urine, and $\log_{10}$ 5.0 $TCID_{50}$ per gram of fecal materials (Donaldson, 1987; Kitching, 1992) in (Animal Health And Welfare, 2006). In situations where cattle herds are mostly kept by pastoral communities with dependence on communal grazing areas and watering points like in Africa (Ismail, 2014), during outbreaks the virus spread get fast as the environment get contaminated easily by saliva, urine and faeces containing viral particles from sick animals (Alexandersen & Mowat, 2005; Cottral, 1969; Kitching, 2002). And this is one of the key constraints to FMD control progress in Africa contrary to how it appears in the developed countries where livestock are grazed in privately owned/managed paddocks and mostly kept indoors in well-designed stalls. (Summary of different bovine tissues versus their FMDV amounts, see Appendix 1).

1.5.12 Foot-and-mouth disease virus modes of transmission

The aerosols containing infectious particles are the principal and most challenging route of FMDV transmission to control (Paton *et al.,* 2018;Brown *et al.,* 2022). Aerosols blown over 250 kilometers from infected pigs in Brittany, France across the English Channel in 1981 caused an epidemic in cattle on the Isle of Wight in the United Kingdom (UK) (Kitching, 2002). Environmental features associated with humid and cold weather enhance airborne transmission by allowing infectious aerosols to be carried over great distances by the wind (Bartley *et al.,* 2002; Colenutt *et al.,* 2020, 2018). In the vast majority of cases, direct contact between susceptible and infected animals has been documented, and carrier cattle in Zimbabwe were identified as being responsible for FMDV transmission and subsequent outbreaks on their destination farms (Thomson, 1996a). Furthermore, susceptible animals can get infected by ingesting animal products (milk or meat), and the virus may spread via fomites such as shoes, vehicle tires, and other inanimate items contaminated with the virus, as well as rats and birds (Alexandersen & Mowat, 2005; Kitching, 2002). In situations where a large proportion of cattle are managed by the traditional sector as in the African context,

communal grazing ranges and water points become contaminated following an FMDV outbreak, and susceptible animals can become infected by ingesting and drinking FMDV-contaminated pastures and water, respectively. Numerous FMD outbreaks have been documented in countries such as Saudi Arabia and Lebanon that import live animals (cattle, sheep, and goats) from Africa and Asia FMDV endemic areas, the origin of the strains from these sources was proved by genetic inference (Brito *et al.,* 2017; Rweyemamu *et al.,* 2008).

Also, milk tankers have been identified as a significant source of the virus' rapid spread (Johnson *et al.,* 2016). However, transmission of FMDV is usually rapid in naïve or unvaccinated herds, as proven in the 2001 United Kingdom where over 90 percent of the herd had clinical signs by the time the disease was firstly detected (Alexandersen *et al.,* 2002).

1.5.13 Pathway of Infection

The vast majority of studies done towards establishing the route of infection in vulnerable hosts have been biasly centered on cattle. Following exposure to FMDV, cattle can become infected with FMDV via oral route by consuming or drinking contaminated feeds or water, by breathing polluted aerosols (respiratory route), iatrogenically, and damaged epithelia (Jiménez-Clavero *et al.,* 2001).

1.5.14 Pathogenesis

Aerosolized virus mostly causes FMDV infection in cattle via the respiratory system (Brown *et al.,* 2022; Colenutt *et al.,* 2020). Infection can also occur through abrasions on the skin or mucous membranes; however, this route is exceedingly ineffective, requiring roughly 10,000 times more virus to be effective (Donaldson, 1987). Contradictory evidence has been uncovered about the place of early viral replication. Some indicate being in the lung or pharyngeal regions (Brown *et al.,* 1992; Brown *et al.,* 2022; Burrows *et al.,* 1981; Sutmoller & McVicar, 1976), the pharynx and not the lungs (Alexandersen *et al.,* 2003; Burrows *et al.,* 1981; Zhang & Kitching, 2001), and nasal mucosa and not in the lungs for pigs (Oleksiewicz *et al.,* 2001). However, the virus strain, aerosol particle size, and

their mode of generation are cited as key variables in determining the location of early viral replication in aerosol-exposed cattle (Alexandersen *et al.,* 2003). Following significant replication in the initial locations, the virus disseminates fast to end point predilection sites (epithelia) in the periphery (oral and pedal epithelial regions) and this is considered to be mediated by monocytes or macrophages (Brown *et al.,* 1992). Occasionally, viruses have been detected in locations that do not show the presence or development of clinical lesions (Brown *et al.,* 1995; Oleksiewicz *et al.,* 2001). Pigs are less vulnerable to aerosol infection than cattle or sheep (Alexandersen *et al.,* 2002; Donaldson & Alexandersen, 2002), and when viraemic, they excrete more aerosolized virus particles than cattle or sheep (Alexandersen *et al.,* 2002; Donaldson & Alexandersen, 2002). They are often infected by consumption of FMDV-contaminated food, direct contact with infected animals, and when in proximity to sites contaminated by FMDV-infected animals (Grubman & Baxt, 2004). Within the first 24 hours after infection, virus had been detected in the bronchiolar epithelium, sub-epithelium, and interstitial regions of the lungs using in-situ hybridization (ISH) experiments on cattle (Brown *et al.,* 1996). Within 72 hours, the signal was identified in the epithelial cells of the soft palate, trachea-bronchial lymph nodes, tonsils, tongue, and feet (Brown *et al.,* 1996).

The incubation period is the interval of time from successful infection to when clinical signs can be seen. In a susceptible host infected with FMDV, the incubation period depends on the virus strain, the viral exposure dosage, the route of infection, the animal species, the susceptibility or immunological status of the animal, and the husbandry practices (Alexandersen & Mowat, 2005; Arzt *et al.,* 2019). Usually, the incubation period ranges from 2 to 3 days to 14 days (Alexandersen & Mowat, 2005), and infected incubating animals may become infectious prior to the manifestation of clinical signs (Stenfeldt *et al.,* 2016). Within-herd transmission of FMDV can occur between 1 and 6 days (Alexandersen & Mowat, 2005), depending on the farm management system and environmental conditions that encourage animal to animal contact and viral survival (Colenutt *et al.,* 2020). During the time of viraemia, animals shade viruses; following the

development of antibodies and the subsequent healing of lesions, animals cease shading viruses.

1.5.14.2 Foot-and-mouth disease virus predilection sites

Except if the virus has gain entry via wounds or abrasions, the pharyngeal region is considered to be a vital initial site of infection (Arzt et al., 2010). The pharyngeal region consists of the dorsal soft palate, the roof of the pharynx, and a portion of the tonsil lined with stratified squamous non-cornified epithelial cells. The non-cornified feature provides living cells for viral replication and also contributes to the persistence of infection in ruminant carrier animals. Virus will remain in the pharynx for one to three days before entering the bloodstream via regional lymph nodes and causing viraemia (Alexandersen et al., 2002;Arzt et al., 2011; Arzt et al., 2010).

1.5.15 Foot-and-mouth disease clinical signs in cattle

Clinical signs of clinically sick animals can range from mild to severe conditions. After the incubation phase, the animal develops a fever of up to 41^0C , anorexia, blister-like vesicles (aphthae) and erosions in the muzzle, nares, tongue, gums, lips, buccal mucosa, around the coronary bands, interdigital cleft, and teats (udder), especially in lactating cows (Stenfeldt et al., 2016;OIE, 2009a). Excessive salivation of stringy or foamy saliva and drooling, lameness, anorexia, loss of body condition, rough hair coat, lacrimation, stagnated growth, decline in milk production, and signs of heat intolerance may develop in convalescent animals. In most cases part, FMD is associated with low mortalities, but myocarditis causes notably high mortality in calves (Grubman & Baxt, 2004). Regardless of whether the condition is severe, the vesicles in the mouth usually heal within seven days, whereas recovery of the tongue papillae takes longer. In the majority of cases, severe lesions occur in regions subjected to trauma or extreme exertion, particularly when animals are in a viraemic state (Hyslop, 1965; Sellers, 1971). Under this circumstance, the most of vesicular lesions are found on the feet and tongue (Grubman & Baxt, 2004).

1.5.16 Foot-and-mouth infection and mortality rates

FMD has been reported to have high incidence and prevalence rates that can approach 100%, with mortalities ranging 1-2% in susceptible adult animals; however, the extremely high mortality rates are found in young calves, lambs, kids, and piglets even before exhibiting vesicles due to virus-induced myocarditis (Grubman & Baxt, 2004).

1.5.17 Foot-and-mouth disease virus spread

Significant quantities of FMD viruses can be disseminated through the secretions and excretions of acutely infected animals. During epidemics, aerosols from acutely infected animals are indicated as the primary means of FMDV transmission (Bartley *et al.,* 2002; Colenutt *et al.,* 2018, 2020). In convalescent animals, infectious virus disappears from most tissues, but low quantities may persist in the oropharynx of certain ruminants (Kitching, 2002). These animals may excrete FMDV, contaminate food, water, formites, humans, birds, rodents, and habitats, and serve as possible vectors for viral transmission to naive animals in new places (Alexandersen & Mowat, 2005; Kitching, 2002). Live virus or viral RNA may be obtained from oesophageo-pharyngeal fluids and mucosal cells of recovered/convalescent animals using a probe cup (Kitching & Donaldson, 1987; Van Bekkum *et al.,* 1959).

1.5.18 Persistent foot-and-mouth disease virus infection

When exposed and subsequently infected with FMDV, the vaccinated or convalescent cattle develop persistent infection (Sutmoller & Casas, 2002). Due to the impracticality of FMD vaccination in buffalo, persistent infection in buffalo is effected via infection and re-infection processes (Cox *et al.,* 2005, 2006; Sallu *et al.,* 2014). FMDV-persistently infected cattle and sheep can become carriers and pose threat for the emergence of new outbreaks; hence, they must be accurately identified after outbreaks (Alexandersen *et al.,* 2002; Grubman & Baxt, 2004). In addition, studies have revealed that African buffaloes may transmit FMDV to cattle herds and initiate new outbreaks (Dawe *et al.,* 1994a; Dawe *et al.,* 1994b; Vosloo *et al.,* 2007). Identifying persistently infected animals is challenging, principally when vaccinations are conducted using impure vaccines. The latter have NSPs analogous to those of wild-type viruses, and

both can stimulate the synthesis of NSP antibodies, thereby making it difficult to differentiate between vaccinated and infected cattle using NSP ELISA (Sorensen *et al.,* 1998). Following infection, viral replication processes are monitored by the expression of immunogenic NSPs (3ABC), and their specific antibodies can be detected using 3ABC-ELISA to distinguish among vaccinated and naturally infected animals (De Diego *et al.,* 1997; Mackay *et al.,* 1998; Sorensen *et al.,* 1998) . FMDV is also persistently maintained in lymph nodes in a non-replicating form, according to studies (Juleff *et al.,* 2008). FMDV is known to be retained in Oesophageo-pharyngeal epithelial cells of persistently infected cattle and buffalo, and these cells may be collected using a probang cup during surveillance (Kitching & Donaldson, 1987; Van Bekkum *et al.,* 1959).

1.5.19 Foot-and-mouth disease diagnosis

The strategic FMD control and eradication initiatives in endemic regions need to be accompanied by plausible disease diagnosis processes (R´emond *et al.,* 2002). FMD diagnosis has mostly been based on viral isolation, detection of FMDV antigen, FMDV nucleic acid materials, and FMDV-specific antibodies in tissue and fluid samples (OIE, 2009). These diagnostic methods can be ranked; the first technique is the identification of the agent by virus isolation, followed by Nucleic acid recognition methods that employ Agarose gel-based RT-PCR assay and Real Time RT-PCR (qRT-PCR) assay, and serological techniques that employ ELISA technique and Complement fixation test (WOAH/OIE, 2009a). However; the recommended method for the tentative diagnosis of FMD incorporates the findings of the clinical examination, post mortem examination, as well as laboratory analysis.

1.5.19.1 Diagnosis of foot-and-mouth disease by Clinical Examination

Infected animals manifest the following clinical signs: anorexia and pyrexia of up to 41^0C, appearance of blister-like vesicles (aphthae) and erosions in the muzzle, nares, gums, lips, buccal mucosa, feet (around the coronary bands and interdigital clefts) and around teats & udder particularly on lactating cows (WOAH/OIE, 2018), as well as excessive salivation of stringy saliva (Grubman & Baxt, 2004). If

reported lately, vesicle lesions within the mouth may be found to have healed within seven days, although the duration for tongue papillae healing often takes longer. However, this diagnostic strategy employs an explicit investigation of the existence of the aforementioned clinical signs and inferences need to be generated regarding the animal's clinical condition.

1.5.19.2 Diagnosis of foot-and-mouth disease in the Laboratory

1.5.19.2.1 Isolation of virus

The virus isolation technique is employed to diagnose FMD by using cell culture or 2-7 day old unweaned mice (House & House, 1989). The most sensitive culture systems for FMDV isolation include primary (pig, calf, or lamb) kidney cells and primary (bovine or calf) thyroid cells (Snowdon, 1966). It's important to exercise caution when employing the latter approach, as cryopreservation of bovine thyroid cells subsequently after trypsinization lowers susceptibility to FMDV (House & House, 1989). The basic stable cell lines, such as IBRS-2 (House *et al.,* 1988), MVPK-1 clone 7 (Dinka *et al.,* 1977), LFBK cell line and BHK-21 (House *et al.,* 1988), are susceptible to FMDV but less sensitive, and are hence not appropriate for detecting low concentrations of infectivity (Clarke & Spier, 1980). Epithelia tissues, vesicular fluids, lymph nodes (submandibular lymph nodes), and oesophageo-pharyngeal fluids are the recommended samples for virus isolation. Only vesicular fluids may be injected directly into cell lines; the rest require further processing. Epithelium and lymph node samples must be removed from PBS/glycerol, wiped dry on absorbent paper to minimize the harmful glycerol content, and weighed. To achieve a 10% suspension, the sample need to be ground in sterile sand using a sterile pestle and mortar with nine times the amount of tissue culture media and antibiotics. In the case of OP fluids, a pretreatment with an equal amount of chlorofluorocarbons is recommended, as viral release from immune complexes may enhance the rate of virus detection. When the sample materials have been adequately homogenized, the supernatant is obtained by centrifuging at 2,000g for 10 minutes. To isolate the targeted virus, the latter can be inoculated into cell cultures or unweaned mice. The sensitivity of any cells utilized should be

evaluated using FMDV standard preparations. The use of IB-RS-2 cells facilitates the distinction of swine vesicular disease virus (SVDV) from FMDV (since SVDV will only develop in cells of pig origin) and is essential for isolating porcinophilic strains such as O Cathay. Upon inoculation and incubation, the cell cultures need to be evaluated for 48 hours for cytopathic effect (CPE). If no CPE is found, the cells should be frozen and thawed, then used to inoculate new cultures and tested for CPE once more 48 hours later. Unweaned mice are an alternative to cell cultures and should be 2–7 days old and of inbred strains that have been carefully chosen. In other instances, earlier passages may be necessary for field viruses to adapt to mice models (Skinner, 1960). A drawback of cell culture is that it is prone to contamination and loses sensitivity in the presence of inhibitors such as interferons and some enzymatic inhibitors.

1.5.19.2.2 Serological techniques

The Enzyme-linked immunosorbent assay (ELISA) technique was invented to accommodate technical variabilities encountered mostly during serological testing. Since 1975, ELISA has been one of the most widely recognized serological tests for the diagnosis of several infectious diseases (Longjam *et al.,* 2011). There are two types of serological tests for FMD; those which detect antibodies to viral structural proteins (SP) and those which detect antibodies to viral nonstructural proteins (NSPs) (Ma *et al.,* 2011). Examples include the virus neutralization test (VNT) (Golding *et al.,* 1976) the solid-phase competition/blocking ELISA (Paiba *et al.,* 2004), and the liquid phase blocking ELISA (Hamblin *et al.,* 1986; Hamblin *et al.,* 1987). Then, SP tests are intended for FMD antibody detection, quantification, and characterization of respective field circulating strains. Following FMDV infection, both anti-SP and anti-NSP antibodies are generated (Brocchi *et al.,* 2006; Sorensen *et al.,* 1998). Genetic studies have enabled the ability to identify and selectively delete genes from a pathogen, enabling the development of "marker vaccines" which then, when coupled with additional diagnostic assays, may discriminate infected animals from vaccinated ones (DIVA) (Uttenthal *et al.,* 2010). DIVA is then conducted through differentiating vaccine-generated antibodies from those elicited by infection with the wild-type virus. The NSP ELISA is used to identify animals with past or present infection

with any of the seven serotypes of the virus, regardless of whether the animal has also been vaccinated (Brocchi *et al.,* 2006). Antibodies for 3AB or 3ABC polypeptides signify natural infection (De Diego *et al.,* 1997; Mackay *et al.,* 1998), and this assay must always be coupled with SP ELISA, i.e. LPBE or SPCE, to determine the FMDV serotype (s) circulating at a particular geographic area if necessary (Ma *et al.,* 2011; Mwiine *et al.,* 2010).

1.5.19.2.3 Polymerase Chain Reaction (PCR) technique

This is currently regarded to be the most effective nucleic-acid-based diagnostic technique since its invention (Bartlett & Stirling, 2003; Mullis & Faloona, 1987) and an alternative system to conventional serological platforms that executes rapid confirmation of clinical diagnosis and possesses high sensitivity and specificity. It is currently considered to be the gold standard for determining nucleic acids in samples from a diversity of origins and has become an indispensable tool in research laboratories (Mackay *et al.,* 2002). PCR has been evaluated as a reliable method for FMD diagnosis (Khan *et al.,* 2021; Rodríguez *et al.,* 1994; Shaw et al.2004) and proven to be more effective than traditional tests (Khan *et al.,* 2021; Shaw *et al.,* 2004). There are advantages connected with the use of PCR as opposed to traditional testing as this reduces the possibility of acquiring a false negative caused by improper sample processing. FMDV in samples can be inactivated during RNA extraction, permitting activities to be performed with a lower biosafety threshold. The most popular PCR platform is Reverse transcription-polymerase chain reaction (RT-PCR), which is described in detail below.

1.5.19.2.4 Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

This technique has been developed and validated for the detection of the polymerase gene (3D) of FMD with an analytical sensitivity 1000 times greater than that of a single passage viral isolation (Moonen *et al.,* 2003). RT-PCR has been demonstrated to have a notably high sensitivity compared to the conventional ELISA assays (Reid *et al.,* 2006; Callahan *et al.,* 2002). Numerous studies have evaluated Reverse Transcription (RT-PCR) techniques with FMDV isolation (Callahan *et al.,* 2002; Khan *et al.,* 2021; Reid *et al.,* 2006), and both

methods matched in terms of specificity and sensitivity for FMDV detection (Callahan *et al.,* 2002; Reid *et al.,* 2014; Shaw *et al.,* 2004). Real-time PCR has outperformed conventional PCR in prominence due to its enhanced rate and sensitivity (Ambagala *et al.,* 2017; Mackay *et al.,* 2002), tackling poor precision, low sensitivity, low resolution, absence of automation, only size-based discrimination, absence of expression of results in numbers, poor quantitative performance (Ethidium bromide staining is not very quantitative), risk of cross-contamination, and post-PCR processing (Black *et al.,* 2002; Nazarenko *et al.,* 1997; Schweiger *et al.,* 2000). Real-time PCR methods approved by the World Organization for Animal Health (WOAH/OIE) for the detection of FMDV use universal primers and fluorescent-labeled probes that target conserved gene areas within the RNA-dependent RNA polymerase gene ($3D^{pol}$) (Callahan *et al.,* 2002). It can be done as one-step RT-PCR, two-step RT-PCR, quantitative (real-time) or in a conventional (Agarose gel electrophoresis) approach. The use of a specific probe improves the specificity of PCR assays as compared to conventional Agarose-gel-based assays (Bustin, 2000; Bustin *et al.,* 2005). However; the current studies on FMDV with the use of PCR have advanced into typing (Knowles *et al.,* 2016) and cycle sequencing protocols to generate more detailed data vital for enhancing knowledge and control strategies (Bachanek-Bankowska *et al.,* 2016; Carrillo *et al.,* 2005; Kasanga *et al.,* 2019).

1.5.20 Foot-and-mouth disease differential diagnosis

FMD is often hard to be differentiated from other vesicular diseases, like Vesicular stomatitis, Vesicular exanthema, and Swine vesicular disease, based on the clinical signs. It may be challenging to differentiate oral lesions in cattle from those of Rinderpest, Bovine viral diarrhea, Infectious bovine rhinotracheitis, Traumatic stomatitis, Malignant catarrhal fever, Epizootic hemorrhagic disease, Foot rot, and Chemical burns (Alexandersen *et al.,* 2002).

1.5.21 Methods of foot-and-mouth disease virus control

The global control and eradication of FMD has been incredibly difficult, and the sustainable global progress in FMD control cannot be attained without engaging and strengthening disease control in

endemic countries (Leforban, 2002; Rweyemamu *et al.,* 2008). The African continent is home to the most FMDV serotypes (A, O, C, SAT1, SAT2, and SAT3) than any other continent (Jean-Francois *et al.,* 2009; Kitching *et al.,* 2007; Leforban *et al.,* 2010; Rweyemamu *et al.,* 2008). The A, O, and C serotypes are for Europe and America, whereas SAT and Asia1 serotypes are exclusive to Africa and Asia, respectively (Brooksby & Rogers, 1957). The Vosloo *et al.,* 2002 study on the state of FMDV in sub-Saharan Africa and Africa in general has demonstrated the role of Africa in the global epidemiology of FMDV and makes Africa considered as threatening reservoir for the global control and eradication of FMDV. Depending on the FMDV status of a country or region, various techniques have been adopted for FMDV control in those specific countries or regions. The FMDV control strategy focuses on preventing the introduction of the disease in free areas and eradicating it in endemic regions via a combination of measures, such as culling of infected livestock, application of quarantine procedures, restrictions on the movement of animals (i.e. fencing) and their products, the study of the virus involved, and vaccination (Bergmann *et al.,* 2005; Saraiva, 2004). However, the detailed clarifications on the status definition of a specified compartment, country, zone, or area in regard to achievement of FMDV freedom status, have been documented in the EU-FMD, 2011 (Fig. 1.4) and additional detailed stipulations in the WOAH/OIE terrestrial code chapter 8.8. Article 8.8.2 to 8.8.38 of 2022 (WOAH, 2022).

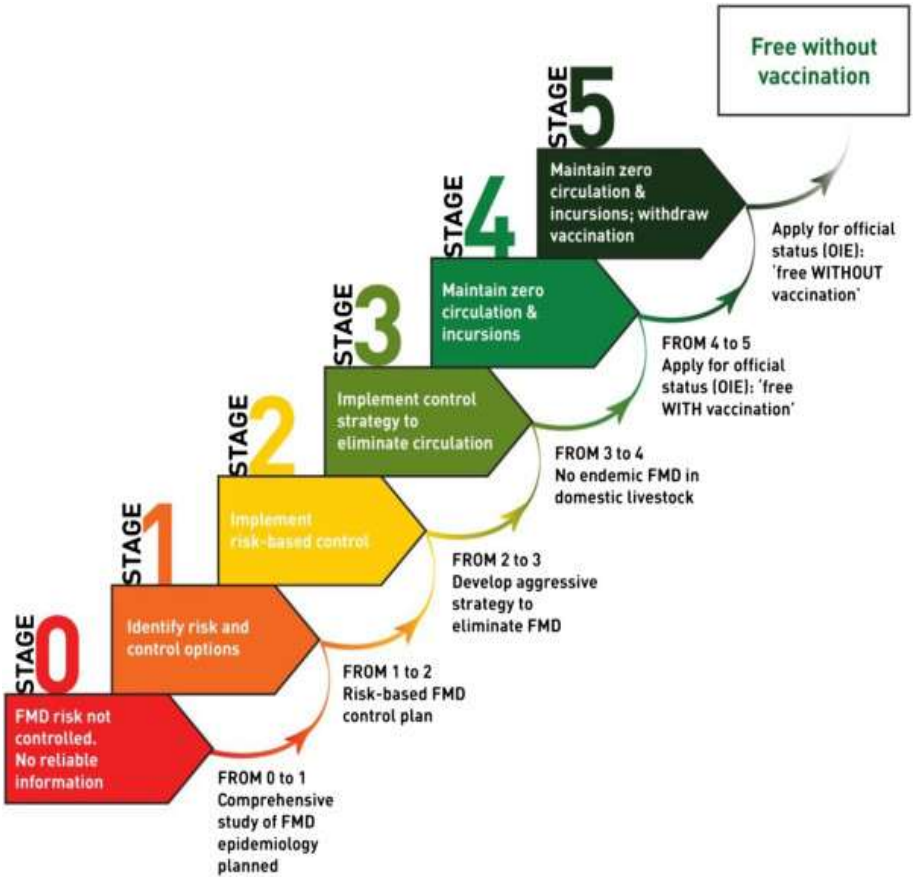

Figure 1.4: **Summarized description for the progressive control pathway stages a country at any FMDV state can undergoes to achieve foot and mouth disease freedom state (EU-FMD, 2011).**

1.5.21.1 Foot-and-mouth disease Control by Vaccination

Vaccines are broadly utilized to control FMD (Doel, 2003; Golde *et al.,* 2005), and the nature of the immune response (Doel, 1999; Doel, 1996) and the prospects for improved vaccines has been reviewed by (Doel, 1996; Parida, 2009). The FMD vaccine is an inactivated (chemically inactivated with aziridine or binary ethyleneimine) preparation that provides protection against challenge for 4–6 months. The vaccine should then constitute representative strains of the serotypes in circulation in the location (Gonzalez *et al.,*

1992); thus, accurate methods for detection and characterization of wild type or field type FMDV in clinical samples are necessary. FMDV detection and characterization in tissues is achieved mostly by serological assays (Kitching *et al.,* 1988; Rweyemamu, 1984), by adopting enzyme linked immunosorbent assay (ELISA) (Longjam *et al.,* 2011; Ma *et al.,* 2011). Vaccines for FMD in ruminants require an oil or aluminum hydroxide/saponin adjuvant, whereas vaccines for pigs have an oil adjuvant. The diverse antigenic and genetic disparities between FMDV serotypes pose challenges for FMD control and eradication by vaccination (WOAH/OIE, 2009a). Therefore, vaccination programs against FMDV will only be effective when FMDV field strains are characterized and antigenically matched to the vaccine strains using CFT, ELISA, or VNT methods (Kitching *et al.,* 1988; Mathion *et al.,* 2009; Rweyemamu *et al.,* 1978). Otherwise, it is reported that, the duration of immunity conferred by vaccines comprising dissimilar strains is quite short (Uttenthal *et al.,* 2010).

1.5.21.2 Foot-and-mouth disease virus Control by Culling

Upon suspected and FMD-confirmed cases, quarantine is implemented whereas animal movements to and from the affected area(s) are restricted or prohibited. Next to that, all infected and in-contact animals are killed and properly disposed (Haydon *et al.,* 2004; Keeling *et al.,* 2003) (Fig. 1.5). In most cases, quarantine is accompanied by ring vaccination to prevent the virus from spreading, and the vaccinated animals may be slaughtered to decrease the time spent in re-establishing freedom status (EU-FMD, 2011). However, the slaughter strategy is most common across Europe (Fig. 1.5), like during the UK FMDV outbreak in 2001 (Leforban, 2002).

Figure 1.5: Culling of infected cattle and in contact cattle (A-B) as well as other animal species (C), disposal of carcasses through burying (C) and burning (D) methods (Lawrence Livermore National Laboratory-UK, 2001).

1.5.21.3 Foot-and-mouth disease virus Control by Movement Control

To regulate animal movements and segregate livestock from wildlife, fences are established, and complimented by vaccination of all susceptible animals as an additional strategy to enhance control measures (EU-FMD, 2011; Ferguson & Hanks, 2012; Vosloo *et al.,* 2002). Southern Africa countries, including Botswana, Namibia, Zimbabwe, and South Africa, have adopted this FMDV control method considerably (Ferguson & Hanks, 2012). Through fences (Fig. 1.6) Botswana, Namibia, and South Africa were able to gain FMD-free certification from the World Organization for Animal Health (WOAH/OIE) for zones within their respective countries. This was achieved via separating domesticated animals (cattle) from wild

animals (buffalo) and establishing buffer zones. Depending on the epidemiological challenges within specified buffer zone areas, the revaccination process of the cattle herds can be conducted at every 4 - 6 months, with vaccines containing all three SAT serotypes (Ferrari *et al.,* 2016). Some of the documented fence designs include single electrified fence lines, similar to the Kruger National Park (KNP) in South Africa, and the double fence lines spaced around 10 meters apart, as is occasionally used in Zimbabwe (Sutmoller *et al.,* 2000). The economic, social, and environmental drawbacks of these fences are considered to outweigh their advantages like obstructing wildlife migration patterns (Mogotsi *et al.,* 2016).

