A STUDY ON THE EPIDEMIOLOGICAL AND MOLECULAR RELATIONSHIPS OF CIRCULATING FOOT AND MOUTH DISEASE VIRUSES IN KENYA

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Signed :   Date: 10th July 2023

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DEDICATION

I dedicate this work to my family, my loving husband David for inspiring me to pursue my dreams, for patience and financial support. To my children Denis, Donald, Donna, Dominic and Davis for warmth, encouragement and for never leaving my side. To my late parents Moses and Rebecca Koech who served as a strong pillar throughout my education and for inculcating in me the invaluable passion for academic excellence. Above all to The Almighty God for divine strength, guidance and success.

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TABLE OF CONTENTS

DECLARATION…………………………………………………………………… 2
ACKNOWLEDGEMENTS………………………………………………………… 4
TABLE OF CONTENTS…………………………………………………………… 6
LIST OF FIGURES…………………………………………………………………12
LIST OF ABBREVIATIONS AND ACRONYMS………………………………..13
ABSTRACT………………………………………………………………………..15
Chapter 1: GENERAL INTRODUCTION…………………………………………18
1.1 BACKGROUND INFORMATION…………………………………………18

1.2 JUSTIFICATION OF THE STUDY…………………………………………...21

1.3. OBJECTIVES OF THE STUDY……………………………………………25

1.3.1 Main Objective……………………………………………………………..25

1.3.2 Specific Objectives…………………………………………………………25

Chapter 2: GENERAL LITERATURE REVIEW…………………………………26
2.1 Foot-and-mouth disease ……………………………….………………. …… .……. 26

2.2 The aetiology……………………………………………………………….....26

2.2.1 Taxonomy of FMD virus ……………………………………………………. 26

2.2.2. Physicochemical properties of FMDV………………………………… 27

2.2.3. FMDV virion properties…………………………………………………28

2.2.4. Serotypes of FMDV……………………………………………………………29

2.3 History of FMD………………………………………………………………….. 31

2.4 Economic importance of FMD…………………………………………………34

2.5 Host range…………………………………………………………………………36

2.6 Incubation period and virus excretion…………………………………………37

2.7 Transmission and Pathogenesis……………………………………………….. 38

2.8. Pathology…………………………………………………………………………41

2.9. Immunity…………………………………………………………………………42

2.10 Geographical Distribution……………………………………………………… 42

2.10.1 Global distribution... 42

2.10.2 FMD in Kenya 43

2.11 The role of wildlife in transmission 44

2.12 Clinical signs, Chronic FMD and The carrier state in animals 46

2.12.1 Clinical signs 46

2.13 Foot-and-mouth disease virus carrier animals 50

2.14 Chronic FMD 51

2.15 Diagnosis of FMD 51

2.16 Control of FMD 54

2.16.1 Control strategies 54

2.16.2 Complexities in FMD control 56

2.16.2.1 Nomadic pastoralism and non-effective management of livestock movement 56

2.16.2.2 Wildlife-Livestock interface 57

2.16.2.3 Vaccines 57

2.16.2.4 Multiplicity of FMDV strains 57

2.16.2.5 Other constraints 58

Chapter 3: EPIDEMIOLOGICAL STUDY ON FOOT-AND-MOUTH DISEASE IN SMALL RUMINANTS : SERO-PREVALENCE AND RISK FACTOR ASSESSMENT IN KENYA

3.1 INTRODUCTION 59

3.2 FOOT-AND-MOUTH DISEASE IN SHEEP AND GOATS 61

3.2.1 Clinical signs 61

3.2.2 Transmission 63

3.2.3 Morbidity and Mortality in sheep and goats 64

3.2.4.The role of sheep and goats in the transboundary spread of FMD 64

3.3 MATERIALS AND METHODS 66

3.3.1 Ethics 66

3.3.2 The study population 66

3.3.3 Description of the study area 67

3.3.4 Study design and methods ... 68
3.3.4.1 Sample size determination ... 68
3.3.4.2 Sampling of herds and animals ... 71
3.3.4.4 Administration of questionnaires ... 73
3.3.4.5 Laboratory sample analysis ... 73
3.3.4.6 Data management and analysis ... 74
3.4 RESULTS 76
3.4.1 Animal and herd level descriptive statistics ... 76
3.4.2 Sero-prevalence of FMD in small ruminants ... 79
3.4.3 Association between FMD sero-positivity and selected potential risk factors ... 84
3.5.DISCUSSION 86
3.6 CONCLUSION 90
Chapter 4: SURVEILLANCE FOR FOOT- AND-MOUTH DISEASE IN DOMESTIC RUMINANTS IN UKAMBANI REGION OF KENYA
4.1 INTRODUCTION 92
4.3. MATERIALS AND METHODS 94
4.3.1 The study population ... 94
4.2.2 Sampling strategy and study area ... 95
4.2.2.1 Study area ... 95
4.2.2.2 Sample size determination ... 96
4.2.2.3 Sample collection: ... 98
4.2.2.4 Clinical examination of active cases of FMD ... 101
4.2.2.5 Laboratory Test: ... 101
4.3 RESULT ... 103
4.4 DISCUSSION ... 107
4.5 CONCLUSION ... 110
Chapter 5: MOLECULAR CHARACTERIZATION AND PHYLOGENETIC EVALUATION OF SEROTYPES O AND A FOOT- AND- MOUTH DISEASE VIRUSES IN CIRCULATION IN KENYA: 2013-2018.
5.1 INTRODUCTION ... 114

5.1.2 Phylogenetic analysis 114

5.1.3 Phylogenetic trees 116

5.1.4 Methods for constructing phylogenies 117

5.1.4.1 Tree construction 117
5.1.4.2 Distance methods... 117
5.1.4.3 Character-based methods... 118
5.1.5 FMD vaccination in Kenya in relation to strains 118

5.3 MATERIALS AND METHODS……………………………………………………119

5.3.1 Selection of field isolates for the study 119

5.3.2 Laboratory Processing steps:... 120

5.3.2.1 Virus Isolation 120
5.3.3.2 Master-mix preparation and RNA Extraction 122
5.3.3.3 Reverse transcription polymerase chain reaction (RT-PCR) 123
5.3.3.4 Purification 123
5.3.3.5 Sequencing work 127
5. RESULTS……………………………………………………………………………127

5.1 Serotype A results 127

5.1.1 Sequence assembly 127
5.1.2 Blast search.. 128
5.1.3 Phylogenetic analysis 128
5.2 Serotype O Results 129

5.2.1 Sequence assembly 129
5.2.2 Blast search.. 129
5.2.3 Phylogenetic analysis 130
5.3 RESULT ANALYSIS…………………………………………………………...130

5.4. DISCUSSION…………………………………………………………………..146

Chapter 6: GENERAL SUMMARY……………………………………………………151
REFERENCES…………………………………………………………………………155
APPENDICES……………………………………………………………………….....181

LIST OF TABLES

Table 2.1: Infectious dose (TCID50) by different routes in the major domestic specie…….41

Table 3.1: DESCRIPTIVE STATISTICS OF SAMPLED INDIVIDUAL ANIMAL VARIABLES INTHE PASTORAL AND SEDENTARY ZONES, KENYA, 2016………...77

Table 3.2: DESCRIPTIVE STATISTICS OF HERD LEVEL VARIABLES IN THE PASTORAL AND SEDENTARY ZONES, KENYA, 2016…………………………………78

Table 3.3: FMD SERO-POSITIVITY PER POTENTIAL INDIVIDUAL ANIMAL RISK FACTOR, KENYA, 2016……………………………………………………………………….81

Table 3.4: SERO-POSITIVITY FOR FMD PER POTENTIAL HERD RISK FACTOR, KENYA, 2016……………………………………………………………………………...82

Table 3.5: ASSOCIATION BETWEEN FMD SERO-POSITIVITY IN SMALL RUMINANTS AND RISK FACTORS STUDIED, KENYA, 2016…………………………85

Table 4.1: DETAILS OF SAMPLING SITES VISITED IN UKAMBANI REGION……….99

Table 4.2: Overall Seroprevalence of FMD in cattle and small ruminants in Ukambani region……………………………………………………………………………………10

4

Table 4.3: Risk factors of FMD in Small Ruminants………………………………………..105

Table 4.4: Sero-prevalence per County per Livestock type in Ukambani Region…………..106

Table 4.5: Risk factor analysis for cattle in Ukambani region………………………………107

Table 5.1: STANDARD THERMOCYCLING PROTOCOL USED FOR RT-PCR AMPLIFICATION OF VP1 REGION OF FMDV SEROTYPE O AND A………………..124

Table5.2: SEROTYPE 'A' SAMPLES METADATA……………………………………..125

Table5.3: SEROTYPE 'O' SAMPLES METADATA…………………………………….125

Table5.4: PRIMERS SUBMITTED FOR SEROTYPE O SEQUENCING……………….127

Table 5.5: PRIMERS SUBMITTED FOR SEROTYPE A SEQUENCING………………127

Table 5.6: SEROTYPE A SEQUENCES

ANALYSED……………………………………131

Table 5.7: SEROTYPE O ANALYSIS……………………………………………………...135

LIST OF FIGURES

Figure 2.1 Genome organization of FMDV and the structure of the virus. 29
Figure 2.2 Maps showing the global distribution of FMD by serotype/Pools 43
Figure 2.3 The Progressive Control Pathway for Foot-and-Mouth Disease (PCP-FMD) 2nd Edition 2018 56
Figure 3.1 FMD Reported and serotyped outbreaks in a period of 15 years (2004-2018)....... 61
Figure 3.2 Foot lesion in the interdigital space and lesions on the dental pad of sheep with FMD. 63
Figure 3.3 Study zones 70
Figure 3.4 Selected sampling sites for the cross-sectional survey,Kenya 2016 71
Figure 4.1 Map of Kenya showing the study area of Ukambani; Kitui, Machakos and Makueni 96
Figure 4.2 Map showing sampling sites visited in Ukambani Region 98
Figure 5.1 The probability of substitution of one nucleotide by another between purines and/or pyrimidines. 116
Figure 5.2 BHK-21 cells before infection and at 48 hours post-infection withb FMDV showing cythopathic effect 122
Figure 5.3 An Electrophoresis picture of the PCR products as seen on 1.5 % agarose gels .. 124
Figure 5.4 A phylogenetic tree representing the relationship between the 53 FMDV isolates, serotype A. 134
Figure 5.5 A phylogenetic tree representing the relationship between the 105 FMDV isolates, serotype O 139
Figure 5.6 A phylogenetic tree representing the relationship between the 118 FMDV isolates, serotype O 142
Figure 5.7 A phylogenetic tree representing the relationship between the 58 FMDV isolates, serotype A. 145

LIST OF ABBREVIATIONS AND ACRONYMS

°C	- Degree Celsius
µl	- Microliter
ASALS	- Arid and Semi-Arid Lands
BHK	- Baby Hamster Kidney
CCPP	- Contagious caprine pleuropneumonia
Chy	-Cathay topotype
DIVA	-Differentiates infected from vaccinated animals
DVS	- Director of Veterinary Services
EA 1-4	-East Africa topotype 1-4
ELISA	- Enzyme linked immunosorbent assay
Euro-SA	- Europe South America topotype
FAO	-Food and Agriculture Organization
FMD	- Foot-and-Mouth disease
FMD LAB	- Foot-and -mouth disease laboratory
FMDV	-Foot-and- mouth disease virus
GF-TADS	- Global Framework for progressive control of Trans-boundary Animal Disease
GDP	- Gross Domestic Product
GLMM	-Generalised Linear Mixed Model
KEVEVAPI	- Kenya Veterinary Vaccines Production Institute
MEA	- Middle East Asia topotype
NACOSTI	- National Commission of Science, Technology and Innovation
NCBI	-National Centre for Biotechnology Information

NSP -Non structural proteins

OD - Optical density

SCVOs - Sub-County Veterinary Officers

OIE - *Office International des Épizooties(*World Organisation for Animal Health)

PCP - Progressive Control Pathway

PPR -Pestes de petit ruminants

RVF -Rift Valley Fever

RNA - Ribonucleic acid

SAT 1-3 - South African Territories1-3

SCVOs - Sub-County Veterinary Officers

SEA - South East Asia

SP -Structural Proteins

TAD - Transboundary Animal Disease

VNT - Virus Neutralisation Test

VP1-4 - Virion Protein 1-4

WA - West Africa topotype

WOAH - World Organization of Animal Health

ABSTRACT

Foot-and-mouth disease (FMD) is endemic in Kenya causing serious economic losses in the livestock sector. The epidemiology of the disease in small ruminants (SR) in Kenya is not well documented and the field dynamics of FMD epidemiology is scarce. The options for vaccine strain selection for emerging FMD outbreaks in endemic countries are yet to be addressed in East Africa. The general objective of this study was firstly to assess and document the FMD sero-prevalence and risk factors associated with SR nationally and a case study in domestic ruminants in Ukambani region. Secondly it aimed to characterize the foot and mouth disease viruses (FMDV) in circulation in 2013-2018 by molecular techniques. To estimate sero-prevalence and associated risk factors, we carried out a national cross-sectional study. Selection of animals used a multistage cluster sampling approach. Sera totaling 7564 were screened for FMD antibodies of non-structural-proteins using ID Screen® NSP Competition ELISA kit. Identification of risk factors used generalized linear mixed model effects (GLMM) logistic regression analysis with county and villages as random effect variables. The country animal level sero-prevalence in SR was 22.5% (95% CI: 22.3%-24.3%) while herd level sero-prevalence was 77.6% (95% CI: 73.9% - 80.9%). FMD sero-positivity in SR was signifcantly associated with multipurpose production type (OR = 1.307; p = 0.042) and negatively associated were male sex (OR = 0.796; p = 0.007), young age (OR = 0.470; p = 0.010) and sedentary production zone (OR = 0.324; p<0.001). There were no statistically significant intra class correlations among the random effect variables but interactions between age and sex variables were statistically significant (p = 0.019). Herds with animals bought from markets or middlemen, with wildlife interaction, reared in low altitude (<1500m above sea level) all had statistically significant higher sero-positivity. Other risk factors identified included unenclosed animals, shared bull, shared watering, communal

grazing, no vaccination and mixed and migratory grazing systems. Ukambani region had a higher seroprevalence in cattle than small ruminants(40% compared to 19%) and also higher seroprevalence in cattle than the National rate, 40% compared to 37.6 % (unpublished study carried out in the same period). The FMD seroprevalence rate in SR was lower than the National rate at 19%. In the molecular characterization study, the nucleotide sequences encoding the capsid protein VP1 (1D) region of FMDV from virus samples were generated by Reverse transcription polymerase chain reaction and sequencing. Study samples were serotype O and A repository isolates banked at the FMD Laboratory, Embakasi collected during FMD outbreaks from cattle in 2013 to 2018. For serotype O, 60 isolates were characterized(n=60), 58 being field viruses and vaccine strain OK77/78 in duplicate and for serotype A were 21 field isolates and one vaccine strain AK5/80 (n= 22). The consensus sequences and additional files obtained from National Centre for Biotechnology Information (NCBI) were aligned using MEGA v11.0.8, employing the ClustalW algorithm. SeaView v5.0.4 was used to edit the alignment and MEGA v11.0.8 was used to construct phylogenetic trees. To increase the robustness of the sequence analysis 9 and 7 sequences were excluded from O and A sequences respectively due to low quality (error rate > 1%). Phylogenetic analysis showed that with few exceptions samples collected around the same time and those from the same county consistently clustered in the same lineage or closer to each other. Serotype O outbreaks were caused by East African topotype 2 viruses (EA-2) except one outbreak in Taita Taveta County whose isolate belonged to EA-1 topotype together with the current vaccine strain. Another vaccine strain not in use currently K82/98 belongs to EA-2 topotype with these recent isolates. For serotype A study isolates, all belong to Africa G-1 topotype though in 3 lineages. All study isolate sequences tended to cluster closely together in one lineage while few others clustered in another lineage with isolates collected 3-7 years earlier. The vaccine strain

belonged to a third lineage together with isolates collected over 20 years and more closely to isolates of 1990s. This study emphasizes the importance of regular surveillance and characterization of circulating strains for development of effective vaccines to support FMD control strategies. Some animal husbandry practices has significant impact on exposure to foot and mouth disease. It's proposed that future vaccine candidate strains selection could consider EA-2 topotype strains for serotype O and recent lineage of G1 topotype for serotype A to control FMDV circulating in Kenya.

Chapter 1: GENERAL INTRODUCTION

1.1 BACKGROUND INFORMATION

Kenya has a livestock population of 18.6 million cattle, 16.8 million sheep, 25.1 million goats and 463,000 pigs (Livestock population as per Kenya Bureau of Statistics 2015). Livestock sub-sector contributes about 12% of the total Gross Domestic Product (GDP) for the country and 40% of the total agricultural GDP. Livestock production is a major socio-economic activity for the communities that live in the high rainfall areas for dairy production and in the arid and semi-arid areas (ASALS) for beef production. In both areas, livestock provides substantial raw material for the local dairy, meat, hides and skins, wool and hair processing industries. The livestock sector accounts for over 30 % of farm gate value of agricultural commodities.

The sector is however burdened by many pests and diseases including Foot-and-mouth disease (FMD) which threaten food security and livelihoods of smallholders and prevent animal husbandry sectors from developing their economic potential. Foot and mouth disease is notorious for its ability to severely affect and indeed disrupt national, regional and international trade in animals and animal products. The burden of FMD on developing countries involving the loss of animals and biological diversity and lowering of production efficiency is underestimated.

Foot and mouth disease is a highly infectious, debilitating viral disease with huge economic implications in livestock production in many developing countries where it's endemic (Arzt, 2011). It affects artiodactyl wild and domestic species (Jamal and Belsham, 2013) including cattle, goats, sheep pigs, camelids, buffalo and deer leading to trade embargoes in countries

free of the disease on livestock and their products and is therefore a major global animal health problem.

The disease causes pyrexia and formation of vesicles in the mouth, nose, teats and interdigital space of the feet which turn into erosions. Clinically this manifests as excess salivation, lips smacking, teeth grinding (due to pain), nasal discharge, mastitis, lameness with lethargy and anorexia (Radostits *et al.*, 2000). This leads to reduced milk production, infertility, eventual loss of weight, loss of draught power and death can be a common sequele in young animals due to degeneration of heart muscle (Kitching, 2002; FAO, 2002). Secondary bacterial infection on the lesions in affected parts of the body complicates the recovery process leading to protracted illness though most animals recover within two weeks. Lesions are not prominent in sheep and goats and unapparent in wild animals like buffaloes (Donaldson and Sellers, 2000)**.** Transmission between animals can occur in various ways including animal to animal contact and contaminated animal products. This is because during the acute phase of the disease the virus is excreted in all body excretions and secretions and these are infectious (Sumption *et al.,* 2012). Peak transmission occurs when vesicles rupture contaminating the environment including pastures and animal watering points. The virus is also found in lymph nodes and bone marrow of slaughtered infected animals with survival being prolonged if the meat is frozen. Mechanical transmission can occur by animals, human, formites and air. These diverse modes of transmission leads to easy virus movement across borders often circumventing control measures in place.

Mortality is low in adult animals, but deaths can be common in young piglets, calves and lambs(Coetzer *et al.,* 1994).

The severity of clinical signs varies with the strain of virus, the exposure dose, the age and breed of animal, the host species and the immunity of the animal. The signs can range from a mild or inapparent infection to one that is severe. Certain strains of the virus may be of low virulence for some species of animals (Donaldson, 2000). It is also difficult to distinguish FMD from other viral vesicular diseases, including swine vesicular disease, vesicular stomatitis and vesivirus infection, solely on the basis of clinical findings. Additionally, other infectious agents can cause stomatitis, e.g. the viruses of mucosal disease , malignant catarrhal fever, rinderpest, peste des petits ruminants, papular stomatitis, orf, blue tongue and epizootic haemorrhagic disease. Thus, a definitive diagnosis requires laboratory investigation. Given the potential of rapid spread of FMD, it is essential that suspected cases are quickly reported and investigated. It's important to use the most rapid and accurate tests, so that control measures can be implemented speedily.

Foot and mouth disease virus has a wide host range, an ability to infect in small doses, a rapid rate of replication, a high level of viral excretion and multiple modes of transmission, including wind. These features make FMD a difficult and expensive disease to control and eradicate(Knight-Jones and Ruston, 2013). It's a disease that is much feared by farmers, veterinarians and those associated with livestock production. Countries free of the disease take great precautions to ensure that the virus does not gain entry. Consequently FMD is a major constraint to international trade in livestock and animal products.

Foot and mouth disease is endemic in Kenya and in most African countries. Infection with FMD quickly spreads in susceptible livestock and the epidemiology of FMD in this region is complicated by the multiplicity of susceptible hosts both wild and domestic. There are also

multiple virus serotypes, poorly informed control measures, inadequacies in the control of movements of livestock and livestock products. Wildlife move freely further complicating zoo-sanitary control measures. Additionally diverse socio-economic factors derail control efforts (Wekesa *et al.,* 2015). Kenya, like many other FMD endemic countries around the world, is in the process of implementing the Progressive Control Pathway (PCP) for Foot-and-mouth disease. This is in line with the Global Framework for the progressive control of Foot-and-Mouth Disease and other Trans-boundary Animal Diseases (GF-TADs) in the country and in the region.

This study was designed to address two areas; understanding the epidemiology of FMD in domestic ruminants for formulation of the most effective control strategies. This was done by surveillance for the disease to estimate the seroprevalence and determine the risk factors associated with the disease. Secondly effective vaccines can be developed by studying the relatedness of virus strains in circulation with the vaccine strains. This study evaluated the relationship of recent circulating FMD field strains with other documented viruses and the vaccine strains in use in the region.

1.2 JUSTIFICATION OF THE STUDY

Influence of FMD incidence on the economy of a country has shown that it is impossible to farm economically in the presence of FMD. The exclusion of a country in regional and international markets is undoubtedly the most serious economic consequence of the presence of FMD. Foot-and-mouth disease has been endemic in Kenya for several decades and for the country to develop effective control strategies certain knowledge gaps have to be addressed.

These include the prevalence of the disease in different susceptible species, farming systems and the risk factors associated with the disease.

Kenya is one of the leading FMD Vaccine producers in the region and the vaccine strains in use were developed over 40 years ago. There is need to match the FMD Vaccine strains with circulating field isolates in Kenya to ensure effective control of the disease. Genetic and antigenic characterization of FMDV field isolates is therefore important for an improved system of virus strain surveillance and vaccine updating.

According to the FMD control strategy being developed in Kenya, vaccination will play a major role in the control of this disease. Like other RNA viruses, the FMD virus continually evolves and mutates, thus one of the difficulties in vaccinating against it is the huge variation between, and even within serotypes (Carrillo *et. al.,* 2007). There is no cross-protection between serotypes (Ludi *et al.,* 2013) and two strains (genotypes) within one serotype may have nucleotide sequences that differ by as much as 15-20% for a given gene (Knowles and Samwel, 2003) and may not cross-protect in vaccination. This means FMD vaccines must be highly specific to the strains being controlled. Vaccination only provides temporary immunity that lasts from months to years and therefore has to be done on routine basis.

Evidence of possible vaccine failure due to introduction of new strain of SAT 1 was experienced in Kenya in 2009 to 2010. Sequencing results obtained from samples sent to World Reference Laboratory for FMD, Pirbright in the United Kingdom showed that the field strain was 10% divergent from the vaccine strain SAT 1 T 155/71 (FMD Laboratory Annual

Report 2012). However, Vaccine matching was not carried out to ascertain that they are antigenically unrelated.

Foot and mouth disease probably has the greatest economic impact on livestock than any other disease (James and Ruston, 2002). The global community under the auspices of the World Organization for Animal Health (WOAH) and the Food and Agricultural Organization (FAO) of the United Nations has also identified FMD as an important economic disease. The occurrence of the disease in a country or zone presents serious socioeconomic consequences such as loss of production of milk and meat, retarded growth rates, infertility as well as deaths in young animals. The impact of FMD is more significant in trade where infected countries and zones face access problems to domestic, regional and international markets for animals and their products thereby threatening livelihoods, jobs and the economy. The GF-TADs has been developed with the objective of guiding the control and eradication of the most significant animal diseases including FMD. Implementation of the Framework will achieve the goals of protecting the livestock industries of developed and developing countries from the repeated shocks of infectious disease.

Foot and mouth disease in Kenya is currently endemic with over 100 reported outbreaks per year. Four of the seven serotypes are currently in circulation in Kenya, i.e., O, A, SAT1 and SAT2 (Wekesa *et al.*, 2013). Short term farm level economic impact study of foot and mouth disease outbreak in a large scale diary farm of 200 heads of cattle in Kiambu County recorded losses of Ksh 1,201,950 equivalent US$15,000 (Mulei *et al.*, 2001). This colossal economic loss within such a short period of time indicates that the control of FMD is of paramount importance in the diary farming sector in Kenya. Another study on the financial impact

assessment of FMD in four large scale farms used benefit cost analysis in Nakuru County and estimated losses of >US$100,000 per farm (Kimani *et al.*, 2005).

Kenya, like many other countries around the world, is in the process of implementing the Progressive Control Pathway (PCP) for Foot-and-mouth disease in the country and in the region in line with the GF-TADs. Kenya is currently in Stage 2 of FMD Progressive control pathway as Risk Based Strategic Plan has been developed. The focus of this stage is to implement risk-based control measures so that the impact of FMD is reduced in one or more livestock sectors.

This study will inform the process and augment the commitment already embedded in the Constitution, the Kenya Vision 2030, the Agricultural Sector Development Strategy, the National Livestock Policy and the Veterinary Policy. Vaccination is widely used to control, eradicate and prevent FMD.

The choice of the most appropriate strains of Foot- and -mouth disease virus vaccines in FMD control programmes and to storage of vaccine antigen reserves are based on the matching of representative field isolates from outbreaks around the world to vaccine strains (Paton *et al.*, 2005). This needs to be done in Kenya as most vaccine strains in use currently were developed over 40 years ago. Additionally in order to reduce the impact of a disease like FMD a risk based approach is necessary and thus determination of the risk factors associated with the disease should be determined so as to institute risk- based control measures.

A recent study on the prevalence of FMD in Kenya was carried out giving an overall prevalence of 52.5% in cattle population (Kibore *et al.*, 2013). Such studies should be carried out periodically to determine the trends of the disease in different farming systems. This will help monitor whether control measures in place have had an impact on reducing disease incidence and also determine which areas to concentrate control. Determination of FMD risk factors and prevalence in Kenya in different farming systems especially sedentary versus pastoral will be key steps to developing a strategy to control the disease. In addition there is need for constant surveillance of circulating strains for appropriate strain selection for vaccine production.

1.3. OBJECTIVES OF THE STUDY

1.3.1 Main Objective

To determine the epidemiological and molecular relationships of circulating Foot-and-mouth disease viruses in Kenya.

.

1.3.2 Specific Objectives

1. To study FMD epidemiology in Sedentary and Pastoral areas of Kenya in domestic small ruminants (SR) through a national cross-sectional survey.
2. To determine the FMD disease dynamics through surveillance for FMD in the Ukambani region in Kenya to document the disease, clinical case manifestation, seroprevalence and risk factor analysis in the region.
3. To determine the genetic characteristics of FMD viruses in circulation in the last six years (2013-2018) and of current vaccine strains in Kenya.

Chapter 2: GENERAL LITERATURE REVIEW

2.1 Foot-and-mouth disease

Foot and mouth disease (FMD) is a highly contagious and usually acute disease of cloven-hoofed animals and camelids. It's a rather complex disease caused by a group of related but distinct viruses, collectively named FMD virus (FMDV) of the genus *Aphthovirus* in the family *Picornaviridae* (Belsham, 1993, Rueckert, 1996). There are seven distinct virus serotypes of FMDV, i.e. A, O, C, Asia-1 and the Southern African Territories (SAT) types 1, 2 and 3 which are found in different geographical distributions. The disease caused by these viruses is clinically indistinguishable but infection with any one serotype does not confer immunity against another. The disease is highly contagious and infection with FMD quickly spreads in susceptible livestock. The epidemiology of the disease in Kenya is complex due to the presence of four of the seven serotypes (O, A, SAT1 and SAT2) and the presence of large numbers of both wild and domestic susceptible animals. Additionally there is inefficient control of animal movements especially livestock and livestock products coupled with inadequate vaccination (Wekesa *et al*., 2015).

2.2 The aetiology

2.2.1 Taxonomy of FMD virus

The causative agent of Foot and mouth disease is the Foot-and-mouth disease virus (FMDV) defined in 1963 by the International Committee of Taxonomy of viruses as belonging to the genus *Aphthovirus*, and of the family *Picornaviridae* (Grubman and Baxt, 2004).The genus name is taken from the Greek word *Aphta*, meaning vesicles in the mouth. The name *Picornaviridae* is derived from the Latin word 'pico' (small) and 'rna' (RNA) which refers to the size.

2.2.2. Physicochemical properties of FMDV

Foot-and-mouth disease virus is a non-enveloped virus and enclosed with a protein shell, a roughly spherical capsid exhibiting an icosahedral symmetry. The capsid consists of polypeptides, which are devoid of lipo-protein, and hence is stable to lipid solvents like ether and chloroform (Cooper *et al.,* 1978). The virus is pH sensitive; and is inactivated when exposed to pH below 6.5 or above 11. Generally picornaviruses are stable between pH 3 - pH 9 but FMDV is distinguished from other picornaviruses by its narrow pH stability range of pH pH 7- 9. Foot and mouth disease virus is very liable in even mildly acid solutions. At pH 6 the rate of inactivation is 90% every 60 seconds while at pH 5 it is 90% per second (Bachrach, 1968). The virus is also liable in alkaline solutions so that at pH 10 its loss of infectivity is 90% every 14 hours. For this reason 2 % Sodium Hydroxide (Na $(OH)_2$) and 4% Sodium Carbonate (Na_2CO_3) are used as cheap and effective disinfectants which can be used FMD contaminated objects (Sahle, 2004). Alkaline solutions are generally more effective than acidic solutions because they are not easily neutralized by organic material such as faeces and blood. However, in milk and milk products, the virion is protected, and can survive at 70 ^{o}C for 15 seconds and pH 4.6. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes (Mckercher and Callis, 1983).

The virus is resistant to alcohol, phenolic and quaternary ammonium disinfectants. However, the FMD virus is also sensitive to a range of other chemicals like trypsin which causes cleavage and denaturation of the vital capsid protein, VP1 (Wild *et al.,* 1969; Rowlands *et al*., 1971). The sizes of droplet aerosols also play an important role in the survival or drying out of the virus; droplet aerosols size of 0.5 - 0.7 μm is optimal for longer survival of the virus in the

air, while smaller aerosols dry out. In dry conditions the virus also survives longer in proteins, e.g., in epithelial fragments (Donaldson, 1987).

2.2.3. FMDV virion properties

It is a small non-enveloped virus, 25 nm in size whose genome consists of a single stranded positive sense RNA, 7.2-8.4 Kb in size (Rueckert, 1996) surrounded by a protein shell or capsid. This FMDV capsid is roughly spherical exhibiting an icosahedral symmetry with the virion consisting of 70% protein, 30% RNA as well as a small quantity of lipids. Virions appear smooth and round in outline in electron micrographs and they appear the same in images reconstructed from X-ray crystallographic analysis. Virions are constructed from 60 copies each of four capsid proteins VP1, VP2, VP3, and VP4 plus a single copy of the genome protein Vpg (Murphy *et al.,* 1999). The VP1, VP2 and VP3 are exposed on the surface, whilst VP4 is located entirely at the inner surface of the capsid, probably in contact with RNA. The genomic RNA is polyadenylated at the 3’ end and is linked to the VPg protein covalently at the 5’ end (King *et al.,* 1982). This genomic RNA is infectious. The virus has a molecular weight of 8.5 x 10^{5} D and a sedimentation rate of 146s.

It’s genome is a single stranded positive sense RNA approximately 8500 bases long and consists of a 5' non-coding region (NCR), a single open reading frame and a short 3' NCR. Therefore the RNA includes three separate parts i.e. the 5′ untranslated region (5′ UTR), a long coding region and the 3′ untranslated region (3′ UTR) (Figure 2.1).The major portion of the genome consists of a single large open reading frame of 6996 nucleotides encoding a polyprotein of the 2332 amino acids (Rueckert, 1996). A small protein (24 or 25 residues

long), termed VPg, which is encoded by the 3B portion of the viral genome region, is covalently linked to the 5′ end of the genome.

