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.

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Yona Sinkala

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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

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Internal Examiner          Signature              Date

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Internal Examiner          Signature              Date

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External Examiner          Signature              Date

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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
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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

\(p\)  \textit{p value}: type I error (probability of wrongly rejecting null hypothesis)
PBS  Phosphate Buffered Saline
PCR  Polymerase Chain Reaction
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<tr>
<td>R₀</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse Transcriptase Polymerase Chain Reaction</td>
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<tr>
<td>rRT-PCR</td>
<td>Real Time Reverse Transcriptase Polymerase Chain Reaction</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>RR</td>
<td>Relative Risk</td>
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<tr>
<td>SADC</td>
<td>Southern Africa Development Community</td>
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<tr>
<td>SAT</td>
<td>Southern African Territories</td>
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<td>SP</td>
<td>Structural Proteins</td>
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<td>Sub Saharan Africa</td>
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<td>Trans-Frontier Conservation Areas</td>
</tr>
<tr>
<td>TPI</td>
<td>The Pirbright Institute</td>
</tr>
<tr>
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<td>Uniform Resource Locater</td>
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<tr>
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<td>United States Dollar</td>
</tr>
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<td>VP1</td>
<td>Viral Protein 1 (Major Capsid Protein VP1)</td>
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</tr>
<tr>
<td>VNT</td>
<td>Virus Neutralisation Test</td>
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<td>WRL</td>
<td>World Reference Laboratory</td>
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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 km² 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 km² (47.5 percent of total land), of which 300,000 km² (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 km² 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](image-url)
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 commercial 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>
<thead>
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<td>906,929</td>
<td>1984</td>
<td>2,036,418</td>
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<td>1952</td>
<td>925,758</td>
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<td>1955</td>
<td>870,680</td>
<td>1991</td>
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<td>1956</td>
<td>891,389</td>
<td>1992</td>
<td>3,095,000</td>
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<td>1957</td>
<td>891,566</td>
<td>1993</td>
<td>3,204,000</td>
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<tr>
<td>1958</td>
<td>917,216</td>
<td>1994</td>
<td>2,525,967</td>
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<td>1959</td>
<td>955,127</td>
<td>1995</td>
<td>2,642,200</td>
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<tr>
<td>1960</td>
<td>1,005,673</td>
<td>1996</td>
<td>2,562,841</td>
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<td>1961</td>
<td>1,051,881</td>
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<td>1962</td>
<td>1,056,184</td>
<td>1998</td>
<td>2,747,175</td>
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<td>1,061,543</td>
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<td>1964</td>
<td>1,069,042</td>
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<td>1965</td>
<td>1,111,858</td>
<td>2001</td>
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<td>1966</td>
<td>1,168,158</td>
<td>2002</td>
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<td>1967</td>
<td>1,242,243</td>
<td>2003</td>
<td>2,375,433</td>
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<td>1968</td>
<td>1,251,057</td>
<td>2004</td>
<td>2,341,970</td>
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<td>1970</td>
<td>1,378,906</td>
<td>2005</td>
<td>2,381,421</td>
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<td>1971</td>
<td>1,444,192</td>
<td>2006</td>
<td>2,444,789</td>
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<td>1972</td>
<td>1,489,332</td>
<td>2007</td>
<td>2,457,279</td>
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<tr>
<td>1973</td>
<td>1,509,871</td>
<td>2008</td>
<td>2,815,583</td>
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<tr>
<td>1974</td>
<td>1,509,871</td>
<td>2012</td>
<td>3,462,357</td>
</tr>
<tr>
<td>1975</td>
<td>1,509,871</td>
<td></td>
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</tbody>
</table>
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)

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

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 account 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 520,000km² 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).](image)

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; Tekleghiorgis et al., 2014).

