Epidemiology of Foot and Mouth Disease in Zambia

By

Yona Sinkala

A thesis submitted to the University of Zambia in fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Veterinary Epidemiology

University of Zambia

Lusaka

2015

Author's Declaration

The work presented in this thesis is entirely my own and carried out with the help of those people mentioned in the acknowledgements. It has not been previously submitted to this or any university for the award of a degree.

_____

Yona Sinkala

_____

Certificate of Approval

This thesis by **Yona Sinkala** is approved as fulfilling the requirements for awarding of degree of Doctor of Philosophy in Veterinary Epidemiology at the University of Zambia

Internal Examiner	**Signature**	**Date**
Internal Examiner	**Signature**	**Date**
External Examiner	**Signature**	**Date**
Supervisor	**Signature**	**Date**

Abstract

Foot-and-mouth disease (FMD) is a highly infectious and devastating disease of livestock that has affected Zambia's potential to boost the economy through trade in livestock commodities. The inadequate understanding, due to limited research based epidemiology data, of the disease dynamics has impacted negatively on its prevention and control. The aim of this study was to determine specific elements of FMD epidemiology that are important for understanding disease dynamics in Zambia. To conduct this study, firstly a retrospective analysis of FMD outbreaks between 1933 and 2012 was carried out in order to elucidate the temporal and spatial patterns that contributed to their occurrence. This was followed by a cross sectional study involving collection of sera and probang samples from cattle and buffalo including administration of a questionnaire at selected livestock/wildlife interface areas that was carried out to determine the seropositivity and associated risk factors. Included was molecular characterization of the circulating foot and mouth disease virus (FMDV) strains in these species. Furthermore, a transmission model incorporating carrier status was developed. Results of the study confirmed that FMD outbreaks remain confined to three high risk areas of Kafue Flats, lower Zambezi basin and northern Zambia. However, the patterns of occurrence of the disease varied in space and time (relative risk range 2.23 to 29.62) as well as in the causative serotypes and topotypes. Namwala district recorded the highest number of outbreaks with SAT 1 being the most predominant serotype in the district and across country (43.7 percent of the recorded outbreaks). And that agro-ecological zone, rainfall, distance away from buffalo migratory routes and international borders were significant factors of FMD occurrence. Furthermore, FMD animal seropositivity ranging from zero to 23 percent and transhumant husbandry practice as the main risk factor were identified. The study further isolated topotype II of SAT 2 from buffalo in Kafue National Park, a strain previously unknown to exist in Zambia. Furthermore, SAT 1 topotype I, closely related to the FMD outbreak virus of 2004/08, was isolated from vaccinated cattle in the livestock/wildlife interface of Monze and Lochnivar National Park. SAT 1 and SAT 2 were also isolated from buffalo in Lower Zambezi and Luambe National Parks. The FMD transmission model predicted an epidemic to peak by day 40 to 100 and die down by day 140 with an inter-epidemic period of nine to 23 years. The study provides significant elements of FMD epidemiology in Zambia including the spatial and temporal patterns, the subclinical infection and characterisation of circulating FMDV in cattle and buffalo at selected livestock/wildlife interface areas. Other elements included the carrier status in cattle, duration of epidemics and inter-epidemic periods. These elements may be considered in targeted risk surveillance, movement control, risk assessments and other disease prevention strategies at national and regional levels. Further work is recommended for better understanding of circulating FMDV in buffalo, carrier status in cattle within ecosystems and the role of livestock value chains in perpetuation of FMD outbreaks. Included is the optimization of the serological tests in use with antigens that are closely matched to the circulating field strains.

Dedication

This work is dedicated to my wife Annie Mwelwa Munsaka, who helped keep body and soul together; my daughters Mwaka and Chifula Nambela, and my son Nkusuwila Jonah Sinkala for their long endurance and sacrifice while I concentrated on my work.

Acknowledgements

This work was carried out at the School of Veterinary Medicine (UNZA), the Royal Veterinary College (RVC), the Central Veterinary Research Institute (CVRI) in Zambia and the Transboundary Animal Diseases Programme of the Agriculture Research Council Onderstepoort Veterinary Institute (TADP ARC-OVI) between 2010 and 2014. The work was financially supported by the Southern African Centre for Infectious Disease Surveillance (SACIDS) through a Wellcome Trust funded Grant number WT087546MA under the Foot and Mouth Disease (FMD) community of practice (CoP).

I would like to thank the Manager at the TADP ARC-OVI, the Principal at the Royal Veterinary College and the Dean at UNZA School of Veterinary Medicine for providing excellent working facilities and conducive environments.

I wish to express my special gratitude to Dr. Martin Simuunza for being passionate about this work, excellent supervision and for devoting his skills to ensuring successful completion of my studies. And to Prof. Dirk Pfeiffer for guidance and critical reviews of manuscripts. Further thanks go to Prof. John Muma for enthusiastic support, positive and excellent supervision, quick and constructive feedback to the manuscripts and Prof. Aaron Mweene for being very supportive to this work as National Coordinator of the Southern Africa Centre for Infectious Disease Surveillance Consortium. I would also like to thank Dr. Christopher Kasanga the SACIDS Postdoctoral Fellow for guidance and leadership of the FMD CoP and Dr. Misheck Mulumba, the Project Coordinator, for funding the capture, sampling and analysis of buffalo samples through the Southern Africa Development Community, Africa Development Bank Project

entitled “Strengthening of Institutions for the Risk Management of Transboundary Animal Diseases”. I am sincerely grateful to Mr. Tingiya Sikombe, Frank Banda and Oliver Chibomba, for their excellent assistance for the laboratory and field work. I am indebted to the Government of the Republic of Zambia through the Ministry of Agriculture and Livestock for granting me paid study leave to undertake this work. Lastly and not the least, to all farmers, veterinary and wildlife officials who in one way or another assisted with the sampling of buffalo and cattle at various livestock-wildlife interface areas. Above all, I thank GOD for enabling me to complete this work.

Yona Sinkala

TABLE OF CONTENTS

Author's Declaration ii
Certificate of Approval iii
Abstract iv
Dedication v
Acknowledgements vi
List of Figures xi
List of Tables xiv
List of Abbreviations and Symbols xvi
CHAPTER ONE 1
1.0 INTRODUCTION 1
1.1 Problem statement 2
1.2 Study justification 3
1.3 Study objectives 4
CHAPTER TWO 5
2.0 LITERATURE REVIEW 5
2.1 Spatial and geographical overview of Zambia 5
2.2 Human demographical parameters and health indicators 8
2.3 Socio economic status 9
2.4 Livestock production system 9
2.5 Wildlife conservation 12
2.6 Foot and mouth disease 15
2.6.5 Foot and mouth disease time periods 29
2.6.6 Clinical manifestation 30
2.6.7 Pathogenesis 31
2.6.8 Immune response to foot and mouth disease virus 32
2.6.9 Diagnosis of foot and mouth disease 33
2.6.10 New field diagnostic tests 37

2.6.11 Socio-economic impact of foot and mouth disease 39
2.6.12 Prevention and control 41
2.6.13 Multiple serotypes 43
2.6.14 Vaccination regimes 44
2.6.15 Foot and mouth disease in Zambia 46
2.6.16 Knowledge gaps of foot and mouth disease in Zambia 54
CHAPTER THREE 56
3.0 MATERIALS AND METHODS 56
3.1 Retrospective study 56
3.1.1. Study area 56
3.1.2 Study design 60
3.1.3 Data analysis 62
3.2 Cross sectional study 64
3.2.1 Study area 64
3.2.2 Study design, sampling and sample size 65
3.2.3 Analysis of samples 70
3.3 Transmission modeling 75
3.3.1 Study area 75
3.3.2 Study design 76
3.3.3 Data collection 78
3.3.4 Model analysis 80
CHAPTER FOUR 81
4.0 RESULTS 81
4.1. Retrospective study results and analysis 81
4.1.1 Descriptive results 81
4.1.2 Cross-sectional study 95
4.1.3 Transmission modeling 104

CHAPTER FIVE 110
5.0 DISCUSSION 110
5.1 Retrospective study 110
5.1.1 Descriptive 110
5.1.2 Spatial and temporal patterns 110
5.1.3 Risk factor analysis 114
5.2 Cross sectional study 116
5.2.1 Sero-prevalence 116
5.2.2 Risk factor analysis 117
5.2.3 Virus Isolation 117
5.3 Transmission modeling 119
CHAPTER SIX 123
6.0 CONCLUSION 123
CHAPTER SEVEN 125
7.0 RECOMMENDATIONS 125
8.0 REFERENCES 126
9.0 APPENDIX 145
Appendix 1 Publications from this thesis 145
Appendix 2 Questionnaire for cross sectional study 146

List of Figures

Figure 1: Zambia's agro-ecological zones and National parks 6

Figure 2: The vegetation of Zambia around the plateau, valley and wetland areas 7

Figure 3: Foot and mouth disease virus structure showing the three exposed surface proteins VP1, VP2 and VP3. 17

Figure 4: Full genome structure of the foot and mouth disease virus with emphasis on the VP1 used for sequencing. 19

Figure 5: Seven conjectured global foot and mouth disease virus pools based on molecular epidemiology 21

Figure 6: Distribution of foot and mouth disease virus serotypes and clusters in Africa. 23

Figure 7: Foot and mouth disease clinical lesions around the mouth and tongue 30

Figure 8: Location of study sites, including the number of buffalo and cattle sampled per site 65

Figure 9: Sampling of buffalo and cattle from the oral-pharyngeal area. 69

Figure 10: Kafue Flats and Namwala District 76

Figure 11: Number of outbreak cases recorded per annum from 1933 to 2012. 81

Figure 12: Cattle density and number of reported foot and mouth disease outbreaks per ward from 1933 to 2012 82

Figure 13: Percentage distribution of outbreaks by serotype in the three agro ecological zones from 1933 to 2012 83

Figure 14: Distribution of foot and mouth disease outbreak cases by serotype and topotype from 1933 to 2012 in the three high risk areas..... 84
Figure 15: Overall percentage distribution of serotypes and topotypes 86
Figure 16: Distribution of topotypes in Kafue Flats, Lower Zambezi basin and Mbala-Isoka area 86
Figure 17: Buffalo migratory routes within the lower Zambezi basin 87
Figure 18: Three significant clusters detected during the period 1933 to 1952 88
Figure 19: Four significant clusters detected during the period 1953 to 1972 89
Figure 20: Seven significant clusters detected during the period 1973 to 1992 90
Figure 21: Five significant clusters detected during the period 1993 to 2012 91
Figure 22: Agarose gel electrophoresis of foot and mouth disease virus isolates from Luambe and lower Zambezi NPs..... 101
Figure 23: Phylogenic tree of SAT 2 isolate from Kafue NP's Ngoma area of Zambia 102
Figure 24: Phylogenic tree of SAT 1 isolate from cattle in Monze's Hakunkula interface with Lochnivar NP in Zambia..... 103
Figure 25: Susceptible Exposed Infected Recovered (SEIR) Model incorporating the carrier class state. 104
Figure 26: Rate of change from susceptible, pre-infectious, immune and infectious states when R_0 =5 107

Figure 27: Rate of change from susceptible, pre-infectious, immune and infectious states when $R_0 = 9$ 107

Figure 28: Susceptible, immune, pre-infectious and infectious states when $R_0 = 9$ and infectious period = 45, taking the proportion of infected that become carriers to be 50 percent 108

Figure 29: The rate of change of infectious if observed for a longer period and the inter-epidemic period. 109

List of Tables

Table 1: Estimates of cattle numbers 11

Table 2: Population estimates of various animal species 13

Table 3: Topotype distribution of foot and mouth disease virus serotypes in Africa from 1990 to 2013 22

Table 4: Duration of viral of persistence in selected domestic animals and wildlife species. 28

Table 5: Variables and parameters used for the transmission of FMD in the Kafue Flats of Zambia 78

Table 6: Results of the univariable logistic regression analysis of potential risk factors for FMD outbreak occurrence 92

Table 7: Cross tabulation of significant predictor variables from univariable analysis 93

Table 8: Results of multivariable logistic regression analysis of potential risk factors for FMD outbreaks 94

Table 9: Number of buffalo and cattle sampled per site 95

Table 10: Foot and mouth disease seropositivity results from cattle conducted at seven study sites 96

Table 11: Univariable logistic regression analysis of risk factors for FMD seropositivity in cattle 97

Table 12: Correlation of significant predictor variables from univariable analysis 98

Table 13: Multivariable logistic regression analysis of potential risk factors for FMD seropositivity at animal level 99

Table 14: Virus isolation, PCR and Antigen Elisa results from buffalo and cattle 99

List of Abbreviations and Symbols

bp	base pair
BVI	Botswana Vaccine Institute
CVRI	Central Veterinary Research Institute
C.I.	Confidence Interval
DNA	Deoxyribonucleic Acid
ECF	East Coast fever
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GDP	Gross Domestic Product
GMA	Game Management Area
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LPBE	Liquid Phase Blocking ELISA
LAMP	Loop Mediated Isothermal Amplification
NP	National Park
NSP	Non Structural Protein
OIE	World Organisation for Animal Health
OR	Odds Ratio
p	*p value*: type I error (probability of wrongly rejecting null hypothesis)
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction

R_0	Basic Reproduction Ratio
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
rRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
RNA	Ribonucleic Acid
RR	Relative Risk
SADC	Southern Africa Development Community
SAT	Southern African Territories
SP	Structural Proteins
SSA	Sub Saharan Africa
TADP ARC-OVI	Transboundary Animal Diseases Programme of Agriculture Research Council Onderstepoort Veterinary Institute
TFCA	Trans-Frontier Conservation Areas
TPI	The Pirbright Institute
URL	Uniform Resource Locater
US$	United States Dollar
VP1	Viral Protein 1 (Major Capsid Protein VP1)
VP2	Viral Protein 2
VP3	Viral Protein 3
VP4	Viral Protein 4
VNT	Virus Neutralisation Test
WRL	World Reference Laboratory

CHAPTER ONE

1.0 INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals such as cattle,, pigs, sheep, goats and about 70 wildlife species such as the African wild buffalo (*Syncerus caffer*) (OIE, 2014b). The disease is caused by the FMD virus (FMDV) with seven known serotypes; A, O, C, Southern Africa Territories [SAT] 1, 2, 3 and Asia 1 (Radostits *et al*., 1994). FMD was present in almost every part of the world where livestock are kept (Jamal and Belsham, 2013). However, it was eradicated in most developed countries, but the risk remains because it is still present in over a 100 countries mainly of Africa, Asia and South America (OIE, 2010b). FMD is such a feared disease that trade in animals and animal products from endemic regions is prohibited because of the risk of introduction into an importing country (Knight-Jones and Rushton, 2013; OIE, 2014b). The restriction to market access has resulted in low productivity and consequently low investment incentives in the livestock sector (Bronsvoort *et al*., 2008). This in many ways condemns the endemic countries (usually resource poor) to further poverty because of limited market access opportunities, thereby depriving the farmers of the much needed household income, food availability for consumption and limited economic growth at national level (Perry and Rich, 2007; Knight-Jones and Rushton, 2013).

Zambia is a sub-Saharan African (SSA) country with potential to boost its economy through trade in livestock commodities (Anonymous, 2011b; WorldBank, 2011). However, this potential is hampered by the presence of numerous disease challenges including FMD. FMD is endemic in Zambia

hindering the economic exploitation of the livestock resource in a country where 80 percent of the population are dependent on agriculture (Anonymous, 2011b). In Zambia, and the rest of SSA, the risk of FMD occurrence is persistent because of (1) the presence of the African wild buffalo which is the reservoir host for SAT type FMD virus coupled with presence of several domestic and wildlife hosts, some with carrier status; (2) The presence of all the seven serotypes except Asia 1; (3) The antigenic and genetic diversity of the six FMDV serotypes combined with the uncontrolled livestock movement, husbandry systems and climatic factors (Haydon *et al*., 2001; Maree *et al*., 2011; Thomson *et al*., 2012). Repeated outbreaks in endemic countries like Zambia have proved difficult to prevent and control because of the inadequate understanding of the epidemiology of the disease (Chilonda *et al*., 1999).

1.1 Problem statement

Although FMD is generally not considered a killer disease, mortality can be high in young animals if infection enters a naive population (Alexandersen *et al*., 2003). Mortality in young naïve calves can reach 100 percent due to myocarditis as a result of inability to suck or walk with their mothers (Falconer, 1972). A proportion of affected animals develop chronic, irreversible, impairment of productive life. In cattle, affected animals are characterized by dyspnoea, long or rough coats and poor body condition stemming from changes to the endocrine system during clinical disease and following recovery (Minett, 1948). In FMD outbreak situations, lesions on the feet and buccal mucosa causes failure to chew and walk to grazing areas, resulting in a drop in production in dairy cattle and very slow weight gain in other livestock (Alexandersen *et al*., 2003). Milk

production has been reported to drop from 25.5 litres a day to 0.5 litres, oxen staying off plough for the whole season when outbreaks occur in a cropping season and inter-calving interval has been reported to be prolonged by 12 months (Bayissa *et al*., 2011).

1.2 Study justification

The presence FMDV in Zambia prevents Zambian farmers from gaining access to both regional and foreign livestock export markets which negatively impacts rural household incomes and hinders national development (Anonymous, 2011b). Therefore, knowledge of the distribution, prevalence, risk factors and transmission of FMDV may provide useful information for surveillance and effective control planning.

A case of FMD will normally be identified at village level where usually the source, extent of spread, level and frequency of contact with wildlife and cattle herds from other villages are not known. Furthermore, the levels of infection in both buffalo and cattle; risk factors that precipitate the maintenance and spread within villages as well as effectiveness of control measures are not known. FMD cases usually subsides when susceptible populations are depleted whether control measures have been effective or not, but the disease may resurface later when the number of naïve susceptible populations reaches the required threshold. Previous FMD epidemics in Zambia have not been systematically studied to gain insight into outbreak causation, transmission patterns and also to predict when the next outbreak would likely occur so that preventive measures could be instituted. Furthermore, most research on FMD in southern Africa has been undertaken in South Africa, Zimbabwe and Botswana. However, findings regarding the

epidemiology of FMD involving wildlife done elsewhere may not be extrapolated to Zambia because of ecological, host and viral variability differences (Vosloo *et al*., 2009). There is therefore a need to carry out a holistic study of the disease in Zambia to understand its epidemiology and gain knowledge needed to assist surveillance and disease control policies and strategies.

1.3 Study objectives

1.3.1 General objectives

To determine the epidemiology of FMD in three agro-ecological areas of Zambia (Kafue Flats, Zambezi basin and Mbala-Isoka area), where the disease is endemic, so as to influence policy of strategic control plans.

1.3.2 Specific objectives

I. To investigate the spatial and temporal patterns of FMD outbreaks in Zambia from 1933 to 2012 and identify potential risk factors that were associated with these outbreaks.

II. To estimate the sero-prevalence of FMD in cattle at selected livestock/ wildlife interface areas.

III. To conduct molecular characterisation of FMD sero-types (prototypes) circulating in the study areas.

IV. To model the transmission dynamics of FMD among villages in the Kafue Flats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Spatial and geographical overview of Zambia

Zambian is a landlocked country covering 752, 610 km^2 between latitudes 8° and 18° south of the Equator and longitudes 22° and 34° East of the Greenwich Meridian. The agriculture land is estimated at 352,890 km^2 (47.5 percent of total land), of which 300,000 km^2 (85 percent of agriculture land) is under pasture. As such the country has more grazing than arable land (FAO, 2005; WorldBank, 2011). A large portion of over 400, 000 km^2 is forest, National parks and game management areas (FAO, 2014).

Zambia comprise of three agro-ecological regions with annual unimodal rainfall averaging 600 mm in region I, 600 to 1000 mm in region II and >1000 mm in region III (Nyemba and Dakora, 2010) (Figure 1). The rainy season is between November and April with December and January usually being the wettest months (Mumba and Thompson, 2005). Zambia has wetlands which are suitable for survival of both wild and domestic animals. Notable is the Kafue Flats, Bangweulu swamps, Barotse, Liuwa and the Simalaha floodplains. The Simalaha is an extension of the Zambezi floodplain of the Caprivi Strip of Namibia. Region I and II are resident to about 60 percent of the estimated 3.4 million cattle, 40 percent of the thousand sheep and 40 percent of the one million goats (Anonymous, 2006, 2011b). Region II which includes parts of Central, Southern, Eastern, and Lusaka provinces has the most favorable rainfall, soil quality, and absence of tsetse fly that allow for a diverse mix of crop and livestock enterprises

(Nyemba and Dakora, 2010). Because of its suitability to support ungulates, sharing of grazing land between wildlife and livestock is common, especially around the park boundaries and areas immediately surrounding National parks (NPs) and other game management areas (GMAs) (Muma J.B., 2006). In the recent past, region I and II have been affected by droughts and floods which apart from stressing the animals, promote increased interaction of livestock herds with wildlife at pasture and water points (Muuka *et al*., 2012). Region III has high rainfall with declining soil fertility by acidity due to leaching. As such the nutritional quality of pasture is usually poor (Nyemba and Dakora, 2010). Two notable wetlands in this region are the Bangweulu Swamps, famous for its black lechwe (*Kobus leche smithemani*) and wild buffalo (Muma *et al*., 2011a) and the Chambeshi plain that is ideal for cattle ranching.

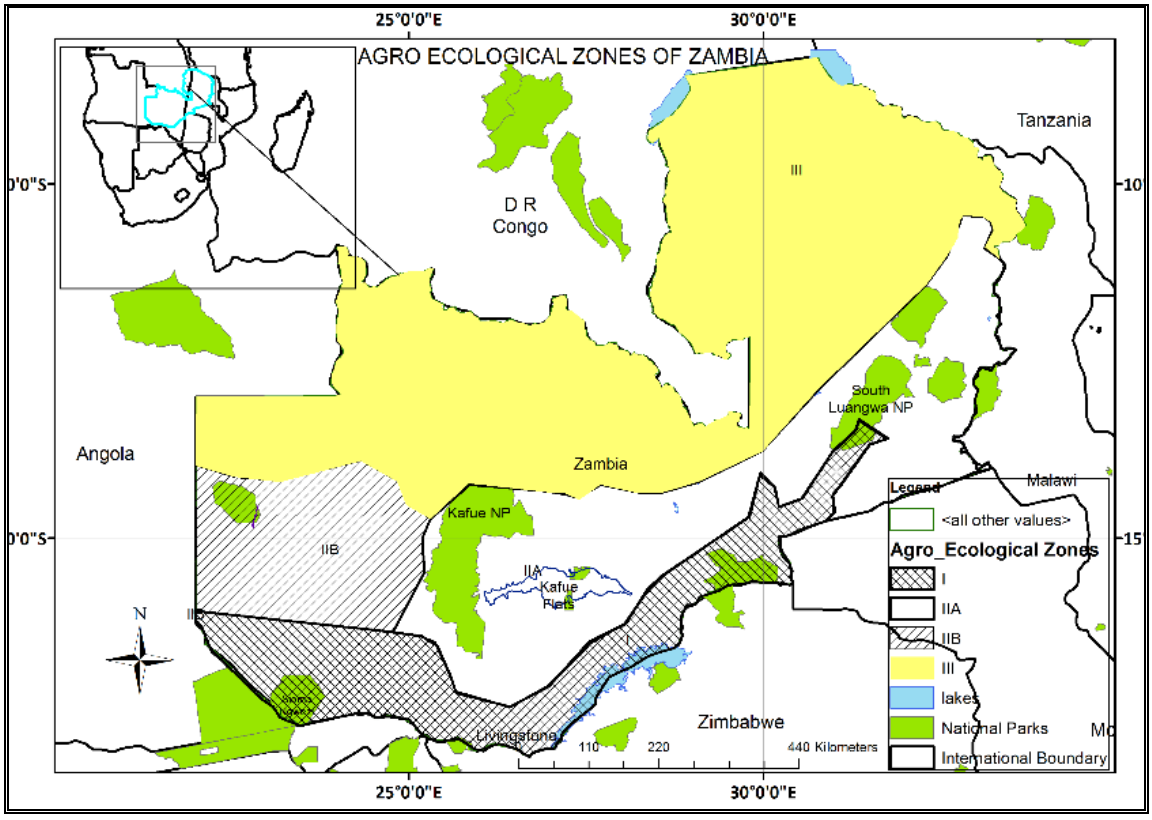

Figure 1: Zambia's agro-ecological zones and National parks

2.1.1 Vegetation and soil types

The vegetation is classified into four major categories: closed forests, woodlands or open forests, termitaria and grasslands (Figure 2) (FAO, 2009). The munga woodlands surrounds the wetlands of Kafue Flats, Lukanga Swamps, Shangombo District and the Luangwa River while, the Mopani is commonly found in the valley areas. Major soil types include the black clays (vertisols) and sandy clays commonly found in the Kafue basin and the dambo areas. Red clays, sand veldt and clay loam soils are common in plateau areas. These soils are generally of moderate fertility status with no salinity problems. The soil types play an important role in water retention and flooding, consequently in human, livestock, wildlife movement and migration (FAO, 2009).

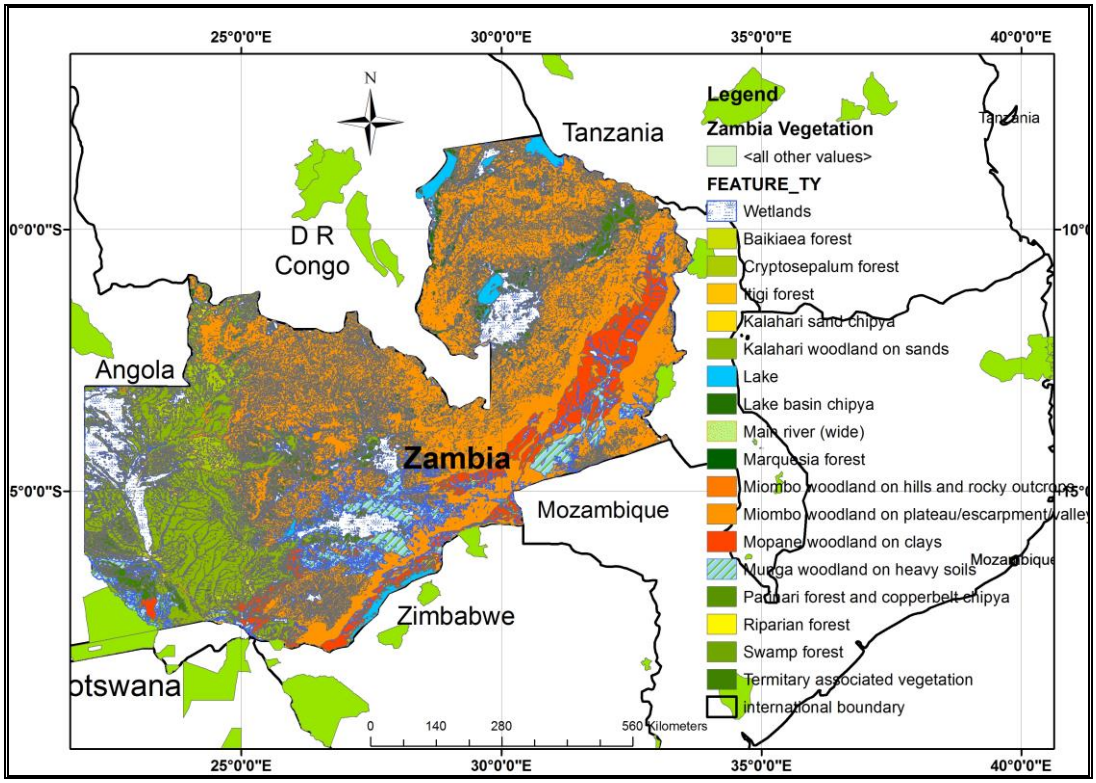

Figure 2: The vegetation of Zambia around the plateau, valley and wetland areas

2.2 Human demographical parameters and health indicators

The Zambia is regarded as one of the least urbanised countries in Africa. It has an estimated total human population of 13.04 million, with majority (60 percent) living in rural areas (CSO, 2010). Traditional farmers occupy the communal areas owning 85 percent of the estimated 3.4 million cattle, 68 percent of the thousand sheep, 97 percent of the one million goats and 90 percent of the 1.5 million pigs (Anonymous, 2011a). The commercial farmers own about 15 percent of the livestock and are concentrated mainly along the line of rail keeping exotic breeds of beef and dairy cattle (Anonymous, 2009; Muma *et al*., 2012). The country's human population growth averages 2.1 percent per annum with majority being below 35 years (CSO, 2012). Maize is the main staple crop grown in most parts of the country and produced using animal draught power (Muma *et al*., 2011b). Livestock further supports the rural communities as a reserve bank, symbol of wealth, transport to move agriculture produce to markets, bridal price, source of manure and animal protein (Perry *et al*., 1984; Anonymous, 2011b). Therefore, outbreaks of animal diseases such as FMD adversely affect livelihoods of Zambian communities (Scoones *et al*., 2010; Ferguson *et al*., 2013; Miguel *et al*., 2013b). The life expectancy of Zambians is estimated at 57 years (WorldBank, 2014), while poverty is at an average of 60.5 percent. In rural areas poverty levels are estimated to be 77.9 percent compared to urban areas where they are estimated at 27.5 percent (CSO, 2012). Further, 14.9 percent of the children under the age of 5 are undeweight (Anonymous, 2013c).

2.3 Socio economic status

Zambia's economy has historically depended on copper mining (Anonymous, 2006). Despite posting relative growth since 1999 with the latest real Gross Domestic Product (GDP) of 7.3 percent for 2012, the growth has not translated into poverty reduction which has remained persistently high (Anonymous, 2006; CSO, 2012; Anonymous, 2014a). The economic growth has been attributed to mining, construction and tourism sectors. However, agriculture upon which 80 percent of the population depends has declined, recording relatively low average growth rates, mainly due to inadequate infrastructure and poor market access (Anonymous, 2006, 2011b, 2014a). Agriculture contributes about 21.5 percent to GDP, out of which 28 percent is from the livestock sector (Muma *et al*., 2009; Anonymous, 2010). Therefore, the livestock sector has been identified as one of the change movers of the anticipated economic growth that is required to reduce poverty (Anonymous, 2011b; WorldBank, 2011; Anonymous, 2014a). The national per capital income is one of the lowest in the world estimated at 1,700 USD for 2012 (Anonymous, 2013c).

2.4 Livestock production system

The livestock production system can broadly be categorised into traditional and commerial sectors (Perry *et al*., 1984).

2.4.1 Traditional cattle

The traditional cattle production systems in Zambia have been described (Perry *et al*., 1984; Muma *et al*., 2006; Munyeme *et al*., 2009). In summary, cattle are raised on communal land under an extensive grazing system. In areas like the

Kafue Flats and Zambezi basin, three linked herding patterns exist: village resident herds (always in the villages), transhumant herds (move between village and the floodplains) and interface herds (permanently stay on the floodplains) (Muma *et al*., 2006; Munyeme *et al*., 2009). In the rest of the country, cattle are grazed in the communal areas as village resident herds and the distances covered are dependent on season and availability of pasture and water (Perry *et al*., 1984). The predominant cattle breeds are Zebu and Sanga with low milk output ranging from two to three litres/day (Muma *et al*., 2009). Cattle density is highest in the Southern, Central, Eastern and Western provinces of Zambia with herd size averaging forty (ten to fifty) in most provinces except, Southern province where herd sizes average 100 (Perry *et al*., 1984; Muma *et al*., 2009; Munyeme *et al*., 2009).

2.4.2 Commercial cattle

The commercial farms are usually private business enterprise keeping exotic breeds of beef and dairy cattle characterised by high production efficiency and high offtake rates to the market. Commercial farms are concentrated along the line of rail between Southern, Copperbelt and Central provinces and are the largest supplier of milk and beef to urban areas (Muma J.B., 2006; Chimana *et al*., 2010). Milk production in the commercial sector is predominantly from Freisian and Holstein cows with average yields of 25 litres/day (Muma *et al*., 2012). Occassionally, disease outbreaks from the traditional sector spill over into these farms. Outbreaks of FMD in this sector causes huge losses in productivity and income from movement bans which in turn affects the national economy through

drop in supply and general rise in prices of beef, dairy and other competing commodities (Perry and Rich, 2007).

The cattle numbers in Zambia have remained relatively low overtime with generally small growth rates (Table 1). The numbers increased significantly in the late 1960s, 1970s to 1980s as a result of creation of Government State Ranches that were later privatized in the 1992-1993 (Anonymous, 1968; Anonymous, 2006).

Table 1: Estimates of cattle numbers according to Department of Veterinary Services Annual Reports

Year	Cattle Numbers	Year	Cattle Numbers
1942	642,959	1976	1,644,786
1943	654,944	1977	1,702,277
1944	708,369	1978	1,742,851
1945	701,788	1979	1,720,757
1947	770,012	1980	1,728,917
1948	807,011	1982	1,951,863
1949	868,599	1983	2,048,260
1950	906,929	1984	2,036,418
1952	925,758	1985	2,075,736
1955	870,680	1991	2,984,000
1956	891,389	1992	3,095,000
1957	891,566	1993	3,204,000
1958	917,216	1994	2,525,967
1959	955,127	1995	2,642,200
1960	1,005,673	1996	2,562,841
1961	1,051,881	1997	2,700,516
1962	1,056,184	1998	2,747,175
1963	1,061,543	1999	2,572,488
1964	1,069,042	2000	2,620,987
1965	1,111,858	2001	2,490,990
1966	1,168,158	2002	2,517,550
1967	1,242,243	2003	2,375,433
1968	1,251,057	2004	2,341,970
1970	1,378,906	2005	2,381,421
1971	1,444,192	2006	2,444,789
1972	1,489,332	2007	2,457,279
1973	1,509,871	2008	2,815,583
1974	1,509,871	2012	3,462,357
1975	1,509,871		

2.5 Wildlife conservation

Conservation of wildlife is through protected areas and game ranches managed and regulated by the Zambia Wildlife Authority (ZAWA), a government institution (Lindsey *et al*., 2013).

2.5.1 Protected areas

The protected areas comprise 19 NPs and 35 GMAs. The GMAs act as buffer zones between NPs and human settlement areas where legal hunting is permitted while NPs are reserved for conservation and education purposes (Muma *et al*., 2006). The distribution of wildlife in these protected areas is provided in Table 2 below. The African wild buffalo, present in most NPs and GMAs contributes over 12.4 percent of the total hunting revenue through safari hunting for game trophies, ecotourism and photographic tourism (Munang'andu *et al*., 2006). The GMAs surrounding the NPs have human settlements, rivers and wetlands with a mosaic of economic activities (livestock grazing, wildlife conservation, fishing and crop cultivation) that contribute to interaction between livestock and wildlife. Before the creation NPs and GMAs between 1972 to 1975, there were people living inside some of the NPs or in close proximity. The NPs and GMAs are not fenced and human encroachment into wildlife areas in search of pasture and water is common especially that most tribes that have settled in these areas are agro pastoral (Muma J.B., 2006).

Table 2: Population estimates of various animal species (Zambia Wildlife Authority Annual Report 2009)

Species	Lower Zambezi Ecosystem	Kafue Ecosystem	Luangwa Ecosystem	Mosi-oa-tunya	Nsumbu Ecosystem	Country wide estimates	Trend
Buffalo (*Syncerus caffer)*	6621	4985	9379	265	180	40,717	Increasing
Bushbuck *(Tragelaphus scriptus)*			60	12	170	460	Stable
Common Duiker (*Sylvicapra grimmia)*		1009	674	25	107	3,449	Stable
Eland (*Taurotragus oryx*)		70	246			600	Decreasing
Elephant (*Loxodonta africana*)	1699	4263	6112	750	160	30,000	Increasing
Giraffe *(Giraffa Camelopardalis)*			94	67	58	416	Stable
Hartebeest (*Alcelaphus buselaphus)*		4053	597		538	9,857	Increasing
Hippopotamus (*Hippopotamus amphibicus*)		2758	20000	100		30,000	Increasing
Impala (*Aepyceros melampus*)	8112	9928	9919	1083		55,180	Increasing
Kudu (*Tragelaphus strepsiceros*),	204	665	476	22		2,597	Increasing
Lechwe Black (*Kobus leche. smithemani*)		52000				60,000	Decreasing
Lechwe Kafue (*Kobus leche kafuensis*)		45000				45,000	Decreasing
Lechwe Red (*Kobus leche leche*)		6108				11,605	Increasing
Puku *(Kobus vardonii)*		9146	3444		3705	18,000	Increasing
Reedbuck *(Redunca)*		543				1,032	Stable
Roan Antelope (*Hippotragus equinus)*		341	944		38	2,514	Decreasing
Sable Antelope (*Hippotragus niger*)	247	8268			129	16,424	Stable
Warthog (*Phacochoerus africanus*)	192	10633	1496	22	302	24,026	Increasing
Water Buck *(Kobus ellipsiprymnus)*	288	4152	738	39		9,912	Stable
Wildebeest (*Connochaetes taurinus*)		33,478	193	9		33,335	Increasing
Zebra (*Equus quagga*)	191	3159	3281	25	22	8,000	Stable

Several studies have shown how co-existence of wildlife with livestock in the GMAs affects transmission of different diseases between livestock and wildlife

(Munang'andu *et al*., 2006; Munyeme *et al*., 2008; Muma *et al*., 2010; Mwacalimba *et al*., 2013). Therefore, it is likely that GMAs which are mostly located in the wetlands such as the Kafue Flats and Zambezi basin where transhumant and floodplain herds share grazing pastures with wildlife serve as interface area for disease transmission between wildlife and livestock (Chilonda *et al*., 1999; Muma *et al*., 2006; Siembieda *et al*., 2011). Hence, this may partly acount for reasons why FMD outbreaks are highest in the Kafue Flats where cattle and buffalo share common grazing pastures.

2.5.2 Game ranching

Game ranches have emerged since 1978 as an alternative conservation strategy to conserve the depleting wildlife resources (McGranahan, 2008). Game ranching is considered environmentally sustainable and economically viable industry because of its ability to integrate eco-tourism and wildlife utilization with livestock keeping (Munang'andu *et al*., 2006). Even though the African buffalo is an attractive collection for game ranchers, the threat of them being long term FMD carriers has prevented rearing on most ranches. Generally, it is becoming mandatory practice that all ungulates captured from national Parks and GMAs known to be endemic with FMD are screened for FMD to ensure they do not carry the virus to game ranches which are located in areas close to livestock production areas. Consequently, this makes FMD a constraint to the expansion of the game ranching industry because of the prohibitive capture operation and FMD screening costs (Bengis *et al*., 2004; Munang'andu *et al*., 2006; Lindsey *et al*., 2013).

2.5.3 Trans-frontier conservation areas

Trans-frontier conservation areas (TFCAs) have been created in Southern and Eastern Africa to conserve wildlife with the primary objective of connecting various protected areas (Thomson *et al*., 2013). This presents unique challenges to the control and prevention of trans-boundary and zoonotic diseases because of the expansion of the human/livestock/wildlife interface (Brückner *et al*., 2002; Thomson *et al*., 2003; de Garine-Wichatitsky *et al*., 2013). Of all TFCAs, the Kavango Zambezi (KAZA) is the largest spanning an estimated 520,000km^2 of which 42 percent is in Zambia (Thomson *et al*., 2013; Anonymous, 2014b). The TFCAs do not only present challenges for management of trans-boundary animal diseases like FMD, but also give rise to increased human wildlife conflicts (Scoones *et al*., 2010). At present there seems to be no agreed strategy within the countries participating in these TFCAs on management of trans-boundary animal diseases like FMD (Thomson *et al*., 2013). Therefore, knowledge of the epidemiology of FMD in these areas makes it the more important if effective evidence based disease control strategies are to be designed and implemented. The linking of livestock and wildlife research to address this knowledge gap was high on the agenda during the Southern Africa Development Community (SADC) workshop of animal health and wildlife experts (Anonymous, 2012).