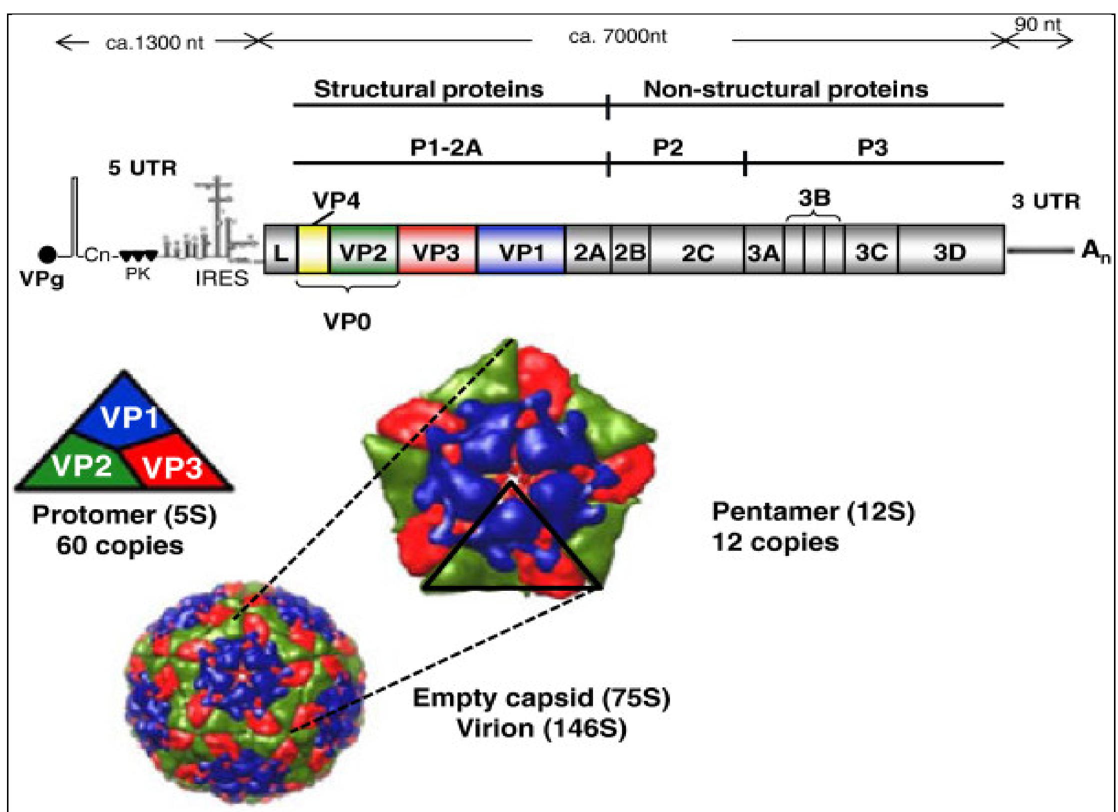


Figure 2.1 Genome organization of FMDV and the structure of the virus.

Source: Jamal and Belsham Veterinary Research 2013, 44:116 http://www.veterinaryresearch.org/content/44/1/116

2.2.4. Serotypes of FMDV

Globally, there are seven immunologically distinct serotypes of FMDV: O (Oise), A (Allemagne), C (Island Riems), SAT1, SAT2 and SAT3 (South African Territories) and Asia 1 (WOAH, 2022) which are found in different geographical locations. All but one of these serotypes (Asia1) are present in sub-Saharan Africa. The epidemiology of the disease is further complicated by the presence of carrier animals (in particular African buffalo) and susceptible wildlife. All the seven serotypes produce a disease that is clinically indistinguishable but immunologically distinct and infection with one serotypes does not

confer immunity against the other serotypes after either infection or vaccination (Domingo *et al.,* 2003). This serological classification is based on the inability of viruses from the different types to induce cross-protection in animals (Pereira *et al*., 1977). Within each one of these serotypes, there are a large number of strains with their own antigenic characteristics, hence there may be only partial cross-immunity between strains of the same serotype (Northumberland, 1968) or no cross-protection at all (Brooksby, 1982). These genetic and antigenic variants occur with different degrees of virulence (Blood & Radostits, 1989; Kitching *et al.,* 1989) and these strains can be identified by biochemical and immunological tests.

Therefore recovery/ from one or vaccination against one serotype does not confer immunity against another or may not confer immunity within the same serotype (Grubman and Mason, 2002). Similarly, a single dose of a monotypic vaccine fails to protect against heterotypic challenge (Cartwright *et al*., 1982). The highly contagious nature of FMDV and the associated productivity losses make it a primary animal health concern worldwide.

Genetically FMDV can be classified based on their geographical origin as topotypes. For example serotype O can be grouped into 10 topotypes : Europe- South America (Euro-SA), Middle East-Asia(SEA), Cathay(chy), West Africa(WA), East Africa(EA-1), East Africa - 2(EA-2), East Africa-3(EA-3), East Africa-4(EA-4), Indonesia 1(ISA-1) and Indonesia-2 (ISA-2) (Knowles *et al.,*2004).

2.3 History of FMD

Foot and mouth disease is probably an ancient disease, because in 1546, H. Fracastorius described a similar disease of cattle in what is now present day Italy which occurred in 1514 (Henning,1956). Almost 400 years later, in 1897, Loeffler and Frosch demonstrated that a filterable agent caused FMD (Van Kamnen, 1999). This was the first demonstration that a disease of animals was caused by a filterable agent and ushered in the era of virology. Prior to this discovery no known micro-organisms were capable of passing through Berkfeild filters and this finding led Loeffler and Frosch to deduce that the agent causing FMD was beyond the resolution of a light microscope. It was not until 1920 that a convenient animal model for the study of FMD virus was established by Waldmann and Pape, using guinea-pigs and later the development of in-vitro cell culture systems for the virus. The chemical and physical properties of FMD virus were elucidated during the remainder of the twentieth century, culminating in 1989 with a complete description of the three-dimensional structure of the virion.

Foot and mouth disease was first described in Africa in 1780 but the disease may have been present in the continent for centuries (Knowles, 2009). Supporting evidence that the disease may have been present in Africa for a long time lies in the fact that SAT types of FMDV are uniquely adapted to long term survival in free living African buffalo populations in South, Central and Eastern Africa. Furthermore these SAT types of FMDV are immunologically and genetically distinguishable from the other four serotypes of FMDV - O, A, C, and Asia 1 which presumably evolved in Asia and Europe. All the FMDV serotypes are endemic in Africa except Asia 1.

Foot and Mouth disease is endemic in Kenya with recorded cases dating back to 1915 although the Maasai community was familiar with the disease prior to the records. It was first characterized in 1932 and typing results have been available in Kenya since 1954 (Wariru, 1994).

Globally, the fear of FMD outbreaks or disease infiltrations since the beginning of the 20th century has led to the establishment of institutes to investigate methods to control the disease. These include the Insel Reims in Germany in 1909, the World Reference Laboratory for FMD in the United Kingdom in 1924, Lindholm Island in Denmark in 1925, the Centro Panamericano de Fiébre Afros' (PanAftosa) in Brazil in 1951, and the Plum Island Animal Disease Centre in the United States in 1953, which were opened specifically to study FMD (Brooksby, 1982). Others in Africa include Onderstepoort in South Africa, Botswana Veterinary Institute in Botswana and the FMD Laboratory at Embakasi, Kenya in 1958.

The logistically difficult and costly efforts required to eradicate the disease resulted in countries, which had achieved eradication becoming wary of reimporting it from endemic areas. They consequently instituted measures to prevent this by placing trade embargoes on livestock and livestock products imports. This applies to countries where efficient control is not practiced or where the epidemiological situation with FMD had not been accurately established (James and Ruston, 2002). This is the main reason why Kenya cannot export livestock or frozen meat to countries like the US and the European Union.

In the late 19th and early 20th Centuries, FMD outbreaks occurred sporadically in Europe, but their occurrence had devastating consequences (Barteling and Vreeswijk, 1991). However, by

the early 1950s, some countries in Western Europe were experiencing numerous outbreaks per year (Brown, 1992). The recent epidemics of FMD in countries such as Taiwan, Japan, S. Korea, the U.K. Ireland, France and Netherlands have re-emphasized the devastating repercussions that FMD can have (Gibbens *et al.,* 2001).

These effects have primarily been economic, for example the direct and indirect costs of the 2001 outbreak in the U.K. may be as high as US dollars 12 billion (Thompson *et al*., 2002). The Taiwan outbreak was reported in 1997 (Yang *et al.,* 1999) and subsequently, starting in late 1999 and 2000, a series of FMD outbreaks were experienced in several countries in East Asia. This was followed by an outbreak in South Africa that culminated in the destructive outbreak in the United Kingdom, which spread to the European continent. The World Reference Laboratory identified a serotype O Pan- Asia lineage virus as the causative agent of all of these outbreaks (Knowles *et al.*,2001). This lineage had originated from India in 1990 and spread through the Middle East, Turkey, and Eastern Europe. It had moved eastward into China in 1999 and then to Taiwan, South Korea in March 2000, Japan, Mongolia, and Far-East Russia. The virus then appeared in South Africa in September 2000 and in the United Kingdom in February 2001 and again in 2007. The outbreak in Surrey in August 2007 was associated with escape of the virus from the nearby Research Laboratories; Institute of Animal Health Ltd. and Merial Animal Health at Pirbright only 4 Km away. The primary control strategy was the slaughter of infected and exposed animals using one kilometer radius from the infected farm as the zone of slaughter and a three kilometer intensive surveillance zone.

In Kenya and most other countries where FMD is endemic, control is by vaccination and quarantine, as slaughter of animals would be too costly for developing countries and have no ability to compensate the farmers.

2.4 Economic importance of FMD

FMD has great impact on economic development both in terms of direct and indirect losses. The disease is endemic in sub-Saharan Africa (Vosloo *et al*., 2002; Sumption *et al*., 2008; Rweyemamu *et al*., 2008). Some regions of the world including the whole of North America and Europe have however been able to eradicate it and been declared disease free by the World Organization for Animal Health (OIE-*Office International des Épizooties*) (OIE, 2012). Nevertheless, some of the disease free regions of the World occasionally suffer sporadic incursions an occurrence that has prompted concerted global control efforts to assist endemic countries control and possibly eradicate this disease (WOAH, 2012). It is estimated that the annual global impact of FMD in terms of production losses and vaccination costs alone are in the region of 5 billion US dollars (Ruston *et al.*,2012). It is listed as a notifiable disease by the World Organisation for Animal Health (WOAH, 2016) in the group of notifiable diseases that affect many species. The impact of FMD is more significant in trade where infected countries and zones face access problems to domestic, regional and international markets for animals and their products thereby threatening livelihoods, jobs and the economy.

Foot and mouth disease ranks highly among the most economically devastating animal diseases in the world (OIE, 2004). Economic losses can be attributed to both direct and indirect costs. Direct effects of the disease include loss of milk production, loss of cart power, growth retardation, abortion in pregnant animals, death in calves and lambs, whereas indirect losses are attributed to the disruption in trade of animals and their products (Blood and Radostits, 1989). Its effects are found to be more important than the acute illness itself (Woodbury, 1995). Taiwan had been free of FMD for 68 years until the 1997 outbreak which culminated into the slaughter of more than 4 million pigs; almost 38% of the entire pig

population at an approximated cost of U.S. $6 billion (Yang *et al.*, 1999). Taiwan was thus declared an FMD-infected zone and lost its pork export market. This reminded the international animal health community of the severe economic consequences that an FMD outbreak could have for a previously disease-free country. Later in 1999 to 2000 another outbreak occurred in Taiwan affecting cattle and goats but was more limited than the 1997 incursion. Nucleotide sequencing of the virus isolated from infected animals revealed that the virus was different from the 1997 virus but closely related to viruses circulating in the Middle East and India (Huang *et al.*, 2001). In March 2000 a large FMD outbreak occurred in South Korea after having been free of the disease for 66 years and a much more limited outbreak occurred in Japan after a 92 year FMD freedom. The Korean outbreak was controlled by the slaughter and vaccination of all cloven-hoofed animals within the affected provinces, resulting in the destruction of over 500,000 animals, mainly cattle (Joo *et al.,* 2002). The virus isolated from infected cows was identified by the World Reference Laboratory as serotype O and was closely related to the 1999 Taiwan isolate. The Japanese outbreak was limited to a few farms and was controlled by slaughter without vaccination. Sequence analysis also placed this virus, in the same lineage as the Taiwan and Korean isolates.

South Africa had also been free of the disease for a long time until September 2000 in which the causative agent was identified as a member of the Pan-Asian type O lineage similar to the Korean and Japanese isolates; a serotype that had never before occurred in South Africa (Sangare *et al.,* 2001). In 2001 and 2007, this pandemic spread to Great Britain, which had also been free of FMD since 1981.

In this outbreak, 2030 cases occurred between February and September 2001, and spread to Ireland, France and the Netherlands (Scudamore and Harris, 2002). As a result, farmers in the UK were compelled to slaughter approximately 4 million infected and in contact animals whose cost was estimated to be more than US $29 billion (Defra, 2005).

These outbreaks re-emphasized the extreme virulence of FMDV in a variety of animal species, the vulnerability of FMD-free countries as well as countries where FMD is enzootic to new viral strains. The effects of globalization on increasing the risks of disease incursion, emphasizes the need for countries to more closely monitor for the presence of exotic diseases.

Mortality rates in adult animals in FMD outbreaks are low but the disease has debilitating effects, while in the young animals, morbidity and mortality rates are high (Kahn and Scottline, 2005). Calves may die even before the appearance of any clinical signs due to viral infection of the developing heart muscles producing a severe myocarditis(Woodbury, 1995). When the disease breaks out in susceptible cattle, it spreads very rapidly and morbidity rates (especially cattle and pigs) approximate 100%. This combined with time and money spent treating animals and their long convalescence contributes to consider it the single most important animal disease in a worldwide contest (Solomon, 1980).

2.5 Host range

The FMD virus naturally affects more than 70 species of cloven-footed animals (Hedger, 1976). All cloven-hoofed animals both domestic and wild are susceptible including cattle, pigs, sheep, goats, and wild animals such as buffalos, deer and antelopes (Blood and Radostits, 1989; Fenner *et al.*, 1993). Cows and pigs are more susceptible and show greater

severity of signs than sheep and goats (Alexandersen *et al*., 2003). Camelids such as llamas and alpacas can also be infected, although, the disease in these species is often subclinical.

Though Foot-and-mouth disease is believed not to be a zoonosis, a few possible cases of human infections have been described (Bauer, 1997). They are very rare in that there has only been one recorded case of FMD in a human being which occurred in Great Britain 1966 (Defra,2005; O.I.E, 2004). The general signs and symptoms of the disease in that case were similar to influenza with some blisters. It is a mild short-lived and self-limiting disease in humans (Bauer, 1997). Human infections if and when they occur are associated with severe epidemics in animals, whereby transmission is commonly through contact among animal handlers or ingestion of infected animal products. Initial signs include fever followed by vesicles on the lips, tongue and pharynx, and in severe cases, lesions are seen around the nails on the fingers, on the conjunctiva and the ears (Brooksby, 1967). Since human infections are exceedingly rare precautions against infection are never taken in the laboratory or field situations to protect against human infection. A more important concern with respect to people is their capability to act as a fomite of the infection. People who are around infected animals can inhale infectious virus and harbor it passively in their pharynx for a day or two, moving from an infected area to a non-infected zone, coughing or sneezing, and passing the virus on to susceptible animals.

2.6 Incubation period and virus excretion

The incubation period of FMD can range from 1-14 days but the incubation period of FMD is most likely to be 2-5 days (Alexandersen *et al.*, 2003).The incubation period depends on pathogen dose, a higher dose is likely to lead to a shorter incubation period. The incubation

period is also affected by: virus strain, species, pre-existing immunity, physiological status and route of transmission.

Virus excretion can begin up to two days prior to or at the appearance of clinical signs, but virus can be detected experimentally in milk up to 4 days before the appearance of clinical signs. Virus excretion usually ceases about 4-5 days after the appearance of vesicles, except in the oesophageal-pharyngeal fluid (Alexandersen *et al.*, 2003; Charleston *et al*., 2011).Virus is present in fluid from ruptured vesicles and in almost all secretions and excretions including serum, oral and pharyngeal fluid, urine, faeces, semen and milk. It can also be detected in bone marrow and lymph nodes of carcass meat.

Large quantities of virus are released in expired air, particularly in pigs. An infectious pig can produce up to 400 million infectious doses (TCID50) per day, ruminants excrete a maximum of 120,000 infectious doses per day. (Alexandersen *et al.,* 2003). For this reason, pigs are seen as important amplifiers of FMD, with the potential to produce vast quantities of airborne virus.

2.7 Transmission and Pathogenesis

Transmission of FMDV is usually direct by contact between animals excreting the virus and susceptible animals but may occasionally be indirectly transmitted by contaminated objects or materials. Contamination is from virus containing secretions, excretions and tissues or by animal products such as milk or by air currents in which virus containing aerosols are suspended. The main route of infection in ruminants is through the inhalation of droplets. Ingestion of infected feed, inoculation with contaminated vaccines, insemination with

contaminated semen, and contact with contaminated clothing, veterinary instruments, and other fomites can all produce infection (Tesfaye, 2006). Infection can also be airborne, especially in temperate zones (up to 60 km overland and 300 km by sea). People, animals, vehicles and birds may serve as mechanical transmitters of infection. Domestic pigs are the most efficient excretors of FMDV into the environment and acquisition of infection other than in pigs, in which it is generally oral, usually occurs by inhalation.

Following contact with other infected animals, the disease takes an incubation period of 1-14 days, during which initial virus multiplication occurs in the infected animal (Sutmoller and McVicar, 1976; Burrows *et al.,* 1981). In animals infected via the respiratory tract, initial viral replication occurs in the pre- pharyngeal area and the lungs followed by viremic spread to other tissues and organs before the onset of clinical disease (Tesfaye, 2006). The virus is then distributed throughout the body, to reach best sites of multiplication such as the epithelium of oro-pharynx, oral cavity, feet, the udder and heart.

Virus probably replicate in the mammary gland of susceptible cow and in the pituitary gland. Viral excretion commences about 24 hours prior to the onset of clinical disease and continues for several days. The virus multiplies in great abundance in superficial tissues of mouth and feet causing vesicles which when they rupture release vast numbers of infectious viral particles which contaminate the environment. Just before appearance of clinical signs the virus is excreted from all secretions and excretions (Kitching, 2002). The virus is also excreted in relatively high levels in droplets in breath, in faeces, urine, milk, semen and may be shed before onset of clinical signs. The acute phase of the disease lasts about one week and viremia usually declines gradually coinciding with the appearance of strong humoral

responses (Murphy *et al.,* 1999). Recovered cattle produce neutralizing antibodies and can resist re-infection by the same subtype of virus for up to one year.

Once inside the infected cell, the RNA is released from the capsid and translated into a single polyprotein that is cleaved into individual proteins. These consist of Structural Proteins (SPs) that will form the viral capsid and Nonstructural Proteins (NSPs), which are involved in viral replication (Belsham, 1993). The virus is capable of replicating fast and spreading at an alarming rate. This phenomenon was demonstrated in the1997 Taiwan outbreak in which the first case was reported in March 1997 and within 3 weeks had spread to almost the entire island (Yang *et al.,* 1999).

Once an outbreak begins, most transmission is by aerosol from one infected animal to another. Pigs especially produce a tremendous amount of aerosol (Table 2.1), their exhalations having 30-100 times more virus than those of sheep and cattle hence termed *amplifier host*. Sheep often do not appear very ill when infected with Foot and mouth disease and have been implicated in much of the transmission. Apparently unaffected sheep are transported from one area to another, carrying the virus with them and so they have been called the *maintenance host*. When these sheep mix with cattle, the cattle develop severe clinical signs of slobbering and lameness, often raising the red flag of infection and so they are referred to as *indicator host*. The reasons for the rapidity of spread to fully susceptible populations is due to the highly infectious nature of the virus, the production of high titer in respiratory secretions and droplets. The virus is stable, has rapid replication cycle and the short incubation period (Sellers, 1971).

During the FMD outbreak that occurred in France and the UK in 1981, virus spread from France to the UK over 250 km. Kitching (1992) also emphasized the possible spread of FMD up to 250 km across the sea and up to 60 km across land if conditions are suitable. At present there are computer models which can predict the most likely wind-borne spread of the virus from infected herds and allow the examination of a variety of control strategies (Dijkhuizen, 1989; Sanson *et al.,* 1991).

Table 2.1: Infectious dose (TCID50) by different routes in the major domestic species

Species	**Inhalation**	**Intradermal**	**Intramuscular**	**Nasal Instillation**	**Oral**
Cattle	10	100	10^4	10^4-10^5	10^5-10^6
Sheep	10	100	10^4	10^4-10^5	10^5-10^6
Pigs	**>800**	100	10^4	Unknown	10^4-10^5

Adapted from (Alexandersen *et al.,* 2003)

2.8. Pathology

In cattle, the diagnostic lesions are single or multiple vesicles ranging from 2 mm to 10 cm. These can occur at all sites of predilection. Usually gross lesions on the tongue progress in the following manner; a small-blanched whitish area develops in the epithelium; fluid fills the area and a vesicle is formed; vesicle enlarges and may coalesce with adjacent ones and then rupture, leaving an eroded red area. Gray fibrinous coating forms over the eroded area that becomes yellow, brown or green till the epithelium is restored (Woodbury, 1995).The vesicle in the interdigital space is usually large because of the stress on the epithelium caused by

movement and weight. The lesion at the coronary band at first appears blanched; then there is separation of the skin and horn. When healing occurs, new horn is formed, but a line resulting from the coronitis is seen on the wall of the hoof. Animals that die may have grayish or yellowish streaking in the myocardium indicating degeneration and necrosis, a condition normally referred to as “tiger heart". Skeletal muscle lesions occur but are rare (Woodbury, 1995).

2.9. Immunity

Immunity to FMD is primarily antibody mediated. Antibodies begin to be detected by ELISA 3-5 days after first appearance of clinical signs, and high levels of antibodies are reached 2-4 days later (5-9 days after the appearance of clinical signs). The antibody titre remains high after infection, and is detectable for several years in ruminants. In contrast, antibodies may only be detectable for a few months in pigs, especially fast growing young animals (Alexandersen *et al.,* 2003).

2.10 Geographical Distribution

2.10.1 Global distribution

According to O.I.E reports the disease is distributed worldwide and with the exception of New Zealand, outbreaks have been reported in almost every livestock containing region of the world (O.I.E, 2004). The disease is currently endemic in all continents except Australia and North America. As shown in Figure 2.1, between 1990 and 2002, almost all the continents were affected in one way or another (O.I.E, 2004). Of the seven Foot and mouth disease virus serotypes O is the most encountered worldwide (Reid *et al.,* 2002). Serotypes A and O have the widest distribution occurring in Africa, Asia and South America. Types SAT 1, 2 and 3

are currently restricted to Africa and Asia 1 to Asia (FAO, 2007). Africa harbours all the serotypes except Asia 1. The disease can spread so rapidly that the question of which types are enzootic or exotic to any one country can never be regarded as static. Furthermore, any virus type breaking into a new area is likely to cause disease on an epizootic scale (Donaldson and Kilm, 1996).

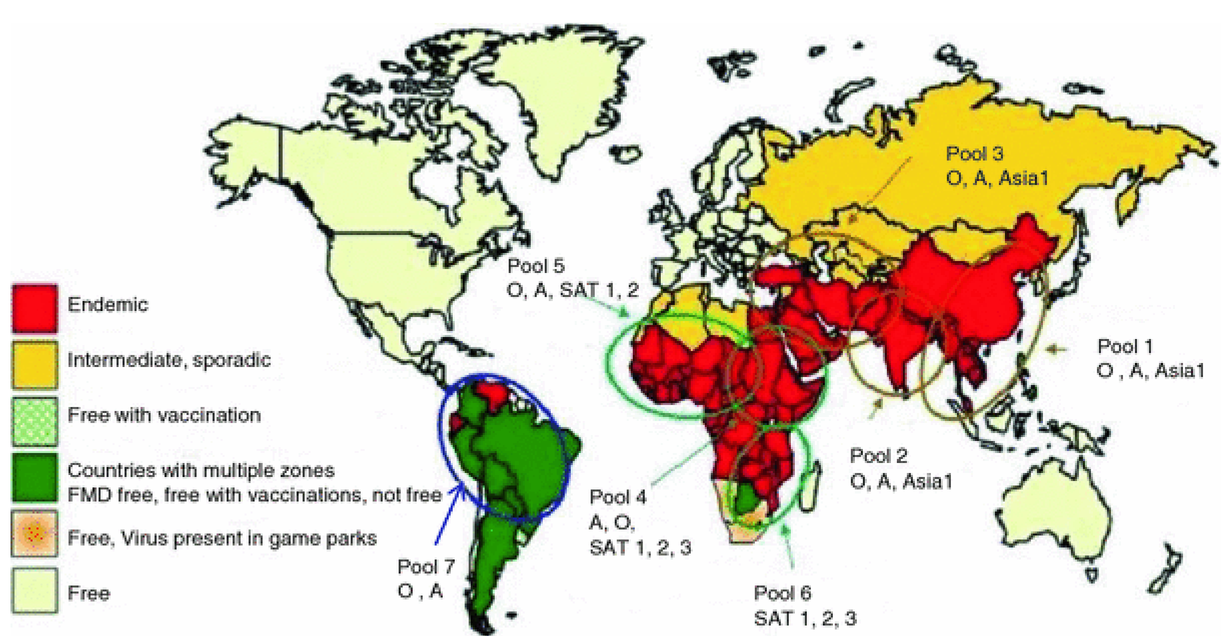


Figure 2.2 Maps showing the global distribution of FMD by serotype/Pools

Source: The conjectured status of FMD in 2010 showing approximate distribution of regional virus pools (Annual OIE/FAO FMD Reference Laboratory Network Report, 2010).

2.10.2 FMD in Kenya

Foot and mouth disease is endemic in Kenya with recorded cases being more than a decade ago in 1915. The five serotypes which have been detected in circulation in the past are O, A, C, SAT1 and SAT2 (Ngichabe, 1984; Vosloo *et al.,* 2002) but serotype C has not been encountered since 2004. Between 1995 -1999 the most prevalent serotype in outbreaks

recorded was SAT2. In most years serotype O has dominated but in 2009 SAT 1 incidence rose steadily (FMD Laboratory Annual Report, 2009) reaching a peak in mid-2010. Phlyogenetic analysis at WRL, Pirbright showed that in the rise in SAT 1 cases were as a result of an incursion new strain which originated from Transmara in 2009 and traversed the country causing major outbreaks even in vaccinated herds in Central and Eastern province in 2010. This strain was divergent from the vaccine strain by about 10 % thus the lack of protection. In the last five years 2017 to 2021 serotype O has been the most common cause of outbreaks in the country followed by SAT 1, SAT 2 and A. Foot and mouth disease was first characterized in Kenya in 1932 and sero-typing results are available since 1954 (Wariru, 1994).

2.11 The role of wildlife in transmission

A general observation has been that wherever in the world FMD has been eradicated from livestock, it has also generally disappeared from wildlife in those regions (Thomson *et al.,* 2003). Similarly, outbreaks of FMD in zoological gardens have coincided with outbreaks of FMD in domestic animals. In Sub-Saharan Africa, wildlife is clearly involved in the maintenance of FMD. Wildlife in South Africa, particularly the Cape buffalo ((*Syncerus caffer)* has been identified as natural hosts for the SAT serotypes of FMDV, although they may be infected by all serotypes (Hedger, 1976). In East Africa, however, little is known about the occurrence and distribution of FMD diversity in wildlife but recent work (Omondi *et al.*, 2020) has highlighted the dynamics of FMD in wildlife species. Cattle in many areas in Africa including Kenya are reared on open rangelands with communal grazing and potential contact with wildlife populations.This wildlife-livestock interface is critical for disease transmission particularly around common watering points and through contamination of

pastures. Other factors include cattle straying into wildlife conservancies especially during the dry seasons in search of pasture and coming in contact with wildlife species.

In South Africa fences have been erected to separate wildlife from domestic animals particularly buffalos and cattle and vaccination in the buffer zones has also helped to improve disease control (Scones *et al*., 2010). The only locality in which overt FMD has been reported regularly in wildlife over the last 60 years is the Kruger National Park of South Africa where there have been 31 outbreaks of FMD in Impalas since 1938. The disease has been reported in several species of wildlife. Examples are the African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*) species, Warthog (*Phacochoerus aethiopicus*), and African savanna /forest elephants (*Loxodanta Africana/Loxodanta cyclotis* respectively). These animals have the ability to both maintain and transmit the disease. The virus can persist in an isolated herd of buffalo for up to 24 years, whilst an individual animal can maintain the infection for up to five years. Furthermore, buffalo have unequivocally been shown to be a source of infection for cattle under both natural and experimental conditions (Sangare, 2002). The mechanism facilitating SAT-type virus transmission from buffalo appears to occur readily when there is close contact between the two species during acute stage of infection and shedding large amounts of virus. Impala (*Aepyceros melampus*) is the most frequent infected species and act as an intermediary in disease transmission of the disease between livestock and buffalos.

Although studies have established that individual impala do not become carriers, it appears that the disease can persist in impala populations for between 6 and 13 months (Vosloo *et al.,* 2002). Kudu (*Tragelaphus strepsiceros)* were shown to be gradually infected, with the carrier

state of between 106-140 days being demonstrated. Experimental infection of warthog (*Phacochoerus aethiopicus*) with SAT2 type virus resulted in severe clinical signs of infection, and transmission to in-contact animals.

Wildlife do not excrete virus to the level of domestic pigs and are not believed to play an important role in the epidemiology of FMD in Africa. Rare case of FMD have also been reported in Indian elephant (*Elephas maximus*) and in the African elephant (*Loxodo africana*) (Thomson, 1994).

2.12 Clinical signs, Chronic FMD and The carrier state in animals

2.12.1 Clinical signs

All cloven-hoofed species and camelids are susceptible to infection with FMDV although the severity of clinical signs varies depending on several factors. These include the level of immunity, the virus serotype, strain, the quantity of infectivity (the exposure dose), host species, age, breed and even variation between individuals of a given herd. Cows and pigs show greater severity of signs than sheep (Alexandersen *et al*., 2003). They sometimes have inapparent infection and when lesions appear in sheep are short -lived. Outbreaks involving indigenous or *Bos indicus* type of cattle as found in the rangelands are often mild and difficult to recognize. FMD is characteristically a rapidly spreading infection. When susceptible animals are in contact with clinically infected animals, clinical signs usually develop in 3 to 5 days (Kitching, 2002), although in natural infection, the incubation period may range from 2-14 days.

Acutely infected cattle salivate profusely and develop a nasal discharge (mucoid and then mucopurulent). Following pyrexia (about 40ºC), vesicles appear on the dorsum of the tongue, hard palate, dental pad, lips, gums, muzzle, coronary bands and interdigital space with consequent salivation and lameness. The morbidity rates in most outbreaks approach 100% of the whole herd. In adult animals mortality rates are low (generally reported as less than 5%) although instances of rates as high as 30% have been recorded in cattle. In young animals however death can occur due to myocarditis and mortality can exceed 50% (Woodbury, 1995). Lameness is usually the first indication of FMD in sheep and goats; however, vesicles in the interdigital cleft are the most common findings in sheep while lesions at other sites are unusual (Hughes *et al.,* 2002). Small ruminants tend to have milder clinical signs that often go unnoticed.

The most obvious symptoms of FMDV infection are elevated temperatures and the appearance of vesicles on the tongue and epidermis adjacent to the hoof, namely, the coronary band and, in ruminants, interdigital skin and mammary glands in females. Lesions first appear within 1 to 5 days, with an average incubation period of 3-14 days. Subsequently, just before the appearance of visible clinical signs, there is the excretion of the virus from all secretions and excretions (Kitching, 2002). Depending on the intensity of contact, the virulence of the virus, and the virus load, infected animals have viremia from around the first day of infection and later vesicles appear on the feet and in and around the mouth (Bachrach, 1968; Alexandersen *et al.,* 2003).

The characteristic appearance of the lesions over time means aging of these lesions can be used to estimate timelines of infection which is helpful in epidemiological investigations (DEFRA, 2005).

The vesicles usually rupture within 24-48 hours leaving shallow erosions and these lesions are susceptible to secondary bacterial infection. At this stage animals are reluctant to eat and move. Other signs include licking of the feet or shifting weight from one leg to the other, holding one hoof off the ground, lagging behind the herd, lying down and reluctance to rise (Brooksby, 1982; Woodbury, 1995). Vesicles may also be seen on the teats of lactating animals. Morbidity is high and young calves may die before the appearance of clinical signs due to virus infection of the developing heart muscle and the production of a severe myocarditis (Woodbury, 1995). However, most animals recover from the disease wthin two weeks.

