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# Epidemiology and impact of foot-and-mouth disease in districts located along the Uganda and Tanzania border

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# EPIDEMIOLOGY AND IMPACT OF FOOT-AND-MOUTH DISEASE IN DISTRICTS LOCATED ALONG THE UGANDA AND TANZANIA BORDER

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**A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology**

**Arusha, Tanzania**

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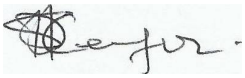
## ABSTRACT

The Uganda-Tanzania border area lies within the main risk areas for foot-and-mouth disease (FMD) circulation. With the introduction of the progressive control pathway for FMD in eastern Africa, reliable information on FMD epidemiology along the Uganda-Tanzania border area is important in informing the pathway such that, strategic collaborative controls are designed. The scarcity of information on FMD impact in both Uganda and Tanzania, leaves a gap in information critical for justification for national and regional expenditures for FMD intervention. The objectives of the present study were to; (i) determine the spatial and temporal distribution of FMD in districts along the Uganda–Tanzania border between 2011 and 2016 (ii) determine genetic relationships between FMD viruses circulating between 2016 and 2017 and, (iii) ascertain the impact of FMD on income and food security. The study was carried out in the border districts of Missenyi and Kyerwa in Tanzania and Rakai and Isingiro in Uganda. For objective (i), retrospective data was compiled and analysed in R and maps were drawn using QGIS. Results showed that 46% of the 82 recorded outbreaks occurred in sub-counties/wards immediately neighbouring the Uganda-Tanzania border and 69.5% of the outbreaks occurred during dry months. For objective (ii), 43 samples were analysed using PCR and 11 were successfully sequenced. Sequences were analysed and trees drawn using MEGA 7. Phylogenetic analysis of the VP1 coding region showed that serotype O viruses obtained belonged to EA-2 topotype and clustered together with an average sequence divergence of 4.9%. Obtained serotype A viruses belonged to Africa-G1 topotype, formed one cluster with a 7.4% sequence divergence. For objective (iii) data was collected from 288 households using a structured questionnaire. Results showed significant reduction in income from livestock and livestock products sales by over 60%, whereas livestock market prices decreased by nearly half. Forty nine percent of farmers reported calf mortalities and milk consumption in households reduced by 57% in Rakai and Isingiro and 48% in Missenyi. These findings provide information helpful for policy reform, and designing better strategies for FMD control. The study recommends comprehensive regional studies to be implemented in border areas.

## AUTHOR'S DECLARATION

I, Susan Diana Kerfua do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.



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Name and signature of candidate

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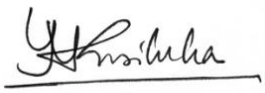
**Name and signature of Supervisor 1**



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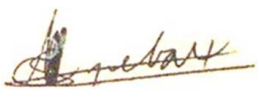

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## CERTIFICATION

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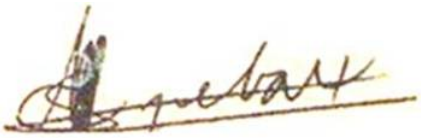

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## DEDICATION

This work is dedicated to my dear late father Hon. Stanley Omwonya Oribdthogwu who relentlessly encouraged me to pursue my PhD studies, I am so saddened that you do not get to share this great achievement with me. I miss you. Rest in peace.

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## LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| AU-IBAR | African Union Inter African Bureau for Animal Resources |
| COMESA | Common Market for East and Southern Africa |
| EA | East Africa |
| EAC | East African Community |
| ELISA | Enzyme Linked Immuno Sorbent Assay |
| FAO | Food and Agriculture Organisation of the United Nations |
| FMD | Foot and Mouth Disease |
| FMDV | Foot and Mouth Disease Virus |
| GDP | Gross Domestic Product |
| g | gram(s) |
| ICPALD | IGAD Center for Pastoral Areas and Livestock Development |
| IRES | Internal Ribosomal Entry Site |
| KEVEVAPI | Kenya Veterinary Vaccine Production Institute |
| MAAIF | Ministry of Agriculture, Animal Industry and Fisheries |
| MEGA | Molecular Evolutionary Genetic Analysis |
| MLFD | Ministry of Livestock and Fisheries Development |
| NADDEC | National Animal Disease Diagnostic and Epidemiology Centre |
| NM-AIST | Nelson Mandela African Institution of Science and Technology |
| OIE | World Organisation for Animal Health |
| ORF | Open Reading Frame |
| PCP | Progressive Control Pathway |
| PCR | Polymerase Chain Reaction |
| RGD | Arginine (R), Glycine (G) Aspartic acid (D) |
| RNA | Ribonucleic Acid |
| RT | Reverse Transcription |
| rRT-PCR | Reverse Transcriptase qualitative PCR |
| SAT | Southern African Territories |
| TADs | Trans-boundary Animal Diseases |
| TZS | Tanzanian Shillings |
| UBOS | Uganda Bureau of Statistics |
| µl | microliters |
| UgX | Ugandan shillings |
| US$ | United States Dollars |
| UTR | Un-translated regions |
| VP1 | Viral protein |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the problem

The livestock industry is one of the important sub-sectors of agriculture and supports the livelihood of billions of people around the world. Livestock provides food, hides and skin, manure, income, draught power and is important in the social and cultural activities of many communities (Devendra, Swanepoel, Stroebel & van Rooyen, 2010). In most African countries, livestock diseases are endemic and present a challenge to the livestock sub-sector, thereby constraining the capacity of the agricultural sector to meet the ever-increasing demands for livestock and livestock products (Food and Agricultural Organisation [FAO], 2004). Most developing countries are plagued by trans-boundary animal diseases (TADs) which easily spread between countries and impede livestock markets by restricting local, regional and international trade. Additionally, there is increased expenditures on prevention and control of these diseases (Otte, Nugent & McLeod, 2004; Perry & Grace, 2009).

Foot-and-mouth disease (FMD) is one of the major TADs of economic importance in sub-Saharan Africa (Otte *et al.*, 2004). The disease causes severe economic consequences in countries that frequently experience outbreaks. Although developed countries rarely experience FMD outbreaks, occasionally they have had incursions that have led to high economic losses. For example, an outbreak of FMD in the United Kingdom in 2001 caused the country a US$ 9 billion (~£7.2 billion) loss and the Netherlands lost about US$1 billion after they experienced an outbreak that had spread from the United Kingdom (Rushton & Knight-Jones, 2012; Knight-Jones & Rushton, 2013). While the impact of FMD is considered to be greatest in Africa (Gall & Leboucq, 2004), studies that document the impact of this disease on the rural communities are very limited (Otte *et al.*, 2004), with the number of studies on FMD impact in sub-Saharan African reported to be less than 30 by 2013 (Knight-Jones & Rushton, 2013). Few studies in sub-Saharan Africa have elucidated on the social and economic consequences of endemic FMD making inference on the losses imposed on households (Rutagwenda, 2003; Pendell, Leatherman, Schroeder & Alward, 2007; Bayissa, Ayelet, Kyule, Jibril & Gelaye, 2011; Baluka, Ocaido & Mugisha, 2014). Thus, there is still limited information on the economic impact of FMD on communities in developing countries yet such studies are critical in decision-making especially in allocation of resources for FMD control.