Figure 1.6: **photos showing established physical fences at livestock-wildlife areas (A and B) to limit FMDV** spread caused by animals movements and interactions (Foundation, 2019)

1.6 General Methodology and Thesis outline

This thesis contains detailed information related to the approach of molecular characterization, molecular and sero-epidemiological survey, as well as the antigenic epitope prediction of foot-and-mouth disease virus strains circulating in Tanzania and Africa as well. The study involved critical literature search that enabled writing of the general literature review that enlightens this study knowledge and a review manuscript describing the challenges of FMD control in Africa as an independent chapter. This was a cross sectional study design, with retrospective and prospective purposive sampling strategy of cattle and African buffalo herds in Tanzania. The study also utilized relevant archived FMDV nucleotides sequences from global rich sources/ databases to fulfill the knowledge gap. The molecular, bioinformatical, serological, and immuno-informatics techniques were used to generate and analyze data of this study. This thesis is comprised of seven chapters, the first being general introduction of FMD, second chapter describes the key challenges for FMD control under the African context, the third chapter describes the molecular epidemiology of the field identified FMDV strains, chapter fourth describes the FMDV types O and A infection status in cattle and buffalo, the fifth chapter describes the FMDV type SAT1 antigenic epitopes prediction, the sixth chapter is on general synthesis of the generated results and chapter seven is on the conclusion and recommendations meant for FMD future studies.

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CHAPTER TWO

2.0 Challenges of Controlling Foot-and-Mouth Disease in Pastoral Settings in Africa

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Review Article

Challenges of Controlling Foot-and-Mouth Disease in Pastoral Settings in Africa

Mkama M. Mashinagu,[1,2,3] **Philemon N. Wambura**,[1] **Donald P. King**,[4] **David J. Paton**,[4] **Francois Maree**,[5] **Sharadhuli I. Kimera**,[2,6] **Mark M. Rweyemamu**,[1,2] **and Christopher J. Kasanga**[1,2]

[1]*Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania*
[2]*SACIDS Foundation for One Health, Africa Centre of Excellence, P.O. Box 3297, Morogoro, Tanzania*
[3]*Tanzania Veterinary Laboratory Agency, Centre for Infectious Diseases and Biotechnology, P.O. Box 9254, Dar es salaam, Tanzania*
[4]*FAO World Reference Laboratory for FMD, The Pirbright Institute, Ash Road, Pirbright, Woking GU24 0NF, UK*
[5]*Clinomics, Ulrich Road, Bainvlei, Bloemfontein 9301, Free State, South Africa*
[6]*Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania*

Correspondence should be addressed to Mkama M. Mashinagu; mathias.mkama@sacids.org

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Foot-and-mouth disease (FMD) is a highly devastating viral disease affecting all cloven-hoofed animals. The disease threatens food security and livelihoods across different parts of the world. FMD is endemic in Africa, where the continuous circulation of the disease impacts the livelihoods of pastoral communities by reducing the quality and quantity of livestock products such as milk and meat, as well as undermining the access of the livestock sector to regional and lucrative global markets. Strategies used to control FMD in Africa, especially tropical Africa, are typically fragmented national-level focused activities with relatively poor outcomes, rather than regionally coordinated initiatives that have been used on other continents (South America, Europe) to successfully reduce and even eliminate virus circulation. Biotechnological advances have improved our ability to detect and characterize FMD virus strains, but more effective approaches to disease control are needed to encourage disease reporting and outbreak investigation. This review of the challenges to FMD control amongst Africa's diverse pastoral communities is intended to provide information and provoke discussion to improve the strategies and approaches for regional FMD control in Africa.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease that affects all cloven-hoofed livestock and wildlife animals, with impacts on food security and the socioeconomics of livestock-dependent communities [1]. The disease is caused by foot-and-mouth disease virus (FMDV) that belongs to the genus *Aphthovirus* of the family *Picornaviridae* [2, 3]. There are seven serotypes of FMDV, namely O, A, C, Southern African Territories (SAT)-1, SAT-2, SAT-3, and Asia-1, five of which have been reported circulating on the African continent (Figure 1(a)–1(e)) [4]. In Africa, FMDV circulates in three main virus pools or ecosystems [5], subdivided further into eight epidemiological clusters (Figure 1(f)) that are dynamic rather than fixed, requiring regular review and updating [6]. FMD is a potential transboundary animal disease (TAD) that requires properly coordinated national, regional, and global progressive control strategies [7–9]. The disease is a major obstacle to both commercial and traditional livestock production systems and even has impacts on the continent's wildlife sector [10, 11].

FMD has a long history in Africa, and countries have struggled for many years to prevent, control, and eliminate the disease through various strategies and initiatives [12]. FMD epidemiological knowledge in livestock and wildlife populations provides vital evidence to design control strategies

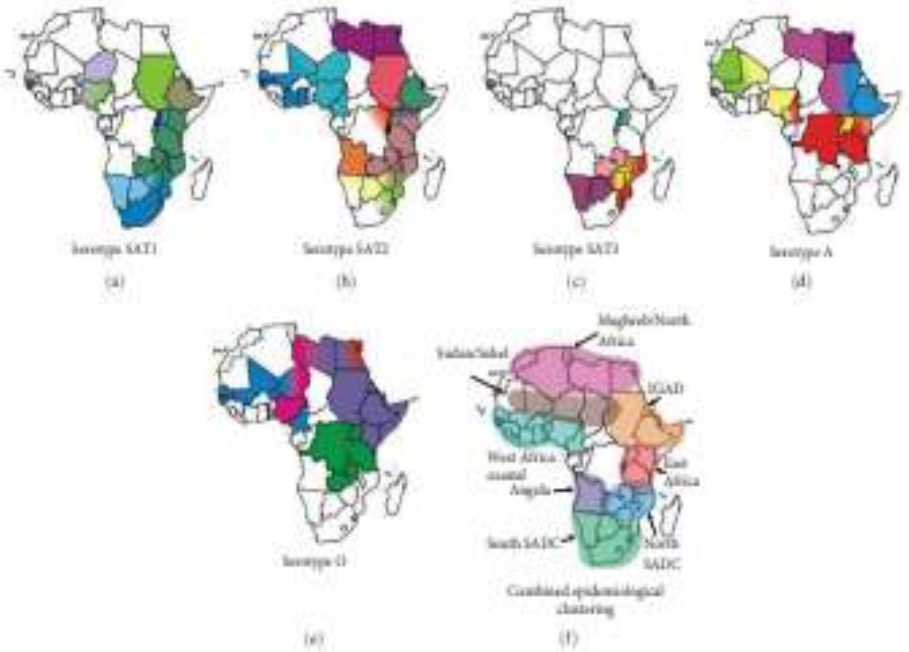


Figure 1: Maps of Africa (a–e) showing the FMDV serotypes and topotypes (color coded) distribution together with the conjectural epidemiological clusters (f). The epidemiological clusters and the color-coded topotypes shown in maps (a-f) do not necessarily show legitimate political borders of the countries. Abbreviations: IGAD, Intergovernmental Authority on Development; SADC, Southern African Development Community; SAT, Southern African territories [6].

[13, 14]. Zoo-sanitary measures, such as quarantine and movement permits and the use of physical fences to separate wildlife from domesticated species, are critically important to break the cycle of infection for highly contagious diseases like FMD. These may be supplemented with vaccination and culling of affected herds, though culling may be appropriate in the final stages of eradication. Conversely, in parts of Africa where FMD is more common and where pastoralists predominate, vaccination has always been regarded as the main FMD control approach [10], since control of animal movements is difficult and there are usually insufficient resources to compensate owners in support of livestock culling. However, vaccination is a complex task, requiring a sound strategy, proper implementation, and regular review [15], in order to be effective, especially where complementary control measures are weak [16, 17]. Where resources are available, new tools can be used for partial genome and whole-genome sequencing of FMDV [18, 19], as well as for serological surveillance using commercial kits. Although these biotechnological advances help to understand the epidemiology of FMD (Figure 1(a)–1(f)), FMD remains a regional and global problem with significant food security and livelihood impacts that affect vulnerable communities.

Surveillance activities continue to monitor the occurrence, emergence, and spread of different FMDV serotypes and lineages, including antigenic characterization, to inform vaccine selection [5] (Figure 1(f)). This knowledge can help direct control efforts properly [20, 21]. In the last 10 years, studies have started to investigate the African pastoral context in which control measures must operate and to consider how social and disease control measures can be reconciled [22]. This review explores the FMD control challenges related to the highly diversified pastoral communities [23] and their implications for the improvement of FMD control strategies across Africa.

2. Africa's Pastoral Systems and Its Impact on FMD

The livestock sector in Africa is predominated by pastoral and agropastoral systems [24]. Pastoralists are found in all regions of Africa with characteristic variable livestock husbandry practices that pertain to their traditional and cultural systems but are influenced by geography, topography, and climatic conditions [25, 26]. According to Robinson [27], there are five main livestock husbandry practices identified in Africa that operate between pastoral and agropastoral systems, namely (i) total nomadism (no permanent place of residence and no regular cultivation), (ii) seminomadism (a permanent place of residence exists and supplementary

(a) (b) (c)

Figure 2: (a) Mixed species of small and large ruminants (goats and cattle) kept together between Dodoma and Morogoro, Tanzania (Source: David Paton, 2019). (b) Livestock and wildlife cograzing during the drought season at the Serengeti ecosystem interface (Source: This study, 2017). (c) Cattle vaccination for FMD in Namibia (Source: David Paton, 2015).

cultivation is practiced, but for long periods of time animals travel to distant grazing areas), (iii) transhumance (a permanent place of residence exists, and herds are sent to distant grazing areas, usually on seasonal cycles), (iv) partial nomadism (farmers live in permanent settlements and have herds at their disposal that graze in the vicinity), and (v) stationary or sedentary animal husbandry (animals remain on the holding in the village throughout the year). In most cases, more than one livestock husbandry practice operates in a particular geographical area, and each has distinct animal movement characteristics [28, 29]. Pastoralists in Africa are unevenly distributed in the savannah lands and experience diverse climatic conditions across the continent. Some of the areas experience floods of varied duration and rivers that are challenging to cross in some seasons [30, 31]. The pastoralists in Africa are unevenly distributed in the savannah lands and experience different climatic conditions across the continent [28, 29]. Some of the areas experience floods of variable duration and rivers that are difficult to cross in some seasons [30, 31]. Pastoral communities that are diverse and always dynamic [32, 33] make FMD surveillance and monitoring activities challenging due to difficulties to trace/track animals [34–36]. Maintenance of FMD infection in these pastoralist settings provides opportunities for spread between herds and is thought to influence the regional virus circulation patterns [6]. The disparities and complexity amongst pastoralist practices across Africa indicate that tailored approaches will be required to achieve FMD control.

3. Tradition Practices of Farming Multiple Species

Unlike highly commercialized farms in many other parts of the world, most of pastoral communities across Africa such as the Maasai in Kenya and Tanzania often keep multiple species of FMD-susceptible livestock [23] including cattle, sheep, and goats [37, 38]. There is uncertainty over the role of some species in the epidemiology of FMD, which likely depends on stocking density and contacts with other species (Figures 2(a) and 2(b)). The tendency of pastoralists to value and care for cattle more than other species also has implications for FMD control strategies. Similarly, cattle have been the main focus of scientific research and veterinary service provision, such as vaccination programs [15, 39] with other animal species often neglected. This underlines knowledge gaps on the relative contribution of different species in the epidemiology of FMD [8, 40].

4. War and Conflicts in Africa Pastoral Communities

Pastoral communities commonly experience conflicts that endanger human and animal lives [41, 42]. Four key categories cover most reported conflicts. The first category involves struggles within pastoral communities [32, 43]; the second involves struggles between pastoralists and the farming communities [44, 45]; the third involves pastoralists being affected by rebellion groups [46, 47]; and the fourth is between pastoralists and government authorities [45, 48]. Access to grazing lands and pastures, water sources, and animal theft often lead to conflicts [49–51]. These problems are all exacerbated by governance problems [52]. In Africa's FMD-endemic areas, civil wars followed by protracted postwar recovery and stabilization have been witnessed [53], and this can often lead to a lack of permanent settlements for pastoralists. The free-roaming pastoral communities distort social programs like vaccination campaigns [32]. Wars and conflicts foster food insecurity, diseases, and vulnerabilities, particularly poverty [23, 54] making animal disease control initiatives a low priority. The disputes in Kenya (al Shabaab, postelection unrest), Uganda, South Sudan, the Central African Republic, the Democratic Republics of the Congo and Chad (Lord's resistance army), and Nigeria (Boko Haram) [46, 47, 55] highlight the type and extent of unrest in Africa. In incidents like these, animal health service providers hesitate to travel to remote areas for fear of their security, hence paralyzing animal health programs. Rweyemamu et al. [56] revealed how insecurity prevented surveying the Southern Somali ecosystem and implicating timely release of global rinderpest eradication declaration reports.

5. Dissimilar Policies on Pastoral Undertakings across African FMD Endemic Countries

Pastoralists in Africa experience a diverse range of policies from their respective governments [57, 58]. For example, Kenya recognizes pastoralism and provides some safeguards as stipulated in the Community Land Act No.27 [59]. In

Tanzania, there is no land legislation specific to pastoralism [34] even the National Land Policy of 1995 [60] does not cover pastoral activities in relation to their land resource use and ownership [61]. In Zambia, most land is held by tribal chiefs and managed in common [62–64]. Land policy in Botswana differs most, with predominantly private ranches for livestock production [65, 66]. Increase in human population with growing demands for cultivation and mining activities are evident in Africa as elsewhere [67–69] and have exerted pressure on many pastoral communities, which often do not have legal papers for land ownership [55, 58, 70, 71]. This pressure has resulted in an increased migration of pastoral communities to areas where they can access land for readjustment of their pastoral activities [72]. These differences in national policies and circumstances call for tailored and strategic approaches to deal with animal health problems. Achievement of TADs control (FMD inclusive), therefore, requires an understanding and adjustment of underlying policies that impact pastoralists and their practices.

6. Animal Movements and FMD Epidemiology in African Pastoral Settings

Most pastoralists keep livestock for prestige and livelihood sustenance. The key reasons for movements of animals involve communal grazing land, transhumance, oxen for transport, bulls for mating, dowry transfers, arable land cultivation, and for other social actions like refraining from unsettled disputes. Furthermore, pastoral communities migrate animals due to excessive drought and rains that destroy habitats, pastures, water sources, and crops [73]. The lack of priority given to pasture management necessitates finding new pastures and water sources [74, 75]. Animal movements influence livestock-wildlife interactions, providing opportunities for subsequent interspecies transmission, and spread of infectious disease pathogens [1]. Kangalawe et al. [76] provided background describing pastoral and agropastoral community movements from the early 1950s in Tanzania, mostly involving Maasai, Barbaig, and Sukuma peoples. The authors also cited the rise in north-to-south migration to Mbeya, Iringa, and other regions of Kigoma, Rukwa, Morogoro, Pwani, and Ruvuma that occurred in the 1980s. The primary drivers were gradual climatic changes, soil or land degradation from overgrazing and overstocking, poor agricultural practices, and population increase in their areas of origin. In Kenya, predominant pastoral communities are Maasai and Turkana, which have limited mobility due to stringent Kenyan government land policies adopted from the British colonial era [77]. In West Africa, Fulani pastoralist groups in the Savannah and Sahel rangelands operate regulated transhumance movements under the Economic Community of West African States (ECOWAS) 1998 Protocol on Transhumance [78] to monitor of risks for animal diseases epidemics like FMD.

Unmonitored movements have potential to spread the FMDV to susceptible hosts, thereby undermining control efforts [79]. For instance, the 2018 FMD serotype O outbreaks that started in Algeria and spread to Tunisia and lastly Morocco in January 2019 were genetically closely related to the O/EA-3 topotype that originates from West Africa. Furthermore, serotype A outbreaks in Algeria in 2017 FMD were genetically closely related to the Nigerian FMDVs [80]. Similarly, the 2006 FMD serotype A outbreaks in Egypt were genetically closely related to the East Africa topotype (G-VII genotype) that had already been reported in Kenya and Ethiopia [81]. Recently, serotypes O and A have spread southward from East Africa to Zambia [82], Malawi, and Mozambique. Animal movements render vaccination campaigns challenging though missed or incomplete vaccination and reduce the efficiency of disease control programs [15]. African animal health authorities deploy three epidemiological strategies to control animal movements. The first is quarantining, which aims to prevent infected animals from infecting nearby herds. The second is issuing movement permits that are approved after acquiring health and vaccination certificates for regulating long-distance movements of animals. Unfortunately, dishonest officials and pastoralists can misuse these permits, reducing their impact to control animal diseases [35]. The third option is based on controlling local movements, e.g., transhumance through the identification of defined areas within which free movements can occur. Animal health and law enforcement officers have to identify target animal herds under all movement control settings and do monitoring (http://www.fao.org/3/w5757e/w5757e12.htm). Some countries such as South Africa, Botswana, Namibia, and Zimbabwe have also erected physical fences to curb animal movements. Game-proof fences restrict both livestock and wild animal movements and their interactions [83]. However, fencing is expensive and creates socioeconomic and environmental concerns and interrupts wildlife migration, and these may outweigh the FMD control benefits [84, 85]. Although fencing has not been widely adopted by other African countries facing similar FMD problems, animal identification is also poorly practiced in most of Africa's FMD-endemic areas.

7. Wildlife Involvement in the Epidemiology of FMD in African Pastoral Settings

Many African countries contain wildlife conservation areas that possess rich pastures and water sources [86]. Most pastoral communities are settled around these conservation areas and strategically graze their animals within the parks during droughts, despite the stringent restrictions imposed by the government authorities [87–89]. Studies have shown that SAT serotypes of FMDV are maintained in African buffalo herds (*Syncerus caffer*) for 24 years or longer [90]. Moreover, transmission of FMDV from infected buffalo in direct contact with cattle can occur under certain conditions of livestock–wildlife interaction, providing a mechanism by which new variants can be introduced into livestock within and around protected areas (Figure 2(b)) [87, 91]. Evidence of long-term FMDV maintenance through virus persistence in infected wildlife of species other than the African buffalo has not been shown, and more efforts are needed to define the FMDV host spectrum in wildlife and their roles in virus spread and maintenance. Such work is hampered by the

practical difficulties of conducting surveillance studies in wildlife essentially due to high expenses [92, 93]. The presence of FMDV persistently infected wildlife complicates the epidemiology of the disease and its eventual eradication prospect [88, 94].

8. Challenges of FMD Surveillance Systems in African Pastoral Settings

Surveillance is essential to understand the risks posed by animal diseases [35, 95]. These activities employ active or passive surveillance to monitor FMDV circulation and identify new virus in the field [6] including high-risk areas, such as the livestock-wildlife interface [35, 87, 94]. Surveillance is expensive since it requires data collection at regular intervals and proper record keeping. Obtaining relevant data on TADs like FMD is difficult as record keeping is a challenge in most Africa countries, though it requires effective coordination of pastoralists at all levels [34]. However, pastoral communities live in isolated, scattered, remote, infrastructure-poor, and underdeveloped areas [96], and some in cross-border ecosystems to access communal grazing areas [97, 98]. At certain periods of the year, livestock and their owners become unavailable for surveillance and disease control programs. Thus, considerable flexibility, commitment, time, patience, and passion are needed for retrieving information when conventional approaches are deployed. Among the different surveillance studies employed in Africa, participatory surveillance remains a cost-effective method of gathering vital data from pastoral communities, especially in remote, FMD high-risk pastoral areas [99]. However, the lack of proper knowledge and commitment to record keeping (i.e., vaccination, vaccines, outbreaks, etc.) for the pastoralists and other key players in diseases control is a notable drawback. The lack of appreciable immediate benefits on animal health surveillance activities exacerbates these challenges [89] especially since pastoral communities have little interest in FMD control programs as they do not appreciate the benefits that may accrue from its successful control [11]. The FMD risk-based animal movement epidemiological surveillance data generated should help to implement vaccination program tactics to improve FMD control effectiveness [100]. Interestingly, most FMD surveillance data from pastoral communities in Africa is generated by research studies rather than routine operational surveillance systems. Such studies are infrequent and need considerable time, resources and expertise to design, implement, and analyse for a specific intended purpose.

9. The Burden of Other Diseases with Human and Animal Health Implications

Apart from FMD, African countries and, in particular, the pastoral communities face significant challenges from other diseases with serious human and animal health consequences [101–103]. Based on the WOAH classification, 15 diseases are considered to be the most contagious and of these, 12 are found in Africa [32]. In the FAO Emergency Centre for Transboundary Animal Diseases reports, it is estimated that about 90% of the WOAH-listed diseases are known to occur in Tanzania [104]. The black-quarter (BQ) [105], contagious bovine pleuropneumonia (CBPP) [106], contagious caprine pleuropneumonia (CCPP) [107], peste des petits ruminants (PPR) [108], and East Coast fever (ECF) [109] are among highly reported animals diseases in different African geographical locations. Considering that over 60% of human diseases are of animal origin, the close association of the pastoral communities with their animals increases their risks of zoonotic infection [110] when compared to other communities, bearing in mind that over 60% of human diseases are of animal origin [111, 112]. Some of the most commonly reported human diseases are brucellosis [110], anthrax [113], tuberculosis (TB) [114], HIV-AIDS [115], rabies [116], malaria [117], cholera [118], and rift valley fever and salmonellosis [119]. New disease(s) epidemics like Ebola [120] further drain resources [121]. Most African countries have weak economies [122] and prioritize the control of human diseases over animal health. The future risks and threats to neglected human and animal diseases are expected to escalate due to changes in trends of disease drivers [52], putting extra strain on budgets for control of TADs. Most African governments have a tendency to express high sensitivity and greatest concern for diseases that cause massive mortalities [123] and condemnation of products. The indiscriminate use of unprescribed drugs in Africa intensifies animal and human health threats from antibiotic-resistant pathogens [52]. These are the reasons why FMD control initiatives in most African countries have hitherto been sluggish and unsuccessful. The budgetary constraints and unsustainable commitment have rendered control efforts on FMD and other TADs as temporary, contrary to the rinderpest eradication program which earned global-level commitment [52].

10. Vaccine Performance and Vaccination Challenges in African Pastoral Settings

In FMD endemic regions, vaccines can be used to protect high-value animals, such as dairy cows, a strategy advocated for countries at the early stages of the progressive control pathway for FMD (PCP-FMD) [15]. The FMD vaccines available in Africa and elsewhere consist of whole virus particles, chemically inactivated with binary ethylene-imine (BEI) and combined with oil-based or aqueous adjuvants [124, 125]. These vaccines induce short-lived immunity and require booster vaccinations in order to provide sustained protection [39, 126]. Therefore, maintaining immunity over time needs repeated vaccinations (prophylactic vaccination) or by one-off vaccination to provide temporary protection against a specific threat such as nearby outbreaks (reactive or emergency vaccination). For prophylactic purpose, naïve animals should get two initial vaccinations 3–4 weeks apart, followed by revaccination at every 4–6 months. FMD control requires a regular vaccination of whole populations within a given zone and measures to prevent virus incursions, especially from animals outside the zone. Unfortunately, due to limited resources, most FMD-endemic countries fail to prioritize vaccination and complement it with other control measures.

Under African status, some manufacturers recommend five vaccinations per annum, depending on the vaccine potency [124, 125, 127] and FMDV challenge weight [15]. Unfortunately, pastoralists cannot afford multiple revaccinations annually [10, 128]. Vaccines sourced from the Kenya Veterinary Vaccine Production Institute (KEVEVAPI), Kenya, and the Botswana Vaccine Institute (BVI), Botswana, (O, A, and SAT1 and 2) cost about 1.80 USD and 2.24 USD per single dose in Tanzania, excluding the logistical costs [129].

A key point with FMD vaccines is that they are thermally sensitive and require intensive cold chain handling to maintain their good quality from leaving the manufacturer until reaching scattered pastoralists in the field [15]; this also has cost implications [10]. Most herders lack permanent physical addresses or settlements making planned vaccination(s) (Figure 2(c)) and postvaccination evaluation(s) challenging [33, 51]. Pastoral communities are insufficiently educated [130, 131] making it more difficult to raise awareness that complement vaccination with other control measures, like biosecurity precautions. For these reasons, emergency vaccination is the often preferred strategy for government intervention. However, this approach requires good surveillance to define the vaccination zone, a rapid response with potent vaccines, good biosecurity to prevent the vaccination teams from spreading infections, and controls on infected livestock being moved beyond the vaccination zone. Additionally, African vaccines are rarely tested or checked for their quality or strain match, and their field performance is seldom monitored or critically reviewed. The Africa Union-Pan African Veterinary Center of the African Union (AU-PANVAC) has a mandate to check the quality of vaccine batches used in African livestock, but this is not yet done as sourcing, immunization, challenging, and monitoring of naive cattle with the FMDV has been major drawback. In the absence of government support, pastoralists may be obliged to pay for their own vaccine and plan their own vaccination schedules which is difficult without proof of cost–benefit, guidance, and coordination [6].

11. Status of Infrastructures Required for FMD Control in African Pastoral Settings

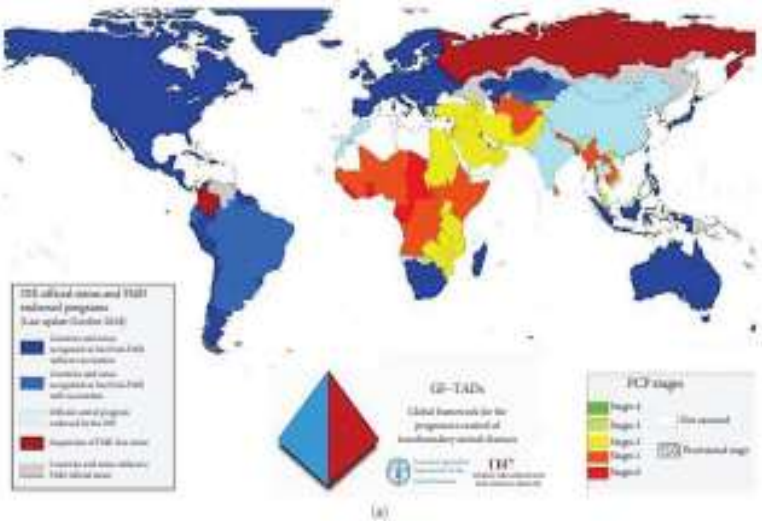
The infrastructure networks and social services necessary for diseases control (roads, electricity, water, banks, veterinary clinics, veterinary laboratories, hospitals, and schools) in most pastoral communities are underdeveloped [132]. Furthermore, the presence of diverse types of obstructive geographical features (rivers, valleys, hills, and steep mountains) in these highly dispersed communities complicates logistics further and increases transport, materials storage, vaccination, and monitoring expenses for disease control [6, 133]. Due to resource constraints, most of the government authorities in Africa give a low priority to disease control in pastoral areas. This makes effective provision of services in these areas even more challenging [130, 134]. Even competent animal health personnel can become discouraged from working in pastoral areas due to this challenge [135]. Therefore, it is difficult for any animal health program (e.g. vaccination programs) to reach pastoral communities in a timely manner and this impacts negatively on the effectiveness of the FMD control programs [6]. Furthermore, the one health approach needs to be emphasized to enable sharing of some pertinent missing services or facilities in remote areas. And finally, the government(s) need to undertake some land policy reforms to accommodate and transform pastoral activities, and suit infrastructures access that facilitates animal diseases control.

12. The Difference in Animal Disease Control Priorities among African Countries and Communities

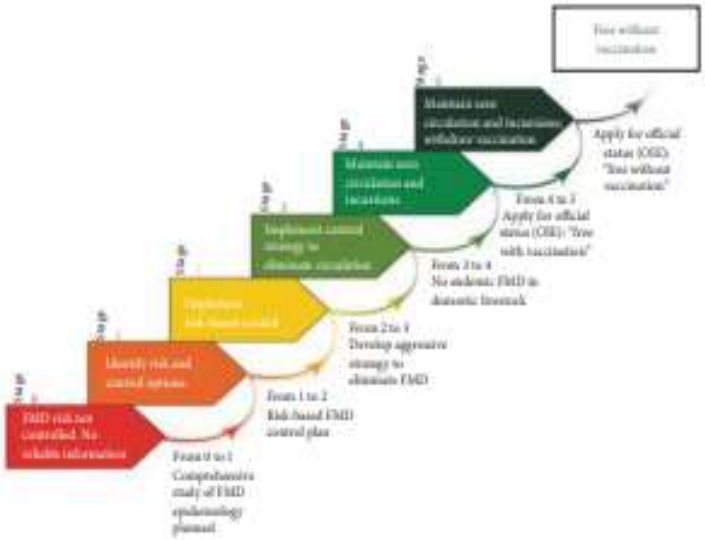
Despite many similarities in the challenges from diseases of human and animal health [101, 102], there are marked differences in priorities for control of FMD and other endemic livestock diseases between communities and countries [136]. For example, a participatory study in the Maasai Mara ecosystem in Southwestern Kenya revealed that pastoralists had different ideas about which animal diseases were most important and how they affected their lives [137]. According to pastoralists who participated, FMD has the highest impact on milk production and is ranked second to CBPP based on ascribed losses instead of ECF. In Tanzania, CBPP is regarded as the highest priority disease among all frequently reported livestock diseases (ECF, trypanosomosis, CBPP, BQ, TB, and anthrax) [138, 139]. In Uganda, FMD stands first in their priority lists for disease control. The economic disparities between countries affect the disease control priorities; for instance, low-income countries experience high challenge of infectious zoonotic disease burden of about 13%, as compared to 1% in high-income countries [136]. The countries with many cattle are likely to be more enthusiastic and ambitious to export animal products to lucrative markets after controlling FMD, but this may be offset by the lack of resources, expertise, and priorities, contrary to the outlook in Southern Africa countries like South Africa and Botswana. Currently, there is no single disease that African countries have decided to control strategically by joint efforts /initiatives under regional agreements as stipulated in the OIE: FAO's [36] document. This lack of harmonization in rankings creates difficulties in agreeing which diseases should be prioritized for control, particularly where joint initiatives among African countries are needed. Perhaps there are lessons to be learnt from the 2011 global Rinderpest eradication success, where all countries were fully engaged in the campaign process [140].