In acute infections in cattle, the initial signs are fever of 103-105° F (39.4-40.6^o C), dullness, anorexia, and reduced milk production. These signs are followed by excessive salivation, smacking of the lips, grinding of the teeth, drooling of saliva, serous nasal discharge, shaking, kicking of the feet or lameness, and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats (Woodbury, 1995; Sahle, 2004).). After vesicle formation, drooling may be more marked, and nasal discharge, lameness, or both may increase, and young calves may die suddenly without developing any vesicle because of inflammation of the heart muscle (Blood *et al.*, 1994). The vesicles in various locations usually rupture leaving shallow erosions that are susceptible to secondary bacterial infection. At this stage animals are reluctant to eat and move. Other signs include licking of the feet or

shifting weight from one leg to the other, holding one hoof off the ground, lagging behind the herd, lying down and reluctance to rise (Brooksby, 1982; Woodbury, 1995). Pregnant cows may abort as a result of fever (Blood *et al.*, 1994). Vesicles may also be seen on the teats of lactating animals and this leads to difficulty in milking properly and thus mastitis is a common complication in cows. A lactating animal may not recover to pre-infection production because of damage to the secretory tissue. Most animals recover within two weeks although bacterial infection may delay recovery of mouth, feet and teat lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight, and breeding problems (Tesfaye, 2006).

In swine other vesicular diseases, such as swine vesicular disease (SVD), vesicular stomatitis, and vesicular exanthema of swine, cause signs so similar to those of FMD making differential clinical diagnosis alone difficult (Bachrach, 1968). In swine lesions often occur on the snout but in other species lesions on the muzzle are rare. In pigs, the initial signs are fever of 104-105°F (40-40.6^{o}C), anorexia, reluctance to move, and squealing when forced to move. These signs are followed by vesicles on the coronary band, heels, inter-digital space, and on the snout. Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle. Pigs and may proceed directly from epithelial necrosis to erosions without vesicle formation. Lesions tend to be a "dry"-type ; there is no drooling; sows may abort, and piglets may die without showing any clinical sign (Blood *et al.*,1994).

In sheep and goats, if the clinical signs occur, they tend, to be very mild, and may include dullness, fever, and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mild lameness may be the only sign. In lame animals, there may be vesicles or erosion on the

coronary band or in the interdigital space. Infected animals may abort and nursing lambs may die without showing any clinical signs (Hughes *et al.,* 2002). Mouth lesions are less common and less pronounced in these species. In sheep and other small ruminants lesions commonly occur on the dental pad where they may be difficult to detect. Usually the mortality in adult animals is negligible (1-2%) although rates as high as 30% have been recorded in cattle, but it may be considerably high in young animals where the virus can affect the heart. Some Pan Asian O lineage has recently been associated with high mortalities in pigs. Small ruminants play an important role in the epidemiology and transmission of Foot-and-Mouth Disease (FMD). The main reasons is that FMD is difficult to diagnose as infected sheep do not always show typical clinical symptoms or as the cardinal signs mimicks other diseases. Sheep and goats may be carriers. Infected herds which practice transhumance or are nomadic can spread the infection to other herds long before diagnosis of the disease is established (Dtsch *et al.,* 2001).

2.13 Foot-and-mouth disease virus carrier animals

Carriers are defined as animals in which FMDV persists in the mucosa of the soft palate, pharynx and oesophagus for more than 28 days after acute infection (Brooksby, 1982). In effect carriers are apparently healthy animals in which the virus is shed in small quantities from basal epithelial cells of the pharynx and dorsal soft palate. Persistent infection occurs in cattle for up to 2 to 3 years (Brooksby, 1982). Sheep may remain carriers up to 9 months and carrier status in buffalo can extend up to 5 years. Transmission of FMDV by livestock in carrier status is at most an extremely rare event. Conversely where African buffalo are concerned such transmission both in cohorts buffalos and cattle has been clearly demonstrated (Hedger, 1976).

It is generally accepted that there is a significant risk of transmission from carrier African buffalo to cattle. The risk of transmission from carrier cattle to other cattle cannot be excluded. Since carrier sheep tend to clear the virus more quickly than carrier cattle, the risk posed by carrier sheep can be considered to be lower than that posed by carrier cattle. Transmission from carrier cattle to other cattle has not been demonstrated experimentally, but it must be noted that if the event occurs only rarely in the field, it will be difficult to replicate in an experimental transmission setting (Tenzin *et al.,* 2008).

2.14 Chronic FMD

Chronic FMD has been encountered historically in cattle both in Europe and South America and is also recognized by pastoralist in East Africa. The condition is usually seen 3-6 months after acute FMD in adult cattle. It is manifested by heat intolerance resulting in an increased respiratory rate, reduced milk production, mastitis, a wooly or hairy coat. General unthriftiness and abortions with sometimes subsequent infertility. It mostly affects yearling cattle due to damage to their glandular tissue such as the thyroid. These animals are sometimes referred to as hairy panters' and may affect up to 5% of cattle in a herd. It has been suggested that heat intolerance is a sequel to FMD and is caused by damage to the endocrine system by the virus (Radostits *et al.,* 1994).

2.15 Diagnosis of FMD

The OIE Manual of Diagnostic Standards has described several techniques in use for the diagnosis of FMD infection (OIE, 2018). The virus can be isolated on cell cultures or the viral antigen detected using ELISA while the presence of viral genomic material can be detected using PCR assays. Other techniques include those that detect the presence of antibodies to the

non-structural proteins in non-vaccinated animals, and those that detect antibodies to the structural proteins in both non-vaccinated and vaccinated animals. In Kenya, as in most other laboratories in the world, two approaches are used to determine the presence of FMDV infection: either cell culture and antigen assays for the detection of the virus or assays for the detection of specific antibodies from sick or recovered animals.

Diagnosis of FMD usually begins with obtaining clinical information, examination of sick animals and sampling, followed by laboratory tests. Due to the number of atypical cases, sub-clinical infections or apparently recovered animals that harbor the infection, clinical diagnosis can present many difficulties. Therefore, other viral infections of the mucous membrane that produce similar clinical signs must first be eliminated, *e.g.,* Vesicular Stomatitis, Rinderpest, Malignant Catarrhal Fever, and Bovine Herpes 1 infections, Exanthema of pigs and Swine Vesicular Disease (Blood & Radostits, 1989). Due to the potentially devastating effects the disease can have on the economies of FMD free countries or zones, an appropriate sample should be collected followed by fast and reliable laboratory diagnosis. Since both the fluid contained in the vesicles and the epithelium covering the vesicles usually contain high concentration of the virus ($\geq 10^6$ infectious dose/ml or gm) these are the specimen of choice in acute cases. The fluid is easily aspirated from un-ruptured vesicles using a needle and syringe while the epithelial fragments can be simply cut free from the edges of the lesion with scissors.

Epithelial specimen contains detectable virus quantities for 4 days after appearance of clinical signs while that of the feet is detectable for 7 days. Fifty percent (50%) glycerol /phosphate buffered saline (PBS) and antibiotics is added to prevent putrefaction of the epithelial

fragments but glycerol cannot be added to vesicular fluid because it is toxic to cell cultures and so PBS is used alone. On reaching the laboratory glycerol may be removed from the specimen by washing with PBS or using absorbent paper. The sample is then processed using World Organization Animal Health standard tests (OIE Manual, 2018).

Traditionally, identification of infected animals has been done by obtaining oesophago-pharyngeal fluid (Probang) samples and subjecting them to virus isolation and identification by ELISA or CFT as per the O.I.E requirements. The latter has proved difficult due to problems associated with obtaining good material as the virus is masked by antibodies present in the material (Sakamoto *et al.,* 2002). For active cases an epithelium sample is taken to the laboratory in 50% glycerol/PBS and ELISA tests are carried out both in the original sample and in tissue culture passaged sample. Molecular science has advanced into quick identification by subjecting the samples to RT-PCR although the technique has low diagnostic sensitivity (Haas and Sorensen, 2002). This has culminated in renewed interest in the use of serological tests such as Virus Neutralization Tests (VNT). However, these tests are cumbersome and time consuming in that they require each serum sample to be tested separately for serotype specific antibodies which however is important in research situations. The use of VNT as used in Kenya requires cell-culture and virus bio-containment facility and this is possible in FMD Laboratory, Embakasi, and takes a minimum of 2-3 days to provide results. In Kenya there exist multiple FMD serotypes in infection among livestock and wildlife presenting challenges in diagnosis (Sangula *et al.*, 2005).

2.16 Control of FMD

2.16.1 Control strategies

In developing countries, control of the disease by eradication is too costly, hence,in Kenya FMD control is mainly through regular vaccination in conjunction with the control of animal movement. In Kenya vaccination is recognized as an effective control strategy to reduce the impact of FMD in endemic areas (Ngichabe 1984). In addition to vaccination the success to FMD control depends on zoo-sanitary measure like effective stock movement control. To effectively control the disease by the vaccination method, the vaccines used must contain the representative strains of the serotype in circulation (Gonzalez *et al.,* 1992). Therefore, in the study of FMDV in Kenya, antigenic relationships between isolates have been determined by serological tests to assess the vaccine strains to be used for the control of outbreaks (Nderitu, 1984). These vaccines are produced by growing the virus in suspension cultures of Baby Hamster Kidney (BHK) cell lines and subsequent inactivation using Binary Ethyleneimine (BEI) as recommended by WOAH (2018). This means efficient methods of diagnosis are required as a prelude to production of effective vaccines, disease surveillance, screening and control. According to Bulut *et al.*, (2002), for countries that use vaccination as their control strategy, it is important to differentiate antibodies resulting from vaccination with those resulting from patent infection. This will serve to detect persistently infected animals (otherwise known as carriers) that may occur either due to poorly inactivated vaccines, general vaccine failure, or lack of vaccination in any herd. This status is important in clearing animals for export to FMD free countries in accordance to standards set by WOAH (OIE, 2018).

According to international standards set by WOAH, vaccine purity is an import/export pre-requisite as the presence of trace amounts of non-structural proteins (NSPs) in vaccines may cause false positive reactions in animals that have been repeatedly vaccinated.

In Kenya these FMD control measures including vaccinations and animal movement control have not been applied at an intensity that could curtail the transmission and maintenance of the disease. The multiplicity of serotypes and low levels of vaccination coverage only serve to complicate the control efforts. The current FMD control strategy has been developed by the Directorate of Veterinary Services is to provide a roadmap for prevention, control and eradication of FMD. It provides the plan on how to manage the disease in the country in a structured and progressive manner such that important gains are made towards its eventual eradication while reducing loses from the occurrence and spread of the disease. The OIE FMD control pathway provides the framework (Figure 2.3). The various Government of Kenya Policy and legal provisions to FMD management in the country are the Kenya Vision 2030, Agricultural Sector Development Strategy, National Livestock Policy and Veterinary policy among others.

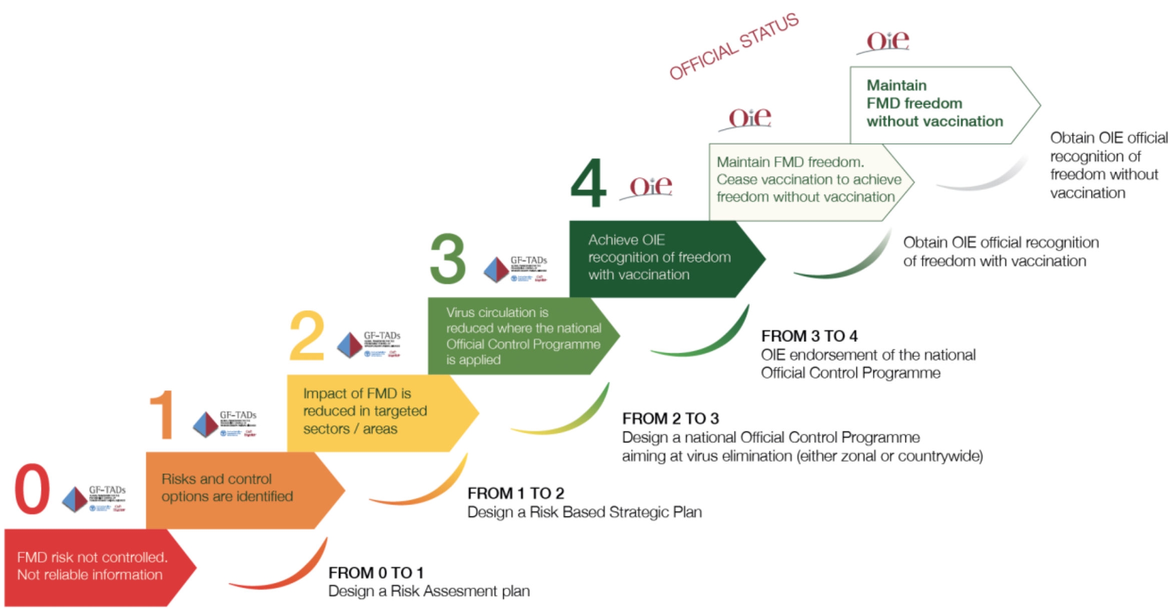


Figure 2.3 The Progressive Control Pathway for Foot-and-Mouth Disease (PCP-FMD) 2nd Edition 2018

2.16.2 Complexities in FMD control

2.16.2.1 Nomadic pastoralism and non-effective management of livestock movement

Cattle and other domestic animals in Kenya are mostly in the hands of pastoralists whose mode of management is by movement of stock in search of pasture and water. In East Africa, different animals are taken to different regions throughout the year, to match the seasonal patterns of precipitation (East African Community, 2010). Illegal stock routes for trade and movement as a result of drought is one of the main causes of disease spread. In the process of these movements, animal diseases like Foot and mouth disease are spread. Illegal livestock movement for marketing together with forced movement of livestock due to cattle rustling within some regions and in neighboring countries also contribute to disease spread. Thus,

uncoordinated livestock movements pose the greatest challenge to disease control in the region. This is coupled with common grazing areas and watering points thus disease spread is normally very dynamic.

2.16.2.2 Wildlife-Livestock interface

Another factor is that most of the wildlife-protected areas are in confluence with livestock grazing areas. Domestic and wild animals share many diseases including FMD. This interface constraints the control of such diseases. Role of wildlife in maintenance and transmission of FMD disease is recognized and the intensive wildlife - livestock interaction enhances disease transmission through contact, sharing of water and pasture(Jori *et al.*, 2021).

2.16.2.3 Vaccines

Current vaccines are expensive, have a narrow antigenic spectrum and since there is no cross-protection between serotypes vaccination has to involve use of multivalent vaccines. This is more complicated where the disease is endemic with several serotypes in circulation like in the Kenyan situation. This further complicates the cost of the vaccination. FMD vaccines also confer short duration of immunity after vaccination. In addition low levels of vaccination coverage make the disease to recur even within herds that are routinely vaccinated when the virus challenge is too high (Singh *et.al.*, 2021)

2.16.2.4 Multiplicity of FMDV strains

FMD virus has several serotypes and within each serotype antigenic variants continue to evolve which makes it necessary to do vaccine matching with the field strains a necessary

practice. Several serotypes, topotypes, lineages and sub-lineages have been found in circulation in East Africa.

2.16.2.5 Other constraints

Other constraints affecting the control of trans boundary diseases in the region include the following: Droughts leading to widespread movement of livestock in search of pasture and water, thereby spreading diseases. Other factors include high levels of insecurity including cattle rustling, inadequate technical personnel willing to work in the generally hostile pastoral environment. Also inadequate legislation and poor enforcement of the laws; low community participation in livestock disease control and inadequate disease diagnosis due to under-reporting of disease outbreaks.

Chapter 3: EPIDEMIOLOGICAL STUDY ON FOOT-AND-MOUTH DISEASE IN SMALL RUMINANTS : SERO-PREVALENCE AND RISK FACTOR ASSESSMENT IN KENYA.

3.1 INTRODUCTION

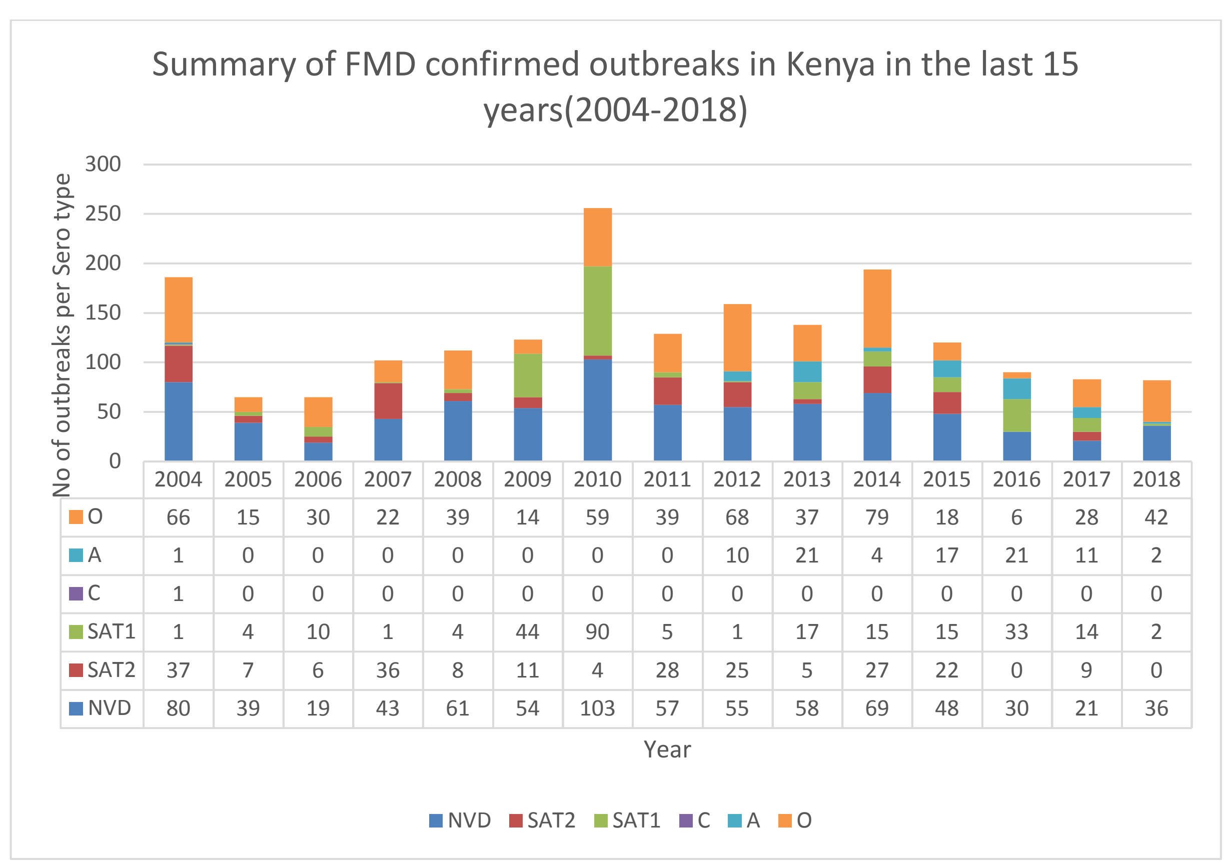
Foot-and-mouth disease (FMD) is endemic in Kenya causing serious economic losses in the livestock sector. The epidemiology of the disease in small ruminants (SR) in Kenya is not well documented.

Foot-and-mouth disease is classified by World Organization for Animal Health to be among the most important notifiable diseases that affects multiple species of animals. Presence of the disease causes a severe restraint to local and international trade of animals and animal products due to sanitary control measures thereby threatening livelihoods, jobs and the economy of a region or a country. It is therefore a disease with serious socio-economic consequences and is considered the most contagious trans-boundary animal disease (TAD) (FAO, 2007). It is estimated that the annual global impact of FMD in terms of production losses and vaccination costs alone are in the region of 5 billion US dollars (Ruston *et al.,* 2012). The disease is notoriously contagious that it can spread as much as 50 miles by wind from one outbreak area to another (Sansbury, 2000). Introduction of virus serotypes or subtypes to regions where they were previously absent can lead to epidemics of varying magnitude (Gibbs,1981). When the disease breaks out in susceptible cattle, it spreads very rapidly and morbidity rates (especially cattle and pigs) approximate 100%. The disease is rarely fatal except in young animals (Kahn and Scottline, 2005).This combined with time and money spent treating animals and their long convalescence contributes to consider it the single most important animal disease in a worldwide contest (Solomon, 1980). The disease generally

occurs in the form of an outbreak that rapidly spread from herd to herd before it is controlled (Radostits *et al.,* 2000).

FMD is a global disease that through the years has affected most of the countries around the world and is endemic in most countries in sub Saharan Africa (Vosloo *et al.,* 2002) with six of the seven serotypes reported to occur in East Africa namely O, A, C, SAT 1, SAT 2 and SAT 3 thus complicating the epidemiology and control of the disease in the region. Serotype SAT 3 has been recorded in Uganda (Vosloo *et al.,* 2002). The disease is endemic in Kenya the several outbreaks occurring all over the Country. In 2002, a study conducted in four dairy farms in Nakuru County during outbreaks revealed colossal losses amounting to a total of US$479105.75 (Kimani *et al.,*2005). Most parts of the country are currently experiencing outbreaks of FMD, which are attributed to at least four serotypes namely O, A, SAT 1 and SAT 2 (Figure 3.1).

In order to control and/or eradicate this disease in the targeted areas, a good understanding of disease epidemiology is important. This can only happen if the disease is traced and regular, and effective surveillance is done together with vaccination regimes being put in place (FAO, 2006). The most important resource in the prevention of FMD is informed animal owners. Livestock owners at all levels of production should be familiarized with basic features of FMD including the recognition of clinical signs of the disease, the need for reporting and management of the disease. Therefore the objective of this study was to determine the prevalence of disease and risks associated with the disease in two different production systems (sedentary and pastoral systems) in Kenya which can help in designing control strategies.



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
O	66	15	30	22	39	14	59	39	68	37	79	18	6	28	42
A	1	0	0	0	0	0	0	0	10	21	4	17	21	11	2
C	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SAT1	1	4	10	1	4	44	90	5	1	17	15	15	33	14	2
SAT2	37	7	6	36	8	11	4	28	25	5	27	22	0	9	0
NVD	80	39	19	43	61	54	103	57	55	58	69	48	30	21	36

Figure 3.1 FMD Reported and serotyped outbreaks in a period of 15 years (2004-2018)

Source: Created from FMD Laboratory Outbreak Records

3.2 FOOT-AND-MOUTH DISEASE IN SHEEP AND GOATS

3.2.1 Clinical signs

Typical clinical signs of FMD in sheep and goats include pyrexia, lameness and oral lesions, which are often mild foot lesions along the coronary band or interdigital spaces, and lesions on the dental pad, but these may go unrecognised, agalactia in milking sheep and goats and death of young stock without clinical signs.

The clinical picture of the disease in sheep and goats is described as less florid and subdued. Therefore it mostly remains unnoticed in these animals. Sheep and goats may also become virus carriers after exposure. Nevertheless, due to their ability to become carriers, poses major

risk of entry of FMD to disease-free countries through trade of these animals. Above all, small ruminants and wildlife species need the major focus for the real control of FMD in the country.

Contrary to the usual occult form of FMD as usually observed in small ruminants, marked clinical lesions and severe form of the disease have also been reported. Sheep have an incubation time of around 3-8 days. Lameness is often the first observed clinical sign in sheep and goats. Affected animals develop fever, show reluctance to walk and may separate itself from the rest of the flock. Vesicles may develop in the interdigital cleft, on the heel bulbs and on the coronary band, but they usually rupture rapidly and their appearance may be hidden by the co-existing presence of foot rot. In sheep, lesions can easily be confused with coronitis seen with bluetongue. Vesicles are formed in mouth which ruptures easily leaving shallow erosions, but commonly seen in the dental pad, adjacent to the incisors, also on the tongue, hard palate, lips and gums.

Up to 25% of affected sheep may not develop clinically apparent vesicles, and 20% have lesions only at one site or develop vesicles visible for less than three days. Vesicles may also be observed on the teats especially of lactating sheep and goats and rarely on the vulva and prepuce. Affected rams are unwilling to work and mate, and lactating animals suffer a temporary loss of milk yield. Compromised epithelium can predispose to other secondary infection and complicate the situation. Ewes may abort and young lambs and kids may die due to heart failure. In some epidemics, large numbers of lambs may fall down dead when stressed.

Figure 3.2 Foot lesion in the interdigital space and lesions on the dental pad of sheep with FMD.

Source : The Institute for Agricultural Protection and Health, Uruguay 2016 Congress on Bovine animal Health tracability

3.2.2 Transmission

Transmission of the virus in sheep and goats are highly prone to infection with FMDV by the aerosol through respiratory route. The virus most often infects these species by direct contact with infected animals. Infection can also occur through abrasions on skin or mucous membranes in contact with contaminated food. Aerosol transmission from infected sheep is unlikely to occur beyond 100 metres. The virus can persist for up to nine months in sheep and up to four months in goats. The pathogenesis of FMD has been studied mainly in cattle and pigs. Acquisition of infection, other than in pigs where it is normally oral, usually occurs by inhalation and the initial site of virus replication is thought to be the respiratory bronchioles of the lung. However, an earlier study showed initial replication of the virus occurred in the mucosa and possibly the lymphoid tissues of the pharynx, particularly in the tonsillar region of the soft palate. The virus then spreads via the bloodstream to Langerhans cells in epithelia, and all epithelial cells in contact with an infected Langerhans cell become infected. In infected animals FMDV is disseminated to many epidermal sites, but lesions only develop in areas subjected to mechanical trauma or physical stress.

3.2.3 Morbidity and Mortality in sheep and goats

The mortality rate is generally less than 1% in adult animals, but it can be much higher in young animals. Mortality rates of 40-94% have been reported in lambs (Donaldson, 2002). A case fatality rate of at least 50% was reported in wild ruminants like mountain gazelles. Clinical disease in young lambs and kids is characterized by death without the appearance of vesicles, due to heart failure. Nursing lambs may die without showing any clinical signs, but with grayish or yellowish streaking in the myocardium with degeneration and necrosis described as 'tiger' stripes, particularly in the left ventricle and interventricular septum ("tiger heart"). On carrier status sheep and goats may also become virus carriers after exposure. Around 50% of convalescent sheep may become persistently infected for up to 9 weeks, and a small number of animals may carry virus for up to 9 months. Cross-bred Southdown sheep can remain carriers for up to 5 months after exposure.

A number of mechanisms have been proposed for persistent infection with FMD virus. The pharynx is an important site of viral persistence. Despite representing the majority of the world's FMD-susceptible livestock, sheep and goats have generally been neglected with regard to their epidemiological role and significance in the spread of FMD. This is partly due to the often inapparent nature of the disease in these animals. Nevertheless, due to their ability to become carriers and act as reservoirs of infection, poses major risk of entry of FMD to disease-free countries through trade in these animals.

3.2.4.The role of sheep and goats in the transboundary spread of FMD

There are reports that, silent nature of FMD in small ruminants transmit the virus causing outbreaks in Asia (India), middle-east (Iran, Iraq, and Turkey), Africa (Libya, Algeria,

Morocco, Tunisia), south-east Asia (Cambodia, Laos and Thailand) and European countries (Bulgaria, UK, Ireland and France). There are many examples of FMD being carried into countries previously disease-free by the movement of infected sheep and goats. In 1983, FMD spread from Spain into Morocco by infected sheep. In 1989, Tunisia in Africa which was previously free from FMD got the disease in cattle with the importation of sheep and goat from the Middle East. The outbreak in Bulgaria in 1991 in cattle due to type 'O' virus was attributed to the alleged introduction of goat from Turkish village. Illegal sheep trade from Turkey into Lesbos in April 1994 was the cause for the origin of FMD in July 1994 in Greece. The outbreak of 1999 due to type 'O' virus in south-east Asia was traced and its origin was considered to be Myanmar from where the goats were imported. In February 2001, FMD outbreak in UK lasted for about 32 weeks, where the role of sheep in the spread of disease was realized. Additional movement of sheep resulted in the dissemination of the virus to Scotland, Wales, Northern Ireland, and France. Sheep products e.g. contaminated frozen lamb from Argentina was blamed as the source of origin of the largest ever recorded type O epidemic in the UK that occurred in 1967-68 (Hugh-Jones and Wright, 1970).

3.2.5. Justification for this study

Sheep and goats in Kenya are reared together with cattle and may present high risk of these small ruminants acting as silent reservoir of virus and silently spreading the infection to cattle which are more affected by the disease. Small ruminants may not manifest clinical signs, therefore they may may spread disease before diagnosis and furthermore the disease may be mistaken for other diseases in these animals, hence the need to determine risk factors and seroprevalence of FMD in small domestic ruminants.

3.3 MATERIALS AND METHODS

3.3.1 Ethics

The research approval for the study was obtained from the University of Nairobi Faculty Biosafety Animal Use and Ethics Committee (Appendix I) and Kenya National Commission For Science, Technology and Innovation (NACOSTI) (Appendix II). Other approvals required for the study were obtained from the State Department of Livestock at National level and from the respective County governments. Additionally, each owner of herds selected for sampling provided verbal consent, once the objectives of the study were explained. Herd owners who did not consent were replaced with the next herd in the random sample list. These set of approvals were also used for the Ukambani surveillance study (Chapter 4).

3.3.2 The study population

A cross -sectional study was carried out which targeted the national small ruminant population in Kenya. The study was conducted from August to September 2016. Kenya is made up of 47 counties and the objective was to primarily measure the sero-prevalence per the major small ruminant production zones. The sampling unit was the smallest administrative unit in record, the village, which was selected after first selecting the second smallest administrative unit, the sub-location. The sampling frame of sub-locations was available from the Kenya 2009 population and housing census (KNBS, 2009). Blood samples were collected from individual small ruminants to identify the presence or absence of antibodies in sera samples. This was done in order to determine what proportions of herds in the country are affected by FMD as indicated by detection of sero-positive animals in the herd. Questionnaires on risk factors

were also used to collect data on sampled herds on potential risk factors at individual animal and herd level. The livestock keepers were interviewed at the time of sample collection.

3.3.3 Description of the study area

Broadly, Kenya can be divided into three ecological zones namely humid, semi-arid and arid areas. About 80% of the country is arid and semi-arid (ASAL) while the humid ecosystem occupies the remaining 20% of the country. The semi-arid areas normally experience short rainfall with prolonged drought while arid areas have long cyclic droughts, thus affecting pasture and water availability. The humid areas have long rain seasons with heavy down pours reaching 2700mm (Sombroek *et al.,*1982). The main small ruminant production systems are pastoralism and agro-pastoralism as well as sedentary/mixed systems. Pastoral systems are in the arid and semi-arid climate zones where about 14 million people are dependent on livestock (Onono *et al.,*2013; Zaal, 1998). Agro-pastoralism, is livestock production which is associated with dryland or rain-fed cropping and animals range over short distances. The average herd size of sheep and goat in pastoralist systems is estimated at 24.9 and 75.2 respectively (Mwanyumba *et al.*, 2015). Sedentary/mixed systems are found in the semi-arid, subhumid, humid and highland zones. This farming system is based on livestock but practiced in proximity to, or perhaps in functional association with, other farming systems based on cropping or is a livestock subsystem of integrated crop-livestock farming. The average herd size of sheep and goats in this system is rarely reported but ranges between three and 10 (Otte and Chilonda, 2002; Zaal, 1998; Humpry *et al.*, 2004).

3.3.4 Study design and methods

3.3.4.1 Sample size determination

For the purpose of this study, the country was divided into two zones; the pastoral zone (PZ) and the sedentary zone (SZ) as in Figure 3.3 below. Sample size calculation was in two stages: number of herds to be sampled and then number of animals to be sampled per herd. A herd included a group of sheep and goats in a farm and animals from neighbouring farms which came into contact (in-contact farms). The formula used was that by Humphry *et al.*, 2004. The assumptions that were made were: number of herds >10,000 in each zone; confidence level = 95%; accepted margin of error = 5%; expected proportion of positive herds in the population = 70% (expert opinion, confirmed by Ahmed *et al.* (2020); intraclass correlation coefficient (measure of variation between clusters) = 0.1 (Otte and Gumm,1997); design effect = 2 (Rowe *et al.*, 2002); test specificity = 99%; test sensitivity = 100% (OIE Manual, 2018) . The calculated sample size was 323 x design effect (2) = 646 herds countrywide. The minimum number of animals to be sampled per herd was determined as summarized in Cannon and Roe table cited by Thrusfield (p.239) (Thrusfield,2007). This was determined by making the following assumptions: expected prevalence of FMD in the herd = 20%; which was an average of two consecutive years in Tanzania (Torsson *et al.*, 2017); confidence level = 95%; average herd size = 100. This yielded a sample size of 13 animals per herd which was increased to 14 to take care of any possible losses. Countrywide therefore, 646 herds resulting from one village per sub-location and one herd per village (or more if necessary to obtain sufficient animals) and 14 animals per herd yielded a sample size of 646 x 14 = 9044 samples. The two zones are quite distinct in structure even if FMD dynamics (in cattle) seem not very different. The SZ has small counties with very many sub-locations while the PZ has large counties with fewer sub-locations which could have led to over sampling in

the SZ and low sampling in the PZ coupled with extensive movement of animals in PZ causing inadequate samples during the study. It was also important to see the difference in the two zones and for this reason we chose to have a complete separation between the two and sample equal number of sub-locations (323) and therefore number of samples (4522) in each zone. Sampling zones and sites were as shown in Figure 3.3 and 3.4 respectively.