Foot-and-mouth disease is caused by the Foot-and-mouth disease Virus (FMDV) which belongs to the family *Aphthoviridae* and the genus *Picornavirus.* It is a non-enveloped RNA virus approximately 8.3 kilobases (kb) long, has four structural proteins and between 8-10 non-structural proteins depending on the serotype (Jamal & Belsham, 2013). There are seven serotypes of the FMDV that have been described globally and these include, A, O, C, Asia 1 and Southern African Territories (SATs) 1 to 3. The serotypes are further divided into 'topotypes' or 'genotypes', 'lineages' and 'variants' based on nucleotide differences within the viral protein 1 (VP1), which is one of the structural proteins that is transcribed by the ID gene (Knowles & Samuel, 2003). Six of the seven serotypes of FMDV have occurred in East Africa, making it the most diverse region in terms of FMDV epidemiology (Sangula *et al*., 2010; Casey-Bryars *et al*., 2018). The high diversity of FMDV serotypes in the region has implications on disease control, especially if vaccination is to be adopted as a means of FMD control. This is because, the vaccine strain used in a country/ region has to be antigenically similar to the virus strains in circulation (Kitching *et al*., 2007).

Epidemiology of foot-and-mouth disease in the border areas is critical, considering its trans-boundary nature. Border areas in eastern Africa have been highlighted as important in FMD circulation (Di Nardo, Knowles & Paton, 2011). Several studies have elucidated increase in trans-border animal and human movement as one of the major factors for introduction and circulation of animal diseases, especially FMD (Otte *et al.*, 2004; Fèvre, Bronsvoort, Hamilton & Cleaveland, 2006) thus making border areas critical in disease spread. Border areas in eastern Africa have been pointed out as important in FMD epidemiology and the border area between Tanzania and Uganda lies within one of the main risk areas for FMD circulation in sub-Saharan Africa (Di Nardo *et al*., 2011). Tanzania and Uganda are members of the East African Community (EAC), situated in the eastern part of Africa and share an international border that is north of Tanzania and south of Uganda. Four districts namely, Isingiro and Rakai in Uganda and Missenyi and Kyerwa in Tanzania, are located along this international border. In both Uganda and Tanzania, FMD outbreaks have been reported annually with little success registered in the control of the disease despite the efforts by the individual governments (Kivaria, 2003; Ministry of Agriculture Animal Industry and Fisheries (MAAIF), 2012; Ministry of Livestock Fisheries Development (MLFD), 2015). According to the Common Market for East and Southern Africa (COMESA), 2009), the increase in cross-border trade in East Africa has facilitated increased movement of livestock, livestock products and people across borders which in turn, may be critical for disease

transmission and circulation. A study by Ayebazibwe *et al.* (2010) showed that in Uganda, sub-counties located along the borders, especially those near to Tanzania, suffered more FMD outbreaks compared to other sub-counties. Also, Kerfua *et al*. (2018) demonstrated that Ugandan and Tanzanian sub-counties/wards located nearer to the international border between Uganda and Tanzania reported more outbreaks between 2011 and 2016 than the other sub-counties/wards. Phylogenetic studies in East Africa provide additional evidence of cross-border transmission of FMDVs from one country to another (Balinda *et al.*, 2010a; Sangula *et al*., 2010). Efforts have been made towards the study of FMD epidemiology in districts along the Uganda and Tanzania area, however these studies were independent and did not involve cross sectional research between the two countries. A study by Namatovu *et al*. (2015) determined the relationship between virus outbreaks in Isingiro. Phylogenetic analysis from the study showed that the viruses obtained from Isingiro were quite similar to Tanzanian viruses that were obtained in other previous studies. Kasanga *et al*. (2013) additionally, observed that the viruses they obtained from Tanzania (O/TAN/16/2008, O/TAN/5/ 2009 and O/TAN/44/2009) were closely similar to Ugandan viruses that had previously been isolated. These study illuminate the possibility of the two countries sharing similar sources of outbreaks and that transmission may most likely be through the international borders. Therefore, trans-border studies regarding virus transmission and general epidemiology provide insights that are pertinent for developing better understanding and interventions that should be collaborative. Otherwise, different control policies in neighbouring countries may constantly place one country at risk, thus increasing disease control expenditures and restrictions to markets (Rushton & Knight-Jones, 2012).

### 1.2 Statement of the problem

The general dearth on availability of data on FMD epidemiology and knowledge of FMDV serotypes and strains circulating in border areas may jeopardise the control of the disease in neighbouring countries. Poor identification of FMD risk points and lack of information on viruses in circulation in such areas demeans development of better disease control strategies. It is critical that FMD control strategies are required to be more focussed for sustainable resources to be allocated disease control.

Impact studies are critical in decision-making especially with regard to allocation of resources for FMD control. However, in most developing countries, including Uganda and Tanzania there have been limited studies on the economic analysis of the impact of the

diseases on livestock production, trade, market access and prices, food security and the well-being of people in the rural communities (Herrero *et al*., 2013).

### 1.3 Rational of the study

Basic information such as prevalence, incidence, serotype and impact of a disease are important in prioritizing and determining the interventions to be implemented and where these interventions can be implemented. The circulation of the FMDV in cThis, in turn, usually affects the outcome on wealth, health and education within communities (Herrero *et al*., 2013), thereby leading to the achievement of sustainable development goals. Information obtained from this study will be helpful in improving the understanding of FMD distribution and spread within the border region between Uganda and Tanzania. The information on the impact of FMD on rural communities is critical in providing evidence that will impact on policies for prevention and control of the disease. Studies on impact of a disease ensures that interventions towards the control of a disease are based on the way a disease influences livelihoods in communities.

### 1.4 Objectives

#### 1.4.1 Overall objective

The overall objective of the present study was to elucidate on the epidemiology of foot-and-mouth disease and its impact in border districts of Uganda and Tanzania with a view of informing regional FMD control strategies.

#### 1.4.2 Specific objectives

(i) To determine the distribution patterns of foot-and mouth disease outbreaks in border districts of Uganda and Tanzania for the years between 2011 and 2016.

(ii) To determine the genetic relationship of outbreak foot-and-mouth disease viruses obtained from cattle in the border districts of Uganda and Tanzania.

(iii) To ascertain the impact of foot-and-mouth disease on household income, livestock market prices and food security in cattle keeping households in the districts located along the border of Uganda and Tanzania.

### 1.5 Research questions

(i) What is the temporal and spatial distribution of foot-and-mouth disease outbreaks in the border districts of Uganda and Tanzania?

(ii) What is the genetic relationship between foot-and-mouth disease virus strains obtained during FMD outbreaks in the districts located along the border of Uganda and Tanzania?

(iii) How do outbreaks of foot-and-mouth disease impact on the income, livestock market prices and food security of households in livestock keeping communities in districts along the Uganda and Tanzania border?

### 1.6 Significance of the study

The study will provide information that will be used to develop better control strategies for FMD in the light of increased trans-boundary activities. Subsequently, there will be improved livelihood for livestock keepers and people along the livestock value chain.