13. Possible Options for Improving FMD Control under the Current Pastoral Settings in Africa

FMD control in the African endemic context faces multiple challenges that need to be addressed systematically to convey tailored solutions concepts. FMD control priorities vary and most countries are in PCP-FMD stages 1 and 2 with only a few in stages 3 and 4 (Figures 3(a) and 3(b)). The FMD free countries avoid new FMDV incursions that can lead to



(a)



(b)

FIGURE 3: (a) Global map showing countries PCP-FMD stages, their respective OIE official statuses and corresponding endorsed programs on FMD control (OIE and GF-TADs, 2018). (b) The summarized stages for the progressive control pathway for FMD control (PCP-FMD) are applicable to all countries endemic to FMD (OIE and GF-TADs, 2018).

suspension of their FMD freedom status and beef exports. These countries are challenged by new FMDV variants, maintaining adequate vaccination immunity, and managing biosecurity measures at FMD high-risk areas like livestock–wildlife interfaces and pastoralists' animal movements. They address animal introduction prevention, prophylactic vaccination, and active monitoring for early detection and rapid response to FMD incursions [9, 141]. The countries at early stages are mostly challenged by the availability of resources, often because of the absence of export markets. PCP-FMD can be executed on a single-country basis but due to porous borders, joint actions are emphasized to enhance outcomes (Figures 3(a) and 3(b)). The countries can progress together by (1) ecosystem-based approaches as described by Marsot et al. [6], (2) geographical-based approaches via defined regions in the continent (East, Central, West, South, and North) or as a whole continent, as in Europe and South America under EuFMD and COLSAFA, respectively, and (3) political-based approaches via multiple countries establishing political communities/forums like The Southern African Development Community (SADC), ECOWAS, and East Africa Federation (EAF).

Sustainable regional and inter-regional trade in Africa needs commodity-based trade (CBT) and other nongeographic FMD control approaches [142]. The latter strategy emphasizes removing the risk of infection from final products or commodities, despite whether the infection has been eradicated from a region's entire livestock population or not. For example, a hazard analysis critical control point may be used to destroy FMDV during beef processing (beef maturation after deboning and lymph nodes removal). Biosecurity measures and vaccinations can be enhanced to protect livestock against FMDV infections in established compartments [142]. Thus, CBT reinforces the 2012 Phuketane Declaration on trade of beef from places where FMD cannot be easily eliminated by the available geographical-based control measures. The integration of movement controls, vaccination strategies, and biosecurity measures in an African pastoral context could minimize infections, and strengthen animal products trade [142]. The adoption of value chain-based approach to CBT when merged with participatory-based studies [143–145] could promote pastoral communities participation to minimizing FMD contamination risk.

During rinderpest surveillance programs in Pakistan and Kenya, pastoralists helped identifying priority disease in various geographical locations to promote pastoralists' prompt response and execution of mitigation measures [146]. Pastoralists can readily adopt disease controls when they experience practical benefits in the livestock production value chain. Adoption of control measures may also aid in managing other diseases that pose a threat to their animals as was the case with HPAI control in Africa [99, 144].

FMDV surveillance programs need to be enhanced to determine spatiotemporal distribution of FMDV strains and to identify FMD risk hotspots based on transmission. The rapid and accurate field deployable diagnostic tools like portable qRT-PCR [147] need to be emphasized to support timely diagnosis during outbreaks. Participatory epidemiology and surveillance programs need to be improved by using digital mobile technologies [148], such as the SACIDS-AfyaData app in Tanzania [149]. This will promote quick access and hasten sharing of data at local, national, and international levels [99]. The mapping of the dominant animal movements needs to be done concurrently to understand animal risk pathways in pastoral regions [142]. With suitable vaccination programs, this will foster opportunities for commercial activities and investment via identified and established FMD-free zones and compartments. Strengthening of public–private partnership approaches to improve FMD vaccine value chain awareness from production, purchasing, distribution, delivery, vaccination, and postvaccination monitoring to FMD endemic and pastoralists predominated countries will enable effective vaccine usage and performance [150].

The countries need to enhance laboratory capacity and networks to diagnose and characterize FMDV in a timely manner. A primary driver should be to encourage countries with unknown FMD status to submit outbreak samples and reports. Laboratories need to take part in FMD mitigation quality control activities like vaccine selection and vaccination monitoring, use of high potency vaccines matched with field circulating strains, recommended vaccination schedule, and coverage are required. Finally, regional strategies and initiatives to control FMD in Africa should be coordinated in multicountry state rather than being centered on individual country efforts, with animal health policies adjusted to accommodate existing disparities among FMD endemic countries [99].

14. Conclusion

This review highlights FMD control challenges across Africa in the context of pastoral communities. African pastoral practices need to be perceived as historical adaptations to survive difficulties along with changing climatic conditions rather than a nuisance. This review reveals how pastoral communities operate in particular ecological contexts that render FMD maintenance and control across Africa more complicated than in any other regions in the world. This calls for tailored mitigation approaches that address:

(i) Reform of land policies in FMD-endemic countries to suit sustainable environmental management practices and enhance pasture and water availability. This will also support the establishment of FMD control infrastructures and promote opportunities for PCP-FMD program.

(ii) The lessons of cooperation via FMD control strategies in Europe and South America. Initiate an AU-FMD platform to foster regional FMD scientific studies, present evidence needed to accelerate CBT, and catalyse PCP-FMD participation.

(iii) Emphasize ecosystem-based disease control approach to account for unique pastoral animal movements, the multiplicity of circulating FMDV field variants, the limited availability of vaccines, vaccination, and monitoring.

(iv) Enhance participatory surveillance systems for gathering pastoral knowledge with quick access and multisectoral sharing of information to improve preparedness and rapid response to epidemic diseases like FMD.

(v) Countries need to consider FMD vaccines as a public good or subsidize, demonstrate their cost-benefits to promote appropriate uptake by pastoralists, and ensure availability of suitable quality vaccines and indicate where they can be effective. In cases where vaccination campaigns experience adequate financing, then the private sector may have to take responsibility for vaccination, as for other endemic diseases.

(vi) The WOAH guidelines (TAHC Chapter 4.4 and Chapter 4.5) for FMD free zones and compartments (OIE, 2014) facilitate exports to FMD free countries, but there is a gap in guidance on appropriate risk mitigation for trade between infected countries and zones, and there are no WOAH guideline for CBT that could accelerate trade amongst FMD challenged countries with roaming pastoral herds and carrier wildlife [87, 88, 142].

The future studies of animal movements across African need to be combined with molecular epidemiological data generated from circulating FMDV strains to improve outbreak predictions and proper vaccine usage. Also, the future vaccines for Africa need to consider thermostability, protection duration, and cross-protection challenges for improving logistical and vaccination expenses.

Ethical Approval

The ethical statement is declared not applicable to this work as no questionnaires, animals, and humans samples were collected in accomplishing this review study.

Disclosure

In this manuscript, we involved articles from peer-reviewed journals, technical reports, open-access data from highly trusted repositories, and chapters from electronic book versions. There were no generalized search criteria, instead only specific keywords were identified for every problem(s) noted under each subheading and used for searching the information objectively. A vast of electronic databases were used for searching the information like Google Scholar, Springer, Wiley, ScienceDirect, etc., and a total of 150 articles were found to have relevant information for inclusion in this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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CHAPTER THREE

3.0 Molecular epidemiology of Foot-and-mouth disease virus in Tanzania during 2020 to 2021 outbreaks

Mkama Mathias[a,b,c], Philemon N. Wambura[a], Sharadhuli Kimera[a,e], Donald P. King[d], David J. Paton[d], Francois Maree[f], Herbertha Mpete[a], Sengiyumva Kandusi[a], Mark M. Rweyemamu[a,b] and Christopher J. Kasanga[a,b]

[a]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.

[b]SACIDS Foundation for One Health, Africa Centre of Excellence for Infectious Diseases of Humans and Animals, P.O. Box 3297, Morogoro, Tanzania.

[c]Tanzania Veterinary Laboratory Agency, Centre for Infectious Diseases and Biotechnology, P.O. Box 9254, Dar es salaam, Tanzania.

[d]FAO World Reference Laboratory for FMD, The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 ONF, United Kingdom.

[e]Department of Veterinary Medicine and public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O Box 3021, Morogoro, Tanzania.

[f]Clinomics, Uitzich Road, Bainsvlei, Bloemfontein, 9301, Free State, South Africa.

*Correspondence: Mkama Mathias *(mathias.*mkama@sacids.org)

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Abstract

Background: Food and mouth disease (FMD) is an endemic disease of cattle and other cloven hoofed animals. The objective of this study was to investigate the genetic characteristics and evolutionary relationships for the 2020 to 2021 field circulating FMDV obtained from reported outbreaks in different parts of Tanzania.

Methods: The epithelial tissues were collected from vesicular lesions (oral, nasal, interdigital) of FMD suspect cases, properly stored and shipped to the laboratory for analysis. In the laboratory, the samples

were prepared for nucleic acid extraction, FMDV detection, typing, sequencing, and phylogeny analysis. The construction of the phylogenetic trees was done by aligning current field strains nucleotide sequences with those from past studies stored in GenBank database.

Results: The study identified three FMDV serotypes (A, O and, SAT1) to be circulating in the field as Africa Topotype G-I lineage, EA-2 Topotype, and Topotype I (NWZ) respectively. The identified field strains showed diverse scores of shared identities among current and past study strains. The generated nucleotide sequences from this study types O and SAT1 field strains were analysed categorically, and showed shared percent identities of 92.0-100.0% and 96.9-98.8% respectively.

Conclusion: The sequencing and analysis of the VP1 coding region enhance FMDV knowledge on the genetic and evolutionary relationships existing among field strains, and commend for improved future strategies for effective national, regional and global FMD control measures.

Key words: FMDV, Serotype A, Serotype O, Serotype SAT1, Tanzania,

3.1 INTRODUCTION

Foot-and-mouth disease (FMD) is a disease of all cloven-hoofed (livestock and wildlife) animals, with severe agricultural and socio-economic implications at national, regional and global community levels (Belsham and Bøtner, 2015, Knight-Jones and Rushton, 2013). FMD undermines the livestock sector by causing production and productivity losses enhanced by trade embargoes and enormous progressive control costs across countries in the world (Knight-Jones et al., 2016). The disease is caused by a highly infectious Foot-and-mouth disease virus (FMDV) that belongs to the genus *Aphthorvirus* of the family *Picornaviridae* (Zell et al., 2017). FMD has been reported worldwide and countries are classified into 6 stages ranging from 0 to 5 depending on the progressive control

pathway-FMD (PCP-FMD) achievements acquired (Sumption et al., 2012). It is a highly contagious disease as its primary spread is through direct contact between infected and naïve animals, aerosols, contaminated environment and fomites (Belsham and Bøtner, 2015). There are 7 worldwide reported FMDV antigenically distinct serotypes O, A, C, Asian1, Southern African Territories (SAT) 1, SAT2 and SAT3 (Brito et al., 2017) circulating as 7 pools of FMDV strains under identified geographic regions globally (Brito et al., 2017). The African continent has the largest number of FMD virus serotypes reported (O, A, C, SAT1, SAT2 and SAT3) than any other continent in the world (Vosloo et al., 2002).

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

In the recent years, there is an increased government interest to control FMD in Tanzania to enhance livestock sector contribution in

the national economy by enabling international lucrative markets access of live animals and their products exports that are currently hindered by the FMD endemic situation (ILRI and CGIAR, 2017, James and Rushton, 2002). Tanzania ranks third in the number of cattle in Africa with a total estimate of over 30 million cattle herds as 1.4% of global and 11% of Africa cattle population (FAO, 2014), therefore FMD control bears feasible socio-economic impacts in the country. FMD is endemic in Tanzania, and four serotypes (O, A, SAT1, and SAT2) have been reported to cause outbreaks on different geographical locations (Kasanga et al., 2012, Kivaria, 2003). The FMD control in endemic countries continues to be challenging due to its prevailing complicated epidemiology instigated by presence of multiple serotypes, subtypes and even topotypes that are widely distributed, higher numbers of livestock herds, biodiversity richness of susceptible host animal species in numerous conservation areas across the country, and uncontrolled animal movements (Tekleghiorghis et al., 2016). This situation implicates on the persistent FMD endemicity in the country and similarly in the other sub Saharan region countries (Tekleghiorghis et al., 2016, Vosloo et al., 2002). The latter could be the reason for the current FMD field situation presenting an increased frequency of FMD outbreaks to the extent of even reporting the disease throughout all seasons of the year. According to FAO and OIE stipulations on FMD control based on a long-term progressive risk reduction approach (Paton et al., 2009, Rweyemamu et al., 2008), the updated knowledge of FMDV circulating field strains is a requirement. Tanzania is at stage one of the PCP-FMD and its advancement to stage 2 requires monitoring of circulating strains to understand the epidemiology of FMD in the country to enhance tailored mitigation options whereby vaccination remains as main FMD intervention of choice under this state. The vast of studies done on FMDV in Africa and other parts of the world deploy VP1 genomic region to infer the molecular characteristics of the virus (Dyirakumunda et al., 2017, Knowles et al., 2016).

Thus, this study was undertaken with the objective of investigating the occurrence and genetic characteristics of FMDV serotypes from 2020 to 2021 field reported FMD outbreaks by deploying molecular analytical techniques for the VP1 genomic region of the FMDV as

target. This study strengthens knowledge on FMD current status in Tanzania based on circulating viral strains at studied geographical areas, their genetic diversity, and existing evolutionary relationships amongst current and previously identified strains. The gathered information is also vital for implementing tailored mitigation measures on FMD and contributes to the PCP-FMD advancement in Tanzania.

3.2 MATERIALS AD METHODS

3.2.1 Samples and Study area

This study aimed to collect cattle tissue samples from districts that reported FMD outbreaks in the duration between 2020 and 2021 across Tanzania. A total of 11 districts (Chalinze, Bagamoyo, Moshi rural, Babati, Mbogwe, Bukombe, Biharamulo, Ngara, Mvomero, Morogoro rural and, Kibaha) reported FMD outbreaks in the stipulated duration (Fig. 3.1). The samples were collected by following World Organisation of Animal Health (OIE) guideline (OIE, 2013). In the field epithelia tissue samples were obtained from well restraint clinically sick animals, stored in cryovials with Viral transport media and stored in liquid nitrogen tank. The obtained samples were shipped to the Department of Veterinary Microbiology, Parasitology and Biotechnology laboratory at Sokoine University of Agriculture, Morogoro and stored at -800C till when analysed. The summary of procedure for epithelial tissue samples collection in cattle is described in Appendix 2.

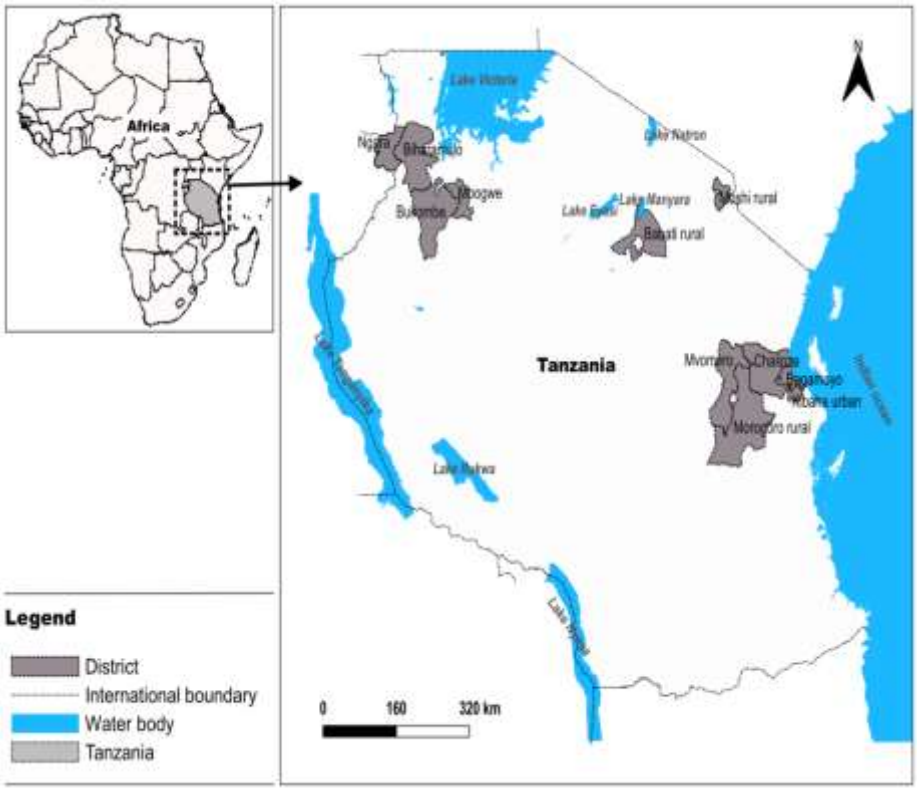

Figure 3. 1: Map of Africa and Tanzania showing areas where samples and previous study nucleotide sequences used in this study were obtained and analysed (Source, This study).

3.2.2 Sample preparation and RNA extraction

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

3.3 Detection and typing of FMDV genome

The extraction RNA obtained were screened to infer the presence of FMD virus genomes in every field samples under study. The screening was done by a one-step RT-PCR using PAN primers (Forward Primer: GCCTGGTCTTTCCAGGTCT; Reverse Primer: CCAGTCCCCTTCTCAGATC) that targets 5'UTR region of the FMD virus genome. The protocol involved 50^0C (30 min.) for reverse transcription, 95^0C (15 min.) for (transcriptase enzyme denaturation, polymerase activation and cDNA unwinding, denaturation 95^0C (1 min.), annealing 55^0C (1 min.), elongation 72^0C (2 min.) for 35 cycles and final elongation 72^0C (5 min.). The PCR amplicons generated were observed under a 1.5% Agarose gel electrophoresis and SafeView™ Classic ladder of 100bp size. The samples that tested positive (328bp band size) for FMDV PAN- Primers were further analyzed by using FMDV serotype specific primers. The serotype specific primers were for FMDV serotypes (A, O, SAT1, 2 and 3) and the analysis was done as previously described (Knowles et al., 2016). The PCR amplification protocol was 50^0C (30 min.) for reverse transcription, 95^0C (15 min.) for (transcriptase enzyme denaturation, polymerase activation and cDNA unwinding), denaturation 95^0C (1 min.), annealing 60^0C (1 min.), elongation 72^0C (2 min.) for 35 cycles and final elongation 72^0C (5 min.). The PCR amplicons generated with diverse band sizes depending on each primer set deployed were observed under a 1.5% Agarose gel electrophoresis with SafeView™ Classic ladder of 100bp size. The properly typed samples were identified and qualified for VP1 amplification process using respective serotype specific primer sets. For detailed FMDV serotypye specific primers see Appendix 5.

3.4 FMDV VP1 amplification

The reaction master-mix was prepared in a separate PCR clean room by adding 8 µl of nuclease-free water, 2.5 µl of FMDV serotype specific forward primer (4 pmol/µl), 5 µl of FMDV serotype specific reverse primer (4 pmol/µl), 5 µl of 5× buffer (containing 2.5 mM $MgCl_2$), 1 µl of dNTPs mix and 1 µl of Qiagen OneStep RT-PCR enzyme mix (QIAGEN OneStep RT-PCR kit (Qiagen, Germany), 2.5 µl of the viral RNA was lastly added to the RT-PCR tube. Template-free amplification controls (RT-PCR tubes with nuclease-free water

only instead of RNA sample) for each primer set were included and amplified parallel to the RNA samples to monitor any chances of cross-contamination in the process. The RT-PCR tubes with reaction mixtures and the control tubes were placed in a thermocycler (Applied Biosystems, ABI 9700; USA) and the appropriate PCR cycling Programme was set based on the serotype and respective primers as described by (Knowles et al., 2016). When the amplification process was done, the tubes were held at 12°C waiting for cycle sequencing processes.

3.5 Sequencing of FMDV VP1 fragment

The VP1 PCR amplicons were purified using Illustra kit and cycle sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies). A total 10 µl reaction mixture was prepared, each with 2 µl 5× sequencing buffer mixed with 0.5 µl BigDye Terminator v3.1 (both reagents are supplied with the kit), 3 µl of FMDV universal reverse sequencing primer (NK72) or serotype/ topotype specific sequencing forward some reverse primers at (1.6 pmol) (Knowles et al., 2016), and 5–20 ng of target DNA. The cycle-sequencing PCR reactions were carried out in each of the primers in 0.2 ml thin-walled tubes by deploying a protocol of 96°C for 1 min and 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min (Applied Biosystems, ABI 9700; USA). After the cycling was done the thermocycler was set to hold the tubes at 4°C while waiting for further procedures. The obtained cycle-sequencing PCR products were cleaned up by ethanol precipitation. The latter used 5 µl containing 125 mM EDTA and 60 µl of 100% ethanol added to each reaction tube containing the sequencing PCR products, then vortexed and incubated for at least 15 min at room temperature to allow precipitation to occur. The precipitation was carried out in the dark enclosure as BigDye Terminator reagent being light-sensitive. After precipitation was done, the tubes were centrifuged at 13,000 rpm for 30 min at 4^0C and the supernatant was pipetted and discarded without disturbing the pellet. Thereafter; the pellets were washed with 60µl of 70% ethanol and centrifuged at 13,000 rpm for 30 min at 4^0C, the supernatant removed and pellets shaded from direct light dried in an oven drier for 60 min to ensure no ethanol remnants. The samples were finally re-suspended in 20µl of Hi-Di Formamide (Life Technologies) and

loaded onto the ABI 3500 DNA Analyser where the sequencing reactions were allowed to run according to the manufacturer's instructions.

3.6 Phylogenetic analysis

The cDNA nucleotides sequences of VP1 origin obtained from different FMDV field isolates that had already been typed into their respective serotypes were assembled using SeqMan Pro (Lasergene package DNAstar Inc., Madison, Wisconsin, USA). The contig nucleotide sequences from each FMDV serotypes were aligned with multiple similar VP1 nucleotide sequences sourced from GenBank (NCBI) database using CLUSTAL W (Thompson et al., 1994). The evolutionary history was inferred by using the Maximum Likelihood method, and the selection of the best model for the construction the phylogenetic tree was achieved by use of aligned multiple cDNA nucleotide sequences in MEGAX (Nei. M and Kumar. S, 2000). The General time reversal (GTR) model combined with gamma distribution and proportion of invariant sites (GTR + G + I) algorithms was applied. The tree was constructed and visualized in MEGAX (Kumar et al., 2018) and the phylogenetic tree obtained was additionally manipulated in FigTree program v1.4.4 for enhanced visualization. For FMDV serotype specific primers and prototype details see Appendix 6 and 7.

3.7 RESULTS AND DISCUSSION

In this study, a total of 113 FMD tissue samples were collected (Table 3.1), of which 48.67% (n = 55) of the FMD tissue samples collected had nucleic acid materials for FMDV when screened by one-step RT-PCR using PAN primers. These results provided evidence of FMDV strains circulating and responsible for the outbreaks in the field. Also the screening results confirmed that, the profile of vesicular lesions manifested in cattle herds in the field were due to FMD outbreaks. This study identified three FMDV serotypes (O, A, and SAT1), to be circulating and causing FMD outbreaks in different geographical locations during the stipulated study period in Tanzania. Unlike in other studies on FMD outbreaks investigation in Tanzania where type O, A, SAT1 and SAT2 were reported (Kasanga et al., 2012, Sallu et

al., 2014).This study did not detect FMDV type-SAT2 but it was lastly detected in 2016 study samples collected in Kilimanjaro, Arusha, Iringa, Morogoro, and Coast Region areas (Mfuru et al., 2018). The study done in 2008 – 2013 showed that, of all outbreaks reported in that study duration SAT2 was accounted for causing 2.85% of all outbreaks (Sallu et al., 2014). Then FMDV type-SAT2 could have probably been circulating in areas that no outbreaks were reported to enable samples to be taken for analysis during study period.

Table 3.1: Summarised data of total samples analysed and their respective detection and serotyping results.

S/N	District	Total samples	PAN-PCR Results (+ve)	Serotype	Topotype
1	Chalinze	3	-	-	
2	Bagamoyo	11	3	SAT1	I(NWZ)
3	Kibaha	5	3	A	G-I
4	Moshi rural	16	10	SAT1	I(NWZ)
5	Babati	1	-	-	-
6	Mbogwe	43	29	O	EA-2
7	Bukombe	2	2	-	-
8	Biharamulo	4	-	-	-
9	Ngara	8	6	O	EA-2
10	Mvomero	15	-	-	-
11	Morogoro rural	5	2	-	-
12	Total	113	55	3	3

3.8 Foot-and-mouth disease virus serotype O

The FMD virus serotype O detected in this study were from outbreak samples obtained from Ngara and Mbogwe districts. The FMD virus type O exhibits a historical cosmopolitan occurrence, and past studies in Tanzania have reported type O to be circulating in the sampled areas for decades (Kasanga et al., 2012). The phylogenetic analysis conducted, inferred the existing genetic and evolutionary relationships amongst GenBank data, and this study nucleotide sequences through expressed clustering patterns (Fig.3.2). In the phylogenetic tree the field identified FMDV serotype O nucleotide sequences with 633nt

size clustered together with reference sequences derived from prototype strains (O/TAN/2/2004 [KF561679.1], O/MAL/1/98 [DQ165074.1], O/UGA/3/2002 [DQ165077.1] and O/KEN/5/2002 [DQ165073]) Tanzania, Malawi, Uganda and Kenya origin isolates, respectively with 100% bootstrap value. The phylogenetic tree topology, the published articles cited in this current study, and the WRLFMD (Pirbright, UK) reports stipulate that, all 2020 to 2021 FMDV serotype O Tanzania field isolates belonged to topotype EA-2. The prototype (O/TAN/2/2004 [KF561679.1]), a 2004 Tanzania origin isolate described a closest relatedness as compared to the other prototypes included in the analysis with shared identity of 95.46 – 96.24% with current study strains. The shared identity of 92.0-100.0% was revealed amongst this study field nucleotide sequences analyzed, whereas the far distant (O/NGR/TZ/03/2021) and (O/MBG/TZ/21/2021) isolates showed the highest identity of 100.0% compared to close distance isolates within Mbogwe district (Fig. 3.2). The identity disparities amongst analyzed sequences portrayed in the phylogenetic tree describe their existing genetic and evolutionary relationships influenced by their geographic locations variabilities (Fig. 3.2). In the phylogenetic tree, the 1998 FMD outbreaks in Tanzania and Malawi had isolates (O/TAN/7/98 [AJ296320.1] and O/MAL/1/98 [DQ165074.1] sharing 97.3% identity, the 2004 and 2005 Kenya and Tanzania isolates (O/KEN/27/2005 [KF135274.1] and O/TAN/2/2004 [KF561679.1]) shared 98.6% identity whilst 2011 Eritrea and Ethiopia had isolates (O/ERI/3/2011 [MK422550.1] and O/ETH/6/2011 [MN987402.1]) sharing 99.8% identity. Also the field strains identified during this study clustered closely to 2005 Kenyan isolate (O/KEN/27/2005 [KF135274.1]) with 98% bootstrap support (Wekesa et al., 2015). These findings inference for the possibility of cross border virus incursions, and are in agreement with Di Nardo's 2011 (Di Nardo et al., 2011) study that described border areas to be experiencing burden of transboundary livestock diseases including FMD fuelled by cross border legal and illegal socio-economic activities. The FMDV strains expressing less than 15% variation in the sequenced VP1 segment are considered to be of the same genotype, and the ones with less than 5% variation are considered to be closely related (Knowles and Samuel, 2001, Samuel et al., 1999). The virus strains under EA-2 cluster have expressed a shared identity of 85.2-

100%. This degree of relatedness in the topotype EA-2 viruses signify that, if FMD vaccines developed from strains belonging to topotype EA-2 identified in this study they are likely to confer suitable protection against viral incursions of EA-2 topotype category.