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Topotype</th>
<th>Genotype/Strain</th>
<th>Representative country/countries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV (EA-1)</td>
<td>Uganda (2007*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>Nigeria (1976), Niger (1976)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td>Nigeria (1981), Sudan (1976)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VII</td>
<td>Uganda (1974)</td>
<td></td>
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<tr>
<td></td>
<td>VIII</td>
<td>Uganda (1997*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IX</td>
<td>Ethiopia (2007)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td>Gambia (1979), Senegal (1983)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VIII</td>
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<td>X</td>
<td>Uganda (2007*)</td>
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<td>XI</td>
<td>Angola (1974)</td>
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<td>XII</td>
<td>Uganda (1976)</td>
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<td>XIII</td>
<td>Sudan (2008), Ethiopia (2010)</td>
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<td></td>
<td>XIV</td>
<td>Ethiopia (1991)</td>
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<td>III (NWZ)</td>
<td>Zimbabwe (1991*)</td>
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<td></td>
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<td>Zambia (1996*)</td>
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</tr>
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<td>Kenya (2010), Uganda (1996)</td>
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</tr>
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<td></td>
<td>ME-SA</td>
<td>Sharquia-SA-72, Egypt (2009)</td>
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</tr>
<tr>
<td></td>
<td>ME-SA</td>
<td>PanAsia-SA-1, South Africa (2000)</td>
<td></td>
</tr>
</tbody>
</table>
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 adequately 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>
<thead>
<tr>
<th>Species/animal</th>
<th>Duration of viral persistence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>2.5 -3.5 years</td>
<td>Hedger 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hargreaves 1994</td>
</tr>
<tr>
<td>Sheep</td>
<td>9 – 12 months</td>
<td>Burrows 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td>McVicar and Sutmoller 1968</td>
</tr>
<tr>
<td>Goats</td>
<td>2-3 months</td>
<td>Singh 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anderson et al., 1976</td>
</tr>
<tr>
<td>Wildlife</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildebeest (Connochaetes taurinus)</td>
<td>28 days</td>
<td>Anderson et al., 1975</td>
</tr>
<tr>
<td>Sable (Hippotragus niger)</td>
<td>28 days</td>
<td>Ferris et al., 1989</td>
</tr>
<tr>
<td>Eland (Taurotragus oryx)</td>
<td>32 days</td>
<td>Anderson 1980</td>
</tr>
<tr>
<td>Fallow deer (Dama dama)</td>
<td>63 days</td>
<td>Forman et al., 1974</td>
</tr>
<tr>
<td>Kudu (Tragelaphus strepsiceros)</td>
<td>104 – 160 days</td>
<td>Hedger 1972</td>
</tr>
<tr>
<td>Water Buffalo (Bubalis bubalis)</td>
<td>2 -24 months</td>
<td>Moussa et al., 1979</td>
</tr>
<tr>
<td>African Buffalo (Syncerus caffer)</td>
<td>5 years</td>
<td>Condy et al., 1985</td>
</tr>
<tr>
<td>Impala (Aepyceros melampus)</td>
<td>7 days</td>
<td>Hedger et al 1972,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anderson et al 1975</td>
</tr>
<tr>
<td>Kafue Lechwe (Kobus leche kafuensis)</td>
<td>Not known</td>
<td>Overby et al 1983</td>
</tr>
</tbody>
</table>

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-clinical 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 bloodstream 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 biocontainment, 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
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; Tekleghiorgis 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 Seshake), and Mbala-Isoka area (Overby and Zymbo, 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
Zynambo, 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 diagnosis

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° to 33° E and latitude 08° to 11° 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 63,000km² 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; Zymbo, 1975; Akafekwa, 1980; Overby and Zymbo, 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.