### 1.7 Delineation of the study

This study was concerned with understanding the epidemiology of FMD and its impact in the districts that are adjacent to international border between Uganda and Tanzania. Epidemiology concerns the study of how a disease is distributed, its incidences and what possible control strategies can be put in place to control its spread or even eliminate disease. Molecular epidemiology involves the use of molecular markers to determine in detail the characteristics of the infectious agent of disease and its possible transmission patterns. The first objective of the study focused on the spatial and temporal distribution of FMD. An outbreak was defined as the presence of two or more clinical signs and symptoms of FMD in one animal in a herd. The second objective of the study focused on molecular epidemiology and utilized the VP1 coding region to deduce relationships between the obtained FMDVs. In this study, deduced amino acid sequences were also compared with the vaccines used in the region. The third objective of the study looked into disease impact at household levels and illustrates the way FMD affects farmers' income, livestock market prices and food security. Food security parameters in this study focused on the changing trends on food prices, food availability and access to food variety. A farmer in study referred to one who kept more than

one head of cattle for the purpose of milk or beef production. The study focused on farmers who had experienced an outbreak twelve months prior data collection. The purpose of this study was to provide information relevant for improvement of FMD control strategies in both countries which could ultimately lead to better incomes and improved healthy livelihoods of livestock keeping communities and persons along the value chain.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Livestock

Over two thirds of the world's population depend on agriculture for livelihood. Livestock husbandry is a major part of agriculture and the theme of the 2009 Food and Agriculture Organization (FAO) annual flagship publication 'The State of Food and Agriculture' was 'Livestock in the Balance'. This alluded to the fact that the livestock sector makes important contributions to food security and poverty reduction (FAO, 2009). An estimated 1.3 billion people worldwide are employed in the different sectors (formal and informal) of the livestock value chain (Herrero, Thornton, Gerber & Reid, 2009) thus is an important source of livelihood. Food from animals are rich in palatable energy sources and quality proteins and are important in the diets of people around the world. Particularly, vulnerable groups of people such as children, pregnant and nursing women, and sick persons benefit from consuming foods such as milk, beef and their products (Murphy & Allen, 2003). Apart from food and income, the livestock industry provides energy in the form of biogas, an important source of fuel used for cooking in developing countries where forests are endangered (Msibi, 2015). Additionally, over half of the total crop area in Africa is cultivated using livestock (Pearson & Vall, 1998; Conroy, Goodman & Kenward, 2010; Upton, 2004) and in social functions such as traditional marriages and cultural virtues livestock are the main commodities used (Otte *et al.*, 2004).

### 2.1.1 Livestock in Tanzania and Uganda

The livestock sector in Eastern Africa accounts for about 10-20% of the region's Gross Domestic Product (GDP). In about two decades (by 2011), this sector had grown at an annual rate of about four percent (FAO, 2004; Pica-Ciamarra, Baker, Morgan & Zezza, 2011). In Tanzania, agriculture employs over 80% of the population and although livestock/livestock products are some of the major agricultural merchandises, the livestock sector contributes only seven percent to the country's national GDP (IGAD Center for Pastoral Areas and Livestock Development (ICPALD), 2013; MLFD, 2015). Tanzania has Africa's second largest livestock population that was estimated at 25 million cattle, 16.7 million goats, 8 million sheep and 2.4 million pigs, with over 3 million households owning at least one of these animals by 2015 (MLFD, 2015).

In Uganda, agricultural sector employs about 73% of the population and the livestock industry's contribution to the national GDP was estimated at 1.7 % by 2014 (Ugandan Bureau of Statistics [UBOS], 2014). The population of livestock in Uganda in 2014 was estimated at 12.1 million cattle, 13.2 million goats, 3.6 million sheep and 3.8 million pigs (UBOS, 2014) and the annual growth of the livestock sector was estimated at 3% in Uganda (UBOS, 2014) and 2.2% in Tanzania (MLFD, 2015). The contribution of livestock sector to the GDP of both countries roughly reflects that the industry is not well-harnessed despite its potential. Growth in the livestock sector ensures improvement in rural livelihood, boosts economies and provides food in terms of livestock products (Upton, 2004). Although the role of livestock in poverty alleviation remains undisputed, most of the livestock farmers in East Africa are resource-poor and usually, keep one or two animals as a source of food and instant cash. About 105 000 livestock keepers in East Africa lived on less than US$ 2 per day (Herrero *et al*., 2013), a reflection that the benefits from keeping livestock are still low. Several factors such as livestock diseases impede the growth of the livestock industry because they pose a threat to productivity and consequently, income and food nutrition (Gall & Leboucq, 2004).

### 2.2 Trans-boundary Animal Diseases

According to Otte *et al*. (2004), TADs are defined as, "Those diseases that are of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries". These diseases (TADs) are a permanent menace to governments, livestock keepers, livestock traders and other people along the livestock value chain. The TADs retard regional and international trade, thereby impeding development of affected countries. Millions of funds have to be spent on prevention and eradication of TADs, making them very expensive to contain. Some of the major TADs include; Foot-and-mouth disease (FMD), Rift Valley fever (RVF), Peste des petits ruminants (PPR), Classical swine fever (CSF), Newcastle disease (NCD), Rinderpest (RP), African swine fever (ASF) and Avian influenza (AI) (Otte *et al.*, 2004). Foot-and-mouth disease remains one of the most important TADs because it is very contagious and has high economic consequences (Rweyemamu & Astudillo, 2002; James & Rushton, 2002). According to the African Union Inter-African Bureau for Animal Resources [AU-IBAR] (2009), for the period of 2010-2014, the cost of resources that was required to

control and prevent TADs in East Africa was estimated at US$ 20 million annually. More specifically, annual costs due to and to prevent FMD outbreaks worldwide were estimated at US$ 5 billion (James & Rushton, 2002). In Uganda, the government spent approximately US$ 58 000 - US$ 1 088 820 on importation of FMD vaccines between 2001-2010 (Muleme *et al.*, 2012) whereas quarantines that were instituted in six districts out of the 56 in 2003 cost the country approximately US$ 8.01 million (Rutagwenda, 2003). In 2007 in Kenya, a Rift Valley fever ban resulted into losses estimated at US$ 32 million in exports and there were other negative local impacts on agriculture and sectors such as transport and services (Rich & Wanyoike, 2010). Countries that are free of TADs make every effort to keep the diseases away however, in cases where there are incursions, enormous losses have been incurred. For example, in the United Kingdom following an FMD outbreak in 2001, the country lost about US$ 9 billion (~ £7.2 billion) and had to cull about 10 000 animals (Thompson *et al.*, 2002). Trans-boundary animal diseases therefore, have major economic implications because they have to be controlled at individual, national, regional and even at international level (Otte *et al.*, 2004).

### 2.2.1 Foot-and-mouth disease

Foot-and-mouth disease is a highly contagious vesicular disease of cloven-hoofed animals which includes both livestock populations and several species of wildlife (Thomson and Bastos, 2004; Coetzer, Thomson & Tustin, 1994). Livestock species such as cattle, goats, sheep and pigs are highly susceptible to FMD when unvaccinated and exposed to the virus (Kitching *et al.*, 2007). The disease usually causes a high morbidity in adult animals and a high mortality in young animals particularly in calves. The young animals often die because the disease affects their heart muscle (Kitching & Hughes, 2002). The symptoms that have been associated with FMD include lameness due to blisters and wounds on the skin between the hooves, salivation, lack of appetite, rise in temperature, blisters in the mouth (Fig. 1) or other areas of tender skin (such as nostrils and udders). Cases of abortions and difficulty in conception in animals that have suffered from FMD have also been reported by some workers (Kitching & Hughes, 2002; Alexandersen, Zhang, Donaldson & Garland, 2003). Clinical signs of FMD in some animals such as sheep and goats can be difficult because of the mild signs they present (Callens, De Clercq, Gruia & Danes,1998; Alexandersen, Zhang, Reid, Hutchings & Donaldson, 2002a; Hughes *et al.*, 2002) and even so, some strains of the virus exhibit low virulence in some animal species (Donaldson, 1972). Nonetheless, some animals

such as pigs act as key FMDV amplifiers and usually suffer the most severe lesions on their feet with the onset symptoms being lameness and blanching of the skin around the coronary bands (Kitching & Alexandersen, 2002).