2.6 Foot and mouth disease

2.6.1 Origins of foot and mouth disease

The origin of FMD in sub-Saharan Africa (SSA) is not very clear. The disease was first reported in southern Africa in 1892 in Mashonaland, Griqualand West, Transvaal, Orange Free State and Natal (Bruckner *et al*., 2002). Some reports

suggest that probably the disease existed as early as 1870 (Thomson and Bastos, 2004). FMD was later reported in 1903 in South Africa suspected to have been due to imported animals from Argentina (Bruckner *et al*., 2002). The disease re-appeared in March 1931 among cattle on Nuanetsi Ranch (southern Zimbabwe) after an absence of several decades and later spread to Botswana (1932) and Zambia in 1933. The absence of FMD between the 1890s and 1930s has been attributed to the advent of the great rinderpest pandemic of 1896 to 1905 which decimated not only the cattle population but also the FMD reservoir host, the African buffalo (Thomson and Bastos, 2004). However, FMDV could have been maintained over long periods of time in small herds of buffalo (Dawe *et al*., 1994a; Dawe *et al*., 1994b).

2.6.2 Taxonomy and phylogeny of foot and mouth disease

2.6.2.1 Taxonomy

Foot and mouth disease is caused by a single stranded RNA virus (FMDV) belonging to the family *Picornaviridae* and genus *Aphthovirus* (Domingo *et al*., 2002; Sáiz *et al*., 2002; Brown, 2003). The name *Picornaviridae* is derived from the Latin word ‘pico’ (small) and ‘rna’ (RNA) refers to the size and genome type while the genus name ‘*aphthovirus*’ refers to the vesicular lesions produced in cloven hoofed animals (Bastos, 2001). The FMDV genome is a molecule of high rate of nucleotide mutation and amino acid substitution because the viral RNA-dependent RNA polymerase lacks proofreading ability resulting in seven immunologically and serologically distinct serotypes O, A, C, Southern African Territories (SAT) 1-3 and Asia 1, with limited cross protection between them (Callis, 1973; Domingo *et al*., 2002; Sáiz *et al*., 2002; Jamal and Belsham, 2013).

The FMDV particle is roughly spherical in shape and about 25-30 nm in diameter (Sáiz *et al*., 2002; Alexandersen *et al*., 2003; Borley *et al*., 2013). It consists of the RNA genome surrounded by a protein shell or capsid (Domingo *et al*., 2002). The capsid is composed of 60 copies of the capsomer or protomeric subunit each comprising the four capsid proteins i.e. VP1, VP2, VP3, and VP4 (Acharya *et al*., 1990). The three surface-exposed proteins, VP1, VP2, and VP3 are external while the smaller VP4 is located internally in contact with the five protomers that assemble to form a pentamer and 12 pentamers subsequently self-assemble to form a complete capsid (Figure 3) (Sobrino *et al*., 2001; Domingo *et al*., 2002; Mateo *et al*., 2008).

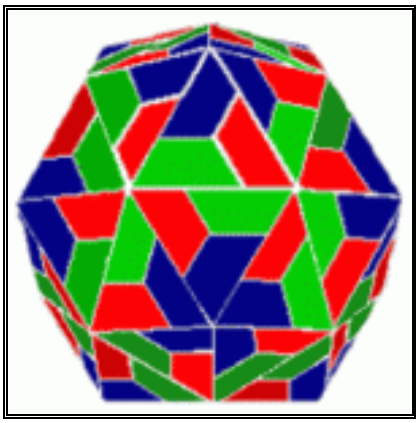

Figure 3: Foot and mouth disease virus structure showing the three exposed surface proteins VP1 (blue colour), VP2 (green colour) and VP3 (red colour) in a protomer, five of which make up a pentamer (Adapted from (Mateo *et al*., 2008).

The International Committee of Taxonomy of Viruses (ICTV) recommends classification of viruses up to serotype level which is equivalent to the species taxonomic unit (Bastos, 2001). The seven immunologically distinct serotypes of FMDV were classified based on serological methods using the criteria of no cross protection between serotypes and ability to infect multiple wildlife hosts

(Alexandersen *et al*., 2002; Clavijo and Kitching, 2003). These serotypes share an approximate 86 percent amino acid identity to each other. However, some of the capsids proteins like VP1 exhibit some variation of 30 to 50 percent between serotypes (Knowles and Samuel, 2003; Borley *et al*., 2013). This variation has impeded the development of vaccines that can provide cross protection both inter and intra-serotypically (Brehm *et al*., 2008; Borley *et al*., 2013).

2.6.2.2 Phylogeny

Classification of FMDV isolates based on phylogenetic methods using VP1 and Reverse Transcriptase Polymerase Chain Reaction (RT PCR) amplification of genomic FMDV RNA isolates and nucleotide sequencing have replaced the serological methods (Sobrino *et al*., 2001; Domingo *et al*., 2002). The FMD RNA genome has a protein capsid composed of four structural viral proteins (VP1, VP2, VP3, VP4) as mentioned above and eight non-structural proteins that are non-enveloped (L, 2A, 2B, 2C, 3A, 3B, 3C and 3D) (Figure 4) (Belsham, 1993; Lubroth, 2002). The VP1, VP2 and VP3 constitute the surface of the virus and are composed of eight anti-parallel B strands linked by loops to form a B barrel. The highly mobile VP1 has a G-H loop that protrudes from the surface of the virus and contains the arginine glycine –aspartic acid (RGD) motif (Fox *et al*., 1989; Borley *et al*., 2013). The viral proteins allow replication of the virus in the infected host cell and infection is usually cytolytic. The VP1 is responsible for antigenic diversity (serotype specificity) because the RGD has a dual function in recognition of Integrins (Domingo *et al*., 2002; Sáiz *et al*., 2002). The G-H loop serve as cellular receptors (virus entry) for FMDV transmission and antibody binding as well as several epitopes involved in FMDV neutralization (immunity)

that has been mapped within the loop (Fox *et al*., 1989; Sáiz *et al*., 2002; Borley *et al*., 2013). This has now made it possible to demonstrate evolutionary dynamics, epidemiological relationship among the genetic lineages and in the authentic tracing of the origin and movement of outbreak strains trans-regionally or trans-continentally (Figure 4) (Vosloo *et al*., 2001; Knowles and Samuel, 2003; Di Nardo *et al*., 2011; Hall *et al*., 2013).

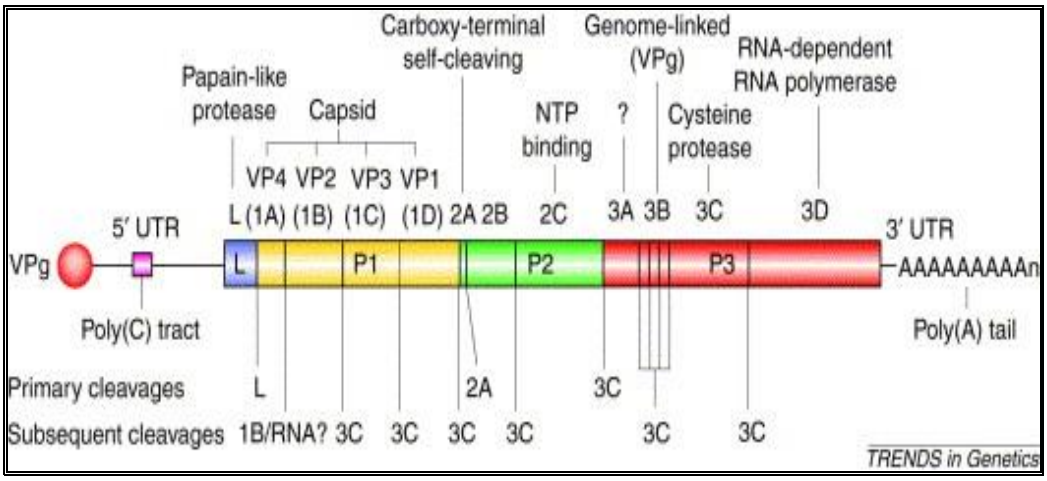

Figure 4: Full genome structure of the foot and mouth disease virus with emphasis on the VP1 used for sequencing (Adapted from (Grubman and Baxt, 2004).

The serotypes are further divided into topotypes and genotypes based on the genetic sequences of their 1D gene or VP1. The genotypes are usually grouped into geographically and genetically distinct lineages (topotypes) of less than 15 percent nucleotide differences in Eurasian and 20 percent for SAT serotypes (Samuel *et al*., 2001; Carrillo *et al*., 2007; Di Nardo *et al*., 2011). However, although emergence of FMDV populations may be attributed to VP1 analysis experimentally, in nature these genotypes are influenced by specific epidemiological and immunological aspects of host-virus interaction (Carrillo *et al*., 2007). Further influence may come from the quasispecies composition of the

viral population (Domingo and Holland, 1997). The quasispecies, antigenic and the genetic variability of the FMDV complicate the selection of FMD vaccine candidates (Haydon *et al*., 2001; Maree *et al*., 2011).

For Africa, six topotypes have been identified for serotype O, two for serotype A, three for serotype C, nine for SAT1, fourteen for SAT 2 and five for SAT 3 (Vosloo *et al*., 2002a; Rweyemamu *et al*., 2008b; Di Nardo *et al*., 2011; Tekleghiorghis *et al*., 2014). The current distribution of these topotypes by country from 1990 to 2013 has been described (Table 3) (Tekleghiorghis *et al*., 2014). Africa has been divided into three FMDV pools with pool four covering East and North Africa with predominance of serotypes A, O, SAT1, SAT2 and, SAT 3. Pool five is restricted to West and Northern Africa with serotypes O, A, SAT1 and SAT2. Pool six is restricted mainly to Southern Africa with SAT1, SAT2 and SAT3 serotypes (Figure 5) (Di Nardo *et al*., 2011; Maree *et al*., 2014; Tekleghiorghis *et al*., 2014). Rweyemamu *et al* (2008) proposed eight epidemiological clusters for Africa based on the serotype and topotype distribution, animal movement patterns, impact of wildlife, and farming systems. Notable from this classification was the division of southern Africa into North and South SADC (Figure 6). The virus pools four and six intersect in Northern Zambia demonstrating viral and antigenic diversity that may need to be investigated to effectively manage FMD by vaccination in this area (Sinkala *et al*., 2014b).

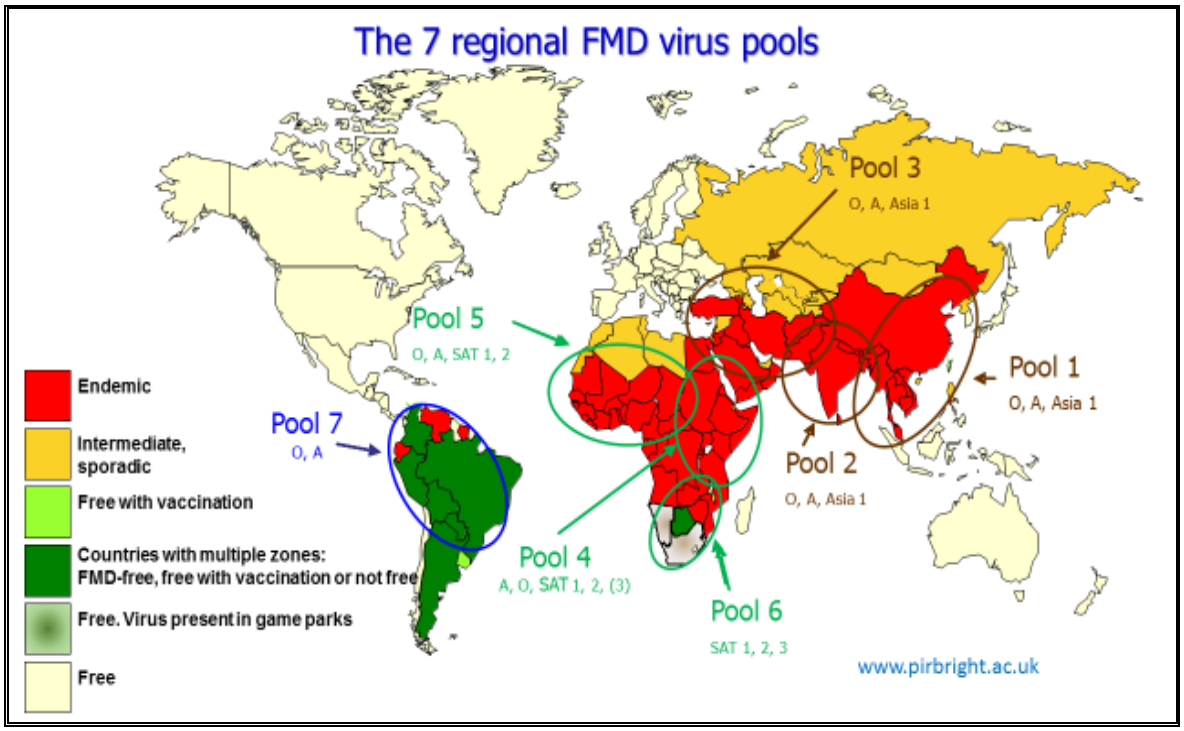

Figure 5: Seven conjectured global foot and mouth disease virus pools based on molecular epidemiology as compiled by the World Reference Laboratory for FMD (Knowles, 2013).

Virus circulation is mainly within these regional pools and strains have evolved specific to the region (in the case of type A and SAT viruses). There is therefore, a requirement for tailored diagnostics and vaccines to subtype level for control (Paton *et al*., 2009). SAT-2 viruses appear to be more diverse in topotypes and prevalence in sub-Saharan African countries with incursion into the Middle East being traced back to Africa (Hall *et al*., 2013). Because of geographic and genetic clustering of FMDVs, ecological adaptation and/or separation has been suggested (Hall *et al*., 2013; Tekleghiorghis *et al*., 2014).

Table 3: Topotype distribution of foot and mouth disease virus serotypes in Africa from 1990 to 2013 (Vosloo *et al*., 2005b; Rweyemamu *et al*., 2008b; Tekleghiorghis *et al*., 2014).

Serotype	Topotype	Genotype/Strain	Representative country/countries
SAT-1	I (NWZ)		Kenya (2011), Tanzania (2010*, 2012), South Africa (2010), Zimbabwe 2003, Mozambique (2009), Zambia (2009), Malawi (2001)
	II (SEZ)		Botswana (1998*), Namibia (2010), Zambia (2010), Zimbabwe (2004), Swaziland (2000), Mozambique (2010*)
	III (WZ)		Zambia (2012), Namibia (2011), Botswana (2006*) Tanzania (1999), Zimbabwe (1997*)
	1V (EA-1)		Uganda (2007*)
	V		Nigeria (1976), Niger (1976)
	VI		Nigeria (1981), Sudan (1976)
	VII (EA-2)		Uganda (1974)
	VIII (EA-3)		Uganda (1997*)
	IX		Ethiopia (2007)
SAT-2	I		Botswana (2011), Malawi (2008), South Africa (2012), Mozambique (2010), Zimbabwe (2010), Burundi (1991), Kenya (1999), Zambia (1996*), Namibia (1998)
	II		Botswana (2008), Namibia (1998*), Zimbabwe (2010), Malawi (2008), Ghana (1991)
	III		Botswana (2006*, 2012), Namibia (2008), Zambia (2009), Zimbabwe (2002), South Africa (2011)
	1V		Kenya (2009), Tanzania (2012), Burundi (1991), Zambia (2012), Ethiopia (1991)
	V		Ghana (1991), Rwanda (2000), Senegal (1975)
	VI		Gambia (1979), Senegal (1983)
	VII		Egypt (2012), Libya (2003, 2012), Cameroon (2005), Eritrea (1998), Niger (2005), Nigeria (2008), Senegal (2009), Sudan (2007, 2010)
	VIII		Rwanda (2001)
	IX		Kenya (1996), Uganda (1995)
	X		Uganda (2007*)
	XI		Angola (1974)
	XII		Uganda (1976)
	XIII		Sudan (2008), Ethiopia (2010)
	XIV		Ethiopia (1991)
SAT-3	I (SEZ)		Zimbabwe (1999), South Africa (1997*), Mozambique (2010*), South Africa (2011),
	II (WZ)		Zimbabwe (1994*), Namibia (1998*), Botswana (1998*), South Africa (2011)
	III (NWZ)		Zimbabwe (1991*)
	1V		Zambia (1996*)
	V		Uganda (1970*), Uganda (1997*), South Africa (2011)
0	EA-1		Kenya (2010), Uganda (1996)
	EA-2		Kenya (2011), Burundi (2003), DRC (2011), Malawi (1998), Rwanda (2004), Tanzania (2009), Uganda (2007), Zambia (2010), Sudan (1999)
	EA-3		Ethiopia (2011), Eritrea (2011), Niger (2007), Nigeria (2009), Somalia (2007), Sudan (2011), Kenya (1987), Libya (2011), Egypt (2012)
	EA-4		Ethiopia (2013), Kenya (2010), Uganda (1999)
	ME-SA	Sharquia-72	Egypt (2009)
	ME-SA	PanAsia-2	Libya (2011), Egypt (2007)
	ME-SA	PanAsia-1	South Africa (2000)

	ME-SA		Algeria (1990), Egypt (1993), Ethiopia (1994), Eritrea (1996), Tunisia (1994), Tanzania (1998), Libya (1994).
	WA		Algeria (1999), Cote d'Ivoire (1999), Burkina Faso (2002), Cameroon (2005), Ghana (1994), Guinea (1999), Gambia (1999), Mali (2007), Mauritania (2001), Niger (2005), Senegal (2006), Togo (2005), Tunisia (1999), Morocco (1999)
A	AFRICA	G-I	Kenya (2009), Tanzania (2012, 2013), Uganda (2002), Zambia (1990), Burundi (1990), DR Congo (2011)
	AFRICA	G-II	Ethiopia (2005)
	AFRICA	G-III	Kenya (2005), Ethiopia (2005), Sudan (2007), Uganda (2002), Cameroon (2005), Egypt (2006)
	AFRICA	G-1V	Egypt (2012), Eritrea (2009), Mali (2006), Nigeria (2009), Togo (2005), Cameroon (2005), Sudan (2006)
	AFRICA	G-V	Ghana (1973)
	AFRICA	G-VI	Mali (1997, 2006), Mauritania (2006), Gambia (1998), Senegal (1996), Burkina Faso (1994), Cote d'Ivoire (1996),
	AFRICA	G-VII	Egypt (2009), Ethiopia (2009), Kenya (2006)
	AFRICA	G-VIII	Kenya (1964)
	ASIA	Iran-05^{BAR-08}	Egypt (2011), Libya (2009)
C	AFRICA (I)	Ken-67	Kenya (2004)
	AFRICA (II)	Eth-71	Ethiopia (1983)
	AFRICA (III)		Angola (1973)

*Foot and mouth disease (FMD) virus isolated from African buffalo

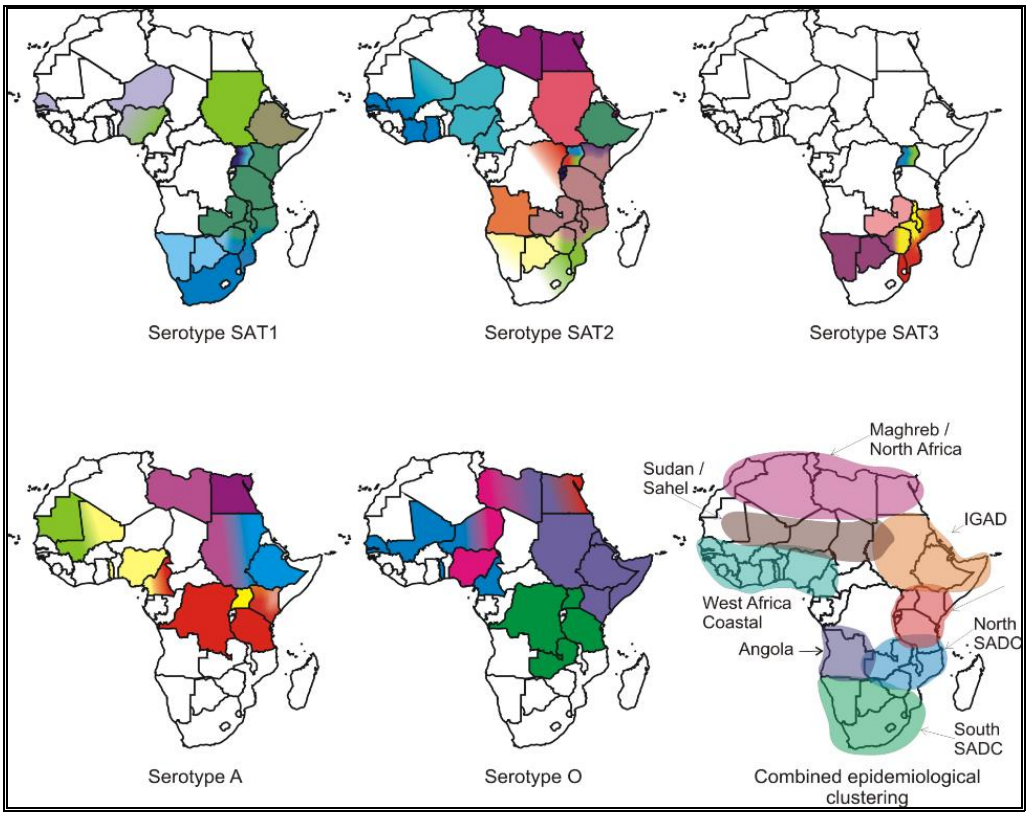

Figure 6: Distribution of foot and mouth disease virus serotypes and clusters in Africa (Adapted from Rweyemamu *et al.*, 2008b).

Foot and mouth disease virus exhibit large intra and inter serotype genetic variability, especially the SAT types. The multiple and repetitive inter subunit interactions appear to have evolved under stringent and selective constraints (Thomson and Vosloo, 2004). Consequently, the viral properties of each serotype are constrained within fundamental structural requirements of the virus capsid (Acharya *et al*., 1990; Mateu *et al*., 1995a; Knowles, 2013). As a result of the genetic and antigenic variants within the SAT serotypes, different degrees of virulence exist as well as regional differences in the distribution and prevalence of serotypes that require updating to effectively control the disease (Kitching *et al*., 1988; Vosloo *et al*., 1995) (Figures 6).

The establishment of a partial VP1 gene sequence database for southern African SAT-type buffalo viruses has been invaluable for accurately tracing the source of SAT1-type outbreaks, and for pinpointing the origin of illegally moved buffalo infected with SAT 3 type viruses (Bastos *et al*., 2001; Vosloo *et al*., 2001). Phylodynamics using both phylogenetics and population genetics data has been used to address fundamental epidemiological questions such as spread into new areas and hypothesizing potential methods of spread (Hall *et al*., 2013). Elsewhere genome analysis shows that serotypes O, A, C and Asia 1 constitute a clear evolutionary lineage distinguishable from the other lineage comprising the Southern African Territories (SAT) types (Acharya *et al*., 1989).

2.6.3 Transmission and infection persistence

Based on the VP1 sequencing described above, two epidemiological forms of FMDV have been described for southern Africa (Vosloo *et al*., 2002a; Vosloo and Thomson, 2004; Rweyemamu *et al*., 2008b). The first form involves SAT 1, SAT

2, and SAT 3 that is maintained and spread by wild buffalo to susceptible cattle without further involvement of buffalo in further spread of the disease within cattle populations (Thomson and Vosloo, 2004). The second form involves Eurasian or South American type involving serotype O and A with incursions of SAT 1 and SAT 2 that is maintained and spread by cattle (Vosloo *et al*., 1996; Thomson *et al*., 2003).

2.6.3.1 Buffalo initiated foot and mouth disease transmission and persistence

The buffalo initiated transmission is suspected to happen at the livestock/wildlife interface areas where sharing of pasture and water is known to exist or sometimes through intermediaries like impala (*Aepyceros melampus*) and kudu (*Tragelaphus strepsiceros*) (Dawe *et al*., 1994a; Bastos *et al*., 2000; Hargreaves *et al*., 2004; Rweyemamu *et al*., 2008b). Although the precise mechanism of transmission of FMDV from buffalo to cattle is not well understood, evidence suggest that transmission does take place but remains irregular, rare and under unknown circumstances (Dawe *et al*., 1994a; Vosloo *et al*., 1996; Bastos *et al*., 2000; Vosloo *et al*., 2002b). One probable theory is that stress is required to initiate at least one adult buffalo to shed viruses that eventually cause infection through horizontal transmission to calves less than 1 year old, referred to as "childhood" epidemics (Thomson *et al*., 1992; Vosloo and Thomson, 2004). It is when these calves are infected and become infectious that livestock (cattle) and other wildlife species are likely to acquire infection (Jori *et al*., 2009). This may happen directly or indirectly through contaminated pasture and stagnant water at drinking points. Direct contact between buffalo and cattle is rare in normal circumstances but extreme weather conditions like drought and floods, production practices such as transhumant grazing and other ecological factors may precipitate such interactions

(Vosloo *et al*., 2009; Miguel *et al*., 2013a). It has also been recognized that the livestock/wildlife interface areas of southern Africa are potential areas of disease transmission from wild buffalo to cattle that need to be further investigated (Tekleghiorghis *et al*., 2014).

The FMDV may persist in wild African buffalo for five years with about 50 to 70 percent becoming carriers post infection (Condy *et al*., 1985; Alexandersen *et al*., 2002) while in a herd persistence may last 24 years and in isolated herds probably up to 80 years (Condy *et al*., 1985; Dawe *et al*., 1994a). Other wildlife species like impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*), sable antelope (*Hippotragus niger*), Kafue lechwe (*Kobus leche kafuensis*) and other antelope species have the potential to spread the disease but none become long term carriers (Vosloo *et al*., 2005a; Vosloo *et al*., 2007) (table 4). Transmission of FMDV from carrier wild buffalo to susceptible cattle has only been demonstrated in a few studies both naturally and experimentally (Gainaru *et al*., 1986; Dawe *et al*., 1994a; Vosloo *et al*., 1996; Bastos *et al*., 2000). In southern Africa, most populations of African buffalo have been shown to harbour all three SAT type simultaneously (Condy *et al*., 1969; Hedger, 1972). However, studies in these species have been less thorough except for South Africa. Until this issue is adquately investigated in the rest of sub Saharan African countries, gaps in understanding FMD epidemiology will continue to exist (Vosloo and Thomson, 2004).

2.6.3.2 Cattle initiated transmission and persistence

Trade initiated livestock movement has been implicated as the main mode of spread of FMD in Africa and beyond (Rweyemamu *et al*., 2008b; Di Nardo *et al*.,

2011; Hall *et al*., 2013; Knight-Jones and Rushton, 2013; Tekleghiorghis *et al*., 2014). In some areas, the nomadic and transhumance tendencies are significant contributors to movement and contact (Di Nardo *et al*., 2011; Tekleghiorghis *et al*., 2014). The growing consensus is that FMD spread in Africa is usually by direct contact from infected to naïve cattle (Hall *et al*., 2013; Miguel *et al*., 2013a; Tekleghiorghis *et al*., 2014). FMD can also be transmitted in a number of ways including long-distance aerosol spread and fomites or inanimate objects, typically fodder and motor vehicles (Alexandersen *et al*., 2003). Cows can also be infected with FMD from the semen of infected bulls (Bastos *et al*., 1999), but the evidence is tenacious (Thomson and Bastos, 2004). The airborne spread reported in Europe is unlikely in Africa except during frost winter mornings when humidity is high with low temperatures and strong winds (Hargreaves *et al*., 2004).

A carrier is any animal from which the FMDV can be recovered from the oropharyngeal area 28 days or more after infection (Salt, 1993; Alexandersen *et al*., 2003). The origin come from the observation by Van Bekkum that live virus could be recovered from oro pharyngeal fluids from cattle during convalescent phase of FMD (Grubman and Baxt, 2004). In cattle, 15 to 50 percent may become carriers following an infection. The viral load declines by seven months although in others it may last up to 3.5 years after infection (Alexandersen *et al*., 2002). The persistence in infected cattle is dependent on the virus strain, breed of cattle and local circumstances (Sutmoller and Olascoaga, 2002). In Zambia, FMDV has been recovered from local cattle 19 months post infection (Perry and Hedger, 1984).

The virus persists in the basal layer cells of the pharyngeal epithelium particularly the dorsal soft palate (Zhang and Kitching, 2001). Persistence is not known to

exist in pigs except for one study that claimed to show persistence (Mezencio *et al*., 1999). In sheep and goats, the virus has been observed to persist for twelve and three months, respectively (Table 4).

Table 4: Duration of viral of persistence in selected domestic animals and wildlife species. (Adapted from (Vosloo *et al*., 2005b)

Species/animal	Duration of viral persistence	Reference
Domestic animals		
Cattle	2.5 -3.5 years	Hedger 1976 Hargreaves 1994
Sheep	9 – 12 months	Burrows 1968 McVicar and Sutmoller 1968
Goats	2-3 months	Singh 1979 Anderson *et al*., 1976
Wildlife		
Wildebeest (*Connochaetes taurinus*)	28 days	Anderson *et al.*, 1975
Sable (*Hippotragus niger*)	28 days	Ferris *et al.*, 1989
Eland (*Taurotragus oryx)*	32 days	Anderson 1980
Fallow deer *(Dama dama)*	63 days	Forman *et al.*, 1974
Kudu (*Tragelaphus strepsiceros*),	104 – 160 days	Hedger 1972
Water Buffalo (*Bubalis bubalis*)	2 -24 months	Moussa *et al.*, 1979
African Buffalo (*Syncerus caffer*)	5 years	Condy *et al.*, 1985
Impala (*Aepyceros melampus*)	7 days	Hedger *et al* 1972, Anderson *et al* 1975
Kafue Lechwe (*Kobus leche kafuensis*)	Not known	Overby *et al* 1983

There is no evidence of FMDV transmission from carrier cattle to naïve animals (Condy and Hedger, 1974; Anderson *et al*., 1976; Grubman and Baxt, 2004; Carrillo *et al*., 2007). Although the long persistence and virus replication in the host may lead to new viral variants (Gebauer *et al*., 1988; Saiz *et al*., 1996; Toja *et al*., 1999), the mechanism employed by the virus to persist and evade the immune elimination from the host is unknown (Salt Jeremy, 2004). How much of the infection in carriers results in effective disease transmission to susceptible cattle is an important aspect to consider in elucidating the epidemiology of FMD in

endemic setting where African wild buffalo are present (Dawe *et al*., 1994a). Sub-clinically infected, vaccinated cattle can transmit infectious dose of FMDV to susceptible animals up to seven days post infection, whereas infection from true carrier cattle remains unproven under controlled conditions (Salt, 1993).

2.6.5 Foot and mouth disease time periods

The important time periods for FMD dynamics include the incubation period defined as the time from infection to the onset of clinical disease; the pre-infectious (also referred to as latent) period defined as the time from infection to when the host is able to transmit the infection to another host; and the infectious period defined as the period from the end of the pre-infectious period until the time when a host is no longer able to transmit the infection to others (Keeling and Rohani, 2008; Mardones *et al*., 2010; Vynnycky and White, 2010a; Charleston *et al*., 2011). For FMD, the pre-infectious period has been reported to be 4.6 days (Charleston *et al*., 2011) and 3.6 days (Mardones *et al*., 2010). The incubation period has been reported to be two to 14 days (average of eight days) (Alexandersen *et al*., 2003) and more recently 4.1 days (Charleston *et al*., 2011) and 5.9 days (Mardones *et al*., 2010). The variability may be due to the strain, route of exposure, intensity of exposure and husbandry conditions (Alexandersen *et al*., 2003). Exposing pigs to high doses of FMDV and keeping a large number of infected animals together shortens the incubation period (Quan *et al*., 2004). For FMD, an infected animal is known to shed viruses two to three days before the appearance of clinical signs referred to as sub subclinical period (Alexandersen *et al*., 2003; Mardones *et al*., 2010).

2.6.6 Clinical manifestation

The disease is characterized by formation of vesicles (blisters) consistently on the mouth (tongue and, gums), hooves, teats and mammary gland (Alexandersen *et al*., 2003) (Figure 7).

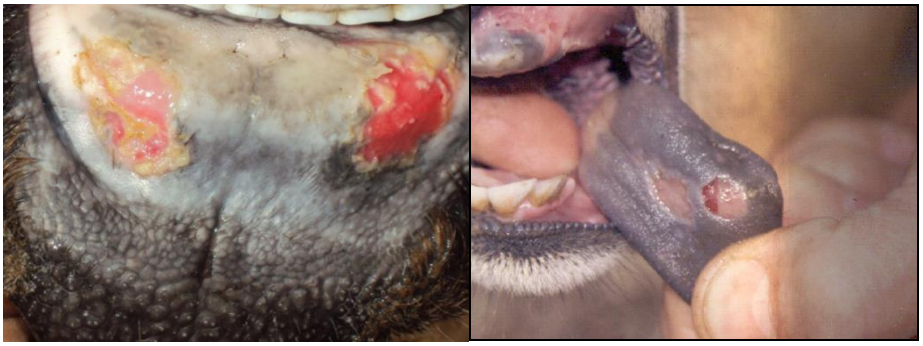

Figure 7: Foot and mouth disease clinical lesions around the mouth and tongue

The pain associated with the lesions cause lameness, anorexia, drooling of saliva from the mouth which is usually pathognomonic (Alexandersen *et al*., 2003; OIE, 2014b). The vesicles develop as a result of separation of the epithelium from the underlying connective tissue and filling of the cavities with vesicular fluid. Sometimes the vesicular fluid may be large and the vesicles visible, while in other cases the fluid is limited and the epithelium may undergo necrosis without forming a vesicle (Kitching, 2002).

Foot and mouth disease is normally acute and easy to detect. However, pigs might initially show mild lameness (Alexandersen *et al*., 2003). Sheep and goats may not show obvious signs at all and rather show non-specific signs that might be confused with a wide range of diseases (Grubman and Baxt, 2004). Mortality is generally low in adult animals but can be high in young animals due to myocarditis (Alexandersen *et al*., 2003).

2.6.7 Pathogenesis

Following infection through the respiratory route, the FMDV replicates locally in the pharyngeal area (Alexandersen *et al*., 2003; Rodriguez and Grubman, 2009; Morelli *et al*., 2013), then spreads through the lymphatic system into the blood stream causing fever and viraemia in cattle 24 to 48 hours after epithelium infection (Sáiz *et al*., 2002; Morelli *et al*., 2013). Consequently, secondary vesicles occur in the mouth and feet due to viral spread to different organs and tissues (Alexandersen *et al*., 2003). However, the predilection sites for the virus are the cornified, stratified squamous epithelia of the tongue and skin (Zhang and Alexandersen, 2004). Development of vesicular lesions is due to the cytopathic effect of the virus on the target cells. Although the FMD virus is detected in smaller amounts in the pharyngeal area than in the tongue and skin during acute infection, viral clearance is much faster in the tongue and skin than in the pharyngeal tissues. Viral RNA in the oro-pharyngeal fluid has been shown to correlate with the presence of infectious virus that lasts seven to eight days (Alexandersen *et al*., 2003; Zhang *et al*., 2004). The acute form gradually declines coinciding with the emergence of a strong humoral response (Alexandersen *et al*., 2003) and convalescence is not always present in recovered animals (Dawe *et al*., 1994a). Following the clearance of viraemia during the recovery period, FMDV continues to be present in high titres at the oral pharyngeal area giving rise to a carrier status. Despite lack of transmission evidence from carrier cattle to naïve animals, the international animal trade policy is still based on the possibility of transmission from asymptomatic carrier animals to naïve ones (Brown, 2003; Arzt *et al*., 2011). Because of the failure of vaccination to prevent carrier state, depopulation following incursions in developed nations remains the norm. Others

have argued that depopulation is not necessary and vaccination should be considered (Brown, 2003; Sutmoller *et al*., 2003).

2.6.8 Immune response to foot and mouth disease virus

The immune response is predominantly humoral with T-cell dependent response (Collen *et al*., 1991; Sáiz *et al*., 2002). The major immunogenic component of FMDV is the surface glycoprotein VP1, especially the G-H loop (Davies, 2002). VP2 and VP3 make little contribution to immune response and are considered as scaffolding proteins. Production of IgM occurs as early as three to four days post-infection or vaccination followed by the major neutralizing antibodies (IgGs) (Sáiz *et al*., 2002). Although IgM is detectable in the secretions of upper respiratory tract during the first ten days of infection, IgA remains the major constituent of local immune response. Despite the chronic stimulation of local IgA that lasts for weeks post infection, the virus appears to persist in the epithelium of the soft palate and oro pharynx (Salt, 1993; Salt Jeremy, 2004). In ruminants, neutralizing specific antibody levels remain high for many months post infection while it remains detectable for about a week in pigs (Alexandersen *et al*., 2003).

Young animals are protected by colostrum-derived antibodies that become undetectable at six months old (Vosloo and Thomson, 2004). The half-life of antibodies in cattle is 21.5 days whilst in pigs it varies with class of immunoglobin that predominate in the colostrum, thus two to eight days for IgM and seven to twenty one days for IgG. Maternal antibodies do not just protect calves from infection until six months of age but may also interfere with vaccination. It is therefore recommended not to vaccinate calves less than six months since it is not cost effective (Kitching and Salt, 1995).

2.6.9 Diagnosis of foot and mouth disease

Techniques for diagnosis of FMDV are available in the OIE Manual of diagnostics and have been used by different laboratories depending on their national priority and level of advancement (OIE, 2014a). Every strategy in the control and prevention of FMD involve use of diagnostic tests of varying application convenience (Namatovu *et al*., 2013), whether it be separation of wildlife and livestock; repeated vaccination of cattle herds exposed to wildlife; control of animals movement; or careful assessment of risk of FMD introduction in non-infected areas (Thomson *et al*., 2003; Namatovu *et al*., 2013).

2.6.9.1 Serological methods

In Africa, diagnosis of FMD mainly relies on use of antibody ELISAs with non-structural protein (NSP) being widely used (Namatovu *et al*., 2013). NSP ELISA is a competitive Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody to NSP. It is relatively simple, cheap, requires low level bio containment, do not need cell cultures and have the advantage of differentiating infected from vaccinated animals (DIVA) (Ferris and Dawson, 1988; Sørensen *et al*., 1998). However, use of non-purified vaccines in Africa makes it difficult to differentiate vaccinated from animals naturally infected, especially where repeated vaccinations are carried out (Jamal and Belsham, 2013; Namatovu *et al*., 2013). Antibodies against NSP do not appear until day eight to nine after infection, as such it is not of much use in the early acute phase of the disease (Lu *et al*., 2007). Antibodies to NSP persist for long periods and therefore positive animals may not necessarily still be infected, but could be carriers (Jamal and Belsham, 2013). Tests for detection of NSP antibodies cannot be used for detection of carrier

animals as some persistently infected animals do not show sero-conversion against NSP (Brocchi *et al*., 2006).

The Liquid phase blocking ELISA (LPBE) detects FMDV antibodies in serum and is used either as a single dilution screening assay or as a quantitative titration assay resulting in end point determination for each serum (Hamblin *et al*., 1986a; Hamblin *et al*., 1986b). The LPBE is a prescribed test for trade because it is appropriate for confirming previous or on-going infection in non- vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field if purified vaccines have been used (Hamblin *et al*., 1986b; Bronsvoort *et al*., 2008). The OIE recommends the LPBE to be optimized to local strains circulating in each country to improve the validity of the results (OIE, 2014a).

The virus neutralization test (VNT) can be used as confirmatory tests in the detection of FMDV-specific antibody in animals previously exposed to the virus (Brocchi *et al*., 2006; Paton *et al*., 2006). However, VNT is slow, subject to contamination and requires restrictive bio containment facilities, and technical skills. The dependence of VNT on cell culture facilities makes it prohibitive for most African countries (Jamal and Belsham, 2013; OIE, 2014a).

All these ELISAs show cross reactions because of repeated vaccination and infection with one or more FMDV serotypes (OIE, 2014a).

2.6.9.2 Virus isolation

Samples for virus isolation and characterisation of FMDV are routinely sent to regional reference laboratories because most countries lack biosecurity level 3 (BSL3) facilities recommended by the World Organization for Animal Health. Virus isolation uses cell cultures to demonstrate cytopathic effect (CPE) to

identity viral activity (OIE, 2014a). However, CPE is not specific to FMDV alone, is not sensitive enough when viral load is very low and is slow and laborious (Kasanga *et al*., 2014b).