3.9 Foot-and-mouth disease virus serotype A

The FMDV serotype A was identified in the analyzed field obtained samples. The FMDV type-A is also widely distributed, having been reported world-wide in the history of the disease (Brito et al., 2017). The virus was detected in outbreak samples obtained from Kibaha district of Tanzania. The current geographic occurrence of type-A are consistent with the past studies that described type A to be circulating in the mentioned areas (Kasanga et al., 2012, Sallu et al., 2014). Also studies have described the Eastern areas to be FMD higher risk area due to frequent reports of multiple types FMD outbreaks of (O, A, SAT1, and SAT2) origin (Kasanga et al., 2012). In this study SAT1 and A types were identified in samples from close distance districts of Bagamoyo and Kibaha districts respectively. The nucleotide sequences for FMDV field identified as serotype A had 621bp size. Based on the phylogenetic tree constructed from FMD virus type A nucleotide sequences, this study nucleotide sequence clustered with a reference nucleotides sequence with (A/KEN/42/66 [KF561699.1]) a Kenyan 1966 isolate (Kasanga et al., 2015). The observed clustering pattern in the phylogenetic tree, the published articles cited in this work as well as the WRLFMD (Pirbright, UK) reports infers that, the FMDV type A Tanzania 2020 field isolates belonged to Africa topotype G-I Lineage. The phylogenetic tree described type A to have a closer clustering with (A/KEN/K39/2015 [MH882570.1]) (Omondi et al., 2015) and (A/UGA/28/2019 [MT602080.1]) (Ludi et al., 2019) Kenyan and Uganda previous studies identified strains respectively, than any other nucleotide sequences from sub Saharan countries (Fig. 3.3).

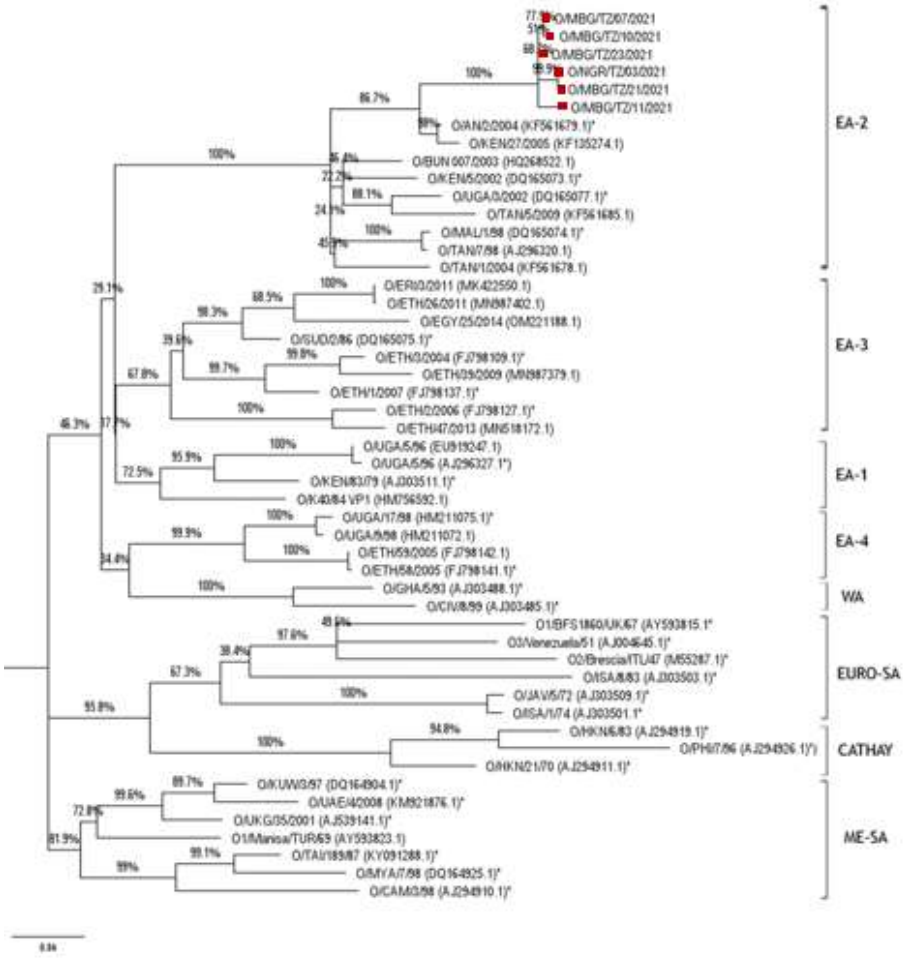

Figure 3.2: Midpoint-rooted maximum likelihood phylogenetic tree showing the genetic relationship between 2020/2021 fields identified FMDV type-O strains and GenBank database archived nucleotide sequences from previous outbreaks in Africa countries. Red dot means this study strains.

The 2015 Kenyan (A/KEN/K39/2015 [MH882570.1]) nucleotide sequence shared 90.8% highest identity followed by 2019 Uganda (A/UGA/28/2019 [MT602080.1]) nucleotide sequence that expressed

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

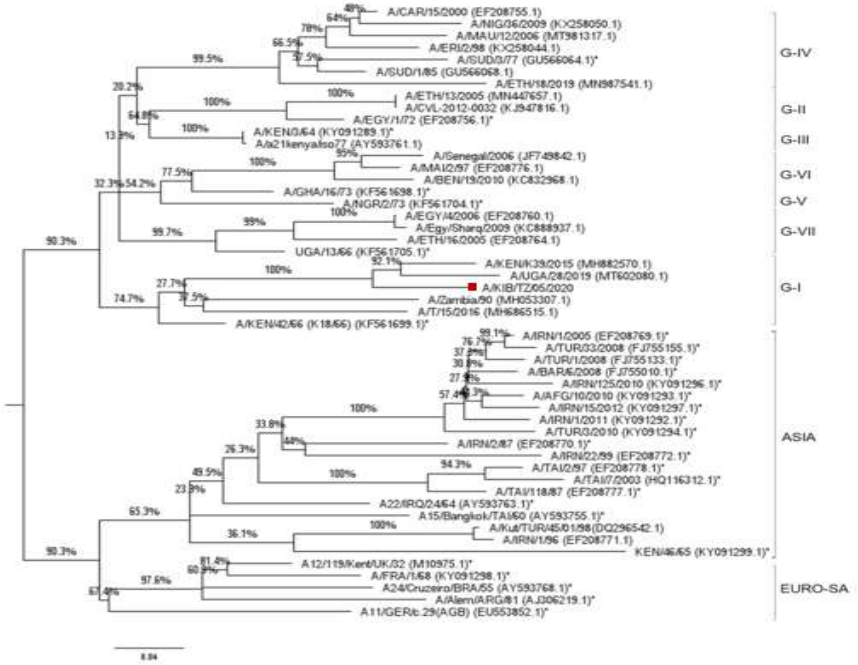

Figure 3.3: Midpoint-rooted maximum likelihood phylogenetic tree showing the genetic relationship between 2020/2021 fields identified FMDV type-A strains and GenBank database archived nucleotide sequences from previous outbreaks in Africa countries. Red dot means this study strains.

3.10 Foot-and-mouth disease virus serotype SAT1

The field identified FMDV type-SAT1 were from outbreak samples obtained from Bagamoyo and Moshi rural districts of Tanzania. The past studies described FMDV type-SAT1 to have been reported in areas it was currently detected in this study (Kasanga et al., 2012). This study identified SAT1 possessed nucleotide sequences with 390 and 655 nucleotides sizes for strains sourced from Moshi Rural and Bagamoyo districts, respectively. The topology of the phylogenetic tree generated from a list of FMD virus type-SAT1 nucleotide sequences of this study and GenBank origin described characteristic clustering pattern (Fig. 3.4). A close relatedness was observed between SAT1 from current study and reference prototypes (SAT1/ZIM/23/2003 [KF219690.1], SAT1/T155/71 [HQ267519.1] and, SAT1/TAN/5/96 [AY442007.1]) 2003 Zimbabwe, 1971 and 1996 Tanzania FMD outbreak strains. The inference made through the generated phylogenetic tree, the published articles cited in this study and the WRLFMD (Pirbright, UK) reports indicated the SAT1 2021Tanzania field strains belonged to topotype I (NWZ). The SAT1 strains of this study had 96.9-98.8% identity and expressed close clustering than other sequences in the SAT1 type list of sequences analysed (Fig. 3.4). These findings are in agreement with Sallu's 2014 (Sallu et al., 2014) study that identified FMDV of topotype I (NWZ) to be circulating and causing outbreaks in different areas in Tanzania. The shared identity of 77.4-99.0% was expressed for nucleotide sequences within same topotype I(NWZ) and least 67.2-70.3% across topotypes I(NWZ) and V. The strains with close geographic relationship had exceptionally higher shared percentage identity (99.0% for SAT1/MOZ/3/02 versus SAT1/ZIM/23/2003) and (93.6% for SAT1/K28/06 versus SAT1/TAN/11/2012). This situation emphasis on enhancing geographical areas/regions based FMD control strategies that execute tailored vaccines based on identified topotypes or lineages rather than generalized vaccines that have failed to confer effective field performances under the current Africa context.

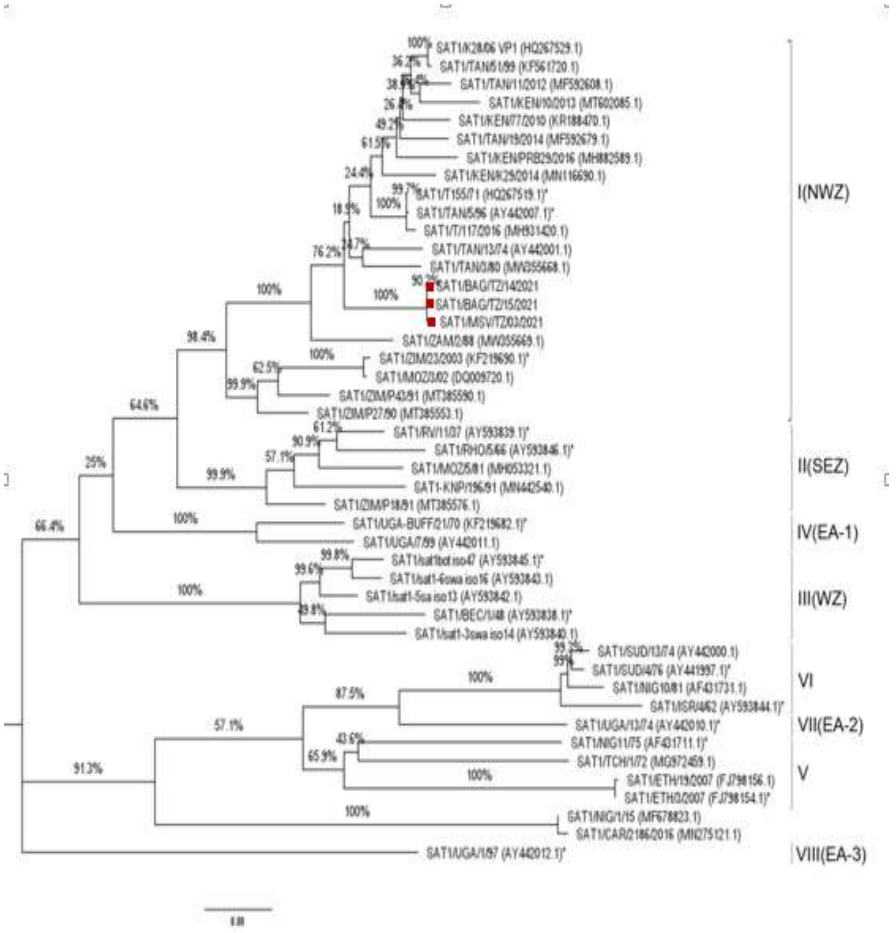

Figure 3.4: Midpoint-rooted maximum likelihood phylogenetic tree showing the genetic relationship between 2020/2021 fields identified FMDV type-SAT1 strains and GenBank database archived nucleotide sequences from previous outbreaks in Africa countries. Red dot means this study strains.

In this study the nucleotide sequences of the current circulating wild type virus strains have been analysed and found no evident new variants circulating in the field. However, the distantly obtained SAT1 and O identified in this study had similar genetic characteristics and these findings provide prospects on feasible FMD control if strategic FMD control mitigations are to be implemented across endemic Africa countries.

3.11 CONCLUSION

The findings of this study provide evidence of FMD presence in Tanzania with multiple outbreaks that implicate food security, livelihoods of communities, and mitigation measures via vaccination. The multiple FMDV types (A, O, and SAT1) identified from samples obtained from diverse geographic locations reveal the epidemiological complexity of FMD in the country, and calls for strategized mitigation measures featured on frequently updated field data. The genetic and evolutionary relationship revealed amongst strains across countries examined during this study, infer the persistence and significance of FMD transboundary consequences. The aspect of uncontrolled animal movements is regarded as the main contributing factor to the viruses spread across districts and even crossing country borders. This state needs to be translated as the landscape for the concerned countries to accord on improving coordinated national, regional, and global FMD control initiatives. The degree of percentage identity expressed within and between FMDV types (A, O, and SAT1) topotypes in this study enhance knowledge for tailored vaccine and vaccination to improve FMD control outcomes. Tanzania has also been involved in progressive control pathway for FMD (PCP-FMD) strategic initiatives and is estimated to be at PCP level 1, the updated knowledge on circulating field strains is vital as it comprehends control strategies (suitable vaccine selection). Therefore, the information of this study significantly advances knowledge on FMDV currently circulating in cattle herds and the underlying molecular and spatial epidemiology of the FMDV in Tanzania and Africa. However, future studies need to be on redefining the FMDV susceptible hosts spectrum due to the richness of livestock and wildlife diversity in the country or region, The knowledge of FMDV whole capsid antigenic characteristics of the circulating strains versus the available vaccine strains, the community level of awareness and attitudes on FMD consequences need to be enhanced for unleashing future participatory control approaches. Though the 5’ UTR FMDV genome target region for PAN primers is highly conserved, but need for revised performance of the primers is a requirement.

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Conflict of interest

The authors declare that they have no conflict or competing interests that may have influenced them in writing this article.

Ethical statement

This research study followed the guidelines and regulations of the Sokoine University of Agriculture on the research proposal approvals, and availability of permits for field works, see Appendix. 8.

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CHAPTER FOUR

4.0 Molecular and Serosurvey of Foot-and-mouth disease virus serotypes O and A in selected livestock-wildlife interface areas of Tanzania

Mkama Mathias[1,2,3], Philemon Wambura[1], Sharadhuli Kimera[3,8], Robert Fyumagwa[4], Sengiyumva Kandusi[1], Raphael Sallu[5], Mark Rweyemamu[3], Francois Maree[7], Donald King[6], Ernest Mjingo[4], Justin Wanda[4], Daniel Mdetele[9], Christopher Kasanga[1,3]

.

[1]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O Box 3019, Morogoro, Tanzania.

[2]Tanzania Veterinary Laboratory Agency, Centre for Infectious Diseases and Biotechnology, P. O Box 9254, Dar es salaam, Tanzania.

[3]SACIDS Foundation for One Health, Africa Centre of Excellence P. O. Box 3297, Morogoro, Tanzania.

[4]Tanzania Wildlife Research Institute, P. O. Box 661, Arusha, Tanzania,

[5]Food and Agriculture Organization, P. O. Box 2, Dar es Salaam, Tanzania,

[6]FAO World Reference Laboratory for FMD, The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 ONF, United Kingdom.

[7]Clinomics, Uitzich Road, Bainsvlei, Bloemfontein, 9301, Free State, South Africa

[8]Department of Veterinary Medicine and public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O Box 3021, Morogoro, Tanzania.

[9]Directorate of Veterinary Service, P. O Box 2870, Dodoma, Tanzania.

*Correspondence: mathias.mkama@sacids.org

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ABSTRACT

Foot-and-mouth disease virus causes a highly devastating FMD to all cloven hoofed livestock and wildlife. The disease implicate food security and livelihoods to vulnerable communities worldwide. The virus circulates worldwide as seven antigenically distinct serotypes (O, A, SAT1-3, C, and Asia1), and African buffalo act as reservoir of SAT1-3 serotypes. It remains unclear whether the buffalo can also act as carrier of the Eurasian serotypes O and A, which occur in parts of East Africa. The screening of FMDV natural infection in buffalo and cattle was done using FMDV NS ELISA then characterized by Solid phase competitive ELISA (SPCE) for FMDV antibodies specific to serotype O and A. The FMDV RNA screening and typing with one-step RT-PCR using PAN primers and serotype specific primers respectively. About 247 sera samples of 330 samples from buffalo and cattle were NSPE positive, whereas 93 were from buffalo and 154 from cattle origin. The SPCE serotype specific results showed that, 83 samples tested positive for FMDV serotype A in cattle and 28 samples for buffalo whereas; 103 sample tested positive for FMDV serotype O in cattle and 51 samples for buffalo. Besides, only one buffalo sample 1.12 %(1) of the 89 probang samples from Serengeti interface reveled FMDV RNA genomic materials and typed as serotype-O by one-step RT-PCR. This study demonstrated serological evidence for FMDV type O and A infections in buffalo, and for the first time FMDV serotype O genomic materials were detected in Tanzania buffalo samples, but no any FMDV serotype A genomic materials were detected from buffalo samples analyzed. This study was important for advancing the knowledge for FMDV epidemiology in order to improve future tailored FMD control strategies in Tanzania.

Keywords: Foot and mouth disease; Foot and mouth disease virus, livestock-wildlife interface areas, Serotype O, Serotype A, Tanzania.

1.0 INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease of even-toed domestic and wild ungulates caused by FMD *virus* (FMDV). It is a disease with potential food insecurity and socio-economic implications to the global community (Knight-Jones & Rushton, 2013). FMDV is a single stranded positive sense RNA virus classified into genus *Aphthovirus* of the family *Picornaviridae* (King, *et al.,* 2000; Zell *et al.*, 2017). The virion is non-enveloped with an icosahedral symmetry comprised of 60 copies of the structural viral proteins VP1, VP2, VP3 and VP4, with an estimated size of 30 nm in diameter (Knowles and Samuel, 2003). The virus exists in the form of seven antigenically distinct serotypes named as A, O, C, Asia1, South African Territories 1 (SAT1), SAT2, and SAT3. Of the seven serotypes, Africa has identified and reported six serotypes (A, O, C, SAT1, SAT2 and SAT3). Except for Asia1 that has never been reported in Africa, serotypes O, A, SAT1 and 2 have been reported circulating and causing outbreaks in Tanzania (Kasanga *et al.,* 2015; Kasanga *et al.,* 2012; Sallu *et al.,* 2014; Vosloo *et al.,* 2002). The SAT 1-3 circulate as restricted to the African continent, and have occasionally been reported to cause outbreaks in the middle east countries (Jamal & Belsham, 2013). The FMDV type Asia1 occurs as restricted to Asia also and have rarely been reported to the western and eastern Eurasia whereas, the FMDV types O and A present an extended distribution, as are reported in Africa, Asia, and South America (Brito et al., 2017; Valarcher et al., 2009; Kitching et al., 2007). There are currently no reports for FMDV type C since 2004 (Brito et al., 2017; Sangula et al., 2011). These virus types circulate in seven global conjectured epidemiological pools where pool 4 - 6 belong to Africa (Brito et al., 2017). There are some pressing factors that make FMD control to be challenging and difficult to achieve in the foreseeable future one of them being its broad host spectrum. Literatures describe FMDV to be potentially infective to over 70 species of livestock and wildlife origin. In the livestock and wildlife animal populations susceptible to FMDV, cattle and buffalo have been identified most as the main species playing role in the transmission and as reservoirs of the virus respectively (Omondi et al., 2018). The large proportion of cattle herds are under pastoral communities, and these keep their animals in the proximity of

conserved lands strategically for grazing their animals during pastures deprived seasons of the year (Bronsvoort *et al.,* 2004; Fè Vre *et al.,* 2006). Uncontrolled movements of animals increase interactions frequency between livestock and wildlife, sustain virus spread and transmissions, thereby complicating the epidemiology of the disease in the country or the entire region as well (Vosloo *et al.,* 2005). The complicated FMD epidemiological situation persisting in various geographic areas for decades, have enabled to the evolution of a large number of subtypes or topotypes within each serotype that portray significant genetic and antigenic distinct characteristics (Martínez, *et al.,* 1992).

Studies carried out in southern Africa for the persistently infected African buffalo have so far been potentially proved that, buffalo (*Syncerus Caffer*) successfully transmit FMDV serotypes SAT1, SAT 2 and SAT 3 to cattle (Thomson *et al.,* 2018). Persistently infected cattle and buffalo (*Syncerus Caffer*) are cited to be the potential sources of new FMD outbreaks in endemic countries (Grubman and Baxt, 2004), and they can maintain the virus for 6 month-3 years and 5-over 24 years respectively (OIE, 2009). In the persistently infected cattle and buffalo FMDV is maintained in Oesophageo-pharyngeal epithelial cells (Longjam *et al.,* 2011; Thomson, 1996). And the major means of viral transmission at interface is through animals interactions that occur either within conserved lands or at close vicinity communal grazing lands (Mkama *et al.,* 2014).

It is not known whether African buffalo can also act as carriers for the Eurasian FMDV serotypes O and A as is the case in cattle. The present study investigated the serotypes O and A FMDV infection status of buffalo (*Syncerus Caffer*) and cattle in sera samples, and evidence of FMDV genomic materials in probang samples from selected livestock-wildlife interface areas of Tanzania. This study deployed both molecular and serological survey methods in obtaining information necessary for fulfilling this knowledge gap. Understanding the epidemiology of FMDV serotypes O and A circulating strains at interface areas animal populations, improve knowledge necessary for strategic control of FMD not only in Tanzania but even the rest of

African FMD endemic countries that are similarly inhabited with a rich spectrum wildlife biodiversity.

4.0 MATERIALS AND METHODS

4.1 Study area

The samples for this study were collected from cattle and buffalo in the select wildlife-livestock interface areas of Mikumi (Morogoro region), Katavi (Rukwa region), Ruaha (Iringa region), Mkomazi (Kilimanjaro region), and Serengeti (Mara and Manyara regions) national parks (Figure 4.1) in Tanzania. The knowledge from FMD outbreaks past studies describing the FMDV serotypes occurrence and their respective distribution in Tanzania, was harnessed to achieve the selection process of the study areas (Kasanga et al., 2012; Kivaria, 2003; Picado et al., 2011; Sallu et al., 2014).

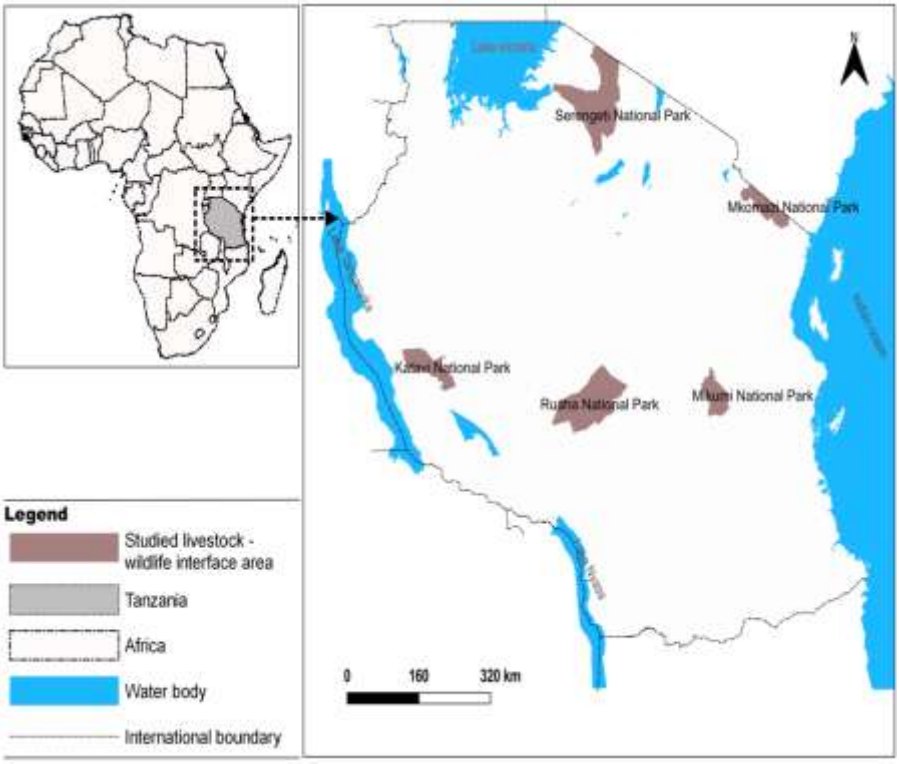

Figure 4.1: Map of Tanzania showing different livestock-wildlife interface areas where buffalo and cattle samples were obtained (Source; This study).

4.2 Study design, sample type(s), and sampling strategy

This was designed as a cross sectional study where by 330 sera (cattle [209] and buffalo [121]) and esophageo-pharyngeal fluid or probang (buffalo [89]) samples were collected in the year (2010 to 2011), and 2018 respectively (Table 4.1). At every interface area, sampling was firstly done on buffalo herds then on cattle herds in their vicinity, that were considered of being intermingling freely with buffalo herds (Figure 4.2). In the field, the obtained sera samples were temporarily stored in labeled sterile cryovials in a cool box with icepacks (+4-6°C) and stored at -20ºC in the laboratory till when analyzed. Whereas, the obtained buffalo probang samples in sterile cryovials with viral transport media (VTM) were properly labeled and temporarily stored in a Liquid Nitrogen dry shipper until when the samples were transported to the Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture laboratory and stored at -80ºC until use. Details on approach to buffalo sampling go to Appendix 3.

Figure 4.2: Summary procedure for sample collection in Serengeti national park (2018); the figure 2A depicts a darted buffalo in a transient state before being fully immobilized. The figures 2B and 2C show probang sample taking and filling of sterile cryovials, respectively. The figure 2D shows blood sample collection from jugular vein of an immobilized buffalo.

4.3 Screening for FMDV infection in buffalo and cattle

Screening of FMDV virus in cattle was done using PrioCHECK® FMDV NS antibody test ELISA kit, a non-species specific kit that detects antibodies directed against non-structural 3ABC proteins of FMDV (Clavijo *et al.,* 2004; Sorensen *et al.,* 1998;Mackay *et al.,* 1998). The test was done as per manufacturer's (PrioCHECK® FMDV NS, Prionics Lelystad B.V, Netherlands) instructions manual supplied with kit of Lot number: F120401L.

4.4 Serotype specific FMDV antibodies characterization

Animals infected or vaccinated with FMDV elicit antibodies against proteins of viral capsids, also called structural proteins (SP). Anti-SP antibodies cannot distinguish between infected and vaccinated animals and are mostly serotype-specific. Different tests are used for each of the seven FMDV serotypes as they are genetically and antigenically distinct (Mackay *et al.,* 2001; Paiba *et al.,* 2004). The Solid phase competitive ELISA (SPCE) assay was deployed to characterize FMDV serotypes O and A. This assay was performed based on the manufacturer's (IZSLER Biotechnology Laboratory, Brescia, Italy) instructions manual supplied with kits of Lot number: 01-2012 120730b.

4.5 Sample preparation and FMDV RNA extraction

The 2018 field obtained probang samples (Figure 4.2) under -80^{0}C storage condition were allowed to equilibrate at room temperature, centrifuged at 12000 rpm and 500µl supernatant collected using 1000µl capacity pipette for RNA extraction. The RNA extraction was done using Qiagen RNeasy® Mini Kit by following the manufacturers' instructions manual, where 50µl RNA was obtained after elution. The extracted RNA products were quantified spectrophotometrically using Nanodrop and all product below the ratio of 2.0 were rejected for further analysis as it contains unfavorable levels of proteins and/or organic contaminants that could impede intended downstream analytical procedures.

2.6 Detection and typing of FMDV type O and A nucleic materials from buffalo probang samples

The extraction products obtained were screened to infer the presence of FMDV genomes in the 89 field samples under study. The screening was done by a one-step RT-PCR using PAN primers (Forward: GCCTGGTCTTTCCAGGTCT; Reverse: CCAGTCCCCTTCTCAGATC) that targets 5'UTR region of the FMDV genome. The protocol involved 50^{0}C (30 min.) for reverse transcription, 95^{0}C (15 min.) for (transcriptase enzyme denaturation, polymerase activation and cDNA unwinding), followed by 35 cycles of denaturation 95^{0}C (1 min.), annealing 55^{0}C (1 min.), elongation 72^{0}C (2 min.) and final elongation 72^{0}C (5 min.). The PCR amplicons

generated were observed under a 1.5% Agarose gel electrophoresis and SafeView™ Classic ladder of 100bp size.

The samples that tested positive for FMDV PAN- Primers were further analyzed by using FMDV serotypes (O & A) specific primers (FMDV type-O, Forward: CCTCCTTCAAYTACGGTG; Reverse: GCCACAATCTTYTGTTTGTG; Probe: [6FAM] CCCTCTTCATGCGGTARAGCAG [BHQ1]; FMDV type-A, Forward: GCCACRACCATCCACGA; Reverse: GAAGGGCCCAGGGTTGGACTC; Probe: [6FAM] CTCGTGCGMATGAARCGGGC[BHQ1]) and the PCR amplification protocol was 50^0C (30 min.) for reverse transcription, 95^0C (15 min.) for transcriptase enzyme denaturation, polymerase activation and cDNA unwinding, 95^0C (1 min.) for denaturation, 60^0C (1 min.)for annealing , 72^0C (2 min.) for elongation amplification repeated for 35 cycles and final elongation 72^0C (5 min.) as described previously (Bachanek-Bankowska et al., 2016).