![Map of study sites](image)

**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;

\[ p = \text{estimated prevalence (Assumed to be 10 percent for both within and between herds because of vaccination.)} \]

\[ q = 1 - p \]

\[ d = \text{the desired absolute precision of the estimate (Assumed desired absolute precision was 5 percent).} \]

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 0.1 percent 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°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 NaN₃. 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°C for 24 hours and re-inoculated into a new monolayer of Instituto Biologico Rim Suino (IBRS)-2 cells in 25cm² and then evaluated after 72 hours. Cultures with CPE were stored at -80°C until processing for typing ELISA. The data analysis and calculation of the log titres was based on TCID₅₀/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 Phylogenetic 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 $\geq 70$ 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:

\[ T = 2\pi \sqrt{\frac{A(D+D')}{R_0-1}} \]

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

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Estimation of parameter and Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth rate</td>
<td>Cattle</td>
<td>For the purpose of this study, birth rate was equal to mortality rate i.e. stable population</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>Cattle</td>
<td>Mortality rate = 1/life expectancy – assume a rectangular population. Animal live and then they dies naturally. Mortality rate = 7.5 percent (Muma et al., 2009)</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>per time</td>
<td>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</td>
</tr>
<tr>
<td>Incubation period</td>
<td>per time</td>
<td>2 -14 days (average = 8) (Alexandersen et al., 2003)</td>
</tr>
<tr>
<td>Latent period</td>
<td>per time</td>
<td>4.1 days (Charleston et al., 2011)</td>
</tr>
<tr>
<td>Infectious period</td>
<td>per time</td>
<td>6 days (Mardones et al., 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6 days (Mardones et al., 2010)</td>
</tr>
<tr>
<td>Basic reproduction ratio</td>
<td>Case</td>
<td>5.4 - 9.1 (Brostoff, 2012)</td>
</tr>
<tr>
<td>Movement period from Infected to</td>
<td>per time</td>
<td>3 days (one per time spent in infectious stage per unit time (Charleston et al., 2011)</td>
</tr>
<tr>
<td>carrier and recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of recovery from carrier to</td>
<td>per time</td>
<td>2.5 – 3.5 (Hedger 1976, Hargreaves et al 1994)</td>
</tr>
<tr>
<td>immune</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Average rates at which individuals become infectious or recover were calculated as follows:

Rate at which individuals become infectious \((f) = \frac{1}{\text{average pre-infectious period}}\) (Vynnycky and White, 2010a).

Rate of recovery/becoming immune \((r) = \frac{1}{\text{(average period of infectiousness)}}\) (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 (χ²=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 to 1982, 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).

![Spatial and temporal clustering](image)

**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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle density per km²*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>3.77</td>
<td>1.35, 10.48</td>
<td>0.011</td>
</tr>
<tr>
<td>11-20</td>
<td>5.51</td>
<td>2.56, 11.84</td>
<td>0.000</td>
</tr>
<tr>
<td>Agro-ecological zone*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4.50</td>
<td>1.92, 10.53</td>
<td>0.001</td>
</tr>
<tr>
<td>III</td>
<td>0.49</td>
<td>0.18, 1.38</td>
<td>0.18</td>
</tr>
<tr>
<td>Small Ruminant density per km²*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>3.06</td>
<td>1.31, 7.15</td>
<td>0.009</td>
</tr>
<tr>
<td>11-20</td>
<td>5.02</td>
<td>2.21, 11.39</td>
<td>0.000</td>
</tr>
<tr>
<td>Buffalo density per km²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1-10</td>
<td>1.92</td>
<td>0.75, 4.89</td>
<td>0.174</td>
</tr>
<tr>
<td>11-20</td>
<td>0.92</td>
<td>0.42, 2.01</td>
<td>0.838</td>
</tr>
<tr>
<td>Distance to National Park (km)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤30</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30-60</td>
<td>2.21</td>
<td>0.96, 5.08</td>
<td>0.062</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0.46</td>
<td>0.19, 1.11</td>
<td>0.085</td>
</tr>
<tr>
<td>Proximity to buffalo migratory route (km)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;25</td>
<td>1.75</td>
<td>0.91, 3.39</td>
<td>0.094</td>
</tr>
<tr>
<td>Buffalo Cattle contact*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No contact</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct, transhumance and migration</td>
<td>1.86</td>
<td>0.87, 3.98</td>
<td>0.109</td>
</tr>
<tr>
<td>No direct contact, transhumance and stray buffalo</td>
<td>5.08</td>
<td>1.87, 13.80</td>
<td>0.001</td>
</tr>
<tr>
<td>Proximity to wetland (km)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;25-50</td>
<td>2.33</td>
<td>0.82, 6.61</td>
<td>0.111</td>
</tr>
<tr>
<td>&gt;50</td>
<td>0.3</td>
<td>0.13, 0.69</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Table 7: Cross tabulation of significant predictor variables from univariable analysis