The success of virus isolation is dependent on the sample quality and requires special transport conditions from the sampling point to the laboratory (Jamal and Belsham, 2013). This presents challenges for most developing countries in areas with high ambient temperatures, long distances from points of sample collection to national laboratory, poor laboratory infrastructure and trained manpower (Belsham *et al*., 2011). Collecting quality samples and having them transported to reference laboratories has proved to be a major setback resulting in number of outbreak FMDVs not being characterized (Namatovu *et al*., 2013). Transportation of samples containing infectious FMDV also represents a significant bio-security hazard and incurs significant costs (Sinkala *et al*., 2014b). Relying on foreign laboratories to manage disease control programmes may not be sustainable because the number of samples analysed is insufficient to get adequate detailed knowledge of the circulating FMDV strains to implement efficient control measures (Rweyemamu *et al*., 2008a). It is now possible to inactivate the virus from clinical field cases at the point of tissue sampling and the preserved RNA of the infectious virus can be recovered subsequently in a biosecurity laboratory environment (Belsham *et al*., 2011). Characterisation is mainly by antigen ELISA, rt RT-PCR and sequencing (OIE, 2014a).

2.6.9.3 Antigen Enzyme Linked Immunosorbent Assay

This is a standard indirect sandwich ELISA for the identification and typing FMDV antigens in tissue samples or cell lines (Hamblin *et al*., 1986a; Hamblin *et*

al., 1986b). It is also not very sensitive when viral load in the samples is very low and is only suitable for epithelial samples (Kasanga *et al*., 2014b).

2.6.9.4 Polymerase chain reaction

Polymerase chain reaction (PCR) involves virus RNA extraction from tissue culture materials followed by reverse transcription where the extracted RNA is converted to cDNA using reverse and forward reverse primers in a thermal cycler (Knowles and Samuel, 1998).

Currently the detection of viral RNA depends on the reverse transcription polymerase chain reaction (Reid *et al*., 2000). Several reverse transcriptase polymerase chain reaction (RT-PCR) and real-time RT-PCR (RT-qPCR) assays have been developed for the detection of FMDV using both universal and serotype specific primers on a range of field samples including epithelial and cell culture isolates (Reid *et al*., 2000; Callahan *et al*., 2002). RT-PCR assays are highly sensitive, rapid and do not require electrophoresis, although they are expensive and out of reach for most developing countries (Kasanga *et al*., 2014b).

2.6.9.5 Sequencing

The viruses are usually propagated on cell cultures before RNA extraction, cDNA synthesis and amplification of the VP1 gene using specific sequence primers that are available as sequencing kits (Knowles and Samuel, 1998; Vosloo *et al*., 2004). RT-PCR amplification of FMDV RNA followed by nucleotide sequencing is the current preferred method for generating sequences (OIE, 2014a). Molecular epidemiology of FMD is based on the comparison of the genetic differences between the VP1 viral protein of the sequenced FMDV and the viruses held in the database at WRL and TADP ARC-OVI (OIE, 2014a). Comparison of whole

genome sequences can provide further discrimination between closely related viruses and help to recreate the transmission pathways between locations within outbreaks (Cottam *et al*., 2008).

2.6.10 New field diagnostic tests

Developing countries of SSA faces many challenges in the diagnosis of FMD. Notable among these challenges is the lack of on the spot field diagnostic tools (Jamal and Belsham, 2013; Namatovu *et al*., 2013). The following tests have been developed to overcome some of these challenges:

2.6.10.1 Lateral flow devices

Lateral flow devices (LFD's) have been developed and evaluated which are either serotype specific (Ferris *et al*., 2009; Yang *et al*., 2013) or can detect all seven FMDV serotypes (Reid *et al*., 2001; Ferris *et al*., 2010). LFDs are immuno-chromatographic tests that allow the diagnosis of FMDV at the site of a suspected outbreak. The LFD can utilise vesicular fluid or vesicular epithelial suspensions but not nasal swabs or sera (Yang *et al*., 2013). The method makes use of capture and detection monoclonal antibodies or specific polyclonal antisera on a strip test. Studies done this far have shown the test to be as sensitive and specific as the antigen ELISA. However, the sensitivity of the strip test may differ for the various FMDV strains (Oem *et al*., 2009). The OIE has not yet received a validation dossier for these tests (OIE, 2014a), as such they have not yet been recommended for use in confirmation of a disease outbreak.

2.6.10.2 Portable real-time polymerase chain reaction platforms

Portable real-time PCR platforms offer many advantages in endemic countries like Zambia. An example of is the Enigma FL field laboratory platform (Enigma diagnostics) capable of nucleic acid extraction, PCR thermocycling and analysis of data without the requirement for user intervention. This test has been tested for FMD diagnosis (King *et al*., 2008). These platforms require to be evaluated as to whether they can detect new viruses as they continue to evolve in sub-Saharan Africa. These platforms can be utilized by non-specialists and are designed to perform all the steps of a RT-PCR test, such as nucleic acid extraction and performing RT-PCR which is valuable for evaluating carriers.

2.6.10.3 Loop-mediated isothermal amplification

The Loop-Mediated Isothermal Amplification (LAMP) assay is an alternative molecular detection technique with similar performance to rt RT-PCR that has been used widely for the detection of RNA and DNA viruses that infect livestock (Dukes *et al*., 2006). LAMP has the capacity to identify on the spot serotypes and carriers in the field. (Yamazaki *et al*., 2013) developed a multiplex RT-LAMP approach to accommodate the high sequence variability encountered in RNA virus genomes particularly for SAT strains and found the analytical sensitivity to be comparable to the singleplex RT-LAMP assays (Dukes *et al*., 2006).

2.6.10.4 Novel diagnostic assays

Additional novel diagnostic assays such as biosensors (Sanchez-Aparicio *et al*., 2009), microarrays (Baxi *et al*., 2006), Gold Nanoparticle (GNP) improved Immuno-PCR (GNP-IPCR**)** (Ding *et al*., 2011), and nucleic acid sequence based amplification (NASBA) (Collins *et al*., 2002) have shown promising ability for

rapid and reliable diagnosis, surveillance screening and strain typing for FMDV in sub-Saharan Africa. Some of the limitations of these assays is that they are yet to be optimised for the FMDV SAT serotypes which have a high degree of sequence variability (OIE, 2014a). The costs involved per test will also determine how widely these assays will be used especially in developing countries. The field-based novel assays will require training of personnel and some laboratory-based tests requires specialised equipment which is not readily available, together with personnel capable of correctly interpreting and analysing the datasets produced.

2.6.11 Socio-economic impact of foot and mouth disease

Foot and mouth disease causes wide spread vesicles that rupture resulting into severe lesions in the mouth, feet, teats, rumen, nares and muzzle. This leads to anorexia, salivation (drooling), lameness, drop in milk production, loss of body condition and abortions which makes FMD such a feared disease by the developed intensive livestock industries (Mahy, 2004). As a result, trade of live animals and animal products from infected to disease free countries is banned. Therefore, the impact of FMD is twofold: impact on the developed economies when disease strikes and effect on the economies where disease remain endemic.

The disease causes huge economic losses when it occurs in developed livestock industries. This is because of the stamping out policy aimed at avoiding development of carrier status in cattle that may jeopardize the regaining of disease free status (Rodriguez and Grubman, 2009). For example, the 2001 UK outbreak was estimated to have costed that country about US$ 13.8 billion (Grubman and Baxt, 2004). The USA projects an economic impact of over US$ 27 billion if FMD strikes that country (Boisvert *et al*., 2012). The recent outbreaks in Japan

(2000, 2010) and Korea (2010, 2011) were estimated to have costed both countries about US$ two billion (Knight-Jones and Rushton, 2013).

South Africa lost over US$ 500 million annually from export bans to the EU following the recent withdraw of the disease free zone status (Thomson *et al*., 2013), while Botswana Meat Corporation recorded a deficit of US$ 51 million from 2009 to 2011 (Thomson *et al*., 2013). The global impact of FMD for year 2011 based on production and vaccination costs was estimated to be US$ five billion (Knight-Jones and Rushton, 2013).

Foot and mouth disease does not only restrict trade in animal and animal products in SSA but also affects productivity and income generation for 70 percent of Africa's rural economies whose backbone is livestock farming (Scoones *et al*., 2010b; Thomson *et al*., 2013). The disease affects production at farm level through production losses from abortions, prolonged inter-calving intervals, failure to drop calves when outbreak occurs in the cropping season, loss of draught power and effect on crop hectarage, and food availability for consumption like milk (James and Rushton, 2002; Perry and Rich, 2007; Jibat *et al*., 2013). It is difficult to estimate the production losses caused during an outbreak, but probably the most serious effect of the disease in indigenous cattle, in which the disease is often rather mild, is due to lameness and thus reduced draught power efficiency (Perry and Rich, 2007). At national level the effect on economic growth and overall poverty has been described in many studies (James and Rushton, 2002; Perry and Rich, 2007; Scoones *et al*., 2010; Jibat *et al*., 2013; Knight-Jones and Rushton, 2013; Thomson *et al*., 2013). In particular, FMD outbreaks affect vulnerable individuals such as women and children since approximately 75 percent of livestock in SSA is raised under the communal smallholder systems

that sustain livelihoods of these groups (Scoones *et al*., 2010; Ferguson *et al*., 2013; Miguel *et al*., 2013a). In Zambia, the losses of income from potential exports of beef is estimated to be over US$ 1.6 billion per annum (WorldBank, 2011). Losses in income from exports of sable antelopes were estimated at US$ 35 million and annual expenditure of US$ three million on preventive measures (Sinkala *et al*., 2014b). From this, it can be seen that endemic FMD constrain investment in agriculture subsequently retarding economic growth (Thomson *et al*., 2013).

2.6.12 Prevention and control

In countries where FMD has been or partially controlled in Africa (South Africa, Botswana and Namibia), control has relied on physical separation of wildlife and livestock, repeated vaccination of cattle herds exposed to wildlife, control of animals movement and careful assessment of risk of FMD introduction in non-infected areas (Thomson *et al*., 2003; Namatovu *et al*., 2013). In the rest of Africa, it is now recognized that animal movement control is difficult under the present production (pastoralist and agro pastoral) and livestock marketing systems (cross border trade) and vaccination remains the main method of control (Di Nardo *et al*., 2011; Tekleghiorghis *et al*., 2014).

2.6.12.1 Vaccination

Vaccination has been used to eliminate FMD from Europe from 1952 until 1991 when it was abandoned, at which time an attenuated vaccine was in use (Brown, 2003). In South America (Uruguay and Argentina) oil adjuvant vaccines were used from 1960s to 2002 to eliminate FMD (Saraiva, 2002). In Africa, vaccination has mainly involved use of the inactivated adjuvant precipitated, non-purified

vaccines. These non-purified vaccines contain NSP and prevent differentiation of infected from vaccinated animals. Further, inactivated vaccine strains have had minimal or no molecular change other than adaptation for growth in the production. Addressing the shortcomings of inactivated vaccines require research that need investment in bio-containment facility, cell culture and human resource (Rodriguez and Grubman, 2009).

2.6.12.2 Inactivated vaccines in use

Vaccines are produced from viruses grown on baby hamster kidney (BHK) cell lines in serum free medium or poly-ethylene glycol (PEG) treated serum using the Frenkel method adapted by Rhone Merieux in Lyons, France (Barteling, 2002; Brown, 2003). Initially they were inactivated with formaldehyde, but from 1985 binary ethylene–imine (BEI) has been in use (Barteling, 2002). The inactivated virus is concentrated on alumina hydroxide, and saponin is added as an adjuvant (Hunter, 1998). The monolayer dose (against one type) is one ml. Inactivation safety tests are performed on inactivated virus concentrated by ultrafiltration, inoculated into monolayer cells (roller bottle) and sub-cultured three times to ensure that inactivation is complete (Barteling, 2002; El-Sayed *et al*., 2012).

2.6.12.3 Purified vaccine

To overcome the problem of presence of cellular protein contaminants and viral NSP in inactivated vaccines, an industrial ultra-filtration and chromatography purification method was developed to allow for differentiation of infected from vaccinated animals (Rodriguez and Grubman, 2009). The purification method was only recently introduced at Botswana Vaccine Institute (BVI), the leading FMD vaccine supplier in southern Africa. But, the cost of these purified vaccines

remains prohibitive to many governments of southern Africa and the conventional alhydrogel saponin precipitated FMD inactivated vaccine has continued to be used (Hunter, 1998; Rodriguez and Grubman, 2009). The continued use of non-purified vaccines apart from precipitating carriers makes it problematic to distinguish between vaccinated and convalescent animals. This impacts negatively on the ability to export livestock and livestock products from FMD controlled regions.

2.6.12.4 Oil based adjuvant

To overcome the problem of short duration of immunity conferred by vaccines with saponin adjuvants, that require repeated vaccinations, some regions like South America have moved to high potency vaccines formulated in oil adjuvants. This is accompanied by continuous selection of new viral strains (Hunter, 1998; Rodriguez and Grubman, 2009).

2.6.13 Multiple serotypes

The antigenic and genetic variability of FMDV and continuous emergence of new mutants that escape the host immune responses as a complicating factor in the selection of vaccine candidates has been described (Haydon *et al*., 2001; Maree *et al*., 2011). Further, SAT 1 and SAT 2 viruses display greater antigenic variation compared to the Eurasian serotypes A, O, C and Asia 1 (Hunter, 1998; Maree *et al*., 2011), although serotype C seem to have disappeared having been last reported in 2004 in Kenya and 2005 in Ethiopia (Di Nardo *et al*., 2011; Tekleghiorghis *et al*., 2014). The development of this antigenic variation is a result of changes within the three major surface capsid proteins (VP1, VP2 and VP3) of the virus (Mateu *et al*., 1995b). The variation is not random but tends to be concentrated at the surface exposed barrel connecting loop and therefore

knowledge of the amino acid residues that comprise the antigenic determinants is important (Dunn *et al*., 1998). FMDV serotypes share an approximately 86 percent amino acid identity to each other (Yang *et al*., 2007). However, some of the capsid proteins exhibit some variation, notably in one non-structural peptide VP1 which varies by 30 to 50 percent between serotypes (Knowles and Samuel, 2003). This variation has impeded the development of vaccines that could provide cross protection both inter and intra-serotypically (Brehm *et al*., 2008).

2.6.14 Vaccination regimes

2.6.14.1 Vaccine selection

Two limitations for vaccine selections have been described (Hunter, 1998). Firstly, some field strains do not have appropriate characteristics for vaccine production and the combination of the baseline vaccine strain (broad spectrum) with a strain which represents less related strains of the same serotype do not always work. Secondly, there is no recognized subtyping system for SAT strains and the r-value is unreliable in predicting intratypic cross protection (Hunter, 1998).

2.6.14.2 Vaccine efficacy considerations

Vaccine efficacy or matching studies are rarely carried out as explained under FMD diagnosis. Currently, in vitro methods to measure cross-reaction between sera elicited by a vaccine and a particular field/outbreak isolate are being explored using neutralization titers to calculate r1-values to determine antigenic relationships (Rweyemamu *et al*., 1978; OIE, 2014a). Using in vitro approach, it has recently been shown that the SAT1 vaccine strains SAR/9/81 and

KNP/196/91 are antigenically relevant for South Africa, Zimbabwe, Mozambique, Botswana, Namibia, Zambia and Tanzania. However, there is lack of knowledge about the antibody response of animals vaccinated with a multivalent vaccine, containing a combination of intra-serotype strains, to cross-react against field viruses (Maree *et al*., 2011).

2.6.14.3 Vaccine matching

Determination of antigenic relationship requires the use of cross neutralization assays of the culture cells and microtitre plates against cattle sera prepared by two consecutive vaccinations (vaccinated on day zero, boosted on day 28 and bled on day 38) with alhydrogel containing vaccine with either one strain per serotype or a combination of both vaccine strain per serotype (Vosloo *et al*., 2004). The r values of the sera against heterologous virus are compared with sera against the homologous virus (Mattion *et al*., 2004; Cox *et al*., 2005). However, routine heterologous challenge studies are rarely performed. They are time consuming, require access to expensive bio-containment facilities and raises serious animal welfare issues when large numbers of animals need to be vaccinated and challenged. If small groups of animals are used, these challenge studies can suffer from low validity (Goris *et al*., 2007). Dendrograms showing the genomic relationship between vaccine and field strains for all seven serotypes based on sequences derived from VP1 gene have been published (OIE, 2014a).

2.6.14.4 Foot and mouth disease improved vaccines

Novel vaccines have since been developed that contain only the portion of the viral genome coding for the viral structural proteins and 3C, the NSP required for virus capsid formation (Mayr *et al*., 1999). Thus animals inoculated with this

immunogens can unequivocally be distinguished from infected or convalescent animals using currently approved diagnostic assays. Most novel vaccines are utilizing reverse genetic approaches and include the chimeric vaccines, recombinant and peptide vaccines, empty capsid or vector vaccines, and DNA vaccines (Rodriguez and Grubman, 2009; Rodriguez and Gay, 2011; Tang *et al*., 2012). These new alternative vaccines do not require infectious virus as well as efforts to understand the role of innate immunity and cytokines to induce protection and boost the immune response offering tremendous potential for the control of FMD in endemic regions, and should target eco-system based synchronization as an FMD control strategy (Rweyemamu and Garland, 2006).

2.6.15 Foot and mouth disease in Zambia

2.6.15.1 Transmission and infection

The origins of FMD in Zambia are not known. The disease was reported in 1933 in Mwandi District of Western Province (formerly part of Sesheke District) (Morris, 1934). At that time the disease was suspected to have been spread by new white settlers moving from South Africa into the hinterland, having been reported earlier in Zimbabwe in 1931 and Botswana in 1932 (Thomson and Bastos, 1994). It was also documented that the locals were not perturbed by the disease indicating that either the disease had existed previously or the less severity in local herds may have caused less worry (Perry and Hedger, 1984). The rinderpest pandemic of 1896 that was suspected to have eliminated FMD from southern Africa did not directly affect Zambia, and the Country was later granted historical freedom from rinderpest in the 1980s by the World Organization for Animal Health (OIE, 2013b). Since then repeated outbreaks have occurred and three high

risk areas of Kafue Flats, lower Zambezi basin and Mbala-Isoka area have been described (Zyambo, 1975; Overby and Zyambo, 1983; Perry and Hedger, 1984; Chilonda *et al*., 1999). However, the risk factors associated with occurrence of outbreaks of the disease and patterns have not been elucidated.

2.6.15.2 Epidemiology of foot and mouth disease in Zambia

The two epidemiological forms of FMD that currently exist in Zambia have been described (Sinkala *et al*., 2014a) (section 2.7.3). The southern Africa form involving SAT 1, SAT 2, and SAT 3 has been observed in Kafue Flats and lower Zambezi basin except that SAT 3 has only been isolated from wild buffalo and once serologically diagnosed in cattle (Chilonda *et al*., 1999). The wetlands of Kafue Flats and Zambezi basins have been observed to contribute to animal disease outbreaks because of sharing of grazing areas between wildlife mainly wild buffalo and cattle (Muma *et al*., 2006; Munang'andu *et al*., 2006).

The Eurasian form involving serotype O and A with incursions of SAT 1 and SAT 2 persist in northern Zambia and is suspected to be maintained and spread by cattle (Kasanga *et al*., 2014a). Furthermore, serotype O and A have so far not been isolated from buffalo (Rweyemamu *et al*., 2008b). The diversity in the circulating FMD virus strains in these two forms as a challenge to the selection of sufficiently cross-protective FMD vaccines because each serotype require the incorporation of more than one strain into a single vaccine has been described (Sinkala *et al*., 2014b).

2.6.15.2.1 Foot and mouth disease in cattle

Most of the cases of FMD in Zambia have been reported in cattle. The disease has mostly been reported in high cattle density areas of Kafue flats (Namwala,

Itezhitezhi, Mumbwa, Monze and Mazabuka), lower Zambezi basin (Livingstone, Kazungula and Sesheke), and Mbala-Isoka area (Overby and Zyambo, 1983; Perry and Hedger, 1984; Sinkala *et al*., 2014a). No breed or sex preference has been observed in these outbreaks, with 70 percent of the cases occurring in winter on the Kafue Flats, while in the lower Zambezi, outbreaks have mostly been reported in wet season (Chilonda *et al*., 1999).

2.6.15.2.2 Foot and mouth disease in buffalo

Wild buffalo are considered by most authorities to be the most important long term reservoir of the SAT type of FMDV and it is likely that some outbreaks of FMD in cattle occur as a result of close contact between the two species at watering points, for instance. The origin of FMD in buffalo is not well known but is suspected to have originated from within Africa (Knowles, 2013). The SAT virus progenitor is suspected to have evolved into type A, O, C and Asia 1 lineage first within the cattle population in eastern Europe or west Asia where cloven hoofed animals were thought to have first been domesticated (Vosloo and Thomson, 2004; Tully and Fares, 2008; Knowles, 2013). The SAT type viruses may have evolved in Africa in association with buffalo. Unfortunately, this theory may never be verified because, the central role of buffalo in FMD epidemiology was only identified in the 1960s during the construction of the Kariba Dam (Vosloo and Thomson, 2004; Anonymous, 2013b). Vaccination of cattle with FMDV that are antigenically closely related to those carried by buffalo form an integral part of FMD control in Southern Africa (Bastos *et al*., 2003). However, research to identify FMDV strains circulating in buffalo in Zambia has been limited and much of the work focused on Kafue basin because of the perceived risk from buffalo given the frequency of outbreaks overtime (Overby and

Zyambo, 1983; Munang'andu *et al*., 2006). The last sampling of buffalo was in the Kafue and Lochnivar National Park in 1993 and 1996 (Munang'andu *et al*., 2006). This sampling initiative to sequence circulating FMDV in buffalo in these areas was done with assistance from Food and Agriculture Organization (FAO) and the Onderstepoort Veterinary Institute (OVI) (Munang'andu *et al*., 2006). Since then, no published work is available on circulating viruses in buffalo.

2.6.15.3 Diagnosis

2.6.15.3.1 Clinical diagnosis

The disease caused by different serotypes is clinically indistinguishable, although they vary somewhat in their epidemiological patterns. The susceptibility to develop infection and clinical disease varies between different species and breeds of animals depending on the level of immunity, the strain and dose of the virus causing infection (Schley *et al*., 2009). In Zambia, the presenting signs of FMD in cattle include lameness, anorexia and drooling of saliva from the mouth. On clinical examination, typical painful vesicles containing colourless or turbid fluid characteristic of FMD have been observed on the tongue. In cattle and pigs, fever and viraemia usually start within 24 to 48 hours after epithelium infection leading to virus spread into other organs and tissues and the production of secondary vesicles preferentially in the mouth, feet, buccal mucosa and mammary glands (OIE, 2010a). The disease may be confused with other vesicular diseases like vesicular stomatitis, vesicular exanthema and swine vesicular disease but these have not been reported in Zambia (OIE, 2013a). Diseases like foot rot are usually eliminated through clinical examinations. The clinical signs may be difficult to observe sometimes because they only last for a few days. Further, the disease is

generally less severe in local breeds that have been previously vaccinated or exposed referred to as the "occult form". Furthermore, cattle are not closely observed in the traditional sector except for dairy animals and convalescence is not always present in recovered animals (Falconer. J., 1972; Dawe *et al*., 1994a). Symptoms of FMD in sheep and goats are usually silent where it goes unnoticed (Hyera *et al*., 2006). Symptoms in pigs are not adequately documented because a large part of the pig population is in Eastern Province where FMD has only once been reported in 2001 in Lundazi District (Sinkala *et al*., 2014a). FMD in wildlife is not documented due to lack of surveillance.

2.6.15.3.2 Laboratory diagnsosis

In Zambia, diagnosis of FMD is often based on clinical suspicion followed by serology and virus isolation. Serology is done locally at Central Veterinary Research Institute (CVRI) while virus isolation is done at regional reference laboratories of Botswana Vaccine Institute (BVI) in Botswana, Transboundary Animal Diseases Programme of Agriculture Research Council Onderstepoort Veterinary Institute (TADP ARC-OVI) in Pretoria, South Africa and The Pirbright Institute (TPI) the world reference laboratory in United Kingdom. For serology, non-structural protein (NSP) and LPBE ELISAs described earlier (section 2.7.9.1) have been used. The 3ABC ELISA kits that have been used include; Cedi test (Ceditest FMDV-NS kit; Prionics Lelystad B.V,) at cut-off of 50 percent; CHEKIT kit (Bommeli Diagnostics CHEKIT FMD-3ABC bo-ov) cut-off taken at 30 percent. Brocchi *et al* (2006) estimated the sensitivity and specificity of 3ABC ELISA to be 90 and 99 percent, respectively, while Bronsvoort *et al* (2008) estimated sensitivity to be 87.7 percent and specificity to be 87.3 percent. The sensitivity and specificity of NSP tests depend on the

immunity of the animals and are less sensitive when animals have been vaccinated prior to becoming infected (Brocchi *et al*., 2006).

The LPBE is serotype specific, highly sensitive, detects antibodies elicited by both vaccination and infection (OIE, 2014a). The LPBE is a prescribed test for trade because it is appropriate for confirming previous or on-going infection in non- vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field if purified vaccines have been used (Hamblin *et al*., 1986b; Bronsvoort *et al*., 2008; OIE, 2014a). The LPBE has a sensitivity of 100 percent and specificity of 95 percent (OIE, 2014a).

Non-structural protein (NSP) testing cannot differentiate between a recovered and a carrier animal, which requires use of virological tests (Jamal and Belsham, 2013). Zambia lacks a biosecurity level three (BSL3) laboratory facility required for virus isolation and characterization and therefore, tissue samples from infected animals in phosphate buffer saline (PBS) are routinely sent to reference laboratories of BVI, ARC-OVI and TPI. However, this involves transportation of samples containing infectious FMDV which represents a significant bio-security risk and is also costly. Further, Zambia is a big country with inadequate transport infrastructure and trained manpower to collect quality virus samples from remote long distant places to the main laboratory. This may contribute to some outbreak causing viruses not being characterized. Virus isolation takes time to confirm the disease and usually by the time vaccines are made available, the FMDV infection would have spread in wider and far areas, increasing the cost and time required for control. Therefore, more rapid on the spot field diagnostic kits are required to mitigate some of these challenges.

2.6.15.4 Intervention

2.6.15.4.1 Vaccination

Methods of control and prevention used in Zambia from 1933 to 1980s have been summarized (Perry and Hedger, 1984) and the challenges of control have recently been reviewed (Sinkala *et al*., 2014b). The current method used for control and prevention in Zambia is bi-annual vaccination of cattle herds in high risk areas using multivalent alhydrogel saponin precipitated FMDV vaccines. A bivalent vaccine is used in the Kafue Flats and lower Zambezi basin while a quadrivalent vaccine is used in northern Zambia.

The shortcomings of this vaccine have been described earlier (section 2.7.11.1), including the contribution to the carrier state. This presents a dilemma for livestock movement because the NSP and LPBE being used for screening prior to movement may not identify carriers and the rt RT-PCR recommended to detect carriers is not available locally.

The control of FMD in Zambia is hindered by lack of tools to identify carriers and prevent the movement of cattle from infected areas to clean areas (Rodriguez and Grubman, 2009; Jamal and Belsham, 2013). Even though carrier cattle may not transmit infection to naïve animals, the long persistence and virus replication in the host may lead to new viral variants (Gebauer *et al*., 1988; Saiz *et al*., 1996; Toja *et al*., 1999). The carrier animals have been shown to be fully infectious and the theoretical risk of spreading the disease is ever present (Alexandersen *et al*., 2003). The cellular protein contaminants and NSP may also be present, especially if multiple vaccination have been carried out, and with possibility of mutation the likelihood of spreading the disease exist although remote (Rodriguez and Grubman, 2009). Therefore, knowledge is required on the risks posed by carrier

animals in the high risk areas of Zambia to assist the PCP-FMD process. As such the diagnostic capacity that is fit for purpose like the LAMP described previously are required.

Current vaccine cocktail may offer protective immunity against most field strains, although breakthroughs have been observed in vaccinated herds (Dawe *et al*., 1994a). It is therefore necessary to monitor field strains of FMD virus in wild buffalo and recovered cattle on a regular basis. There is a growing concern about effectiveness of the current vaccines. The recent Global FMD Research Alliance congress (GFRA, 2013) highlighted the urgent need for new SAT vaccine strains with good immunogenicity for use in Africa. History has shown us that vaccination alone is not enough to control FMD (Brown, 1992).

2.6.15.4.2 Movement control

Wherever FMD has been controlled, animal movement restrictions have played a significant role (Brown, 2003; Thomson and Vosloo, 2004). Movement control remains a huge challenge in Zambia because of the agro-pastoral production system that involves transhumance and communal grazing (Muma *et al*., 2006; Muuka *et al*., 2012), and cross border livestock trade (Banda *et al*., 2014; Sinkala *et al*., 2014b). Marketing of livestock cannot be separated from FMD control in Africa and therefore research on market structures in various regions may provide sustainable solutions to control of FMD (Knight-Jones and Rushton, 2013; OIE, 2014c).

2.6.16 Knowledge gaps of foot and mouth disease in Zambia

Despite the knowledge of presence of FMD in Zambia for close to a century, gaps in epidemiological understanding of the disease still exist that may be affecting the effective control of the disease. In summary, the gaps include:

- The inadequate understanding of viral and antigenic diversity of virus pools 4 and 6 that intersect in northern Zambia to effectively manage FMD by vaccination in this area;
- The lack of understanding of the genetic and antigenic variants within the SAT serotypes and the different degrees of virulence that exist as well as regional differences in the distribution and prevalence of serotypes required to effectively control the disease;
- Lack of understanding of how FMD is transmitted at livestock wildlife interface areas of southern Africa;
- The level of subclinical infection in both cattle and wild buffalo at the interface and the rest of the country is unknown;
- The mechanism employed by the virus to persist and evade the immune elimination from the host is unknown;
- Inadequate knowledge of how much of the infection in carriers results in effective disease transmission to susceptible cattle;
- Lack of information on optimization of the 3ABC ELISA and LPBE serological tests to circulating local strains;
- Lack of information on the magnitude and duration of carrier status in cattle in the respective high risk areas to guide movement control;

- Lack of information on the diversity of FMD topotypes (strains) circulating in cattle and wildlife in Zambia that may be included in vaccines to prevent outbreaks;
- Lack of knowledge of the marketing of livestock in Zambia and how this influences the FMD epidemiology;
- Lack of risk assessments along the livestock value chains to identify critical points of interventions to assist the PCP-FMD process.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This study was performed in three parts as follows: a retrospective study, cross sectional study and FMD transmission modeling**.**

3.1 Retrospective study

3.1.1. Study area

This study was carried out in areas of Zambia that are known to be endemic for FMD which included the Mbala-Isoka area on the border with Tanzania; the game management areas in the Kafue Flats; and lower Zambezi basin and Luangwa ecosystem.

3.1.1.1 Mbala-Isoka area

The Mbala-Isoka area is a stretch of about 300 km from longitude 31^{o} to 33^{o} E and latitude 08^{o} to 11^{o} S along the Zambia northern region bordering Tanzania. The area borders the southern highland regions of Rukwa and Mbeya of Tanzania and together make up a productive agricultural belt with similar annual rainfall of >1000mm (Nyemba and Dakora, 2010). The tribes of this area are pastoralists practicing mixed farming and often use oxen as the main source of draught power. Therefore, cattle movement is mainly across the border from north to south because of the high cattle densities in Tanzania compared to Zambia. For instance, Mbeya and Rukwa regions of Tanzania have over two million cattle compared with 60, 000 in northern Zambia (Anonymous, 1997, 2008, 2011a). Livestock

markets called *Munada* exist along the Tanzania/Zambia border (Banda *et al*., 2014). This has caused an influx of the Masai and Sukuma pastoralists from northern Tanzania resulting in spread of trans-boundary animal diseases like FMD and CBPP (Wilson, 2003). Previous attempts to stop this movement through control regulations have not been successful (Anonymous, 1968).

3.1.1.2 Kafue Flats

The Kafue Flats has previously been described (Muma *et al*., 2007; Munyeme *et al*., 2009; Haller, 2012; Chabwela and Wanga, 2013). Briefly, the Kafue Flats is an extensive seasonal flood plain of about 255 kilometres long and 60 kilometres wide covering more than 6,500 km². The wetland is a mosaic of human activities including hydropower generation, livestock grazing, wildlife conservation, fish production, irrigation for sugar plantation and ecological life for wild birds and other species.

The Kafue Flats are divided into three main terrains comprising the flood plains (savannahs and grasslands) near the river front followed by intermediate termitaria and the Mopani woodlands in the upper most part (Haller, 2012). Livestock grazing follows a similar pattern with three herd management systems described earlier (section 2.4.1**)** as flood plain herds, transhumant herds and villages herds. As such, exchange of disease is common especially during winter transhumance when over 600,000 cattle converge on the floodplain (Chilonda *et al*., 1999). The Kafue Flats is a GMA host to several wildlife species including over 5,000 wild buffalo (*Syncerus caffer),* Impala (*Aepyceros melampus*), Kafue lechwe (*Kobus leche kafuensis*), Kudu (*Tragelaphus strepsiceros*), Warthog *(Phacochoerus aethiopicus)* and Bush pig *(Potamochoerus porcus)* (Table 1). The

transhumant and floodplain herds share grazing pastures with wildlife and disease transmission between wildlife and livestock is common (Chilonda *et al*., 1999; Muma *et al*., 2006; Siembieda *et al*., 2011). Fluctuations in the flooding patterns of the Flats have adversely affected pasture availability and sometimes increased contacts between cattle and wildlife (Sheppe, 1985; Mumba and Thompson, 2005; Chansa and Kampamba, 2009; Muma *et al*., 2010; Haller, 2012).

3.1.1.3 Lower Zambezi basin

The lower Zambezi basin has previously been described (Chilonda *et al*., 1999). Briefly, it's the southern border area from Livingstone westwards to a point beyond Sesheke where the Zambian border with Angola turns to the northwest (Chilonda *et al*., 1999). This area is both a wildlife corridor and trade route for livestock from Caprivi Strip of Namibia into Zambia and *vice-versa* (Perry and Hedger, 1984). The area between the rivers of Zambezi and Chobe forms the Zambezi floodplain, a low basin subject to constant flooding in the rainy season. This floodplain extends into Zambia forming the Simalaha plain from Kazungula (junction of Namibia, Zambia, Zimbabwe and Botswana) to Sesheke where similar grazing patterns to that of Kafue Flats described above exist (Muma *et al*., 2009). This area of the Zambezi River also has islands that are an attraction for winter grazing to both livestock and wildlife from Namibia and Zambia. The basin is part of Kavango-Zambezi trans-frontier conservation area described previously which was created to promote free movement of wildlife (mainly buffalo and elephant) between national parks (section 2.5.3).

The lower Zambezi basin is characterized by sandy soils with typical savannahs and grasslands which are flooded and interspaced with Mopani woodlands with

rainfall averaging 600 mm per annum (Nyemba and Dakora, 2010). During flooding times, cattle graze on the upland but converge on the plains of the Zambezi River in the dry months (April to November). During times of excessive flooding, the Mambova fault together with the Impalila Island located on the eastern side of the Zambezi floodplain become the main access point to the upland for both cattle and wildlife (NASA, 2010; Hogan, 2012).

3.1.1.4 Luangwa Valley ecosystem

The Luangwa Valley ecosystem has also previously been described (Timberlake, 2000; Munang'andu *et al*., 2012). Briefly, the Luangwa Valley ecosystem is made of the valley which stretches for a distance of 700 km with an average width of 200 km. The valley covers a total area of 63,000km^2 being the southern end of the Great Rift Valley and the montane biome with annual average rainfall of 800mm. It is from the montane biome that the Luangwa and Chambeshi rivers originate. The valley is bordered by the Muchinga Escarpment to the east, Mafinga Mountains to the north and the Nyika Plateau to the east. The banks of the Luangwa River are made of thick Miombo forests while the adjacent slopes are composed of Mopane woodlands. The valley floor comprises four NPs and six GMAs namely north Luangwa, south Luangwa, Luambe and Lukusuzi. The area has the highest concentration of wild buffalo in Zambia (Table 2). The presence of wildlife in Miombo woodlands renders the area an ideal ecological niche for trypanosomiasis transmission.

The main activities within the valley are wildlife tourism, agro-pastoral agriculture and fishing. However, because of the presence of tsetse flies, most agro-pastoral agriculture activities and human settlements are on the plateau areas.

Cattle are grazed communally near the villages with no transhumance practice. The confluence of the Luangwa and Zambezi rivers forms a floodplain which is part of the Lower Zambezi National Park in Zambia and Manna Pools in Zimbabwe.

3.1.2 Study design

Information on FMD outbreaks was gathered from databases at Ministry of Agriculture and Livestock which included CVRI and National Livestock Epidemiology and Information Unit for the period 1980 to 2012. For the period 1933 to 1979, Ministry of Agriculture and Livestock archived files were used that included outbreaks investigation reports, disease status reports, annual reports, and case and laboratory files. A few published journal articles were also used as data sources (Morris, 1934; Zyambo, 1975; Akafekwa, 1980; Overby and Zyambo, 1983; Perry and Hedger, 1984; Chilonda *et al*., 1999; Vosloo *et al*., 2002a). The dates of outbreak reporting were cross checked with district reports and reference laboratory records to provide a close estimate. Animal disease outbreaks are reported at veterinary camp level, but to allow for spatial analysis wards were used because currently, veterinary camps have not been digitized into polygons. Therefore, outbreak data was segregated into spatial resolution of wards being the smallest administrative unit (n=1287), into those that were affected (n=168) and not affected (n=1119). Zambia is administratively divided into ten provinces, which are divided into 106 districts. The districts are further divided into 152 constituencies that are further subdivided into 1287 wards.

Case data was segregated into the first reports of the disease referred to as primary and subsequent reports of the same disease and serotype as secondary based on

the reporting temporal sequence. During each outbreak, an index case was considered as primary and all subsequent cases of the same serotype and in the same locality as secondary. In compiling case data, national records were cross checked with reference laboratory databases. Visits were also made to the respective districts to verify the records from the case files. The case data were further segregated by serotype and topotype whenever molecular data was available. The outcome of interest was a ward reporting at least one primary case of FMD during the period.

The risk factors under consideration were husbandry system, trade and environmental factors relevant to cattle farming, agro ecological zone, cattle density, small ruminant density, presence of trade routes, rainfall, proximity to national parks, buffalo density, proximity to wetland, proximity to buffalo migratory route, livestock movement type, proximity to international border and FMD vaccination history. Information on cattle and small ruminants density were extracted from FAO database on predicted livestock density of the world (Robinson *et al*., 2007). Maps locating wildlife protected areas in Zambia and the bordering countries were obtained from the World Database on Protected Areas (http://www.wdpa.org/) and http://protectedplanet.net/. The ward level risk factors of proximity to national park, buffalo migratory routes, international border were calculated using ArcGIS. Other factors like presence of trade routes and cross border cattle trade were obtained from the districts. The case data was segregated by location (agro ecological zone) to elucidate the risk factors association with FMD occurrence. The historical annual rainfall for the years 1935 (oldest record) to 2012 were collected from the Zambia Meteorological Department. The rainfall data was categorized into normal, sub normal (drought) and above normal (floods)

based on region. All the data was consolidated into a database to show ward FMD history for the period. All geographical data were projected to UTM zone 34, 35 and 36S coordinate system (datum WGS84 and represented using ArcGIS v 10.0 (Environmental Systems Research Institute, Redlands, California, USA).

3.1.3 Data analysis

3.1.3.1 Descriptive

The month, year and geographical locations of the districts and wards that reported FMD outbreaks between 1933 and 2012 were entered into a Microsoft Excel® spreadsheet. The data was aggregated at ward level, sorted and checked for consistency and duplication. The cases with missing values were included in the database using a unique code for all missing values.

The data analysis focused on spatial and temporal description of primary outbreaks at ward level resolution. It was assumed that without primary cases, secondary ones would not occur. The case data was segregated and frequencies and summary statistics were calculated using SPSS 16.0 (IBM, USA).