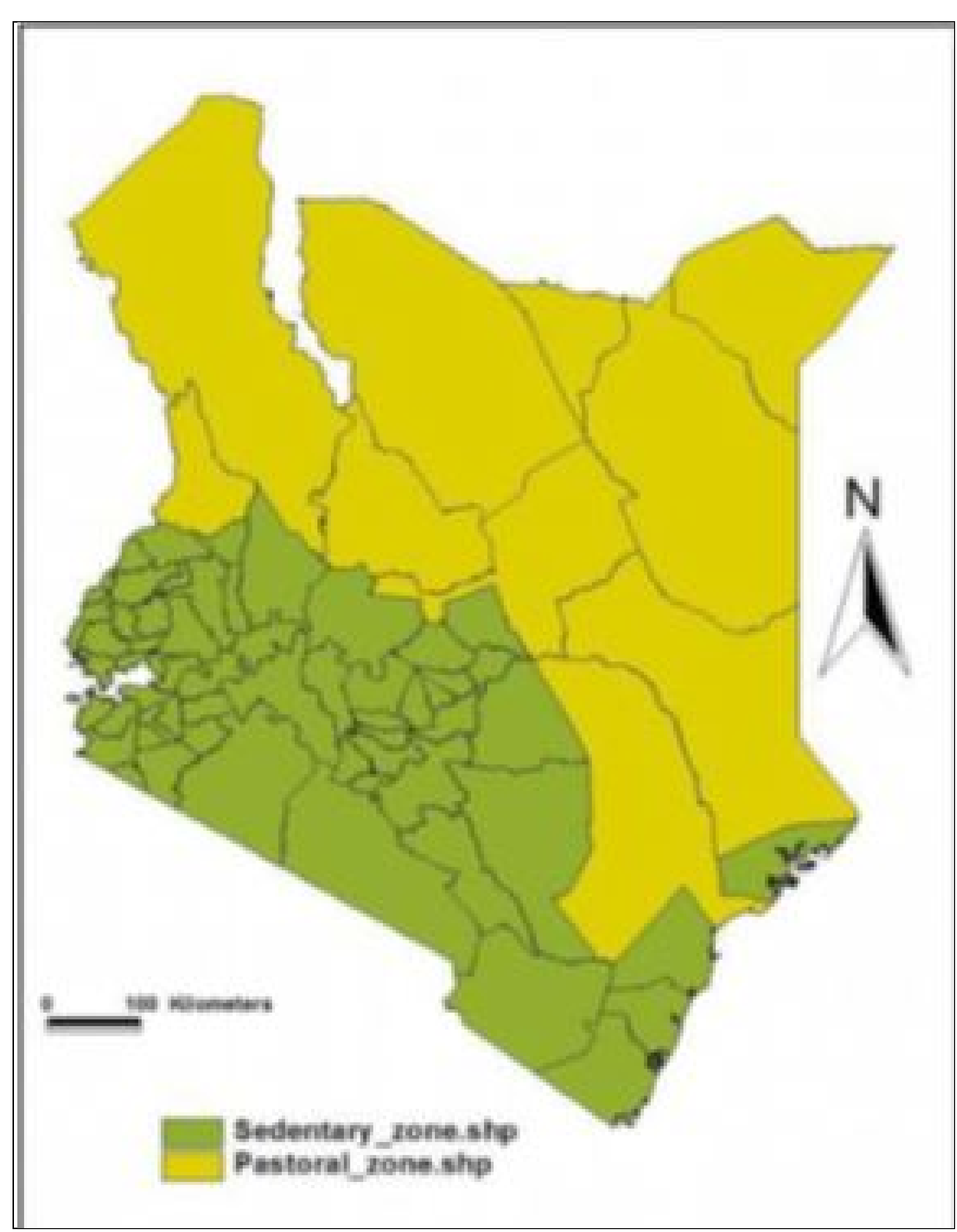


Figure 3.3: Study zones

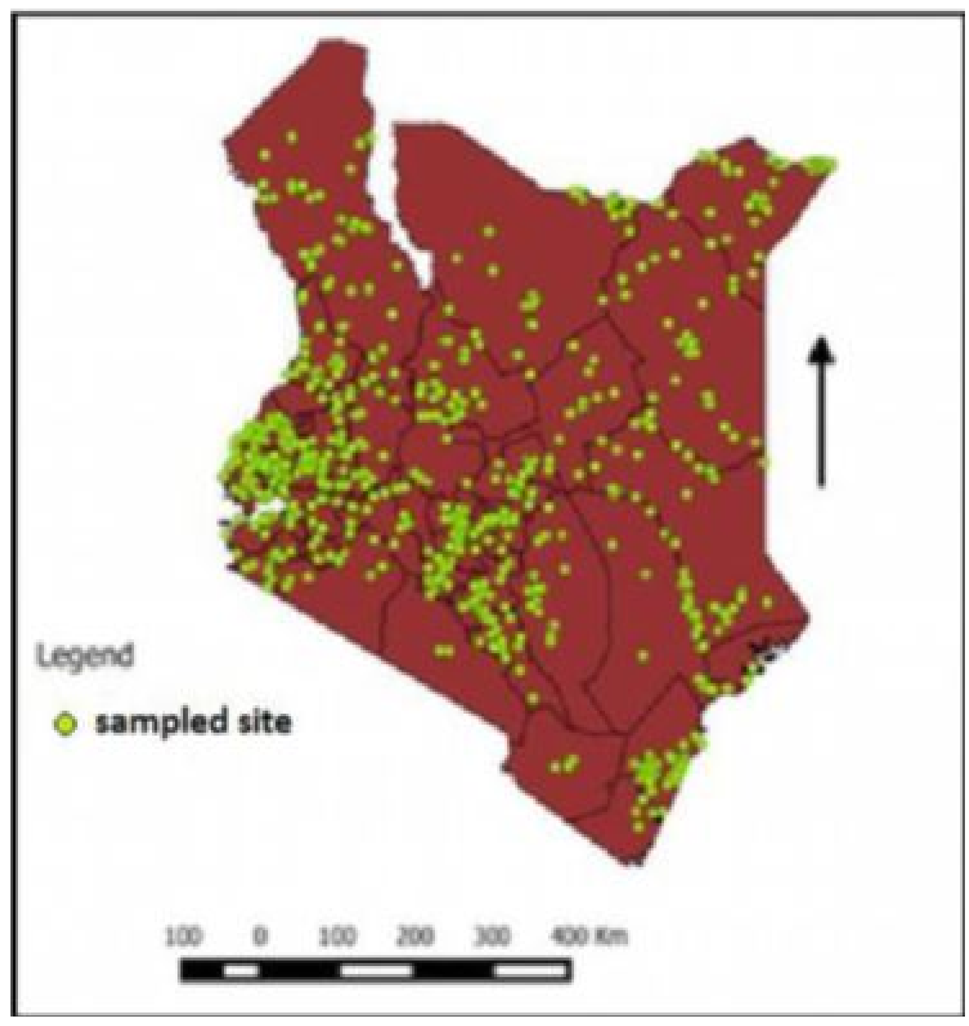


Figure 3.4: Selected sampling sites for the cross-sectional survey,Kenya 2016

3.3.4.2 Sampling of herds and animals

The sampling frame consisted of the 6796 sub-locations, as obtained from the national census of 2009 (KNBS, 2009). The sampling frame was used to randomly select the sub-locations to be sampled. However, sub-locations can be quite large and therefore once in the field the teams obtained the list of villages in the sub-locations and randomly selected villages within the sub-locations. A herd was then considered as all animals within the village from which the individual animals sampled were randomly selected. If one herd could yield all the animals required, only that herd was sampled, otherwise additional herds close to the selected herd

were sampled until the required number to be sampled in the sub-location was reached. Therefore, a multistage cluster sampling was employed to select the animals from the two zones (Niewiesk, 2014). Age was determined by examining the dentition of each animal and information from the owner for young animals with no permanent incisors (Eubanks,2012). In the sampling stage animal level variables (biodata) were collected into a sampling form. (Appendix III) and included species (ovine or caprine), breed, age and the sex of the animal and origin (whether born in herd or brought in). The blood samples were collected from a jugular vein, using 10 ml sterile vacutainer tubes and gauge 21 needles and labeled with a unique identification (county code/sub-location/animal number/sex/age). The samples were then allowed to clot in cool-boxes. Once the blood clot had retracted after 12 to 24 hours the vials were centrifuged in the laboratories in the field to obtain serum which was placed in two 2ml cryovials (two aliquots) labeled with corresponding identification codes. In areas where laboratories or centrifuges were unavailable, serum was separated using sterile disposable pipettes (one per sample) and transferred into the cryovials.

Samples were stored at -20˚C in freezers located in the areas sampled in the field until the end of the sampling period (which was not more than 20 days per team) and were transported on ice using cool-boxes to the Central Veterinary Laboratories (CVL), Kabete, Kenya. At the CVL, the samples were held at -86˚C until testing after which they were placed in a serum bank at the same temperature. Sample collection was part of a larger national survey for Rift Valley Fever (RVF) and Peste de Petits Ruminants (PPR) antibodies in small ruminants under a project titled "Improving Animal Disease Surveillance in Support of Trade in IGAD Member States", in short "Surveillance of Trade Sensitive Diseases–STSD." One aliquot was used to test for the presence or absence of antibodies for Rift Valley Fever (RVF) and Peste de

Petits Ruminants.(PPR) antibodies according to the objective of the STSD project. The second aliquot was moved to the FMD National Laboratory, Embakasi, Kenya and stored at -20˚C until laboratory investigation for FMD antibodies.

3.3.4.4 Administration of questionnaires

A pre-tested semi-structured questionnaire (Appendix III) was administered in-person by trained enumerators to owners of sampled herds following the guidelines at the time of sample collection to capture herd-level variables. Herd level variables were production zone, whether the herd owners brought in animals in the last one year, whether the herd owners purchased animals from the market/middlemen, interaction with wildlife, production type, production system, housing type, grazing system, watering system, breeding method and altitude/elevation. Also based on Geographic Positioning System (GPS) technology, GPS coordinates and elevations were recorded for each herd location and this information was recorded in each questionnaire form which was labeled with the unique herd identification code.

3.3.4.5 Laboratory sample analysis

Individual animal serum samples were analysed using the foot and mouth disease virus 3 ABC- ELISA ID Screen® FMD NSP Competition kit (ID-VET, Grabels, France) to detect specific antibodies against the non-structural protein (NSP) of FMDV regardless of sero-type. This was done according to the manufacturer's protocol. The test has specificity of 99% and sensitivity of 100% (OIE Manual, 2018). A herd was considered as positive if one or more animals in the herd were seropositive for FMD.

3.3.4.6 Data management and analysis

Individual animal laboratory data generated during testing along with individual animal biodata data obtained during sample collection (species, breed, sex, age, origin) were entered in Microsoft Excel 2010 spreadsheet. Questionnaire data which included mainly herd data (county type, production zone, whether animals were brought into the herd, whether animals were purchased from markets and middlemen, wildlife interaction, production type, production system, housing system, grazing system, watering system, breeding method and altitude/elevation) were entered in Microsoft Access 2010 due to the large amount of data and need to link the data tables. The required data columns from each data set were then brought together in a Microsoft Excel Spreadsheet, data cleaned and coded before being exported for descriptive analysis using IBM Statistical Package for Social Science (SPSS) Statistics for Windows Version 20 (IBM Corp., Armonk, N.Y., USA) and R version 4.0.3 (2020-10-10) for regression analysis. Descriptive analysis generated sums, means, proportions and confidence intervals. Descriptive statistics were also generated for the seroprevalence in the two different production zones (pastoral and sedentary), for each county and for the other potential risk factors. Apparent prevalence was calculated using Equation 1 (Thrusfield, 2007) while true prevalence was calculated using Equation 2 (Rogan and Gladen,1978). Confidence interval of the true prevalence was calculated using Equation 3 (Greiner and Gardner,2000; Reiczigel and Ozsva´ ri, 2010).

$$Apparent\ prevalence = \frac{No.of\ \ animals\ testing\ positive}{Total\ number\ of\ animals\ in\ the\ group\ tested}\ x\ 100 \qquad (1)$$

$$True\ prevalence = \frac{apparent\ prevalence + specificity - 1}{sensitivity + specificity - 1}\ x100 \qquad (2)$$

(95% *CI of true prevalence* = $p \pm 1.96\ \sqrt{(pq/nJ^2\ )}$ (3)

Where, p is apparent prevalence; q is 1-p; n is sample size and J^2 is Youden's index (Se+Sp-

1) where Se is test sensitivity and Sp is test specificity.

Chi-squared test as recommended by Campbell (Altman, 2000; Richardson, 2011) was used for pairwise comparison of proportions while the confidence intervals of the proportions were calculated using the method recommended by Altman et al. Coding in regression analyses was such that the lowest code (0), the reference, was the factor which exhibited the highest proportion/ Wald statistic (Grace-Martin). Test for collinearity of the variables was by testing for correlation. Simple correlation coefficients for pairs of independent variables are determined and a value of more than 0.3 was considered reasonable collinearity among a pair of independent variables and one was dropped (Kennedy,1985).This was done systematically until only those with correlation of 0.3 or less remained. Multivariable generalized linear mixed effects logistic regression analysis (GLMM) with county and villages as random effect variables was used to test the strength of association between the potential risk factors and FMD sero-positivity. This made use of backward fitting of variables and generated odds ratio (OR) and p values. Interaction between variables was also tested. The interpretation of odds ratios less than one were after obtaining their inverse (Bland and Altman,2000). Scaled residuals and fitted values were generated and used to evaluate the final models developed. In all the analysis, confidence level was kept at 95% and $\rho \leq 0.05$ was set for significance. The goodness of fit test used for the regression models was the Akaike Information Criterion (AIC) which maximizes the likelihood function. The model with the lowest AIC was considered as the most parsimonious (Burnham and Anderson, 2004; Cavanaugh and Neath, 2019).

Each owner of a herd selected for sampling provided verbal consent, once the objectives of the study were explained. Herds whose owners did not consent were replaced with the next herd in the random sample list.

3.4 RESULTS

The cross-sectional study was carried out from August to September 2016 cross-nationally. In the study, 898 herds were sampled yielding 8201 samples. The herds were more than the number calculated since, especially in the sedentary area, it was difficult to find sufficient animals in one herd. However, only 7564 samples from 872 herds were available for testing for FMD sero-prevalence as 637 were already depleted while testing for other diseases or had spilled or were grossly contaminated. Sheep samples were 2560 (33.8%) while goat samples were 5004 (66.2%). Of these 3909 (51.7%) were from the PZ and 3655 (48.3%) were from the SZ. Of the 44 counties investigated (samples from three out of 47 counties were not available), 11 (25.0%) were in the PZ and 33 (75.0%) were in the SZ.

3.4.1 Animal and herd level descriptive statistics

The table below shows the number of small ruminants sampled, the individual animal variable descriptive statistics in both the PZ and SZ and overall (Table 3.1). The the mean herd size in the PZ was about ten times that in the SZ. The animals in both zones were mainly females older than one year, about three-quarters of the total number. About two-thirds of the SR sampled was of caprine species. Nearly half of the animals were of local breed. Majority of the animals sampled were mature (97.0%) and born in the herds (82.9%).

Table 3.1: DESCRIPTIVE STATISTICS OF SAMPLED INDIVIDUAL ANIMAL VARIABLES INTHE PASTORAL AND SEDENTARY ZONES, KENYA, 2016.

Variable	No. in Pastoral Zone	% in Pastoral Zone	No. in Sedentary Zone	% in Sedentary Zone	Total	% of Total
Species						
Caprine	2694	68.9	2310	63.2	5004	66.2
Ovine	1215	31.1	1345	36.8	2560	33.8
Total	3909	100.0	3655	100.0	7564	100.0
Breed						
Local	1714	43.8	1596	43.7	3310	43.8
Cross-breed	431	11.0	430	11.8	861	11.4
Exotic	178	4.6	516	14.1	694	9.2
Unidentified	1586	40.6	1113	30.5	2699	35.7
Total	3909	100.0	3655	100.0	7564	100.0
Sex						
Female	3010	77.0	2912	79.7	5922	78.3
Male	894	22.9	742	20.3	1636	21.6
Unidentified	5	0.1	1	0.0	6	0.1
Total	3909	100.0	3655	100.0	7564	100.0
Age						
Mature (>1 year)	3879	99.2	3456	94.6	7335	97.0
Young (<1 year)	11	0.3	187	5.1	198	2.6
Unidentified	19	0.5	12	0.3	31	0.4
Total	3909	100.0	3655	100.0	7564	100.0
Origin						
Born in herd	3145	80.5	3128	85.6	6273	82.9
Brought in	50	1.3	267	7.3	317	4.2
Unidentified	714	18.3	260	7.1	974	12.9
Total	3909	100.0	3655	100.0	7564	100.0

The herd level variable descriptive statistics in both zones and overall are represented in Table 3.2. Although these were herd level variables, the numbers are of actual number of animals involved as most analyses in the study were at individual animal level. Overall about two-thirds of animals were in herds where SR were brought into the herds in the last one year, in herds which purchased SR from markets or middlemen, in herds under communal grazing, in herds which shared watering and in herds at altitude of less than 1500m above sea level. Just

over a half of the animals were from herds which had interaction with wildlife and from herds which utilized own-male breeding method. Majority of animals were in meat and multi-purpose production type (86.1%), in the sedentary and pastoral production system (75.5%) and were enclosed at night (77.0%).

Table 3.2: DESCRIPTIVE STATISTICS OF HERD LEVEL VARIABLES IN THE PASTORAL AND SEDENTARY ZONES, KENYA, 2016.

Variable	No. in PZ	% in PZ	No. in SZ	% in SZ	Total	% of Total
Herds brought in SR?						
No	2494	63.8	2273	62.2	4767	63.0
Yes	1387	35.5	1382	37.8	2769	36.6
Unidentified	28	0.7	0	0.0	28	0.4
Total	3909	100.0	3655	100.0	7564	100.0
Buy SR from market or middlemen?						
No	1733	44.3	3020	82.6	4753	62.8
Yes	2176	55.7	635	17.4	2811	37.2
Total	3909	100.0	3655	100.0	7564	100.0
SR interaction with wildlife						
Yes	3246	83.0	1053	28.8	4299	56.8
No	209	5.3	1242	34.0	1451	19.2
Unidentified	454	11.6	1360	37.2	1814	24.0
Total	3909	100.0	3655	100.0	7564	100.0
SR Production type						
Meat	1594	40.8	1747	47.8	3341	44.2
Multipurpose	1876	48.0	1294	35.4	3170	41.9
Mixed	228	5.8	138	3.8	366	4.8
Dairy	20	0.5	170	4.7	190	2.5
Unidentified	191	4.9	306	8.4	497	6.6
Total	3909	100.0	3655	100.0	7564	100.0
SR Production System						
Sedentary/mixed	265	6.8	2850	78.0	3115	41.2
Pastoral	2481	63.5	112	3.1	2593	34.3
Agro-pastoral	782	20.0	266	7.3	1048	13.9
Multiple	138	3.5	28	0.8	166	2.2
Unidentified	243	6.2	399	10.9	642	8.5
Total	3909	100.0	3655	100.0	7564	100.0
SR Housing						

Enclosed at night	3061	78.3	2760	75.5	5821	77.0
None	794	20.3	592	16.2	1386	18.3
Enclosed day and night	54	1.4	303	8.3	357	4.7
Total	3909	100.0	3655	100.0	7564	100.0
SR grazing						
Communal	1611	41.2	1113	30.5	2724	36.0
Fenced	318	8.1	1799	49.2	2117	28.0
Mixed	1070	27.4	198	5.4	1268	16.8
Migratory	744	19.0	42	1.1	786	10.4
Unidentified	124	3.2	234	6.4	358	4.7
Zero-grazing	42	1.1	269	7.4	311	4.1
Total	*3909*	100.0	*3655*	100.0	7564	100.0
SR watering						
Shared	3529	90.3	1011	27.7	4540	60.0
On-farm	199	5.1	2226	60.9	2425	32.1
Unidentified	181	4.6	274	7.5	455	6.0
Mixed	0	0.0	144	3.9	144	1.9
Total	3909	100.0	3655	100.0	7564	100.0
SR breeding method						
Own male	2059	52.7	2370	64.8	4429	58.6
Mixed	1014	25.9	230	6.3	1244	16.4
Common-use male	556	14.2	244	6.7	800	10.6
Unidentified	183	4.7	395	10.8	578	7.6
Male from another farm	64	1.6	382	10.5	446	5.9
Artificial insemination	33	0.8	34	0.9	67	0.9
Total	3909	100.0	3655	100.0	564	100.0
SR location elevation						
<1500m	3130	80.1	1754	48.0	4884	64.6
>1500m	641	16.4	1828	50.0	2469	32.6
Unidentified	138	3.5	73	2.0	211	2.8
Total	3909	100.0	3655	100.0	7564	100.0

3.4.2 Sero-prevalence of FMD in small ruminants

At the time of sampling, none of the SR animals in the surveyed herds showed FMD clinical symptoms. Sero-prevalence of non-structural FMDV protein (antibodies) for a total of 7564 sera collected from the whole country was determined. The overall true sero-prevalence of FMD in small ruminants was 22.5% (95% CI: 22.3–24.3%). The sero-prevalence was significantly higher (p = 0.021) in the PZ at 31.2% (95% CI: 29.8–32.7%) compared to that in the SZ which had a sero-prevalence of 14.7% (95% CI: 13.4–15.7%). The distribution of

FMD sero-positives among SR was near ubiquitous with nearly every county registering some positives. Variations in spatial distributions of FMD sero-prevalence were observed across the country with true sero-prevalence levels higher than the national average of 22.5% recorded in 11 (25%) of Counties among them Mandera, Kilifi, Lamu, Kajiado, West-Pokot, Garissa, Turkana, Wajir, Kwale Tana River and Isiolo counties, mainly in the PZ except for Kilifi, Lamu and Kwale counties. Some counties (14), mainly in the SZ namely Embu, Kisii, Nakuru, Elgeiyo- Marakwet, Kiambu, Bungoma, Kirinyaga, Vihiga and Murang'a counties had low sero-prevalence of less than 10.0%. Other counties (19 in number) in sedentary zone had sero-prevalence above 10.0% but lower than the national average. The sero-prevalence for Mombasa and Nyamira was 0.0% but the number of samples tested was too small (5 and 14 respectively) to give any meaningful interpretation. The FMD sero-positivity per potential individual animal risk factor was as shown in Table 3.2. For variables with more than two categories, chi-square reported in Tables 3.3 and 3.4 is for sero-prevalence for all categories. The chi-square reported in the ensuing text are for pair-wise comparison of sero-prevalence. Thus at individual animal level, the sero-positivity of FMD in caprine (goats) compared to that in ovine (sheep) was significantly higher ($p<0.001$). That for exotic breeds was significantly lower than that for local breeds ($\chi2 = 14.43$; $p<0.001$) and cross breeds ($\chi2 = 9.13$; $p = 0.003$). Sero-prevalence in mature animals was significantly higher than in young animals ($p<0.001$) while that in animals that were born in the herd was significantly higher than that of animals that were brought in ($p<0.001$).

The herd level prevalence, a measure of sero-prevalence in herds where at least one animal in a herd tested positive, for all the 872 herds tested was 77.6% (95% CI: 73.9–80.9), which was significantly higher than overall animal level sero-prevalence (22.5% (95% CI: 22.3-24.3).

The sero-positivity per potential herd risk factor was as in Table 3.4. Herds which had brought in SR in the last one year had significantly lower sero-prevalence than those that had not ($p<0.001$). Herds in which animals were bought from the market or middlemen had significantly higher sero-positivity than in those herds where this was not the case ($p<0.001$). Similarly, herds which had wildlife interaction had significantly higher seropositivity than those without such interaction ($p<0.001$). The sero-positivity of herds at low altitude (<1500m above sea level was significantly higher ($p<0.001$) than that of herds at higher altitude (>1500m above sea level).

Table 3.3: FMD SERO-POSITIVITY PER POTENTIAL INDIVIDUAL ANIMAL RISK FACTOR, KENYA, 2016.

Variable	Total tested	Positive	% Positive	95%CI of % positive	$\chi2$	p-value
Species						
Caprine	5004	1202	24.0	22.9–25.2	789.68	<0.001
Ovine	2560	560	21.9	20.3–23.5		
Breed						
Local	3310	807	24.4	22.9–25.9	2728.79	<0.001
Cross-breed	861	207	24.0	21.2–27.1		
Exotic	694	123	17.7	15.0–20.8		
Unidentified	2699	25	0.9	0.6–1.4		
Sex						
Female	5922	1403	23.7	22.6–24.8	7406.90	<0.001
Male	1636	356	21.8	19.8–23.9		
Unidentified	6	3	50.0	14.0–86.1		
Age						
Mature (>1 year)	7335	1731	23.6	22.6–24.6	13790.73	<0.001
Young (<1 year)	198	20	10.1	6.4–15.4		
Unidentified	31	20	35.5	19.8–54.6		
Origin						
Born in herd	6273	1474	23.5	22.5–24.6	8459.147	<0.001
Brought in	317	54	17.0	13.2–21.7		
Unidentified	974	234	24.0	21.4–26.9		

Unidentified means that the variable was not indicated for those samples. The chi-square and p value are overall values for the differences in proportions.

Table 3.4: SERO-POSITIVITY FOR FMD PER POTENTIAL HERD RISK FACTOR, KENYA, 2016.

Variable	Total tested	Positive	% Positive	95%CI of % positive	χ2	p-value
Production Zone						
Pastoral (PZ)	3909	1220	31.2	29.8–32.7	5.29	0.021
Sedentary (SZ)	3655	531	14.7	13.4–15.7		
Herds brought in SR?						
No	4767	1160	24.3	23.1–25.6	4490.11	<0.001
Yes	2769	598	21.6	20.1–23.2		
Unidentified	28	4	14.3	4.7–33.6		
Buy SR from market or middlemen?						
No	4753	1023	21.5	20.4–22.7	498.59	<0.001
Yes	2811	739	26.3	24.7–28.0		
SR interaction with wildlife						
Yes	4299	1201	27.9	26.6–29.3	1906.15	<0.001
No	1451	183	12.6	11.0–14.5		
Unidentified	1814	378	20.8	19.0–22.8		
SR Production type						
Meat	3341	700	21.0	19.6–22.4	6732.83	<0.001
Multipurpose	3170	816	25.7	24.2–27.3		
Mixed	366	70	19.1	15.3–23.6		
Dairy	190	31	16.3	11.5–22.5		
Unidentified	497	145	29.2	25.3–33.4		
SR Production System						
Sedentary/mixed	3115	463	14.9	13.6–16.2	4311.26	<0.001
Pastoral	2593	799	30.8	29.0–32.6		
Agro-pastoral	1048	305	29.1	26.4–32.0		
Mixed	166	37	22.3	16.4–29.5		
Unidentified	642	158	24.6	21.4–28.2		
SR Housing						
Enclosed at night	5821	1377	23.7	22.6–24.8	6687.38	<0.001
None	1386	346	25.0	22.7–27.3		
Enclosed day and night	357	39	10.9	8.0–14.7		
SR grazing						
Communal	2724	713	26.2	24.5–27.9	3820.75	<0.001
Fenced	2117	335	15.8	14.3–17.5		
Mixed	1268	351	27.7	25.3–30.3		
Migratory	786	232	29.5	26.4–32.9		
Unidentified	358	96	26.8	22.4–31.8		
Zero-grazing	311	35	11.3	8.1–15.4		
SR watering						
Shared	4540	1290	28.4	27.1–29.8	6566.08	<0.001

On-farm	2425	330	13.6	12.3–15.1		
Unidentified	455	124	27.3	23.3–31.6		
Mixed	144	18	12.5	7.8–19.3		
SR breeding method						
Own male	4429	1020	23.0	21.8–24.3	10157.63	<0.001
Mixed	1244	325	26.1	23.7–28.7		
Common-use male	800	215	26.9	23.9–30.1		
Unidentified	578	131	22.7	19.4–26.3		
Male from another farm	446	63	14.1	11.1–17.8		
Artificial insemination	67	8	11.9	5.7–22.7		
SR location elevation						
<1500m	4884	1277	26.2	24.9–27.4	4332.06	<0.001
>1500m	2469	419	17.0	15.5–18.5		
Unidentified	211	66	31.3	25.2–38.1		

Multipurpose production type herds had significantly higher sero-positivity than meat (χ2 = 20.00; p<0.001), mixed (χ2 = 7.62; p = 0.006) and dairy (χ2 = 8.41; p = 0.004) production type herds. The pastoral production system showed significantly higher sero-positivity than sedentary (χ2 = 207.61; p<0.001), agro-pastoral (χ2 = 104.96;p<0.001) and mixed (χ2 = 5.34; p = 0.021) production systems. The sero-prevalence in the sedentary production system was significantly higher than that in the mixed production system (χ2 = 6.67; p = 0.001). Herds which were not enclosed or enclosed only at night had a significantly higher sero-positivity than herds which were enclosed by day and by night (χ2 = 32.75; p<0.001 and χ2 = 31.15; p<0.001 respectively). Communal grazed herds had significantly higher sero-prevalence than fenced (χ2 = 75.94; p<0.001) and zero-grazed herds (χ2 = 33.33; p<0.001). Fenced herds had significantly higher sero-prevalence than herds with mixed grazing (χ2 = 69.50; p<0.001), herds with migratory grazing (χ2 = 68.49) and zero-grazed herds (χ2 = 4.25; p = 0.04). Herds with mixed and migratory grazing systems had significantly higher sero-prevalence than zero-grazed herds (χ2 = 36.32; p<0.001 and χ2 = 40.04; p<0.001 respectively). Small ruminant herds that had shared watering had significantly higher sero-positivity than those with on farm watering and mixed type watering (χ2 = 194.02; p<0.001; χ2 = 17.76; p<0.001 respectively).

The statistically significant higher sero-prevalence with regard to breeding method were observed only between herds utilizing all other breeding methods (own male, mixed methods, common use male) and those utilizing a male from another farm ($\chi2 = 18.57; p<0.001$, $\chi2 = 26.73; p<0.001$, $\chi2 = 27.04; p<0.001$ respectively) as well as those utilizing AI ($\chi2 = 4.61$; $p = 0.032$), $\chi2 = 6.77; p = 0.009$, $\chi2 = 7.27; p = 0.007$ respectively).

3.4.3 Association between FMD sero-positivity and selected potential risk factors

Pairwise Spearman correlation of all the potential risk factors in Tables 4.3 and 4.4 showed significant moderate to strong correlation between many factors except age, sex, production zone, whether herds brought in SR, production type, breeding method and elevation. These were retained in the group of potential risk factors for FMD sero-positivity risk factor analysis. The most parsimonious mixed effects logistic regression model showing the association between FMD sero-positivity in small ruminants and risk factors as well as the relevant interactions are in Table 3.5. In this final model obtained, the AIC was 7079.6 and the log likelihood was -3532.8 which indicated good fit for the data. Only multipurpose production type showed statistically significant positive association when compared with meat production type. Thus multipurpose production type was 1.307 times more likely to be associated with FMD sero-positivity when compared with meat production type ($p = 0.042$). Interpretation of OR for risk factors that were negatively associated with FMD sero-positivity was after finding the inverse of OR (1/OR) as specified by Bland and Altman (Bland and Altman,2000). Therefore with reference to female animals, male animals were 1.238 times less likely to be seropositive for FMD ($p = 0.008$). Compared to mature animals, young animals were 3.436 times less likely to be seropositive for FMD ($p = <0.001$). Animals in the

sedentary zone were 3.597 times less likely to be sero-positive when compared with those in the pastoral zone (p<0.001). County and village IDs (with village IDs nested within counties) were used in the model as random effects variables but both effects were non-existent. The variances and Sd associated with observations within a village and county were 1.11 (Sd = 1.05) and 0.41(Sd = 0.67), respectively. Scaled residuals ranged between -2.30 and 5.29. A model used to investigate interactions showed that interaction between age and sex was significant (p = 0.019). An animal that was mature and female was 3.436 times more likely to be sero-positive in contrast to being young and female (p = 0.004) and 1.238 times more likely to be seropositive in contrast to being mature and male (p = 0.041). An animal that was young and female was 2.78 (1/0.360) times less likely to be sero-positive in contrast to one that was mature and male (p = 0.029). Thus an interaction with matureness or femaleness increased the risk of FMD sero-positivity above that for sex or age alone respectively.

Table 3.5: ASSOCIATION BETWEEN FMD SERO-POSITIVITY IN SMALL RUMINANTS AND RISK FACTORS STUDIED, KENYA, 2016.