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>Chi2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximity to buffalo migratory route and distance to NP</td>
<td>34.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Proximity to buffalo migratory route and proximity to wetland</td>
<td>97.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Proximity to buffalo migratory route and cattle density</td>
<td>62.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Buffalo density and buffalo-cattle contact</td>
<td>77.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Proximity to wetland and distance to NP</td>
<td>63.20</td>
<td>0.001</td>
</tr>
<tr>
<td>Cattle density and agro-ecological zone</td>
<td>124.91</td>
<td>0.001</td>
</tr>
<tr>
<td>Cattle density and distance to NP</td>
<td>92.78</td>
<td>0.001</td>
</tr>
<tr>
<td>Cattle density and buffalo-cattle contact</td>
<td>88.73</td>
<td>0.001</td>
</tr>
<tr>
<td>Cattle density and proximity to wetland</td>
<td>47.85</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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

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 Itelhitezhzi’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>
<thead>
<tr>
<th>Location</th>
<th>Number examined</th>
<th>3ABC (95% CI)</th>
<th>LPBE (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dundumwezi</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lundazi</td>
<td>101</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Livingstone</td>
<td>20</td>
<td>5.0 (0, 17.6)</td>
<td>5.0 (0, 17.6)</td>
</tr>
<tr>
<td>Sesheke</td>
<td>99</td>
<td>0</td>
<td>2.6 (0.9, 6.1)</td>
</tr>
<tr>
<td>Kazungula</td>
<td>51</td>
<td>2.0 (0, 13.2)</td>
<td>33.3 (20.4, 46.3)</td>
</tr>
<tr>
<td>Monze</td>
<td>53</td>
<td>11.3 (0.1, 22.6)</td>
<td>26.4 (14.5, 38.3)</td>
</tr>
<tr>
<td>Itenzhitezhi</td>
<td>62</td>
<td>11.2 (0.9, 21.6)</td>
<td>22.6 (12.2, 32.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>413</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Ref</th>
<th>1.88, 23.2</th>
<th>0.003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>6.62</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Distance to NP

<table>
<thead>
<tr>
<th></th>
<th>&lt;30km</th>
<th>Ref</th>
<th>0.42, 2.82</th>
<th>0.856</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥30km</td>
<td></td>
<td>1.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Distance to road network

<table>
<thead>
<tr>
<th></th>
<th>&lt;35 km</th>
<th>Ref</th>
<th>0.43, 2.91</th>
<th>0.810</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;35 km</td>
<td></td>
<td>1.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proximity to buffalo*

<table>
<thead>
<tr>
<th></th>
<th>&lt;25km</th>
<th>Ref</th>
<th>0.09, 1.12</th>
<th>0.093</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;25km</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>Chi2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle density and proximity to buffalo migratory route</td>
<td>90.71</td>
<td>0.0001</td>
</tr>
<tr>
<td>Small ruminant density and proximity to buffalo migratory route</td>
<td>139.45</td>
<td>0.0001</td>
</tr>
<tr>
<td>Transhumant grazing and FMD vaccination status</td>
<td>196.42</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cattle density and transhumant grazing</td>
<td>413.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

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
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)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transhumant grazing</td>
<td>No (ref)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>16.91</td>
<td>0.001</td>
<td>3.58, 79.76</td>
</tr>
<tr>
<td>Age</td>
<td>≤ 2 years (Ref)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;2 – 4 years</td>
<td>0.98</td>
<td>0.98</td>
<td>0.32, 3.06</td>
</tr>
<tr>
<td></td>
<td>&gt;4 years</td>
<td>0.46</td>
<td>0.48</td>
<td>0.52, 4.07</td>
</tr>
</tbody>
</table>

**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**
The rt-PCR amplification of the five isolates from lower Zambezi and two from Luambe NPs yielded expected bands sizes of 490 bp (Figure 2), 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.