The case data was analysed using spatial scan statistic for the presence of spatial clusters (Kulldorff and Nagarwalla, 1995) using the SaTScan version 9.1 software (http://www.satscan.org). A prospective space time permutation scan statistic that does not require population at risk (PAR) data was used for the analysis (Kulldorff *et al.*, 2005). This made it convenient to analyse outbreak cases in Zambia where determination of population at risk is problematic. Temporal, spatial and space time scan statistics are now commonly used for disease cluster detection and evaluation for a variety of diseases including avian influenza, rift valley fever and FMD among others (Perez *et al.*, 2005; Pfeiffer *et al.*, 2007;

Pfeiffer *et al*., 2008; Sindato *et al*., 2014). PAR or census population numbers may be less relevant for surveillance data for diseases like FMD in endemic settings where the catchment area may be undefined (Kulldorff *et al*., 2005). The analysis was conducted using individual case coordinates using the space-time permutation model based on 9999 Monte-Carlo simulation (Kulldorff and Nagarwalla, 1995; Rivas *et al*., 2006). The outbreak data prior to being analysed was segregated into twenty year successive periods (1933-1952, 1953-1972, 1973-1992, 1993-2012) based on cattle census data. During these periods the cattle population remained fairly stable to account for the population shift bias (table 1) (WorldBank, 2011; Kulldorff, 2014). This resulted in four separate data sets that allowed for the detection and comparison of clusters across populations in different time periods (Pfeiffer *et al*., 2007). Relative risk (RR) will be estimated to compare the risk of FMD within the cluster and surrounding areas. The greater the RR from one the greater the risk within the cluster in comparison to surrounding areas. A RR closer to one would be indicative of similar risks within and outside the cluster (Kulldorff *et al*., 2005). The significant clusters were then mapped and represented using ArcGIS version 10.1 (Environmental Systems Research Institute, Redlands, California, USA).

The association between potential risk factors and FMD outbreak occurrence at ward level were investigated using multivariable logistic regression. The outcome variable of interest was a report of at least one primary outbreak within each ward between 1933 and 2012. The risk factors potentially associated with the occurrence of FMD were based on information published in the scientific literature (Zyambo, 1975; Overby and Zyambo, 1983; Perry and Hedger, 1984; Bastos *et al*., 2003; Rivas *et al*., 2006; Hamoonga *et al*., 2014).

The analysis was conducted first by screening all potential risk factors for statistical significance at p-value of <0.25 in a univariable logistic regression analysis. Variables that were not statistically significant during the univariable analysis but likely to biologically contribute to FMD outbreaks were included in the multivariable analysis. Initially, all continuous- and ordinal-scale explanatory variables had to be grouped into categorical variables to test for trends. Secondly, the significant risk factors were included in a multivariable logistic regression based on a forward variable selection approach using the likelihood ratio statistic and p-value ≤0.05. The variables included in the model were those that did not show significant collinearity. This enabled exclusion of confounding effects among the factors. During model building statistically significant variables were adjusted for the other explanatory variables present in the model in relation to primary and secondary outbreaks. The Hosmer and Lemeshow test was used to assess the goodness of fit of the final model. The discrimination ability of the final model was assessed using the area under the curve (AUC).

The univariable and multivariable analysis of significant risk factors for FMD infection was performed using Stata version SE 12.0 (Stata Corp. College Station, TX, USA) and SPSS 16.0.

3.2 Cross sectional study

3.2.1 Study area

The cross sectional study was carried out as part of the SADC Trans-boundary Animal Diseases Project at the livestock wildlife interface areas, where buffalo and cattle were suspected to share grazing and water. These included the Kafue Flats, the southern Kafue NP, lower Zambezi basin and the Luangwa Valley

ecosystem (Figure 8). Except for southern Kafue NP, the Kafue Flats, lower Zambezi basin and the Luangwa Valley ecosystem have all been described in section 3.1 above. The southern Kafue NP is surrounded by Sichifulo and Bilili GMAs. In these buffer zones, coexistence of wildlife, humans and livestock is common. These buffer zones have a mean annual rainfall of 800mm and cattle are grazed communally as village herds since wetlands are absent. The area has sandy clay soils, with typical savannahs interspaced with Mopani woodlands.

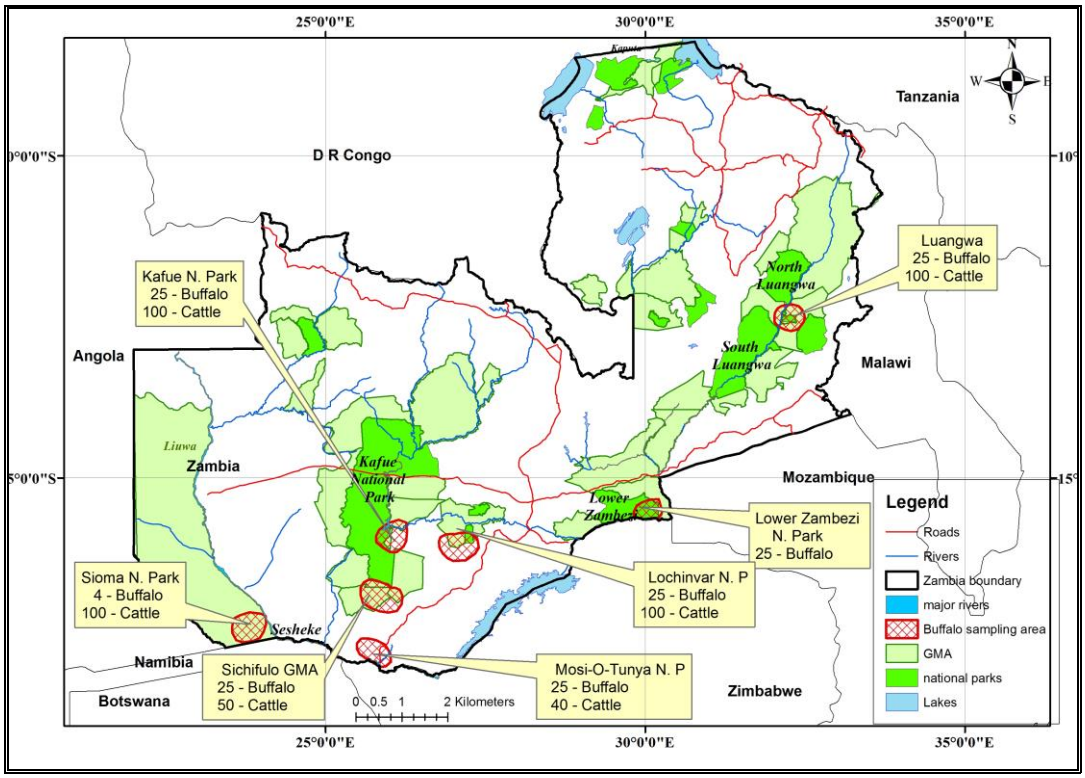

Figure 8: Location of study sites, including the number of buffalo and cattle sampled per site.

3.2.2 Study design, sampling and sample size

3.2.2.1 Buffalo sampling

The sampling of buffalo was purposive based on sightings of herds. The sample size for estimating the herd level prevalence of FMDV in buffalo in the respective areas was predetermined (n=25 per site) by the project on account of logistics.

The study unit was the individual animal. Therefore in Lochnivar NP that has an estimated buffalo population of 1000, sampling was from a herd of about 250. Similarly in Kafue NP with about 4,000 buffalo, sampling was from a herd of about 200 in Ngoma area and 150 in Sichifulo GMA. In Mosi-oa-tunya NP sampling was from a herd of about 265; In Sioma NP with an estimated 500 buffalo population sampling was from a herd of 20. In Lower Zambezi NP with about 6621 buffalo population sampling was from a herd of 200. In Luambe NP of the Luangwa ecosystem with about 9400, sampling was from a herd of about 500 (Table 2). Other wildlife present in these national parks included kudu, impala, and Kafue lechwe among other range of antelopes (Table 2).

3.2.2.2 Cattle sampling

The study was cross-sectional, with multistage sampling of villages as primary unit, herds as secondary and individual cattle as tertiary units. The cattle population estimates in the Kafue Flats was 600, 000, Lower Zambezi basin 100, 000 and Lundazi district 40,000. Villages within the GMA adjacent to the buffalo sampling site in the respective NPs were identified. Within these villages, cattle herds were purposively selected on the basis of willingness and convenience to sample. From each herd at least ten cattle (individual animal being the study unit) were randomly selected and sampled. Cattle were put in a kraal ‘‘randomly’’ captured, cast down and single probang and blood were collected. In addition**,** hooves of sampled animals were examined for growth arrest lines as clinical evidence of FMD convalescence (Sammin *et al.,* 2007). Prior to sampling of the animals, consent was obtained from the owner of the animals.

The number of animals sampled in each area was determined based on the following (Eng, 2003);

$n = 1.96^2pq/d^2$

Where n = sample size;

p = estimated prevalence (Assumed to be 10 percent for both within and between herds because of vaccination.)

q = 1- p, and

d = the desired absolute precision of the estimate (Assumed desired absolute precision was 5 percent).

The sample size for cattle was initially fixed at 50 per site by the project because of logistics but later adjusted to 100 per site to accommodate this study despite the calculated sample size for cattle per site being 139.

At the time of sampling, a pre-tested close-ended structured questionnaire was used to collect information about potential risk factors for FMDV infection in cattle and buffaloes. An initial visit was made to one of the study areas, where a few farmers were interviewed and the easiness of completion of the questionnaire and ubiquity of questions was noted and revised. Where necessary translation of the questions and answers into the local language was done by the local veterinary extension officer, but all recording were done in English. The information gathered using the questionnaire included GIS coordinates, cattle and buffalo densities, husbandry system, small ruminant densities, sharing of water and pasture between cattle and buffalo, presence of trade routes, proximity to National parks, proximity to wetlands, proximity to buffalo migratory routes, proximity to international border and FMD vaccination history (appendix 2).

The Zambia Wildlife Authority officers were also interviewed during buffalo sampling. The wildlife questionnaire included; demographic data including GIS coordinates, numbers of wildlife species, wildlife movements into cattle grazing areas, contact with cattle, number of buffalo clusters, level of poaching, disease burden and any human activities that could have been affecting wildlife habitats.

3.2.2.1 Buffalo capture

Buffalo herds were sighted using aerial (helicopter) survey and driven into open grassland for darting. Although the target were those in the midrange of three to four years because the virus is more likely to be isolated from this age group (Perry and Hedger, 1984), it was not possible to estimate the age from the helicopter. The animals were chemically immobilized by darting using Thianil 10mg/ml (Thiafentanil oxalate 10mg with preservative methylparaben 0.1percent m/v® from MC Pharma (Pty) Ltd, South Africa), a super potent opioid agonist that causes narcosis with analgesia in combination with Azeperone loaded in a single dart. Following sample collection (probang and blood) and age estimation by tooth development and wear (Jolles, 2007), as well as horn development (Grimsdell, 1973), the procedure was reversed using an analgesic antagonist, Trexonil (naltrexone®) at standard dosages as recommended by the manufacturer (MC Pharma (Pty) Ltd, South Africa). The used darts and all disposable materials were collected and later incinerated at CVRI. After the capture all personnel and vehicles were sprayed with citric acid used as disinfectant.

3.2.2.2 Cattle and buffalo blood collection

From each immobilised animal, five to ten ml of blood was collected aseptically through the jugular vein using a sterile vacutainer needle into a plain vacutainer

tube. The blood samples were held in a box to prevent haemolysis and left overnight at room temperature to coagulate. The serum was harvested into sterile vials after centrifugation at 2500 rpm for five minutes to separate the serum, labelled to identify the animal source and location, and then packed on ice in a cooler box for transportation to the laboratory where the samples were kept at -20 degrees Celsius until needed for analysis.

3.2.2.3 Cattle and buffalo probang sampling

Epithelial scrapping's from the oral pharyngeal area were collected from all buffalo and cattle using a probang cup (see figure 9a and 9b) following standard recommended protocol (Kitching and Donaldson, 1987). Between each sampling, the probang cups were disinfected using citric acid (0.2 percent, wt/vol) and rinsed three times in water then in phosphate buffer. Each probang sample was mixed with phosphate buffer supplied from TADP ARC-OVI in a conical tube, stored at +4 degrees Celsius for about one to two hours, after which the mixture was transferred into a cryotube and preserved in liquid nitrogen. The probang samples were later dispatched to TADP ARC-OVI for analysis.

Figure 9: Sampling of (a) buffalo and (b) cattle from the oral-pharyngeal area to collect epithelia tissue using a probang cup.

3.2.3 Analysis of samples

3.2.3.1 Laboratory analysis of samples

Cattle sera were analysed jointly at CVRI using non-structural protein (NSP) ELISA and the structural protein (SP) Liquid Phase Blocking ELISA (LPBE) and TADP ARC-OVI (LPBE). Briefly, the NSP ELISA procedure was as follows:

The 3ABC ELISA was performed using commercially available 3ABC ELISA Cedi test (Ceditest FMDV-NS kit; Prionics Lelystad B.V) at cut-off of ≥50 percent and following the manufacturer's instructions (Prionics, Netherlands). Briefly, the 3ABC ELISA kits microplate pre-coated with recombinant 3ABC antigen was incubated with an equal mixture of serum and monoclonal antibodies Horse Radish Peroxidase (HRP) (1:100 dilution in the conjugate diluent) for 90 minutes at 37^{o}C. During the first incubation, if antibodies against 3ABC were present in the test sample, the antibodies and the HRP conjugated monoclonal antibodies against 3ABC competitively bound to the antigens in the well. Following this incubation, all unbound material were removed by aspiration and washing before the addition of a substrate solution. The reaction was stopped by addition of the stop solution and calorimetric reading was performed by using a spectrophotometer (PerkinElmer) at 450nm and 620nm.

The LPBE was conducted based on the method of (Hamblin *et al*., 1986a; Hamblin *et al*., 1986b), at a cut-off point of >1.9 log titre as positive using TADP ARC OVI protocol and Manufacturer's instructions (Prionics, Switzerland). Briefly, flat bottomed microplates were used with each plate for each particular FMD serotype (SAT1, SAT2 and SAT 3). Test and control sera were added to the plates in 1/5 dilution in ELISA buffer with ten percent normal calf serum (NCS)

and 0.05 percent NaN_3. After overnight incubation 50µl of rabbit antisera specific for the different serotypes of FMDV at an optimum dilution in ELISA buffer, with ten percent NCS, was passively adsorbed to polystyrene microplates and incubated for one hour. Enzyme-labelled Horse radish peroxidase (HRP) conjugated to swine antiguinea pig IgG antibody diluted at 1/1000 in ELISA buffer with ten percent NCS and one percent normal guinea pig serum, was added and incubated for 30 minutes. 50µl of substrate /chromogen solution was added to all the plates and incubated for 15 minutes. Following colour development 50µl of acid stopper solution (1.25M sulphuric acid) was added to the wells of all plates to stop the reaction. The plates were read using Labsystems, Multiskan EX microplate reader with an interference filter of 450nm connected to a personal computer with relevant software. The Optimal densities generated by the reader were converted to log titres using the computer software. Log titres of >1.9 were considered positive, >1.6 and <1.9 as doubtful and <1.6 as negative.

3.2.3.2 Virus isolation and molecular analysis

Foot and mouth disease virus isolation from probang sampling was performed at TADP ARC-OVI, a reference laboratory for FMD. This is because Zambia lacks an approved FMD bio-security level three containment facility. The virus isolation was done following the standard procedure (OIE, 2014a). Briefly, confluent monolayer primary pig kidney cell cultures in 10ml tubes were used. These cell cultures were inoculated with the processed probang sample and diluted with media (phosphate buffer saline) from 10^{-1} to 10^{-7} according to the standard protocol. The cell cultures were also inoculated with a positive control diluted with media from 10^{-1} to 10^{-7} accordingly in order to get to the end point

where the cells were no longer susceptible to the virus. A negative control, where cell cultures were inoculated with media alone, was used. The cell cultures were then incubated and checked for cytopathic effect (CPE) every 24 hours for three days. Cultures that did not develop CPE were lysed by freezing at -80^{o}C for 24 hours and re-inoculated into a new monolayer of Instituto Biologico Rim Suino (IBRS)-2 cells in 25cm^{2} and then evaluated after 72 hours. Cultures with CPE were stored at -80^{o}C until processing for typing ELISA. The data analysis and calculation of the log titres was based on $TCID_{50}$/ml - 50 percent tissue culture infective dose per ml for the calculation of the end point (Karber, 1931). An aliquot of 140 micro litres from each probang sample was used for PCR. For cost reasons, cattle samples (sera) were first subjected to LPBE and only probang samples from those that were positive were subjected to virus isolation.

3.2.3.3 Antigen Enzyme Linked Immunosorbent Assay

Antigen ELISA was performed on the aliquots from virus isolations according to TADP ARC-OVI protocol adapted from Hamblin *et al.*, (1986a). The test is a standard indirect sandwich ELISA for the identification and typing of FMDV antigens in tissue samples or cell cultures. Briefly, rabbit antisera specific for each of the three SAT types (SAT-1, SAT-2, and SAT-3) as well as three European types (A, O and C) were passively adsorbed to polystyrene micro wells. With the addition of the test sample, FMD antigen (if present) was trapped by the immobilized antibodies to one of the serotypes. Serotype specific guinea-pig antisera of the same type as the rabbit trapping antisera was added to react with the trapped antigen. The bound guinea-pig antibodies were then detected by an anti-guinea-pig IgG conjugated to the enzyme peroxidase. With the addition of

substrate/ chromogen, a coloured product developed that qualitatively identified the type of FMD virus present in the sample. Micro titre plates were washed between each step of the test with washing buffer in an automated plate washer. The Plates were analysed using Labsystems®, Multiskan EX microplate reader with an interference filter of 450nm connected to a personal computer with relevant software. For each sample, the values of the negative control antigen were first subtracted then the values recorded for each type compared.

3.2.3.4 Reverse transcription polymerase chain reaction and sequencing

Polymerase Chain Reaction (PCR) was performed in three stages, which involved RNA extraction, rt-PCR and gel electrophoresis.

The RNA was extracted from cell culture specimens using QIAamp® Viral RNA kit (Qiagen, Hilden, Germany). Beside the samples, extraction was done to additional samples that had a positive extracted control (serotype O Kenya vaccine strain) and a negative extracted control. The pure nucleic acid (RNA) was stored at -81 degrees Celsius until needed for rt-PCR.

The extracted RNA above was converted to cDNA using AMV- reverse transcriptase with the forward and reverse primers and using the 2A/B Junction primer in the presence of random hexanucleotides for those intended for sequencing. One-step reverse transcription and PCR was performed as previously described by (Knowles *et al*., 2009) using Ready-to-Go One-step RT-PCR beads (Amersham Pharmacia Biotech) and 5 µl of RNA on a PTC-100™ thermal cycler (MJ Research®). The thermal profile for all the RT-PCR's was 42 degrees Celsius for 30 minutes, 94 degrees Celsius for five minutes, 35 cycles of 94 degrees Celsius for one minute, 60 degrees Celsius for one minute, 72 degrees Celsius for

1.5 minutes and final extension at 72 degrees Celsius for five minutes. These were then held at four degrees Celsius until processed or stored at -20 degrees Celsius. The oligonucleotide primers used for PCR amplification were SAT 1-P1-1228F with SAT-2B208R as a reverse and SAT2 P1-1223F as a forward primer (Kasanga *et al*., 2014a).

The RT-PCR amplicons were separated by agar gel electrophoresis on a 1.5 percent agarose gel, using Tris-acetate EDTA (TAE) buffer solution and their size estimated relative to a 100 bp ladder (Gene Ruler 100 bp DNA Ladder Plus, ready-to-use; MBI Fermentas). A positive and negative control was included in each reaction. Agarose gel electrophoresis was performed based on the method previously described (Knowles and Samuel, 1998; OIE, 2014a).

Sequencing of part of the PCR fragment was done based on the method of (Knowles and Samuel, 1996) and using the sequencing kits as recommended by the Promega. Following treatment with ExoSAP-IT (Exonuclease I and Shrimp Alkaline Phosphatase; USB Corporation) the amplicons were cycle-sequenced with the primers NK72, SAT1-1C559F, SAT1U-OS, SAT1-ID394R and SAT1-ID200F for serotype SAT 1 and SAT2 -1C445F, SAT2VP3-AB, SAT2-1C523F, SAT-ID209F and SAT2-D for SAT 2 and the rest was as earlier described (Kasanga *et al*., 2014a).

3.2.3.5 Epidemiological data analysis

The test results for the LPBE, 3ABC, cattle bio data together with the questionnaire data were entered into a Microsoft Excel® spreadsheet. The data was sorted and checked for consistency and duplication. The entries with missing values were included in the database using a unique code for all missing values.

Data analysis focused on seroprevalence of FMDV at animal level. The frequencies and summary statistics were calculated using STATA version 12 (Statacorp, College Station, TX, USA). The test performance comparison between the LPBE and the 3ABC ELISA was evaluated using the Kappa test.

FMDV seroprevalence in various cattle population was estimated by using the seropositive cases against the sampled population at a 95 percent confidence interval. The outcome variable of interest was whether at least one animal (cattle) was seropositive for FMDV. The logistic regression analysis was carried out as described earlier (section 3.1.3.3) to identify potential risk factors.

3.2.3.6 Phylogenic analysis

VP1 gene nucleotide sequences were translated and the deduced amino acid sequences aligned using the Clustal_X Omega software. These alignments were used to construct phylogenetic trees using midpoint rooted neighborhood joining (NJ) tree method in Mega 6.06, with the bootstrap method for test of phylogeny. The bootstrap replications were set at 1000 and bootstrap values ≥ 70 percent. Gaps and missing values in the data set were set at complete deletion.

3.3 Transmission modeling

3.3.1 Study area

This study was conducted in the Kafue Flats and specifically in Namwala District. The Kafue Flats has been described earlier (section 3.1.1.2). Namwala District has the highest cattle population density in the country, with over 300, 000 cattle congregating on the floodplain during transhumance between April and November (Malama *et al*., 2014) (Figure 10). It has been documented that some

transhumant herds come from villages as far as 50 to 60 km. A village size in Namwala averages 80 households, with cattle herd size averaging 400 to 500 (CSO, 2012). On average the productive life span of cow is 12 to 15 years (Charles Maseka, personal communication).

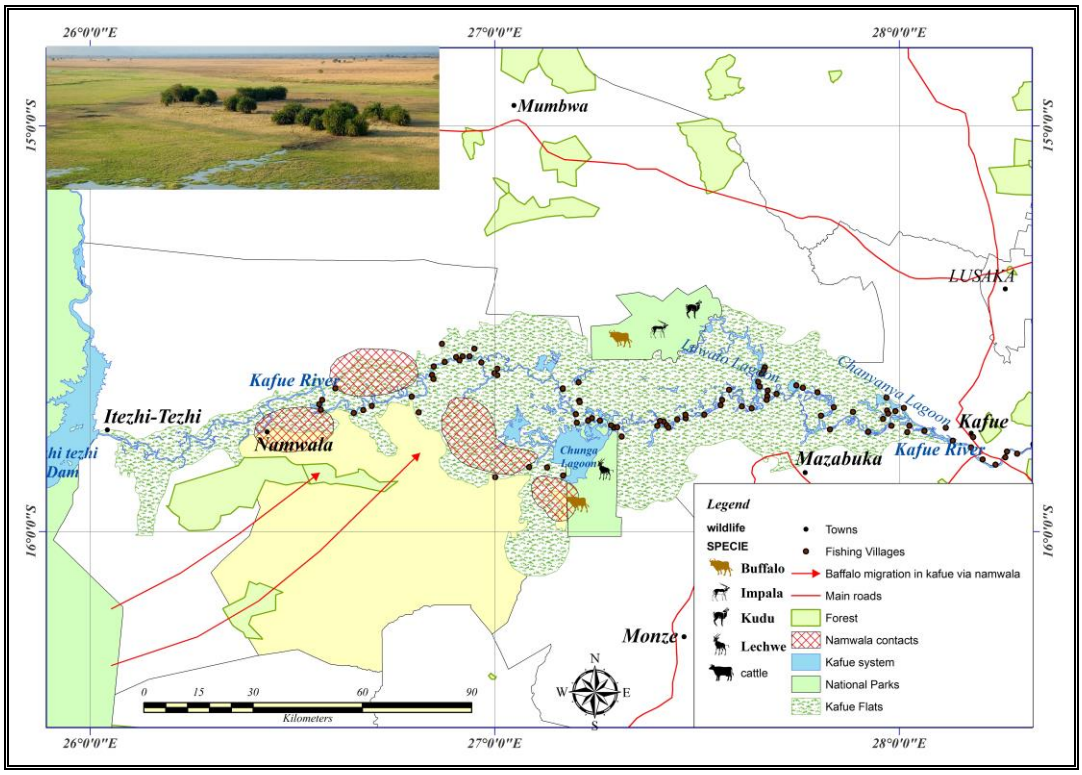

Figure 10: Kafue Flats and Namwala District (yellow shade), the area of annual transhumance from April to November

* Red lines indicate the conjectured buffalo migratory routes (Southern Kafue NP through the forest reserve areas towards the Kafue Flats. Inserted in the top left is a picture of the Kafue Flats

3.3.2 Study design

A susceptible, exposed, infectious and recovered (SEIR) model developed during the 2001 UK FMD outbreak was adapted to describe the transitional states in the cattle population (Woolhouse, 2004; Vynnycky and White, 2010a). The SEIR model was chosen because of the short latent and incubation periods of FMD and

to account for the time lag between infection and infectiousness. A deterministic model set up using differential equations and a discrete time step of one day as an appreciable period of time to notice the transitions was used. One day is appreciable as it is smaller than both the latent and the infectious periods of FMD and the most accurate descriptions of transmission dynamics are obtained using smallest time space (Woolhouse, 2004; Vynnycky and White, 2010a). The homogenous mixing that exist under communal grazing was taken into account by assuming mass action whilst accepting the heterogeneous aspect that herds in adjacent villages mix frequently compared to far off herds (Keeling, 2000, 2005).

Carrier class was introduced into the model assuming a stable population to account for the endemic setting. The rates of change of the population following the introduction of one infectious animal from susceptible to infectious, then infected and recovered were derived from literature and expert opinion. A portion of the infected animals may become carriers and susceptible animals can either be infected by carriers or acutely infectious animals (Keeling and Rohani, 2008a). The recovered animals were either removed due to death or become susceptible again. The duration of being a carrier and the proportion that become carriers were calculated based on parameters from published literature.

Based on the above model and using the formula of (Vynnycky and White, 2010), the inter-epidemic period (T) of FMD was given by:

$$T = 2\pi\frac{\sqrt{A(D+D')}}{R0-1}$$

Where A= Average age at infection
D = infectious period
D' = Preinfectious period
R_0 =Basic reproduction ratio

3.3.3 Data collection

The important time periods for FMD rates of change of the population following introduction of one infectious animal are provided (see table 5). Included were the incubation period defined as the time from infection to the onset of clinical disease; the pre-infectious (also referred to as latent) period defined as the time from infection to when the host was able to transmit the infection to another host; and the infectious period defined as the period from the end of the pre-infectious period until the time when a host was no longer able to transmit the infection to others (Keeling and Rohani, 2008; Mardones *et al*., 2010; Vynnycky and White, 2010a; Charleston *et al*., 2011).

Table 5: Variables and parameters used for the transmission of FMD in the Kafue Flats of Zambia

Description	Unit	Estimation of parameter and Variables
Birth rate	Cattle	For the purpose of this study, birth rate was equal to mortality rate i.e. stable population
Mortality rate	Cattle	Mortality rate = 1/life expectancy – assume a rectangular population. Animal live and then they dies naturally. Mortality rate = 7.5percent (Muma *et al*., 2009)
Life expectancy	per time	15 years (based on the assumption that cows start producing at 3 years. On average they produce six calves and with inter-calving interval of almost two years) – expert opinion from veterinary field officer and farmers
Incubation period	per time	2 -14 days (average = 8) (Alexandersen *et al*., 2003) 4.1 days (Charleston *et al*., 2011) 6 days (Mardones *et al*., 2010)
Latent period	per time	3.6 days (Mardones *et al*., 2010)
Infectious period	per time	For non-carriers 4.2 (Charleston *et al*., 2011) 4.4 (Mardones *et al*., 2010) For carriers = (pre infectious period + disease duration)
Basic reproduction ratio (R0)	Case	5.4 - 9.1 (Brostoff, 2012)
Movement period from Infected to carrier and recovery	per time	3 days (one per time spent in infectious stage per unit time (Charleston *et al*., 2011)
Rate of recovery from carrier to immune (viral	per time	2.5 – 3.5 (Hedger 1976, Hargreaves *et al* 1994)

persistence in oral pharyngeal area)		0.1076/month (Tenzin *et al*., 2008)
Proportion or percent of Infected who become carriers	dimensionless	0.5 (Sutmoller and Gaggero, 1965) 0.52 (Tenzin *et al*., 2008) 0.043 (Brostoff, 2012)
Total population for Namwala District	Cattle	150,000

Average rates at which individuals become infectious or recover were calculated as follows:

Rate at which individuals become infectious (f) = 1/average pre-infectious period (Vynnycky and White, 2010a).

Rate of recovery/becoming immune (r) =1/ (average period of infectiousness) (Vynnycky and White, 2010a).

These equations assumed that the distribution of pre-infectious and infectious periods are exponential.

3.3.4.1 Assumptions and the complexity of foot and mouth disease

The following assumptions were made:

- Assumed a single strain while in reality there are five serotypes circulating in Zambia (three active in buffalo –SAT 1, SAT 2, SAT 3 and two in cattle - SAT 1, SAT 2);
- No viral evolution while in reality antigenic drift and shift exist;
- No maternal immunity (in reality maternal immunity exists);
- A single host species while in reality other potential hosts are present;
- Assumed homogenous mixing whilst accepting the heterogeneous mixing that exist;
- Because of repeated outbreaks we assumed endemic setting for our model. Although the numbers of infectious cattle is not known; and
- Stable population (births = deaths).

3.3.4 Model analysis

The modeling was done using Berkeley Madonna Version 8.3.18, a mathematical modeling software (http://www.berkeleymadonna.com).

CHAPTER FOUR

4.0 RESULTS

4.1. Retrospective study results and analysis

4.1.1 Descriptive results

The total number of FMD outbreak cases recorded from 1933 to 2012 was 1,568, distributed in 168 wards across 20 districts (Figure 11). It can be noted from this figure that there was a generally quiet period in the late 1980s and 1990s whilst the last decade recorded an upswing in the number of outbreak cases.

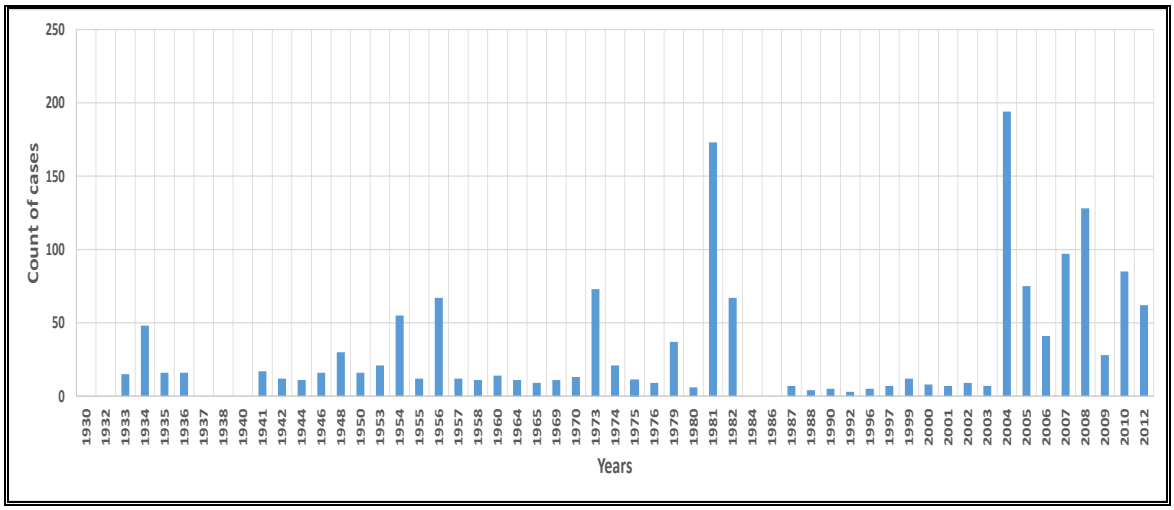

Figure 11: Number of outbreak cases recorded per annum from 1933 to 2012. There was no incidence of foot and mouth disease for the years not reported

The FMD outbreak case distribution were confined to the three known high risk areas of Mbala-Isoka, Kafue Flats and Lower Zambezi basin, except for the year 2000 SAT 1 outbreak in the Eastern Province (Figure 12).

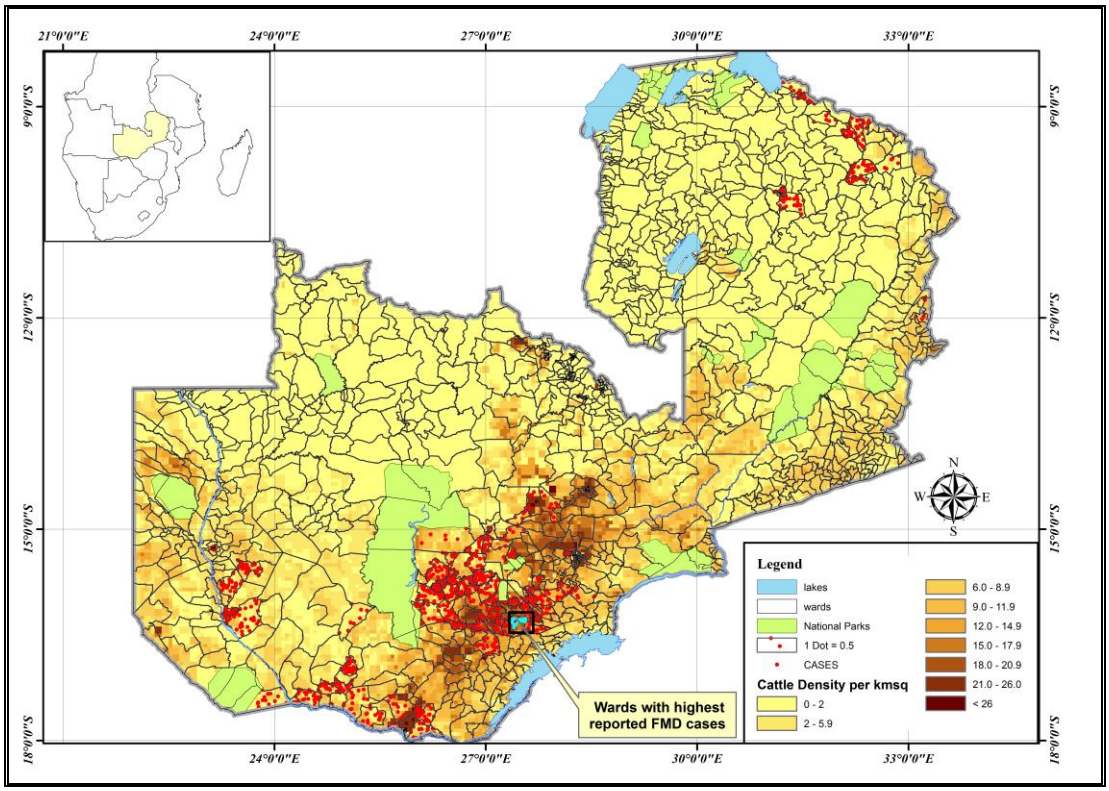

Figure 12: Cattle density and number of reported foot and mouth disease outbreaks per ward from 1933 to 2012

The Kafue Flats recorded the highest number of outbreaks followed by lower Zambezi basin and Mbala-Isoka area and the difference was significant (chi2=29.45, p=0.0001) Furthermore, there was a significant difference in the distribution of serotypes between these areas (chi2=16.22, p=0.001) (Figure 13).

Between the districts, Namwala had the highest number of outbreaks (16.9%), followed by Mazabuka (13.9%), Monze (12%), Mbala (11.4%), Mumbwa (7.2%), Sesheke (7.0%), Kazungula (6.8%) and Livingstone (6.5%). Forty three wards reported at least a case each while Manungu and Mayaba wards in Monze recorded the highest number of cases (eleven each) in 1982 (Figure 12).

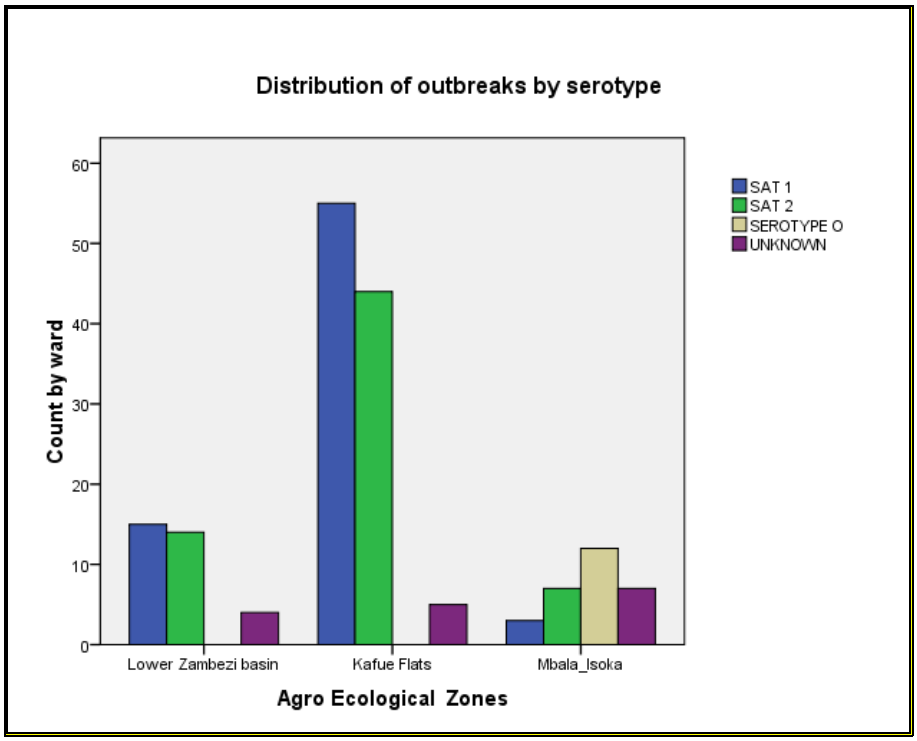

Figure 13: Percentage distribution of outbreaks by serotype in the three agro ecological zones from 1933 to 2012 and the difference was statistically significant (chi2=16.22, p=0.001)

4.1.1.1 Foot and mouth disease epidemics

The period between FMD outbreaks of the same serotype in the respective high risk areas varied, with generally long periods of disease inactivity (Figure 14). For example, there was no reported outbreak of SAT 1 in the Kafue Flats for 17 years from 1956 to 1973 and for another period of 30 years from 1974 to 2004. Similarly, there was no reported outbreak of SAT 2 in this area for 12 years from 1948 to 1960, for 16 years from 1965 to 1981 and for 14 years from 1982 to 1996. Since the last reported case in 1996, it has now been absent for 18 years.

In the lower Zambezi basin, no outbreak of SAT 1 was reported for seven years from 1981 to 1988, for the period of 12 years from 1988 to 2000 and for another period of 11 years from 2001 to 2012. There was no report of SAT 2 in the lower

Zambezi basin for five years from 1970 to 1975, for 22 years from 1975 to 1997 and for another 10 years from 1997 to 2007.

In northern Zambia, serotype O was not reported for six years from 1976 to1982, for 19 years from 1982 to 2001 and for eight years from 2002 to 2010. No outbreak of SAT 1 was reported for four years from 2004 to 2008. No SAT 2 outbreak was reported for three years from 1999 to 2002, for seven years from 2002 to 2009 and for three years from 2009 to 2012.

Serotype C was only recorded once in 1981 in the Kafue Flats. The source of this outbreak remains unknown, although it was suspected to have been due to vaccine reaction. Serotype A was also recorded once in 1990 in Mwenzo, Nakonde and it was suspected to have been introduced from Malawi.