4.7 Data management and statistical analysis

The FMD percentage seropositivity (PS) were calculated by dividing the total number of serotype specific (type O, A, or O&A) positive samples to SPCE test to the total number of non-structural protein ELISA (NSPEs) positive samples tested (Mwiine *et al.,* 2010). This study information was managed using Microsoft excel v.2013, and descriptive analyses, chi-square test, as well as independent t-test level were deployed to analyze and compare the PS variations for detected FMDV type O, A and O&A from cattle and buffalo sera samples plus their significance at 95% confidence.

4.8 RESULTS

In this study, a total of 247 3ABC-NSP ELISA positive serum samples from buffalo (n = 93) and cattle (n = 154) herds were serotyped by SPCE assay for detection of antibodies specific to FMDV serotype O and A and the SPCE results obtained were summarized in Table 4.1.

Table 4.1: Serological characterisation of cattle and buffalo sera samples using SPCE for detection of antibodies specific to FMDV types O and A

National park	Animal Spp.	Samples Tested *(Mkama etal., 2014)*	NSPE +Ves	SPCE			Type-A&O -Ves(%)
				Type-A (%)	Type-O (%)	Type-A&O(%)	
Katavi	Cattle	61	49	27(55.1)	39(75.6)	21(42.9)	4(8.2)
	Buffalo	29	29	13(44.8)	25(86.2)	11(37.9)	4(13.8)
Ruaha	Cattle	53	41	13(31.7)	33(80.5)	11(26.8)	6(14.6)
	Buffalo	31	29	9(31.0)	20(69.0)	8(27.6)	9(31.0)
Mikumi	Cattle	35	29	17(58.6)	14(48.3)	11(37.9)	9(31.0)
	Buffalo	30	28	4(14.3)	5(17.9)	4(14.3)	23(82.1)
Mkomazi	Cattle	60	35	26(74.3)	17(48.6)	13(37.1)	5(14.3)
	Buffalo	31	7	2(28.6)	1(14.3)	1(14.3)	6(85.7)
Total	Cattle	209	154	83(53.9)	103(66.9)	56(36.4)	24(15.6)
	Buffalo	121	93	28(30.1)	51(54.8)	23(24.7)	42(45.2)
		330	247	106/247	154/247	79/247	66/247

+Ves = positive samples; -Ves = negative samples; NSPE = Non-structural protein detection ELISA; SPCE = Solid phase competitive ELISA.

Table 4.2: Descriptive analysis of FMDV serotypes A and O in buffalo and cattle at selected livestock-wildlife interface areas in Tanzania

Species	FMDV serotypes	Frequency positives	%age	Standard Error	95.0% Lower CL	95.0% Upper CL
Buffalo	Type-A	28	30.10%	5	19	39
	Type-O	51	54.80%	6	39	64
	Type- A&O	23	24.70%	5	15	33
	Total	93	100.00%	8	79	108
Cattle	Type-A	83	53.90%	7	69	98
	Type-O	103	66.90%	8	88	118
	Type- A&O	56	36.40%	7	44	70
	Total	154	100.00%	8	139	168

Chi-square test of df=2, p <.001

The findings portrayed in table 4.1 elaborate the different score levels for antibodies specific to FMDV type O and A on buffalo and cattle

NSPE positive sample tested. The scores show the counts together with their corresponding percentages. The columns of type O&A mixed seroreactions and that of the samples that did neither test positive for FMDV type O nor A were also included. Results from table 4.2 shows that out of the 93 analyzed NSPE positive buffalo samples, 30.1% (28) of CI (19 – 39) had antibodies specific to FMDV Type A. Also 54.8% (51) of CI (39 – 64) and 24.7% (23) of CI (15 – 33) had antibodies specific to FMDV type O and those of mixed sero reaction respectively. Similarly, out of 154 NSPE positive cattle samples analyzed 53.9% (83) of CI (69 – 98), 66.9% (103) of CI (88 – 118) and 36.4% (56) of CI (44 – 70) had FMDV antibodies specific to type A, O and mixed seroreactions respectively. The results for the molecular screening of probang samples obtained from clinically normal buffalos of the Serengeti national park showed that, 1 (1.12%) of the 89 probang sample extraction products indicated presence of FMDV genomic materials (Figure 4.6) and when typed using FMDV serotype O and A specific primers, FMDV serotype O was revealed (Figure 4.7). The different statuses of FMDV infections across buffalo and cattle herds' samples were summarized in Figure 4.3 – 5, and statistical significance of observed results were described in the contingency Table 4.3. Detailed result sheet for NSPE, RT-PCR and RT-qPCR read Appendices 4 and 5.

Table 4.3: Contingency table with observed, expected and chi-square statistic for each cell of buffalo and cattle FMDV different infection statuses

S/n	Species	Detection Categories					Total
1		FMDV type-A	FMDV type-O	FMDV Mixed-O&A	FMDV-NEG. O&A	FMDV-POS. NSP	
2	Cattle	27 (19.63) [2.76]	47 (46.87) [0.00]	56 (50.67) [0.56]	24 (41.80) [7.58]	55 (50.03) [0.49]	209
3	Buffalo	4 (11.37) [4.77]	27 (27.13) [0.00]	24 (29.33) [0.97]	42 (24.20) [13.09]	24 (28.97) [0.85]	121
4	Totals	31	74	80	66	79	330

X^2 (df = 4, N = 330) = 31.0876, p = .00001. The results is significant at p<.05.

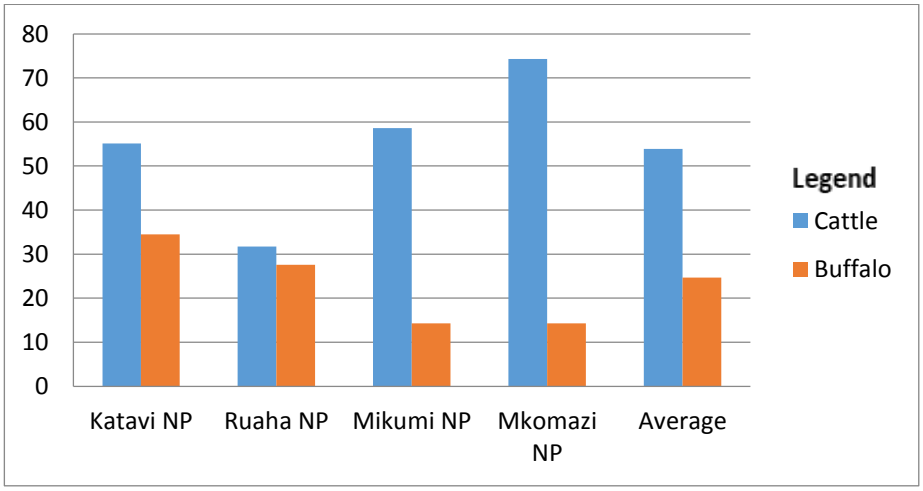

Figure 4.3: The overall infection rates for FMDV serotype A across buffalo and cattle at the studied livestock-wildlife interface areas in Tanzania.

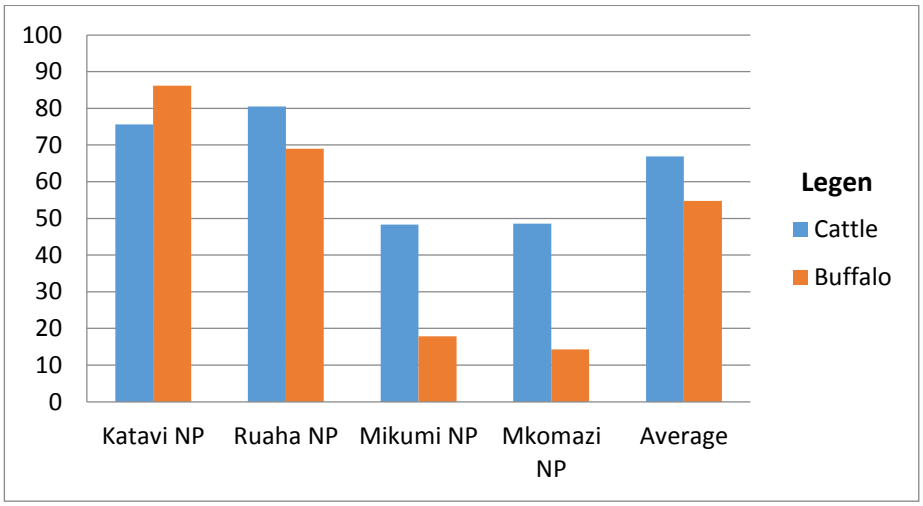

Figure 4.4: The overall infection rates for FMDV serotype O across buffalo and cattle at the studied livestock-wildlife interface areas in Tanzania.

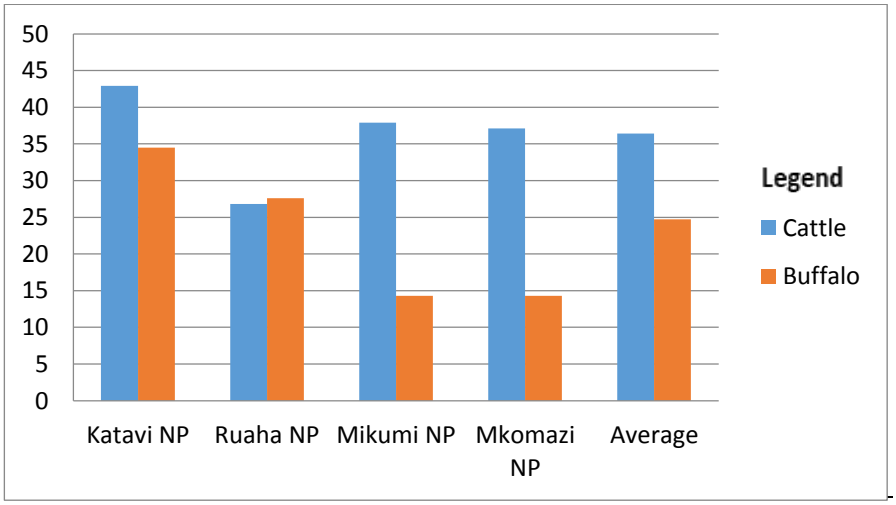

Figure 4. 5: The overall FMDV serotypes O&A mixed infections across buffalo and cattle at the livestock-wildlife interface areas in Tanzania.

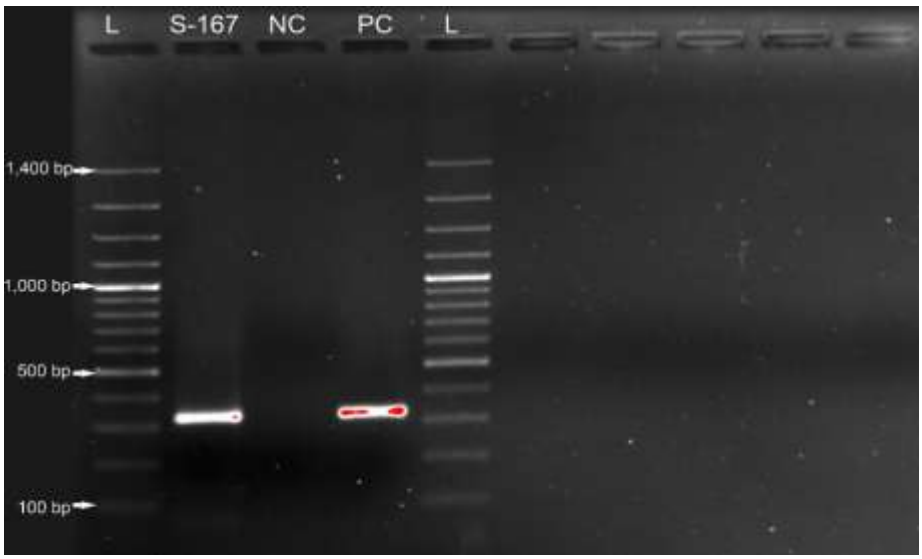

Figure 4.6: The agarose gel electrophoresis image showing 328bp band size amplified using FMDV PAN-primers on buffalo probang sample S-167.

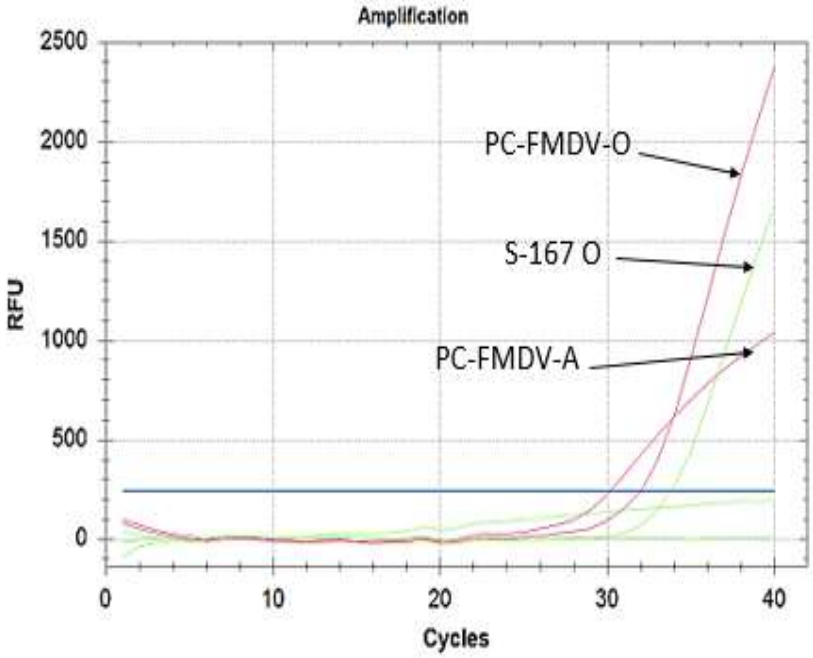

Figure 4.7: M**olecular typing amplification curves derived from qRT-PCR assay using FMDV serotype O and A specific primers. The abbreviations PC-FMDV-O, PC-FMDV-A and S-167 means positive control for FMDV type O and A together with sample S-167 amplification curve positive for FMDV type O specific primers respectively.**

4.9 DISCUSSION

Significant interactions between buffalo and cattle herds exist at each livestock-wildlife interface where this study was carried out. Traditionally, the pastoral cattle herds are frequently grazed within or around national parks and intermingle with buffalo. The interactions between cattle and buffalo increase during drought periods or when most of the communal grazing areas get cultivated. This state has turned into a chronic problem not only for conservation progresses in Tanzania (Michael *et al.,* 2015) but also for animal diseases strategic control initiatives as in the case for FMD (Thomson *et al.,* 2018). Despite the strict illegal entry restrictions imposed by TANAPA and

TAWA on conserved areas, the challenge remains due to the fact that pastoralists inhabiting villages surrounding the parks have huge numbers of cattle herds that cannot solely be sustained through communal grazing lands.

In this study, the FMDV infection in cattle and buffalo was investigated using NSPE, and the results revealed FMDV to be prevalent in all livestock-wildlife interface areas studied. None of the sampled cattle and buffalo had a history of being vaccinated against any of the FMDV serotype(s) suggesting that, all seroreactions resulted from FMDV natural infection. All sera samples from buffalo and cattle livestock-wildlife interface areas demonstrated the presence of both FMDV serotypes O and A at different percentage seropositivity (PS) levels (Figures 4.3–5). At every interface area studied, the FMDV serotypes O or A detected in either buffalo or cattle sera samples, was similarly detected in the counter side herds of their vicinity. This study serological inference indicated the co-occurrence and circulation of FMDV serotypes O and A in the field as it has been similarly stated in the Vosloo *et al.* (2002) study. An overall higher PS for FMDV serotypes O and A, in cattle than in buffalo at every livestock-wildlife interface area studied, except for Katavi interface (Table 4.1 - 2). The Katavi buffalo expressed a higher PS of 86% to FMDV serotype O than cattle in their vicinity, which showed a PS value of 76% (Table 4.1, Figure 4.4). The distinctive PS disparity expressed by Katavi interface buffalo could be attributed by probable extent of active virus activity at time of sampling, their reported large numbers (TAWIRI, 2019) enabling close interactions that facilitate virus spread, and FMDV infections amongst buffalo herds. The descriptive analysis for the serotype-specific seroreactions statuses revealed an overall high percentage count for type O across all species, followed by type A, and the mixed seroreactions being the least (Table 4.2). However; at 95% CI, the analyzed cattle and buffalo NSPE positive samples expressed overall higher scores of (41.7%; 20.65%), (33.6%; 11.34%) and (22.67%; 9.31%) on type O, type A and mixed seroreactions respectively (figure 4.3-5). Inferential statistics were done via Chi-square test of independence at the 0.05 level of significance to examine the relation between species difference (cattle and buffalo) and FMDV infection statuses of types

O, A, and O & A mixed (Table 4.2). The inferential statistical analysis had a null hypothesis stating as "there was no relation between spp difference and FMDV infection statues observed" in table 4.1 and 4.2 results. The analysis at 0.05 level of significance revealed a significant statistical relation, X^2 (4, N = 330) = 31.0867, p = .00001 across analyzed seroreactions scores on cattle and buffalos as the p-value (p=.00001) qualified the criterion (p<0.05) (Table 4.3). Despite of FMDV being infective to over 70 spp, FMDV infection rates are more likely to be influenced by the susceptible spp variations state, and that could reflect the probable variable roles of each spp in the epidemiology of the disease.

The results also presented FMDV serotypes O and A as predominant in the far southern and southern highlands (Katavi and Ruaha) and eastern and northern parts (Mikumi and Mkomazi) national parks of Tanzania (Table 4.1, Figure 4.3-4). A study in the Kenya ecosystems (Maasai-Mara, Tsavo, and Meru) showed that only FMDV types O, A, SAT1, and SAT2 were detected (RT-qPCR) from cattle tissue samples, where SAT1 and SAT2 were detected via virus isolation and Ag-ELISA in probang obtained from clinically normal buffalo between 2008 and 2012, but no type O was detected in buffalo (Wekesa et al., 2015). Studies in Uganda's Queen Elizabeth, Lake Mburo, Murchison Falls, and Kidepo Valley national parks demonstrated similar serological findings to those of this current study in Tanzania. The Uganda studies showed that most buffalo sera samples tested positive for antibodies against FMDV NSP; and upon serotype-specific SPBEs on the sera samples, FMDV serotypes O, SAT1, SAT2, and SAT3 were found circulating (Ayebazibwe *et al.,* 2010; Ruhweza, 2014). Though the Uganda studies did FMDV isolation and RT-qPCR, no buffalo sample enabled the isolation, detection, and typing of Eurasian FMDV RNA materials apart from the already known types SAT1–3. Of the sera samples that tested positive to NSPE, 66/247 (26.7%) serum samples did not test positive to FMDV serotypes O and A by SPCE (Table 4.1). This group of samples represented other FMDV serotypes (SAT1–3, Asia1 and C) apart from FMDV serotypes O and A. The magnitude of samples falling into this group varied, with Mikumi and Mkomazi buffalo (Table 4.1) describing the highest percentage of negative samples for FMDV

serotypes O and A (Table 4.1). This study detected FMDV genomic materials from clinically normal buffalo probang samples with 328bp band size upon agarose gel electrophoresis analysis (Figure 4.6). Using a panel of FMDV serotype-specific primers for types O and A, the sample was identified as FMDV type O (Figure 4.7). The small number of probang positives samples and low CT value expressed in the type O amplification curve of about 33.79 could be attributed by buffalo infection status, samples salivary enzymes and cofactors that interfere the biochemical reactions during genomic materials isolation process resulting into reduced products. No sample expressed evidence for the FMDV type-A genomic materials presence. Apart from the reports on SAT 1, SAT 2, SAT 3, O, and A, there has been no FMD outbreak in Tanzania caused by FMDV serotypes Asia1 or C (Bronsvoort, et al., 2006; Vosloo et al., 2002). Probably the observed findings of low infection rate statuses from Mikumi and Mkomazi livestock-wildlife interface areas could be due to FMDV SAT serotypes predominance, an aspect that had not been examined in this study.

4.10 CONCLUSION

This study was the first to document the molecular and serological survey of Eurasian FMDV serotypes O and A at livestock-wildlife interface areas in Tanzania. The results from this study were important for understanding FMDV epidemiology in Tanzania. The detection of FMDV serotype O in probang samples of buffalo origin provides evidence for the advancing complexity of FMD epidemiology in the wildlife species that have been previously cited as potential hosts for SAT types. According to the findings, FMD continue to press a negative impact on livestock sector growth, and to a considerable extent on wildlife conservation. Current control efforts should focus on reducing FMDV circulations by limiting interactions not only between cattle and buffalo herds but also between other cloven-hoofed livestock and wildlife species, which appear to be neglected in most cases. Further studies need to be directed towards Eurasian viruses surveillance in buffalo herds inhabiting Mwanza, Shinyanga, Mara, Kagera, Tabora, Kigoma, and Geita regions where type O predominates, and those of Morogoro, Tanga, and Coast region where type A is common. The surveillance samples need to be

analyzed in cell culture (virus isolation) to increase chances of Eurasian types O and A detection, then genetic and antigenic characterizations to enrich FMD knowledge necessary for tailored control options.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict or competing interests that may have influenced them in writing this article.

Ethical statement

This research study followed the guidelines and regulations of the Sokoine University of Agriculture on the research proposal approvals, and availability of permits for field works, see Appendix. 8.

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CHAPTER FIVE

5.0 Genetic-antigenic characterizations of VP1 polypeptides from Africa select FMDV type-SAT1 strains. Insilco - based study

Mkama Mathias[1,2,3], Philemon N. Wambura[1], Sharadhuli Kimera[1,2], Raphael S. Sallu[3], Francois Maree[4], Sengiyumva Kandusi[1], Mark M. Rweyemamu[1,2] and Christopher J. Kasanga[1,2]

[1]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.

[2]SACIDS Foundation for One Health, Africa Centre of Excellence for Infectious Diseases of Humans and Animals, P.O. Box 3297, Morogoro, Tanzania.

[3]Tanzania Veterinary Laboratory Agency, Centre for Infectious Diseases and Biotechnology, P.O. Box 9254, Dar es salaam, Tanzania.

[4]Clinomics, Uitzich Road, Bainsvlei, Bloemfontein, 9301, Free State, South Africa.

Correspondence: Mkama Mathias *(*mathias.mkama@sacids.org)

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Abstract

Multiple factors complicate the Foot and Mouth Disease (FMD) strategic control initiatives, but antigenic heterogeneity among field-circulating strains represents the most significant factor. The field's infinite variants poses a challenge to the effectiveness of vaccinations and suggests quick, reliable, and cost-effective methods for obtaining relevant information to enhance vaccine performance. In light of this, it is vital to understand not only the spatio-temporal features of FMDV, but also the B-cell epitope mapping insights, in order to improve the development of tailored vaccines and disease control measures. This study's computational prediction and mapping of linear and conformational B-cell epitopes is feasible as it circumvents the time and cost constraints associated with conventional experimental methods. The publicly available immuno-informatics was harnessed to analyze B-cell epitopes present on the FMDV type SAT1 VP1 polypeptides for this study. In order to maximize the authenticity of the prediction goals, this study combined multiple immuno-informatics tools ranging from those employing propensity scales to the most recent machine learning and artificial intelligence algorithms. Through this approach, a total of five and six consensus linear and conformational epitope sites were revealed as prediction outcome from all amino acid sequences analyzed respectively. This study commends for BepiPred-2.0 and Ellipro as combined tools that can be deployed in predicting and mapping B-cell epitopes for other FMDV serotypes and even for future researches covering other viral infectious pathogens.

Keywords: FMDV, Epitope mapping, Immuno-informatics tools, Serotype SAT1, VP1.

5.1 INTRODUCTION

The Foot and Mouth Disease Virus (FMDV) cause a highly contagious Foot-and-mouth disease (FMD) throughout all cloven-hoofed livestock and wildlife (Borley et al., 2013; Mason et al., 2003). The disease exerts catastrophic agricultural and socioeconomic impacts at the national, regional, and international levels(Belsham &

Bøtner, 2015). Seven antigenically unique FMDV serotypes (A, O, C, SAT1, SAT2, SAT3, and Asia1) have been documented worldwide (Sanyal et al., 2003; Vosloo et al., 2002), and they circulate in seven conjectured epidemiological pools (Pool 1-7) (Brito et al., 2017). Africa has documented six FMDV serotypes (O, A, C, SAT1, SAT2, and SAT3) causing epidemics in various geographical regions (Vosloo et al., 2002). Despite the existence of multiple serotypes and subtypes with indicated genetic divergences in the field (Carrillo, 2012), the FMDV serotypes share around 86% of their identity with one another (Yang et al., 2007).

FMDV contains a positive-sense, single-stranded RNA genome that is about 8.5kb in size (Figure 5.1) (Lloyd-Jones et al., 2017; Mahapatra et al., 2015). The 60 copies of each one of the four structural proteins (SPs) (VP1, VP2, VP3, and VP4) generated from P1 segment of an open reading frame enclose the FMDV genome (Mason et al., 2003). FMDV SPs may contribute to capsid assembly and stability, viral attachment, as well as antigenicity (Wright et al., 2011). It is the three-dimensional (3D) configurations of viral SPs VP1, VP2, VP3, and the inner VP4 provide surface-exposed antigenic sites that induce immune responses to vaccination or infection (Jackson et al., 2003). The cores of proteins VP1, VP2, and VP3 of a vast number of icosahedral viruses contain a highly conserved eight-stranded β-barrel (Acharya et al., 1989; Borley et al., 2013). All FMDV serotypes feature an extremely mobile antigenic G-H loop which protrudes from the surface of the virus and an arginine-glycine-aspartic acid (RGD) motif that is essential for binding to the host integrins (Borley et al., 2013). Among the capsid proteins, VP1 has 30–50% variability across serotypes (Knowles & Samuel, 2003), followed by VP3, VP2, and VP2 with least variation (Carrillo, 2012). The genetic variations of the virus influence its antigenic characteristics at a specific time and location (Belsham & Bøtner, 2015). When VP1 protective antigenic characteristics of viral SPs vaccine strains are properly exploited, they can provide adequate protection versus field viruses with identical antigenic properties. Vaccination seems to be the most effective method for mitigating FMD contrasted to culling (Belsham & Bøtner, 2015; Haydon et al.,

2004) under the current epidemiological and socioeconomic circumstances.

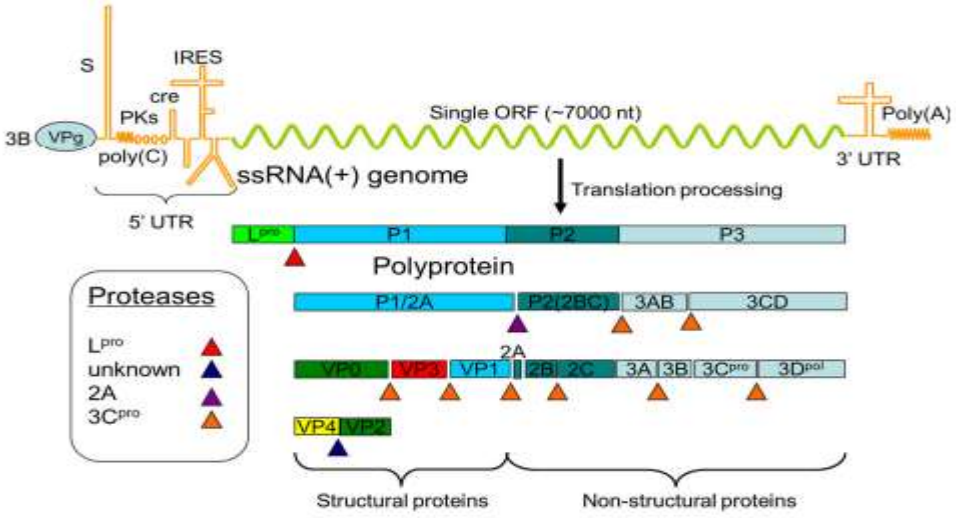

Figure 5.1: **The schematic diagram of the FMDV genome showing various structural and non-structural proteins regions** (Han et al., 2015) **the drawing was adapted from Mason et al., 2003**

The antigenic heterogeneity of circulating strains in the field poses a challenge to vaccines because it implicates both inter- and intra-typic cross-protectionability of vaccination or infection genesis (Lyons et al., 2017). Higher multiplication and mutation rates in the field lead in an infinite number of antigenic variants, primarily across VP1 polypeptides (Meloeni & Barteling, 1986). VP1 region genetic-antigenic information must be often be updated to accommodate field viral changes (Di Nardo et al., 2011; Rweyemamu & Hingley, 1984), as an essential component in achieving tailored vaccines development (Nadine et al., 2010). The traditional epitope mapping experimental platforms such as crystallography-based techniques, mass spectrometry-based techniques, nuclear magnetic resonance, the surface plasmon resonance and binding tests are time-consuming and expensive(Ahmad et al., 2016). In recent years, a growing number of immune-informatics techniques have been developed that predict relevant B cell epitope residues (Soria-Guerra et al., 2015;

Streatfield, 2005; Tomar & De, 2010) by using the existing molecular data available in global databases including those of VP1 nucleotides sequences for FMDV type-SAT1 used in this study.