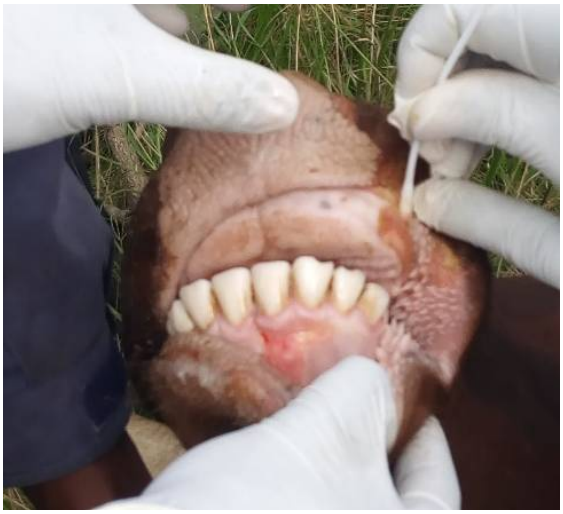


Figure 1: Lesions on the gums of a cow suspected to have FMD (Susan Diana Kerfua)

### 2.2.2 Carrier status and subclinical infections with FMD

Carrier animals can be defined as those from which the virus can be isolated from their oesopharynx 28 days after they have recovered from infection. The virus can persist for up to four months in goats and nine months in sheep. Most cattle can live with the virus for up to six months and stay persistently infected for up to 3.5 years (Kitching, 2002). Studies have documented wildlife species like buffalo, deer, gazelles, elands, wildebeests, impalas, hartebeests, waterbucks and giraffes, and elephants as carriers for FMDV and their roles in FMD epidemiology in eastern and southern Africa have been often cited (Anderson, Doughty, Anderson & Paling, 1979; Vosloo, Bastos, Sangare, Hargreaves & Thomson, 2002; Vosloo *et al*., 2005; Bengis, 2005; Ayebazibwe *et al.*, 2010; Miguel *et al*., 2013). In the late 1970s, Anderson *et al*. (1979), documented the recovery of the FMDV from over 50% of oesophageal-pharyngeal samples from selected wild animals in Kenya. The wildlife species included buffalo, elands, gazelles, impala, giraffes, waterbucks and wildebeests. Additionally, serological evidence of infection after exposure to the virus was detected from samples from buffalo, elands, gazelles, wildebeest and topi. Although buffaloes have long been implicated for spreading FMD because of their carrier status, no experimental evidence has been

established to this effect (Anderson *et al*., 1979; Kitching *et al*., 2007). Subclinically-infected animals are those that are infected with the disease but do not show any clinical signs or lesions and yet are shedding the virus (Sutmoller, Barteling, Olascoaga & Sumption, 2003; Kitching & Hughes, 2002). In some instances, it may occur when some viruses have low virulence or when there is exposure of partially immune animal populations to infection (low herd immunity) (Van Bekkum, Frenkel, Frederiks & Frenkel, 1959). In some cases, an animal may suffer an acute stage of FMD infection that may cause a symptomless and persistent infection leading to the carrier status (Sutmoller & Gaggero, 1965; Alexandersen, Zhang & Donaldson, 2002b). Domesticated animals such as goats and sheep that hardly show clinical signs of FMD when infected, and have been implicated in some of the major global outbreaks that occurred in the United Kingdom and Denmark in early 2000s (Sutmoller *et al*., 2003; Kitching & Hughes, 2002). Sutmoller *et al*. (2003) suggested that carriers may actually have a lower rate of FMDV transmission compared to animals that are in the subclinical state of infection. He further argued that the 'carriers' that have been implicated in disease spread could have done so when they were in subclinical state of infection as previously postulated by Gainaru *et al*. (1986). However, there is still limited research on the role of carrier and subclinically-affected animals in FMD epidemiology calling for more research. However, in developed countries stringent and strategic control measures such as the stamping out policy is enforced. This is where, total slaughter of all animals, both affected and apparently normal in an infected premise in the event of an outbreak is done in order to eliminate the virus completely. Otherwise, the perception would be that some animals that may be apparently normal could be carriers and pose a continuous risk for outbreaks (Alexandersen *et al.*, 2003).

### 2.3 Economic impact of FMD

Several studies have demonstrated that FMD is one of the most economically-devastating diseases worldwide because of the high economic consequences it imposes on affected countries (James & Rushton, 2002; FAO, 2012; Knight-Jones & Rushton, 2013). The disease is of global importance given that both FMD-free countries have to spend enormous resources to keep the disease out of their countries whereas those that have an endemic status have to spend on controlling its spread. Additionally, countries where FMD is endemic are not allowed to participate in world livestock and livestock product markets and therefore, forfeit the foreign exchange that would otherwise boost their economies (Rushton & Knight-Jones, 2012), roughly reflecting on their low GDPs. Although adult mortalities due to FMD

are low, productivity of infected animals is immensely affected leading to low milk and meat yields, and decreased draught power (James & Rushton, 2002) and even in some cases delay in reproduction, thus affecting the farmers income and creating food insecurity that can lead to malnutrition (Rushton & Knight-Jones, 2012).

The impacts of FMD may be complex to quantify because they are highly variable but can be divided mainly into direct and indirect impacts (Knight-Jones & Rushton, 2013) which affect farmers, governments, livestock traders, market chains and communities in general (Fig. 2). Furthermore, direct impacts are divided into visible and invisible losses whereas the indirect impacts are divided into additional costs and foregone revenue.

### 2.3.1 Direct impacts

In endemic settings, visible impacts such as reduction in milk yield has been shown to account for up to 33% of the losses incurred during an FMD outbreak and a chronic case of FMD can reduce milk yield by about 80%. Mortality in young animals was estimated between 2%-5% (James & Rushton, 2002) and reduction of traction power in draught animals was observed to significantly affect ploughing of land and harvesting of crops (James & Ellis, 1978; Perry *et al*., 1999). Direct invisible impacts due to FMD are not as obvious. In Bolivia, it was observed that although problems with fertility were not as obvious as abortion losses, they were longer lasting and immensely impacted on the ability of an animal to conceive. Thus, a farmer had to spend more on investng in breeding stock per kilo of meat or milk (Rushton, 2009).

### 2.3.2 Indirect impacts

Indirect costs involve losses incurred due to resources spent on disease control and foregone revenue. Control measures such as vaccination, outbreak control, qaurantines, culling and compensation are expenses that tax payers have to bear. It was estimated that 2.6 billion doses of FMD vaccines were used annually in the world with costs for the drug and delivery ranging between US$ 0.4 to US$ 3 per dose, thus a total cost of $US 5 billion (Sutmoller *et al*, 2003; Barasa *et al*., 2008; Forman *et al*., 2009). In Africa, by 2004, most of the resources were spent on preventing FMD than any other livestock disease (Le Gall & Leboucq, 2004). Direct impact losses due to FMD across Africa have been estimated at $US 830 million annually and yet these estimates do not even include losses due to both local and international market restrictions (Rushton & Knight-Jones, 2012). Also, it was observed that the Ugandan

government spent approximately US$ 4 million on importation of FMD vaccines between 2001-2010 (Muleme *et al.*, 2012); and FMD quarantines that were instituted in six districts out of the 56 in 2003, cost the country US$ 8.01 million. Moreover, countries that were unable to participate in international markets because of their FMD endemic status, lost about US$ 17 per kg of beef. Additionally, it was shown that livestock market prices dropped by half in the districts of Kumi and Isingiro due to an FMD outbreak (Rutagwenda, 2003). Indirect impacts are also realised when market access and rural economies are disrupted. Impact at farmer level influences livestock producers and the entire market chain such as dairies, abattoirs and markets is affected (Le Gall & Leboucq, 2004). Other observations indicate that, FMD affects the export of other goods such as fruits and vegetables to FMD-free countries (James & Rushton, 2002).