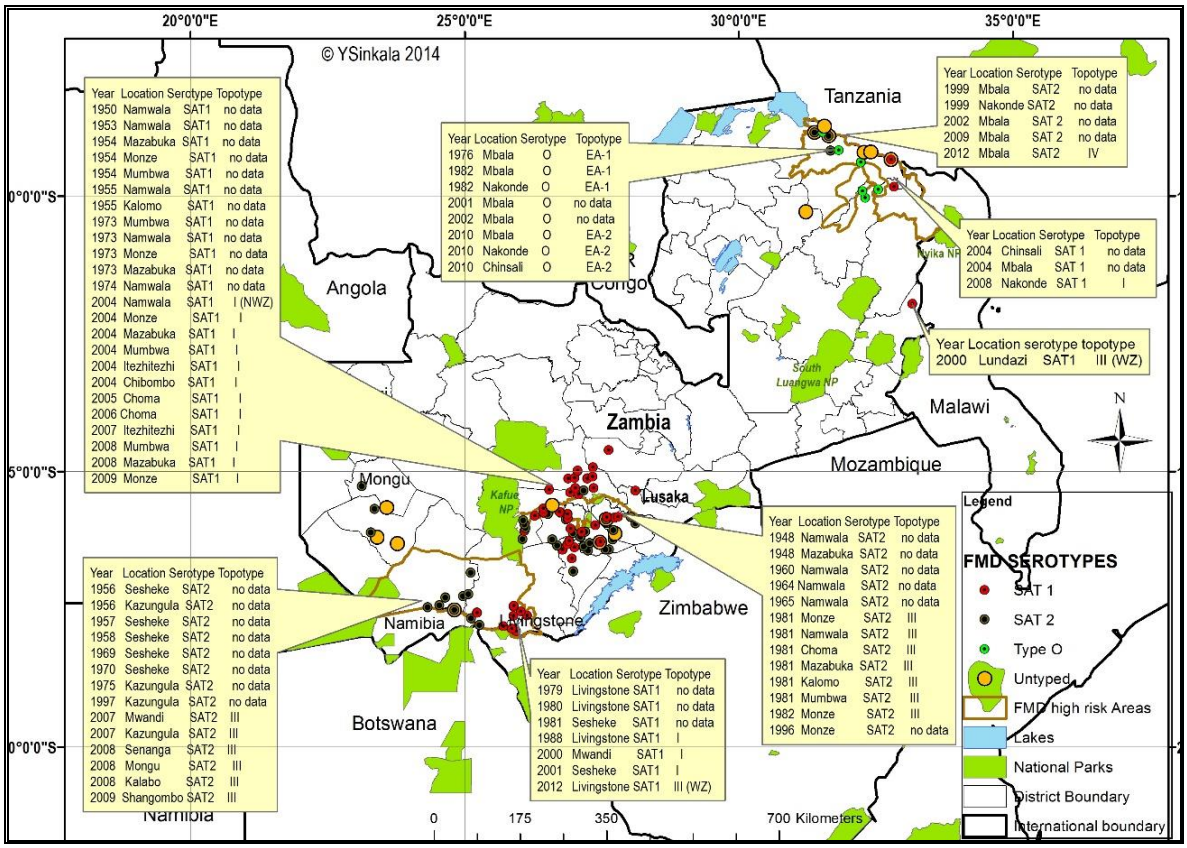

Figure 14: Distribution of foot and mouth disease outbreak cases by serotype and topotype from 1933 to 2012 in the three high risk areas

The Kafue Flats recorded more SAT 1 (55.8 percent, n=24) in comparison to SAT 2 (30.2 percent, n=13), while the lower Zambezi basin recorded more SAT 2 (56 percent, n=14) in comparison to SAT 1 (28 percent, n=7), while 16 percent of the outbreaks were untyped. In northern Zambia, serotype O dominated with 41.4 percent of the outbreaks, followed by SAT 2 (24.1 percent) and SAT 1 (10.3 percent), while the rest (31.8 percent) were untyped. Overall SAT 1 was highest followed by SAT 2 and serotype O (chi2=16.22, p=0.001) (Figure 15a).

The topotypes identified to be associated with outbreaks included topotypes I and III within SAT 1; topotypes I, III and IV within SAT 2; topotypes EA-1 and EA-2 within serotype O; and topotype III within serotype A. However, some outbreak viruses were not characterized to topotype level (36 percent) (Figure 15b). In the Kafue Flats, topotype I of SAT 1 dominated followed by topotype III of SAT 2. However, no topotype information was available on majority of outbreaks (55.8 percent) (Figure 16a). In the lower Zambezi basin, topotypes I and III of SAT 1 and topotype III of SAT 2 accounted for 40 percent of the outbreaks while no topotype information was available on majority of outbreaks (60 percent) (Figure 16b). In northern Zambia, topotypes EA-1 and EA-2 of serotype O, topotypes I of SAT 1, IV of SAT 2 and III of serotype A accounted for 40.9 percent of the outbreaks, while there was no topotype information on majority of outbreaks (59.1 percent) (Figure 16c).

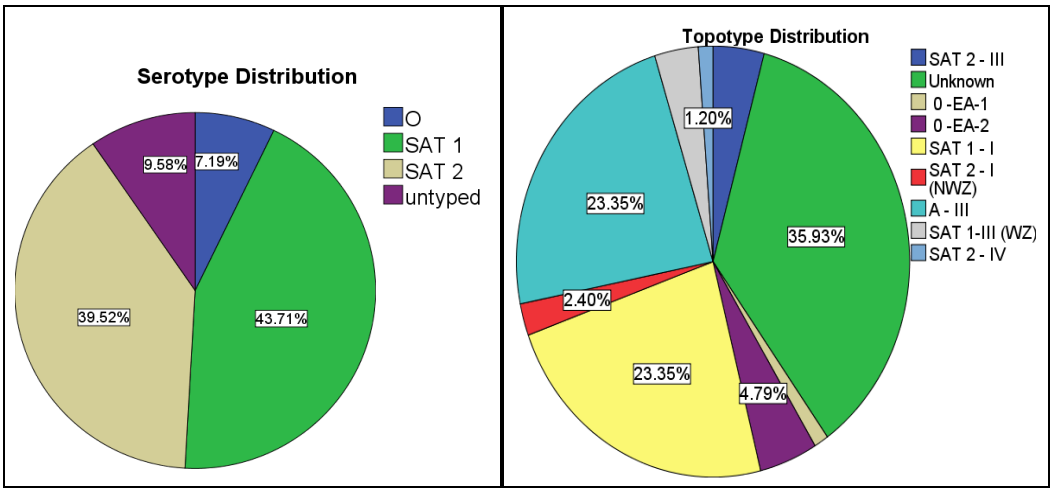

Figure 15: Overall percentage distribution of (a) serotypes and (b) topotypes

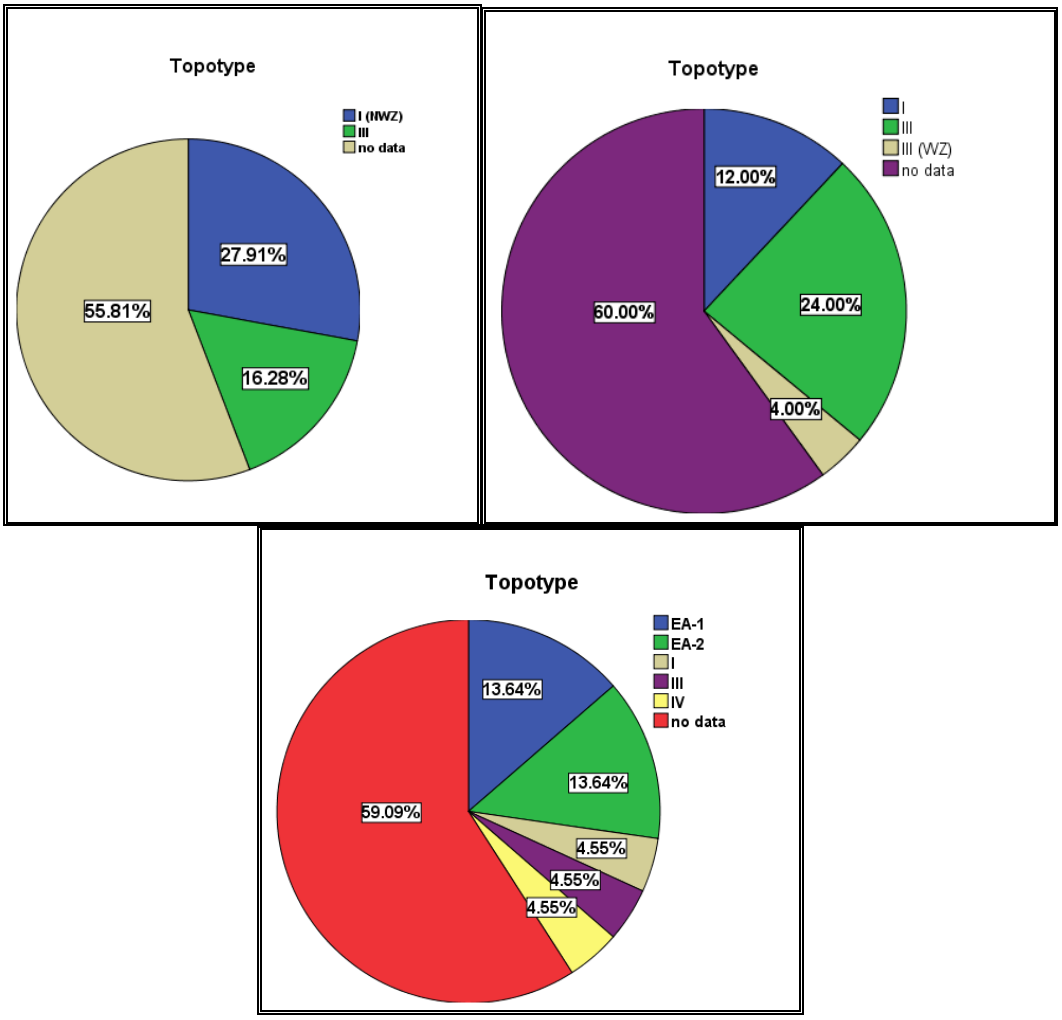

Figure 16: Distribution of topotypes in (a) Kafue Flats (b) Lower Zambezi basin and (c) Mbala-Isoka area

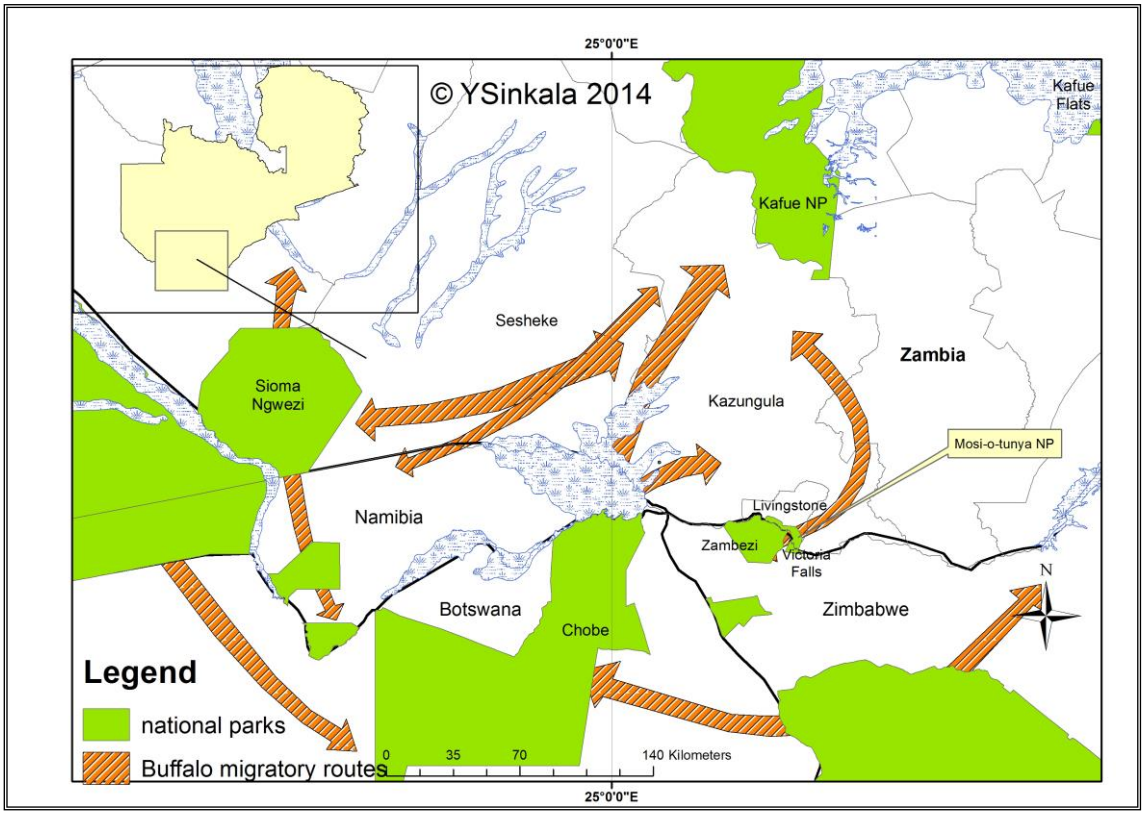

Figure 17: Buffalo migratory routes within the lower Zambezi basin to illustrate the likelihood of contact with cattle as most areas are occupied by small holder farmers

* Migratory routes data courtesy of Peace Parks GIS team and from Africa Wildlife Foundation

4.1.1.2 Temporal and spatial patterns

For the analysis of temporal trends, the outcome variable was whether at least one primary outbreak was reported in a ward in a given year. Data on the 1568 outbreak cases in 168 wards from 20 districts was used for the analysis.

4.1.1.2.1 Period 1933 to 1952

The space-time permutation model identified three significant clusters during the period 1933 to 1952. The relative risk (RR) was calculated as a comparison of the likelihood of a case of FMD occurring inside the cluster in comparison to areas outside of it (Kulldorff *et al*., 2005; Kulldorff, 2014). The most likely clusters

were located in Namwala (n=6) with relative risk (RR) of 6.48 between 1942 and 1950, Mongu-Senanga (n=4) with RR of 2.54 between 1935 and 1936 and Sesheke (n=6) with RR of 2.23 in 1933 (Figure 18).

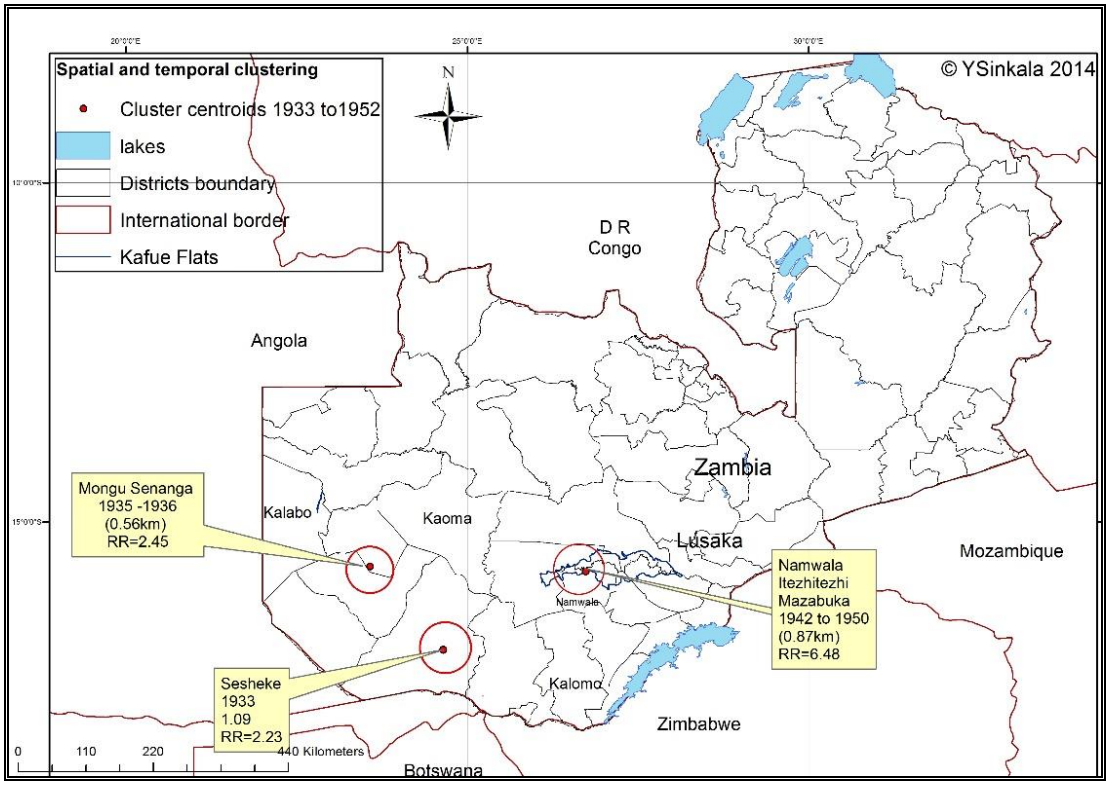

Figure 18: Three significant clusters (p=0.001) detected during the period 1933 to 1952. Included is the RR and the radius of the clusters based on the 210 outbreak cases in 16 wards

4.1.1.2.2 Period 1953 to 1972

The model identified four significant clusters during the period. The most likely clusters were located in Namwala (n=4) with RR of 6.05 in 1953, Mazabuka (n=10) with RR of 2.36 in 1954, Sesheke (n=4) with RR of 5.51 from 1956 to 1957 and Itezhitezhi (n=5) with RR of 10.78 in 1960 to 1965 (Figure 19).

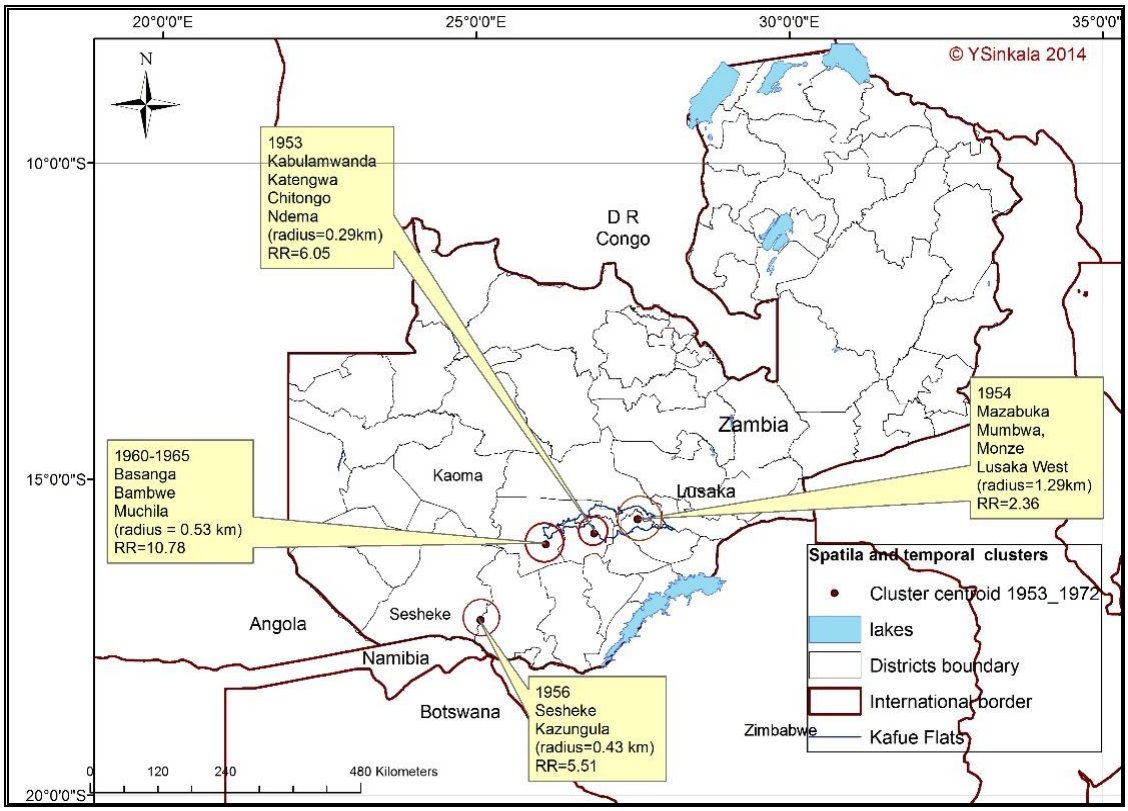

Figure 19: Four significant clusters (p=0.001) detected during the period 1953 to 1972. The number of cases used for this analysis was 248 cases in 23 wards

4.1.1.2.3 Period 1973 to 1992

The model identified eight significant clusters during the period. The most likely clusters were located in Kazungula (n=6) with RR of 13.75 between 1979 and 1980; Mumbwa (n=14) with RR of 3.03 between 1973 and 1974; Mbala (n=4) RR of 3.4 in 1976; Choma (n=10) with RR of 2.25 in 1981; Monze (n=4) with RR of 5.73 in 1982; Nakonde (n=5) with RR of 4.90 in 1982; Mazabuka (n=6) with RR of 19.25 in 1987; and Monze (n=6) with RR of 29.6 in 1992 (Figure 20).

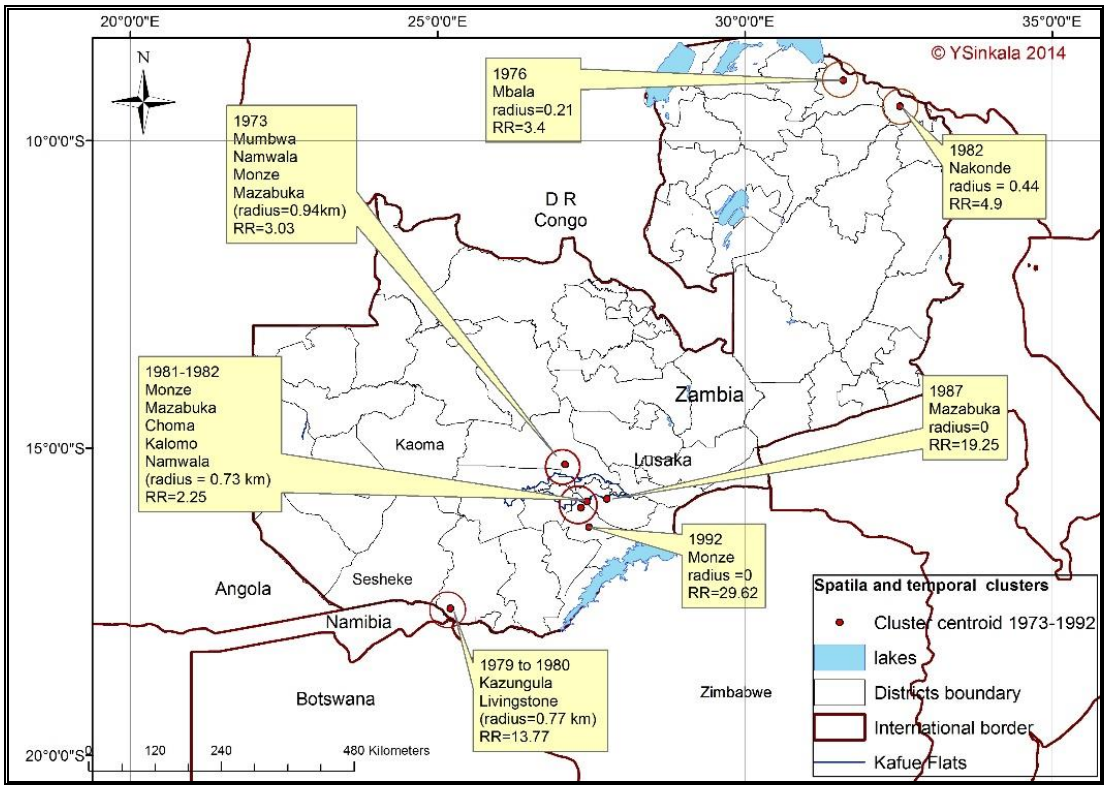

Figure 20: Seven significant clusters (p=0.001) detected during the period 1973 to 1992 based on the 385 outbreak cases in 46 wards

4.1.1.2.4 Period 1993 to 2012

The model identified five significant clusters during the period. The most likely clusters were located in Livingstone-Kazungula (n=8) with RR of 10.46 in 2012; Namwala/Mumbwa (n=15) with RR of 3.47 in 2004; Mbala-Isoka (n=13) RR of 3.75 in 2009 to 2011; Choma/Itezhitezhi (n=6) with RR of 12.71 in 2005; Monze (n=2) with RR of 14.14 in 1996; and Mongu-Sesheke (n=4) with RR of 7.61 in 2008, (Figure 21).

The five clusters detected during the period demonstrated the continued persistence and variation in risk within the three high risk areas.

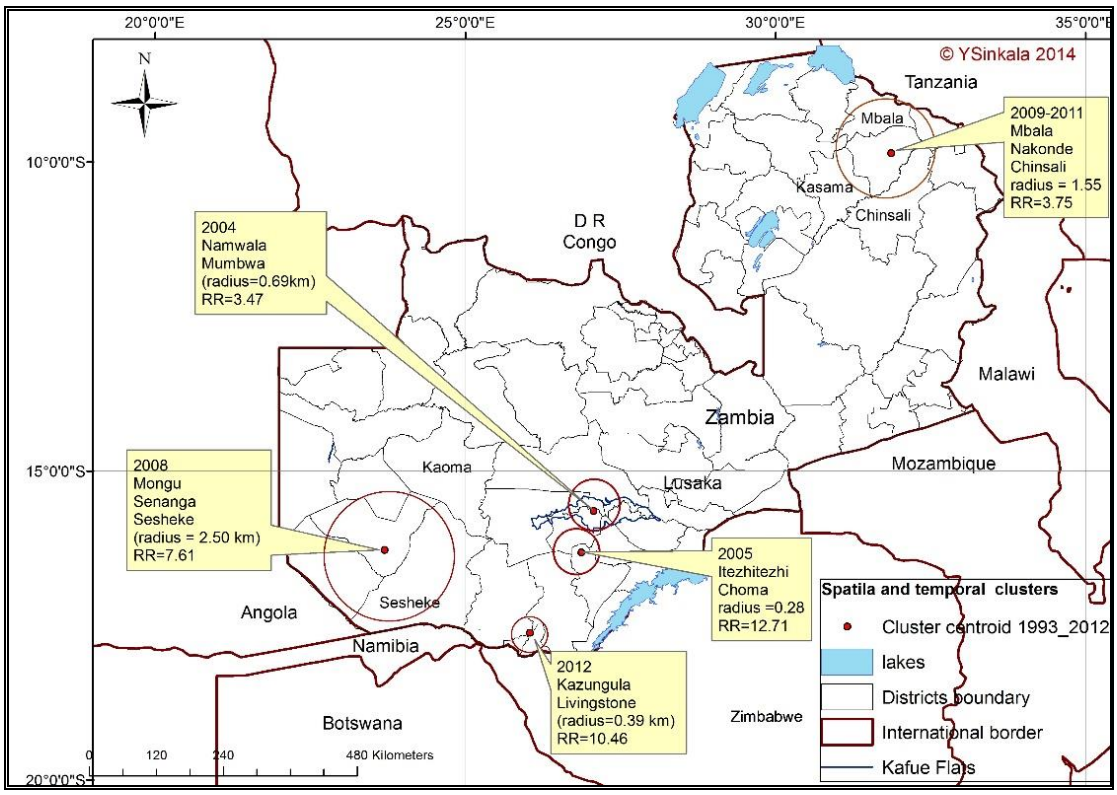

Figure 21: Five significant clusters (p=0.001) detected during the period 1993 to 2012 based on 1004 outbreak cases in 63 wards

The FMD risk of exposure remained persistent in the lower Zambezi basin and Kafue Flats throughout the all the four periods while risks of exposure in northern Zambia emerge during the 1973 to 1992 period and persist into the 1993 to 2012 period.

4.1.1.3 Risk factor analysis

The results of the univariate analysis indicated that cattle density, agro-ecological zone, small ruminant density, proximity to buffalo migratory route, proximity to wetlands, rainfall, and proximity to international border were significant predictors of FMD occurrence (Table 6). However, cattle density and small ruminant density had similar odds ratios. Therefore, small ruminant density was

excluded from further analysis. There was positive collinearity between several predictor variables (Table 7). Therefore, cattle density, proximity to wetlands, distance to NPs, and buffalo-cattle contact were excluded from the final model. This enabled exclusion of confounding effects among the factors.

Table 6: Results of the univariable logistic regression analysis of potential risk factors for FMD outbreak occurrence at ward level from 1933 to 2012

Variable	Odds Ratio	95% CI	P-value
Cattle density per km^2*			
≤5	Ref		
6-10	3.77	1.35, 10.48	0.011
11-20	5.51	2.56, 11.84	0.000
Agro-ecological zone*			
I	Ref		
II	4.50	1.92, 10.53	0.001
III	0.49	0.18, 1.38	0.18
Small Ruminant density per km^2*			
≤5	Ref		
6-10	3.06	1.31, 7.15	0.009
11-20	5.02	2.21, 11.39	0.000
Buffalo density per km^2			
≤1	Ref		
>1-10	1.92	0.75, 4.89	0.174
11-20	0.92	0.42, 2.01	0.838
Distance to National Park (km)*			
≤30	Ref		
>30-60	2.21	0.96, 5.08	0.062
>60	0.46	0.19, 1.11	0.085
Proximity to buffalo migratory route (km)*			
≤25	Ref		
>25	1.75	0.91, 3.39	0.094
Buffalo Cattle contact*			
No contact	Ref		
Direct, transhumance and migration	1.86	0.87, 3.98	0.109
No direct contact, transhumance and stray buffalo	5.08	1.87, 13.80	0.001
Proximity to wetland (km)*			
≤25	Ref		
>25-50	2.33	0.82, 6.61	0.111
>50	0.3	0.13, 0.69	0.005

Rainfall *			
Normal and flooding	Ref		
Drought	4.18	2.09, 8.32	0.000
Livestock movement*			
Transhumance	Ref		
Cross Border cattle trade	0.18	0.08, 0.37	0.000
Cross border cattle theft	2.87	0.35, 23.61	0.324
Proximity to international Border (km)*			
≤25	Ref		
>25	9.44	4.44, 20.08	0.000

Note: * These values had Fisher's exact p-value <0.25 and were identified as potential risk factors for inclusion in the multivariable analysis.

Table 7: Cross tabulation of significant predictor variables from univariable analysis

Predictor variables	Chi2	P-value
Proximity to buffalo migratory route and distance to NP	34.89	0.001
Proximity to buffalo migratory route and proximity to wetland	97.51	0.001
Proximity to buffalo migratory route and cattle density	62.26	0.001
Buffalo density and buffalo-cattle contact	77.25	0.001
Proximity to wetland and distance to NP	63.20	0.001
Cattle density and agro-ecological zone	124.91	0.001
Cattle density and distance to NP	92.78	0.001
Cattle density and buffalo-cattle contact	88.73	0.001
Cattle density and proximity to wetland	47.85	0.001

4.1.3.1 Multivariable analysis

The multivariable logistic regression analysis indicated that there were differences in outbreak risk at ward level during the period 1933 to 2012 within the three agro-ecological zones. Of the six variables considered in the final multiple binary logistic regression model, agro-ecological zone, rainfall, proximity of ward away from buffalo migratory route and proximity of ward away from international

border were identified as significant predictors of FMD outbreaks (Table 8). The risk of FMD in wards in Agro-ecological zone III was reduced by 12.0 percent (95% CI: 3, 49) compared to those in Agro-ecological zone I, when the effect of other variables were controlled (p =0.003). Similarly, drought in a ward increased the risk of FMD by 2.54 (95% CI: 1.09, 5.89) compared to those that had floods or normal rainfall when the risk of other variables were controlled (p=0.030). Further, wards that were more than 25km from buffalo migratory routes were 3.18 (95% CI: 1.27, 7.98) times more likely to have an FMD outbreak than those that were less than or equal to 25 km from the buffalo migratory route when the other three variables present in the model were controlled (p = 0.013). Equally, wards that were more than 25 km from the international border were found to be 4.60 times (95% CI: 1.72, 12.27) more likely to have an outbreak of FMD than those that were less than or equal to 25km from the international border, when the effect of other variables was controlled (p = 0.002). The Hosmer Lemeshow test suggested a good fit to the data (Chi2=3.95, 6df, p=0.684 at 95% CI) and the predictive accuracy of the multivariable model suggested a good final model discrimination (AUC=0.83).

Table 8: Results of multivariable logistic regression analysis of potential risk factors for FMD outbreak wards between 1933 and 2012 (at cut off p-value = 0.05)

Variable	Level	b	SE (b)	P-value	Odds Ratio	95% CI
Constant		-2.18	0.1689	0.03	0.38	0.16, 0.91
Agro-Ecological Zone	I				Ref	
	II	-0.96	0.37	0.337	0.46	0.95, 2.24
	III	-2.97	0.086	0.003	0.12	0.03, 0.49
Rainfall	Normal and flooding				Ref	
	Drought	2.17	1.09	0.030	2.54	1.09, 5.89
Ward proximity to buffalo migratory route (km)	≤25				Ref	

	>25	1.49	2.47	0.013	3.18	1.27, 7.98
Ward proximity to international Border (km)	≤25				Ref	
	>25	3.04	2.3	0.002	4.6	1.72, 12.27

4.1.2 Cross-sectional study

4.1.2.1 Number of animals sampled per study site

Table 9 provides the number of cattle sampled at each site. In southern Kafue NP (Dundumwezi) buffalo could not be located and only cattle were sampled. In Sioma Ngwezi only four buffalo were sampled from a herd of twenty as the rest buffalo population had crossed the border into Angola. In Lower Zambezi NP, no cattle were sampled as the Zambezi escarpment forms a natural barrier.

Table 9: Number of buffalo and cattle sampled per site

Study site	Cattle		Buffalo	
	Probang	Sera	Probang	Sera
Lochnivar NP- Monze (Hakunkula)	50	53	25	25
Kafue NP - Itezhitezhi (Basanga plain)	50	100	25	25
Kafue GMA – Sichifulo	50	80	25	25
Kafue GMA – Dundumwezi	50	60	none	none
Sioma NP - Sesheke	50	100	4	4
Lower Zambezi NP	none	none	25	25
Luangwa (Chanjuzi GMA) - Lundazi	50	101	25	25
Mosi-oa-tunya NP – Livingstone	50	50	25	25

4.1.2.2 Sero-prevalence in cattle

The FMDV seropositivity varied significantly amongst the study sites. The animals were first screened using the LPBE however, because of routine vaccinations that were conducted, the 3ABC ELISA was used as a confirmatory test. Therefore, the seropositivity was 11.2 percent (95% CI: 0.9, 21.6) in Itezhitezhi's Basanga area, 11.3 percent (95% CI: 0.1, 22.6) in Monze's

Hakunkula area, 2.0 percent (95% CI: 0, 13.2) in Kazungula's Moomba area and 5.0 percent (95% CI: 0, 17.6) in Livingstone based on the 3ABC ELISA (Table 10). No cattle were seropositive in Sesheke, Lundazi and Dundumwezi area of Kalomo district. The difference in seropositivity between these locations was statistically significant (Chi2=26.18, p= 0.001).

Table 10: Foot and mouth disease seropositivity results from cattle conducted at seven study sites between 2010 and 2012

Location	Number examined	3ABC (95% CI)	LPBE (95% CI)
Dundumwezi	29	0	0
Lundazi	101	0	0
Livingstone	20	5.0 (0, 17.6)	5.0 (0, 17.6)
Sesheke	99	0	2.6 (0.9, 6.1)
Kazungula	51	2.0 (0, 13.2)	33.3 (20.4, 46.3)
Monze	53	11.3 (0.1, 22.6)	26.4 (14.5, 38.3)
Itezhitezhi	62	11.2 (0.9, 21.6)	22.6 (12.2, 32.9)
Total	413		

Kappa (K) agreement between the LPBE and the 3ABC ELISA was 0.115, indicative of slight agreement (K<0.20).

4.1.2.3 Risk factor analysis for cattle seropositivity

According to univariable analysis age, cattle density, small ruminant density, transhumant grazing, proximity to international border, presence of livestock trade route, FMD vaccination status and proximity to buffalo migratory route were significant predictors of FMD seropositivity (Table 11). However, small ruminant density, proximity to international border and presence of livestock trade route had similar odds ratios. Therefore, proximity to international border and presence

of livestock trade route were excluded from further analysis. There was positive collinearity between proximity to buffalo migratory route and cattle density; proximity to buffalo migratory route and small ruminant density; transhumant grazing and cattle density and cattle density and FMD vaccination status (Table 12). Therefore, cattle density, proximity to buffalo migratory route, small ruminant density, and FMD vaccination status were excluded from the final model. This enabled exclusion of confounding effects among the factors. FMD seropositivity was not affected by sex (Chi2=3.34, p=0.067).

Table 11: Univariable logistic regression analysis of risk factors for FMD seropositivity in cattle

Risk factor variable	Odds Ratio	95% CI	P-Value
Age*			
< 2 years	Ref		
>2-4 years	0.57	0.21,1.54	0.273
> 4 years	0.13	0.02, 1.02	0.053
Cattle density*			
<2/km2	Ref		
2-5/km2	0.3	0.05, 1.85	0.198
>5-10 /km2	3.9	1.07, 14.15	0.038
Small ruminant density*			
<5/km2	Ref		
>5 /km2	3.7	0.83, 16.35	0.084
Sharing of water and grazing with wild animals			
No	Ref		
Yes	2.6	0.34, 20.20	0.351
Transhumant grazing*			
No	Ref		
Yes	7.4	2.5, 21.5	0.001
Proximity to international border*			
≤50km	Ref		
>50 km	4.8	1.56, 14.93	0.006
Presence of livestock trade route*			
No	Ref		
Yes	3.7	0.84, 16.35	0.084

FMD vaccination status*			
No	Ref		
Yes	6.62	1.88, 23.2	0.003
Distance to NP			
<30km	Ref		
≥30km	1.09	0.42, 2.82	0.856
Distance to road network			
<35 km	Ref		
>35 km	1.12	0.43, 2.91	0.810
Proximity to buffalo* migratory route			
<25km	Ref		
>25km	0.34	0.09, 1.12	0.093

Note: * These values had Fisher's exact p-value ≤0.25 and were identified as potential risk factors for inclusion in the multivariable analysis.

Table 12: Correlation of significant predictor variables from univariable analysis

Predictor variables	Chi2	P-value
Cattle density and proximity to buffalo migratory route	90.71	0.0001
Small ruminant density and proximity to buffalo migratory route	139.45	0.0001
Transhumant grazing and FMD vaccination status	196.42	0.0001
Cattle density and transhumant grazing	413.0	0.0001

4.1.2.4 Multivariable analysis

Transhumant grazing was found to be a significant predictor for cattle seropositivity in the final multiple logistic regression model (Table 13). Despite age being marginally associated with seropositivity, it was included in the final model because it had no effect on the ORs, and p-value. Therefore, animals that were involved in transhumant grazing were 16.91 times more likely to be seropositive in comparison to those that were not involved. The Hosmer Lemeshow suggested a good fit to the data (Chi2=0.96, 3df, p=0.812) and the

predictive accuracy of the multivariable model suggested a good final model discrimination (AUC=0.83) compared with transhumance alone (AUC=0.81).

Table 13: Multivariable logistic regression analysis of potential risk factors for FMD seropositivity at animal level (at cut off p-value = 0.05)

Variable	Level	Odds Ratio	P-value	95% CI
Transhumant grazing	No (ref)			
	Yes	16.91	0.001	3.58, 79.76
Age	≤ 2 years (Ref)			
	>2 – 4 years	0.98	0.98	0.32, 3.06
	.>4years	0.46	0.48	0.52, 4.07

4.1.2.5 Virus Isolation

A total of 154 probang samples collected from buffaloes in GMAs were subjected to virus isolation using primary pig kidney cell cultures. From these samples, the FMD virus was successfully isolated from buffalo samples collected from Kafue NP (16 percent), lower Zambezi NP (20 percent) and Luambe NP (8.3 percent). For cattle, virus isolation was only successful in Lochnivar NP Monze's Hakunkula interface (2 percent) (Table 14). The isolated virus were subjected to antigen ELISA, PCR and sequencing for serotype identification.

Table 14: Virus isolation, PCR and Antigen Elisa results from buffalo and cattle collected in 2010, 2011 and 2012

NP/GMA	Prop	VI -CPE% (CI)	PCR	Serotype
Ngoma – Kafue	4/25	16.0 (8.0 -25.4)	Pos	SAT 2
Lochnivar	0/26	Nil	Nil	Nil
Lower Zambezi	5/25	20.0 (8.0 - 35.2)	Pos	SAT 1
Luambe – Chanjuzi	2/25	8.3 (0-19.3)	Pos	SAT 2
Mosi-oa-Tunya	0/25	Nil	Nil	Nil
Sichifulo	0/20	Nil	Nil	Nil
Sioma	0/4	Nil	Nil	Nil
Monze - Hakunkula *	1/53	2.0 (1.8 - 5.8)	Pos	SAT 1

Note: * isolate from cattle at the interface

The rt-PCR amplification of the five isolates from lower Zambezi and two from Luambe NPs yielded expected bands sizes of 490 bp (Figure 22), confirming the presence of the FMDV genome in the samples. The amplicons were subjected to sequencing. However, the resulting sequences for samples from lower Zambezi and Luambe NPs were not very informative due to low DNA quality and thus were not used in the phylogenetic analysis.