Risk factor	**Variable**	**P**	**OR**	**95%CI of OR**
Intercept	Included	<0.001	2.459	2.047–2.872
Sex	Female	Ref		
	Male	0.008	0.808	0.650–0.966
Age	Mature	Ref		
	Young	<0.001	0.291	-0.420–1.002
Production zone	Pastoral	Ref		
	Sedentary	<0.001	0.278	-0.225–0.780
Production type	Meat	Ref		
	Multipurpose	0.042	1.307	1.049–1.566
	Mixed	0.608	0.876	0.373–1.380
	Dairy	0.416	1.351	0.626–2.076
Sex * age interaction	Male * young	0.019	3.671	3.671–4.754
	Mature Female versus young female	0.004	3.436	2.725–4.147

	Mature female versus mature male	0.041	1.238	1.079–1.396
	Mature female versus young male	0.986	1.158	0.322–1.995
	Young female versus mature male	0.029	0.360	-0.363–1.084
	Young female versus young male	0.193	0.337	-0.735–1.409
	Mature male versus young male	0.999	0.936	0.090–1.782

3.5.DISCUSSION

The mean SR herd sizes in the PZ and SZ were 27.5 and 2.7 respectively. This is consistent with what has been reported in sub-saharan sedentary production systems (Chilonda *et al.*,2000). The herd structure in the pastoral zone is similar to what has been reported in Somalia (Abdullahi,1990). For the PZ, this is within the range reported recently in Kenya (Jahnke, 1982) but lower than that reported by Zaal (Zaal,1998), probably due to dwindling land available for livestock keeping and other changes in farming systems. According to this study, the bulk of SR in Kenya are held in the PZ. Only 7564 samples from 872 herds were available for testing for FMD sero-prevalence compared to a calculated sample size of 9044. Though slightly lower than the calculated sample size, due to sample loss and lack of usability of some samples for the test (16%), these samples were deemed sufficient for determination of the sero-prevalence of FMD in the SR herds given that there was sufficient design effect (2) consideration and provision for sample loss in sample size calculation.

The country sero-prevalence of FMD in SR to be 22.5% similar to what has been reported in other countries where FMD is endemic (Ur-Rehman, 2014; Farooq,2017). It is however higher than that reported in Ethiopia, Israel, Libya and Sudan (Mesfine, 2019; Abdela, 2017; Elnekave, 2016; Eldaghayes, 2017; Raouf, 2017) but about half of what has been reported in Tanzania and Myanmar (Phyoe, 2014; Torsson, 2017; Casey-Bryars,2016). A previous study in cattle in Kenya showed much higher sero-prevalence in cattle at 52.5% (Kibore *et al.,*2013) and unpublished data obtained at the same time with this current study in Kenyan cattle revealed a sero-prevalence of 37.6%. This means sheep and goats in Kenya could be less susceptible to FMDV compared to cattle despite the fact that they are normally herded together in endemic settings of Kenya as was also observed in Ethiopia (Mesfine, 2019; Abdela, 2017).

In the absence of vaccination, sero-prevalence to FMDV can be an indicator of presence of FMD though the method used for testing was non-structural proteins ELISA which demonstrates seropositivity in animals that have been exposed to the virus. Sero-prevalence was significantly higher in the PZ (31.5%) than in the SZ (14.5%). This may be attributed to a high level of herd mobility, contact of animals at grazing and watering points, dynamism of herds (frequent additions) and frequent contact with the livestock of neighbouring countries through cross-border contact in the PZ. These animals move across the boundaries for grazing, watering and also for illegal trade thus promoting the concept that FMD outbreaks are associated with animal movement. In the process of movement they also come in contact with other animals from different areas which are an important factor for the transmission of the disease. The livestock in pastoral areas also end up in some sedentary zones during the dry season, potentially spreading disease (Wanyoike, 1999). Foot and mouth being a disease

spread due to movement of animals closer together makes sedentary zone have lower incidence of spread between herds. This is important because most of the SR are in the PZ where they are more often herded together with cattle and cross-transmission may be the reason for the observed sero-positivity. The sero-prevalence in counties within the PZ or bordering the PZ such as Lamu were significantly higher than those in the counties in the SZ as also reported by others (Chepkwony *et al.*, 2012; Kibore *et al.*, 2013). This might be due to differences in the movement and distribution of livestock, the level of contact between herds and ungulate wildlife, proximity to stock routes, the grazing patterns and watering sources in each County.

A significant difference was observed in sero-prevalence of FMD among mature (23.6%) and young sheep and goats (10.1%). This is in agreement with the results of others (Torsson, 2017; Casey-Bryars, 2016) although the sero-positivity levels in our study were lower. The difference in sero-positivity between age groups may be due to the fact that mature animals may have experienced more exposures to FMD at grazing, watering point and at market than in age group less than one year. Therefore, adult animals might have acquired infection from multiple strains and serotypes thus producing antibodies against multiple virus incursions of FMD. It could also be due to cumulative sero-positivity through repeated infection in their longer life time. The low prevalence in young animals may also be indicative of persistent passive immunity and less frequency of exposure of the animal to the disease as the farmers keep their lambs and kids in the homesteads. Females showed higher sero-prevalence at 23.8% than males (21.9%). However, these results are in contrast to Ethiopian studies where 15.7% and 8.3% seroconversions were reported in male and female animals respectively (Jenbere *et al.,* 2011) and 8.9% in female while 3.0% in male(Mesfine *et al.,*2019).

Sero-prevalence was significantly higher in the multipurpose production type than in all the other production types (meat, mixed, dairy). This may be possible because this production type is found mainly among pastoral and agro-pastoral systems where purchase of animals is from the market or middlemen and which in each case had high sero-prevalence. Other researchers have demonstrated higher FMD sero-prevalence in production types resembling the multi-purpose production type than in meat, mixed and dairy production types (Elnekave et al., 2016 Casey-Bryars, 2016). However, researchers (Mesfine *et al.*, 2019) in Ethiopia have demonstrated lower sero-prevalence in pastoral and agro-pastoral production systems than in sedentary production systems. Further, there was high sero-prevalence in animals whose production type was not identified hence the need for further investigation of sero-prevalence between the production types.

The proportion of herds with at least one animal sero-positive for FMD (herd prevalence) was high at 77.6% similar to 74.7% reported by Ahmed (Ahmed *et al.*, 2020) in Ethiopian cattle but higher than that in the reports in southern Ethiopian cattle and in Omani cattle with sero-prevalence of 48.1% and 55.2%, respectively (Megersa *et al.*, 2009; Hussain *et al.*, 2019).This comparison of SR herds and cattle herds is relevant given possible transmission from cattle to SR. The high prevalence of FMD at the herd level in our study might be due to the common practice of communal grazing and watering in the study area as was also alluded to by Ahmed et al (Ahmed *et al.,* 2020). In spite of many variables showing differences in proportions of seropositive animals across the categories, only multipurpose production type showed a statistically significant positive association with FMD sero-positivity.

Male sex, young age and sedentary production zone showed a statistically significant negative relationship. Thus some husbandry related variables showed significant relationship with sero-positivity as has also been alluded to by (Balinda *et al*., 2009) in Uganda. These results demand for risk-based surveillance which considers the significant risk factors. They also call for extension services and policies for small ruminant keepers to advice on interventions and husbandry practices which could limit the circulation of FMDV among SR herds which could also reduce cross-transmission with cattle herds. Vaccination of small ruminants against FMD in Kenya is non-existent due to scarce vaccine and cost implications (Nyaguthii *et al.*, 2019). It may be worthwhile to vaccinate SR in some scenarios, given the identified risk factors. The possibility of transmission of FMDV from cattle to SR needs to be researched.

3.6 CONCLUSION

The bulk of small ruminants in Kenya are held in the pastoral zone. Small ruminant FMD sero-positivity established in most counties countrywide shows that FMD may be present in the species in majority of herds but this needs to be authenticated through isolation of the FMD virus. The study has shown that the sero-prevalence in small ruminants in Kenya is estimated at 22.5% with sheep and goats having almost equal sero-prevalence. Given the near ubiquitous distribution of sero-prevalence, it is possible that the FMD virus may be circulating in a significant proportion of closed SR herds. There is also possible cross transmission of FMDV across the species which needs investigation. The pastoral zone had higher sero-positivity as compared to the sedentary zone. This shows the importance of concentrating control efforts in the pastoral zone where sero-positivity is high but without neglecting the sedentary areas which usually suffer the highest production and productivity losses in case of FMD outbreaks. Besides, livestock in the pastoral zone also end up in some counties in the

sedentary zone during the dry season. Past efforts for control of FMD in Kenya centered on compulsory vaccination of cattle in areas mostly located in the sedentary areas. The findings of this study should be considered in the development of FMD risk-based surveillance and control plans in small ruminants alongside those of the cattle population with due consideration of the established risk factors. More risk factors should be identified through planned studies.

Chapter 4: SURVEILLANCE FOR FOOT- AND-MOUTH DISEASE IN DOMESTIC RUMINANTS IN UKAMBANI REGION OF KENYA

4.1 INTRODUCTION

Ukambani Region is located in the South Eastern part of Kenya comprising three counties of Kitui, Machakos and Makueni home to the Kamba community of Kenya. The area is in equatorial climate classified as Arid and Semi -Arid Lands Agroecology with 30-84% aridity. Rainfall is bimodal, long rains usually received in April-May while short rains are in November to December. Agriculture in Kitui provides 87% of household income giving farmers sustainable employment though drought is an impediment to sustainable production. In Machakos agriculture employs 73% and provides 70% of the household income. Makueni has mostly low lying terrain except the hilly areas of Kilungu, Mbooni and Chyulu hills which receive 800-1200mm of rain while the lowlands receive an average of 300mm. The temperatures range from 20.2-35.8^{o}C, the hills being relatively colder. In Makueni agriculture employs 78% of the population giving 70% of the household income.

Ukambani is situated on an eastward-facing slope, which becomes progressively lower and drier to the east. It is part of Kenya's Eastern Foreland Plateau, an eroded basement complex broken by residual hill masses and occasionally overlain by Tertiary volcanics (Bernard *et al.,*1989; Wisner, 1977). The Machakos Hills area are described as hills dropping down to a series of plains, separated by steep slopes.

Livestock production in Ukambani involves keeping of dairy, beef cattle, goats, sheep, poultry, bee- keeping and fish farming. Cattle population comprises both local (97% zebu and 3% boran and sahiwal combined) and exotic breeds; Friesians, Aryshire, Guernsey and Jersey.

Goat breeds are small East African goat, Gala and their crosses. Dairy goats are also kept mainly Toggenburg and a few German Alpine breed.

After the devolution of 2013 in Kenya, integration among counties has emerged to form blocs largely due to their historical, political and economic similarities. South Eastern Kenya Economic Bloc comprising of three (3) counties namely Kitui, Machakos and Makueni was formed mainly to address economic development in the three counties and to spur economic growth within the respective counties through policy harmonization and resource mobilization. FMD is one of the major diseases impacting the Livestock industry in the country and in this region. Kenya is in the process of developing Risk Based FMD Control strategy the Proposed Control Pathway in line with the GF-TADs to guide the country, where the risk of introduction, infection and spread of the disease is effectively managed. The findings of this research may provide useful information on the development of this control strategy for this region.

In order to control and/or eradicate this disease in the targeted areas, a good understanding of disease epidemiology is important and this can only happen if the disease is traced and regular, and effective surveillance is done together with vaccination regimes being put in place (FAO, 2005, 2006). Therefore the objective of this study was to determine clinical prevalence of FMD, the sero-prevalence in large (cattle) and small domestic ruminants and to identify the risks associated with the disease in Ukambani region of Kenya.

4.2 JUSTIFICATION FOR THE STUDY

Foot-and-mouth disease outbreaks occur in Ukambani region affecting domestic ruminants. Surveillance for Foot-and-moth disease important in order to understand the epidemiological dynamics in the field for development of effective control strategies. This requires understanding disease occurrence, clinical manifestation and risk factors in both large and small ruminants.

4.3. MATERIALS AND METHODS

4.3.1 The study population

A cross -sectional study was carried out from August to September 2016. Ukambani region is made up of Kitui, Machakos and Makueni counties. The objective was to primarily trace active cases of FMD and sample for FMD confirmation in the region and to measure the sero-prevalence risk factors in domestic ruminants.The sampling unit was the smallest administrative unit in record, the village, which was selected after first selecting the second smallest administrative unit, the sub-location. The sampling frame of sub-locations was available from the Kenya 2009 population and housing census(KNBS, 2009). Animals with clinical signs were carefully examined and sampled to confirm FMD by laboratory diagnosis. Blood samples were collected from both bovine and small domestic ruminants to identify the presence or absence of antibodies from the serum. This was done in order to determine what proportions of herds in the region that are affected by FMD as indicated by detection of sero-positive animals in the herd. Pre-tested questionnaires was used to collect data on sampled herds on potential risk factors at individual animal and herd level and to verify the occurrence of Foot and mouth disease in the region, knowledge of the disease and frequency of outbreaks. The livestock keepers were interviewed at the time of sample collection. Together with

random sera sampling of selected herds, clinical examination and sampling of suspected infected animals was carried out.

4.2.2 Sampling strategy and study area

4.2.2.1 Study area

The objective of carrying out cross-sectional survey was to determine the prevalence of Foot and mouth disease in regional population of cattle, SR and herds. The sampling method was two stage, sub-location and individual animals. This is because from the Kenya National Bureau of statistics (National Population Census, 2009), sampling frame of Sub-locations was available. From the available data of Sub-Locations a list of herds was prepared and a simple random sample of herds was selected. On the Second stage, within selected herds, a simple random sample of animals was selected.

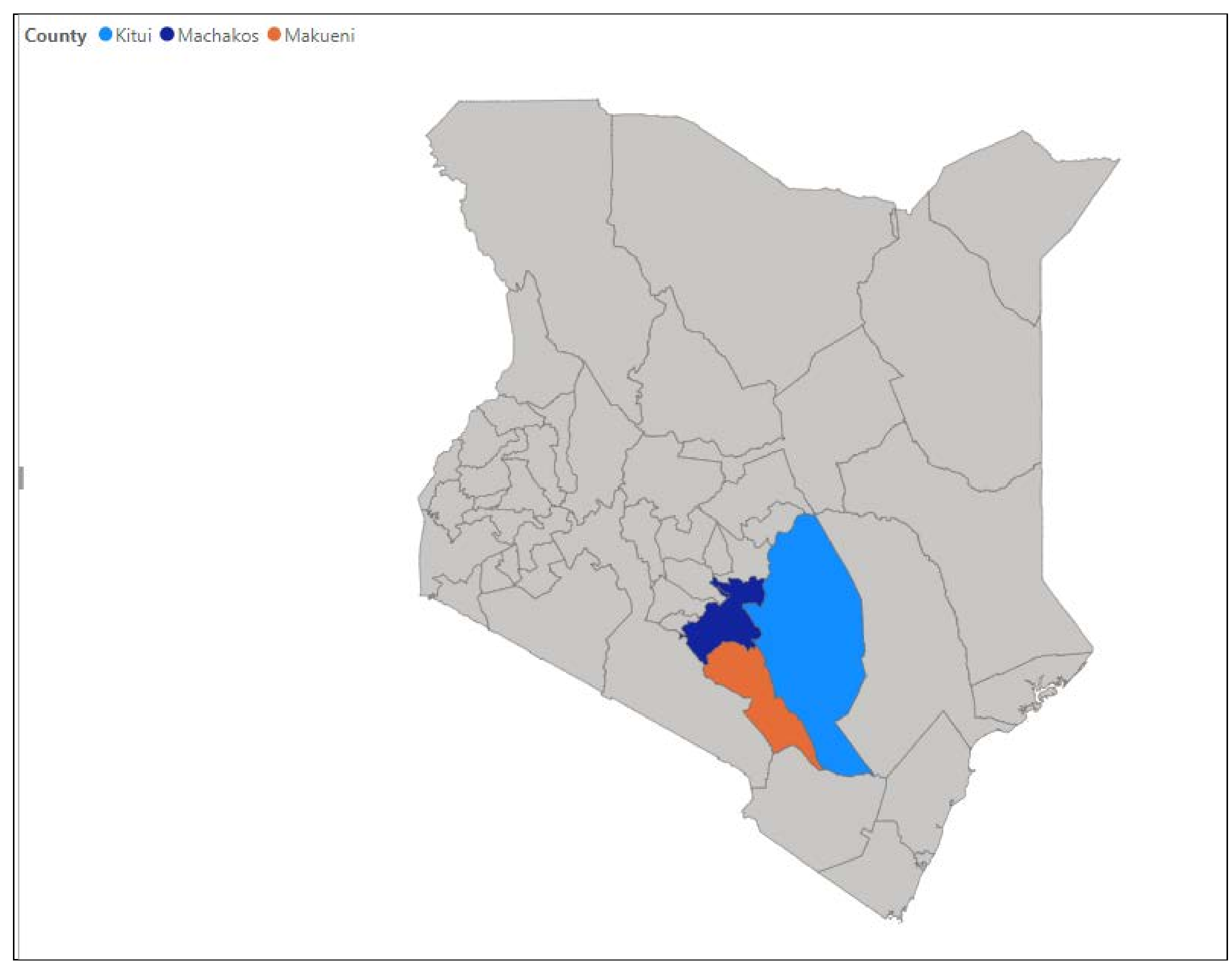


Figure 4.1 Map of Kenya showing the study area of Ukambani; Kitui, Machakos and Makueni

4.2.2.2 Sample size determination

The minimum sample size for cross-sectional survey was calculated using the formula by Dohoo *et al.,* (2003) as shown below:

$n=Z^2 p q/L^2$

Where:

n = minimum sample size

Z = the standard normal deviate set at 1.96 for a confidence interval of 95%

p = the estimated proportion of animals in the area testing positive for Foot-and-mouth disease

$q = 1 - p$

From previous prevalence studies an average FMD prevalence rate in Kenya of 0.3 was used.

L = is the degree of accuracy desired and was set at 0.05

Therefore the minimum sample size was:

$n = (1.96)^2 \times 0.3 \times 0.7 / (0.05)^2$

= 323

To take care of design effect this was multiplied by 2

Total minimum samples = 646

A random sample of sub-locations from a list of sub-locations was selected. From a list of villages in each sub-location one village randomly was selected. One household herd was also randomly from the village selected with one or two others identified in case the number of cattle/small ruminants in the first herd would be inadequate. Within the herd random selection of 14 cattle and 14 small ruminants was selected was done and where the selected herd had less than 14 cattle a second and third herd was selected to top up the number.

The number 14 was arrived at using Win Episcope 2.0(samples →detection of disease→sample size), confidence interval 95%.

14 cattle were sampled per herd.

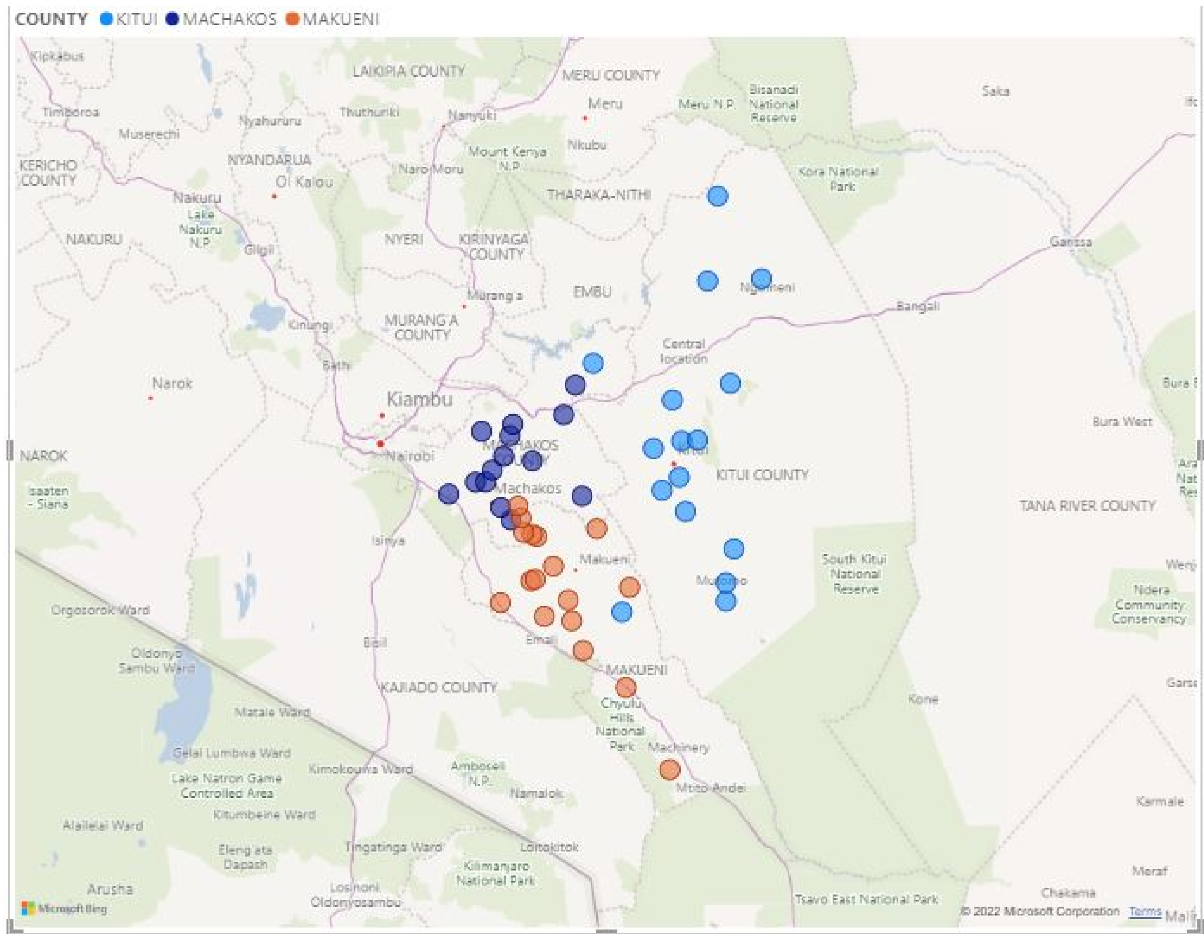


Figure 4.2 Map showing sampling sites visited in Ukambani Region

From the 47 randomly sampling sites with 14 samples per species, a total of 658 cattlesamples and 658 small ruminants. Kitui had 17 herds while both Machakos and Makueni had 15 herds each.

4.2.2.3 Sample collection:

Cattle aged twelve (12) months and above were sampled to avoid those with maternal antibodies. Age was determined by examining the dentition of each animal and information from the owner for young animals with no permanent incisors.

GPS coordinates and elevations were recorded for each herd location. Each questionnaire was recorded for each herd location and each given a unique herd number and GPS coordinates. Blood was collected from each selected animal from the jugular vein aseptically using a sterile vacutainer needle into plain 10mls vacutainer tubes. At the time of sampling the details of individual animals were taken; breed, sex, dentition, origin, clinical signs if any and vaccination status and this was recorded in the sample form and unique code recorded on the vacutainer tubes to match the record on the form.

The blood sample was allowed to clot in cool boxes with ice in a cool place. Once the blood clot had retracted after 12-24 hours the vials were centrifuged in the field laboratories to obtain serum or in areas where laboratories were unavailable serum was separated using a sterile pipette and transferred into 2mls cryovials labeled with corresponding identification codes. Samples were transported using iceboxes to National Foot and mouth disease Laboratory, Embakasi and stored at -20ºc until laboratory investigation.

Table 4.1: DETAILS OF SAMPLING SITES VISITED IN UKAMBANI REGION

S/ NO	COUNTY	SUB-LOCATION	GPS CORDINATES	ELEVATION (m)	CATTLE	GOAT	SHEEP	TOTAL
1	KITUI	KYAMBITI	S:01.41532º E:038.037788º	11144	14	14	0	28
2	KITUI	MBITINI	S: 01.96435º E:037.80345º	11111	14	14	0	28
3	KITUI	KATWALA	S:01.55518º E:038.06274º	967	14	0	14	28
4	KITUI	KALINDILO	S:01.29750º E:037.93209º	1194	14	12	2	28
5	KITUI	NZAKAME	S:01.46862º E:037.96659º	1092	14	14	0	28
6	KITUI	KAVETA	S:00.95012º E:037.68553º	1148	14	14	0	28
7	KITUI	MUNG'ANG'A	S:01.26399º E:038.11241º	737	14	14	0	28
8	KITUI	KAVELU	S:01.84600º E:038.22811º	713	14	14	0	28
9	KITUI	KITOO	S:01.92086º E:038.22811º	833	14	13	1	28
10	KITUI	IMALE	S:01.70722º	631	14	14	0	28

			E:038.26104°					
11	KITUI	NDETANI	S:00.60572° E:038.37358°	725	14	14	0	28
12	KITUI	KASYATHUNI	S:00.26856° E:038.19461°	532	14	14	0	28
13	KITUI	KIMU	S :00.61517° E:038.15258°	913	14	14	0	28
14	KITUI	KYANDALI	S :0069150° E:038.0601°	951	14	14	0	28
15	KITUI	KAMANDIO MALILI	S:01.26710° E:038.04641°	1248	14	14	0	28
16	KITUI	KYAMBOO	S:01.10027° E:038.00951°	1249	14	14	0	28
17	KITUI	KYANGATI	S :01.03209° E:038.24638	720	14	14	0	28
18	MACHAKOS	KIVUTINI	S:01.43463° E:037.24638°	1774	14	14	0	28
19	MACHAKOS	MUA	S:01.43538° E:037.20515°	2066	14	14	0	28
20	MACHAKOS	KIVANDINI	S:01.53841° E:037.30801°	1495	14	13	1	28
21	MACHAKOS	NZUINI	S:01.58940° E:037.35021°	1480	14	14	0	28
22	MACHAKOS	MATHUNYA	S:01.38632° E:037.27347°	1495	14	14	0	28
23	MACHAKOS	KAWATHEI	S:01.32951° E:037.31896°	1525	14	14	0	28
24	MACHAKOS	MWATATI	S:01.24614° E:037.34555°	1545	14	10	4	28
25	MACHAKOS	MATUU	S:01.22849° E:037.22971°	1515	14	12	2	28
26	MACHAKOS	KALULUINI	S:01.16039° E:037.56517°	1522	14	14	0	28
27	MACHAKOS	KINYAATA	S:32401° E:037.61977°	1277	14	14	0	28
28	MACHAKOS	KIBAU	S:01.34841° E:037.43759°	1276	14	14	0	28
29	MACHAKOS	KATITU	S:01.19920° E:037.35790°	1331	14	14	0	28
30	MACHAKOS	ITUMBULE	S:01.49204° E: 037.64013°	1151	14	14	0	28
31	MACHAKOS	MUSINGINI	S:01.03910° E:037.61313°	1176	14	14	0	28
32	MACHAKOS	ATHI RIVER NORTH	S:01.48436° E:037.09612°	1648	14	7	7	28
33	MAKUENI	MALUNDA	S:01.62500° E:037.70041°	1215	14	14	0	28
34	MAKUENI	IIANI	S:01.53284° E:037.37839°	1361	14	14	0	28
35	MAKUENI	ITETANI	S:01.58205° E:037.39084°	1434	14	9	5	28
36	MAKUENI	NZEVENI 1	S:01.64881° E:037.43784°	1902	14	13	1	28
	MAKUENI	NZEVENI 2	S:01.64002° E:037.40086°	1662				
37	MAKUENI	MUTITU	S:01.65768° E:037.45525°	1926	14	11	3	28
38	MAKUENI	UTAATI	S:01.77770° E:037.52315°	1189	14	12	2	28
39	MAKUENI	NDOLO 1	S:01.83182° E:037.44827°	1394	14	11	3	28
	MAKUENI	NDOLO 2	S:01.83762° E:037.43178°	1406				
40	MAKUENI	KIONGWANI	S:01.92690° E:037.30666°	1418	14	14	0	28
41	MAKUENI	KAWALA	S:02.00106° E:037.59833°	1025	14	14	0	28
42	MAKUENI	KALILI	S:01.91640°	1130	14	14	0	28

			E: 037.58405°					
43	MAKUENI	KALIINI	S:01.98270° E:037.48572°	1369	14	14	0	28
44	MAKUENI	MUSAMUKYE	S:02.12172° E:037.64447°	1023	14	14	0	28
45	MAKUENI	MANYATA	S:02.27274° E:037.81917°	993	14	14	0	28
46	MAKUENI	MUTHINGINI	S:02.60742° E:037.99847°	942	14	13	1	28
47	MAKUENI	IVINGA NZIA	S:01.86433° E:037.83440°	988	14	13	1	28

4.2.2.4 Clinical examination of active cases of FMD

In the 47 sites two active FMD outbreaks were encountered in Athi -River, Mavoko Sub-County Machakos and Makindu Sub-County in Makueni (Figure . Epithelium sampling was carried out after clinical examination of all affected cattle and samples submitted to FMD National Reference Laboratory for sero-typing using methodology described in the World Organization of Animal Health Terrestrial Manual (OIE, 2018). Antigen detection ELISA yielded SAT 1 serotype as the cause of the outbreak in Athi River but no virus was detected in the Makueni outbreak samples as the lesions were aging because the animals were in the healing stage but FMD was confirmed by Real -Time PCR test. For laboratory diagnosis, the tissue of choice is epithelium or vesicular fluid. Ideally, at least 1 g of epithelial tissue was collected from an unruptured or recently ruptured vesicle, usually from the tongue, buccal mucosa or feet. To avoid injury to personnel collecting the samples, as well as for animal welfare reasons, it is recommended that animals be sedated before any samples are 4.2.2.5 Laboratory Test.

4.2.2.5.1 Antigen detection

Where clinical manifestation of FMD was encountered, epithelium samples were collected from freshly infected animals and laboratory analysis was done by first isolating the virus on BHK-21 cells and serotyping by antigen detection ELISA (OIE, 2018) using ISZLER kit.

4.2.2.5.2Antibody detection

Sera collected were submitted to FMD National Reference Laboratory for antibody assays. They were analyzed by ID Screen FMD NSP competition foot and Mouth disease virus 3 ABC- EILSA (ID Vet) kit to detect specific antibodies against the non structural protein of Foot and Mouth Disease virus (OIE, 2018) in bovine and small ruminant serum samples.

A total of 658 cattle and 658 small ruminants sera were screened using the kit procedures were based on a solid phases indirect ELISA. In this procedure, samples were exposed to non structural FMDV antigen (NSP 3 ABC) coated wells on micro titer plates. Samples to be tested and the controls were added to the microwells. Anti NSP antibodies if present form an antigen-antibody complex which masks the virus epitopes. Anti- horseradish peroxidase (HRP) conjugate is added subsequently to the microwells and it fixes the remaining free epitopes forming an antigen-conjugate HRP-complex. The excess conjugate is removed by washing and the substrate solution (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the sample being tested. In the absence of antibodies a blue solution appears which becomes yellow after addition of stop solution. In the presence of antibodies no coloration appears. Within 15 min in the dark, the result was read by micro plate photometer, where the optical density (OD) was measured at 450 nm. The diagnostic relevance of the result was obtained by comparing the optical density (OD) which develops in wells containing the samples with the OD from the wells containing the positive control as it was read by the ELISA reader. Mean OD value of negative control is greater than 0.7 while mean OD for positive control is less than 30%. The competition percentage S/N% = OD sample /OD negative control x100. S/N % less than or equal to 50% are considered positive. Greater 50% are considered negative.

The 3ABC ELISA has an advantage over the conventional serological diagnosis of FMD as it has the ability to identify vaccinated animals from infected animals based on the detection of the non-structural protein (NSP) that is secreted during infection but not during vaccination .

4.3 DATA ANALYSIS

The data collected was entered into Microsoft Excel, cleaned and coded for analysis, the laboratory investigation for prevalence result were analyzed using R statistical package. For each individual herd, pre-tested questionnaire surveys were conducted for assessment of the associated risk factors using odds ratio.

Variation for the prevalence in the three counties, species, breed, sex, age and vaccination status in cattle were analyzed. In all the analysis, confidence level was at 95% and $\rho<0.05$ was set for significance.

4.3 RESULT

Active cases of FMD were encountered in two sites,one in Athi-River, Machakos and Makueni. Test by Antigen detection ELISA yielded SAT 1 serotype as the cause of the outbreak in Athi River but no virus was detected in the Makueni outbreak samples as the lesions were aging because the animals were in the healing stage but FMD was confirmed by Real -Time PCR test.