Foot And Mouth Disease free wealthy countries suffer occasional outbreaks of the disease and such events have led to high costs incurred in the containing the disease. For example, the 2001 FMD outbreak that occurred in United Kingdom cost US$ 9 billion (~7.2 billion pounds) (Thompson *et al.*, 2002) and when the outbreak spread to Netherlands, the country lost over US$ 1billion in control expenses (Rushton & Knight-Jones, 2012). The FMD-free countries endeavor to keep their territories FMD-free and in doing so they have to spend on:

(i) Ensuring that they can be able to detect and control the disease early enough. This would include setting up surveillance systems, imposing permanent restrictions on livestock and livestock product movement and ensuring veterinary services are sufficient and organised.

(ii) Handling outbreaks and this may involve ensuring movement restrictions, culling and vaccinations.

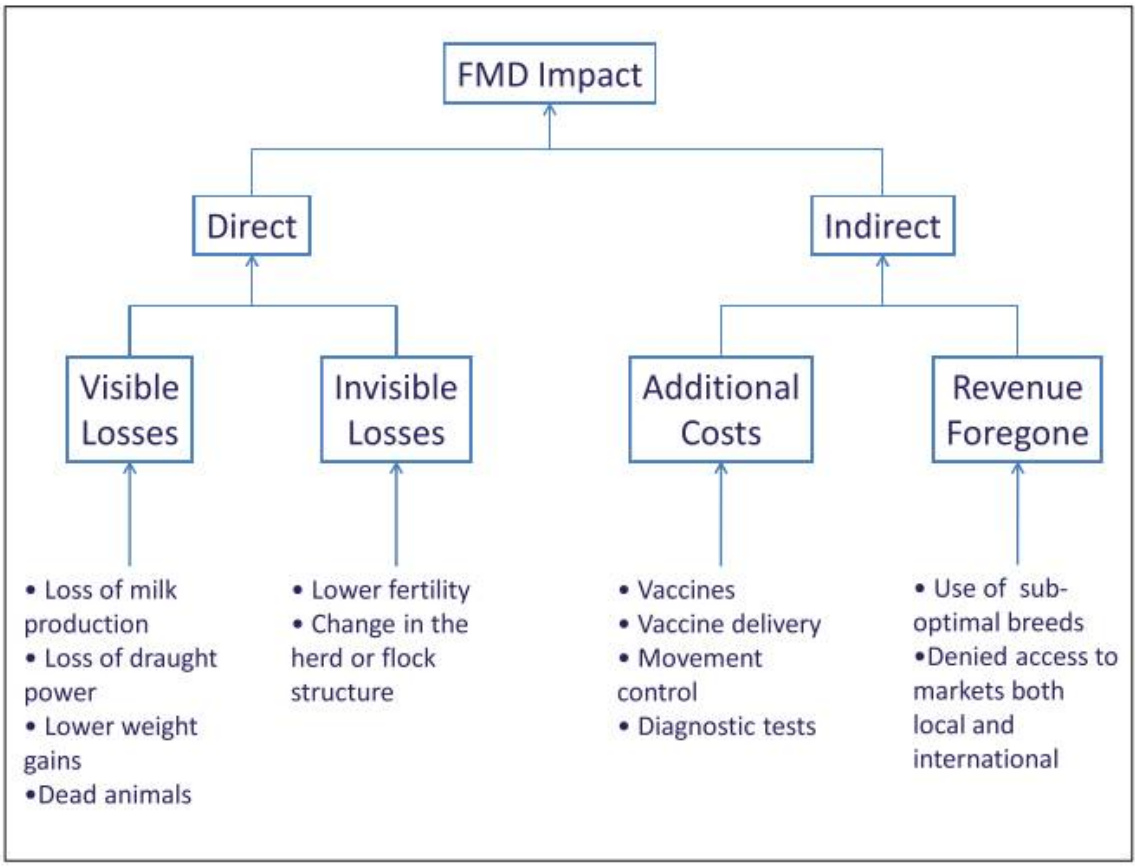



Figure 2: Impacts of FMD (Rushton & Knight-Jones, 2012)

Foot and Mouth Disease free countries receive much more attention during an FMD outbreak than developing countries which suffer the FMD endemic status. Endemic countries carry much of the global FMD burden and despite the conventional control measures such as ring vaccination and imposed quarantines, the disease remains persistent (FAO, 2012). Household income studies that have been carried out around the world have shown that low income households experience some of the greatest losses during outbreaks and lose about 12% of their income (Shankar, Morzaria, Fiorucci & Hak, 2012). In Ethiopia, losses for a lactating cow were put at US$ 137 while losses per herd were estimated at US$ 2175 (Beyi, 2012). Additionally, Jemberu, Mourits, Woldehanna and Hogeveen (2014) showed that crop and livestock farmers in Ethiopia lost about US$ 76 of their income and that losses due to milk yield ranged from US$ 0 to US$ 176 depending on severity of milk reduction and how long the animal had been ill.

Such economic studies show that farmers usually suffer the burden of the disease with the cattle and pig sectors being the most affected (Otte *et al*., 2004; Rushton & Knight-Jones, 2012; Knight-Jones & Rushton, 2013; Baluka *et al*., 2014; James & Rushton, 2002; Rushton & Knight-Jones, 2012; Casey-Bryars *et al*., 2018). However, impact of FMD on the small holder farmer has been neglected, is poorly quantified with limited studies (Knight-Jones, McLaws & Rushton, 2017), while in FMD-free countries, these studies are well defined. Interestingly though, many of the impact studies on FMD in non-endemic countries have been carried out based mostly on expert opinion and simulations and not on observation because animals are quickly culled off (James & Rushton, 2002).

A review by Knight-Jones and Rushton (2013) identified only 30 country and regional FMD impact studies published prior to 2013 for all of Africa (Knight-Jones & Rushton, 2013). Yet, in order to justify national and regional policy formulations, existing policy evaluations and resource allocation for FMD control, knowledge of FMD impact is important. In Europe and America, impact studies were central to the control and prevention of FMD (Knight-Jones & Rushton, 2013). For example, the policies on stamping out and vaccinations were key in the eradication of FMD in Europe and other FMD-free countries (James & Rushton, 2002). For that reason, accurate assessment of the socio-economic impacts of FMD on households is important to both regional and national governments to consider policy reforms regarding FMD prevention and control.