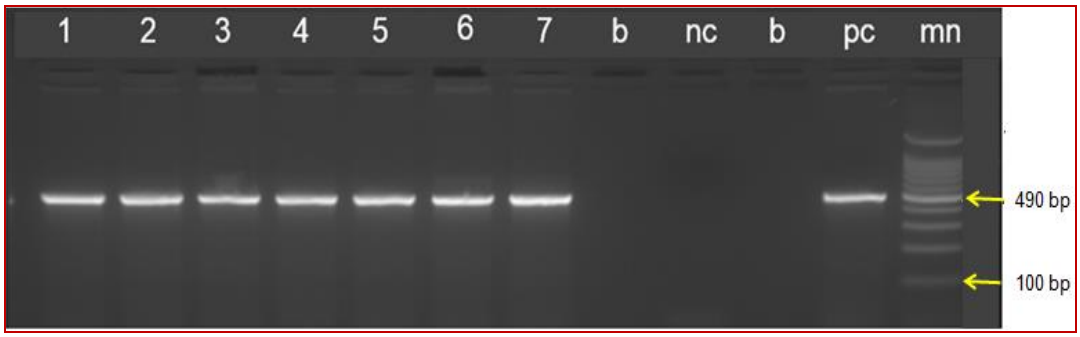

Figure 22: Agarose gel electrophoresis of foot and mouth disease virus isolates from Luambe and lower Zambezi NPs

***** PCR bands 1 to 5 samples from lower Zambezi NPs and 6 to 7 samples from Luambe NPs. The b is the buffer, nc is the negative control, pc is the positive control, and mn is the 100 bp ladder

However, partial VP1 sequence of the SAT1 from Lower Zambezi NP was 506 nucleotides determined from a single reverse sequence. The virus probably belonged to topotype I (NWZ) and was more than ten percent different to SAT 1 viruses from Tanzania and northern Zimbabwe. The partial VP1 sequence of the SAT 2 from Luambe NP was 318 nucleotides, determined from a single reverse sequence and the virus probably belonged to topotype IV, but not closely related to any other viruses in the ARC OVI TADP database.

Phylogenetic analysis of the SAT 2 virus isolate from buffalo in Kafue NP's Ngoma area showed that the virus belonged to topotype II and was different from the 1993 and 1996 viruses' isolates from the Kafue NP (Figure 23).

The SAT 1 virus isolate from cattle in Monze's Hakunkula interface area with Lochnivar NP belong to topotype I. It clustered closely with FMD virus responsible for 2004 to 2008 outbreak that was recorded in this area (Figure 24).

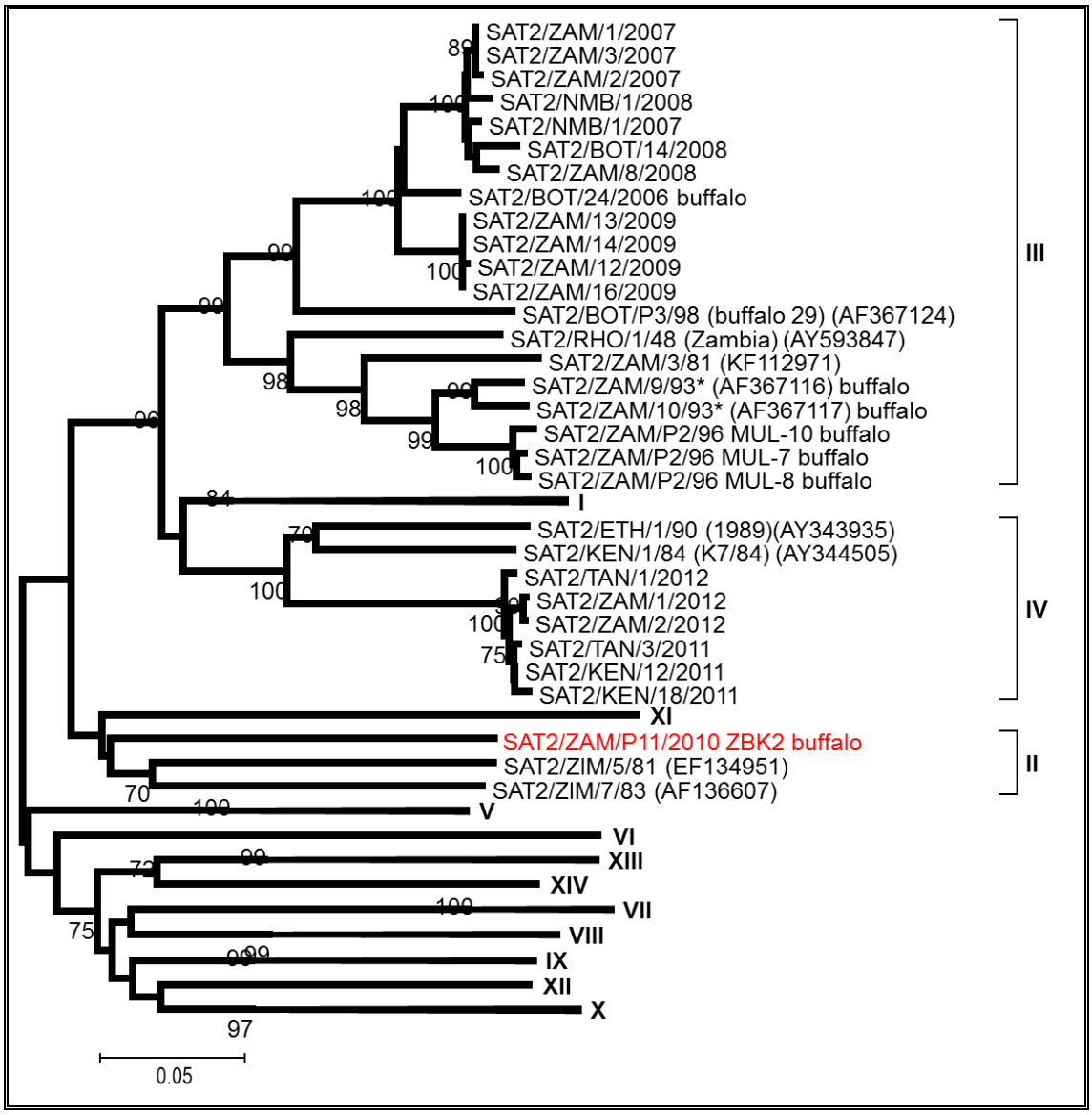

Figure 23: Phylogenic tree of SAT 2 isolate from Kafue NP's Ngoma area of Zambia

* The statistical significance of this SAT 2 isolate (SAT2/ZAM/P11/2010 ZBK2 buffalo) in relation to previous SAT 2 outbreak isolates from cattle and buffalo isolates from Zambia and the region is provided by the numbers on the left side of the tree. The phylogenic tree was produced using The Pirbright Institute (TPI) database courtesy of Dr Nick Knowles.

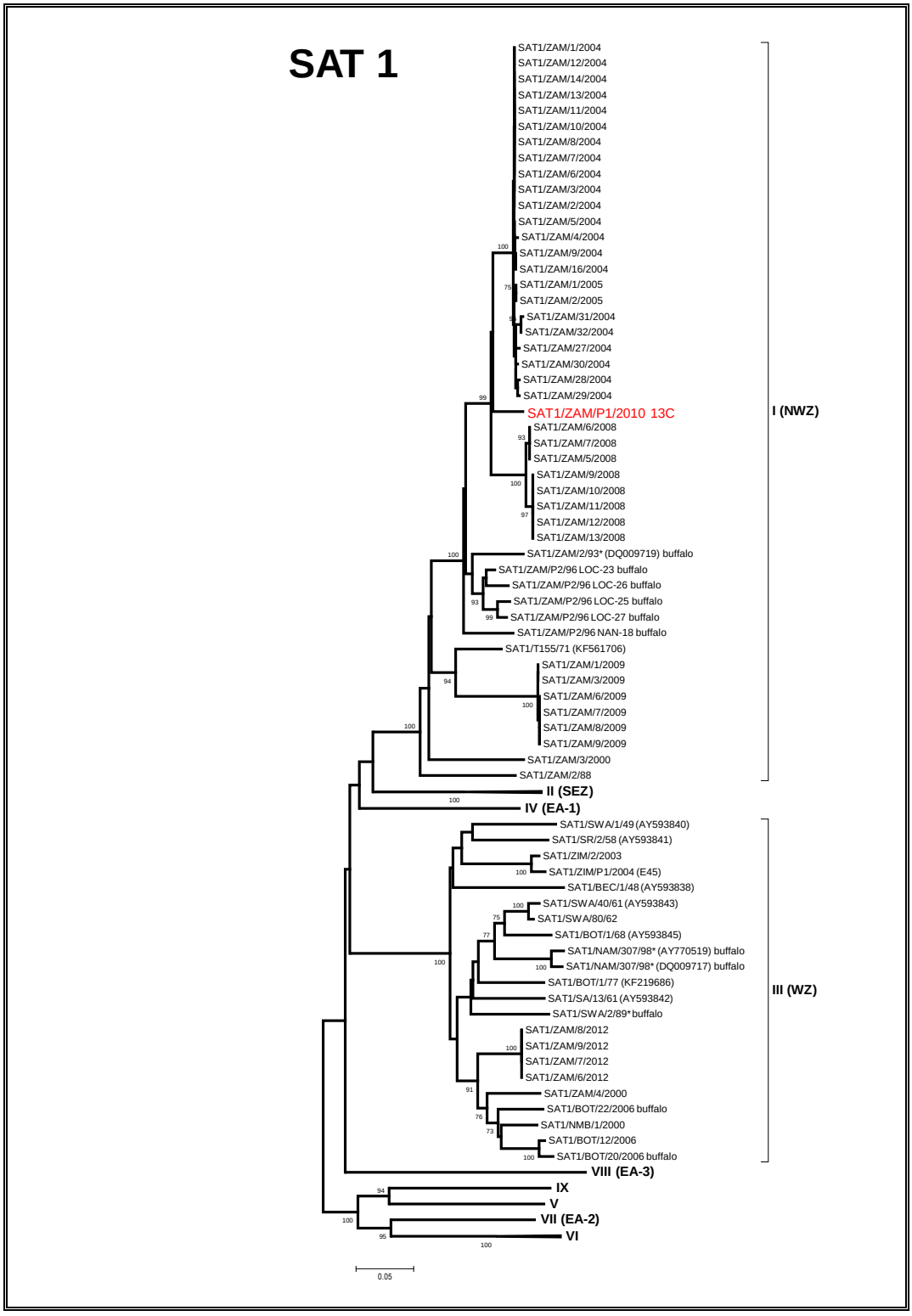

Figure 24: Phylogenic tree of SAT 1 isolate from cattle in Monze's Hakunkula interface with Lochnivar NP in Zambia

***** The statistical significance of this SAT 1 isolate (SAT1/ZAM/P1/2010.13C) in relation to SAT 1 Kafue Flats outbreak isolates of 2004 to 2009 and other SAT 1 outbreak FMD viruses from cattle as well as buffalo isolates from Zambia and the region is provided by

the numbers on the left side of the tree. The phylogenic tree was produced using The Pirbright Institute (TPI) database courtesy of Dr Nick Knowles.

4.1.3 Transmission modeling

Figure 25 describes the flow chart for the susceptible exposed infectious and recovered (SEIR) model developed for the analysis. The model incorporates a carrier class to reflect the endemic state of the disease in Namwala District of the Kafue Flats.

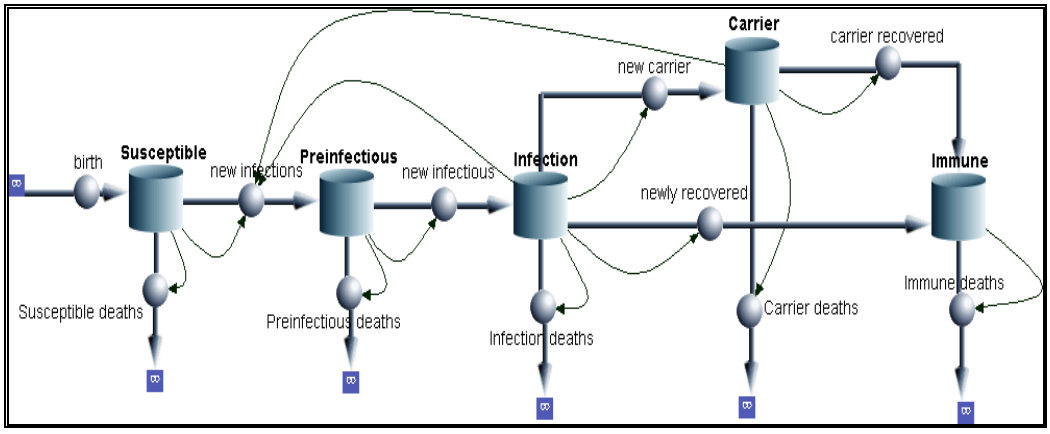

Figure 25: Susceptible Exposed Infected Recovered (SEIR) Model incorporating the carrier class state. The population was fixed (birth equals death rate)

*****The SEIR model was adapted from Ferguson *et al.*, 2001 and Woolhouse, 2004

A summary of the differential equations describing the transformation of the reservoir states (blue cylinders in figure 25) is described below:

d/dt (Susceptible) = - new_infections + birth - susceptible_deaths
d/dt (Preinfectious) = + new_infections - new_infectious - preinfectious_deaths

d/dt (Infection) = + new_infectious - new_carrier - infection_deaths – new infectious carrier

d/dt (Carrier) = + new_carrier - newly_recovered - carrier_deaths

d/dt (Immune) = + newly_recovered - immune_deaths + new infectious carriers

The equations of parameters and variables used in the arrows are described below:

new_infections = beta * susceptible * (infection + carrier)

new_infectious = preinfectious * infectious rate

new_carrier = infection * carrier rate * carrier_percent

newly_recovered = carrier*carrier recovery rate

birth = total population * birth rate

Susceptible_deaths = susceptible * mortality rate

Preinfectious_deaths = preinfectious * mortality rate

Infection_deaths = infection * mortality rate

Carrier_deaths = carrier * mortality rate

Immune_deaths = immune * mortality rate

Infected carriers= infection * carrier_rate * (1-carrier_percent)

The data sources for parameters and variables used in the equations were described earlier (Table 5). Therefore:

Total population = 150,000

Preinfectious period = 8 days

Infectious period = 7 days

$R_0 = 5$

Infectious rate = 1/Preinfectious period

Recovery rate = 1/infectious period

Beta = R_0/ (total population * infectious period)

Susceptible at start = total population - 1

Preinfectious at start = 0

Infectious at start = 1

Carrier at start = 0

Immune at start = 0

Precarrier period = 3 days

Carrier rate = 1/precarrier period

Carrier percent = 0.5

Carrier persistence yrs = 2.5

Carrier recovery rate = 1/ (carrier persistence yrs*365)

Life expectancy_yrs = 15

Mortality rate = 1/ (life expectancy_yrs*365)

Birth rate = mortality rate= $1.83x10^{-4}$

Proportion susceptible = Susceptible/total population

Proportion immune = 1- proportion susceptible

$R_n = R_0$ * proportion susceptible

The model assumes introduction of one infectious animal into Namwala a totally susceptible population of 150,000 cattle. According to the theory, the rate of change ∧ **$=R_0-1/D$**

Where D is the duration of infectiousness.

Assuming R_0 = 5 or 9 and D=7 as applied in this study, then:

Rate of change = (5-1/7) or (9-1/7) gives range 0.57 to 1.14 new infections per day. Therefore the infection prevalence increases by 0.6 to 1.1 per day. There are other variants, this is only reasonably reliable during the early stages of an epidemic (Vynnycky and White, 2010a).

The model predicted that it would take about 73 days for the epidemic to reach its peak at R_0=5 and by day 120, almost the entire population would have been affected by the disease and would have either recovered or become carrier (Figure 26).

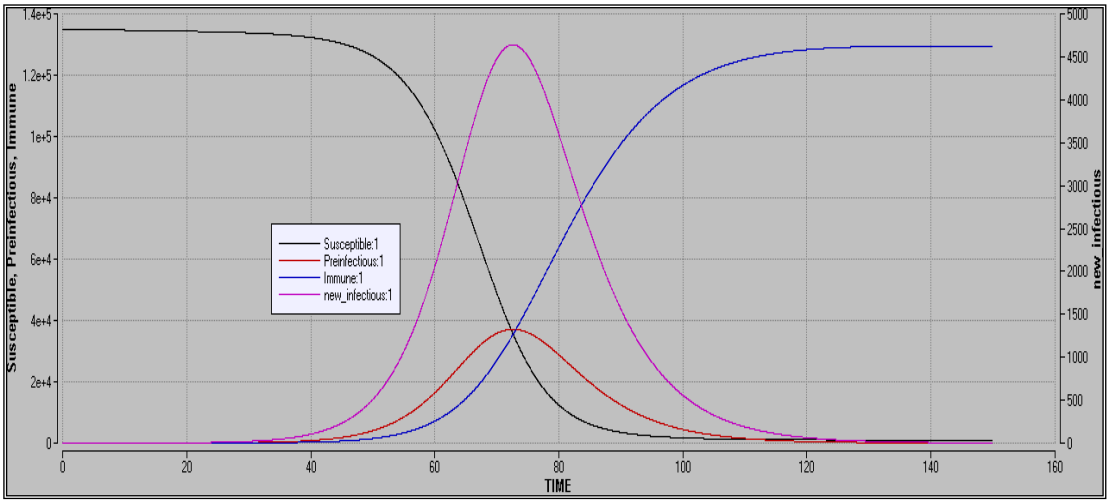

Figure 26: Rate of change from susceptible, pre-infectious, immune and infectious states when R_0 =5 following introduction of one infectious animal in Namwala, assuming mass action the outbreak dies down at day 120.

But assuming R_0=9, the epidemic peaks by day 45 and dies down by day 80 (Figure 27).

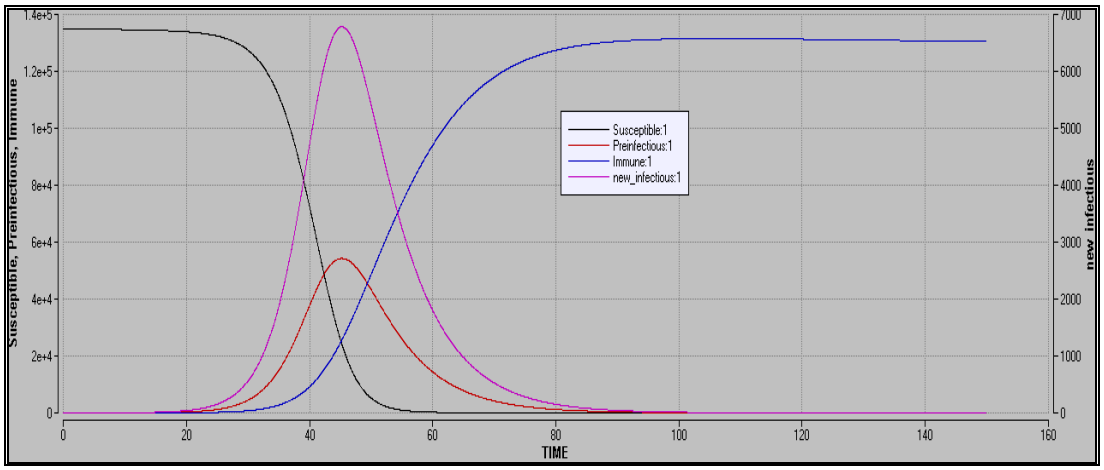

Figure 27: Rate of change from susceptible, pre-infectious, immune and infectious states when R_0 = 9 following introduction of one infectious animal in Namwala, assuming mass action the outbreak dies down at day 80.

When R_0 = 9 and the infectious period is 45 days (duration before animals recover), the susceptible, pre-infectious and infectious populations would be depleted by day 135 and what would remain would be the immune and carriers (Figure 28).

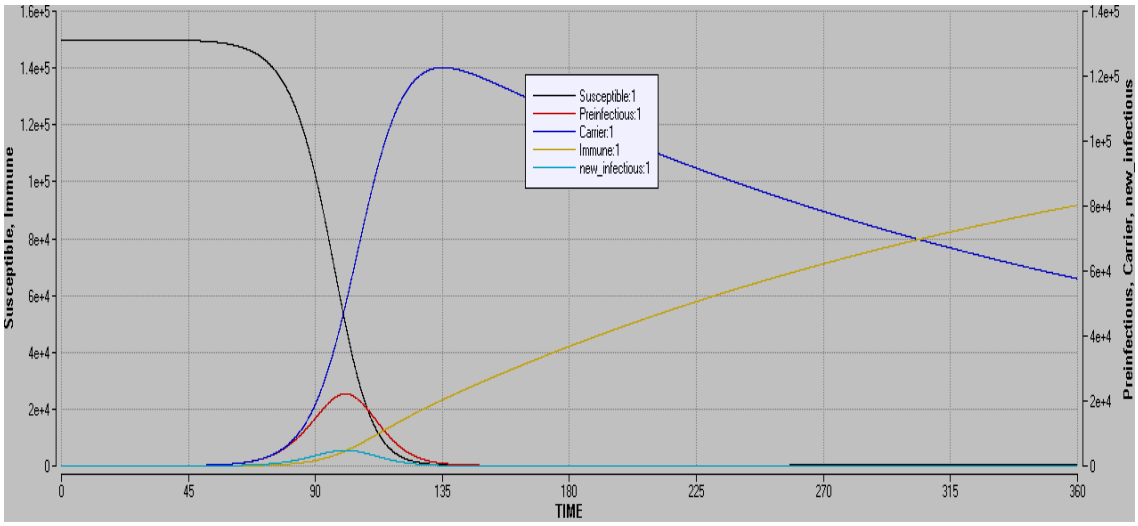

Figure 28: Susceptible, immune, pre-infectious and infectious states when R_0 =9 and infectious period = 45, taking the proportion of infected that become carriers to be 50 percent of infected. By day 135 all the susceptible, pre-infectious and infectious would be depleted and what would remain are the immune and carriers.

The inter epidemic results showed that an outbreak would be expected every 9.6 years if age of infection was three years and 11 years if age of infection was four years. This calculation did not take into account carrier status and vaccination. Assuming that 50 percent of the recovered animals become carriers, the model predicted that the disease would die down by day 135 and depending on the rate at which susceptible builds up, the disease may cycle after 23 years before going into equilibrium (Figure 29).

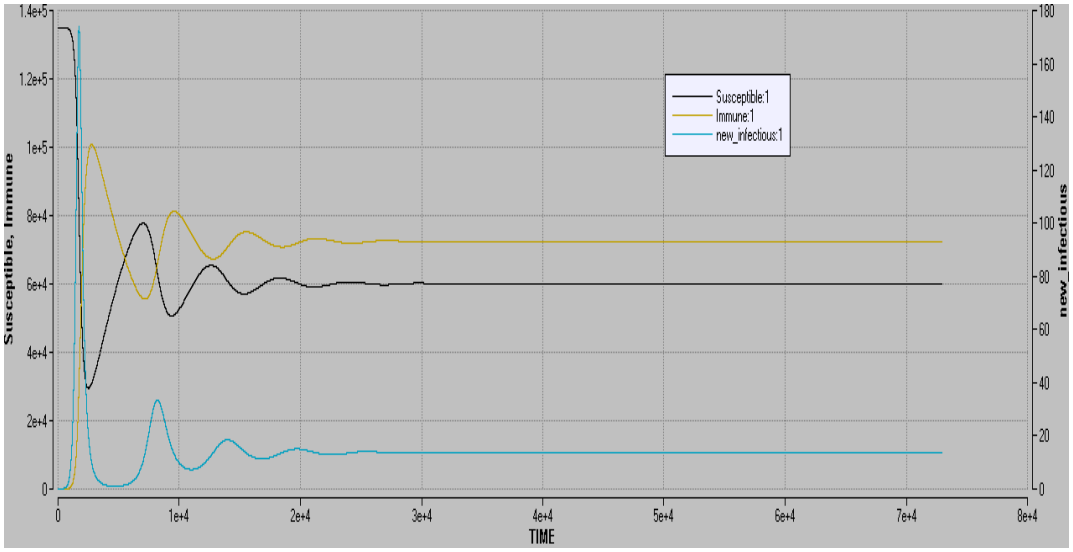

Figure 29: The rate of change of infectious if observed for a longer period and depending at the rate at which susceptible builds up a possible epidemic may occur after 23 years.

Therefore, depending on the average age at infection, infectious period, basic reproduction ratio, carrier status and rate of new susceptible, a new epidemic may occur after 9 to 23 years. A higher R_0 and lower Infectious period depletes susceptible quickly.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Retrospective study

5.1.1 Descriptive

The spatial and temporal distribution of FMD outbreak cases in Zambia for the period 1933 to 2012 showed that there were fluctuations in the occurrence. The high number of FMD outbreaks in the 21st Century following quiescent period of the 1980s and 1990s, may be attributed to the fluctuating cattle density, cattle husbandry and marketing system. other probable reasons for the reduction in FMD incidences include the aphthization that was introduced in 1940s to 1950s; the introduction of vaccinations in the 1960s, 1970s and 1980s (Perry and Hedger, 1984; Sinkala *et al*., 2014b), especially during the 1980s when, through a French project, vaccination of cattle in the lower Zambezi basin was intensified (Debaste, 1987).

5.1.2 Spatial and temporal patterns

The spatial and temporal analysis identified significant clusters in different time periods with variable probabilities signifying the presence of hotspots in Mbala and Nakonde in northern Zambia; around the wetlands of Lochnivar, Blue Lagoon and southern Kafue National Parks; the Sesheke-Kazungula area and Livingstone within the lower Zambezi basin. These areas could be targeted for risk based disease surveillance and prevention.

The identified hotspots within the Kafue Flats make biological sense in terms of risk of exposure to FMD virus. For example, within the Kafue Flats there are areas along the river banks where pastures remain green during the dry season. In such areas, there is aggregation of cattle and wildlife during transhumance providing an opportunity for the transmission of FMD. Furthermore, the identified cluster centroids were within areas suspected to have been the initial sources of outbreaks. It was observed that the reduction in cattle numbers in the Kafue Flats as a result of the East Coast fever/Corridor disease outbreak of 1978 coupled with heavy poaching may have reduced the frequency of contact between cattle and buffalo (Chilonda *et al*., 1999).

However, with the cattle population increase of the early 2000s following successive immunization of calves against Corridor disease (Anonymous, 2009), coupled with the severe droughts of 2001 and 2002, the frequency of contact between cattle and buffalo may have increased due to competition for water and pasture. Despite the area receiving normal rainfall in 2003, the water was not adequate to avert the draught and the threat to the ecosystem biodiversity. This resulted in the opening of the floodgates at Itezhitezhi dam in July 2004 (NASA, 2004). However, this did not prevent the occurrence of SAT 1 outbreak that was reported within three weeks after opening the floodgates. Therefore, within the Kafue Flats, FMD outbreaks usually coincide with periods of drought and high cattle density (Anonymous, 2013a).

The study further identified two distinct statistically significant clusters in lower Zambezi basin, one cluster of SAT 1 in Livingstone and the other cluster of SAT 2 in Kazungula-Sesheke area. The area from Mosi-oa-tunya NP in Livingstone is a known migratory route for buffalo to the upland once the Zambezi River banks

are flooded (Sinkala *et al*., 2014a) (Figure 17). Therefore, the Livingstone cluster identified in this study may be as a result of infected buffalo coming into contact with cattle along these migratory routes. The area from Kazungula to Sesheke is both a wildlife corridor and a livestock trade route promoting back and forth movement between Namibia's Caprivi Strip and Zambia (Perry and Hedger, 1984; Debaste, 1987)(Figure 17). On both sides of the Zambezi River between Kazungula and Sesheke, cattle share the same islands for grazing with wildlife during the dry season. Furthermore, the flooding of the Zambezi basin shrinks the grazing land and pushes the animals to the upland mainly through the Mambova fault (NASA, 2007, 2009). This may explain why Mambova is a hot spot for outbreaks. It has been observed that the SAT 2 outbreaks that have dominated in this area have been largely due to illegal cattle movement from the Caprivi Strip (Perry and Hedger, 1984; FAO-WRLFMD, 2012). Despite vaccination being carried out twice a year in this area, outbreaks have remained frequent and difficult to control (Sinkala *et al*., 2014b). However, on the western side of Sesheke, FMD outbreaks are rarely reported. This maybe because in Katima Mulilo on the Namibia side, there is a double barrier fence intended to prevent spread of contagious bovine pleura pneumonia (CBPP) restricting cattle movements and prevents incursion of the disease into this area of Sesheke district.

Northern Zambia recorded a high frequency of FMD outbreaks due to serotypes O, A, SAT 1 and SAT 2. SAT 2 was mainly confined to Mbala while SAT 1 and serotype A were confined to Nakonde with serotype O cutting across the Mbala-Isoka area (Figure 14). The SAT 2 cluster that was identified in Mbala may be a result of the spread of the disease from the Rukwa plains where transmission between buffalo and cattle is suspected to occur (Chilonda *et al*., 1999). SAT 1

and serotype A outbreaks in Nakonde were suspected to be from illegal movement of cattle from northern Malawi (Vosloo *et al*., 2002a). Serotype O may be a result of movement of cattle from across Tanzania including the northern border areas with Kenya and Uganda described earlier (section 2.7.12.2). It is suspected that the increased volume of cattle movement from neighboring Tanzania into northern Zambia aided by the creation of livestock markets along the Tanzania/Zambia border may have contributed to the outbreaks (Banda *et al*., 2014). Most outbreaks in this area coincided with the beginning of the rainy season, a period when demand for oxen draught power for field ploughing was highest (Sinkala *et al*., 2014a). The rise in human population and commercial activities in northern Zambian Districts of Nakonde, Kasama, Mpulungu and Mporosoko may also have contributed to the increased demand for animal protein and the resultant trans-boundary cattle movement. The multi serotypes involved in these areas have also been a challenge for disease control by vaccination, especially that most of the outbreak virus strains (36 percent) were not subtyped to genome level to be useful for vaccine development (Figure 23b).

It has also been suggested that differences in the patterns of geographical clustering of SAT serotypes of FMD virus may result from the differing behaviour of these serotypes in cattle (Knowles, 1994). Most outbreaks in Zambia from 1933 to 2012 have been due to SAT1. This is contrary to observations elsewhere where SAT 2 has been observed to be more widely spread with a probable longer carrier status in cattle (Knowles, 1994).

Space time permutation is usually used in outbreak data analysis with a short time periods where the population at risk (PAR) does not change and also to analyse cases over a long time period in situations where the PAR remained stable

(Hanson and Wieczorek, 2002; Sudakin and Power, 2009). In this study, the cases were segregated into time periods where the PAR remained fairly stable. Generally, in Zambia the cattle population has not changed much over the years and as such population shift bias was minimal (WorldBank, 2011).

5.1.3 Risk factor analysis

This study identified agro ecological zone, average annual rainfall and ward's being away from buffalo migratory route and to international border as risk factors for reporting a primary FMD outbreak. These findings are consistent with a previous study that reported proximity to wildlife sanctuaries, distances to the major international border crossing, distance to the nearest major road, wetness index and elevation as predictors of FMD occurrence (Hamoonga *et al*., 2014), with the exception of the high risk of FMD in wards away from the buffalo migratory route and international border. It may be due to the fact that animals near the NPs are frequently exposed to the virus and therefore immune. Most human settlements (hence livestock numbers) are usually some distance away from the NPs and buffalo migratory routes. Further, although outbreaks may start on the floodplains, reporting has been when cattle return to home villages after having been grazing on the Kafue Flats during the dry season (Overby and Zyambo, 1983). Equally, the high risk of FMD outbreak in wards away from the international boundary may be because of settlements (livestock density) that are usually further inland from the borders. Usually cross border cattle movement is towards these same settlements as was observed during the 1956 Sesheke outbreak (Perry and Hedger, 1984). This analysis supports the hypothesis that although proximity to buffalo migratory route and international border may be a

risk factor, outbreaks may get established further away around settlements where cattle density is high. No apparent general seasonal predilection was observed in this study, however, FMD outbreaks have been previously reported (70 percent) to occur in the dry season (Perry and Hedger, 1984).

The number of cases reported in this study may be an under estimation given the underreporting that exist in the country. However, FMD is a notifiable disease in Zambia and usually attracts resources from central government to the affected district and therefore the incentive to report is very high among field officers. Even when this is the case, beef animals are not closely observed and its possible that occult outbreaks of the disease in such farming systems were missed (Anonymous, 2009). Due to repeated vaccinations that exist some outbreaks may go unnoticed and besides not all veterinary camps were manned (Muuka *et al*., 2012).

The outbreak case data used in this study was readily available from the Department of Veterinary Services archives. However, obtaining information on some of the risk factors was not straight forward. Information had to be cross checked and sometimes expert opinion were solicited which may have introduced recall bias into the study. Missing values were included in the analysis but assigned a unique number as recommended (Dohoo *et al*., 2003). Despite all this, the presented data gives an accurate account of the FMD situations in the period under review. As pointed out elsewhere (Hamoonga *et al*., 2014) the consistency of outbreak distribution within the known high risk areas provides indirect evidence of an absence of selection or misclassification bias in the 1933 to 2012 data as well as little systematic error in disease reporting. Further, the spatial

persistency of the identified outbreak areas implies that risk factors for FMD incursions in Zambia have remained relatively constant over time.

5.2 Cross sectional study

5.2.1 Sero-prevalence

Seropositivity of FMD in cattle at animal level was observed to be high especially in cattle from Itezhitezhi and Monze (Table 10). These findings are consistent with animal level sero-prevalence studies done in other endemic areas of Ethiopia and Bhutan where similar values were observed (Megersa *et al*., 2009; Dukpa *et al*., 2011; Jenbere *et al*., 2011).

The poor agreement observed in cattle between the LPBE and the 3ABC ELISA may be because of the routine vaccinations that are carried out. As mentioned earlier, the LPBE measures SP which may be high in vaccinated populations while the 3ABC measures NSP from past or ongoing infection. The NSP reaction as a result of non-purified vaccine in use is extremely low (< 0.01 percent) based on validation of the Priocheck NSP assays performed by the ARC-OVI (Melanie Chitray, personal communication). This is further confirmed by the slightly better agreement (Kappa=0.296) observed between the two tests in buffalo (Sikombe *et al*., 2015, in press). Therefore, there is validity in the observed results. The observed seropositivity in Sesheke in an area where vaccination is not practiced was suspected to be a result of vaccinated animals that may have been moved from areas under vaccination.

5.2.2 Risk factor analysis

In this study, the difference in sero-prevalence in cattle among the study sites could be attributed to transhumant grazing similar to pastoral system found in Ethiopia (Megersa *et al*., 2009). Therefore, the relatively high seropositivity in Itezhitezhi and Monze may have been due to transhumant grazing, a practice that was absent in the study areas in Lundazi, Livingstone, Kalomo, Kazungula and Sesheke. In these areas, the absence of this practice may have resulted into reduced contact between cattle and carrier buffalo hence the low seropositivity in these areas. Therefore, transhumant grazing may have to be considered during risk surveillance and disease control strategies.

In Lundazi, the absence of seropositivity makes biological plausibility because while wildlife was in the valley, most cattle are kept on the plateau for fear of tsetse flies (Mubanga Joseph, personal communication). Furthermore, movement of cattle from Lundazi is usually towards the Malawi market thereby reducing the contact between buffalo and cattle.

Because vaccination is mandatory in high risk areas and no records existed to differentiate vaccinated from non-vaccinated animals, vaccinated animals were included in the study. The effect of vaccination in confounding the results was taken into account by segregating the animals by age. However, the seropositivity was not significantly different by age in this study (Table 11 and Table 13) contrary to other studies (Megersa *et al*., 2009; Jenbere *et al*., 2011).

5.2.3 Virus Isolation

From the seven study sites virus isolation from probang samples in buffalo was only successful in three out of seven places sampled that is Kafue NP's Ngoma

area, Lower Zambezi and Luambe NPs, while for cattle success was only recorded in Hakunkula in Monze (Table 14). These were also the same areas that recorded high seropositivity in cattle except for lower Zambezi NP where no cattle were sampled and Lundazi where seropositivity in cattle was absent. The poor success rate with virus recovery from probangs is well known (OIE, 2014a). The poor virus recovery rates may have been compounded also by the long logistical time delay of a year before sample analysis could be conducted.

The SAT 2 topotype II isolate from Ngoma buffalo is probably the first record of this topotype in Kafue NP. Previous buffalo isolates from this park were topotype III of SAT 2 from Mulangu and Nanzhila areas conducted in 1993 and 1996. This finding may be an indication that there may be several other topotypes circulating in buffalo and other wildlife populations emphasizing the need to continuously monitor the viruses circulating in these species. Determination of the r value in relation to the vaccine used in this area (vaccine matching studies) are yet to be conducted for this strain. It's plausible that this topotype II may have been introduced through buffalo migration that is known to exist between Kafue NP and the NPs across the Zambezi river in Zimbabwe, Botswana and Namibia (Debaste, 1987) or it could be that it has been in existence in this herd of buffalo for many years and was not picked up due to the inadequate surveillance (Dawe *et al*., 1994a; Dawe *et al*., 1994b).

The SAT 1 topotype I isolate from vaccinated cattle in Hakunkula provides evidence of the existence of the carrier state 24 months after the last recorded outbreak in the area. The 99 percent bootstrap agreement in clustering between this virus isolate and the previous outbreak virus of 2004 to 2008 provides strong evidence of a carrier state from natural infection and not from vaccination (Figure

24). It has been suggested that probably the serial passage of the FMDV in the same species (carrier animal) overtime may result in strengthening of the virus and subsequent transmission to naïve cattle resulting in an outbreak (Condy and Hedger, 1974; Grubman and Baxt, 2004; Carrillo *et al*., 2007). However, although this is probable, supportive evidence is lacking. This finding has implications for animal movements from the high risk areas to low risk areas as this may result in the spread of the disease. Therefore, the carrier status of such animals would require to be ascertained before any animal could be moved to low risk areas (Zhang and Alexandersen, 2003). This finding is consistent with previous findings in Nakonde in 1977 where serotype O was isolated 19 months after the outbreak of 1976 (Perry and Hedger, 1984).

5.3 Transmission modeling

A deterministic transmission model of FMD that incorporated a carrier class in the Kafue Flats of Namwala was developed and used for the analysis. The model predicted that following introduction of one infectious animal in Namwala, it would take about 73 days for the epidemic to reach its peak assuming a basic reproduction number of five ($R_o = 5$). The epidemic would take a shorter period (45 days) to reach its peak if the basic reproduction number was high ($R_o = 9$). When the infectious period was adjusted to 45 days to account for the duration of the disease before recovery and assuming the proportion of infected that become carriers to be 50 percent, the epidemic took about 100 days to reach its peak. Therefore, we conclude that depending on the basic reproduction number and duration of the infectious period, the epidemic may take 45 to 100 days to peak. These findings are consistent with other FMD modeling studies where epidemics

lasted <100 days (Ward *et al*., 2009) and 30 to 109 days (Schoenbaum and Terry Disney, 2003). The findings make biological plausibility because usually the disease would have been circulating for some time before it was diagnosed and peaks much later after the first case has been reported. The basis reproduction numbers used in this study were based on studies done elsewhere, therefore, locally derived estimates are required. The model also agrees with current evidence that carriers will continue post epidemic although the duration and magnitude have not been determined. For example, this study identified a SAT 1 carrier in a vaccinated cattle herd in Monze, two years after the last FMD case was reported in the area. Therefore, knowledge of the level and duration of carriers in the Kafue Flats is required to improve model predictions.