Figure 4.3 Lesions in the mouth and foot of an infected cow in Mavoko Sub-County

Seroprevalence result of total 658 cattle, 824 small ruminants sera processed from Ukambani region were assessed for the presence of non-structural FMDV protein (antibodies) demonstrating sero-positivity (Table 4.2). The overall sero prevalence of FMD in cattle in the region was 40% (263/658) and 19% in SR (112/824). There is a 40% chance of cattle selected at random being infected with FMD and 19% chance in SR. (Table 4.3)

Table 4.2: Overall Seroprevalence of FMD in cattle and small ruminants in Ukambani region

Livestock category	Total samples tested (n)	Negative result	Positive result	% seropositivity	Odds Ratio (95% CI)	p-value
Cattle	658	395	263	40		
Small ruminants	864	356	112	19	0.2393	0.1931

Table 4.3: Risk factors of FMD in Small Ruminants

Risk Factor	Variable	P value	OR	95% CI of OR	
Sex	Female		100.00%	NA	NA
	Male	0.107	64.17%	37.31%	106.62%
Age	Mature(above 1yr)		100.00%	NA	NA
	Young(below 1 yr)	0.057	27.00%	3.97%	93.17%
Source	Born in herd		100.00%	NA	NA
	Brought in	0.190	68.42%	38.41%	116.86%
Sex * Age Interaction	Male /Young				
	Mature female		100.00%		
	Young female	0.052	18.80%	0.77%	93.74%
	Mature female		100.00%		
	Mature male	0.100	62.50%	35.85%	105.09%
	Mature female		100.00%		
	Young male	0.456	39.51%	1.55%	224.83%
	Mature male		100.00%		
	Young female	0.305	30.17%	1.21%	161.64%
	Young female		100.00%		
	Young male	1.000	206.16%	4.86%	8729.04%
	Mature male		100.00%		
	Young male	1	63.32%	2.42%	382.96%

Age specific seroprevalence study revealed a higher prevalence in adults SR (OR=100%) as compared to young stock (OR=27%). The proportion of mature SR infected with FMD is greater than the proportion of young SR with FMD (p-value= 0.057) (Table 4.2). The same applied to cattle where mature cattle had higher chances of being seropositive for FMD (Table 4.4). In SR sex analysis as a risk factor for FMD, the proportion of female SR infected with FMD is greater than the proportion of males at OR=100% and OR= 64.17% respectively (p<0.057). In cattle the male animals had higher chance of being seropositive (OR=111.35%) than female animals(OR= 100%, p=0.517).

Animals that were born in herd were more likely to be seropositive(OR= 100%) than animals that were brought in small ruminants(68.42%, p=0.190). The overall seroprevalence in cattle in the region was 40% with Machakos posting the highest prevalence in the region of 47.1%, Makueni 41.9% Kitui level was lower at 31.1%. On SR seroprevalence on the region was 19%, Makueni had the highest rate at 22.7% while Machakos had 19% and Kitui maintained a lower rate at 16%.

Table 4.4: Sero-prevalence per County per Livestock type in Ukambani Region

County	**Livestock type**	**NSP antibody Positive samples**	**NSP antibody Negative samples**	**Total samples tested**	**% Sero-positivity**
Kitui	Cattle	164	74	238	31.1
Machakos		111	99	210	47.1
Makueni		122	88	210	41.9
Kitui	Small ruminants	29	152	181	16.0
Machakos		40	170	210	19.0
Makueni		43	146	189	22.7

Table 4.5: Risk factor analysis for cattle in Ukambani region

Risk Factor	Variable	P value	OR	95% CI of OR	
Sex	Female		100.00%		
	Male	0.5171281	111.35%	80.98%	153.00%
Age	Mature(>2 yrs)		100.00%		
	Young(1-2 yrs)	0.4884584	52.92%	6.96%	240.33%
Sex * Age Interaction	Male /Young				
	Mature female		100.00%		
	Young female	1.000	81.73%	10.00%	450.18%
	Mature female		100.00%		
	Mature male	0.568	110.91%	80.48%	152.70%
	Mature female				
	Young male				
	Mature male		100.00%		
	Young female	1	73.72%	8.98%	408.23%
	Young female				
	Young male				
	Mature male				
	Young male				

4.4 DISCUSSION

According to this study the Ukambani sero- prevalence of FMD was 40% in cattle. The study showed lower overall Country cattle sero- prevalence from previous findings (Kibore *et. al.*2013) in which Country seropositivity of 52.5% was reported and in a study done at the same time as this regional surveillance in the country had a seroprevalence of 37.6% (unpublished work, 2016) which is also slightly lower than the region's seroprevalence. Machakos had higher seroprevalence compared to Makueni and Kitui which agrees with these earlier findings. This is probably due to it's proximity to Nairobi and Kenya's big abattoirs animals from all over the country converge to the large slaughter abbatiors. There is also rampant movement of animals from many surrounding counties towards the highlands during the dry season in search of pastures and water and in the process transmitting FMD.

In small ruminants the 19% seroprevalence in Ukambani region in this study is slightly lower than the country seroprevalence in small ruminants(22.5%) carried out at the same time as this study (Chepkwony *et al.,*2021).This study demonstrates that FMD has been circulating in all areas in Ukambani region and majority of cases are unreported or investigated. Encounter of two active unreported outbreaks in the region within three weeks attests to this finding.The serological profile of FMD in all the three counties of Kitui, Machakos and Makueni shows that FMD is a real impediment to the livestock industry and needs to be controlled to improve farmers livelihood through better yields and heightened trade in this economic block. Of the 47 of the farmers, 46 in the area (97.9%) are conversant with clinical signs of FMD and rate the disease among the three most important diseases citing productivity losses and fast spread for their rating. The majority of herd owners (85.1%, 40/47) interviewed alluded to increased cases of FMD in the dry seasons pointing to the association of FMD with animal movement. These animals move across the boundaries for grazing and watering, and also by illegal trade thus promoting the concept that FMD outbreak peaks in domestic ruminants being associated with cattle movement. There was also higher prevalence in animals which were born in herd than those which had been moved in from markets and other farms. These moving animals come in contact with other animals from different areas which is an important factor for the transmission of the disease but the opposite was true.

A significant difference was observed in sero-prevalence of FMD among mature than young cattle and small ruminants. This may be due to the fact that mature animals had experienced more exposures to FMD at grazing, watering point and at market than in age group less than 2 years for cattle and less than 1 year for small ruminants. Therefore, adult animals might have acquired infection from multiple strains and serotypes thus producing antibodies against

multiple virus incursions of FMD. The low prevalence in young animals may be indicative of persistent passive immunity and less frequency of exposure of the animal to the disease as the farmers keep their calves and young ruminants in pens and enclosures with minimal contact with other animals near the homestead. Similar findings were observed in other studies (Khan *et al*.,2002, Gelaye *et al.*,2009, Ochi *et al.,* 2014)). Some studies also suggest cumulative exposure of adult animals with FMD viruses leading to high seroprevalence (Murphy *et al.*, 1999). However few studies have shown higher FMD prevalence in calves than in adult cattle (Perry *et al.,* 2003, Rufael *et al.*, 2008). Unlike small ruminants higher seroprevalence in males than females in cattle was recorded. This may be due to the fact that in this region males are kept for long because they are used for ploughing land and beef trade and tend to move from farm to farm in the planting season. Unlike many other regions of Kenya male animals in Ukambani region form a significant part of the herd structure.

Another variable that was found to be important predictor of cattle being sero-positive to FMD was vaccination status. Varied vaccination histories were collected with some cattle being recently vaccinated and others vaccinated too long before. Vaccination carried out once in more than two years is unlikely to offer reasonable protection so all cattle reported to have been vaccinated once in more than two years without booster doses were regarded as unvaccinated. Those which had been vaccinated within the last one year were considered as the vaccinated group. The rest were pooled with those which were reported to be unvaccinated. Those whose vaccination statuses were unknown were included in the analysis as the third category. In this study cattle that were not vaccinated had 1.168 times the risk of FMD infection compared to cattle that were vaccinated (in a year or less before i.e 2015, 2016). Thus 14% of the FMD infections in the group that were infected can be attributed to

being un-vaccinated. Vaccinations though in most cases sampled were not regular showed that it plays a key role in preventing exposure to FMD infection.

Extensive movement of livestock and high rate of contact among animals at commercial markets, in communal grazing areas and at watering points undoubtedly contributed to the high prevalence rate in the areas, these being the main risk factors in the region from the questionnaire surveys. Most of the Ukambani region is semi-arid and most resources like water and pastures are shared leading to quick spread of the disease during outbreaks. This was witnessed in two outbreaks encountered during the surveillance.

Therefore vaccination and movement control can have an impact on reducing the economic impact of the disease on the country's economy.

4.5 CONCLUSION

The obtained seroprevalence 40% of FMD in Ukambani region shows that FMD is one of the economically important diseases in the area. There is higher seroprevalence in cattle compared to small ruminants at 40% and 19% respectively. This could mean the risk in cattle is higher than SR and possibly infection normally moves from cattle to SR but further studies in field situations like this need to be carried out. Risk factors associated with the disease are production system, age, sex and vaccination status. Based on this study high prevalence of FMD and its presence in all parts of the region means concerted efforts should be applied to control the disease. Since this area forms an economic block joint efforts of disease control can be applied.

Vaccination of cattle, early detection of FMD outbreaks as well as movement controls is a method of choice to prevent future outbreaks in the region.As the Risk Based FMD Control Strategy is currently being developed, findings of this study could be considered to move the region on the Proposed Control Pathway which requires that risk factors identification and Surveillance output as part of the activities necessary to progress.

Surveillance forms an important tool in FMD control as it gives us a clear indication of whether the virus is circulating, in which specific areas and in what levels. It can also help to detect virus circulation to allow early interventions and it could be used as an indicator for monitoring the effectiveness of instituted control measures.

Control of an economically important disease like FMD is important in alleviating poverty and growing the economy of the region like Ukambani where Livestock production contributes over 70% of the household income. This study highlighted the benefits of conducting serological and questionnaire surveys, simultaneously, to ascertain clinical occurrence of FMD, the sero-prevalence and risk factors of associated with the disease. Some of the findings from this study could be considered for strengthening of the current FMD control program in the region as the Risk based Control strategy.

4.6 RECOMMENDATIONS

1. Presence of foot and mouth disease in livestock population affects the economy at large by limiting international and local trade of live animals and animal by products, and consideration of this situation is important in controlling the disease. Two active cases of unreported FMD outbreaks were detected in this surveillance. More active

and passive surveillance work in the region to help in detecting virus circulation and developing FMD control strategies.

2. Identification of more risk factors to FMD is important for developing Risk Based Control Strategies recommended for present day management of FMD. This study concentrated on FMD epidemiology in domestic ruminants. More work on other susceptible animals like wildlife and pigs in the region can be carried out understand seroprevalence and risk factors in other species. This could be intergrated to develop risk based control measures to ensure the little resources available for disease control is utilized in best cost effective manner. Control measures through risk based vaccination and restriction of animal movements remains the most important strategy to minimize the risk of FMD in a region or Country.
3. Governors/ Politicians, Policymakers and Economists have to be provoked to put their relentless effort in the control of FMD in this Economic block, a disease that has serious impact on trade. Disease control is a devolved function of the Counties therefore sensitization of the Governors on spending more money on disease control especially vaccination and proper management of animal movements and especially livestock markets to reduce spread of the disease. Lack of vaccination and buying animals from markets were significant risk factors for seroprevalence in this study.
4. These outbreaks were unreported so training of Veterinary service providers should be carried out to help the field personnel in early detection, proper sampling, reporting, shipping of samples and FMD Biosecurity. This will help in instituting early interventions and reducing spread of the disease.
5. There was significant relationship between wildlife-livestock interaction and seroprevalence. More FMD studies should be done on other susceptible species

including wild ruminants and pigs which have potential contact with domestic ruminants so as to understand their role in the epidemiology of the disease.

Chapter 5: MOLECULAR CHARACTERIZATION AND PHYLOGENETIC EVALUATION OF SEROTYPES O AND A FOOT- AND- MOUTH DISEASE VIRUSES IN CIRCULATION IN KENYA: 2013-2018.

5.1 INTRODUCTION

5.1.2 The basis of molecular characterization

The molecular characterization of virus isolates is an important requirement in control of FMD. Sequencing the region of FMDV genome encoding the capsid proteins of the virus provide the most detailed information about isolates as this region is variable between serotypes and subtypes (Dominigo *et al.,*1990). The hypervariable region of FMDV genome that is responsible for this antigenic diversity lies in the VP1 gene segment. Therefore FMDV antigenic diversity is due to nucleotide and amino acid substsitution of VP1.

The determination of FMDV nucleotide sequences and phylogenetic analysis is definitive technique for characterizing individual strains of FMDV(Knowles and Samwel,1995). Nucleotide sequencing was first used for epidemiology of FMDV by Beck and Strohmaier in 1987 who investigated the origin of outbreaks of types O and A in Europe over a 20 year period. Since then genetic variability has been used to individually characterize strains of FMD and track their movement across international borders(Knowles and Samwel,1998). Vaccines must be carefully matched to outbreak strains and continually updated to ensure efficacy (Samwel and Knowles, 2001).

5.1.2 Phylogenetic analysis

Phylogenetic analysis of the VP1 region of FMD viruses has been used extensively to investigate the molecular epidemiology of the disease worldwide. These techniques have assisted in studies of the genetic relationships between different FMD virus isolates,

geographical distribution of lineages and genotypes, the establishment of genetically and geographically linked topotypes and tracing the source of virus during outbreaks (Samuel and Knowles, 2001a, b, Knowles *et al*., 2000; Knowles and Samuel, 2003; Sangare *et al.,* 2001, 2003). Sequence differences of 30 to 55% of the VP1 gene were obtained between 7 serotypes of FMD while different subgroups (genotypes, topotypes) were defined by differences of 15 to 20% (Knowles and Samuel, 2003). Since 1987, the analysis of the genetic distance and phylogenetic resolution of the sequence of VP1 encoding gene have provided crucial epidemiological information covering different degree of genetic relationships between field isolates (Vosloo *et al*., 1992; Samuel *et al*., 1997; Samuel *et al*., 1999; Bastos *et al*., 2001; Samuel and Knowles, 2001a,b; Knowles and Samuel, 2003) as follows:

Virus isolates from the same epizootic differ by $\leq$ 1%

Viruses belonging to the same epizootics (common origin) differ by < 7%

Viruses of the same genotype differ up to 15%

Viruses from different genetic lineages differ by ($\geq$ 20%)

The evolutionary changes of viruses are determined by comparing genomic material from more than one virus with each other. The basic process in the evolution of DNA/RNA sequence is the substitution of one nucleotide for another over evolutionary time. Changes in nucleotide sequences are used in molecular evolutionary studies both for estimating the rate of evolution and for re-constructing the evolutionary history of organisms (Graur and Wen-Hsiung, 2000). At present, DNA sequencing and phylogenetic trees are widely used to illustrate the genetic relationships between viruses. In order to construct evolutionary trees assumptions are made about the substitution process and these assumptions are stated in the form of a model.

Several assumptions exist regarding the probability of substitution of one nucleotide by another. For example the one parameter model of Juke and Cantor (1969) is based on the assumption that substitutions occur with equal probability among the four nucleotide types (Figure 5.1) while Kimura's two-parameter model (Nei and Kumar, 2000) assumes transitions are generally more frequent than transversions. A simple measure of the extent of sequence divergence is the proportion (p) of nucleotide sites at which the two sequences are different.

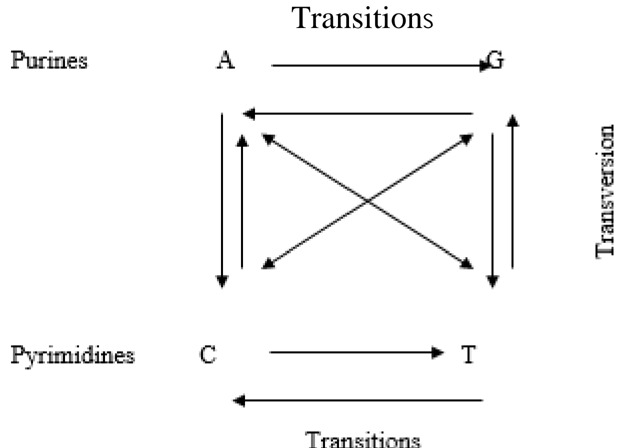


Figure 5.1 The probability of substitution of one nucleotide by another between purines and/or pyrimidines.

5.1.3 Phylogenetic trees

The evolutionary relationships among a group of organisms are illustrated by means of phylogenetic trees where the phylogeny is the branching history of route of inheritance of species populations or genes and is microevolutionary informative (Maddison *et al.*, 1992). Phylogeny is misleading unless it is based on a reasonable alignment of the sequences used in the analysis and computer programs are available for obtaining optimal alignment of sequences.

5.1.4 Methods for constructing phylogenies

5.1.4.1 Tree construction

There are a variety of methods available to construct trees from sequence data which use two primary approaches to tree construction: algorithmic and tree-searching. The algorithmic approach uses an algorithm to construct a tree from the data while the tree-searching method constructs many trees and then uses some criterion to decide which is the best tree or best of trees. Currently, three primary methods for constructing phylogenies from nucleic acid alignments *viz*., Neighbour-joining (NJ), Maximum Parsimony (MP) and Maximum likelihood (ML) methods are in use (Nei and Kumar, 2000). The method of choice depends both on what you want to learn and on the size and complexity of the data set. It also depends on the speed of your computer and the ease of implementing the particular method. However, some criteria like efficiency, robustness, computational speed and discriminating ability are considered to select the best method for constructing evolutionary trees.

5.1.4.2 Distance methods

Distance methods convert the aligned sequences into a distance matrix of pair-wise differences (distances) between the sequences. The NJ and Unweighted Pair-Group Method (UPGMA) using arithmetic average methods are currently in use which are both algorithmic methods *i.e.*, they use a specific series of calculations to estimate a tree. The calculation involves manipulations of a distance matrix that is derived from multiple alignments. Starting with the multiple alignments, both programmes calculate for each pair of taxa the distance, or the fraction of differences, between the two sequences and write that distance to a matrix.

5.1.4.3 Character-based methods

Character-based methods include Parsimony, Maximum Likelihood, and Bayesian methods; all use the multiple alignments directly by comparing characters within each column (each site) in the alignment. Parsimony looks for the tree or trees with the minimum number of changes (Parsimony-informative sites). Maximum likelihood tries to infer an evolutionary tree, under some model of evolution, by finding that tree that maximizes the probability of observing the data. The Bayesian analysis is a recent variant of Maximum Likelihood. This method, instead of seeking the tree that maximizes the likelihood of observing the data, seeks those trees with the greatest likelihoods given the data.

5.1.5 FMD vaccination in Kenya in relation to strains

Control of FMD in the country has been through vaccination both as a means to contain an active outbreak and also for building immunity using locally produced inactivated vaccine. Control strategies are impeded by the presence of multiple serotypes and strains in the country and rampant animal movement in search of pastures/water and also for trade. Vaccines are available from the local producer Kenya Veterinary Vaccines Production Institute (KEVEVAPI) and vaccines can be sourced for prophylaxis and also for ring vaccination to contain an outbreak after confirming FMDV and serotyping by laboratory diagnosis. Control measures currently are not consistent and vaccination is often done to contain an outbreak rather than being risk-based or following a structured regime. Vaccines of good quality are critical in controlling the disease. Vaccine failures are sometimes reported probably due to emergence of new strains in the field which may not phenotypically and antigenically relate to the vaccine strains. This study was designed to evaluate the molecular and phenotypic

relationship of the field strains of serotype O and A in the period 2013-2018, other documented strains in the gene bank and the current Kenyan vaccine strains.

5.2 Justification of the study

The FMD virus has high mutation frequencies leading to new lineages and this presents the importance of studying evolutionary changes that can occur in the viral strains. The VP1 is pivotal capsid protein important in the replication of viral particles. It's highly polymorphic having receptor-mediated attachment and humoral immune responses with major neutralization antigenic sites. In many past studies VP1 nucleotide sequence has been used in the epidemiology of field outbreak investigations, selection of appropriate vaccine, the development of engineering vaccines, improving diagnostic techniques, to trace outbreaks and their spread and also for serotyping and sub-typing of the viral strains.

5.3 MATERIALS AND METHODS

5.3.1 Selection of field isolates for the study

Epithelial samples and original suspension samples which had serotyped O and A were obtained from the archived samples at FMD National Laboratory sample storage. The samples were basically diagnostic samples collected by field Sub-County Veterinary Officers (SCVOs)/ Field Officers from cattle with active FMD oubreaks from the year 2013 to 2018. During this study period several samples were collected which typed different serotypes but for the purpose of this study those which tested positive for serotype O and A were used. The initial purpose of submission of these samples was for FMD confirmation and serotype identification in order to institute control measures. Upon collection of samples in the field they were preserved in transport medium consisting of proportionate amounts of glycerol with

0.04 M phosphate buffered saline plus neomycin sulphate antibiotics and antifungal agent mycostatin each 100 I.U and maintained at neutral pH 7.2-7.6 (OIE, 2018). This is kept cool until it arrives in the testing laboratory by use of ice. The required quantity was processed and subsequently aliquoted and preserved at -20°C during the testing period. Upon completion of testing, the extra epithelia samples were preserved in glycerol and banked in -80°C freezers for longer preservation.

In the molecular characterization, the nucleotide sequences encoding the capsid protein VP1 (1D) region of FMDV from virus samples were generated by RNA extraction and Reverse transcription polymerase chain reaction and sequencing. For serotype O, 60 isolates were characterized, 58 being field viruses and serotype O vaccine strain (OK77/78) in duplicate and for serotype A were 21 field isolates and one vaccine strain AK5/80 (n= 22).

5.3.2 Laboratory Processing steps:

5.3.2.1 Virus Isolation

Viruses in preserved epithelium and original suspension were isolated on Baby Hamster Kidney cells (BHK-21) which are more commonly used in the FMD Laboratory, Kenya or where there was unsuccessful isolation primary bovine thyroid (BTY) cells were utilized (OIE, 2018) for isolation then extraction. Metadata of samples successfully retrieved are in Tables 5.2 and 5.3. The archival samples generated after initial diagnostic processing were mostly epithelium tissues which had been preserved in -80°C ultra-freezer with glycerol. Upon removal thawing was done at room temperature, glycerol was removed by blotting on absorbent paper to ensure the toxicity of glycerol doesn’t cause unnecessary harm to cells. The required quantity was weighed and ground and original suspension was made by adding a

small volume of pre-warmed Eagles minimum essential media culture medium with protein hydrolysate and antibiotics to obtain a suspension of 10%. The suspension was clarified using a bench centrifuge and re-inoculated into cell cultures to passage the viral titres. In samples where no virus was recovered, the samples were inoculated into LFBK cells which contain a principal *cellular* receptor of *FMDV (bovine* αVβ6 integrin) and observed for cytopathic effect within 48 hours and this improved the chances of isolating the virus. Lack of development of cythopathic effect meant the *vir*us titres had deteriorated in long storage therefore samples in this category were excluded in the study. Most of the selected samples were however positive and therefore processing continued. From the positive samples the virus was harvested by freezing and thawing the cell culture and centrifugation. The harvested infected fluid was tested by FMDV antigen detection ELISA for confirmation of virus presence and serotyping as O and A.

Original suspension stocks of virus recovered were clarified and kept at -70°C and until required for extraction step in Polymerase chain reaction test. In this study, the samples selected were 58 field samples and vaccine isolate in duplicate for serotype O, and 21 field isolates for serotype A and the vaccine strain. The purpose of studying these strains was to genetically characterize the circulating field strains and to relate to this vaccine strain and other documented strains.

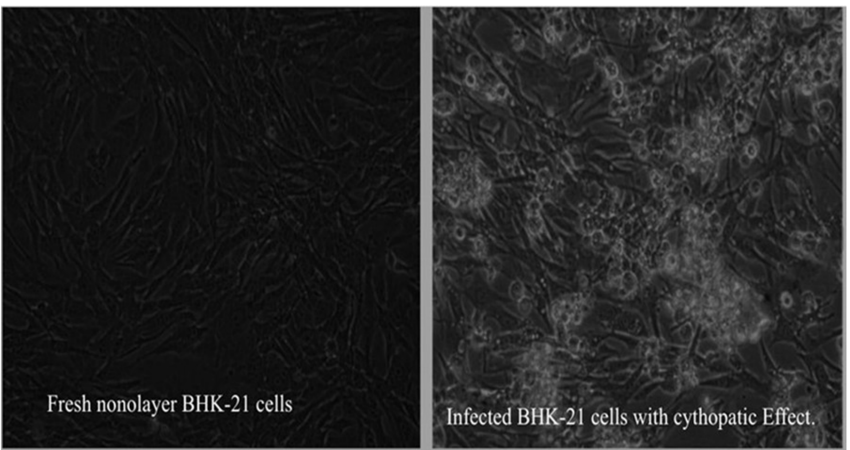


Figure 5.2 BHK-21 cells before infection and at 48 hours post-infection withb FMDV showing cythopathic effect

5.3.3.2 Master-mix preparation and RNA Extraction

In this study to avoid possible contamination, the master-mix was prepared in a separate PCR clean room combined with following good work habits. The process followed manufacturer's procedure QIAGEN One-Step RT-PCR kit (Qiagen, Germany) was used and the master mix tube of 50 µl consisted of the following:13 µl of nuclease-free water, 4 µl forward primer O-1C499F or A-1C612 (for serotype A) - (5' TAC GCG TAC ACC CGC GTC 3' or 5' TAG CGC CGG CAA AGA CTT TGA 3') at a concentration of 0.6 µlM, 4 µl of reverse primer EUR–2B52R (5'-GAA GGG CCC AGG GTT GGA CTC- 3 ' (21 mer)0.6 µlM, 10.0 µl of 5x PCR Buffer made of 12.5 mM of $MgCl_2$), 2.0 µl of deoxyncleoside Triphosphate mix consisting of 10 mM 400 µM of each dNTPs), 2.0 µl Enzyme Mix and 10 µl of 5x Q-solution to make a total master mix of 50 µl with 5 µl of the sample which is added in the extraction room.

In another dedicated extraction room samples were assembled and total RNA was extracted using the manufacturer's instructions available on the Qiagen QIAamp viral RNA Minikit

(Qiagen, Hilden, Germany). RNA was carefully extracted from each of these tissue samples using 50 µl of nuclease free water and kept on ice to await the RT-PCR or in -20°C if this step was not immediate.

5.3.3.3 Reverse transcription polymerase chain reaction (RT-PCR)

After preparing the master mix and extracting the RNA, 5 µl of the viral RNA prepared as described in step 2 was added to the mastermix. To check and ensure no cross contamination the primer sets were set up without templates/samples substituted with nuclease free water. The PCR cycling programme was chosen according to the primer sets as shown in Table 5.1. To prevent evaporation of the reaction mix the lid of the thermocycler was preheated before the cycling process. After completion of the reverse transcription step the tubes were placed in the fridge at -20°C until the next step. The PCR was run in a thermocycler (Gene Amp® PCR system 3700 version 3.0 - Applied Biosystems) using the touchdown method for 1 cycle; for serotype O we used a programme of 50°C for 30 minutes reverse transcription step, 1 cycle 95°C for 15 minutes Inactivation step, 35 cycles of three steps namely 95°C 60 seconds denaturation, 60°C 60 seconds primer annealing and 72°C 120 seconds extension steps, 1 cycle final extension for 5 minutes and 4°C holding step. For serotype A samples the temperature was adjusted to 55°C for annealing step.

5.3.3.4 Purification

The resulting cDNA products were purified using the QIAquick PCR purification kit (Qiagen). To quantify the PCR products for cycle sequencing a NanoDrop® 1000 Spectrophotometer (Thermo Scientific) was used. The purified PCR products were tested to

ascertain presence of VP1 protein of expected length according to procedure described by Knowles *et al*., 2016.

Table 5.1: STANDARD THERMOCYCLING PROTOCOL USED FOR RT-PCR AMPLIFICATION OF VP1 REGION OF FMDV SEROTYPE O AND A

Cycling parameter	Temperature value used	Duration	Cycles
Reverse Transcription	50°C	30 min	1
Inactivation	95 °C	15 min	1
Denaturation	95 °C	60 sec	35
Primer annealing	60 °C for **O** and 55 °C for **A**	60 sec	
Extension	72 °C	120 sec	
Final Extension	72 °C	5 min	1

The PCR products (ca. 885 bp) were viewed by electrophoresis on 1.5 % agarose gels (Seakem GTG agarose in 1 X TAE - low EDTA buffer) at 120 volts for 1 hour in parallel with a molecular weight marker ΦX 174-RF DNA(Fig 5. 2).