B-cell epitopes are linear or conformational (Jespersen et al., 2019) sites on the surface of an antigen that specific antibodies can recognize and bind with, initiating subsequent immune response pathways (Van Regenmortel, 2009). Existing data on epitope mapping suggest that more than 90% of B-cell epitopes contain conformational epitopes (Kringelum et al., 2013; Van Regenmortel, 2009). Despite the existing tiny proportion of linear epitopes, the vast majority of existing prediction algorithms are designed for linear epitopes, whereas only a small fraction are designed for conformational epitopes (Galanis et al., 2021).

This work focused on the holistic prediction of B cell antigenic epitopes present in the FMDV type-SAT1 VP1 polypeptide using a spectrum of freely accessible immuno-informatics epitope prediction tools. The deployed analysis process taken into account the incorporation of multiple immuno-informatics models, from the earliest inventions employing physicochemical propensity scales such as hydrophilicity, flexibility, β-turns, antigenicity, polarity, as well as surface accessibility (Andersen et al., 2006) to the most recent machine learning and artificial intelligence algorithms. This strategy attempted to maximize the process of predicting linear and conformational epitopes present within type-SAT1 VP1 polypeptides that were obtained as consensus reads of all tools prediction findings. The outcomes of this research are anticipated to improve the prospects for designing tailored vaccines and encourage FMD studies envisioned to use of publicly available molecular data from rich global databases.

5.2 MATERIALS AND METHODS

5.1 Study areas and samples selection

This study intended to analyze a list of five diverse sequences. The five sequences for analysis were selected from 3 VP1 FMDV type-SAT1 topotype I (NWZ) nucleotide sequences 2020/2021 Tanzania (Mkama et al., 2022[Unpublished]), and 20 sequences of vaccine

strains and known SAT1 prototypes (Knowles et al., 2016) retrieved from the GenBank (NCBI) database archives collected between 1937 – 2015 years (Table 5.1). The phylogenetic analysis was done after aligning sequences (Table 5.1) using CLUSTAL W (Thompson et al., 1994). The evolutionary relationships existing amongst nucleotide sequences were inferred using the Maximum Likelihood method, and the best model for the phylogenetic tree construction was achieved by use of aligned multiple cDNA nucleotide sequences in MEGAX (Nei & Kumar, 2000). The General time reversal (GTR) model combined with gamma distribution and proportion of invariant sites (GTR + G + I) algorithms was applied to construct the phylogenetic tree in MEGAX (Kumar et al., 2018) then identify and select the five diverse study sequences.

Table 5.1: **The different FMDV serotype SAT1 nucleotide sequences obtained database. from this study and GenBank**

S/N	Sample ID	Topotype	Accession#	Country	Year	Description
1	SAT1/MSV/TZ/03/2020	I(NWZ)	-	Tanzania	2020	This study
2	SAT1/BAG/TZ/14/2021	I(NWZ)	-	Tanzania	2021	This study
3	SAT1/BAG/TZ/15/2021	I(NWZ)	-	Tanzania	2021	This study
4*	SAT1/KEN/80/2010	I(NWZ)	MZ868203	Kenya	2010	Vaccine strain
5*	SAT1/BOT/1/68	-	AY593845	Botswana	1968	Vaccine strain
6	**SAT1/T155/71**	I(NWZ)	KF561706	Tanzania	1971	Kasanga *et al.*, 2013
7	SAT1/ZIM/23/2003	I(NWZ)	KF219690	Zimbabwe	2003	Knowles *et al.*, unpub.
8*	**SAT1/RV/11/37**	II(SEZ)	AY593839	Rhodesia	1937	Carrillo *et al.*, 2005
9*	SAT1/RHO/5/66	II(SEZ)	AY593846	Rhodesia	1966	Carrillo *et al.*, 2005
10*	**SAT1/BEC/1/48**	III(WZ)	AY593838	Bechuanaland	1948	Carrillo *et al.*, 2005
11	**SAT1/UGA BUFF/21/70**	IV(EA-1)	KF219682	Uganda	1970	**Knowles *et al.*, unpub.**
12	**SAT1/NIG/11/75**	V	AF431711	Nigeria	1975	Sangare *et al.*, 2003
13*	SAT1/SUD/3/76	VI	DQ009725	Sudan	1976	Maree *et al.*, unpub.
14	**SAT1/UGA/13/74**	VII(EA-2)	AY442010	Uganda	1974	Sahle *et al.*, 2007
15	**SAT1/UGA/1/97**	VIII(EA-3)	AY442012	Uganda	1997	Sahle *et al.*, 2007
16	**SAT1/ETH/3/2007**	IX	FJ798154	Ethiopia	2007	Ayelet *et al.*, 2009
17	**SAT1/NIG/1/2015**	X	KX822796	Nigeria	2015	Ehizibolo *et al.*, 2017
18*	**SAT1/TCH/1/72**	XI	MH053323	Chad	1972	**Knowles *et al.*, unpub.**
19	**SAT1/ANG/9/74**	XII	MG972460	Angola	1974	**Knowles *et al.*, unpub.**
20	SAT1/MOZ/P13/2010_BUF	XIII	KF219691	Mozambique	2010	Kasanga *et al.*, unpub.

* Contains whole genome nucleotide sequences

5.3 Prediction of B cell antigenic on epitopes on FMDV type- SAT1 VP1 polypeptides

The list of softwares used for predicting linear and conformational epitopes was established. To maximize the chances of predictions on amino acids sequences residues, the predictions output results were merged to obtain a consensus prediction output result. The prediction softwares were selected by considering their free accessibility, format of input and output data, performance, linear or/both conformational epitope platform, approach of analysis/ mode of action and predictors with multiple/combined modes were highly prioritized. The following five B-cell epitope prediction tools were identified for this study (Table 5.2).

Table 5.2: **The different B-cell epitope predictors platforms deployed, and their respective properties.**
TEP: type of epitope predicted, SVM: support vector machine, AAP: amino acid pair.

Predictors	Mode Of Action/Description	Institution	TEP	Input	Link	Reference
BepiPred-2.0	Random forest algorithm trained on epitopes derived from crystal structures	Department of Bio and Health Informatics, Technical University of Denmark, Denmark	Linear	Amino acids Sequences in Fasta format	http://www.cbs.dtu.dk/ services/BepiPred/	(Jespersen et al., 2017)
BcePred	Physico-chemical propensity scales	Institute of Microbial Technology, Chandigarh, India	Linear	Amino acids Sequences in Fasta format	http://crdd.osdd.net/ raghava/bcepred/ index.html	(Saha & Raghava, 2004)
LBtope	SVM & Physicochemical propensity scales & AAP	Institute of Microbial Technology, Chandigarh, India	Linear	Amino acids Sequences in Fasta format	http://crdd.osdd.net/ raghava/lbtope/ protein.php	(Singh et al., 2013)
Ellipro	Structure based (approximation of the protein shape as an ellipsoid, calculation of the residue protrusion index (PI), and clustering of neighboring residues based on their PI values.)	Department of Bio and Health Informatics, Technical University of Denmark, Denmark	Linear & discontinuous	3D structures in pdb file format & Amino acids Sequences in Fasta format	http://tools.immuneepitope.org/tools/ElliPro.	(Ponomarenko et al., 2008)
Discotope-2.0	amino acid statistics, spatial information, and surface exposure Random forest, SVM, Physicochemical propensity scale, AAP, aa statistics, surface exposure	Department of Health Technology Ørsteds Plads, Building 345C DK-2800 Kgs. Lyngby Denmark	Conformational	3D structures in pdb file format	https://services.healthtech.dtu.dk/ service.php?DiscoTope-2.0	(Haste Andersen et al., 2006)

5.4 Amino acids sequences editing and manipulations

The FMDV type-SAT1 VP1 aa residues reads predicted as B-cell epitopes by multiple tools were properly organized and submitted to the BioEdit tool for alignment and editing/manipulation. It was from the aligned amino acids residues reads, consensus sites were determined.

5.5 Transforming amino acid sequences to 3D structural form

Some of the B-Cell epitope prediction tools input polypeptides in the form pdb file format (3D structures). The amino acids sequences in Fasta formats had to be converted to pdb file formats by using a free accessible online I-Tasser server (Zhang, 2008). The readable pdb file format allows the immune-informatics tools that input pdb file formats to synthesize different 3D-manipulative epitopic structures that can be observed under compatible visualization model (ie. JSmol).

5.6 Data management and analysis

The gathered data for the linear and conformational epitopes prediction from deployed softwares were stored, and organized in the Microsoft Excel 2010 to provide summary tables pertinent for this study.

5.7 RESULTS AND DISCUSSION

5.7.1 Phylogenetic analysis

The constructed phylogenetic tree visualized in MEGAX (Kumar et al., 2018) described close and distant genetic and evolutionary relationships amongst analysed sequences. The five VP1 sequences selected from constructed phylogenetic tree were; SAT1/MSV/TZ/03/2020 [I-NWZ] (current field strain), two vaccine strains (SAT1/BOT/1/68 [III-WZ] and SAT1/KEN/80/2010 [I-NWZ]) and two SAT1 prototypes (SAT1/ZIM/23/2003 [I-NWZ] and SAT1/MOZ/P13/2010_BUF_B16 [XIII]) (Figure 5.2). The selection approach aimed to explore the antigenic epitopes characteristics available in a spectrum of highly diversified SAT1 study strains circulating in different geographic locations (Figure 5.2).

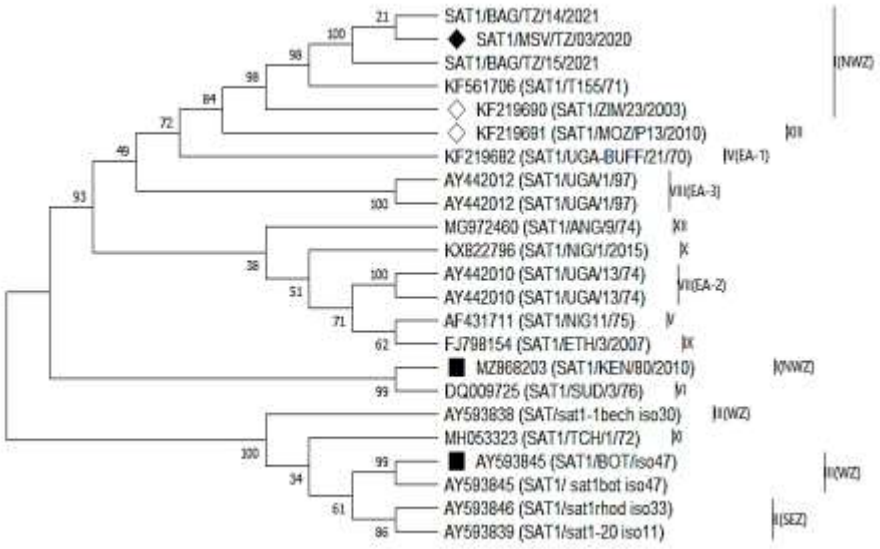

Figure 5.2: Phylogenetic tree constructed to aid selection of diversified FMDV type-SAT1 sequences for this study. Symbols meaning ♦ current study identified strain, ■ vaccine strains, and ◊ FMDV type SAT1 prototypes

5.8 FMDV type-SAT1 VP1 polypeptides conserved regions identification

The five analyzed select FMDV SAT1 aa sequences in a Fasta format file were aligned by Clustal W in the BioEdit and then submitted to the Protein Variability Server (PVS) for identifying conserved and highly conserved regions across the aligned aa sequences. The threshold of 1 was defaultly adopted under Shannon variability model for selecting the conserved fragments (Figure 5.4), whereas variable fragments with consecutive residues (aa≥6) were identified and selected (Table 5.3) as conserved region. About 8 fragments were recognized to be conserved with variable aa sequence sizes, whereas fragments 3 and fragment 8 being largest and the smallest sizes respectively. The large sized conserved fragments were positioned mostly on the first half from the 5' UTR end and sizes ideally become smaller in sizes as heading 3'UTR of VP1 polypeptides (Figure 5.3). FMD vaccines are challenged by the frequently changing FMDV antigenic profiles of circulating field

strains, and identifying regions of conserved antigenic epitopes landscapes for enhancing future vaccines prospects by increasing their field longer duration effectiveness.

Table 5.3: T**able showing positions of different conserved fragments properties across the analyzed SAT1 VP1 polypeptides.**

S/N	Fragment	Start	End	Sequence
1	1	1	23	TTSAGEGAEPVTTDASQHGGDRR
2	2	34	45	FLLDRFTLVGKT
3	3	48	98	NKLTLDLLQTKEKALVGAILRAATYYFSD-LEVACVGTNKWVGWTPNGAPEL
4	4	100	129	EVGDNPVVFSHNGTTRFALPYTAPHRCLAT
5	5	131	139	YNGDCKYKP
6	6	149	156	RGDLATLA
7	7	185	195	RMKRAELYCPR
8	8	215	220	KPAKQL

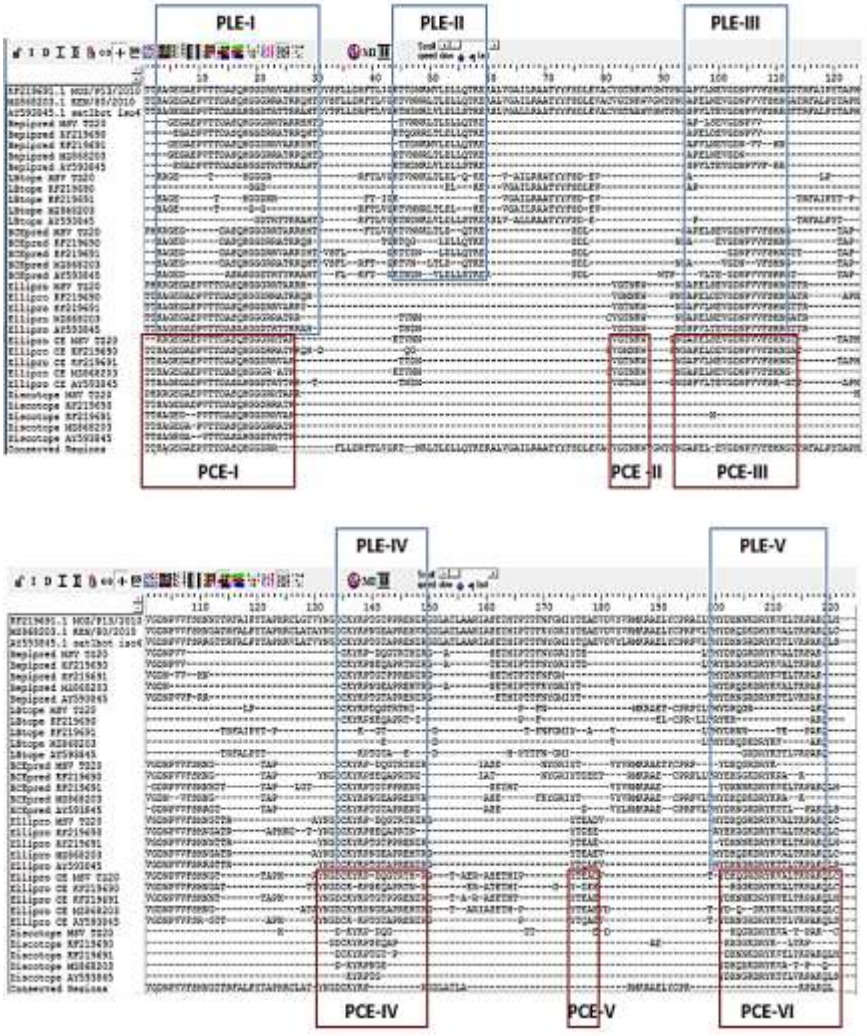

Figure 5.3: showing regions of predicted B cell epitopes from analyzed VP1 aa sequences and a single last sequence that shows the conserved regions across all VP1 polypeptides. PCE means predicted conformational epitope, and PLE means predicted linear epitope.

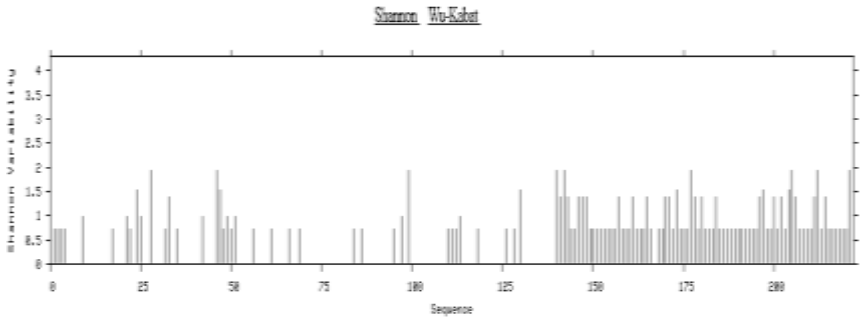

Figure 5.4: Shows the plotted variability score indices under Shannon Variability model of aa across FMDV SAT1 VP1 consensus sequence analyzed in the protein variability Server (PVS)

5.9 Prediction of B-cell antigenic epitopes on FMDV type-SAT1 VP1 polypeptides

This study deployed a combinatorial approach of available technological and methodological advances on B-cell antigenic epitopes prediction softwares to enhance the prediction likelihood authenticity for antigenic epitope residues/regions on FMDV SAT1 VP1 polypeptides. According to the literatures, most of the epitopes have residues length sizes between 4 to 20 residues, and only few exceed the 40 residues in size (Gupta et al., 2013). Following analysis of the select aa sequences, each of the deployed prediction tool generated results as detailed below.

5.9.1 BepiPred-2.0 B-cell antigenic epitopes prediction tool

The *BepiPred-2.0* immuno-informatics tool is trained to use a Random Forest algorithm to predict B-cell antigenic epitopes from polypeptide sequence (Jespersen et al., 2017). The tool inputs aa sequence in a Fasta format and predicts linear epitope residues available on polypeptides (Jespersen et al., 2017). Of the 5 analyzed polypeptides, Bepipred-2.0 identified an average of 108 (48.9%) aa as epitope residues across analyzed polypeptides (Table 5.4). The lowest and highest scores of 105 (47.5%) and 111(50.2%) were expressed by (KF219691 and MZ868203) and KF219690 respectively (Table 5.4). The BepiPred-2.0 predictor identified six consensus regions as antigenic epitopes, and the largest possessing 28 and smallest with 13 aa residues positioned at (5 - 31) and (95 – 107)

respectively. Two regions had 16 aa residues (position: 44 to 59 and 161 to 176), 17 aa (position: 134 to 150) and 21 aa residues (position: 198 to 218). Amongst analyzed polypeptides, three had aa residue A that was solitarily positioned at 153 except in the KF219691 and AY593845 polypeptides which did have that aa residue. The *BepiPred-2.0* generated fragments that had almost equal characteristic sizes across each sequence analyzed, except for MZ868203 and KF219691 sequences, that had 3 and 4 missing aa at third and fifth fragments respectively (Figure 5.3). The tool described good consistence in identifying residues predicted for being B-cell epitopes across VP1 polypeptides analyzed.

Table 5.4: **Summarized prediction results of analyzed VP1 selected sequences from FMDV type-SAT1 using BepiPred 2.0 B-cell linear epitope prediction algorithm**

S/n	Sample ID	Sequence Size (aa)	TAE	TES	Percentage (TAE)
1	MSV_TZ_2020	220	108	6	49.1
2	KF219690	221	111	6	50.2
3	KF219691	221	105	6	47.5
4	MZ868203	221	105	6	47.5
5	AY593845	221	109	6	49.3
	Average	221	108	6	48.7

TAE: Total amino acids identified as epitopes; TES: Total epitope sites/reads identified

5.9.2 LBtope B-cell antigenic epitopes prediction

The LBtope immuno-informatics tool is trained to operate on Support vector machine (SVM), physicochemical propensity scales and amino acids pair (AAP) algorithms to predict the B-cell antigenic epitopes aa residues from polypeptides. It inputs aa sequence in a Fasta format to predict linear epitope residues available on polypeptides (Singh et al., 2013). The tool operates under five different models and this study preferenced “LBtop_Confirm” model as it predicts different sized epitopes with default window length of 15(Kringelum et al., 2013), and the epitopes under this model were verified by default at least twice. Of the 5 analyzed aa sequences, LBtope identified

average (33.3%) aa as epitope residues across analyzed polypeptides. The KF219690 and AY593845 showed the lowest and highest scores of 56 (25.3%) and 92(41.6%) respectively (Table 5.5). The tool identified seven consensus regions predicted as B-cell epitopes across polypeptides analyzed (Figure 5.3). The predicted consensus epitopes positioned at 3 - 6 and 190 – 209 had the smallest and the largest aa residues of 4 and 30 respectively. Most epitopes had less than 20 residues except those at positions 38 – 59 and 190 – 209. Upon each sequence analyzed, at the first epitope (position: 3 – 6) sequences KF219690 and AY593845 did not express any residues as B cell epitopes.

Table 5.5: Summarized prediction results from analyzed VP1 sequences from select FMDV type-SAT1 using LBtope B-cell linear epitope prediction algorithm.

S/n	Sample ID	Sequence Size (aa)	TAE	TES	Percentage (TAE)
1	MSV_TZ_2020	220	88	5	40.0
2	KF219690	221	56	5	25.3
3	KF219691	221	70	5	31.7
4	MZ868203	221	62	4	28.1
5	AY593845	221	92	5	41.6

TAE: Total amino acids identified as epitopes; TES: Total epitope *sites/reads identified*

5.9.4 BCEpred B-cell antigenic epitopes prediction

The *BCEpred* immuno-informatics tool is trained to use a combined residue properties physic-chemical propensity scales to predict B-cell antigenic epitopes from aa sequences. The tool inputs aa sequence in one letter code (No header line as in a Fasta format), for predicting linear epitope residues available on polypeptides with a prediction accuracy of 58.7% (Saha & Raghava, 2004). The BCEpred recognized an average 117 (52.9%) aa as epitope residues, whereas KF219691 and AY593845 described the lowest (50.7%) and highest (55.2%) scores respectively (Table 5.6). The tool identified nine consensus regions as predicted antigenic epitopes across analyzed aa sequences. The smallest predicted regions possessed 6 aa residues at positions 2 – 7 and 43 – 48, whereas the largest being 22

aa residues at position 193 - 215. The rest of the predicted epitopes were of variable aa reads and sequentially located at positions14-30(17);52-59(8); 102-112(11); 134-148(15); 170-176(7) and182-190(9) respectively.

Table 5.6: **Summarized prediction results from analyzed VP1 sequences from select FMDV type-SAT1 using BCEpred B-cell linear epitope prediction algorithm.**

S/n	Sample ID	Sequence Size (aa)	TAE	TES	Percentage (TAE)
1	MSV_TZ_2020	220	119	7	54.1
2	KF219690	221	116	7	52.5
3	KF219691	221	112	7	50.7
4	MZ868203	221	118	7	53.4
5	AY593845	221	122	7	55.2

TAE: Total amino acids identified as epitopes; TES: Total epitope sites/reads identified

5.9.5 Ellipro B-cell antigenic epitopes prediction

The Ellipro immuno-informatics tool deploys a structure based algorithm that involves approximation of the protein shape as an ellipsoid, calculation of the residue protrusion index (PI), and clustering of neighboring residues based on their PI values in predicting the B-cell antigenic epitopes from aa sequences (Ponomarenko et al., 2008). The tool inputs 3D structures in pdb file format and aa sequences in Fasta format to for predicting both linear and conformational epitope residues available on aa sequences (Ponomarenko et al., 2008). The tool identified (48.4%) of analyzed aa sequences as linear epitopes. The KF219691 aa sequences expressed 102 (46.2%) as the lowest score while (MZ868203 and AY593845) had the highest scores of 110(49.8%) (Table 5.7). The predictor identified six regions as antigenic epitopes, with the largest predicted regions possessing 28 aa residues at position 1 - 28 whereas the smallest ones had 6 aa residues at positions 82 – 87 and 175 - 180. The other epitopes were sequentially positioned at 93-115(23); 131-150(30), and 200-221(22). The tool also predicted CE, and eight regions were identified as predicted CE from aa sequences analyzed. The number of aa identified as CE was higher compared to those of linear epitopes. The smallest predicted epitope size at

position 45-48 had 4 aa residues whereas the largest had 26 aa residues and positioned at 1-26. Based on the aa residues length sizes, 50% of the predicted antigenic epitopes had their aa sequences 4≤20, and 50% had their sequences with aa sequences 20≤26. Apart from the smallest and the largest predicted epitope, the rest of the predicted antigenic epitopes were sequentially positioned 82-87(6); 92-113(22); 130-150(21); 157-166(12); 175-180(6); 199-221(23).

Table 5.7: Summarized prediction results from analyzed VP1 sequences from select FMDV type-SAT1 using ElliPro B-cell linear and conformational epitopes prediction algorithm

S/n	Sample ID	Sequence Size (aa)	TAE	TES	Percentage (TAE)
1	MSV_TZ_2020	220	105	6	47.5
2	KF219690	221	109	6	49.3
3	KF219691	221	102	6	46.2
4	MZ868203	221	110	6	49.8
5	AY593845	221	110	6	49.8
6	MSV_TZ_2020_CE	220	119	8	54.1
7	KF219690_CE	221	113	8	51.1
8	KF219691_CE	221	116	8	52.5
9	MZ868203_CE	221	116	8	52.5
10	AY593845_CE	221	112	8	50.7

TAE: Total amino acids identified as epitopes; TES: Total epitope sites/reads identified

5.9.6 DiscoTope 2.0 B-cell antigenic epitopes prediction

The DiscoTope 2.0 is quoted/cited to be the first immuno-informatics tool to predict conformational B-cell antigenic epitopes from aa sequences/ polypeptides. The tool deploys amino acid statistics, their spatial information, as well as their surface accessibility scores in a compiled data set of conformational epitopes determined by X-ray crystallography of antibody/antigen complexes. The tool inputs pdb file format that contains 3D structures of the respective polypeptides under study (Andersen et al., 2006). The DiscoTope 2.0 identified 23.5% of analyzed polypeptides as epitope residues. MZ868203 expressed the lowest 48 (21.7%), whereas the strains from this study

MSV_2020 and KF219691 expressed the highest 55 (24.9%) scores (Table 5.8). The DiscoTope 2.0 predictor identified three regions with higher likelihood of encompassing antigenic epitopes across aa sequences analyzed. The predicted regions had variable sizes with the largest possessing 26 aa residues (position: 1 - 26) where the smallest with 9 aa residues (position: 134 – 142) and the third one with 18aa (position: 202 – 219).

Table 5.8: **Summarized prediction results from analyzed VP1 sequences from select FMDV type-SAT1 using DiscoTope 2.0 B-cell linear epitope prediction algorithm**

S/n	Sample ID	Sequence Size (aa)	TAE	TES	Percentage (TAE)
1	MSV_TZ_2020	220	55	3	25
2	KF219690	221	53	3	24
3	KF219691	221	55	3	25
4	MZ868203	221	48	3	22
5	AY593845	221	51	3	23

TAE: Total amino acids identified as epitopes; TES: Total epitope sites/reads identified

5.10 The consensus B cell linear epitopes predicted from FMDV type-SAT1 VP1 polypeptides

The four Linear B cell epitope immuno-informatics tools created 20 rows aligned in BioEdit as results of analysis (Figure 5.3). The manual percentage analysis across every column was done and columns with prediction aa residues >50% (n=10/20) were qualified as part of the consensus epitope region. The first region with consensus predicted epitope possesses 28aa (position:3-30) at columns of 55 – 100% aa residues. The 75% of this epitope falls in the first conserved area and columns (position: 24- 30) (25%) in non-conserved area of the polypeptide. The second region (position: 44-59) is composed of three short read epitope fragments of 4-5aa residues (positions: 44-48, 51-54, and 56-59) and they had (50-60%, 50-65%, and 50-70%) respectively. It is flanked by two conserved regions, where approximately 13% and 75% of the columns fall in the second and third conserved regions respectively. The two columns

with approximately 13% of this region were in the non-conserved columns. The third consensus epitope region was positioned between columns 95 and 112 and this region columns had 50-75% aa residues. It was estimated that, 22% and 72% of this region belong to columns in the third and fourth conserved regions respectively and leaving column 99 floating in a non-conserved site. The fourth consensus epitope region was positioned between columns 134 and 149 of the aa sequences. The region had columns with estimated aa residues of about 55 -100% and approximately 38% of the columns were located in the fifth conserved region and the last column 149 in the sixth conserved region. The remaining 56% of this region was in the non-conserved region. The fifth consensus epitope region was positioned at columns 199 and 219. The large part (76%) of this region is floating in a non-conserved region and the estimated 4% of the remaining columns in the eighth conserved region of the aa sequences.