The model prediction of the inter-epidemic period seems to be in agreement with the retrospective study. Probably the serotype specific inter-epidemic periods observed in the retrospective study (section 4.1.1.1) may be explained by the model inter-epidemic predictions of nine to 23 years. The long periods between outbreaks may be due to cyclicity of an immunising infection, basic reproduction number and changes in susceptible population from the effect of birth and mortality rates (Vynnycky and White, 2010a). Therefore, knowledge of these parameters is critical to understanding the epidemiology of disease in this area where outbreaks have been suspected to be from carrier buffalo to cattle during transhumance (Zyambo, 1975; Perry and Hedger, 1984; Sinkala *et al*., 2014a).

The main purpose of deterministic models is to aid understanding and increase knowledge, an aspect that has been achieved in this study. For example, the knowledge generated on the duration of epidemics and inter-epidemic periods is important in developing surveillance and control strategies for FMD (Carpenter *et*

al., 2004). However, this model like all others, is a simplification of a complex process and should be questioned against the assumptions made. The transmission dynamics may change depending on the virus antigenicity and virulence (emergence of new mutants) which may be due to the host factors within the wild buffalo reservoir or carrier cattle. Further, influence may come from the movement behaviour of buffalo, transhumance and cattle density influence on the rate of contact as changes in these may result in shorter inter epidemic periods (Tekleghiorghis *et al*., 2014). The environmental changes on the Kafue Flats such as drought or over flooding may shorten the inter-epidemic period due to reducing pasture thereby increasing contact between buffalo and cattle (Sheppe, 1985; Haller, 2012). Further, a higher basic reproduction number and lower infectious period depletes susceptible quickly thereby shortening the inter-epidemic period.

This study assumed one infected animal being introduced in the susceptible population, but in reality there may be several animals that get infected at the same time. Also individuals are infected or develop disease on a continuous rate and not at discrete times (Vynnycky and White, 2010a). The frequency of transmission of FMD from buffalo to cattle is not known and is believed to be a rare event under unknown circumstances (Anderson, 1986; Gainaru *et al*., 1986; Dawe *et al*., 1994a; Bastos *et al*., 2000; Vosloo *et al*., 2002b; Rweyemamu *et al*., 2008b). Gainaru *et al*., (1986) reported that under normal field conditions, only buffalo in acute stages of infection with SAT types were likely to provide a direct potential source of infection to cattle and only when close contact occurs. Therefore, the probability of an effective contact between buffalo and cattle per unit time, where an effective contact is defined as one sufficient to lead to transmission if it occurred between a susceptible and infectious individual is

unknown. Quantification of within buffalo and between buffalo and cattle transmission is not possible at the moment because of lack of knowledge.

The study also assumed homogenous mixing, mass action but in reality there is heterogeneity in the mixing for example animals in the neighboring *lutanga* (super-herd) are likely to mix regularly than animals in distant *lutanga*. The distance covered during grazing is dependent on the availability of green lush pasture which is dependent on the flooding and period within the dry season. The proportion of calves born with maternal antibodies that lasts until six months when they contribute to the susceptible population under existing vaccination regimes is not known.

The government carries out biannual vaccination in the Kafue Flats. As indicated previously, vaccination in endemic settings of resource poor countries like Zambia faces several challenges including low vaccination coverage and vaccine failure that may arise due to the many factors (Sinkala *et al*., 2014b). The herd immunity threshold (HIT) for FMD has been estimated to be 80 percent (Hunter, 1998). From the estimated population of 150,000 used in this study, to achieve the required 80 percent HIT 120,000 cattle must be vaccinated and at least 70 percent may have to seroconvert. Currently, between 125,000 and 130,000 cattle are vaccinated biannually in Namwala which is sufficient for the required HIT (Anonymous, 2014b). But If the assumption that the cattle population of Namwala during transhumance is about 300, 000 (Malama *et al*., 2014), then 240, 000 would require to be vaccinated. Reliable census figures that can be used to validate the model is therefore required.

CHAPTER SIX

6.0 CONCLUSION

FMD occurrence in Zambia has remained confined to three high risk areas of Kafue Flats, lower Zambezi basin and Mbala-Isoka area. In southern Zambia, the southern African form is dominant with a prominence of both SAT 1 and SAT 2 in Kafue Flats, while in lower Zambezi basin SAT 1 is prominent in Livingstone and SAT 2 in Kazungula/Sesheke. In northern Zambia, the Eurasian or South American type prevail, dominated by serotype O with incursions of SAT 2 that has remained confined to Mbala, while SAT 1 and serotype A have remained confined to Nakonde. The risk factors for FMD occurrence identified in this study included rainfall (droughts and floods), agro-ecological zone, proximity to buffalo migratory route and international border.

The FMDV seropositivity in cattle varied at animal level within the interface areas evaluated. Seropositivity was highest in cattle in Basanga Plains of Itezhitezhi district, Hakunkula of Monze district and absent in Lundazi district and Dundumwezi area of Kalomo district. The transhumant husbandry system was identified as the main risk factor for seropositivity. Molecular characterization of FMDV circulating in buffalo and cattle was only successful at three sites for buffalo and one site for cattle. A new isolate of SAT 2 topotype II previously not identified in Zambia was isolated from buffalo in Ngoma area of Kafue NP. SAT 1 was identified in Lower Zambezi NP and SAT 2 in Luambe NP, however the resulting sequences from these were not very informative due to low DNA quality for phylogenetic analysis. SAT 1 topotype I similar to FMD virus responsible for

the 2004 to 2008 outbreaks in the Kafue Flats was isolated from a vaccinated cow suggestive of carrier status.

The transmission model predicted that following introduction of one infectious animal in Namwala District of the Kafue Flats, the infection incidence would increase by 0.6 to 1.1 per day and an epidemic would peak between days 45 to 109 depending on basic reproduction number and infectious period and would die out by day 135 with a possible inter-epidemic period of nine to 23 years. Although no model can capture reality, the transmission model developed in this study predicted inter-epidemic periods that are in agreement with the serotype specific inter-epidemic periods predicted by the retrospective study that was based on outbreak cases. Even though the model is yet to be validated with parameters developed from Zambian disease situation, the predictions that have been made describe the FMD disease progression in the Kafue Flats of Zambia with some degree of confidence in the results.

This study has described significant elements of FMD epidemiology in Zambia including the spatial and temporal patterns, sero-prevalence of FMDV in cattle at selected livestock/wildlife interface areas. Furthermore, associated risk factors, evidence of an additional circulating topotype and carrier status that exist, duration of epidemics and inter-epidemic periods have been determined. These elements should be considered when developing risk surveillance and FMD control strategies at national and regional levels in line with the progressive control pathway for FMD.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

Considering that the ultimate aim of elucidating the epidemiology of a disease is to provide information relevant for prevention and control, the following recommendations are made based on findings of this FMD study in Zambia:

1. Surveillance should consider ecosystem epidemiological variability including effects of droughts, trade initiated livestock movement, buffalo migration routes, existence of other wildlife antelopes and small ruminants and proximity to international boundaries
2. Optimization of the 3ABC ELISA and LPBE serological tests so that antigens used in the tests are closely matched to the field strains is urgently required.
3. Knowledge of the risks associated with the livestock value chains and marketing in Zambia and how this influences the FMD epidemiology need to be investigated.
4. There is need to locally generate reliable FMD transmission variables and parameters such as cattle census data, basic reproduction number, duration of carrier status, infectious period to improve model prediction. The variables and parameters once generated could also be used to test the effectiveness of the control measures especially vaccination.

8.0 REFERENCES

Acharya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D., Brown, F., 1989. The three-dimensional structure of foot-and-mouth disease virus at 2.9 A resolution. Nature 337, 709-716.

Acharya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D., Brown, F., 1990. The structure of foot-and-mouth disease virus: implications for its physical and biological properties. Veterinary Microbiology 23, 21-34.

Akafekwa, G., 1980. The sanitary position and methods of control used in Zambia - 1979 Bulletin de l'Office International des Epizooties 92 (7/8), 647-653.

Alexandersen, S., Quan, M., Murphy, C., Knight, J., Zhang, Z., 2003. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. J. Comp. Pathol. 129, 268-282.

Alexandersen, S., Zhang, Z., Donaldson, A.I., Alexandersen, S., Zhang, Z., Donaldson, A.I., 2002. Aspects of the persistence of foot-and-mouth disease virus in animals—the carrier problem. Microbes and Infection 4, 1099-1110.

Anderson, E.C., 1986. Potential for the Transmission of Foot-and-Mouth-Disease Virus from African Buffalo (Syncerus-Caffer) to Cattle. Res Vet Sci 40, 278-280.

Anderson, E.C., Doughty, W.J., Anderson, J., 1976. The role of sheep and goats in the epizootiology of foot and mouth disease in Kenya. Journal of Hygiene 76, 395-402.

Anonymous, 1968. Annual Report for the Department of Veterinary and Tsetse Control Services for the year 1968. Government of Republic of Zambia, Ministry of Land and Natural Resources. Printed by Government Printer 1969. Page 4

Anonymous, 1997. Mbeya District Socio Economic Profile. United Republic of Tanzania, The Planning Commisssion Dar es salaam and Mbeya District Council. http://www.tzonline.org/pdf/Mbeyadis.pdf, Accessed: 28th October 2013.

Anonymous, 2006. Fifth National Development Plan for 2006 to 2010. Republic of Zambia, Ministry of Finance and National Planning, Published December 2006 Lusaka.

Anonymous, 2008. Productive Sectors. Rukwa Regional Commissioners Office, http://www.rukwa.go.tz/productivesc.html, Accessed: 28th October 2013.

Anonymous, 2009. Disease Free Zone Concept Paper. Ministry of Agriculture and Cooperatives, Department of Veterinary and Livestock Development, Lusaka.

Anonymous, 2010. Annual Report. Republic of Zambia. Bank of Zambia (http://www.boz.zm/AnnualReports/); Accessed: 22nd January 2013.

Anonymous, 2011a. Annual report for the Department of Veterinary Services for 2010. Government of Zambia - Ministry of Livestock and Fisheries Development, Lusaka.

Anonymous, 2011b. Sixth National Development Plan for Zambia 2011 to 2015. Republic of Zambia, Ministry of Finance and National Planning, www.mofnp.gov.zmpublished, Accessed: 11th June 2013.

Anonymous, 2012. Resolution by the Southern African Development Community (SADC) Calling for Adoption of Commodity-Based Trade and Other Non-Geographic Approaches for Foot and Mouth Disease Management as Additional Regional Standards for Trade in Animal Products. In, Joint SADC/AHEAD Workshop: Reconcilling Livestock Health and Wildlife Conservation goals in Southern Africa: Strategies for Sustainable Economic Development, Phakalane Golf Estates, Gaborone, Botswana.13-15 November 2012.

Anonymous, 2013a. Annual rainfall and temperature database for selected stations in Zambia from 1932 to date, *unpublished*. Meteorological Department under the Ministry of Transport, Work, Supply and Communication, Lusaka.

Anonymous, 2013b. Operation Noah, A Zambia Safari Rescue Story. http://operationnoah.blogspot.com/, Accessed: 30th September 2013.

Anonymous, 2013c. World Fact Book - Zambia. www.cia.gov/library/publications/the-world-factbook/geos/za.htm, Accessed: 15th August 2013.

Anonymous, 2014a. Bank of Zambia anuual report 2013. BOZ, http://www.boz.zm/publishing/39/39_BOZ%20ANNUAL%20REPORT%202013.pdf, Accessed: 24th July 2014.

Anonymous, 2014b. Department of Veterinary Services Annual Report for 2013.

Arzt, J., Juleff, N., Zhang, Z., Rodriguez, L.L., 2011. Pathogenesis of Foot-and-Mouth Disease I: Viral Pathways in Cattle. Pathogenesis of Foot-and-Mouth Disease I: Viral Pathways in Cattle 58, 291-304.

Banda, Christopher J. Kasanga, Raphael Sallu, Yona Sinkala, Tingiya Sinkombe, Misheck Mulumba, Mark Rweyemamu, Philemon Wambura, 2014. Investigation of foot-and-mouth disease outbreaks in the Mbala and Kazungula districts of Zambia. Onderstepoort J Vet Res 81.

Barteling, S., 2002. Development and performance of inactivated vaccines against foot and mouth disease. Revue scientifique et technique-Office international des épizooties 21, 577-583.

Bastos, A., 2001. Molecular epidemiology and diagnosis of SAT-type foot-and-mouth disease in southern Africa. PhD thesis, University of Pretoria.

Bastos, A.D., Boshoff, C.I., Keet, D.F., Bengis, R.G., Thomson, G.R., 2000. Natural transmission of foot-and-mouth disease virus between African buffalo (Syncerus caffer) and impala (Aepyceros melampus) in the Kruger National Park, South Africa. Epidemiology and infection 124, 591-598.

Bastos, A.D., Haydon, D.T., Forsberg, R., Knowles, N.J., Anderson, E.C., Bengis, R.G., Nel, L.H., Thomson, G.R., 2001. Genetic heterogeneity of SAT-1 type foot-and-mouth disease viruses in southern Africa. Arch Virol 146, 1537-1551.

Bastos, A.D., Haydon, D.T., Sangare, O., Boshoff, C.I., Edrich, J.L., Thomson, G.R., 2003. The implications of virus diversity within the SAT 2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. J Gen Virol 84, 1595-1606.

Bastos, A.D.S., Bertschinger, H.J., Cordel, C., Van Vuuren, C.d.W.J., Keet, D., Bengis, R.G., Grobler, D.G., Thomson, G.R., Bastos, A.d.s., Bertschinger, H.j., Cordel, C., Van Vuuren, C.D.W.j., Keet, D., Bengis, R.g., Grobler, D.g., Thomson, G.r., 1999. Possibility of sexual transmission of foot-and-mouth disease from African buffalo to cattle. Possibility of sexual

transmission of foot-and-mouth disease from African buffalo to cattle 145, 77-79.

Baxi, M.K., Baxi, S., Clavijo, A., Burton, K.M., Deregt, D., 2006. Microarray-based detection and typing of foot-and-mouth disease virus. Vet J 172, 473-481.

Bayissa, B., Ayelet, G., Kyule, M., Jibril, Y., Gelaye, E., 2011. Study on seroprevalence, risk factors, and economic impact of foot-and-mouth disease in Borena pastoral and agro-pastoral system, southern Ethiopia. *Trop Anim Health Prod* 43, 759-766.

Belsham, G.J., 1993. Distinctive features of foot-and-mouth disease virus, a member of the picornavirus family; aspects of virus protein synthesis, protein processing and structure. . *Progress in Biophysics and Molecular Biology* 60, 241-260.

Belsham, G.J., Jamal, S.M., Tjørnehøj, K., Bøtner, A., 2011. Rescue of foot-and-mouth disease viruses that are pathogenic for cattle from preserved viral RNA samples. PloS one 6, e14621.

Bengis, R., Kock, R., Thomson, G., Bigalke, R., 2004. Infectious diseases of animals in sub-Saharan Africa: The wildlife/livestock interface. Infectious diseases of livestock, 225-238.

Boisvert, R.N., Kay, D., Turvey, C.G., Boisvert, R.N., Kay, D., Turvey, C.G., 2012. Macroeconomic costs to large scale disruptions of food production: The case of foot-and-mouth disease in the United States. Econ. Model. 29, 1921-1930.

Borley, D.W., Mahapatra, M., Paton, D.J., Esnouf, R.M., Stuart, D.I., Fry, E.E., 2013. Evaluation and use of in-silico structure-based epitope prediction with foot-and-mouth disease virus. PloS one 8, e61122.

Brehm, K.E., Kumar, N., Thulke, H.H., Haas, B., Brehm, K.E., Kumar, N., Thulke, H.H., Haas, B., 2008. High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus. Vaccine 26, 1681-1687.

Brocchi, E., Bergmann, I.E., Dekker, A., Paton, D.J., Sammin, D.J., Greiner, M., Grazioli, S., De Simone, F., Yadin, H., Haas, B., Bulut, N., Malirat, V., Neitzert, E., Goris, N., Parida, S., Sorensen, K., De Clercq, K., 2006. Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. Vaccine 24, 6966-6979.

Bronsvoort, B.M., Parida, S., Handel, I., McFarland, S., Fleming, L., Hamblin, P., Kock, R., 2008. Serological survey for foot-and-mouth disease virus in wildlife in eastern Africa and estimation of test parameters of a nonstructural protein enzyme-linked immunosorbent assay for buffalo. Clin Vaccine Immunol 15, 1003-1011.

Brostoff, N.A., 2012. Endemicity and the Carrier Class: Modeling Foot-and-Mouth Disease in the Lake Chad Basin, Cameroon. The Ohio State University, Ohio, https://etd.ohiolink.edu/ap/10?0::NO:10:P10_ACCESSION_NUM:osu1338388013, Accessed: 13th June 2014.

Brown, F., 1992. New approaches to vaccination against foot-and-mouth disease. Vaccine 10, 1022-1026.

Brown, F., 2003. The history of research in foot-and-mouth disease. Virus Research 91, 3-7.

Bruckner, G.K., Vosloo, W., Du Plessis, B.J., Kloeck, P.E., Connoway, L., Ekron, M.D., Weaver, D.B., Dickason, C.J., Schreuder, F.J., Marais, T., Mogajane, M.E., 2002. Foot and mouth disease: the experience of South Africa. Rev Sci Tech 21, 751-764.

Callahan, J.D., Brown, F., Csorio, F.A., Sur, J.H., Kramer, E., Long, G.W., Lubroth, J., Ellis, S.J., Shoulars, K.S., Gaffney, K.L., Rock, D.L., Nelson, W.M., 2002. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J. Am. Vet. Med. Assoc. 220, 1636-1642.

Callis, J.J., 1973. Nature of foot-and-mouth disease. In, 2 Internationalnd Conference on Foot-and-mouth Disease, New York, 9-10.

Carpenter, T.E., Thurmond, M.C., Bates, T.W., 2004. A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. Journal of Veterinary Diagnostic Investigation 16, 11-16.

Carrillo, C., Lu, Z., Borca, M., Vagnozzi, A., Kutish, G., Rock, D., 2007. Genetic and phenotypic variation of foot-and-mouth disease virus during serial passages in a natural host. Journal of virology 81, 11341-11351.

Chabwela, H.N.W., Wanga, M., 2013. Integrating Water Conservation and Population Strategies on the Kafue Flats: Case Study: Zambia. Lusaka. http://www.aaas.org/international/ehn/waterpop/zambia.htm, Accesed: 16th July 2013.

Chansa, W., Kampamba, G., 2009. The population status of the Kafue Lechwe in the Kafue Flats, Zambia. African Journal of Ecology 48, 837-840.

Charleston, B., Bankowski, B.M., Gubbins, S., Chase-Topping, M.E., Schley, D., Howey, R., Barnett, P.V., Gibson, D., Juleff, N.D., Woolhouse, M.E., 2011. Relationship between clinical signs and transmission of an infectious disease and the implications for control. Science 332, 726-729.

Chilonda, P., Woodford, J.D., Ahmadu, B., Samui, K.L., Syakalima, M., Mlangwa, J.E., 1999. Foot and mouth disease in Zambia: a review of the aetiology and epidemiology and recommendations for possible control. Rev Sci Tech 18, 585-592.

Chimana, H.M., Muma, J.B., Samui, K.L., Hangombe, B.M., Munyeme, M., Matope, G., Phiri, A.M., Godfroid, J., Skjerve, E., Tryland, M., 2010. A comparative study of the seroprevalence of brucellosis in commercial and small-scale mixed dairy–beef cattle enterprises of Lusaka province and Chibombo district, Zambia. Tropical animal health and production 42, 1541-1545.

Clavijo, A., Kitching, P., 2003. The nature and diagnosis of foot-and-mouth disease. Clinical Microbiology Newsletter 25, 81-88.

Collen, T., Dimarchi, R., Doel, T.R., 1991. A T-Cell Epitope in Vp1 of Foot-and-Mouth-Disease Virus Is Immunodominant for Vaccinated Cattle. J Immunol 146, 749-755.

Collins, R.A., Ko, L.S., Fung, K.Y., Lau, L.T., Xing, J., Yu, A.C.H., 2002. A method to detect major serotypes of foot-and-mouth disease virus. Biochem Biophys Res Commun 297, 267-274.

Condy, J.B., Hedger, R.S., 1974. The survival of foot and mouth disease virus in African Buffalo with non-transference of infection to domestic cattle. Research Veterinary Science 16, 182-185.

Condy, J.B., Hedger, R.S., Hamblin, C., Barnett, I.T.R., 1985. The duration of the foot-and-mouth disease virus carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd. Comparative Immunology, Microbiology and Infectious Diseases 8, 259-265.

Condy, J.B., Herniman, K.A., Hedger, R.S., 1969. Foot-and-mouth disease in wildlife in Rhodesia and other African territories. A serological survey. J Comp Pathol 79, 27-31.

Cottam, E.M., The´baud, G.l., Wadsworth, J., Gloster, J., Mansley, L., Paton, D.J., King, D.P., Haydon, D.T., 2008. Integrating genetic and epidemiological data to determine transmission pathways of foot-and-mouth disease virus. Proc.R.Soc.B 275, 887-895.

Cox, S.J., Voyce, C., Parida, S., Reid, S.M., Hamblin, P.A., Paton, D.J., Barnett, P.V., 2005. Protection against direct-contact challenge following emergency FMD vaccination of cattle and the effect on virus excretion from the oropharynx. Vaccine 23, 1106-1113.

CSO, 2012. Living Conditions and Monitoring Survey. Central statistics Office, Lusaka, http://www.zamstats.gov.zm/report/Lcms/2006-2010%20LCMS%20Report%20Final%20Output.pdf.

CSO, 2010. Zambia 2010 Census of Population and Housing Lusaka Central Statistics Office, http://unstats.un.org/unsd/demographic/sources/census/2010_phc/Zambia/PreliminaryReport.pdf; Accessed: 22nd October 2012.

Davies, G., 2002. Foot and mouth disease. Research in Veterinary Science 73, 195-199.

Dawe, P.S., Flanagan, F.O., Madekurozwa, R.L., Sorensen, K.J., Anderson, E.C., Foggin, C.M., Ferris, N.P., Knowles, N.J., 1994a. Natural transmission of foot-and-mouth disease virus from African buffalo (Syncerus caffer) to cattle in a wildlife area of Zimbabwe. Vet Rec 134, 230-232.

Dawe, P.S., Sorensen, K., Ferris, N.P., Barnett, I.T., Armstrong, R.M., Knowles, N.J., 1994b. Experimental transmission of foot-and-mouth disease virus from carrier African buffalo (Syncerus caffer) to cattle in Zimbabwe. Vet Rec 134, 211-215.

de Garine-Wichatitsky, M., Miguel, E., Mukamuri, B., Garine-Wichatitsky, E., Wencelius, J., Pfukenyi, D.M., Caron, A., 2013. Coexisting with wildlife in transfrontier conservation areas in Zimbabwe: cattle owners' awareness of disease risks and perceptions of the role played by wildlife. Comp Immunol Microbiol Infect Dis 36, 321-332.

Debaste, H., 1987. Foot and mouth disease: Zambia. 17th Conference of the OIE foot and mouth disease Commission. Paris, 1-3 October, 1986, 50-52.

Di Nardo, A., Knowles, N.J., Paton, D.J., 2011. Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. Rev Sci Tech 30, 63-85.

Ding, Y.Z., Liu, Y.S., Zhou, J.H., Chen, H.T., Zhang, J., Ma, L.N., Wei, G., 2011. A highly sensitive detection for foot-and-mouth disease virus by gold nanopariticle improved immuno-PCR. Virol J 8, 148.

Dohoo, I., Martin, W., Stryhn, H. (Eds.), 2003. Veterinary Epidemiologic Research. AVC.

Domingo, E., Baranowski, E., Escarmis, C., Sobrino, F., Domingo, E., Baranowski, E., Escarmis, C., Sobrino, F., 2002. Foot-and-mouth disease virus. Comp. Immunol. Microbiol. Infect. Dis. 25, 297-308.

Domingo, E., Holland, J., 1997. RNA virus mutations and fitness for survival. Annual Reviews in Microbiology 51, 151-178.

Dukes, J.P., King, D.P., Alexandersen, S., 2006. Novel reverse transcription loop-mediated isothermal amplification for rapid detection of foot-and-mouth disease virus. Arch Virol 151, 1093-1106.

Dukpa, K., Robertson, I.D., Ellis, T.M., 2011. The seroprevalence of foot-and-mouth disease in the sedentary livestock herds in four districts of Bhutan. Preventive Veterinary Medicine 100, 231-236.

Dunn, C.S., Samuel, A.R., Pullen, L.A., Anderson, J., 1998. The biological relevance of virus neutralisation sites for virulence and vaccine protection in the guinea pig model of foot-and-mouth disease. Virology 247, 51-61.

El-Sayed, E., El-Din, W.M.G., Rizk, S.A., El-Aty, M.A., 2012. Effect of Different Storage Temperatures on the Efficacy of the Bivalent Foot and Mouth Disease Oil Vaccine. Journal of Advanced Veterinary Research 2, 198-205.

Eng, J., 2003. Sample size estimation: how many individuals should be studied? Radiology 227, 309-313.

Falconer, J., 1972. The Epizootiology and Control of Foot-and-Mouth Disease in Botswana. . *Vet Rec* 91, 354 - 359.

FAO-WRLFMD, 2012. OIE/FAO Reference Laboratory Contract Report January - March FAO World Reference Laboratory for FMD, Pirbright. http://www.wrlfmd.org/ref_labs/ref_lab_reports/OIE-FAO%20FMD%20Ref%20Lab%20Report%20Jan-Mar%202012.pdf, Accesed: 13th December 2013.

FAO, 2005. Livestock Sector Brief on Zambia Livestock Information, Sector Analysis and Policy Branch, www.fao.org/ag/againfo/resources/en/publications/sector_briefs/lsb_ZMB.pdf, Accessed: 16th September 2013.

FAO, 2009. Country Pasture/Forage Resource Profiles. FAO, http://www.fao.org/ag/agp/AGPC/doc/Counprof/PDF%20files/Zambia.pdf , Accessed 31st December 2013.

FAO, 2014. Country Statistics on Zambia Food and Agriculture Organisation of the United Nations, http://www.fao.org/countryprofiles/index/en/?iso3=ZMB.

Ferguson, K.J., Cleaveland, S., Haydon, D.T., Caron, A., Kock, R.A., Lembo, T., Hopcraft, J.G., Chardonnet, B., Nyariki, T., Keyyu, J., Paton, D.J., Kivaria, F.M., 2013. Evaluating the potential for the environmentally sustainable control of foot and mouth disease in Sub-Saharan Africa. Ecohealth 10, 314-322.

Ferris, N.P., Dawson, M., 1988. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases 16, 201-209.

Ferris, N.P., Nordengrahn, A., Hutchings, G.H., Paton, D.J., Kristersson, T., Brocchi, E., Grazioli, S., Merza, M., 2010. Development and laboratory

validation of a lateral flow device for the detection of serotype SAT 2 foot-and-mouth disease viruses in clinical samples. J Virol Methods 163, 474-476.

Ferris, N.P., Nordengrahn, A., Hutchings, G.H., Reid, S.M., King, D.P., Ebert, K., Paton, D.J., Kristersson, T., Brocchi, E., Grazioli, S., Merza, M., 2009. Development and laboratory validation of a lateral flow device for the detection of foot-and-mouth disease virus in clinical samples. J Virol Methods 155, 10-17.

Fox, G., Parry, N.R., Barnett, P.V., McGinn, B., Rowlands, D.J., Brown, F., 1989. The cell attachment site on foot-and-mouth disease virus includes the amino acid sequence RGD (arginine-glycine-aspartic acid). The Journal of general virology 70 (Pt 3), 625.

Gainaru, M.D., Thomson, G.R., Bengis, R.G., Esterhuysen, J., Bruce, W., Pini, A., 1986. Foot and mouth disease and the African buffalo (*Syncerus Caffer*). II. Virus Excretion and the transmission during acute infection Onderstepoort J Vet Res 53, 75-85.

Gebauer, F., de la Torre, J.C., Gomes, I., Mateu, M.G., Barahona, H., Tiraboschi, B., Bergmann, I., de Mello, P.A., Domingo, E., 1988. Rapid selection of genetic and antigenic variants of foot-and-mouth disease virus during persistence in cattle. The Journal of Virology 62, 2041.

GFRA, 2013. Global Foot and Mouth Disease Research Alliance Workshop Resolutions In, 2013 GFRA Scientific Workshop, Epidemiology, Surveillance, Transmission and Control of Foot-and-Mouth Disease in Endemic Settings of Africa. Hosted by Sokoine University of Agriculture and Southern Africa Centre for Infectious Disease Surveillance, 8 to 10 October 2013, Arusha, Tanzania

Goris, N., Merkelbach-Peters, P., Diev, V., Verloo, D., Zakharov, V., Kraft, H.-P., De Clercq, K., 2007. European Pharmacopoeia foot-and-mouth disease vaccine potency testing in cattle: between test variability and its consequences. Vaccine 25, 3373-3379.

Grimsdell, J.J.R., 1973. Age determination of the African buffalo, Syncerus caffer sparrman. *East African Wildlife J* 11, 54.

Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. Clin Microbiol Rev 17, 465-493.

Hall, M.D., Knowles, N.J., Wadsworth, J., Rambaut, A., Woolhouse, M.E., . 2013. Reconstructing Geographical Movements and Host Species Transitions of Foot-and-Mouth Disease Virus Serotype SAT 2. MBio. 2013 Oct 22 4.

Haller, T., 2012. The Contested Floodplain. Institutional Change of Commons in the Kafue Flats of Zambia. Lexington, Lahman.

Hamblin, C., Barnett, I., Crowther, J., 1986a. A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus: II. Application. Journal of immunological methods 93, 123-129.

Hamblin, C., Barnett, I., Hedger, R., 1986b. A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus I. Development and Method of ELISA. Journal of Immunological Methods 93, 115-121.

Hamoonga, R., Stevenson, M.A., Allepuz, A., Carpenter, T.E., Sinkala, Y., 2014. Risk factors for foot-and-mouth disease in Zambia, 1981–2012. Preventive Veterinary Medicine 114, 64-71.

Hanson, C.E., Wieczorek, W.F., 2002. Alcohol mortality: a comparison of spatial clustering methods. Social Science & Medicine 55, 791-802.

Hargreaves, S.K., Foggin, C.M., Anderson, E.C., Bastos, A.D., Thomson, G.R., Ferris, N.P., Knowles, N.J., 2004. An investigation into the source and spread of foot and mouth disease virus from a wildlife conservancy in Zimbabwe. Rev Sci Tech 23, 783-790.

Haydon, D.T., Samuel, A.R., Knowles, N.J., 2001. The generation and persistence of genetic variation in foot-and-mouth disease virus. Preventive Veterinary Medicine 51, 111-124.

Hedger, R.S., 1972. Foot-and-mouth disease and the African buffalo (Syncerus caffer). J Comp Pathol 82, 19-28.

Hogan, C., 2012. Zambezi River. http://www.eoearth.org/view/article/51cbf2e97896bb431f6ab2de, Accessed: 26th October 2014.

Hunter, P., 1998. Vaccination as a means of control of foot-and-mouth disease in sub-saharan Africa. Vaccine 16, 261-264.

Hyera, J.M., Letshwenyo, M., Monyame, K.B., Thobokwe, G., Pilane, A.R., Mapitse, N., Baipoledi, E.K., 2006. A serological survey for antibodies to foot-and-mouth disease virus in indigenous Tswana goats and sheep in Kasane, Maun and Shakawe districts in northwestern Botswana. Onderstepoort J Vet Res 73, 143-147.

Jamal, S.M., Belsham, G.J., 2013. Foot-and-mouth disease: past, present and future. Veterinary research 44, 116.

James, A.D., Rushton, J., 2002. The economics of foot and mouth disease. Rev Sci Tech 21, 637-644.

Jenbere, T.S., Etana, M., Negussie, H., 2011. Study on the Risk Factors of Foot and Mouth Disease in Selected Districts of Afar Pastoral Area, Northeast Ethiopia. J. Anim. Vet. Adv. 10, 1368-1372.

Jibat, T., Admassu, B., Rufael, T., Baumann, M., Pötzsch, C., Jibat, T., Admassu, B., Rufael, T., Baumann, M., Pötzsch, C., 2013. Impacts of foot-and-mouth disease on livelihoods in the Borena Plateau of Ethiopia. Pastoralism: Research, Policy and Practice 3, 1-11.

Jolles, A.E., 2007. Population biology of the African buffalo (Syncerus caffer) at Hluhluwe-Mfolozi Park, South Africa. *Afric J Ecology* 45, 398-406.

Jori, F., Vosloo, W., Du Plessis, B., Bengis, R., Brahmbhatt, D., Gummow, B., Thomson, G., 2009. A qualitative risk assessment of factors contributing to foot and mouth disease outbreaks in cattle along the western boundary of the Kruger National Park. Revue scientifique et technique 28, 917.

Karber, G., 1931. 50% end -point calculation *Arch. Exp. Pathol. Pharmak* 162, 480-483.

Kasanga, C.J., Wadsworth, J., Mpelumbe-Ngeleja, C.A., Sallu, R., Kivaria, F., Wambura, P.N., Yongolo, M.G., Rweyemamu, M.M., Knowles, N.J., King, D.P., 2014a. Molecular Characterization of Foot-and-Mouth Disease Viruses Collected in Tanzania Between 1967 and 2009. Transbound Emerg Dis.

Kasanga, C.J., Yamazaki, W., Mioulet, V., King, D.P., Mulumba, M., Ranga, E., Deve, J., Mundia, C., Chikungwa, P., Joao, L., Wambura, P.N.,

Rweyemamu, M.M., 2014b. Rapid, sensitive and effective diagnostic tools for foot-and-mouth disease virus in Africa. Onderstepoort J Vet Res 81, 5 pages.
Keeling, M.J., 2000. Metapopulation moments: coupling, stochasticity and persistence. Journal of Animal Ecology 69, 725-736.
Keeling, M.J., 2005. Models of foot-and-mouth disease. Proceedings of the Royal Society B: Biological Sciences 272, 1195-1202.
Keeling, M.J., Rohani, P., 2008. Modeling Infectious Diseases in Humans and Animals. Princeton University Press, Chapter 2 page 44.
King, D.P., Dukes, J.P., Reid, S.M., Ebert, K., Shaw, A.E., Mills, C.E., Boswell, L., Ferris, N.P., 2008. Prospects for rapid diagnosis of foot-and-mouth disease in the field using reverse transcriptase-PCR. Vet Rec 162, 315-316.
Kitching, R., Donaldson, A., 1987. Collection and transportation of specimens for vesicular virus investigation. Rev. sci. tech. Off. int. Epiz 6, 263-272.
Kitching, R.P., 2002. Future research on foot and mouth disease. Rev Sci Tech 21, 885-889.
Kitching, R.P., Rendle, R., Ferris, N.P., 1988. Rapid correlation between field isolates and vaccine strains of foot-and-mouth disease virus. Vaccine 6, 403-408.
Kitching, R.P., Salt, J.S., 1995. The interference by maternally-derived antibody with active immunization of farm animals against foot-and-mouth disease. British Veterinary Journal 151, 379-389.
Knight-Jones, T.J.D., Rushton, J., 2013. The economic impacts of foot and mouth disease – What are they, how big are they and where do they occur? Preventive Veterinary Medicine 112, 161-173.
Knowles, N., 2013. Foot-and-Mouth Disease Virus Variation and Molecular Epidemiology in Africa. Global Foot and Mouth Disease Research Alliance Workshop. http://www.ars.usda.gov/GFRA/events/2013%20GFRA%20workshop%20AGENDA.pdf, , 8 -10th October 2013, Arusha, Tanzania.
Knowles, N., Samuel, A., 1998. RT-PCR and sequencing protocols for the molecular epidemiology of exotic virus diseases of animals. Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright.
Knowles, N.J., 1994 Phylogenetic analysis of SAT strains of foot-and-mouth disease virus. . In, OIE International Scientific Conference on the Control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia,, Gaberone, Botswana, 20-23 April 1994.
Knowles, N.J., Nazem Shirazi, M.H., Wadsworth, J., Swabey, K.G., Stirling, J.M., Statham, R.J., Li, Y., Hutchings, G.H., Ferris, N.P., Parlak, U., Ozyoruk, F., Sumption, K.J., King, D.P., Paton, D.J., 2009. Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. Transbound Emerg Dis 56, 157-169.
Knowles, N.J., Samuel, A.R., 1996. RT-PCR and Sequencing Protocols for Molecular Epidemiology of Virus Diseases. In: Group, W.-F.M.E. (Ed.) Institute for Animal Health, Pirbright Laboratory, Surrey.
Knowles, N.J., Samuel, A.R., 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Research 91, 65-80.
Kulldorff, M., 2014. SatScan User Guide for Version 9.3, page 9. http://www.satscan.org, Accessed: 12th August 2014.

Kulldorff, M., Heffernan, R., Hartman, J., Assunção, R., Mostashari, F., 2005. A Space–Time Permutation Scan Statistic for Disease Outbreak Detection. PLoS Med 2, e59.

Kulldorff, M., Nagarwalla, N., 1995. Spatial disease clusters: Detection and inference. Statistics in Medicine 14, 799-810.

Lindsey, P.A., Barnes, J., Nyirenda, V., Pumfrett, B., Tambling, C.J., Taylor, W.A., Rolfes, M.t.S., 2013. The Zambian Wildlife Ranching Industry: Scale, Associated Benefits, and Limitations Affecting Its Development. PLoS ONE 8, e81761.

Lu, Z., Cao, Y., Guo, J., Qi, S., Li, D., Zhang, Q., Ma, J., Chang, H., Liu, Z., Liu, X., 2007. Development and validation of a 3ABC indirect ELISA for differentiation of foot-and-mouth disease virus infected from vaccinated animals. Veterinary microbiology 125, 157-169.

Lubroth, J., 2002. Foot and Mouth Disease A review for the practitioner *Vet Clin Food Anim* 18, 475 - 499.

Mahy, B.W., 2004. Overview of Foot-and-Mouth Disease and Its Impact as a Re-emergent Viral Infection. In: Domingo, E., Sobrino, F. (Eds.), Foot and Mouth Disease: Current Perspectives CRC Press, 438-446.

Malama, S., Johansen, T.B., Muma, J.B., Munyeme, M., Mbulo, G., Muwonge, A., Djonne, B., Godfroid, J., 2014. Characterization of Mycobacterium bovis from Humans and Cattle in Namwala District, Zambia. Veterinary medicine international 2014, 187842.

Mardones, F., Perez, A., Sanchez, J., Alkhamis, M., Carpenter, T., 2010. Parameterization of the duration of infection stages of serotype O foot-and-mouth disease virus: an analytical review and meta-analysis with application to simulation models. Vet Res 41, 45.

Maree, F.F., Blignaut, B., Esterhuysen, J.J., de Beer, T.A., Theron, J., O'Neill, H.G., Rieder, E., 2011. Predicting antigenic sites on the foot-and-mouth disease virus capsid of the South African Territories types using virus neutralization data. J Gen Virol 92, 2297-2309.

Maree, F.F., Kasanga, C., Scott, K., Opperman, P., Chitray, M., Sangula, A., Sallu, R., Sinkala, Y., Wambura, P., King, D., 2014. Challenges and prospects for the control of foot-and-mouth disease: an African perspective.

Mateo, R., Luna, E., Rincon, V., Mateu, M.G., Mateo, R., Luna, E., Rincon, V., Mateu, M.G., 2008. Engineering Viable Foot-and-Mouth Disease Viruses with Increased Thermostability as a Step in the Development of Improved Vaccines. The Journal of Virology 82, 12232.

Mateu, M.G., Andreu, D., Domingo, E., Mateu, M.g., Andreu, D., Domingo, E., 1995a. Antibodies raised in a natural host and monoclonal antibodies recognize similar antigenic features of foot-and-mouth disease virus. Antibodies raised in a natural host and monoclonal antibodies recognize similar antigenic features of foot-and-mouth disease virus 210, 120-127.

Mateu, M.G., Camarero, J.A., Giralt, E., Andreu, D., Domingo, E., 1995b. Direct evaluation of the immunodominance of a major antigenic site of foot-and-mouth disease virus in a natural host. Virology 206, 298-306.

Mattion, N., König, G., Seki, C., Smitsaart, E., Maradei, E., Robiolo, B., Duffy, S., León, E., Piccone, M., Sadir, A., 2004. Reintroduction of foot-and-mouth disease in Argentina: characterisation of the isolates and

development of tools for the control and eradication of the disease. Vaccine 22, 4149-4162.