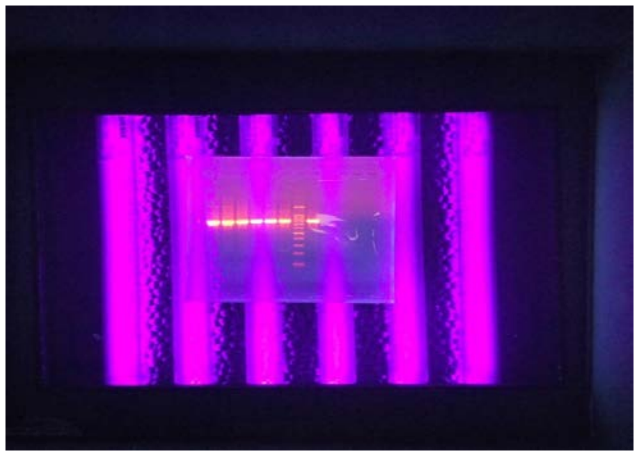


Figure 5.3 An Electrophoresis picture of the PCR products as seen on 1.5 % agarose gels

Table5.2: SEROTYPE 'A' SAMPLES METADATA

SEQUENCING CODE	FMD LAB CODE	MONTH AND YEAR OF COLLECTION	SOURCE/ SUB-LOCATION	LOCATION	DIVISION	SUB-COUNTY	COUNTY	SEROTYPE
A1	K74/15	Aug-15	Bogetenga	Mugirago	Bogetenga	Gucha South	KISII	A
A2	K74/17	Nov-17	Darga	Fafi	Fafi	Fafi	GARISA	A
A3	K75/17	Nov-17	Darga	Fafi	Fafi	Fafi	GARISA	A
A4	K76/16	Nov-16	Kaplomboi	Ndanai	Ndanai	Sotik	KERICHO	A
A5	K86/15	Sep-15	Otonglo	Central Kisumu	Korando "B"	Kisumu West	KISUMU	A
A6	K11/16	Mar-16	Maji Tamu	Arusto	Solai	Rongai	NAKURU	A
A7	K12/16	Mar-16	Ndundori	Bahati	Bahati	Bahati	NAKURU	A
A8	K13/16	Mar-16	Ndundori	Bahati	Bahati	Bahati	NAKURU	A
A9	K17/16	Mar-16	Kabolet	Makutano	Cherangany	Trans nzoia	TRANS NZOIA	A
A10	K74/16	Oct-16	Subukia East	Subukia East	Subukia	Subukia	NAKURU	A
A11	P2535	Dec-17	PTS sample					A
A12	K8/16	Feb-16	Tabuga	Tabuga	Rongai	Rongai	NAKURU	A
A13	K68/16	Sep-16	Ngoisa	Kabenes	Soy	Eldoret	UASIN GISHU	A
A14	K23/17	May-17	Ridge ways	Roy-sambu	Kasarani	Kasarani	NAIROBI	A
A15	K73/17	Nov-17	Darga	Fafi	Fafi	Fafi	GARISA	A
A16	K72/17	Nov-17	Darga	Fafi	Fafi	Fafi	GARISA	A
A17	K73/15	Jul-17	Kisumu	Municipality	Kisumu	Kisumu west	KISUMU	A
A18	K31/18	Jan-18	Mutira	Mutira	Karatina	Kirinyaga	KIRINYAGA	A
A19	K62/17	Nov-17	Kigaa/Gikuuri	Runyenjes East	Runyenjes	Runyenjes	EMBU	A
A20	K10/16	Mar-16	Chepareria	Kipkomo	Chepareria	Pokot South	WEST POKOT	A
A21	K15/16	Mar-16	Kiptulwa	Kiptulwa	Kipsonoi	Sotik	BOMET	A
A22	AK5/80	Jan-80	In use for vaccine prodcuction at KEVEVAPI					A

Table5.3: SEROTYPE 'O' SAMPLES METADATA

SEQUENCING CODE	FMD LAB CODE	MONTH AND YEAR OF COLLECTION	SOURCE/ SUB-LOCATION	LOCATION	COUNTY	COUNTY CODES	SEROTYPE DETECTED
O1	K9/18	Jan-18	Kapiti	Mathatani	MACHAKOS	16	O
O2	K12/18	Jan-18	Kapiti	Mathatani	MACHAKOS	16	O
O3	K14/18	Jan-18	Salgaa	Salgaa	NAKURU	32	O
O4	K18/18	Jan-18	Laikipia East	Sweetwaters	LAIKIPIA	31	O
O5	K20/18	Jan-18	Langas	Langas	UASIN-GISHU	26	O
O6	K21/18	Jan-18	Ituka	Ituka	MAKUENI	17	O
O7	K22/18	Jan-18	East Narasha	Olkinyei	NAROK	33	O
O8	K42/17	Sep-17	Bukengi	Bukhayo West	BUSIA	40	O

O9	K27/17	Jun-17	Kasikeu	Kasikeu	MAKUENI	17	O
O10	K28/17	Jun-17	Township	Thika West	KIAMBU	22	O
O11	K29/17	Jul-17	Township	Municipality	UASIN-GISHU	26	O
O12	K33/17	Aug-17	Kapiyet	Kapiyet	NANDI	29	O
O13	K34/17	Aug-17	Kebulonik	Sangalo	NANDI	29	O
O14	K37/17	Aug-17	Kanyariri	Kanyariri	KIAMBU	22	O
O15	K40/17	Aug-17	Westlands	Lower kabete	NAIROBI	47	O
O16	K53/17	Oct-17	Endebes	Endebes	TRANS-NZOIA	27	O
O17	K59/17	Nov-17	Township	Township	KIAMBU	22	O
O18	K63/17	Nov-17	Makongi	Segero	UASIN-GISHU	26	O
O19	K79/17	Dec-17	Nyathona	Kabatini	NAKURU	32	O
O20	K81/17	Dec-17	Aporodo	Ahero	KISUMU	42	O
O21	K67/16	Sep-16	Juja	Juja	KIAMBU	22	O
O22	K17/17	Mar-17	Bruynsha	Bruynsha	KIAMBU	22	O
O23	K39/17	Aug-17	Gitaru	Gitaru	KIAMBU	22	O
O24	K41/17	Sep-17	Merewet	Merewet	UASIN-GISHU	26	O
O25	K56/17	Oct-17	Kauti	Lower Kaewa	MACHAKOS	16	O
O26	K61/17	Nov-17	Ruai	Ruai	NAIROBI	47	O
O27	K64/17	Nov-17	Gichagi	Mountain View	NAIROBI	47	O
O28	K67/17	Nov-17	Kipkenyo	Kipkenyo	UASIN-GISHU	26	O
O29	K11/18	Jan-18	Kapiti	Mathatani	MACHAKOS	16	O
O30	K25/18	Jan-18	OlPajeta	Sweetwaters	LAIKIPIA	31	O
O31	K26/18	Jan-18	Withare	Withare	LAIKIPIA	31	O
O32	K27/18	Jan-18	Urudi	Urudi	KISUMU	42	O
O33	K28/18	Jan-18	Wasare	Wasare	KISUMU	42	O
O34	K30/18	Jan-18	Sukut	Kishaunet	WEST	24	O
O35	K33/18	Feb-18	Kituluni	Kituluni	MAKUENI	17	O
O36	K34/18	Feb -18	South Kochongo	Kochongo	KISUMU	42	O
O37	K24/14	Feb-14	Central	Kiwanjani	KAJIADO	34	O
O38	K22/14	Feb-14	Yathui	Yathui	MACHAKOS	16	O
O39	K44/18	Feb-18	Litein	Chesingoro	KERICHO	35	O
O40	K25/14	Jan-14	Muhoroni	Koru	KISUMU	42	O
O41	K39/14	Feb-14	Rarieda	West Asembo	SIAYA	41	O
O42	K43/14	Feb-14	Malili	Malili	MACHAKOS	16	O
O43	K46/14	Feb-14	Kanduyi	East Sang'alo	BUNGOMA	39	O
O44	K50/14	Feb-14	Soy	Kabulgey	UASIN GISHU	26	O
O45	K55/14	Mar-14	Assa	Assa	TANA RIVER	4	O
O46	K61/14	Mar-14	Ngata	Ngata	NAKURU	32	O
O47	K84/14	May-14	Gilgil	Gilgil	NAKURU	32	O
O48	K86/14	May-14	Wote	Wote	MAKUENI	17	O
O49	K112/14	Jul-14	Ruai	Shujaa	NAIROBI	47	O
O50	K148/14	Oct-14	Eldama Ravine	Ravine	BARINGO	30	O
O51	K35/13	May-13	Juja	Kiahuria	KIAMBU	22	O
O52	K82/13	Oct-13	Lanet	Lanet	NAKURU	32	O
O53	K61/15	Jul-15	Soy	Kiplombe	UASIN GISHU	26	O
O54	K16/15	Jan-15	Kikoe	Kikoe	KIAMBU	22	O
O55	K44/15	Mar-15	Kilibwoni	Kilibwoni	NANDI		
O56	K35/16	Jun-16	Kikobe	Gilgil	NAKURU	32	O
O57	K37/16	Jun-16	Kikobe	Gilgil	NAKURU	32	O
O58	K45/18	Feb-18	Mabasi	Kisiara	KERICHO	35	O
O59	OK77/78	May-78	Vaccine strain	KEVEVAPI			O
O60	OK77/78	May-78	Vaccine strain	KEVEVAPI			O

5.3.3.5 Sequencing work

After obtaining the PCR products, they were sent to Macrogen, Amsterdam in The Netherlands for sequencing. We sent a total of 82 samples (O and A) together with specific forward and reverse primers for each samples set i.e. for each serotype. Table 5.4 and 5.5 shows the primers used.

Table5.4: PRIMERS SUBMITTED FOR SEROTYPE O SEQUENCING

Primers used	Sequence(5'-3')	Genome direction	Gene
Forward Primer O-1C 499F	Seq 5'-TAC GCG TAC ACC CGC GTC-3'	+	VP 1
Reverse Primer NK	Seq: 5'-GAA GGG CCC AGG GTT GGA CTC- 3 '(21 mer)	-	2B

Table 5.5: PRIMERS SUBMITTED FOR SEROTYPE A SEQUENCING

Primers used	Sequence(5'-3')	Genome direction	Gene
Forward Primer A-1C 612	Seq 5'-TAG GCG CGG CAA AGA CTT TGA ACC CGC GTC-3'	+	VP 1
Reverse Primer NK	Seq: 5'-GAA GGG CCC AGG GTT GGA CTC- 3 '(21 mer)	-	2B

5. RESULTS

5.1 Serotype A results

5.1.1 Sequence assembly

There were originally 22 samples of Serotype A. Upon receiving the sequence data from macrogen, the AB1 files were exported to the online tool DNA Subway: https://dnasubway.cyverse.org/, which would be used to assemble the sequences of the FMD

virus. First, all the sequences were automatically trimmed. However, samples A4_1F, A7_1F, A7_1R, A9_1F, A9_1R, A11_1F, A12_1F, A17_1F and A21_1F had very low qualities and were subsequently excluded from subsequent analysis. The average error rates for these sequences were greater than 1%, indicating they are of low quality and may produce erroneous analysis results. The number of samples that were considered for further analysis were 15. The forward and reverse reads were paired for each sample. The resulting consensus sequence of each of the 15 samples was then edited by manually trimming the low-quality reads from the 3` and 5` ends.

5.1.2 Blast search

The consensus sequences were then blasted against the nucleotide NCBI database on the International Livestock Research Institute (ILRI) high-performance computer (HPC) using blast/2.10.0+. The objective of the BLAST search was to search for homologous nucleotide sequences from the nucleotide database, using the FMD virus sequences as the query sequences.

5.1.3 Phylogenetic analysis

The original files were renamed, taking into account the metadata. The naming took on the format of the country of collection + the code/county code/year of collection. Additional data was obtained from NCBI https://www.ncbi.nlm.nih.gov/popset/619324022. The consensus sequences and the additional files obtained from NCBI were aligned using MEGA v11.0.8, employing the ClustalW algorithm. SeaView v5.0.4 was used to edit the alignment. MEGA v11.0.8 was used to construct a phylogenetic tree. The evolutionary history between the samples was inferred using the Neighbor-Joining method with 1000 bootstrap replications.

The optimal tree is shown in Figure 5.3 and 5.4. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved 53 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

5.2 Serotype O Results

5.2.1 Sequence assembly

There were originally 60 samples of Serotype O. The AB1 files were also exported to the online tool DNA Subway: https://dnasubway.cyverse.org/, which would be used to assemble the sequences of the FMD virus. First, all the sequences were automatically trimmed. However, samples 10_OR, 26_OR, 34_OF, 36_OF, 41_OR, 42_OR, 52_OF, 55_OR and 59_OF, had very low qualities and were subsequently excluded from subsequent analysis. The average error rate for these sequences was greater than 1%, indicating low quality and to make the analysis more robust we excluded them. The number of samples that were considered for further analysis was 51. The forward and reverse reads were paired for each sample. The consensus sequence of each of the 51 samples was then edited by manually trimming the low-quality reads from the 3` and 5` ends.

5.2.2 Blast search

The consensus sequences were then blasted against the nucleotide NCBI database on the International Livestock Research Institute (ILRI) high-performance computer (HPC) using

blast/2.10.0+. The objective of the BLAST search was to search a homologous nucleotide sequence(s) from the nucleotide database, using the FMDV sequences as the query sequences.

5.2.3 Phylogenetic analysis

The original files were renamed, taking into account the metadata. The naming took on the format of the country of collection + code/county code/year of collection. Additional data was obtained from NCBI https://www.ncbi.nlm.nih.gov/popset/300791303. The consensus sequences and the additional files obtained from NCBI were aligned using MEGA v11.0.8, employing the ClustalW algorithm. SeaView v5.0.4 was used to edit the alignment. MEGA v11.0.8 was used to construct a phylogenetic tree. The evolutionary history between the samples was inferred using the Neighbor-Joining method with 1000 bootstrap replications. The optimal tree is shown in Figure 5.3 and 5.4. The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved 105 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

5.3 RESULT ANALYSIS

In this study, 79 circulating FMD viruses (O: n=58, A:n=21) collected in the span of six years (2013 -2018), including the current vaccine strains in Kenya, were processed in the National Foot and Mouth Disease Laboratory, Embakasi, Nairobi and the cDNA products were sent to Macrogen, Amsterdam in The Netherlands for sequencing. They represented two serotypes which after sequencing , the good sequences included in the analysis reduced to 66(O: n = 51

and A: n = 15). These, together with 92 sequences (O: n = 54 and A: n = 38) selected from publicly available sources (NCBI), constitute a list of 158 sequences for the two serotypes (O: n = 105 and A: n = 53). This is shown in Tables 5.6 and 5.7.

Serotype A

Table5.6: SEROTYPE A SEQUENCES ANALYSED

S/NO	LAB CODE	MONTH AND YEAR OF COLLECTION	COUNTY	COUNTRY	COUNTY CODES	SERO TYPE DETECTED	ACCESSION NUMBER	REFERENCE
1	K74/15	Aug-15	Kisii	Kenya	45	A	Not yet available	This study
2	K74/17	Nov-17	Garisa	Kenya	7	A	Not yet available	This study
3	K75/17	Nov-17	Garisa	Kenya	7	A	Not yet available	This study
4	K86/15	Sep-15	Kisumu	Kenya	42	A	Not yet available	This study
5	K11/16	Mar-16	Nakuru	Kenya	32	A	Not yet available	This study
6	K13/16	Mar-16	Nakuru	Kenya	32	A	Not yet available	This study
7	K74/16	Oct-16	Nakuru	Kenya	32	A	Not yet available	This study
8	K68/16	Sep-16	Uasin-Gishu	Kenya	26	A	Not yet available	This study
9	K23/17	May-17	Nairobi	Kenya	47	A	Not yet available	This study
10	K73/17	Nov-17	Garisa	Kenya	7	A	Not yet available	This study
11	K72/17	Nov-17	Garisa	Kenya	7	A	Not yet available	This study
12	K31/18	Jan-18	Kirinyaga	Kenya	20	A	Not yet available	This study
13	K62/17	Nov-17	Embu	Kenya	14	A	Not yet available	This study
14	K10/16	Mar-16	West pokot	Kenya	24	A	Not yet available	This study
15	AK5/80	Jan-80	Nairobi	Kenya	47	A	Not yet available	This study
16	K3/13	2013	Thika	Kenya	22	A	KJ440876.1	https://pubmed.ncbi.n

			East					lm.nih.gov/24368254/
17	K154/12	2013	Koibatek	Kenya	30	A	KJ440875.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
18	K148/12	2013	Nakuru North	Kenya	32	A	KJ440874.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
19	K143/12	2013	Naivasha	Kenya	32	A	KJ440873.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
20	K138/12	2013	Gilgil	Kenya	32	A	KJ440872.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
21	K63/09	2013	Narok South	Kenya	33	A	KJ440871.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
22	K73/08	2013	Loitokto k	Kenya	34	A	KJ440870.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
23	K44/05	2013	Nakuru	Kenya	32	A	KJ440869.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
24	K129/03	2013	Kajiado	Kenya	34	A	KJ440868.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
25	K60/01	2013	Nairobi	Kenya	47	A	KJ440867.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
26	K73/01	2013	Meru Central	Kenya	12	A	KJ440866.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
27	K1/97	2013	Meru North	Kenya	12	A	KJ440865.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
28	K64/95	2013	Garissa	Kenya	7	A	KJ440864.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
29	K9/94	2013	Narok	Kenya	33	A	KJ440863.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
30	K15/92	2013	Kakamega	Kenya	37	A	KJ440862.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
31	K11/92	2013	Meru North	Kenya	12	A	KJ440861.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
32	K87/91	2013	Meru North	Kenya	12	A	KJ440860.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
33	K83/85	2013	Mombasa	Kenya	1	A	KJ440859.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
34	K67/85	2013	Isiolo	Kenya	11	A	KJ440858.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
35	K49/84	2013	Narok	Kenya	33	A	KJ440857.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
36	K36/84	2013	Meru Central	Kenya	12	A	KJ440856.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
37	K293/83	2013	Taita Taveta	Kenya	6	A	KJ440855.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
38	K288/83	2013	Kericho	Kenya	35	A	KJ440854.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
39	K51/81	2013	Kwale	Kenya	2	A	KJ440853.1	https://pubmed.ncbi.n

								lm.nih.gov/24368254/
40	K48/81	2013	Kilifi	Kenya	3	A	KJ440852.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
41	K7/81	2013	Teso	Kenya	40	A	KJ440851.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
42	K4/81	2013	Bungoma	Kenya	39	A	KJ440850.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
43	K50/81	2013	Trans-Nzoia	Kenya	26	A	KJ440849.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
44	K5/80	2013	Kajiado	Kenya	34	A	KJ440848.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
45	K16/80	2013	Kajiado	Kenya	34	A	KJ440847.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
46	K35/80	2013	Embu	Kenya	14	A	KJ440846.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
47	K158/80	2013	Laikipia	Kenya	31	A	KJ440845.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
48	K151/79	2013	Thika	Kenya	22	A	KJ440844.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
49	K131/79	2013	Kiambu	Kenya	22	A	KJ440843.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
50	K61/78	2013	Kajiado	kenya	34	A	KJ440842.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
51	K28/78	2013	Meru Central	kenya	12	A	KJ440841.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
52	K179/71	2013	Kajiado	Kenya	34	A	KJ440840.1	https://pubmed.ncbi.nlm.nih.gov/24368254/
53	K18/66	2013	Kericho	Kenya	35	A	KJ440839.1	https://pubmed.ncbi.nlm.nih.gov/24368254/

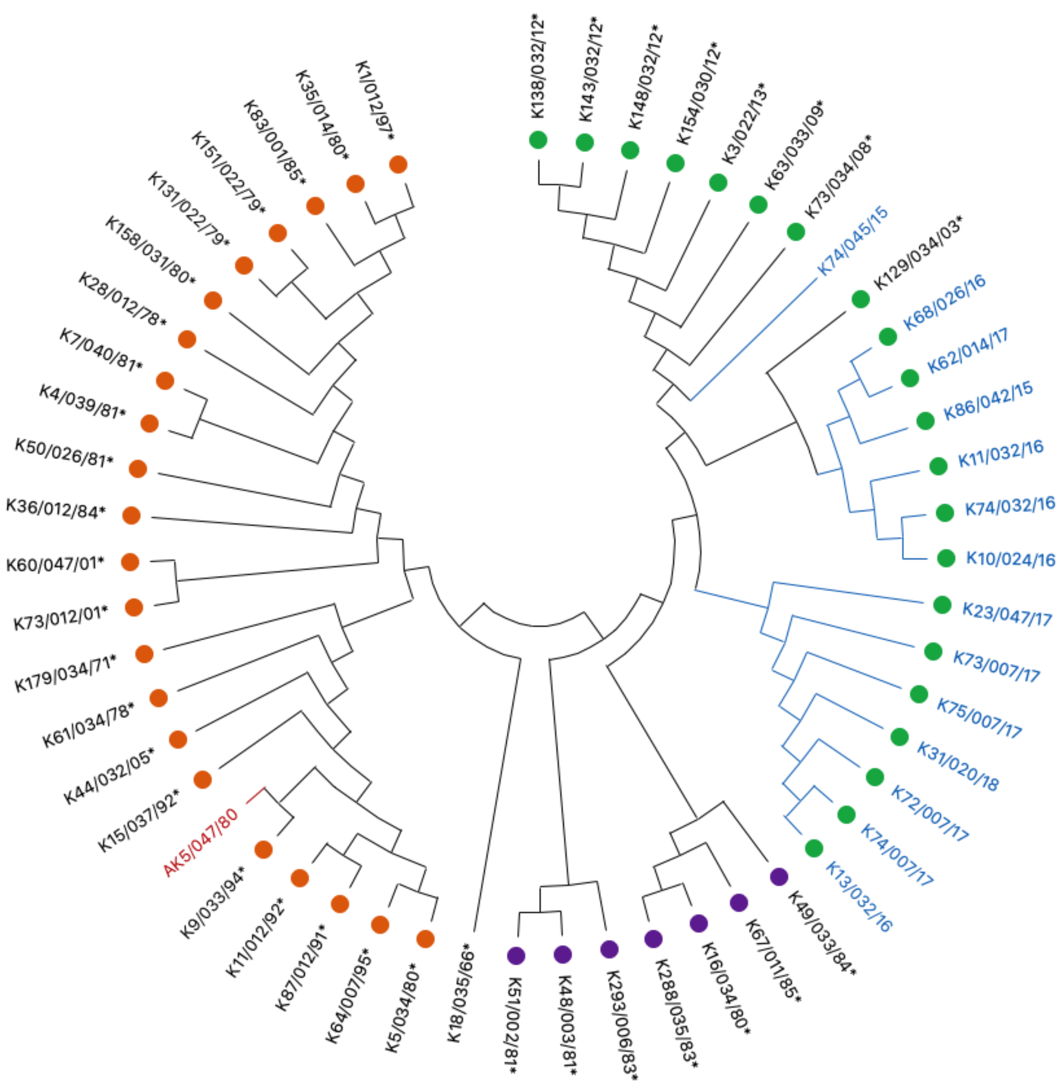


Figure 5.4 A phylogenetic tree representing the relationship between the 53 FMDV isolates, serotype A.

Parameters included: pairwise deletion, 1000 replicates for bootstrap analysis, neighor-joining method for tree construction. All the FMDV isolates were organised into three separate clades: Clade 1 (red), clade 2 (purple) and clade 3 (green), The samples number codes coloured blue were generated in this study, the ones coloured red are the vaccine strains and those coloured black with an asterisk at the end are the samples obtained from NCBI.

All the samples used to construct this phylogenetic tree were collected from different counties in Kenya. In phylogenetic tree construction, all the FMDV isolates were organised into three

separate clades, with 23 isolates grouped in clade 1(red), 7 isolates in clade 2(purple) and 22 isolates in clade 3(green).

All the samples from this study clustered in clade 3, where they formed two sub-clusters. The first cluster contains five samples collected in 2017, 1 in 2018 and 1 in 2016, and the second cluster contains four samples collected in 2016, 1 collected in 2017 and 1 collected in 2015. The vaccine strains consistently cluster with the older samples obtained from NCBI. The vaccine strains lie in clade 1(current vaccine strain A K5/80 together with AK 35/80 and AK 179/71). In this clade 1, the current vaccine strain AK5/80 clustered with the much older samples collected between 1970 and 1990s and closely to vaccine strain AK179/71. The third vaccine strain under this study AK35/80 clustered with samples collected between 1978-1980s. Samples collected around the same time and those collected in the same county consistently cluster in the same clade or closer to each other.

Table5.7: SEROTYPE O ANALYSIS

S/NO	SEQUENCE NAME	MONTH / YEAR OF COLLECTION	COUNTY	COUNTRY	COUNTY CODES	SEROTYPE DETECTED	ACCESSION NUMBER	REFERENCE
1	K9/18	Jan-18	MACHAKOS	Kenya	16	O	Not yet available	This study
2	K12/18	Jan-18	MACHAKOS	Kenya	16	O	Not yet available	This study
3	K14/18	Jan-18	NAKURU	Kenya	32	O	Not yet available	This study
4	K18/18	Jan-18	LAIKIPIA	Kenya	31	O	Not yet available	This study
5	K20/18	Jan-18	UASIN-GISHU	Kenya	26	O	Not yet available	This study
6	K21/18	Jan-18	MAKUENI	Kenya	17	O	Not yet available	This study
7	K22/18	Jan-18	NAROK	Kenya	33	O	Not yet available	This study
8	K42/17	Sep-17	BUSIA	Kenya	40	O	Not yet available	This study
9	K27/17	Jun-17	MAKUENI	Kenya	17	O	Not yet available	This study
10	K29/17	Jul-17	UASIN-GISHU	Kenya	26	O	Not yet available	This study
11	K33/17	Aug-17	NANDI	Kenya	29	O	Not yet available	This study
12	K34/17	Aug-17	NANDI	Kenya	29	O	Not yet	This study

							available	
13	K37/17	Aug-17	KIAMBU	Kenya	22	O	Not yet available	This study
14	K40/17	Aug-17	NAIROBI	Kenya	47	O	Not yet available	This study
15	K53/17	Oct-17	TRANS-NZOIA	Kenya	27	O	Not yet available	This study
16	K59/17	Nov-17	KIAMBU	Kenya	22	O	Not yet available	This study
17	K63/17	Nov-17	UASIN-GISHU	Kenya	26	O	Not yet available	This study
18	K79/17	Dec-17	NAKURU	Kenya	32	O	Not yet available	This study
19	K81/17	Dec-17	KISUMU	Kenya	42	O	Not yet available	This study
20	K67/16	Sep-16	KIAMBU	Kenya	22	O	Not yet available	This study
21	K17/17	Mar-17	KIAMBU	Kenya	22	O	Not yet available	This study
22	K39/17	Aug-17	KIAMBU	Kenya	22	O	Not yet available	This study
23	K41/17	Sep-17	UASIN-GISHU	Kenya	26	O	Not yet available	This study
24	K56/17	Oct-17	MACHAKOS	Kenya	16	O	Not yet available	This study
25	K64/17	Nov-17	NAIROBI	Kenya	47	O	Not yet available	This study
26	K67/17	Nov-17	UASIN-GISHU	Kenya	26	O	Not yet available	This study
27	K11/18	Jan-18	MACHAKOS	Kenya	16	O	Not yet available	This study
28	K25/18	Jan-18	LAIKIPIA	Kenya	31	O	Not yet available	This study
29	K26/18	Jan-18	LAIKIPIA	Kenya	31	O	Not yet available	This study
30	K27/18	Jan-18	KISUMU	Kenya	42	O	Not yet available	This study
31	K28/18	Jan-18	KISUMU	Kenya	42	O	Not yet available	This study
32	K33/18	Feb-18	MAKUENI	Kenya	17	O	Not yet available	This study
33	K24/14	Feb-14	KAJIADO	Kenya	34	O	Not yet available	This study
34	K22/14	Feb-14	MACHAKOS	Kenya	16	O	Not yet available	This study
35	K44/18	Feb-18	KERICHO	Kenya	35	O	Not yet available	This study
36	K25/14	Jan-14	KISUMU	Kenya	42	O	Not yet available	This study
37	K46/14	Feb-14	BUNGOMA	Kenya	39	O	Not yet available	This study
38	K50/14	Feb-14	UASIN GISHU	Kenya	26	O	Not yet available	This study
39	K55/14	Mar-14	TANA RIVER	Kenya	4	O	Not yet available	This study
40	K61/14	Mar-14	NAKURU	Kenya	32	O	Not yet available	This study
41	K84/14	May-14	NAKURU	Kenya	32	O	Not yet available	This study
42	K86/14	May-14	MAKUENI	Kenya	17	O	Not yet available	This study
43	K112/14	Jul-14	NAIROBI	Kenya	47	O	Not yet available	This study
44	K148/14	Oct-14	BARINGO	Kenya	30	O	Not yet available	This study
45	K35/13	May-13	KIAMBU	Kenya	22	O	Not yet available	This study
46	K61/15	Jul-15	UASIN GISHU	Kenya	26	O	Not yet available	This study

47	K16/15	Jan-15	KIAMBU	Kenya	22	O	Not yet available	This study
48	K35/16	Jun-16	NAKURU	Kenya	32	O	Not yet available	This study
49	K37/16	Jun-16	NAKURU	Kenya	32	O	Not yet available	This study
50	K45/18	Feb-18	KERICHO	Kenya	35	O	Not yet available	This study
51	OK77/78	May-78		Kenya	47	O	-	This study
52	K32/08	2008	THIKA	Kenya	22	O	HM756640.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
53	K31/08	2008	KAJIADO	Kenya	34	O	HM756639.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
54	K14/08	2008	BARINGO	Kenya	30	O	HM756638.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
55	K11/08	2008	KIAMBU	Kenya	22	O	HM756637.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
56	K4/08	2008	THIKA	Kenya	22	O	HM756636.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
57	K1/08	2008	NAIROBI	Kenya	47	O	HM756635.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
58	K82/07	2007	MURANG'A	Kenya	21	O	HM756634.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
59	K31/07	2007	KIAMBU	kenya	22	O	HM756633.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
60	K30/07	2007	LAIKIPIA	Kenya	31	O	HM756632.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
61	K28/07	2007	LAIKIPIA	Kenya	31	O	HM756631.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
62	K6/07	2007	KOIBATEK	Kenya	30	O	HM756630.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
63	K2/07	2007	KIAMBU	Kenya	22	O	HM756629.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
64	U25/06	2006	MPIGI	Uganda		O	HM756628.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
65	U18/06	2006	MPIGI	Uganda		O	HM756627.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
66	K50/06	2006	UASIN GISHU	Kenya	26	O	HM756626.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
67	U12/05	2005	WAKISO	Uganda		O	HM756625.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
58	K48/05	2005	KIAMBU	Kenya	22	O	HM756624.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
59	K31/05	2005	LAIKIPIA	Kenya	31	O	HM756623.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
70	K5/05	2005	LAIKIPIA	Kenya	31	O	HM756622.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
71	U20B/04	2004	HOIMA	Uganda		O	HM756621.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
72	U17B/04	2004	HOIMA	Uganda		O	HM756620.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
73	U14B/04	2004	HOIMA	Uganda		O	HM756619.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
74	U13B/04	2004	HOIMA	Uganda		O	HM756618.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
75	K55/03	2003	NAKURU	Kenya	32	O	HM756617.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
76	K79/02	2002	NAKURU	Kenya	32	O	HM756616.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
77	K61/01	2001	MOMBASA	Kenya	1	O	HM756615.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
78	K45/01	2001	NAKURU	Kenya	32	O	HM756614.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
79	K150/00	2000	UASIN GISHU	Kenya	26	O	HM756613.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
80	K147/00	2000	TRANS NZOIA	Kenya	27	O	HM756612.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
81	K145/00	2000	LAIKIPIA	Kenya	31	O	HM756611.1	https://pubmed.ncbi.nlm.nih.gov/20619358/

82	K141/00	2000	WEST POKOT	Kenya	24	O	HM756610.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
83	K131/00	2000	NAIROBI	Kenya	47	O	HM756609.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
84	K130/00	2000	TRANS NZOIA	Kenya	27	O	HM756608.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
85	K117/00	2000	NYERI	Kenya	19	O	HM756607.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
86	K109/00	2000	UASIN GISHU	Kenya	26	O	HM756606.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
87	K63/00	2000	TRANS NZOIA	Kenya	27	O	HM756605.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
88	U97/99	1999	N/A	Uganda		O	HM756604.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
89	K117/99	1999	Nakuru	Kenya	32	O	HM756603.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
90	K82/98	1998	Kiambu	Kenya	22	O	HM756602.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
91	K56/95	1995	Kiambu	Kenya	22	O	HM756601.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
92	K29/95	1995	Kiambu	Kenya	22	O	HM756600.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
93	K34/93	1993	Laikipia	Kenya	31	O	HM756599.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
94	K11/93	1993	Kiambu	Kenya	22	O	HM756598.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
95	K52/92	1992	Kiambu	Kenya	22	O	HM756597.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
96	K51/92	1992	Nakuru	Kenya	32	O	HM756596.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
97	K121/91	1991	Kiambu	Kenya	22	O	HM756595.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
98	K114/87	1987	Kiambu	Kenya	22	O	HM756594.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
99	K131/85	1985	Kiambu	Kenya	22	O	HM756593.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
100	K40/84	1984	Kiambu	Kenya	22	O	HM756592.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
101	K11/84	1984	Kiambu	Kenya	22	O	HM756591.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
102	K103/82	1982	Thika	Kenya	22	O	HM756590.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
103	K101/80	1980	Laikipia	Kenya	31	O	HM756589.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
104	K77/78	1978	Nakuru	Kenya	32	O	HM756588.1	https://pubmed.ncbi.nlm.nih.gov/20619358/
105	K120/64	1964	Laikipia	Kenya	31	O	HM756587.1	https://pubmed.ncbi.nlm.nih.gov/20619358/

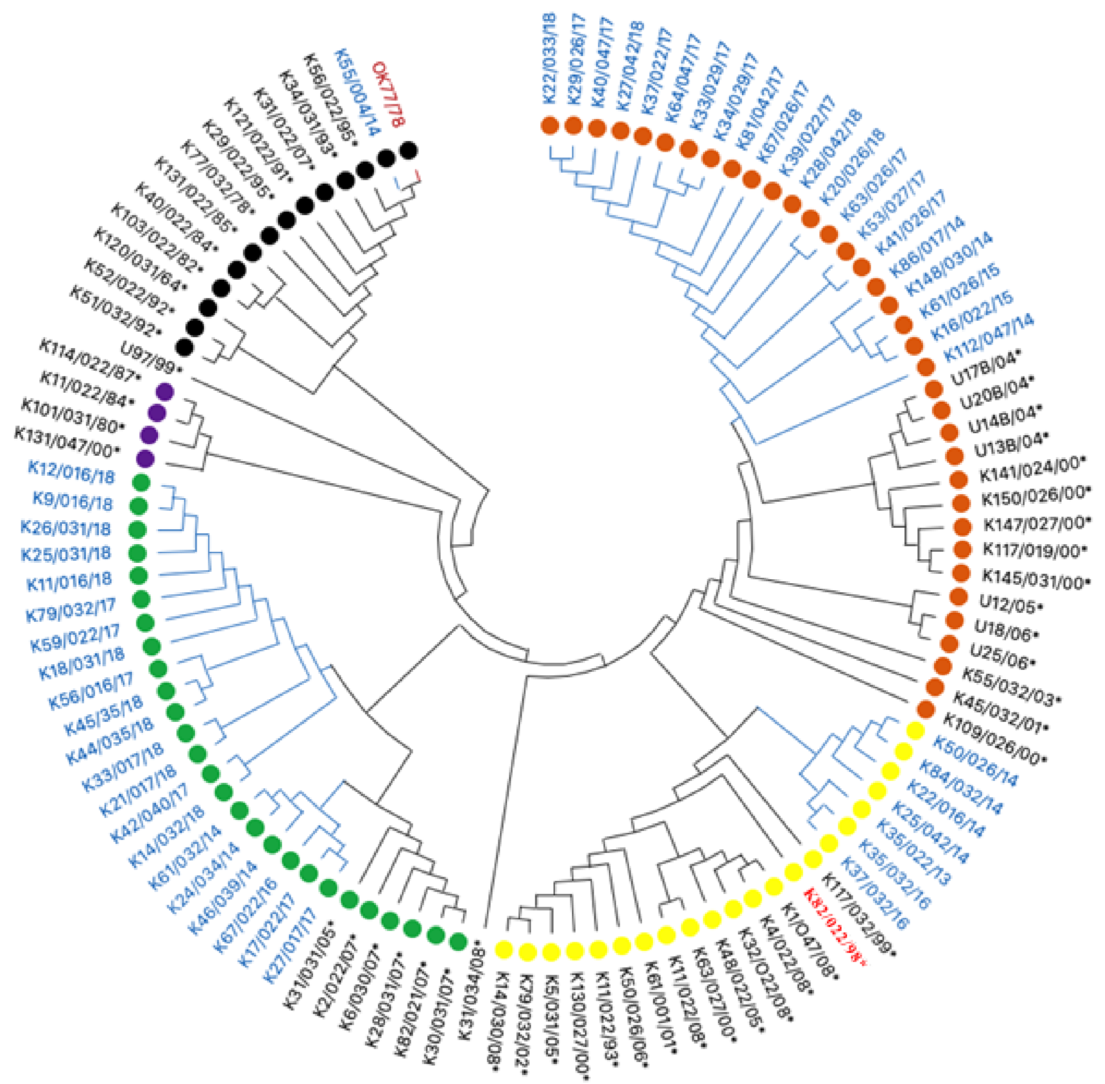


Figure 5.5 A phylogenetic tree representing the relationship between the 105 FMDV isolates, serotype O

All the FMDV isolates were organised into five separate clades: Clade 1 (black), clade 2 (purple) and clade 3 (green), clade 4 (yellow) and clade 5 (orange). The samples codes coloured blue are those generated in this study, the ones coloured red are the vaccine strains and those coloured black with an asterisk at the end are the samples obtained from NCBI.