5.11 The consensus B-cell conformational epitopes predicted

The B-cell conformational epitopes (CE) were predicted by deploying Ellipro and Discotope-2.0 tools. The columns with prediction aa residues >50% (n=5/10) were qualified being part of the consensus CE region. The tools identified six regions as consensus predicted epitopes at positions 1-26; 82-87; 93-114; 131-149; 175-179; and 201-221. Approximately 89% of the first region was under the first conserved region. The second consensus epitope region had its 100% size located in the third conserved region. The third consensus epitope region was positioned between third and fourth conserved regions by 35% and 60% respectively. The fourth consensus epitope had its 53% epitope on the fifth conserved region and 47% floating in a non-conserved area. The fifth consensus epitope had its 100% size in a non-conserved area. The sixth Consensus epitope region had 19% its columns in the eighth conserved area and estimated 71% of size in the non-conserved area. The predicted epitopes aa reads variations were due tothe technical disparities of deployed tools in identifying aa markers necessary for setting a starting point of the antigenic area prediction. Some of the consensus predicted antigenic epitopes regions (first, fourth and sixth) were commonly identified by almost all predictors across all aa sequences analyzed. These sites

are likely to be encompassing aa with higher likelihood properties for CE prediction. The prediction tools were also capable to identify antigenic epitopes from both conserved and hypervariable regions across study sequences, and the hypervariable domains possessed a higher likelihood of being part of antigenic epitope compared to conserved domains aa reads.

5.12 CONCLUSION

This study finding describes the predicted B-cell epitopes sites available within FMDV type SAT1 VP1 polypeptides. The study identified six predicted consensus epitope sites/ regions of conformational and linear category. Characteristic overlaps between conformational and linear predicted epitopes were revealed signifying existence of dual role of some aa on immune responses. Based on polypeptides aa variability characteristics, most of the predicted consensus epitopes were located at conserved regions and this study designate epitopes of conserved region(s) as priority landscapes for future vaccines for curbing frequently emerging field viral variants that escape vaccine antigenic scope. These results are important for the development of future new technology tailored vaccines like sub-unit vaccines/ recombinant protein vaccines, nucleic acid vaccines RNA vaccines ie. COVID -19 Pfizer BioNTech and Moderna, and most preferable the replicating viral vectored vaccines as they assure longer duration immunity contrary to others. Further studies are needed to improve tools to unveil additional undisclosed features to enhance discrimination of epitope and non–epitope motifs to improve the prediction results, extend study to whole capsid sequences of FMDV serotypes versus available vaccines in Africa, and deploy rational techniques to verify further the **predicted** epitopes and their immunologic role for tailored vaccine development and improvement of the available vaccines.

5.13 Acknowledgements

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5.14 Conflict of interest

The authors declare that they have no conflict or competing interests that may have influenced them in writing this article.

5.15 Ethical statement

This research study followed the guidelines and regulations of the Sokoine University of Agriculture on the research proposal approvals, and availability of permits for field works, see Appendix. 8.

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CHAPTER SIX

6.0 GENERAL DISCUSSION

The findings of this study revealed the presence of FMDV in Tanzania especially in the areas where samples were collected. There were multiple FMDV strains identified circulating in the field at different geographic locations in the country. The FMDV serotypes O, A and SAT1 were identified from samples collected from the field and were suggested to be responsible for the outbreaks observed in the field during this study period. This study analysed vesicular lesions samples of clinically sick cattle herds, and 48.67% (n=55) had FMDV nucleic materials. The preceding FMD studies on FMD in Tanzania identified type O, A, SAT1 and SAT2 from field outbreak samples (Kasanga *et al.,* 2012 ; Sallu *et al.,* 2014; Mfuru *et al.,* 2018), this study did not detect FMDV type-SAT2). The 2008 – 2013 FMD study in Tanzania indicated SAT2 was accounted for causing 2.85% of all reported outbreaks. During this study, FMDV type-SAT2 could have probably been circulating in persistently infected animals that were not part of this study sampling strategy. The FMDV serotype O detected in this study were from Ngara and Mbogwe districts outbreaks samples. Characteristically, the FMDV type O exhibits a historical cosmopolitan distribution, and preceding studies in Tanzania indicated type O to be predominantly circulating in the sampled areas including some other areas of the country (Kasanga *et al.,* 2012). The currently detected FMDV type O nucleotide sequences revealed close clustering together with reference sequences derived from prototype strains (O/TAN/2/2004 [KF561679.1], O/MAL/1/98 [DQ165074.1], O/UGA/3/2002 [DQ165077.1] and O/KEN/5/2002 [DQ165073]) Tanzania, Malawi, Uganda and Kenya origin strains, respectively with 100% bootstrap value, and were inferred to belong to the topotype EA-2 (Fig. 3.2). The high percentage identity expressed (92 – 100%) amongst current study and neighbouring countries strains suggest for the possibility of cross border virus incursions. These results are in agreement with Di Nardo *et al.,* (2011) who described border areas to be burdened with transboundary livestock diseases like FMD, that are exacerbated by cross border legal and illegal socio-economic activities. The FMDV of same genotype need to have <15% variation in the sequenced VP1

where those with <5% variation are considered to be closely related (Samuel *et al.,* 1999; Knowles & Samuel, 2001). The degree of relatedness of 85.2-100% expressed within topotype EA-2 cluster virus strains suggest that, if FMD vaccines were to be developed from strains under the topotype EA-2 cluster described, they are likely to confer suitable protection against viral incursions of EA-2 topotype category only if suitably tailored in geographical basis.

The FMDV type-A was detected in outbreak samples obtained from Kibaha district of Tanzania. The FMDV type-A has also been reported to exhibit a world-wide historical occurrence (Brito *et al.,* 2017). The current geographic occurrence of type-A are consistent with the past studies that described type A to be circulating in the cited areas (Kasanga *et al.,* 2012; Sallu *et al.,* 2014). The past studies have also designated the Tanzania Eastern zone to be FMD higher risk areas due to frequent reports of multiple types FMD outbreaks of O, A, SAT1, and SAT2 origin (Kasanga *et al.,* 2012). Similarly, it was from Eastern zone close distance Bagamoyo and Kibaha districts samples, where SAT1 and A types were identified during this study respectively. In the constructed phylogenetic tree, the Tanzania 2020 FMDV type A field strains were inferred as Africa topotype G-I Lineage. This study type A sequences described closer clustering with 2015 Kenyan (Omondi *et al.,* 2015) and 2019 Uganda (Ludi *et al.,* 2019) than any other nucleotide sequences from sub Saharan countries (Fig. 3.3). The 2015 Kenyan (A/KEN/K39/2015 [MH882570.1]) strain shared 90.8% whereas the 2019 Uganda (A/UGA/28/2019 [MT602080.1]) strain had 89.7% shared identity with A/KIB/TZ/05/2020 strain. These being the highest percentage identity levels expressed in the cluster list of the Africa topotype G-I Lineage that ranged from 82.3-90.8%. The phylogenetic analysis results indicates FMDV type A multiple topotypes and lineages (G-I to G-VII) to be present in the sub Saharan region with G-I to G-III lineages being limited to East and Central part of Africa (Knowles *et al.,* 2016). The phylogenetic tree also depicted the genetic evolutionary relationships existing between the EURO-SA, Asia and African topotypes as they are distinct and have been evolving and circulating in different geographic areas (Brito *et al.,* 2017). The FMDV type-A multiple topotypes state signifies its existing antigenic richness, which

complicates vaccine performance and call for in-depth studies to generate information enabling for suitable vaccine strain(s) selection amongst local strains that could be capable of controlling incursions of self-lineage and others-lineages effectively.

The FMDV SAT1 strains were also identified from Moshi Rural and Bagamoyo districts sourced samples. The current occurrence of SAT1 strains support the past studies that described FMDV type-SAT1 to have been reported in those areas (Kasanga *et al.,* 2012). The phylogenetic analysis results from this study indicated that SAT1 strains belonged to topotype I (NWZ), as had close relatedness with type SAT1 reference prototypes (SAT1/ZIM/23/2003 [KF219690.1], SAT1/T155/71 [HQ267519.1] and, SAT1/TAN/5/96 [AY442007.1]) 2003 Zimbabwe, 1971 and 1996 Tanzania FMD outbreak strains. This study SAT1 strains had 96.9-98.8% shared identity and revealed close clustering amongst SAT1 sequences analysed (Fig. 3.4). These findings are in agreement with Sallu et al. (2014) study that identified FMDV of topotype I (NWZ) to be circulating and causing outbreaks in different areas in Tanzania particularly coast regions. The shared identity of 77.4-99.0% was expressed for nucleotide sequences within same topotype I(NWZ) and least 67.2-70.3% across topotypes I(NWZ) and V. The strains with close geographic relationship had exceptionally higher shared percentage identity (99.0% for SAT1/MOZ/3/02 versus SAT1/ZIM/23/2003) and (93.6% for SAT1/K28/06 versus SAT1/TAN/11/2012). These findings suggest for enhancing geographical based FMD control strategies that execute tailored vaccines from identified topotypes or lineages rather than generalized vaccines that have failed to confer effective field performances for decades under the current Africa context.

The serological NSP ELISA results indicated FMDV is prevalent in all areas where samples were collected. None of the sampled cattle and buffalo had a history of FMDV vaccination against any of the FMDV serotype(s). Then it can be established that, the positive reactions observed in this study resulted from FMDV natural infections. Significant interactions between buffalo and cattle exist at each livestock-wildlife interfaces where this study was carried out. Despite of the imposed strict illegal entry restrictions, grazing cattle in the

national parks has turned into a chronic problem for conservation developments in Tanzania (Michael *et al.,* 2015) as well as disease control measures. At least all tested buffalo and cattle serum samples obtained from each of the interface areas demonstrated presence of both FMDV serotypes O and A at different PS levels (Fig. 4.3 – 4.5). Both FMDV serotypes O and A were detected in serum samples obtained from buffalo and corresponding cattle in their vicinity. These results collaborate the findings reported by Vosloo et al. (2002) on FMDV serotypes O and A on their field common occurrence and circulation. Results showed overall higher PS for FMDV serotypes O and A in cattle than in buffalo at every livestock-wildlife interface area, except Katavi interface (Table 4.1). Katavi buffalo expressed high PS of 86% to FMDV serotype O than cattle in the vicinity that showed PS value of 76% (Table 4.1). The higher PS disparity expressed by Katavi interface buffalo could be due to active virus activity in persistently infected buffalo at time of sampling, the large numbers and size of buffalo herds (TAWIRI, 2019) that facilitate close interactions, virus spread, and FMDV infections amongst buffalo herds. The results described FMDV serotypes O and A predominate in the far Southern and Southern highlands (Katavi and Ruaha) and Eastern and Northern parts (Mikumi and Mkomazi) of Tanzania respectively (Table 4.1). A study in the Kenya ecosystems (Maasai-Mara, Tsavo and Meru) showed that only FMDV type O, A, SAT1 and SAT2 were detected by RT-qPCR from cattle tissue samples, and SAT1 and SAT2 in probang (Virus isolation and Ag-ELISA) obtained from clinically normal buffalo between 2008 – 2012 and did not detect type O in buffalo (Wekesa *et al.,* 2015). Whereas studies in Uganda (Queen Elizabeth, Lake Mburo, Murchison Falls and Kidepo Valley) National Parks demonstrated similar serological findings to those of this current study in Tanzania. The Uganda studies showed that most buffalo samples tested positive for antibodies against FMDV NSP; when serotype-specific SPBEs were done on the samples, FMDV serotypes O, SAT1, SAT2 and SAT3 were found circulating (Ayebazibwe *et al.,* 2010; Ruhweza Simon, 2014). Though the Uganda studies did FMDV isolation and RT-qPCR but no buffalo sample enabled isolation, detection and typing of Eurasian FMDV RNA materials apart from already known types SAT1-3. Of the serum samples that tested positive to NSP ELISA, 66/247 (26.7%) serum

samples did not test positive to FMDV serotype O and A by SPCE (Table 4.1). This group of samples represented other FMDV serotypes (SAT1, SAT2, SAT3, Asia1 and C) apart from FMDV serotypes O and A. Results showed variations on the magnitude of samples falling under this group where by Mikumi and Mkomazi buffalo (Table 4.1) described the highest percentage for negative samples to FMDV serotypes O and A (Table 4.1). The probang samples obtained from clinically normal buffalo analyzed, 1/89 (1.12%) had FMDV genomic materials (Table 4.2). The panel of FMDV serotypes specific primers for type (O & A) characterized the PAN primer positive sample (Fig. 4.6) as FMDV type-O (Fig. 4.7). No sample expressed evidence for presence of FMDV type-A genomic materials. However; the recommended conventional approach on probang samples is through virus isolation then PCR but this could result into loss of some of the wild type characteristics of the virus under study, but removes PCR inhibitors. The low level of FMDV nucleic materials detection in probang samples could be influenced by health status of sampled buffaloes, and presence of PCR inhibitors in probang samples. The findings of this study revealed a narrow correlation between rate of FMDV nucleic materials detection in probang samples and high titer level of NSP ELISA results. Despite the probang sample positive to PAN primers RT-PCR had corresponding high titer of 90.3%, but other samples (n=25) with >90% titer did not show any evidence of FMDV nucleic materials presence by RT-PCR detection. The overall FMDV serotype O higher PS in buffalo could be suggestive to the revealed detection of FMDV type O in buffalo probang samples and not for FMDV type A. Since there is no any reported FMD outbreak in Tanzania that has been caused by FMDV serotypes Asia 1 or C (Leforban *et al.,* 2010; Rweyemamu *et al.,* 2008; Vosloo *et al.,* 2002) apart from O, A, SAT 1, SAT 2 (Kasanga *et al.,* 2012; Sallu, 2016) and the unconfirmed SAT 3 (Fè Vre *et al.,* 2006). Then, the low infection rates at Mikumi and Mkomazi livestock-wildlife interface areas could be due to small number and size of buffalo herds that are not facilitative to excessive virus spread, and being predominated by FMDV SAT serotypes that had not been examined in this study.

The antigenic epitope prediction results obtained from the four immuno-informatics tools for linear B cell epitope prediction revealed the first region of consensus predicted epitope with 28 aa reads with 75% of the epitope size from the 5' end under the first identified conserved region of the polypeptide (Fig. 5.3). The second consensus epitope region is located at 44 to 59 aa position of the polypeptide sequences and was composed of three short aa reads epitope fragments of 4-5aa residues flanked by two conserved regions, and approximately 88% specified as 13% and 75% of the columns fall in the second and third conserved regions respectively. The third consensus epitope region was positioned between columns 95 and 112 and was estimated that 94%, 22% and 72% of this region belong to the third and fourth conserved regions respectively. The fourth consensus epitope region was positioned between columns 134 and 149 of the aa sequences with approximately 44% conserved where (38%) in the fifth conserved region and (6%) in the sixth conserved region. The fifth consensus epitope region was positioned at columns 199 and 219 and (76%) of this region is floating in a non-conserved region and only 24% of the part in the eighth conserved region of the aa sequences. As the vaccines are challenged by the rapidly evolving FMDV in the field, then unveiling conserved epitope domains for development of tailored FMDV vaccines need to be prioritized as could enhance their long-term effectiveness in the field. Also, the conserved epitopes are priority landscapes for the new generation peptide-based vaccines for their enhanced prospects. The first, second and third predicted epitopes possess 75%, 88%, and 94% of their aa reads conserved respectively and primarily suggested to be epitope regions of priority for immunity-based studies on protective epitopes. The fourth and fifth had 44 % and 24% of their aa reads conserved and regarded as second and last priority respectively because of being prone to variations. For the B cell conformational epitopes predicted, the first consensus epitope region was located at 1 to 26 positions (Fig. 5.3). This epitope region had aa residues with 70% to 100% of likelihood of being epitope, and approximately 89% of its size belonged to the first conserved region. The second consensus epitope region was located at 82 to 87 and had 100% of its size conserved. The third consensus epitope region was located at 93 to 112 and conserved (95%) between third (35%)

and fourth (60%) conserved regions. The fourth consensus epitope region is located at 131 to 147 with 53% of its aa residues in the fifth conserved region. The fifth consensus epitope had its 100% size in a non-conserved area. The sixth consensus epitope region occupied 201 to 221 with 29% its size in the eighth conserved area and estimated 71% of size in the non-conserved area. Unlike the fifth and sixth predicted epitopes that were 100% and 29% conserved, the rest of the B cell conformational epitopes were highly conserved with higher aa prediction scores of 71 – 100% levels. These first, second, third, and fourth regions of predicted B cell conformational epitopes were designated to be priority epitope regions. They were considered priority for future vaccines as they can offer protection prospects of an extended period of time as compared to those with high level of hypervaribale domains. The B-cell epitope identified at a particular region expressed variable sizes in terms of aa reads corresponding to similar or variable starting and ending positions across the aa sequences analyzed. The characteristic flexile sizes observed on predicted epitopes aa reads was caused by the technical variations existing amongst deployed tools in identifying aa markers necessary for setting a starting point of the antigenic area. Whereas some of the antigenic epitopes were strongly identified by almost all predictors across all aa sequences analyzed than others, this defines their higher likelihood of being significant antigenic epitopes but this need to be inferred further by conventional epitope mapping approaches.

CHAPTER SEVEN

7.0 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusion

This study has shown FMDV current circulating strains in Tanzania. The findings of this study have described the presence of multiple outbreaks caused by multiple circulating FMDV types (A, O, and SAT1) of variable topotypes and lineages implicating animal health, food security and livelihoods of the communities. The observed broad and diverse geographic distribution of the virus strains evidenced from analyzed samples reveal the epidemiological complexity of FMD in the country, and calls for strategized mitigation measures including frequently updated field data. The diverse genetic and evolutionary relationship revealed amongst strains across countries with their sequences examined during this study, inference for the persistence consequences involved in mitigating FMD in Africa. Unlike the past studies that reported Eurasian FMDV serotypes O and A to be solely infectious in cattle, this study for the first time demonstrated FMDV type O infection with genomic inferences in buffalo samples from Serengeti ecosystem in Tanzania through combined molecular and sero-assays. The uncontrolled animal movements are cited to be the main contributing factor to the viruses spread across districts, country borders, and even across established national parks borders at livestock-wildlife interface areas. This phenomenon could be facilitative to the frequently broadening genetic and antigenic diversity in the field circulating virus strains though multiple factors can be accounted for this. The rapid and frequent changes in genetic-antigenic characteristics of wild type FMDV challenge vaccines performance in the field. This challenge needs to be addressed with the use of techniques that are quick, reliable, cost effective and accommodative to the African context in predicting antigenic epitopes as unveiled in this study SAT1 VP1 polypeptides. The predicted linear and conformational epitopes with conserved domains have vital role for the future tailored vaccines development to improve their field performance duration. However; the BepiPred-2.0 and Ellipro tools are the commended combined platforms with this study for the prediction and mapping of B-cell epitopes for other FMDV serotypes and even for future researches covering other viral infectious

pathogens. The results from this study were important for understanding FMDV molecular epidemiology in Tanzania; demonstrate the genetic and predicted antigenic relatedness of viral strains amongst regional and other countries in Africa. These findings need to be translated as the landscape for countries to accord on enhancing coordinated national, regional, and global FMD control initiatives as stipulated in progressive control pathway for FMD (PCP-FMD). Currently under the PCP-FMD program, African countries exhibit varying PCP levels related to their invested FMD control efforts, and Tanzania is estimated to be at PCP level 1. In most of the PCP levels, the updated knowledge on circulating field strains is a key requirement as it comprehends control strategies (suitable vaccine selection). Therefore, the information of this study significantly advances knowledge on FMDV currently circulating in cattle and buffalo herds and the underlying molecular and spatial epidemiology of the FMDV in Tanzania and Africa in general.

7.2 Recommendations

i. Continued monitoring of field circulating FMDV strains from reported outbreaks across different geographical areas in the region to identify variants.
ii. Continued surveillance of field circulating FMDV strains in clinically normal animals at identified hot spots across different geographical areas in the region.
iii. Surveillance studies need to be done on both buffaloes and cattle herds at livestock-wildlife interface areas in Tanzania and other wildlife rich countries to enable further isolation and molecular characterization of FMDV Eurasian types O and A to enhance tailored control options.
iv. Conduct strategic serological surveillance to understand where, when and what animal species essentially in the wildlife FMDV is likely to be maintained for their continued investigation or monitoring.
v. Studies need to be done to unveil the underlined descriptive mechanism exhibited by FMDV enabling its movement from buffalo to cattle and vice versa.
vi. Further serological testing of the FMDV NSP ELISA positive samples for detection of other FMDV serotypes (C, SAT1,

SAT2, SAT3 and Asian1) need to be done in achieving a comprehensive understanding of what is circulating in the fields.

vii. The prediction knowledge of the antigenic epitopes for the whole capsid sequences of FMDV serotypes versus available vaccine strains in Africa.

viii. Studies to unveil additional undisclosed features of aa residues necessary for enhanced epitope and non –epitope motifs identification/predict, to discriminate linear and conformational clearly for improved prediction results.

ix. The community level knowledge, awareness, and attitudes on FMD consequences and control need to be quantified to enhance future participatory control approaches.

x. Studies on antigenic matching between field circulating strains and vaccine strains to unveil critical areas to be addressed during prospective tailored vaccines development.

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APPENDICES

Appendix 1: past studies indicating amount of FMDV present in animal tissues and excreta during viraemia in bovine animals.

S/n	Tissue:	Amount of virus	References:
1	Blood	$10^{5.0}$ PFU/ml	(Burrows, 1968; Cottral, 1969)
2	Urine	$10^{4.9}$ LD_{50}/ml	(Cottral *et al.*, 1968; Donaldson, 1987)
3	Semen	$10^{6.2}$ $TCID_{50}$/ml	(Donaldson, 1987)
4	Milk	$10^{6.6}$ $TCID_{50}$/ml	(Donaldson, 1987)
5	Feces	$10^{5.0}$ $TCID_{50}$/ml	(Donaldson, 1987)
6	Bone marrow	$10^{5.9}$ PFU/g	(Cottral, 1969)
7	Lymph nodes	$10^{8.2}$ PFU/g	(Burrows *et al.*, 1981)
8	Heart muscle	10^{10} PFU/g	(Burrows *et al.*, 1981)
9	Adrenal gland	$10^{10.6}$ PFU/g	(Burrows *et al.*, 1981)
10	Thyroid gland	$10^{6.0}$ PFU/g	(Cottral, 1969)
11	Pancreas	$10^{6.4}$ PFU/g	(Cottral, 1969)
12	Liver	$10^{3.6}$ PFU/g	(Cottral, 1969)
13	Rumen	$10^{6.4}$ PFU/g	(Cottral, 1969)
14	Spleen	$10^{3.1}$ PFU/g	(Cottral, 1969)
15	Kidney	$10^{4.0}$ PFU/g	(Cottral,1969)
16	Skin	$10^{6.0}$ PFU/g	(Gailiunas and Cottral, 1966, 1967)
17	Pituitary	$10^{6.8}$ PFU/g	(Scott *et al.*, 1965)
18	Pineal body	$10^{4.3}$ PFU/g	(Scott *et al.*, 1965)
19	Spinal cord	$10^{3.2}$ PFU/g	(Scott *et al.*, 1965)
20	Cerebrum	$10^{2.5}$ PFU/g	(Scott *et al.*, 1965)
21	Cerebellum	Virus not detected	(Scott *et al.*, 1965)
22	Medulla	Virus not detected	(Scott *et al.*, 1965)
23	Hippocampus	Virus not detected	(Scott *et al.*, 1965)
24	Cerebrospinal fluid	$10^{3.4}$ PFU/ml	(Scott *et al.*, 1965)
25	Saliva	$10^{8.5}$mouse ID_{50}/ml	(Hyslop, 1965)

Source: Animal Health And Welfare, 2006

Appendix 2: Procedures for Blood and epithelial tissue samples Collection in Cattle

1.1: Introduction

Cattle blood samples were collected in accordance with this protocol. The latter had been intended to serve as a guide for ensuring that all activities were conducted ethically in terms of animal welfare and personnel safety. The purpose of this document was to outline the procedures for blood (via jugular vein) and tissue samples collection in cattle. In advance of participating in fieldwork, all engaged personnel must be taught in the right approach to cattle restraint techniques. Training must be conducted whenever new members join the sampling team.

Animal handlers should be cautious at all times to prevent injuring animals or themselves as a result of head-butting and curve of swing, and being injured by an animal on solid surfaces like wall, fence, chute etc. handlers should avoid being kicked or stepped on:

i. Front feet pecking;
ii. Rear foot forward and sideways ("cow kick")

1.2: Safety and Sanitation

a. Ensure the use of proper Protective Equipment (PPE) to protect the handler from unintentional injury or exposure to blood and other bodily fluids, wear:
    i. coveralls
    ii. steel-toed shoes or boots
b. Hands should be cleansed and/or gloves should be replaced between animals; ideally, vacutainer tube holder should be sanitized between barns or other animal holding locations.
c. Carefully dispose off discarded sharps in the leak-proof, puncture-resistant sharps container provided.

1.3: Materials and Equipments

a. Blood collection tubes (such as syringes and vacutainer tubes)
b. Needles (18 gauge x 1.5 inches) or vacutainer needles with holder
c. Restraint device (e.g., halter)
d. Antiseptic
e. Gauze
f. Cool box with ice packs
g. Liquid nitrogen tank or Dry shipper
h. Pair of scissors and tissue forceps

1.4: Methodology

A. BLOOD SAMPLES

a. Restrain the animal with a stanchion or v-crash, halter with a quick-release knot, with head held up, for the jugular vein to be revealed.
b. Remove superficial filth and debris from the regions to be pierced using antiseptic gauze, then disinfect with methylated spirit.
c. Locate the jugular vein by applying pressure to the base of the jugular groove, and observe the enlarged vein.
d. With the bevel facing up, firmly enter the needle into the skin and vein at a 20° angle.
e. If using a vacutainer, steady the needle after insertion and press the vacutainer tube into the holder. If the vein has been struck, blood will flow easily into the tube.
   Filling several tubes is possible by removing the filled tube and replacing it with a new one.

NOTE: Do not remove the needle from the vein while the vacutainer tube is still connected, as this will release the vacuum in the vacutainer. If you have missed the vein, reinsert the needle with the vacutainer connected until the vessel is pierced. Generally, no more than two or three attempts

should be done at a time to reduce animal suffering and potential vein damage.

f. You may also use a needle and syringe. Before usage, break the syringe's seal by gently drawing back. With the needle connected to the syringe and the air removed, enter the needle at a 20° angle and aspirate the syringe to confirm insertion and collect blood.

g. Once collection is complete, remove the vacutainer tube and withdraw the needle by applying pressure to the injection site and place the tube with blood in rack packed in cool box.

h. Place needle in an approved Sharps container.

i. To establish sufficient hemostasis, apply gauze pressure for 30 to 60 seconds.

j. Serial samples can be collected by alternating sides and changing insertion sites cranially, as long as no hematoma development occurs.

B. TISSUE SAMPLES

a. Examine the animal for late lesions

b. Clean the lesion with portable water

c. With forceps and scissors take a sufficient amount of tissue sample and put into tube with viral transport media

d. put the tube containing the sample into Dry shipper and ship to the lab for analysis.

1.5: Potential Adverse Effects, Countermeasures, and Treatment

a. Hematoma or blood clot

i. Insert needle at an angle of no more than 30 degrees

ii. Employ a needle with a lower gauge than the vein

iii. Apply pressure on the wound until it stops bleeding (1+ minutes).

b. Pain at the blood collection site

i. Use a smaller gauge needle than the vein.

ii. Before working on a living animal, practice on vein models.

c. Infection at the site of blood collection.

i. Use only single-use, sterilized equipment.
ii. Disinfect all surfaces of the workplace.
iii. Wear gloves and wash your hands.

Note 1: when there is excessive trauma and bleeding after tissue sample has been taken, spray with Oxytetracycline spray and cover the animal with broad spectrum antibiotic to ward off secondary bacterial infections.

Note 2: Consult a licensed veterinarian for therapeutic guidance if you see any of the following. Initial observation of heat, pain, and swelling at the site of blood draw insertion, with purulent material streaming from the site of insertion, Pyrexia, infections local or systemic and septic shock.

Appendix 3: Blood and Probang Samples Collection Procedures in African Buffalo

Introduction:

Before taking samples from buffalo, the animals must be immobilized. M^{99} (Etorphine + Azaperone) was employed for immobilization and M^{2525} (Diprenorphine) was used for revival at dosages of (8-12mg and 80-100mg) for immobilization and 16-24mg as an antidote, respectively. The darting can be accomplished through helicopter (aerial approach) or automobiles. In this investigation, blood and probang samples were collected from field buffalo using vehicles. Before entering the field, the buffalo sampling team had to prepare all necessary materials for immobilization and sample collection. Regarding the well-being of the animals and the safety of the personnel, all activities were conducted ethically. The purpose of this document was to describe the procedures for collecting blood and probang samples from buffaloes via the jugular vein and as per os respectively.