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

*Note: * isolate from cattle at the interface
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 phylogenetic 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)](image)

*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:

\[
\frac{d}{dt} \text{(Susceptible)} = - \text{new}_\text{infections} + \text{birth} - \text{susceptible}_\text{deaths} \\
\frac{d}{dt} \text{(Preinfectious)} = + \text{new}_\text{infections} - \text{new}_\text{infectious} - \text{preinfectious}_\text{deaths} \\
\frac{d}{dt} \text{(Infection)} = + \text{new}_\text{infectious} - \text{new}_\text{carrier} - \text{infection}_\text{deaths} - \text{new}_\text{infectious}_\text{carrier} \\
\frac{d}{dt} \text{(Carrier)} = + \text{new}_\text{carrier} - \text{newly}_\text{recovered} - \text{carrier}_\text{deaths}
\]
\[
d/dt (\text{Immune}) = + \text{newly\_recovered} - \text{immune\_deaths} + \text{new infectious carriers}
\]

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

\[
\text{new\_infections} = \beta \times \text{susceptible} \times (\text{infection} + \text{carrier})
\]

\[
\text{new\_infectious} = \text{preinfectious} \times \text{infectious rate}
\]

\[
\text{new\_carrier} = \text{infection} \times \text{carrier rate} \times \text{carrier\_percent}
\]

\[
\text{newly\_recovered} = \text{carrier} \times \text{carrier recovery rate}
\]

\[
\text{birth} = \text{total population} \times \text{birth rate}
\]

\[
\text{Susceptible\_deaths} = \text{susceptible} \times \text{mortality rate}
\]

\[
\text{Preinfectious\_deaths} = \text{preinfectious} \times \text{mortality rate}
\]

\[
\text{Infection\_deaths} = \text{infection} \times \text{mortality rate}
\]

\[
\text{Carrier\_deaths} = \text{carrier} \times \text{mortality rate}
\]

\[
\text{Immune\_deaths} = \text{immune} \times \text{mortality rate}
\]

\[
\text{Infected carriers} = \text{infection} \times \text{carrier\_rate} \times (1 - \text{carrier\_percent})
\]

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 = \( R_0 / \text{total population} \times \text{infectious period} \)
- 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 \(\Delta = R_0 - 1/D\)

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.](image)

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.](image)
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.](image)

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.
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 Ssheke-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 Itezhitzezi 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, Mbulungu 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 Zymbo, 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

117
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
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 ($R_0 = 5$). The epidemic would take a shorter period (45 days) to reach its peak if the basic reproduction number was high ($R_0 = 9$). 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., 2014a).
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.
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9.0 APPENDIX

Appendix 1 Publications from this thesis

Published papers


## QUESTIONNAIRE FOR THE CROSS-SECTIONAL SURVEY OF FOOT AND MOUTH DISEASE AT THE LIVESTOCK WILDLIFE 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

<table>
<thead>
<tr>
<th>Date of visit</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Name of Village/</td>
<td></td>
</tr>
<tr>
<td>Dip tank/crushpen</td>
<td></td>
</tr>
<tr>
<td>Location:</td>
<td></td>
</tr>
<tr>
<td>Constituency/District</td>
<td></td>
</tr>
<tr>
<td>/Province</td>
<td></td>
</tr>
<tr>
<td>GPS coordinates</td>
<td>Northern</td>
</tr>
<tr>
<td></td>
<td>Eastern</td>
</tr>
<tr>
<td>Owners name</td>
<td></td>
</tr>
<tr>
<td>Name of person interviewed</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---</td>
</tr>
<tr>
<td>Relation of the person interviewed</td>
<td>Owner</td>
</tr>
<tr>
<td></td>
<td>Family member</td>
</tr>
<tr>
<td></td>
<td>Care taker</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goats</th>
<th>Pigs</th>
<th>Others (Specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**Give a breakdown of herd structure**