All the sequences used to construct this phylogenetic tree were collected from different counties in Kenya and Uganda. The Uganda sequences were included in the blasting stage as they tended to cluster together with Kenyan isolates. During phylogenetic tree construction,

all the FMDV isolates were organised into five separate clades, with 14 isolates getting grouped into clade 1, 4 isolates grouped into clade 2, 27 isolates into clade 3, 22 isolates into clade 4, 36 isolates into clade 5. The samples from this study clustered in three different clades throughout the phylogenetic tree: clade 3, clade 4 and clade 5. In clade 3, the samples collected in 2018 and 2017 clustered together, while those collected in 2016 and 2017 are more closely related to those collected in 2005 and 2007 and obtained from NCBI. In clade 4, four samples collected in 2014, 2 collected in 2016 and 1 collected in 2013 clustered together, while those collected in the early 2000's group together in a sub-cluster.

In clade 5, most samples collected in 2017 and 2018 clustered together. Samples from NCBI collected in the early 2000s form a sub-cluster.The vaccine strain lies in clade 1 and is more closely related to the samples collected in the '90s. Samples collected around the same time and those collected in the same county consistently cluster in the same clade or closer to each other.

Identifying where the study isolates belong by topotype, prototype and strains

FMDV sequences are organized by serotype and, within each serotype are topotypes. For the serotype A and O isolates in this study, we can find which specific topotype and prototype they belong to in terms of lineage and sub-lineage.

Serotype O

Serotype O in East Africa exist in four topotypes. The main serotype O topotypes found in East Africa are **EA-1, EA-2, EA-3 and EA-4.** Analysis was done to check whether our set of isolates belongs to one topotype or different topotypes.

We downloaded sequences from https://www.foot-and-mouth.org/FMDV-nomenclature-working-group/prototype-strains. Among the downloaded samples, the isolates belonging to the EA-1 topotype include UGA/5/96 from Uganda, K40/84 from Kiambu in Kenya and K83/79 from Kenya. The sequences belonging to the EA-2 topotype include O/MAL/1/98 from Malawi, O/UGA/3/2002 from Uganda, O/KEN/5/2002 from Kenya and TAN/2/2004 from Tanzania: Kibaha District, Pwani Region. The isolates belonging to the EA-3 topotype include O/SUD/2/86 from Sudan and ETH/3/2004, ETH/1/2007 & ETH/2/2006 from Ethiopia. The isolates belonging to the EA-4 topotype include UGA/17/98 from Uganda and ETH/58/2005 from Ethiopia.

The sequences obtained from this study, those from NCBI and those from this site: https://www.foot-and-mouth.org/FMDV-nomenclature-working-group/prototype-strains were aligned and a phylogenetic tree constructed using MEGA v11.0.8. The evolutionary history between the samples was inferred using the Neighbor-Joining method with 1000 bootstrap replications. The optimal tree is shown in Figure 5. 5. The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved 118 nucleotide

sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

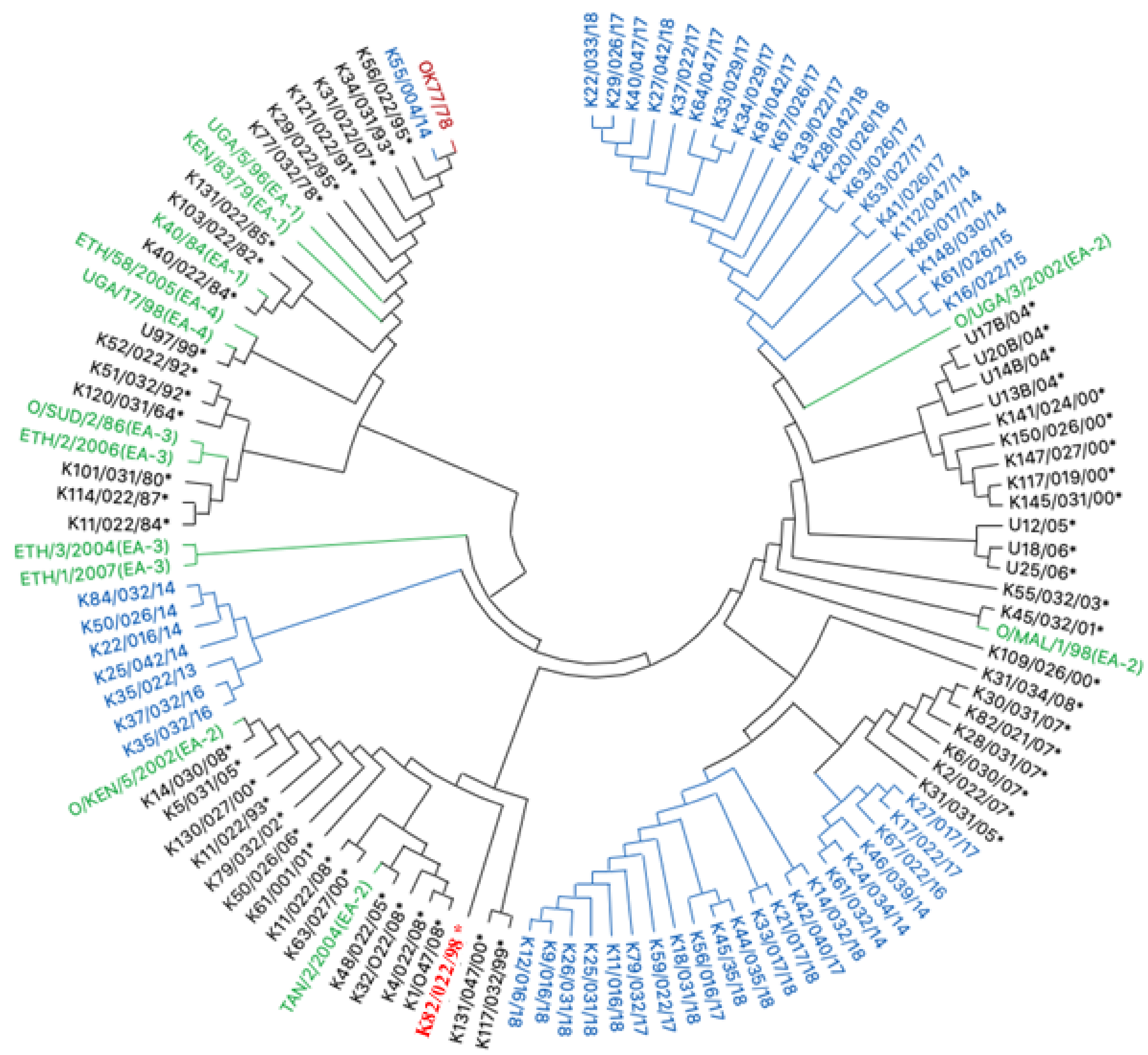


Figure 5.6 A phylogenetic tree representing the relationship between the 118 FMDV isolates, serotype O

Parameters included: pairwise deletion, 1000 replicates for bootstrap analysis, neighor-joining method for tree construction. The samples coloured blue are those generated in this study, the ones coloured red are the vaccine strains, the ones colored green are those with a known topotype and those coloured black with an asterisk at the end are the samples obtained from NCBI.

Based on the findings from the phylogenetic analysis Figure 5.5, all the serotype O viruses isolated in this study except the current vaccine strain: OK77/78 and K55/004/14, clustered within the EA-2 topotype. All the isolates and another vaccine strain OK82/98 belong to EA-2 topotype.

Serotype A

Serotype A in Africa exists in 1 topotype with several sublineages. The main serotype A sublineages found in Africa are **G-I to G-VII.** For this analysis, we left out G-V and G-VI which are found in Nigeria and Ghana respectively. We then proceeded to identify whether our set of isolates belongs to one sublineage or different sublineages.

We downloaded sequences from https://www.foot-and-mouth.org/FMDV-nomenclature-working-group/prototype-strains. Among the downloaded sequences, the sequence belonging to the G-I sublineage was KEN/42/66 from Kenya. The isolate belonging to the G-II sublineage was A/EGY/1/72 from Egypt: Alexandria. The isolate belonging to the G-III sublineage was A_{21}/Lumbwa/KEN/3/64 from Kenya. The isolate belonging to the G-IV sublineage was SUD/3/77 from Sudan. The isolate belonging to the G-VII sublineage was UGA/13/66 from Uganda.

The sequences obtained from this study, those from NCBI and those from this site: https://www.foot-and-mouth.org/FMDV-nomenclature-working-group/prototype-strains were aligned and a phylogenetic tree constructed using MEGA v11.0.8. The evolutionary history between the samples was inferred using the Neighbor-Joining method with 1000 bootstrap replications. The optimal tree is shown in Figure 3.6. The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The

evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved 58 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

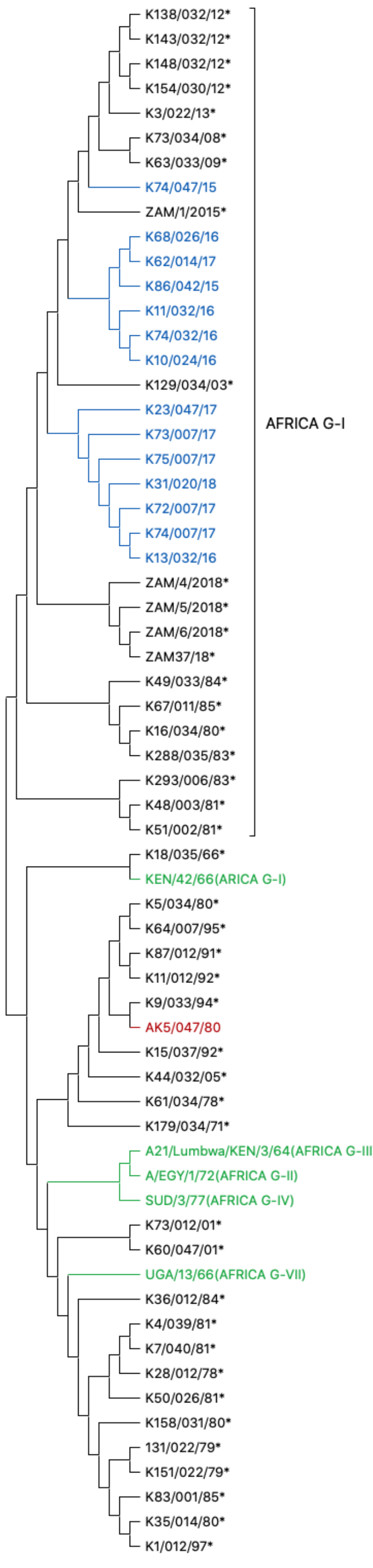


Figure 5.7 A phylogenetic tree representing the relationship between the 58 FMDV isolates, serotype A.

Parameters included: pairwise deletion, 1000 replicates for bootstrap analysis, neighor-joining method for tree construction. The samples coloured blue are those generated in this study, the ones coloured red are the vaccine strains, the ones colored green are those with a known topotype and those coloured black with an asterisk at the end are the samples obtained from NCBI.

Based on the findings from the phylogenetic analysis Figure 5.6, all the serotype A viruses isolated in this study clustered within the Africa G-I sublineage. We included 6 FMD samples from Zambia (https://www.mdpi.com/1999-4915/13/11/2195) to determine the sublineage in which the samples in this study belong. These Zambian samples had already been classified under the Africa G-1 sublineage. The rest of the samples downloaded from (https://www.foot-and-mouth.org/FMDV-nomenclature-working-group/prototype-strains) clustered with the older samples obtained from NCBI and the vaccine strain.

5.4. DISCUSSION

For serotype A study isolates, all belong to Africa G-1 though in 3 lineages. All study isolate sequences tended to cluster closely together in one lineage while few others clustered in another lineage with an isolates collected 3-7 years earlier. The vaccine strain belonged to a third lineage together with isolates collected over 20 years and more closely related to those collected in early 90s.

Based on the findings from the phylogenetic analysis (Figure 3.5) all the serotype O viruses isolated in this study except the strain currently used for serotype O vaccines OK77/78 and field isolate K55/004/14 clustered within the EA-2 topotype. The OK77/78 and K55/004/14 vaccine strain and isolate from Tana River collected in an outbreak in 2014 belong to EA-1. All the VP1 sequences of isolates in this study with many other isolates previously sequenced isolates (from NCBI) belong to EA-2 topotype together with another vaccine OK82/98. These

field strains belonging to EA-2 topotype were in circulation in Kiambu in 2013, in 2014 outbreaks were detected in Nairobi, many parts of the Rift Valley area; Nakuru, Baringo, Kajiado and Trans Nzoia and moving into Western region in Bungoma. They continued to cause outbreaks in Kiambu in 2015 and 2016 and moved to neighbouring Nakuru. Several outbreaks were recorded of EA-2 topotype in subsequent years of 2017-2018 in Nairobi, Kiambu, Machakos, Makueni and in the Rift Valley in Trans Nzoia, Nandi, Nakuru and affected Kisumu and Busia.

Serotype O outbreaks continue to cause outbreaks in many counties all over the country. This study shows that outbreaks in neighbouring counties and occurring in the same period of time are closely related. Some strains across national boundaries like Uganda cluster together with circulating strains in Kenya. Animal movement is likely to play a key role in onward transmission of the strains around the country.

According to this study of 51 serotypes of FMD type O isolated from 2013 to 2018, Serotype O EA-2 topotype continues to be the most prevalent strain causing outbreaks in Kenya resulting in serious economic losses in the livestock industry. This is in agreement with Lloyd-Jones et al, (2017) who found similar dominance of EA-2 and EA-3 from 80 isolates of FMD type O collected in 1993-2012. Work carried out in 2010 and 2011(Wekesa *et al.,*2015) also consistently found EA-2 to be the most common topotype detected together with EA-4 but no detection of EA-1 and EA-3. This shows the dynamic changes that occur in circulating strains at different periods in the Country. Similar recent work by Kerfua *et al.* in 2019 demonstrated limited nucleotide divergence in viruses circulating in the Ugandan/Tanzania border and belonging to topotype EA-2. The divergence of the current circulating strains in

East Africa underscores the need for regular genetic characterization and vaccine matching as outlined by other researchers(Knowles *et al*., 2003;Namatovu *et al.*, 2015; Swai *et al.,* 2009).

EA-1 topotype still occurs albeit in lower prevalence and the current vaccine strain OK77/78 belongs to this group. Future vaccine candidate can be selected from the EA-2 group of viruses banked in the FMD National Laboratory repository which consist of several isolates successfully sequenced in this study. The particular strain to be selected will depend on results of vaccine matching tests and adaptability of the strain to tissue culture cells. It is to be noted that Lloyd-Jones (2017) had established that the vaccine strain O/KEN/77/78 , showed low antigenic matches on in-vitro neutralization experiments with recently circulating EA-2 and 3 viruses compared to O/PanAsia-2 and O/Manisa vaccines though belonging to a different topotype(ME-SA topotype) . Developing new vaccine candidates from these isolates with closer matches is therefore important to enhance effective control of FMD in the East African region. Vaccine strain OK82/98 can be re-activated for use after vaccine match tests are carried out as it clusters with the recent isolates.

It's recommended however that the original vaccine strain OK77/78 be retained as one of the vaccine antigen reserves so that it can be activated whenever increase of cases of EA-1 strains are detected as seen in this study that some recent strains cluster with past strains in some lineages coupled with high antigenic match with this strains. It's also possible that this strain can protect against future circulating strains which are distantly related if they have high antigenic matches and this be determined by carrying out in-vitro-neutralization tests.

4.6 CONCLUSION

Recently circulating isolates for serotype A belong to G-1 topotype but spread out in three lineages. Though the vaccine strain AK5/80 belongs to this topotype, it's in a third lineage. It's therefore proposed that a vaccine candidate can be developed from a new lineage related to the circulating strains. Fuirther testing for vaccine match and adaptability to tissue culture cells will be done to determine the appropriate candidate.

From this study most recently circulating serotype O viruses in 2013-2018 belong to EA-2 topotype. Viruses circulating in the same time span and in neighbouring counties tend to belong to one lineage. Past strains however appear to cluster with recent virus in one lineage in some instances. Only one isolate of the study isolates (from Tana River) belong to EA-1 topotype which has the vaccine strain OK77/78. Five lineages were recognized in this study with four having clusters of viruses isolated between 2013 and 2018.

This study emphasizes the importance of regular surveillance and characterization of virus strains in circulation for selection of effective vaccine strains to aid in development of FMD control strategies. It's proposed that since many circulating strains belong to EA-2, a virus strain from this group be recruited as a vaccine candidate for future use in FMD control. Such a candidate can be identified by antigenic matching tests and ability to adapt to tissue culture cells. A selection can therefore be made from related sequences identified in this study. It's however prudent to maintain the current vaccine strain as a reserve antigen as EA-1 strains may be detected in future surveillance as has been seen in this study. Virus strains isolated more than 10 years earlier tended to cluster with current circulating viruses. It's proposed that

future research work could centre on antigenic matching tests to ascertain the ability of current vaccines and proposed candidate strains to protect against the circulating strains.

Chapter 6: GENERAL SUMMARY

The endemicity of Foot-and-mouth disease (FMD) in Kenya has been recognized for over a century, with the first recorded cases dating back to 1915. Foot-and-mouth disease in Kenya requires concerted efforts to control due to the major economic losses associated outbreaks typically occurring throughout the year in different regions of the country. This research work aimed to understand the epidemiology and molecular characteristics of the causative agent of this disease, foot and mouth disease virus in circulation in the recent past. Understanding these characteristics will aid in vaccine updating and development of effective strategies for control of the disease.

From existing Foot-and-mouth disease epidemiological studies the disease in small ruminants (SR) in Kenya is not documented. We carried out a cross-sectional study, the first in Kenya, to estimate the sero-prevalence of FMD in SR and the associated risk factors nationally and a case study in large and small ruminants in Ukambani region.

To identify the risk factors, generalized linear mixed effects (GLMM) logistic regression analysis with county and villages as random effect variables was used. The country animal level sero-prevalence was 22.5% (95% CI: 22.3%-24.3%) while herd level sero-prevalence was 77.6% (95% CI: 73.9%-80.9%). The risk factor that was significantly positively associated with FMD sero-positivity in SR was multipurpose production type (OR = 1.307; p = 0.042). The risk factors that were significantly negatively associated with FMD sero-positivity were male sex (OR = 0.796; p = 0.007), young age (OR = 0.470; p = 0.010), and sedentary production zone (OR = 0.324; p<0.001). There were no statistically significant intra class correlations among the random effect variables but interactions between age and sex

variables among the studied animals were statistically significant (p = 0.019). Herds with animals bought from markets or middlemen, with wildlife interaction, in low altitude (<1500m above sea level) all had statistically significant higher sero-positivity. Other risk factors identified included lack of animal enclosures, shared bull, shared watering, communal grazing, no vaccination and mixed and migratory grazing systems. Ukambani region had a higher seroprevalence in cattle than small ruminants (40% compared to 19%) and also higher seroprevalence in cattle than the National rate, 40% compared to 37.6 % (unpublished study carried out at the same period). The FMD seroprevalence rate in SR was lower than the National rate at 19%. Two active FMD outbreaks were encountered in Ukambani surveillance which was confirmed by laboratory diagnosis. These epidemiological studies showed that there may be widespread undetected virus circulation in SR indicated by the near ubiquitous spatial distribution of significant FMD sero-positivity in the country. Strengthening of risk-based FMD surveillance in small ruminants is recommended. Adjustment of husbandry practices to control FMD in SR and in contact species is suggested. Cross-transmission of FMD among different species and more risk factors need to be researched.

Production of effective vaccines against incursions of infection in endemic areas is achieved by evaluating the genetic and antigenic characteristics of the circulating viruses. The molecular study aimed to isolate, serotype, and molecularly characterize FMDV from Kenya from 2013-2018. Nucleotide sequences encoding the capsid protein VP1 (1D) region of FMDV from virus samples were generated by Reverse transcription polymerase chain reaction and sequencing. Study samples were serotype O and A repository isolates banked at the FMD Laboratory, Embakasi collected during FMD outbreaks from cattle in 2013 to 2018. Isolation was done from 58 field samples on BHK-21 cells, and serotyping of the isolated

viruses was carried out using antigen ELISA. Isolated viruses were also analyzed using reverse transcription PCR, and the PCR products were subjected to sequencing. Based on the quality of obtained sequence spectra, only 51 isolates were aligned using MEGA v11.0.8, employing the ClustalW algorithm. SeaView version 5.0.4 was used to edit the alignment, and MEGA 11.0.8 was used to construct the phylogenetic tree and align it with the commercially used vaccinal strains (OK77/78 and OK82/98). With a few exceptions, isolates collected over the same period and those from the same regions consistently clustered in the same lineage or closer to each other. A total of 50/51 strains belong to the East African-2 (EA-2) topotype together with the vaccine strain OK82/98. Tana River County belongs to the EA-1 topotype together with the current vaccine strain (OK77/78). None of these isolates was found to belong to the EA-3 and EA-4 topotypes. This study emphasizes the importance of regular surveillance and characterization of circulating virus strains for developing effective vaccines against FMD. It's proposed that future vaccine candidate strains selection could consider EA-2 topotype strains for serotype O including re-activation of vaccine strain OK82/98 and recent lineage of G1 topotype for serotype A to control FMDV circulating in Kenya.

General Recommendations

1. Development of future FMD vaccines candidates based on circulating strains to replace historic strains to centre on East -African Topotype 2(EA-2). Further work to centre on vaccine matching tests so as to get the appropriate strains to be adopted.
2. There is need for regular surveillance and characterization of the ever mutating circulating FMDV strains to aid effective strain selection for vaccine updating.
3. Concentrate efforts of FMD control in Pastoral Zone without neglecting the Sedentary Zone by vaccinating animals before seasonal movements. Law enforcement to monitor

herd mobility and also ensure zoo-sanitary measures of disease control include small ruminants.

4. Improve active and passive surveillance among field personnel in all Counties so as improve disease investigation, sampling and reporting for instituting control measures including quarantine and vaccination. This should be supported by training programmes for field personnel by FMD experts.
5. Concerted efforts including control of animal movement and risk based vaccination should be applied.

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APPENDICES

Appendix I : Approval of Proposal by the Faculty Biosafety, Animal Use and Ethics Committee-University of Nairobi.

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2020/262

Dr. Eunice Cherono Chepkwony
University of Nairobi
Dept. Veterinary Pathology, Microbiology & Parasitology
13/01/2020

Dear Dr. Chepkwony
RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee
Molecular epidermiology and Antigenic relationship study of Foot and Mouth disease viruses in Kenya. 2010-2016.

Dr. Eunice Cherono Chepkwony

We refer to your PhD proposal submitted to our committee for review and your application letter dated June 2019. We have reviewed your application for ethical clearance for the study.
The number of cows to be sampled, sera sample collection, preservation and analysis at the FMD Laboratory in Embakasi meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.
We have also noted that registered veterinary surgeons will supervise the work.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.
Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix II(i): National Commision for Science, Technology and Innovation Research Authorization

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/57224/31389**

Date: **21st August, 2019**

Dr Eunice Cherono Chepkwony
University of Nairobi
P.O Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Molecular, Epidemiological and antigenic relationships study of foot and mouth disease viruses, contagious bovine pleuro-pneumonia, Peste des Petits Ruminants (PPR) and Rift Valley Fever in Kenya: 2013-2018."*** I am pleased to inform you that you have been authorized to undertake research in **all Counties** for the period ending **19th August, 2020.**

You are advised to report to **the County Commissioners, and the County Directors of Education, all Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA., MSc, MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioners
All Counties.

The County Directors of Education
All Counties.

National Commission for Science, Technology and Innovation is ISO9001:2008 Certified

Appendix II(ii) National Commision for Science, Technology and Innovation Research License

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014.

CONDITIONS

1. The License is valid for the proposed research, location and specified period.
2. The License and any rights thereunder are non-transferable.
3. The Licensee shall inform the County Governor before commencement of the research.
4. Excavation, filming and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
5. The License does not give authority to transfer research materials.
6. NACOSTI may monitor and evaluate the licensed research project.
7. The Licensee shall submit one hard copy and upload a soft copy of their final report within one year of completion of the research.
8. NACOSTI reserves the right to modify the conditions of the License including cancellation without prior notice.

National Commission for Science, Technology and innovation
P.O. Box 30623 - 00100, Nairobi, Kenya
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REPUBLIC OF KENYA

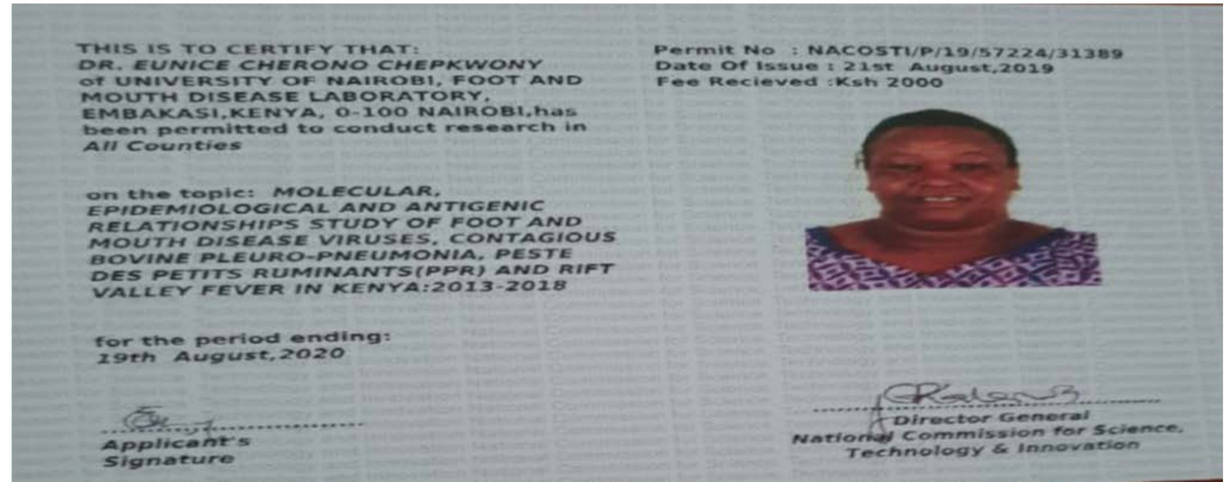
NACOSTI

National Commission for Science, Technology and Innovation

RESEARCH LICENSE

Serial No.A 26379

CONDITIONS: see back page


THIS IS TO CERTIFY THAT:
DR. EUNICE CHERONO CHEPKWONY
of UNIVERSITY OF NAIROBI, FOOT AND MOUTH DISEASE LABORATORY, EMBAKASI,KENYA, 0-100 NAIROBI,has been permitted to conduct research in
All Counties

on the topic: MOLECULAR, EPIDEMIOLOGICAL AND ANTIGENIC RELATIONSHIPS STUDY OF FOOT AND MOUTH DISEASE VIRUSES, CONTAGIOUS BOVINE PLEURO-PNEUMONIA, PESTE DES PETITS RUMINANTS(PPR) AND RIFT VALLEY FEVER IN KENYA:2013-2018

for the period ending:
19th August,2020

Applicant's Signature

Permit No : NACOSTI/P/19/57224/31389
Date Of Issue : 21st August,2019
Fee Recieved :Ksh 2000

Director General
National Commission for Science, Technology & Innovation

Appendix III: Data Collection Questionnaire Crossectional Survey

DATA COLLECTION QUESTIONNAIRE_CROSSECTIONALSURVEY

Serial No.................... Enumerator Name...

A. GENERAL INFORMATION

Herd number				Name of livestock keeper *(2or 3 Names)* and Mobile Tel. No.	
Date				Name of village	
GPS coordinates	Lat:			Sub-Location	
	Long:				
Elevation				Location	
Distance to main road	Km				

B: PRODUCTION SYSTEM

1. Herd size: *how many animals of different species and ages are in the herd?*

Species	Young (<1 year sheep, goats, pigs. <2 years cattle and camels)		Mature (1 year or more - sheep, goats, pigs. 2 years or more - cattle and camels)		Total
	Male	Female	Male	Female	
Cattle					
Sheep					
Goats					
Pigs					
Camels					

2. Herd dynamics*: in the past year, how many animals have been added to the herd - births, purchases, gifts etc.? In the past year, how many animals have left the herd - death, sale, slaughter, gifts etc.?*

Species	No. born	No. died	No. bought	No. sold	No. slaughtered	No. gifted or loaned	No. gifts received or borrowed	Other reasons for leaving herd (stolen, lost, predator)	Other reas for joining (stray anin breeding b
Cattle									
Sheep									
Goats									

Species	8. Breeding method			
	AI	Own bull ram or he - goat	Own bull /ram or he goat common use	bull /ram or he goat from another farm
Cattle				
Sheep				
Goats				

Species	3. Production type: *what is the main reason*			4. Production system: *what is the main farming*			5. Housing: *are the animals enclosed?*	
	Dairy	Meat	Multi-purpose	Sedentary/ mixed farming	Agro-pastoralist	Pastoralist	Enclosed at night	Enclosed during day
Cattle								
Sheep								
Goats								

Species	6. Grazing: *what type of grazing is practised?*				7. Water source: *where do the animals drink?*	
	Zero-grazed	Fenced	Communal	Migratory	On the farm	Shared water source

9. Selling live animals: *in the past one year, what methods have you used to sell animals?*

Species	Livestock market	Direct local sale to neighbour	Direct sale to middle man/trader	Other (specify)
Cattle				
Sheep				
Goats				

10. Buying live animals: *in the past one year, what methods have you used to buy animals?*

Species	Livestock market	Direct purchase from neighbour or nearby village	Direct purchase from middle man/trader	Other
Cattle				
Sheep				
Goats				

11. Wildlife –Livestock Interaction

Do your animals come in close contact with wildlife at any time *(Tick)*	If yes which type of wild animals *(Name them)*	Interaction points: 1=Grazing grounds 2=Watering points 3= others(specify)

12. Personnel, equipment and farm products movement at farm level

Do you share Equipment		Milk collection in farm		Buy or sell hay 1=buy 2=sell	
Do you Share Laborers with neighbors		AI Attendant/ Vet visit		Buy or sell compost: 1=buy 2=sell	

C: DISEASE SITUATION

13. *What diseases have affected the herd in the past one year?*

Local disease name	Species affected	No. affected	Main clinical signs	No. affected	No. Dead	Duration of disease	Season(s) when occurred	Suspected diagnosis

14. Rank the 5 most important cattle diseases/conditions and give the criteria for ranking (*1 is most important and 5 is least important)*

Rank	Disease	Criterion
1		
2		
3		
4		
5		

15. Current diseases affecting the herd: (using the local terms for FMD) *ask if there are any animals in the herd that are currently sick with any of these syndromes?*

If there is no local name for any of these diseases, describe the clinical signs of the disease and ask if they have seen anything like this in the last one year (fill details above), and if they currently have any sick animals that might have any of these diseases (fill details below).

Local disease	Species affected	Number affected	Main clinical signs	Season(s) when	Suspected diagnosis

Ask the owner to show you the clinical cases after you have finished this interview, and fill in the clinical examination form.

16. *If an animal becomes sick, what do you usually do? (tick all that apply)*

Do nothing	Treat it myself (or a family member)	Consult a traditional healer	Consult a community animal health worker	Consult an extension officer	Consult a private vet	Consult a government vet	Consult an NGO	Others (specify)

Reasons for choice above...

17. *Where do you get medicines from?* (tick all that apply)

Collect or make myself	Traditional healer	Community animal health	Pharmacy/ Agro-vet	General shop or market	Private vet	Government vet	NGO	Others (specify)

20. *When did you last have vaccinations against the following diseases?*

Species	Vaccine	Date of vaccination	Source of vaccine	*Reasons for vaccinating
	FMD			
	PPR			
	RVF			
	CBPP			

*Reasons for vaccinating: 1=Routine 2=Rumor of disease nearby 3=Outbreak within herd 4=Ring vaccination 5=others (specify)

Use of insecticides or acaricides:

Species	21. *Do you use tick or*		22. *If yes, what method do you use?*				23. *If you dip, is it private or Communal?*	
	Yes	No	Hand dressing	Spray	Dip	Others (specify)	Private	Communal
Cattle								
Sheep								
Goats								