### 2.4 Foot-and-mouth disease virus

The FMDV belongs to the genus Aphthovirus and family *Picornaviridae*. The virion when viewed through an electron microscope, is an icosahedral capsid, non-enveloped round particle with a diameter of around 25 nm. The 8.0-8.3 kb long RNA virus has single stranded positive sense genome. Its open reading frame (ORF) has structured 5’ and 3’ un-translated regions (5’UTRs and 3’ UTRs) which are about 1300 and 90 nucleotides long, respectively (Carrillo *et al.*, 2005; Jamal & Belsham, 2013). The 5’UTR is made up of the nucleotide short fragments (S), a poly C tract (Cn), and about 700 nucleotide terminus of the genomic long fragment (L). Three tandemly repeated pseudo-knots, a stem loop cis-acting replication element (cre) and a type II Internal Ribosomal Entry Site (IRES) compose the L fragment. The 5’UTR is crucial in cap independent translation initiation of viral polyprotein and viral genome replication. The VPg peptide found at the extreme end of the 5’ UTR encodes for three different peptide forms, each acting as the primer for RNA synthesis such that each

RNA genome is covalently linked to a VPg after its synthesis (Jamal & Belsham, 2013). The 90 nucleotides long 3'UTR is thought to contain cis-acting elements for genome replication. The s-fragment and the IRES have been observed to interact with the 3' UTR (Serrano *et al.*, 2006). Based on four cleavage sites, the single FMDV ORF indicated by the shaded grey rectangle (Fig. 3) can be divided into four regions which are; Lpro, structural protein (P1) and non-structural proteins P2 and P3 (Robertson *et al*., 1985). The region Lpro encodes for two L proteins (Lab and Lb) as it has two in-frame AUG initiation codons. Regions ID, IB, IC and IA code for structural viral proteins, VP1, VP2, VP3 and VP4, respectively. Protomeric and pentameric subunits of the virus are assembled as indicated in the Fig. 3 and assembled particles comprise of one copy of the RNA and 60 copies of the four capsid proteins VP1-VP4. The proteins VP1-VP3 are exposed whereas the VP4 protein is internal (Jamal & Belsham, 2013). The ID region that encodes for the VP1 protein is 627-639 nucleotides long depending on the serotype and produces a protein of containing 209-213 amino acids. The antigenic variation in the VP1 protein has been used to group serotypes and topotypes. Topotypes are virus variants that are found within the serotypes. According to Knowles and Samuel (2003), a 15% or less difference in VP1 nucleotides between a virus and a prototype would mean they do belong to the same lineage. A difference higher than 15% would render the virus in another lineage. The VP1 protein, is the main immunogenic viral protein (Bachrach, 1968) and is used in serotype specific polymerase chain reaction (PCR) and nucleotide sequencing to distinguish type, subtype and antigenic variants of the FMDV. The VP1 protein possesses the conserved arginine–glycine–aspartic acid (RGD) motif through which the virus binds to the αvβ integrin receptors on the epithelia of the animal cells (Monaghan *et al*., 2005). Since the VP1 protein is immunogenic, it plays a significant role in FMDV antibody neutralization (Chenwen *et al*., 2007) and FMD vaccines have been designed based on this feature (Domingo *et al*., 2003).

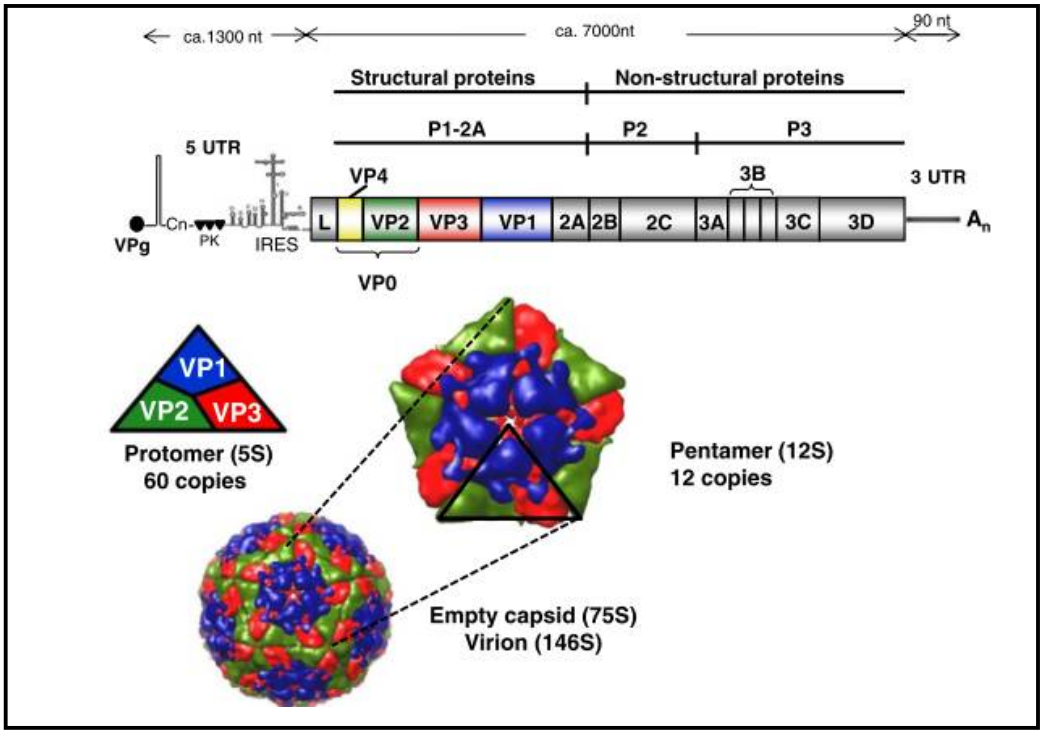



Figure 3: Genomic illustration of FMDV. In blue rectangle is the VP1 region that determines antigenic variability of FMDVs (Jamal & Belsham, 2003)

### 2.4.1 FMDV cell entry

Under field conditions, the virus enters the cells via receptor-mediated cytosis. The virus attaches to the receptor integrin such as αvβ6, αvβ3 and αvβ8 (Jackson, King, Stuart & Fry, 2003; Jackson, Sheppard, Denyer , Blakemore & King, 2000; Monaghan *et al*., 2005) found on the epithelia cells. Foot-and-mouth desease virus replication occurs within the cytoplasm of the infected cells similar to that of the poliovirus which is also a *Picornavirus* (Follett, Pringle & Pennington, 1975). The RNA virus particle alone is infectious when it gets into the cell. Thus, upon entry, the genome is immediately translated and a polyprotein is produced. The polyprotein is then cleaved and gives rise to structural and non-structural proteins which facilitate further replication of the genome.

### 2.4.2 Viral proteins and their roles in genome replication

The P1 region of genome encodes the viral capsids whereas regions P2 and P3 encode for the non-structural proteins (NSP). The NSP proteins are involved in genome replication (2B, 2C, 3AB, 3B, 3CD and 3D) and protein processing (2A, 3C). Protein 2A facilitates viral RNA replication and auto cleavage of its C terminus whereas 2B and 2C are the most conserved

genes within serotypes (Carrillo *et al*., 2005). The proteins expressed by these genes are crucial in the coordination for clearing the pathway for protein transportation from the endoplasmic reticulum to the Golgi apparatus (Moffat *et al*., 2007). The L protein cleaves the host initiation factor elF4G and this leads to the shutdown of the host cap dependent translation (Medina, Domingo, Brangwyn & Belsham, 1993). The 3CD precursor is cleaved by the 3D and this assists in RNA synthesis. The translation of a poly protein followed by the rate of proteolytic processes allows for control of protein expression.