Important Note: whenever handling buffalo in the wild, utmost caution must be exercised for the safety of the team and the immobilized animal. Care must be taken to avoid not only surrounding buffalo herds, but also other dangerous animals such as lions, elephants, and rhinoceroses. The handlers must maintain constant vigilance to avoid injuring animals or themselves by a voiding being kicked or stepped on.

Safety and Hygiene

a. Ensure proper Personal Protective Equipment (PPE) is deployed to protect handler from accidental injury or exposure to blood and other body fluids, such as:
    i. coveralls
    ii. Steel-toed shoes or boots
    iii. wear disposable gloves (e.g., latex, nitrile) for sample taking
b. Hands must always be washed and/or gloves must all be changed between animals; if vacutainer needle holders are used, they must be disinfected between animals and when relocating to other buffalo herds.

c. Dispose of used sharps immediately in the leak-proof, puncture-resistant sharps container

Equipments and Materials

a. Blood collection containers (such as syringes and vacutainer tubes)
b. Needles (18 gauge x 1.5 inches) or vacutainer needles and holders
c. Restraint (e.g., halter, squeeze chute)
d. Spray bottle
e. Probing cup (for large animals)
f. Gauze
g. M99 (Etorphine hydrochloride + Azaperone hydrochloride)
h. M2525 (Diprenorphine hydrochloride)

Procedures

a. Identify the buffalo herd to be sampled
b. Scan the surrounding area to determine the terrain, safety of the sampling team, and immobilized buffalo versus predators
c. Identify the best buffalo(s) in the herd for the intended samples and
d. immobilize them with darts of immobilon
e. Chase away other buffalos to create a good working atmosphere
f. Cover the buffalo eyes with a piece of cloth,
g. Identify the jugular groove and occlude the jugular vein, then see the elevated vein and pierce it at a 20° angle in order to extract blood into plain vacutainer tubes.

NOTE: If using a vacutainer, steady the needle after insertion and place the vacutainer tube into the vacutainer needle holder. If the vein has been struck, blood will flow easily into the tube. Filling several tubes is possible by removing the filled tube and replacing it with a new one. Do not remove the needle from the vein with the vacutainer tube still connected, since this will cause the vacutainer to lose its suction.

h. Once collection is complete, remove the vacutainer tube and withdraw the needle by applying pressure to the injection site.
i. Place needle in an authorized Sharps container.

j. Restrict the head well and open the mouth to allow the probang cup to touch the oesophageo-pharyngeal regions.
k. Move the cup in an inside-out way for >6X to scrape the epithelial membrane cells and fluid.

Potential Adverse Effects, Countermeasures, and Treatment

a. Hematoma or blood clot
   i. Insert needle into vessel at an angle of 30 degrees or less.
   ii. Apply pressure until bleeding ceases (1+ minutes).

b. Pain at the site of blood collection
   i. Use a needle with the correct gauge for the vein size.
   ii. Practice on vein models before working on a live animal.

c. Infection at the site of blood collection
   i. Use only sterile single-use devices
   ii. Disinfect work surfaces
   iii. Wear gloves and wash hands

Photo describing blood sampling in an immobilized buffalo (Source; This study)

Appendix 4: NSP-ELISA (ID Screen ®FMD NSP cELISA), PAN PCR, and Molecular Typing (RT-qPCR) results for types O and A. from Serengeti national park buffalo sera and probang sample (2018)

S/n	sample ID	Lab. No	NSPE, =<50"POS"	reversed, >=50"POS"	probang	Type O	Type A
1	SGT/2018/1	96	21.6	78.4	neg		
2	SGT/2018/2	97	17.2	82.8	neg		
3	SGT/2018/3	98	74.6	25.4	neg		
4	SGT/2018/4	99	16.1	83.9	neg		
5	SGT/2018/5	100	8.1	91.9	neg		
6	SGT/2018/6	101	78.9	21.1	neg		
7	SGT/2018/7	102	66.2	33.8	neg		
8	SGT/2018/8	103	68.4	31.6	neg		
9	SGT/2018/9	104	78.3	21.7	neg		
10	SGT/2018/10	105	18.5	81.5	neg		
11	SGT/2018/11	106	12.0	88.0	neg		
12	SGT/2018/12	107	13.9	86.1	neg		
13	SGT/2018/13	108	6.8	93.2	neg		
14	SGT/2018/14	109	13.1	86.9	neg		
15	SGT/2018/15	110	20.0	80.0	neg		
16	SGT/2018/16	111	62.6	37.4	neg		
17	SGT/2018/17	112	79.9	20.1	neg		
18	SGT/2018/18	113	10.0	90.0	neg		
19	SGT/2018/19	114	74.4	25.6	neg		
20	SGT/2018/20	115	10.1	89.9	neg		
21	SGT/2018/21	116	8.9	91.1	neg		
22	SGT/2018/22	117	45.5	54.5	neg		
23	SGT/2018/23	118	83.8	16.2	neg		
24	SGT/2018/24	119	9.6	90.4	neg		
25	SGT/2018/25	120	78.2	21.8	neg		
26	SGT/2018/26	121	46.7	53.3	neg		
27	SGT/2018/27	122	8.0	92.0	neg		
28	SGT/2018/28	123	53.6	46.4	neg		
29	SGT/2018/29	124	10.1	89.9	neg		
30	SGT/2018/30	125	37.2	62.8	neg		
31	SGT/2018/31	126	8.4	91.6	neg		
32	SGT/2018/32	127	8.7	91.3	neg		
33	SGT/2018/33	128	10.9	89.1	neg		
34	SGT/2018/34	129	18.8	81.2	neg		
35	SGT/2018/35	130	67.6	32.4	neg		
36	SGT/2018/36	131	9.9	90.1	neg		
37	SGT/2018/37	132	10.7	89.3	neg		
38	SGT/2018/38	133	81.5	18.5	neg		
39	SGT/2018/39	134	8.0	92.0	neg		
40	SGT/2018/40	135	18.8	81.2	neg		
41	SGT/2018/41	136	22.1	77.9	neg		
42	SGT/2018/42	137	8.7	91.3	neg		
43	SGT/2018/43	138	14.1	85.9	neg		
44	SGT/2018/44	139	7.7	92.3	neg		
45	SGT/2018/45	140	12.8	87.2	neg		
46	SGT/2018/46	141	19.2	80.8	neg		

47	SGT/2018/47	142	12.5	87.5	neg		
48	SGT/2018/48	143	31.5	68.5	neg		
49	SGT/2018/49	144	13.9	86.1	neg		
50	SGT/2018/50	145	72.3	27.7	neg		
51	SGT/2018/51	146	7.3	92.7	neg		
52	SGT/2018/52	147	45.6	54.4	neg		
53	SGT/2018/53	148	23.6	76.4	neg		
54	SGT/2018/54	149	68.3	31.7	neg		
55	SGT/2018/55	150	81.4	18.6	neg		
56	SGT/2018/56	151	85.5	14.5	neg		
57	SGT/2018/57	152	77.5	22.5	neg		
58	SGT/2018/58	153	70.8	29.2	neg		
59	SGT/2018/59	154	45.4	54.6	neg		
60	SGT/2018/60	155	7.1	92.9	neg		
61	SGT/2018/61	156	81.4	18.6	neg		
62	SGT/2018/62	157	81.4	18.6	neg		
63	SGT/2018/63	158	61.8	38.2	neg		
64	SGT/2018/64	159	83.4	16.6	neg		
65	SGT/2018/65	160	13.7	86.3	neg		
66	SGT/2018/66	161	7.5	92.5	neg		
67	SGT/2018/67	162	7.7	92.3	neg		
68	SGT/2018/68	163	7.2	92.8	neg		
69	SGT/2018/69	164	83.4	16.6	neg		
70	SGT/2018/70	165	56.7	43.3	neg		
71	SGT/2018/71	166	8.0	92.0	neg		
72	SGT/2018/72	167	7.7	92.3	**pos**	**pos**	**neg**
73	SGT/2018/73	168	22.3	77.7	neg		
74	SGT/2018/74	169	10.5	89.5	neg		
75	SGT/2018/75	170	6.9	93.1	neg		
76	SGT/2018/76	171	9.7	90.3	neg		
77	SGT/2018/77	172	53.5	46.5	neg		
78	SGT/2018/78	173	13.5	86.5	neg		
79	SGT/2018/79	174	19.7	80.3	neg		
80	SGT/2018/80	175	36.4	63.6	neg		
81	SGT/2018/81	176	25.6	74.4	neg		
82	SGT/2018/82	177	10.9	89.1	neg		
83	SGT/2018/83	178	9.1	90.9	neg		
84	SGT/2018/84	179	7.6	92.4	neg		
85	SGT/2018/85	180	13.3	86.7	neg		
86	SGT/2018/86	181	11.9	88.1	neg		
87	SGT/2018/87	182	10.7	89.3	neg		
88	SGT/2018/88	183	13.9	86.1	neg		
89	SGT/2018/89	184	10.3	89.7	neg		
90	SGT/2018/90	185	11.5	88.5	neg		
91	SGT/2018/91	186	11.6	88.4	neg		
92	SGT/2018/92	187	15.9	84.1	neg		
93	SGT/2018/93	188	71.4	28.6	neg		
94	SGT/2018/94	189	6.7	93.3	neg		
95	SGT/2018/95	190	12.7	87.3	neg		
96	SGT/2018/96	191	82.4	17.6	neg		

Appendix 5: Reverse-transcription polymerase chain reaction (RT-PCR) primers specific to each of the FMDV serotypes O, A, SAT1, SAT2, and SAT3

Primer name	Sequence (5′ – 3′)	Genome direction	Gene	Product size
Serotype O				
O–1C272F	TBGCRGGNCTYGCCCAGTACTAC	FP	VP3	1,135
O–1C244F	GCAGCAAAACACATGTCAAACACCTT	FP	VP3	1,165
O–1C283F	GCCCAGTACTACACACAGTACAG	FP	VP3	1,124
Serotype A				
A–1C562F	TACCAAATTACACACGGGAA	FP	VP3	866
A–1C612F	TAGCGCCGGCAAAGACTTTGA	FP	VP3	814
Serotypes O/A/C/Asia 1				
EUR–2B52R	GACATGTCCTCCTGCATCTGGTTGAT	RP	2B	
Serotype SAT 1				
SAT1–1C559F	GTGTATCAGATCACAGACACACA	FP	VP3	1,043
SAT1U–OS	GTGTACCAGATCACTGACAC	FP	VP3	1,043
SAT1–P1–1228F	AACCTGCACTTCATGTACAC	FP	VP3	1,286
Serotype SAT 2				
SAT2–1C445F	TGGGACACMGGIYTGAACTC	FP	VP3	1,145
SAT2–P1–1223F	TGAACTACCACTTCATGTACACAG	FP	VP3	1,279
Serotype SAT 3				
SAT3–1C559F	CTGTACCAAATYACAGACAC	FP	VP3	1,034
SAT3–P1–1222F	AATCTGCATTTCATGTACAC	FP	VP3	1,277
Serotypes SAT 1–3				
SAT–2B208R	ACAGCGGCCATGCACGACAG	RP	2B	

Source: Knowles et al., 2016 FP forward primer; RP reverse primer

Appendix 6: The cycle-sequencing primers specific to the three detected FMDV types O, A, and SAT1

Primer name	Sequence (5′ – 3′)	Genome direction	Gene
All serotypes			
NK72	GAAGGGCCCAGGGTTGGACTC	RP	2A/2B
Serotype O			
O–CRH2F	GAYTACGCSTACACSGCGTC	FP	VP3
O–1C605F	TGGCTAGTGCTGGTAAAGACTTTGAG	FP	VP3
O–1D293F	TGGAYAACACCACYAAYCCAAC	FP	VP1
O–1D296F	ACAACACCACCAACCCAAC	FP	VP1
O–1D628R	GTTGGGTTGGTGGTGTTGT	RP	VP1
Serotype A			
A–1C612F	TAGCGCCGGCAAAGACTTTGA	FP	VP3
A–1D202F	TCAGCCACCTACTATTTCTCTGA	FP	VP1
A–1D478R	CAGTGCTCCGTAGTTAAAGGATGA	RP	VP1
Serotype SAT 1			
SAT1–1C559F	GTGTATCAGATCACAGACACACA	FP	VP3
SAT1–1D200F	TGCGYGCIGCCACGTACTAYTTCTC	FP	VP1
SAT1–1D394R	GGYTTGTACTTRCARTCACCGTTGTA	RP	VP1

Source: Knowles et al., 2016

Appendix 7: A list of reference prototypes sequences, whereby the prototype under each serotype-topotype of FMDV are in bolded fonts.

Topotype	Lineage	Sub-lineage	Serotype/isolate name	Accession no.	Reference
CATHAY	**–**	**–**	**O/HKN/21/70**	**AJ294911**	**(Knowles et al., 2001)**
CATHAY	–	–	O/HKN/6/83	AJ294919	(Knowles et al., 2001)
CATHAY	–	–	O/PHI/7/96	AJ294926	(Knowles et al., 2001)
CATHAY	–	–	O/Yunlin/Taiwan/97	AF308157	(Beard & Mason, 2000)
EA–1	**–**	**–**	**O/K83/79* (Kenya)**	**AJ303511**	**(Samuel & Knowles, 2001)**
EA–1	–	–	O/K40/84* (Kenya)	KY091280	(Knowles et al., 2016)
EA–1	–	–	O/UGA/5/96	AJ296327	(Samuel & Knowles, 2001)
EA–2	**–**	**–**	**O/MAL/1/98**	**DQ165074**	**(Knowles et al., 2005)**
EA–2	–	–	O/UGA/3/2002	DQ165077	(Knowles et al., 2016)
EA–2	–	–	O/KEN/5/2002	DQ165073	(Knowles et al., 2016)
EA–2	–	–	O/TAN/2/2004	KF561679	(Kasanga et al., 2015)
EA–3	**–**	**–**	**O/SUD/2/86**	**DQ165075**	**(Knowles et al., 2005)**
EA–3	–	–	O/ETH/3/2004	FJ798109	(Ayelet et al., 2009)
EA–3	–	–	O/ETH/2/2006	FJ798127	(Ayelet et al., 2009)
EA–3	–	–	O/ETH/1/2007	FJ798137	(Ayelet et al., 2009)
EA–4	**–**	**–**	**O/UGA/17/98**	**HM211075**	**(Ayelet et al., 2009)**
EA–4	–	–	O/ETH/58/2005	FJ798141	(Ayelet et al., 2009)
EURO–SA	**–**	**–**	**O1/BFS 1860/UK/67**	**AY593815**	**(Carrillo et al., 1990)**
EURO–SA	–	–	O2/Brescia/ITL/47	M55287	(Krebs., 1991)
EURO–SA	–	–	O3/Venezuela/51	AJ004645	(Leister et al., 1993)
EURO–SA	–	–	O/Corrientes/ARG/06	DQ834727	(Malirat et al., 2007)
ISA–1	**–**	**–**	**O11/ISA/1/62**	**AJ303500**	**(Samuel & Knowles, 2001)**
ISA–1	–	–	O/ISA/9/74	AJ303502	(Samuel & Knowles, 2001)
ISA–1	–	–	O/ISA/8/83	AJ303503	(Samuel & Knowles, 2001)
ISA–2	**–**	**–**	**O/JAV/5/72**	**AJ303509**	**(Samuel & Knowles, 2001)**
ISA–2	–	–	O/ISA/1/74	AJ303501	(Samuel & Knowles, 2001)
ME–SA	**–**	**–**	**O1/Manisa/TUR/69**	**AY593823**	**(Carrillo et al., 1990)**
ME–SA	–	–	O/R2/75* (India)	KP822947	(Biswal et al., 2015)
ME–SA	–	–	O/IND/53/79	AF292107	Hemadri et al., unpublished
ME–SA	Ind–2001	a	O/KUW/3/97	DQ164904	(Knowles et al., 2005)
ME–SA	Ind–2001	b	O/OMN/7/2001	DQ164941	(Knowles et al., 2005)
ME–SA	Ind–2001	c	O/UAE/4/2008	KM921876	(Knowles et al., 2014)
ME–SA	Ind–2001	d	O/BHU/3/2009	KM921814	(Knowles et al., 2014)
ME–SA	PanAsia	–	O/UKG/35/2001	AJ539141	(Mason et al., 2003)
ME–SA	PanAsia–2	–	O/IRN/8/2005	KY091281	(Knowles et al., 2016)
ME–SA	PanAsia–2	ANT–10	O/IRN/88/2009	KY091282	(Knowles et al., 2016)
ME–SA	PanAsia–2	BAL–09	O/IRN/18/2010	KY091283	(Knowles et al., 2016)

ME–SA	PanAsia–2	FAR–09	O/IRN/31/2009	KY091284	(Knowles et al., 2016)
ME–SA	PanAsia–2	PUN–10	O/PAK/16/2010	KY091285	(Knowles et al., 2016)
ME–SA	PanAsia–2	SAN–09	O/TUR/2/2009	KY091286	(Knowles et al., 2016)
ME–SA	PanAsia–2	TER–08	O/TUR/38/2008	KY091287	(Knowles et al., 2016)
SEA	**–**	**–**	**O/TAI/189/87***	**KY091288**	**(Knowles et al., 2016)**
SEA	Cam–94	–	O/CAM/3/98	AJ294910	(Knowles et al., 2001)
SEA	Mya–98	–	O/MYA/7/98	DQ164925	(Knowles et al., 2005)
WA	**–**	**–**	**O/GHA/5/93**	**AJ303488**	**(Samuel & Knowles, 2001)**
WA	–	–	O/CIV/8/99	AJ303485	(Samuel & Knowles, 2001)
AFRICA	G–I	–	A/KEN/42/66	KF561699	(Kasanga et al., 2015)
AFRICA	G–II	–	A/EGY/1/72	EF208756	(Knowles et al., 2007)
AFRICA	**G–III**	**–**	**A_{21}/Lumbwa/KEN/3/64**	**KY091289**	**(Knowles et al., 2016)**
AFRICA	G–IV	–	A/SUD/3/77	GU566064	(Habiela et al., 2010)
AFRICA	G–V	–	A/NGR/2/73	KF561704	(Kasanga et al., 2015)
AFRICA	G–VI	–	A/GHA/16/73	KF561698	(Kasanga et al., 2015)
AFRICA	G–VII	–	A/UGA/13/66	KF561705	(Kasanga et al., 2015)
ASIA	**A22**	**–**	**A_{22}/IRQ/24/64**	**AY593763**	**(Carrillo et al., 1990)**
ASIA	Irn–87	–	A/IRN/2/87	EF208770	(Knowles et al., 2007)
ASIA	Irn–96	–	A/IRN/1/96	EF208771	(Knowles et al., 2007)
ASIA	Irn–99	–	A/IRN/22/99	EF208772	(Knowles et al., 2007)
ASIA	Irn–05	–	A/IRN/1/2005	EF208769	(Knowles et al., 2007)
ASIA	Iran–05	AFG–07	A/AFG/6/2007	FJ755007	(Knowles et al., 2009)
ASIA	Iran–05	ARD–07	A/TUR/1/2008	FJ755133	(Knowles et al., 2009)
ASIA	Iran–05	BAR–08	A/BAR/6/2008	FJ755010	(Knowles et al., 2009)
ASIA	Iran–05	ESF–10	A/IRN/9/2010	KY091290	(Knowles et al., 2016)
ASIA	Iran–05	EZM–07	A/TUR/33/2008	FJ755155	(Knowles et al., 2009)
ASIA	Iran–05	FAR–09	A/IRN/78/2009	KY091291	(Knowles et al., 2016)
ASIA	Iran–05	FAR–11	A/IRN/1/2011	KY091292	(Knowles et al., 2016)
ASIA	Iran–05	HER–10	A/AFG/10/2010	KY091293	(Knowles et al., 2016)
ASIA	Iran–05	KSS–09	A/TUR/3/2010	KY091294	(Knowles et al., 2016)
ASIA	Iran–05	QAZ–11	A/IRN/9/2011	KY091295	(Knowles et al., 2016)
ASIA	Iran–05	SIS–10	A/IRN/125/2010	KY091296	(Knowles et al., 2016)
ASIA	Iran–05	SIS–12	A/IRN/15/2012	KY091297	(Knowles et al., 2016)
ASIA	A15	–	A_{15}/Bangkok/TAI/60	AY593755	(Carrillo et al., 1990)
ASIA	Sea–97	–	A/TAI/2/97	EF208778	(Knowles et al., 2007)
ASIA	Sea–97	–	A/TAI/7/2003	HQ116312	(Abdul-Hamid et al., 2011)
ASIA	Thai–87	–	A/TAI/118/87*	EF208777	(Knowles et al., 2007)
EURO–SA	**A12**	**–**	**A_{12}/119/Kent/UK/32**	**M10975**	**(Robertson et al., 1985)**
EURO–SA	A24	–	A_{24}/Cruzeiro/BRA/55	AY593768	(Carrillo et al., 1990)
EURO–SA	A5	–	A5/FRA/1/68 (1960)	KY091298	(Knowles et al., 2016)
EURO–SA	A–81	–	A/Alem/ARG/81	AJ306219	(Konig et al., 2001)
Unassigned	**G–VIII**	**–**	**A_{23}/Kitale/KEN/64 (KEN/46/65)**	**KY091299**	**(Knowles et al., 2016)**
Unassigned	**A11**	**–**	**A_{11}/Germany/c.29 (AGB)**	**EU553852**	**(Valarcher et al., 2008)**
AFRICA	**–**	**–**	**C/K267/67 (KEN/32/70)**	**KY091300**	**(Knowles et al., 2016)**
AFRICA	–	–	C/ETH/1/71	FJ798151	(Ayelet et al., 2009)
ASIA	**–**	**–**	**C/N65/Tadjikistan/USSR/67**	**KY091302**	**(Knowles et al., 2016)**
ASIA	–	–	C/IND/51/79 (1977)	KY091301	(Knowles et al., 2016)
EURO–SA	**–**	**–**	**C_3/Resende/BRA/55**	**M90381**	**(Martínez et al., 1992)**

EURO–SA	–	–	C1/Santa Pau/Spain/70 (C-S8c1)	AJ133357	(Toja et al., 1999)
EURO–SA	–	–	C_3/Indaial/BRA/71	M90376	(Martínez et al., 1992)
EURO–SA	–	–	C/PHI/7/84	KY091303	(Knowles et al., 2016)
Unassigned	**–**	**–**	**C/Germany/c.26 (CGC)**	**EU553893**	**(Valarcher et al., 2008)**
Unassigned	**–**	**–**	**C/UK/149/34**	**EU553905**	**(Valarcher et al., 2008)**
ASIA	**–**	**–**	**Asia1/PAK/1/54**	**AY593795**	**(Carrillo et al., 1990)**
ASIA	G–I	–	Asia1/AFG/1/2001	DQ121109	(Valarcher et al., 2005)
ASIA	G–II	–	Asia1/IRN/10/2004	DQ121119	(Valarcher et al., 2005)
ASIA	G–III	–	Asia1/ IND/762/2003*	DQ101240	(Valarcher et al., 2005)
ASIA	G–IV	–	Asia1/HKN/19/74	FJ785230	(Valarcher et al., 2005)
ASIA	G–V	–	Asia1/IND/18/80	DQ121116	(Valarcher et al., 2005)
ASIA	G–VI	–	Asia1/IND/14/95	AF390678	Gurumurthy et al., unpublished
ASIA	Sindh–08	–	Asia1/PAK/8/2008	KY091304	(Knowles et al., 2016)
ASIA	–	–	Asia1/Shamir/ISR/89	JF739177	Lee et al., unpublished
ASIA	–	–	Asia1/IND/63/72*	Y09949	(Reddy et al., 1999)
ASIA	–	–	Asia1/YNBS/China/58	AY390432	Chang et al., unpublished
I (NWZ)	**–**	**–**	**SAT1/T155/71***	**KF561706**	**(Kasanga et al., 2015)**
I (NWZ)	–	–	SAT1/ZIM/23/2003	KF219690	(Knowles et al., 2016)
II (SEZ)	**–**	**–**	**SAT1/RV/11/37**	**AY593839**	**(Carrillo et al., 1990)**
II (SEZ)	–	–	SAT1/RHO/5/66	AY593846	(Carrillo et al., 1990)
III (WZ)	**–**	**–**	**SAT1/BEC/1/48**	**AY593838**	**(Carrillo et al., 1990)**
III (WZ)	–	–	SAT1/BOT/1/68	AY593845	(Carrillo et al., 1990)
IV (EA–1)	**–**	**–**	**SAT1/UGA BUFF/21/70**	**KF219682**	**(Knowles et al., 2016)**
V	**–**	**–**	**SAT1/NIG/11/75**	**AF431711**	**(Sangare et al., 2003)**
V	–	–	SAT1/ETH/3/2007	FJ798154	(Ayelet et al., 2009)
VI	**–**	**–**	**SAT1/ISR/4/62**	**AY593844**	**(Carrillo et al., 1990)**
VI	–	–	SAT1/SUD/4/76	AY441997	(Sahle et al., 2007a)
VII (EA–2)	**–**	**–**	**SAT1/UGA/13/74**	**AY442010**	**(Sahle et al., 2007a)**
VIII (EA–3)	**–**	**–**	**SAT1/UGA/1/97***	**AY442012**	**(Sahle et al., 2007a)**
I	**–**	**–**	**SAT2/SA/106/59**	**AY593848**	**(Carrillo et al., 1990)**
I	–	–	SAT2/ZIM/14/2002	KF219689	(Knowles et al., 2016)
II	**–**	**–**	**SAT2/ZIM/7/83***	**AF540910**	**(Van Rensburg & Nel, 1999)**
II	–	–	SAT2/ZIM/5/81	EF134951	(Hall et al., 2013)
III	**–**	**–**	**SAT2/RHO/1/48**	**AY593847**	**(Carrillo et al., 1990)**
III	–	–	SAT2/ BOT/P3/98 (VUM-29)	KY091305	(Knowles et al., 2016)
IV	**–**	**–**	**SAT2/KEN/1/84**	**AY344505**	**(Sahle et al., 2007)**
IV	–	–	SAT2/ETH/1/90	AY343935	(Sahle et al., 2007)
V	**–**	**–**	**SAT2/NIG/2/75**	**AF367139**	**(Bastos et al., 2003)**
V	–	–	SAT2/GHA/2/90	AF479415	(Bastos et al., 2003)
VI	**–**	**–**	**SAT2/GAM/8/79**	**AF479410**	**(Bastos et al., 2003)**
VII	**–**	**–**	**SAT2/SAU/6/2000**	**AF367135**	**(Bastos et al., 2003)**
VII	–	–	SAT2/CAR/8/2005	JX570616	(Ahmed et al., 2012)
VIII	**–**	**–**	**SAT2/ZAI/1/74**	**DQ009737**	**Maree et al., unpublished**
VIII	–	–	SAT2/RWA/1/00*	AF367134	(Bastos et al., 2003)
IX	**–**	**–**	**SAT2/KEN/3/57**	**AJ251473**	**Newman et al., unpublished**

IX	–	–	SAT2/KEN/2/84	AY343941	(Sahle et al., 2007)
X	**–**	**–**	**SAT2/ZAI/1/82**	**AF367100**	**(Bastos et al., 2003)**
X	–	–	SAT2/UGA/19/98	AY343969	(Sahle et al., 2007)
XI	**–**	**–**	**SAT2/ANG/4/74**	**AF479417**	**(Bastos et al., 2003)**
XII	**–**	**–**	**SAT2/UGA/51/75**	**AY343963**	**(Sahle et al., 2007)**
XIII	**–**	**–**	**SAT2/SUD/6/77**	**AY343939**	**(Sahle et al., 2007)**
XIII	–	–	SAT2/ETH/2/2007	FJ798161	(Ayelet et al., 2009)
XIV	**–**	**–**	**SAT2/ETH/2/91**	**FJ798159**	**(Ayelet et al., 2009)**
I (SEZ)	**–**	**–**	**SAT3/SA/57/59**	**AY593850**	**(Carrillo et al., 1990)**
I (SEZ)	–	–	SAT3/KNP/10/90/3	AF286347	(Van Rensburg et al., 2002)
II (WZ)	**–**	**–**	**SAT3/BEC/1/65**	**AY593853**	**(Carrillo et al., 1990)**
II (WZ)	–	–	SAT3/BEC/20/61	AY593851	(Carrillo et al., 1990)
III (NWZ)	**–**	**–**	**SAT3/ZIM/P25/91 (UR-7)**	**KY091306**	**(Knowles et al., 2016)**
IV	**–**	**–**	**SAT3/ZAM/P2/96 (MUL-4)**	**KY091307**	**(Knowles et al., 2016)**
V (EA)	**–**	**–**	**SAT3/UGA BUFF/27/70 (aka UGA/92/70)**	**KF219685**	**(Knowles et al., 2016)**
V (EA)	–	–	SAT3/UGA/10/97 (UGA/2/97/3)	KY091308	(Knowles et al., 2016)

Source: Knowles et al., 2016; *Not a WRL-FMD reference number

Appendix 8: University Research proposal, and Field work Permit Approvals