Mayr, G.A., Chinsangaram, J., Grubman, M.J., 1999. Development of replication-defective adenovirus serotype 5 containing the capsid and 3C protease coding regions of foot-and-mouth disease virus as a vaccine candidate. Virology 263, 496-506.

McGranahan, D., 2008. Managing private, commercial rangelands for agricultural production and wildlife diversity in Namibia and Zambia *Biodivers Conserv*.

Megersa, B., Beyene, B., Abunna, F., Regassa, A., Amenu, K., Rufael, T., 2009. Risk factors for foot and mouth disease seroprevalence in indigenous cattle in Southern Ethiopia: the effect of production system. Trop Anim Health Prod 41, 891-898.

Mezencio, J.M.S., Babcock, G.D., Kramer, E., Brown, F., 1999. Evidence for the persistence of foot-and-mouth disease virus in pigs. Veterinary Journal 157, 213-217.

Miguel, E., Grosbois, V., Caron, A., Boulinier, T., Fritz, H., CornÃ©lis, D., Foggin, C., Makaya, P.V., Tshabalala, P.T., de Garine-Wichatitsky, M., 2013a. Contacts and foot and mouth disease transmission from wild to domestic bovines in Africa. Ecosphere 4(3), 51.

Miguel, E., Grosbois, V., Caron, A., Cornelis, D., Boulinier, T., Foggin, C., Makaya, P., Fritz, H., de Garine-Wichatitsky, M., 2013b. Wildlife-livestock contacts: frequency of interactions contact and foot-and-mouth disease dynamic in cattle populations at the periphery of Transfrontier Conservation Areas in Southern Africa. Ecosphere 4, art51.

Minett, F.C., 1948. Panting in cattle: a sequel to foot-and-mouth disease. *Amer Vet Medical Assoc J* 113, 545-550.

Morelli, M.J., Wright, C.F., Knowles, N.J., Juleff, N., Paton, D.J., King, D.P., Haydon, D.T., 2013. Evolution of foot-and-mouth disease virus intra-sample sequence diversity during serial transmission in bovine hosts. Veterinary research 44, 12.

Morris, J.P.A., 1934. Annual report for the Department of Veterinary Services for the year 1933. Government of Northern Rhodesia, Printed by Government Printers, Lusaka, 71 pp Lusaka.

Mulumba, M., Speybroeck, N., Billiouw, M., Berkvens, D.L., Geysen, D.M., Brandt, J.R.A., Mulumba, M., Speybroeck, N., Billiouw, M., Berkvens, D.L., Geysen, D.M., Brandt, J.R.A., 2000. Transmission of theileriosis in the traditional farming sector in the Southern Province of Zambia during 1995-1996. Trop. Anim. Health Prod. 32, 303-314.

Muma, e.a., Samui, K.L., Siamudaala, V.M., Oloya, J., Matope, G., Omer, M.K., Munyeme, M., Mubita, C., Skjerve, E., 2006. Prevalence of antibodies to Brucella spp. And individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. Trop. Anim. Health Prod. 38, 195-206.

Muma J.B., 2006. Epidemiology of brucella infections in livestock − wildlife interface areas in Zambia. Department of Food Safety and Infection Biology. Oslo, Norway: Norwegian School of Veterinary Science, Oslo, Norway: Norwegian School of Veterinary Science.

Muma, J.B., Lund, A., Siamudaala, V.M., Munang'andu, H.M., Munyeme, M., Matope, G., Nielsen, K., Djonne, B., Godfroid, J., Tryland, M., Skjerve,

E., 2010. Serosurvey of Brucella spp infection in the Kafue Lechwe (Kobus leche Kafuensis) of the Kafue Flats in Zambia. J. Wildl. Dis. 46, 1063-1069.

Muma, J.B., Munyeme, M., Matope, G., Siamudaala, V.M., Munang'andu, H.M., Matandiko, W., Godfroid, J., Skjerve, E., Tryland, M., 2011a. Brucella seroprevalence of the Kafue lechwe (Kobus leche kafuensis) and Black lechwe (Kobus leche smithemani): Exposure associated to contact with cattle. Prev. Vet. Med. 100, 256-260.

Muma, J.B., Munyeme, M., Samui, K.L., Siamudaala, V., Oloya, J., Mwacalimba, K., Skjerve, E., 2009. Mortality and commercial off-take rates in adult traditional cattle of Zambia. Tropical Animal Health and Production 41, 783-789.

Muma, J.B., Munyeme, M., Simuunza, M., Mwacalimba, K., Mwaanga, E., Siamudaala, V.M., Mbozi, A., Pandey, G.S., 2011b. Contribution of ox draught power towards crop production: a case study of southern province. UNZA Journal of Science and Technology 15 (2), 29-35.

Muma, J.B., Pandey, G.S., Munyeme, M., Mumba, C., Mkandawire, E., Chimana, H.M., 2012. Brucellosis among smallholder cattle farmers in Zambia. Tropical animal health and production 44, 915-920.

Muma, J.B., Samui, K.L., Oloya, J., Munyeme, M., Skjerve, E., 2007. Risk factors for brucellosis in indegenious cattle reared in livestock -wildlife interface areas of Zambia. Preventive Veterinary Medicine 80, 306-317.

Mumba, M., Thompson, J.R., 2005. Hydrological and ecological impacts of dams on the Kafue Flats floodplain system, southern Zambia. Physics and Chemistry of the Earth, Parts A/B/C 30, 442-447.

Munang'andu, H.M., Siamudaala, V., Munyeme, M., Nalubamba, K.S., 2012. A Review of Ecological Factors Associated with the Epidemiology of Wildlife Trypanosomiasis in the Luangwa and Zambezi Valley Ecosystems of Zambia. Interdisciplinary Perspectives on Infectious Diseases 2012.

Munang'andu, H.M., Siamudaala, V.M., Nambota, A., Bwalya, J.M., Munyeme, M., Mweene, A.S., Takada, A., Kida, H., 2006. Disease constraints for utilization of the African buffalo (Syncerus caffer) on game ranches in Zambia. Jpn J Vet Res 54, 3-13.

Munyeme, M., Muma, J., Skjerve, E., Nambota, A., Phiri, I., Samui, K., Dorny, P., Tryland, M., 2008. Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia. Preventive veterinary medicine 85, 317-328.

Munyeme, M., Muma, J.B., Samui, K.L., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Rigouts, L., Tryland, M., 2009. Prevalence of Bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas. Tropical Animal Health Production 41, 345-352.

Muuka, G., Songolo, N., Kabilika, S., Hang'ombe, B.M., Nalubamba, K.S., Muma, J.B., 2012. Challenges of controlling contagious bovine pleuropneumonia in sub-Saharan Africa: a Zambian perspective. Trop Anim Health Prod 45, 9-15.

Mwacalimba, K.K., Mumba, C., Munyeme, M., 2013. Cost benefit analysis of tuberculosis control in wildlife–livestock interface areas of Southern Zambia. Preventive Veterinary Medicine 110, 274-279.

Namatovu, A., Wekesa, S.N., Tjornehoj, K., Dhikusooka, M.T., Muwanika, V.B., Siegismund, H.R., Ayebazibwe, C., 2013. Laboratory capacity for diagnosis of foot-and-mouth disease in Eastern Africa: implications for the progressive control pathway. BMC Vet. Res. 9.

Nambota, A., Samui, K., Sugimoto, C., Kakuta, T., Onuma, M., Nambota, A., Samui, K., Sugimoto, C., Kakuta, T., Onuma, M., 1994. Theileriosis in Zambia: Etiology, epidemiology and control measures. Theileriosis in Zambia: Etiology, epidemiology and control measures 42, 1-18.

NASA, 2004. Floodwaters Renew Zambia's Kafue Wetland. NASA Earth Observatory, http://earthobservatory.nasa.gov/IOTD/view.php?id=4992&src=ve, Accesed: 15th May 2014.

NASA, 2007. Flooding in the Zambezi Valley. NASA- Earth Observatory, http://earthobservatory.nasa.gov/NaturalHazards/view.php?id=18078&eocn=image&eoci=related_image, Accessed:15th May 2014.

NASA, 2009. Deadly Flooding of the Zambezi Food Plain. NASA-Earth Observatory, http://eoimages.gsfc.nasa.gov/images/imagerecords/38000/38212/Caprivi_TMO_2009104_lrg.jpg, Accessed: 15th May 2014.

NASA, 2010. Zambezi Flood Plain NASA - Earth Observatory, http://earthobservatory.nasa.gov/IOTD/view.php?id=44132, Accessed: 15th May 2014.

Nyemba, R., Dakora, F., 2010. Evaluating N2 fixation by food grain legumes in farmers' fields in three agro-ecological zones of Zambia, using 15N natural abundance. Biology And Fertility Of Soils 46.

Oem, J.K., Ferris, N.P., Lee, K.N., Joo, Y.S., Hyun, B.H., Park, J.H., 2009. Simple and rapid lateral-flow assay for the detection of foot-and-mouth disease virus. Clin Vaccine Immunol 16, 1660-1664.

OIE, 2010a. Foot and Mouth Disease chapter 2.1.5. OIE, Paris.

OIE, 2010b. Members having an FMD free zone where vaccination is not practised, according to the provisions of Chapter 8.5. of the Terrestrial Code

OIE, 2013a. World Animal Health Information Database (WAHID) Interface. World Organisation for Animal Health www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation, Accessed: 9th September 2013.

OIE, 2013b. Zambia Rinderpest, Handistatus II, Multiannual Animal Disease Status. OIE, http://web.oie.int/hs2/sit_pays_mald_pl.asp?c_pays=219&c_mald=5, Accessed: 17th November 2013.

OIE, 2014a. Chapter 2.1.5 of the Manual of Diagnostics on Foot and Mouth Disease adopted May 2012. OIE, http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf, Accessed: 3rd August 2014.

OIE, 2014b. Foot and Mouth Disease, Chapter 8.7. OIE, http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/chapitre_fmd.pdf, Accessed: 2nd August 2014.

OIE, 2014c. Impact of foot and mouth disease In: Rushton, J., Knight, J. (Eds.), http://www.oie.int/doc/ged/D11888.PDF, Accessed: 8th August 2014.

Overby, E., Zyambo, G.C.N., 1983. Foot and mouth disease outbreaks in Zambia. Rev Sci. Tech. Off. Int. Epiz 2, 189-197.

Paton, D.J., de Clercq, K., Greiner, M., Dekker, A., Brocchi, E., Bergmann, I., Sammin, D.J., Gubbins, S., Parida, S., 2006. Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. Vaccine 24, 6503-6512.

Paton, D.J., Sumption, K.J., Charleston, B., 2009. Options for control of foot-and-mouth disease: knowledge, capability and policy. Philos T R Soc B 364, 2657-2667.

Perez, A.M., Thurmond, M.C., Grant, P.W., Carpenter, T.E., 2005. Use of the scan statistic on disaggregated province-based data: foot-and-mouth disease in Iran. Preventive veterinary medicine 71, 197-207.

Perry, B.D., Hedger, R.S., 1984. History and epidemiology of foot-and-mouth disease in Zambia: a review. Trop Anim Health Prod 16, 107-114.

Perry, B.D., Mwanaumo, B., Schels, H.F., Eicher, E., Zaman, M.R., Perry, B.D., Mwanaumo, B., Schels, H.F., Eicher, E., Zaman, M.R., 1984. A study of health and productivity of traditionally managed cattle in Zambia. Preventive Veterinary Medicine 2, 633-653.

Perry, B.D., Rich, K.M., 2007. Poverty impacts of foot-and-mouth disease and the poverty reduction implications of its control. Veterinary Record 160, 238-241.

Pfeiffer, D., Robinson, T., Stevenson, M., Stevens, K., Rogers, D., Clements, A. (Eds.), 2008. Spatial Analysis in Epidemiology. Oxford University Press New York.

Pfeiffer, D.U., Minh, P.Q., Martin, V., Epprecht, M., Otte, M.J., 2007. An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occurrence in Vietnam using national surveillance data. The Veterinary Journal 174, 302-309.

Quan, M., Murphy, C.M., Zhang, Z., Alexandersen, S., 2004. Determinants of Early Foot-and-Mouth Disease Virus Dynamics in Pigs. Journal of Comparative Pathology 131, 294-307.

Radostits, O., Blood, D.C., Gay, C.C. (Eds.), 1994. Foot-and-mouth disease (FMD, Aphthous fever). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. . W.B. Saunders Company Ltd, page 965-974 London

Reid, S.M., Ferris, N.P., Bruning, A., Hutchings, G.H., Kowalska, Z., Akerblom, L., 2001. Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen. J Virol Methods 96, 189-202.

Reid, S.M., Ferris, N.P., Hutchings, G.H., Samuel, A.R., Knowles, N.J., 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J. Virol. Methods 89, 167-176.

Rivas, A.L., Kunsberg, B., Chowell, G., Smith, S.D., Hyman, J.M., Schwager, S.J., Rivas, A.L., Kunsberg, B., Chowell, G., Smith, S.D., Hyman, J.M., Schwager, S.J., 2006. Human-mediated foot-and-mouth disease epidemic dispersal: Disease and vector clusters. J. Vet. Med. Ser. B-Infect. Dis. Vet. Public Health 53, 1-10.

Robinson, T., Franceschini, G., Wint, W., 2007. The Food and Agriculture Organization's Gridded Livestock of the World. Veterinaria Italiana 43, 745-751.

Rodriguez, L.L., Gay, C.G., 2011. Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert review of vaccines 10, 377-387.

Rodriguez, L.L., Grubman, M.J., 2009. Foot and mouth disease virus vaccines. Vaccine 27, Supplement 4, D90-D94.

Rweyemamu, M., Garland, A.J.M., 2006. The design of vaccines and diagnostics for use in endemic FMD settings. In: Global Endemic FMD roadmap workshop; Agra, India. In: Perry, B.D., Sones, K.R. (Eds.), Global roadmap for improving the tools to control foot and mouth disease in endemic setting from 29th November to 1st December 2006, Agra, India.

Rweyemamu, M., Roeder, P., MacKay, D., Sumption, K., Brownlie, J., Leforban, Y., 2008a. Planning for the progressive control of foot-and-mouth disease worldwide. Transboundary and emerging diseases 55, 73-87.

Rweyemamu, M., Roeder, P., Mackay, D., Sumption, K., Brownlie, J., Leforban, Y., Valarcher, J.F., Knowles, N.J., Saraiva, V., 2008b. Epidemiological patterns of foot-and-mouth disease worldwide. Transbound Emerg Dis 55, 57-72.

Rweyemamu, M.M., Booth, J.C., Head, M., Pay, T.W.F., 1978. Microneutralization tests for serological typing and subtyping of foot-and-mouth disease virus strains. Microneutralization tests for serological typing and subtyping of foot-and-mouth disease virus strains. 81, 107-123.

Saiz, J.C., Domingo, E., Saiz, J.C., Domingo, E., 1996. Virulence as a positive trait in viral persistence. The Journal of Virology 70, 6410.

Sáiz, M., Núñez, J.I., Jimenez-Clavero, M.A., Baranowski, E., Sobrino, F., Sáiz, M., Núñez, J.I., Jimenez-Clavero, M.A., Baranowski, E., Sobrino, F., 2002. Foot-and-mouth disease virus: biology and prospects for disease control. Microbes and Infection 4, 1183-1192.

Salt Jeremy, 2004. Persistence of Foot-and-Mouth Disease Virus. In: Domingo, E., Sobrino, F. (Eds.), Foot and Mouth Disease: current perspectives CRC Press, 104-143.

Salt, J.S., 1993. The carrier state in foot and mouth disease—an immunological review. British Veterinary Journal 149, 207-223.

Sammin, D.J., Paton, D.J., Parida, S., Ferris, N.P., Hutchings, G.H., Reid, S.M., Shaw, A.E., Holmes, C., Gibson, D., Corteyn, M., Knowles, N.J., Valarcher, J.F., Hamblin, P.A., Fleming, L., Gwaze, G., Sumption, K.J., 2007. Evaluation of laboratory tests for SAT serotypes of foot-and-mouth disease virus with specimens collected from convalescent cattle in Zimbabwe. Vet Rec 160, 647-654.

Samuel, A.R., Knowles, N.J., Samuel, A.R., Knowles, N.J., 2001. Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). J. Gen. Virol. 82, 609-621.

Sanchez-Aparicio, M.T., Rosas, M.F., Ferraz, R.M., Delgui, L., Veloso, J.J., Blanco, E., Villaverde, A., Sobrino, F., 2009. Discriminating foot-and-mouth disease virus-infected and vaccinated animals by use of beta-galactosidase allosteric biosensors. Clin Vaccine Immunol 16, 1228-1235.

Saraiva, V., 2002. Vaccines and foot-and-mouth disease eradication in South America. Developments in biologicals 114, 67-77.

Schley, D., Paton, D.J., Cox, S.J., Parida, S., Gubbins, S., 2009. The Effect of vaccination on undetected persistence of foot and mouth disease virus in cattle herds and sheep flocks. Epidemio. Infect. 137, 1494 -1504.

Schoenbaum, M.A., Terry Disney, W., 2003. Modeling alternative mitigation strategies for a hypothetical outbreak of foot-and-mouth disease in the United States. Preventive Veterinary Medicine 58, 25-52.

Scoones, I., Bishi, A., Mapitse, N., Moerane, R., Penrith, M.L., Sibanda, R., Thomas, G., Wolmer, W., 2010. Foot-and-mouth disease and market access: challenges for the beef industry in southern Africa. Pastoralism 1, 135-164.

Sheppe, W.A., 1985. Effects of human activities on Zambia's Kafue Flats ecosystems. Environmental Conservation 12, 49-57.

Siembieda, J.L., Kock, R.A., McCracken, T.A., Newman, S.H., Siembieda, J.L., Kock, R.A., McCracken, T.A., Newman, S.H., 2011. The role of wildlife in transboundary animal diseases. Anim. Health. Res. Rev. 12, 95-111.

Sindato, C., Karimuribo, E.D., Pfeiffer, D.U., Mboera, L.E.G., Kivaria, F., Dautu, G., Bernard, B., Paweska, J.T., 2014. Spatial and Temporal Pattern of Rift Valley Fever Outbreaks in Tanzania; 1930 to 2007. PLoS ONE 9, e88897.

Sinkala, Y., Simuunza, M., Muma, J.B., Pfeiffer, D.U., Kasanga, C.J., Mweene, A., 2014a. Foot and mouth disease in Zambia: Spatial and temporal distributions of outbreaks, assessment of clusters and implications for control. Onderstepoort Journal of Veterinary Research 81, Art. #741, 746 pages.

Sinkala, Y., Simuunza, M., Pfeiffer, D.U., Munangandu, H.M., Mulumba, M., Kasanga, C.J., Muma, J.B., Mweene, A.S., 2014b. Challenges and Economic Implications in the Control of Foot and Mouth Disease in Sub-Saharan Africa: Lessons from the Zambian Experience. Veterinary medicine international 2014, Article ID 373921, 12 pages 12 pages.

Sobrino, F., Sáiz, M., Jiménez-Clavero, M.A., Núñez, J.I., Rosas, M.F., Baranowski, E., Ley, V., Sobrino, F., Sáiz, M., Jiménez-clavero, M.A., Núñez, J.I., Rosas, M.F., Baranowski, E., Ley, V., 2001. Foot-and-mouth disease virus: a long known virus, but a current threat. Veterinary research 32, 1.

Sørensen, K., Madsen, K., Madsen, E., Salt, J., Nqindi, J., Mackay, D., 1998. Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. Archives of virology 143, 1461-1476.

Sudakin, D., Power, L.E., 2009. Regional and temporal variation in methamphetamine-related incidents: applications of spatial and temporal scan statistics. Clinical Toxicology 47, 243-247.

Sutmoller, P., Barteling, S.S., Olascoaga, R.C., Sumption, K.J., 2003. Control and eradication of foot-and-mouth disease. Virus Research 91, 101-144.

Sutmoller, P., Gaggero, A., 1965. Foot-and mouth diseases carriers. Vet Rec 77, 968-969.

Sutmoller, P., Olascoaga, R.C., 2002. Unapparent foot and mouth disease infection (sub-clinical infections and carriers): implications for control. Revue Scientifique Et Technique-Office International Des Epizooties 21, 519-524.

Tang, H., Liu, X.S., Fang, Y.Z., Pan, L., Zhang, Z.W., Zhou, P., Lv, J.L., Jiang, S.T., Hu, W.F., Zhang, P., Wang, Y.L., Zhang, Y.G., 2012. Advances in Studies on Vaccines of Foot-and-mouth Disease. Asian J. Anim. Vet. Adv. 7, 1245-1254.

Tekleghiorghis, T., Moormann, R., Weerdmeester, K., Dekker, A., 2014. Foot-and-mouth Disease Transmission in Africa: Implications for Control, a Review. Transboundary and emerging diseases.

Tenzin, Dekker, A., Vernooij, H., Bouma, A., Stegeman, A., 2008. Rate of Foot-and-Mouth Disease Virus Transmission by Carriers Quantified from Experimental Data. Risk Analysis 28, 303-309.

Thomson, G., Bastos, A., 1994. Foot-and-mouth disease. Infectious diseases of livestock with special reference to southern Africa 2, 825-852.

Thomson, G., Penrith M-L., Fosgate G, 2012. Technical challenges associated with the eradication of TADs in southern Africa with special reference to FMD. In, Joint SADC/AHEAD Workshop: Reconcilling Livestock Health and Wildlife Conservation goals in Southern Africa: Strategies for Sustainable Economic Development, Phakalane Golf Estates, Gaborone, Botswana. 13-15 November 2012.

Thomson, G., Penrith ML, Atkinson MW, Atkinson SJ, Cassidy D, SA., O., 2013. Balancing livestock production and wildlife conservation in and around southern Africa's transfrontier conservation areas. Transbound Emerg Dis 60, 492-506.

Thomson, G.R., Bastos, A.D.S., 2004. Viral Disease Picornaviridae - Foot-and-mouth disease. In: Coetzer, J.A.W., Tustin, R.C. (Eds.), Infectious diseases of livestock with special reference to southern Africa. Oxford University Press Southern Africa, Oxford, Cape Town, page 1324 -1365.

Thomson, G.R., Vosloo, W., 2004. Natural Habitats in Which Foot-and-Mouth Disease Virus is Maintained. Foot and Mouth Disease: current perspectives CRC Press, page 384-410.

Thomson, G.R., Vosloo, W., Bastos, A.D., 2003. Foot and mouth disease in wildlife. Virus Res 91, 145-161.

Thomson, G.R., Vosloo, W., Esterhuysen, J.J., Bengis, R.G., 1992. Maintenance of foot and mouth disease viruses in buffalo (Syncerus caffer Sparrman, 1779) in southern Africa. Rev Sci Tech 11, 1097-1107.

Timberlake, J., 2000. Biodiversity of the Zambezi basin. Biodiversity Foundation for Africa, www.biodiversityfoundation.org/documents/BFA%20No.9_Zambezi%20Basin%Biodiversity.pdf, Accessed: 1st August 2013.

Toja, M., Escarmís, C., Domingo, E., 1999. Genomic nucleotide sequence of a foot-and-mouth disease virus clone and its persistent derivatives: Implications for the evolution of viral quasispecies during a persistent infection. Virus Research 64, 161-171.

Tully, D.C., Fares, M.A., 2008. The tale of a modern animal plague: Tracing the evolutionary history and determining the time-scale for foot and mouth disease virus. Virology 382, 250-256.

Vosloo, W., Bastos, A.D., Boshoff, C.I., 2005a. Retrospective genetic analysis of SAT-1 type foot and mouth disease outbreaks in southern Africa. Archives of Virology 151, 285-298.

Vosloo, W., Bastos, A.D., Kirkbride, E., Esterhuysen, J.J., Janse van Rensburg, D., Bengis, R.G., Keet, D.W., Thomson, G.R., 1996. Persistent infection

of African buffalo (Syncerus caffer) with SAT-type foot-and-mouth disease viruses: rate of fixation of mutations, antigenic change and interspecies transmission. Journal of General Virology 77, 1457- 1469.

Vosloo, W., Bastos, A.D., Michel, A., Thomson, G.R., 2001. Tracing movement of African buffalo in southern Africa. Rev Sci Tech 20, 630-639.

Vosloo, W., Bastos, A.D., Sangare, O., Hargreaves, S.K., Thomson, G.R., 2002a. Review of the status and control of foot and mouth disease in sub-Saharan Africa. Rev Sci Tech 21, 437-449.

Vosloo, W., Bastos, A.D.S., Sahle, M., Sangare, O., Dwarka, R.M., 2005b. Virus topotypes and the role of wildlife in foot and mouth disease in Africa (Chapter 10). In: Osofsky, S.A. (Ed.), Proceedings of the Southern and Eastern Africa Experts panel on designing successful conservation and Development Interventions at the wildlife Livestock interface: Implication for wildlife, Livestock and Humans health. IUCN Vth World Parks Congress. Conservation and Development 6 Interventions at the Wildlife-Livestock interface: Implications for Wildlife, Livestock and Human Health, IUCN, pp. 67-73., Durban, South Africa, 14 and 15th September 2003.

Vosloo, W., Boshoff, K., Dwarka, R., Bastos, A., 2002b. The possible role that buffalo played in the recent outbreaks of foot-and-mouth disease in South Africa. Annals of the New York Academy of Sciences 969, 187.

Vosloo, W., de Klerk, L.M., Boshoff, C.I., Botha, B., Dwarka, R.M., Keet, D., Haydon, D.T., 2007. Characterisation of a SAT-1 outbreak of foot-and-mouth disease in captive African buffalo (Syncerus caffer): clinical symptoms, genetic characterisation and phylogenetic comparison of outbreak isolates. Vet Microbiol 120, 226-240.

Vosloo, W., Dwarka, R.M., Bastos, A.D.S., Esterhuysen, J.J., Sahle, M., Sangare, O., 2004. Molecular epidemiological studies of foot-and-mouth disease virus in sub-Saharan Africa indicate the presence of large numbers of topotypes: implications for local and international control. Proceedings of the Open Session of the EU-FMD Research Group, 2-15 October. FAO, http://www.fao.org/ag/againfo/commissions/docs/greece04/App22.pdf, Accessed 2nd February 2014, Crete, Greece, 149-115.

Vosloo, W., Kirkbride, E., Bengis, R.G., Keet, D.F., Thomson, G.R., 1995. Genome variation in the SAT types of foot-and-mouth disease viruses prevalent in buffalo (Syncerus caffer) in the Kruger National Park and other regions of southern Africa, 1986-93. Epidemiology and infection 114, 203-218.

Vosloo, W., Thompson, P.N., Botha, B., Bengis, R.G., Thomson, G.R., 2009. Longitudinal study to investigate the role of impala (Aepyceros melampus) in foot-and-mouth disease maintenance in the Kruger National Park, South Africa. Transbound Emerg Dis 56, 18-30.

Vosloo, W., Thomson, G., 2004. Natural habitats in which foot and mouth disease virus is maintained In: Sobrino, F., Domingo, E. (Eds.), Foot and mouth disease; current perspectives Horizon bioscience, Horizon bioscience, 383-410.

Vynnycky, E., White, R., 2010. An introduction to Infectious Disease Modeling. Oxford University Press. Chapter 4, page 82 - 83

Vynnycky, E., White, R., 2010a. An Introduction to Infectious Disease Modeling. Oxford University Press. Chapter 2, page 13 - 61

Ward, M.P., Highfield, L.D., Vongseng, P., Graeme Garner, M., 2009. Simulation of foot-and-mouth disease spread within an integrated livestock system in Texas, USA. Preventive veterinary medicine 88, 286-297.

Wilson, T.R., 2003. Animal Health and Disease Control in the Usangu Wetlands of southwestern Tanzania Tropical Animal Health Production 35 (1), 47-67.

Woolhouse, M.E.J., 2004. Mathematical Models of the Epidemiology and Control of Foot and Mouth Disease. In: Sobrino, F., Domingo, E. (Eds.), Foot and Mouth Disease: Current Perspectives Horizon Bioscience, Norfolk, page 355 - 381.

WorldBank, 2011. What Would it Take for Zambia's Beef and Dairy Industries to Achieve Their Potential? . WorldBank. © World Bank. https://openknowledge.worldbank.org/handle/10986/2771,License Creative Commons Attribution CC BY 3.0." Accessed: 30th January 2014.

WorldBank, 2014. Country Profile WorldBank, http://data.worldbank.org/country/zambia, Accessed: 24th July 2014.

Yamazaki, W., Mioulet, V., Murray, L., Madi, M., Haga, T., Misawa, N., Horii, Y., King, D.P., 2013. Development and evaluation of multiplex RT-LAMP assays for rapid and sensitive detection of foot-and-mouth disease virus. J. Virol. Methods 192, 18-24.

Yang, M., Clavijo, A., Li, M.Y., Hole, K., Holland, H., Wang, H., Deng, M.Y., 2007. Identification of a major antibody binding epitope in the non-structural protein 3D of foot-and-mouth disease virus in cattle and the development of a monoclonal antibody with diagnostic applications. J Immunol Methods 321, 174-181.

Yang, M., Goolia, M., Xu, W., Bittner, H., Clavijo, A., 2013. Development of a quick and simple detection methodology for foot-and-mouth disease virus serotypes O, A and Asia 1 using a generic RapidAssay Device. Virology journal 10, 125.

Zhang, Z., Alexandersen, S., 2003. Detection of carrier cattle and sheep persistently infected with foot-and-mouth disease virus by a rapid real-time RT-PCR assay. Journal of virological methods 111, 95.

Zhang, Z., Alexandersen, S., 2004. Quantitative analysis of foot-and-mouth disease virus RNA loads in bovine tissues: implications for the site of viral persistence. J Gen Virol 85, 2567-2575.

Zhang, Z., Alexandersen, S., Zhang, Z., Alexandersen, S., 2004. Quantitative analysis of foot-and-mouth disease virus RNA loads in bovine tissues: implications for the site of viral persistence. The Journal of general virology 85, 2567.

Zhang, Z., Kitching, R., 2001. The localization of persistent foot and mouth disease virus in the epithelial cells of the soft palate and pharynx. Journal of Comparative pathology 124, 89-94.

Zyambo, G.C.N., 1975. Foot and mouth disease outbreaks in Zambia Bull. Off. Int. Epiz 83, 19-25.

9.0 APPENDIX

Appendix 1 Publications from this thesis

Published papers

- **Sinkala**, Y., Simuunza, M. , Pfeiffer, D.U., Munang'andu, H.M., Mulumba, M., Kasanga, C. J., Muma, J.B., Mweene, A.S., 2014 ·Challenges and economic implications in the control of foot and mouth disease in Sub-Saharan Africa: Lessons from the Zambian experience *Veterinary Medicine International*, vol. 2014, Article ID 373921, 12 pages, 2014. doi:10.1155/2014/373921
- **Sinkala**, Y., Simuunza, M., Muma, J.B., Pfeiffer, D.U., M., Kasanga, C., Mweene, A., 2014; Foot and mouth disease in Zambia: Spatial and temporal distributions of outbreaks, assessment of clusters and implications for control; Onderstepoort Journal of Veterinary Research 81 (2), Art. #741, 6 pages, http://dx.doi: 10.4102/ojvr.v81i2.741

Appendix 2 Questionnaire for cross sectional study

QUESTIONAIRE FOR THE CROSS-SECTIONAL SURVEY OF FOOT AND MOUTH DISEASE AT THE LIVESTOCK WILDIFE INTERFACE

Department of Disease Control, School of veterinary Medicine, University of Zambia, P.O Box 32379, Lusaka Zambia, Tele-fax 292737

SURVEY ON FOOT AND MOUTH DISEASE

SECTION ONE: **IDENTIFICATION**

Date of visit		
Name of Village/ Dip tank/crushpen		
Location: Constituency/ District /Province	.	
GPS coordinates	Northern	
	Eastern	
Owners name		

Name of person interviewed	
Relation of the person interviewed	⭘ Owner ⭘ Family member ⭘ Care taker

Q1 What type of animals do you have on your farm?

Cattle	Sheep	Goats	Pigs	Others (Specify)

Give a breakdown of herd structure

Cattle	Number	Small Ruminants	Number
1.Number of cows		Does/Ewes	
2.Number of bulls		Bucks/Rams	
3.Number of heifers >1 yr		Females > 8 Months	
4.Number of female calves <1yr		Female kids <8 months	
5.Number of males calves <1 yr		Male kids <8 months	
6. Male cattle between 2-3 years			
7. Female cattle between 2-3 years			

8. Female cattle over 4 years			
9. Male cattle over 4 years			
10. Average herd size			

SECTION TWO: **FARM OWNERSHIP AND CARE**

Q2. What type of animal breeds do you have on your farm?

Cattle	**Breed type**	**Small Ruminants**	**Breed type**
1. Exotic		1. Exotic	
2. Local		2. Local	
3. Mixed		3. Mixed	

Pigs

Q3. What kind of breeding methods do you use on this farm?

0. Artificial insemination……..... ☐

1. Natural methods……..... ☐

2. Both 1 and 2..…….....☐

Q4. If you use bulls, where do you get your bulls?

0. Use own bull……..... ☐

1. Use hired bulls…….....☐

Q5. What is the source of your stock?

0. Animal market……..... ☐

1. Any farm……..... ☐

2. Others specify…….....

Q6. Have you brought any animal onto your farm during the past 3 years?

0. Yes……..... ☐

1. No……..... ☐

Q7. If you brought animals on the farm, which sex were they?

0. Male……..... ☐

1. Female……..... ☐

2. Male and Female……..... ☐

If yes specify the numbers…….....

Q8. Do you hire out your bull/buckram to other farmers for breeding? ☐

0.Yes …….....☐

1. No…….....☐ ☐

If yes specify…….....

Q9. How do you get rid of manure from the kraal/animal houses? ☐

0. Left to dry and collected later.....…….....☐

1. Drained through sewerage…….....…….☐☐

2. Others specify…….....

Q10. Who is primarily responsible for looking after the animals?

0. Owner/ family member…..…….…….....☐

1. Hired caretaker…….....☐

2. Both 1 and 2…….....☐☐

3. Others (specify)…….....

Q11. Do you keep any written records for the animals?

0. Yes ……..…….....☐

1. No…….…….....☐

Q12. Do you receive any veterinary supportive services?

0. Yes…….....☐

1. No…….…….....……..☐

If yes specify……..

SECTION THREE**: FEEDING**

Q13. How do you feed your animal?

0. Pasture plus grain supplements all year…….....☐

1. Mainly concentrate feeding…….....☐

2. Pasture plus grain supplement occasionally…….....☐

3. Tethering (zero grazing)…….....☐

4. Free grazing/browsing…….....☐

Q14. If you practice grazing what type of grazing is it?

0. Own pastures…….....☐

1. Only communal pastures…….....☐

2. Own and communal pastures…….....☐

SECTION FOUR: **ANIMAL MOVEMENT PATTERNS**

Q15. Do you graze your animal in one place whole year round or you change with seasons? (Transhumance)

0.Yes …….....☐

1. No…….....….....☐

If yes descried location (e.g. in the plains) where you take the animals? Must include the furthest point

Month	**Location**
January	
February	
March	
April	
May	
June	
July	
August	
September	
October	
November	
December	

Q16. Do your animals come into contact with wild animals?

0. Yes…….....☐

1. No…….....☐

If yes specify the type of wild animals….....

Q17. For how long and which periods of the year do you animals come into contact with wild animals?

0. Less than a month …….....☐

1. 3-6 months …….....☐

2. All year round…….....☐

Q18. Do you hire animals to fertilize your fields?

0.Yes …….....☐

1. No…….....☐

Q19. Do you use oxen for draught power/ transport purpose?

0.Yes …….....☐

1. No…….....☐

Q20.Are animals moved out of your area?

0.Yes …….....☐

1. No…….....☐

If Yes please fill Table below

Details of live animals moved and their destination for year 2010/2011

Species	Volume	Seasonality	Destination
		⭘ Constant ⭘ Seasonality (circle month(s) of higher trade activity): J F M A M J J A S O N D	

		❍ Constant ❍ Seasonality (circle month(s) of higher trade activity): J F M A M J J A S O N D	
		❍ Constant ❍ Seasonality (circle month(s) of higher trade activity): J F M A M J J A S O N D	
		❍ Constant ❍ Seasonality (circle month(s) of higher trade activity): J F M A M J J A S O N D	
		❍ Constant ❍ Seasonality (circle month(s) of higher trade activity): J F M A M J J A S O N D	

Q21 How are the animals moved?

1. Trekking ……..... ☐
2. Vehicle…… ☐

SECTION FIVE: **MARKETING**

Q22. Have you sold any animal in the last 12 months?

0.Yes …….....☐

1. No…….....☐

If yes specify…….....

Q23. If you sold, where did the buyers come from?

0. Within the neighbourhood…….....…..☐

1. Within town…….....…..☐

2. Within the province…….....…..☐

3. Outside the province…….....…..☐

4. Both within and outside the province…….....…...☐

5. Others …….....☐

Q24. Where do you sell your maize?

1. Milling company ...…….....☐

2. Food Reserve Agency…….....☐

3. Middle men …….....☐

4. Others …….....☐

SECTION SIX**: DISEASE AND DISEASE CONTROL**

Q25. Did you notice lameness or failing to walk in your herd?

0. Yes…….....☐

1. No…….....….☐

If yes, what was the cause?…….....

Q26. Did you notice any lesions in the mouth, feet, teats in your herd in the last 12 months?

0. No…….....☐

1. Yes…….....☐

Q27. Have you heard of a disease called foot and mouth disease?

0. Yes …….....☐

1. No…….....☐

Q28 Have your animals received any vaccinations in the last one year?

0. No……. ☐

1. Yes…….. ☐

If YES name disease

1. FMD……. ☐

2. CBPP…….. ☐

3. ECF……. ☐

4. OTHERS (specify)…… ☐

Q29. How often do you conduct disease preventive measures such as dipping?

0. None……..…….....☐

1. Regular (At least once per year)…….....☐

2. Irregular (once after many years).....☐

Q30. Did any animal die in your herd in the last 12 months?

0. No……☐

1. Yes……☐

If yes what is the likely cause?

☐

0. Tick borne…….....

1. Worms ……..... ☐

2. Emaciation ……..... ☐

3. Others specify…….....☐

SECTION SEVEN**: ECONOMICS**

Q31 Has your crop hectarage increased in the last 3 years?

1. No…… ☐
2. Yes…… ☐

If yes, state of increase.....

Q32 Do you have problems selling your animals?

If yes, state reasons .....

Q33 How much do you sell your animals?

Cattle	**Price**	**Small Ruminants**	**Price**
Cow		Doe	
Bull		Buck	
Heifers		Ram	
Ox		Bull	
Calf		Pig (sow and boar)	

Q34 What is the annual offtake from your farm?

Species	**number**	**Species**	**Number**
Cow		Goat	
Bull		sheep	
Heifer		Others (specify)	
Ox		milk	
Calf		pig	

Manual, drought power etc

Q34 How does FMD affect you as a farmer?

BUFFALO SURVEY

SECTION ONE: **IDENTIFICATION**

Date of visit		
Name of wildlife camp		
Location: Constituency/ District/ Province	.	
GPS coordinates	Northern	
	Eastern	
Name of National Park		
Name of person interviewed		

Q1 What type of animals do you have In your Park?

Species	Number

Q2 How many clusters of buffalo are there?

Cluster	Herd size

Q3 What is the calving period for the buffalo? J F M A M J J A S O N D

Q4 How many buffalo are culled every year on average?

Q5 Do know the pattern of movement of the buffalo during the year?

Month	**Location**
January	
February	
March	
April	
May	

June	
July	
August	
September	
October	
November	
December	

Q6 Have the activities in the park increased?

1. No……. ☐
2. Yes…….. ☐

If yes state the reasons

i. Encroachment …….....

ii. Lodges ……..... ☐

iii. Tour operations ……..... ☐

iv. Hunting……..... ☐

v. Poaching……..... ☐

Q7 What is the level of poaching against buffalo (in percent).....

Q8 Do farmers bring their cattle into the park for grazing

1. No……. ☐
2. Yes…….. ☐

If the answer is yes, state what periods of the year? J F M A M J J A S O N D

Q9 Is the buffalo populations increasing or decreasing?

State reasons for the increase or decrease? ☐

i. Disease…….....

ii. Culling/hunting ……..... ☐

iii. Production ……..... ☐

iv. Poaching……..... ☐

v. Others specify…….....