Genome replication is mediated by the 3D protein (RNA polymerase) and the *cre* which serves as a template for uridylation of the Vpg (Bedard & Semler, 2004). Thus, the Vpg protein acts as primers for RNA replication and a double stranded RNA intermediate is formed. Additional replications give rise to negative strand templates from which positive templates are formed. Ribonucleic Acid synthesis is unbalanced because there are 30-50 times more positive strands than negative RNA strands (Novak & Kirkegaard, 1991).

### 2.4.3 Serotypes and other variants

Globally, FMDV exists in seven serologically distinct serotypes; A, C, O, Southern African Territories (SAT) 1-3 and Asia 1; with multiple variants existing within each serotype. The serotypes grouped according to the serological characteristics exhibited by the viruses, while its variants are grouped based on the nucleotide differences within the (VP1) coding region of the virus and their antigenic characteristics (Jamal & Belsham, 2013). The distribution of FMDV serotypes globally is such that, serotypes O and A are found in Asia, South America and Africa; Asia 1 is restricted to Asia and the Middle East while SAT serotypes are mostly found within Africa. Within each serotype, there are topotypes and variants that are geographically distinct however occasional incursions of another serotype variant may occur in another region (Rweyemamu *et al.*, 2008).

Serotype O has 11 geographical topotypes in circulation. These include Indonesia-1 (ISA-1), Indonesia-2 (ISA-2), Europe-South America (Euro-SA), East Africa-1 (EA-1), East Africa-2 (EA-2), East Africa-3 (EA-3), East Africa-4 (EA-4), West Africa (WA), Middle East-South Asia (MESA) and South-East Asia (SEA) (https://www.wrlfmd.org/fmdv-genome/fmd-prototype-strains). The main topotypes that have been documented in circulation in East Africa include East Africa-1 (EA-1), East Africa-2 (EA-2), East Africa-3 (EA-3), East Africa-4 (EA-4). Serotype A viruses comprises three main topotypes, Asia, Africa and

Europe-South America (Euro-SA) and has over 26 lineages. The main lineages that have been documented in circulation in East Africa include Africa-G-I, Africa G-II, Africa G-IV and Africa-G-VII (Bari *et al*., 2014). Serotype C occurs in three topotypes, Euro-SA, Asia and Africa while SAT 1 has thirteen topotypes (I-XIII), SAT 2 has fourteen topotypes (I-XIV) and SAT 3 has five topotypes (I-V) (http://www.wrlfmd.org/fmdv-genome/fmd-prototype-strains).

### 2.4.4 FMDV evolution

The high variation in FMDVs is attributed to the high rate of mutation of the virus among many other factors such as natural selection, recombination and genetic drift. Because replication of the virus is mediated by the RNA dependent polymerase which has a low fidelity, mutant progeny emerge and these can have mutations of 1 per $10^3$ to $10^5$ nucleotides copied (Holland *et al.*, 1982; Sobrino, Davila, Ortin & Domingo, 1983). Mutation rate determines the speed and degree to which a virus can adapt to its environment. Over the years, the rate of change has been observed to be between 0.0004 – 0.045 substitutions/nucleotide/year (Haydon, Samuel & Knowles, 2001). These changes make it possible for the virus to additionally evade immune system and they become less susceptible to vaccines.

## 2.5 Diagnosis of FMD

Proper laboratory diagnosis of FMD is important because other vesicular diseases such as vesicular stomatitis, swine vesicular disease and vesivirus infection may be mistaken for FMD if diagnosis is based solely on clinical signs (Holliman, 2005). Yet, most importantly, because the disease is highly contagious, immediate and accurate diagnosis of FMD is critical such that control measures are quickly put in place to reduce the spread of and the impact of the disease. The disease can be diagnosed by clinical signs and animals may exhibit signs like fevers (high temperature), salivation, vesicle formation on the mouth and gums, on the nose, udders and the inter-digital spaces and coronary bands on the feet. The challenge with clinical diagnosis is that and some livestock species such as goats and sheep rarely show symptoms of the disease (Jamal & Belsham, 2013). Laboratory diagnosis in eastern Africa remains a challenge as observed by Namatovu *et al.* (2013) because of the limited laboratory facilities and personnel. Additionally, Kerfua *et al*. (2018) observed that most FMD cases that were reported by District Veterinary Officers (DVOs) in selected districts in Uganda and Tanzania

were not based on laboratory findings but on clinical diagnosis. Although farmers' descriptions of FMD had a high correlation with positive ELISA results obtained from FMD outbreaks in the Lake zone region of Tanzania (Genchwere & Kasanga, 2014), it is imperative that more reported cases should be confirmed by laboratory analysis. Clinical diagnosis limits the type of information that would be derived from a suspect case and information on serotype in circulation is important in informing control strategies particularly vaccination (Kitching *et al*., 2007; Jamal & Belsham, 2013). In the next paragraphs, various methods for FMD diagnosis that are recommended by the OIE are briefly described.

### 2.5.1 Virus neutralization

This test involves the detection of antibodies to the structural proteins of the virus and is considered the gold standard. This test is also recommended for import and export certifications of animals/ animal products. However, this test is time consuming, variable (because of different cell lines used), prone to contaminations and requires a high degree of bio-containment facilities (OIE, 2012) because it involves culturing viruses.

### 2.5.2 Virus isolation

In this test, the virus is grown in susceptible cell cultures such as bovine thyroid cells and porcine or ovine kidney cells or in cell lines like baby hamster kidney (BHK) or Instituto Biologico-Rim Suino-2 (IBRS2). This is an expensive test and time-consuming and still requires a high level of bio-containment. The quality of the sample will also dictate as to whether there will be infectious material that can be grown. It may take about 4 days to demonstrate the presence of the virus using this method and sometimes the virus may fail to grow because of a specific cell type (OIE, 2012). This test, however, has to be used in combination with either ELISA or PCR in order to confirm that the virus has grown in the cell lines.

### 2.5.3 Enzyme linked immuno-sorbent assay (ELISA)

Antigen ELISAs were developed following complications due to pro and anti-complement activities while using the complement fixation test (CFT). High titre antisera were raised against a purified 146S FMDV particle for antigen capture and detection. This test is 125 times more sensitive than CFT. Its sensitivity is at about 70-80 percent and thus, sometimes the virus may have to be grown in cells lines and then tested using antigen ELISA (Jamal &

Belsham, 2013). Monoclonal antibody (MAb) ELISAs have also been developed for FMD diagnosis using the recombinant integrin αvβ6 for virus capture (Morioka *et al*, 2009; Chen, Peng, Zhang & Liu, 2012). The intergrin MAb ELISA has a wide antigenic and molecular diversity and not all FMDVs were detected but it maintained its sensitivity and the specificity compared to the conventional poly clonal ELISA (Ferris, Grazioli, Hutchings & Brocchi, 2011).

### 2.5.4 Reverse transcription-polymerase chain reaction (RT-PCR)

This test is known for its fast, sensitive and reliable results. Rodriguez *et al.* (1992) was the first to type FMDV by RT-PCR and differentiated serotypes O, A and C. Since then, serotype specific primers have been developed for all the seven serotypes of the virus (Vangrysperre & De Clercq, 1996; Callens *et al.*, 1998). Several primers have been developed to target several regions of the virus including the 5' UTR, ORF and the 3'UTR, however, no single primer set can targ*et al*l the seven serotypes thus multiplex assays and incorporation of more than one set of primers have been developed for a certain group of isolates making RT-PCR not that sensitive or specific (Jamal & Belsham, 2013). Also, recently, real time/quantitative RT-PCR (rRT-PCR) methods that do not require gel analysis have been developed and indicators can be observed directly as the target molecule is being amplified thus and making it easy to quantify the virus. The most recent primers and probes were developed by Knowles, Wadsworth and King (2016).