<table>
<thead>
<tr>
<th>Cattle</th>
<th>Number</th>
<th>Small Ruminants</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Number of cows</td>
<td>Does/Ewes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Number of bulls</td>
<td>Bucks/Rams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Number of heifers &gt;1 yr</td>
<td>Females &gt; 8 Months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Number of female calves &lt;1yr</td>
<td>Female kids &lt;8 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Number of males calves &lt;1yr</td>
<td>Male kids &lt;8 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Male cattle between 2-3 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Female cattle between 2-3 years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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?

<table>
<thead>
<tr>
<th>Cattle</th>
<th>Breed type</th>
<th>Small Ruminants</th>
<th>Breed type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Exotic</td>
<td>1. Exotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Local</td>
<td>2. Local</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Month</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td></td>
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<tr>
<td>April</td>
<td></td>
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<tr>
<td>May</td>
<td></td>
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<tr>
<td>June</td>
<td></td>
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<tr>
<td>July</td>
<td></td>
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<tr>
<td>August</td>
<td></td>
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<tr>
<td>September</td>
<td></td>
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<tr>
<td>October</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td></td>
</tr>
<tr>
<td>December</td>
<td></td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Species</th>
<th>Volume</th>
<th>Seasonality</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>● Constant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Seasonality (circle month(s) of higher trade activity):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>J F M A M J J A S O N D</td>
<td></td>
</tr>
</tbody>
</table>
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................................................................. □
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?

<table>
<thead>
<tr>
<th>Cattle</th>
<th>Price</th>
<th>Small Ruminants</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td></td>
<td>Doe</td>
<td></td>
</tr>
<tr>
<td>Bull</td>
<td></td>
<td>Buck</td>
<td></td>
</tr>
<tr>
<td>Heifers</td>
<td></td>
<td>Ram</td>
<td></td>
</tr>
<tr>
<td>Ox</td>
<td></td>
<td>Bull</td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td>Pig (sow and boar)</td>
<td></td>
</tr>
</tbody>
</table>

Q34 What is the annual offtake from your farm?

<table>
<thead>
<tr>
<th>Species</th>
<th>number</th>
<th>Species</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td></td>
<td>Goat</td>
<td></td>
</tr>
<tr>
<td>Bull</td>
<td></td>
<td>sheep</td>
<td></td>
</tr>
<tr>
<td>Heifer</td>
<td></td>
<td>Others (specify)</td>
<td></td>
</tr>
<tr>
<td>Ox</td>
<td></td>
<td>milk</td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td>pig</td>
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</tbody>
</table>

Manual, drought power etc
Q34 How does FMD affect you as a farmer?

BUFFALO SURVEY

SECTION ONE: IDENTIFICATION

<table>
<thead>
<tr>
<th>Date of visit</th>
<th></th>
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<tbody>
<tr>
<td>Name of wildlife camp</td>
<td></td>
</tr>
<tr>
<td>Location: Constituency/ District/ Province</td>
<td></td>
</tr>
<tr>
<td>GPS coordinates</td>
<td>Northern Eastern</td>
</tr>
<tr>
<td>Name of National Park</td>
<td></td>
</tr>
<tr>
<td>Name of person interviewed</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td></td>
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</tbody>
</table>

157
Q2 How many clusters of buffalo are there?

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Herd size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>

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 you know the pattern of movement of the buffalo during the year?

<table>
<thead>
<tr>
<th>Month</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
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<tr>
<td>February</td>
<td></td>
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<tr>
<td>March</td>
<td></td>
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<tr>
<td>April</td>
<td></td>
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<tr>
<td>May</td>
<td></td>
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<tr>
<td></td>
<td>June</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
</tr>
</tbody>
</table>

**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.................................................................................................