### 2.5.5 Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

Field molecular diagnosis is enabled by having portable equipment for rRT-PCR in the field and thus, loop-mediated amplification (LAMP) was developed. The assay amplifies DNA by the auto-cycling strand displacement reaction principle and has two sets of specific inner and two outer primers and a DNA polymerase. The DNA polymerase has a very high displacement activity. The assay is fast and visual interpretation without other instruments required makes it an ideal for use in the field and in endemic countries (Dukes *et al.*, 2006). However, the assay has not been extensively evaluated in order to replace or evaluate the assays in place (Jamal & Belsham, 2013).

### 2.5.6 Chromatographic strip test

Fast and rapid assays for FMD detection can allow for quick interventions to be set up to alleviate disease spread. Reid *et al*. (2001) developed a MAb based chromatographic strip for the detection of FMDV and it was found to be as sensitive as the antigen ELISA and highly sensitive from supernatants derived from cell cultures. Further studies are being carried out to develop serotype specific test strips (Jamal & Belsham, 2013).

### 2.5.7 Sequencing of 3D, 5-UTR and/or VP1 region of FMDV

Sequencing of the FMDV allows for further analysis of the virus in order for comparative genomics of the FMDVs Sequencing of the cDNA is usually carried out after performing PCR assays and is performed to determine which serotype or genotype and even strain the FMDV belongs to. Analysis of the 3D and 5' UTR has been shown to be quite successful in determine comparative genomics of FMDVs (Manju *et al*., 2001; Carrillo *et al*., 2005). However these two regions are more conserved compared to the ID region (that codes for the VP 1) that has only 26% of its nucleotide residues as invariant and has been mostly used to determine the genetic analysis of viruses (Carrillo *et al*., 2005). Therefore the VP 1 is still widely used in sequencing of FMDVs for comparative genomics (Jamal & Belsham, 2013)

## 2.6 Prevention and control of FMD

### 2.6.1 Prevention and control of FMD in East Africa

Prevention and control of FMD spread in East Africa has been mainly through vaccination, control of movement of livestock and their products (Rushton & James, 2002). Vaccination against FMD is the most commonly used method to prevent and control FMD outbreaks in East Africa (Muleme *et al*., 2012; Wekesa *et al*., 2015). However, FMD vaccination is complex because of multiplicity of antigenic types and subtypes given that there is no cross protection between serotypes or subtypes within the given serotypes (Kitching *et al*., 2007). Additionally, vaccination is complicated by the short period the vaccine elicits immunity (6 months) and the high cost of the vaccine as well (Kitching *et al*., 2007). Substantial progress has been made towards control and eradication of FMD in several regions of the world, notably Europe, and some parts of South America and Asia. However, sporadic outbreaks have occurred in FMD free countries like Great Britain, Netherlands, Japan, Taiwan, North Korea, Greece, Argentina and Brazil with serious economic consequences (James & Rushton,

2002; Pendell *et al.*, 2007). In large parts of the world, particularly in sub-Saharan Africa, eradication can only be viewed as a long term objective (Kitching *et al.*, 2007). In Tanzania and Uganda, control of FMD spread is mainly through the use of vaccines that are usually supplied by Kenya Veterinary Vaccine Production Institute (KEVEVAPI) and Botswana Vaccine Institute (BVI). In Uganda, the trivalent vaccine containing strains for serotypes O, SAT1 and SAT 2 has been commonly used whereas in Tanzania strains for serotype O, A, SAT1 and SAT2 are incorporated in the vaccine that has been used. Although the restriction of livestock and product movement is also emphasized by the Ugandan government during outbreaks, it still lacks proper enforcement (EAC, 2006; Balinda *et al*., 2010a).

### 2.6.2 Progressive Control Pathway - FMD

The progressive control pathway for FMD was introduced by the FAO to enable countries that are still FMD endemic to develop strategies based on the available information on the FMD status in the countries or regions. Uganda and Tanzania are still in the initial stages of the pathway. The initial stages of the pathway are critical because comprehensive information on FMD is collected in order to move from stage 0 to 1 after which, appropriate strategies are developed and implemented accordingly in order for that country/region to move from stages 1-2. For countries in stages 1-4 of implementation, vaccination is one of the major control strategies and thus basic information on serotypes and even topotypes in circulation are important. The pathway has five steps through which a region/country should go until they reach a free status without vaccination. The pathway works on fundamental principles of:

(i) Active monitoring and understanding FMD epidemiology
(ii) Having activities at each stage of the PCP that are fit for the required reduction of virus in circulation
(iii) Having each activity and impact measurable at each stage that are comparable
(iv) Using resource optimally especially in critical risk points areas in order to achieve FMD control (FAO, 2011).

The five critical stages of the PCP-FMD are hereby discussed below:
In stage 1, according to FAO (Fig. 4) the focus is “To gain an understanding of the epidemiology of FMD in the country and develop a risk-based approach to reduce the impact of FMD”. For a country or region to be included in this stage, the minimum requirement is to have a comprehensive plan that allows them to gain information on the epidemiology and the

socio-economics of FMD. In this stage, it is important to have key outputs such as, having all husbandry systems, livestock marketing networks and socio-economic drivers well-described and understood, have a working hypothesis on the epidemiology of FMD in the country, estimate socio-economic impacts of FMD on different stakeholders, having most common strains identified, important risk spots are identified, among others.

To move on to stage 2 where the focus is "To implement risk based control measures such that the impact of FMD is reduced in one or more livestock sectors and/or in one or more zones", a country or region has to have a strategic FMD control plan developed for at least a zone or a husbandry sector. The plan should be based on the risks identified through stage 1 activities. Some of the key outcomes from stage 2 are monitoring circulating viral strains and risk in the different husbandry systems, implementing risk based control measures in the zone based on the strategic control plan developed, and ensuring that there is reduced impact of FMD in areas/zones where the control has been implemented and further expansion of an environment that enables control activities. To advance to stage 3, it is important that a country or region develops a revised and more aggressive control strategy for at least one zone of the country, with the aim of eliminating the disease.

Stage 3 focuses on "Progressive reduction in outbreak incidence followed by elimination of FMDV circulation in domestic animals in at least one zone of the country". The minimum requirement for inclusion into this stage is completion of the previous stages and ongoing monitoring of circulating strains as well as implementation of strategic control plan that was developed in stage 2. To enter into stage 4, there should be evidence that FMD is not endemic in a certain zone or within the country.

The focus of stage 4 is "To maintain 'zero tolerance' of FMD within the country/zone and eventually achieve OIE recognition of 'FMD free with vaccination'. The key outcomes for this stage include continued monitoring of circulating strains of the virus and development of a plan to meet the OIE requirements for recognition of "FMD-free with vaccination" status. Additionally, the country should be able to show that the risk for FMD entering into it is mitigated and that there is low FMD incidence and occasional incursions of the disease. To move to stage 5, the country/region should ensure that they meet all the OIE requirements for recognition of "free with vaccination" and that a report is submitted to OIE such that they are recognised for this status.

In stage 5, the focus is “To maintain ‘zero incidence’ of FMD within the country/zone and eventually achieve OIE recognition of ‘FMD free without vaccination’. The minimum inclusion for this stage is completion of stage 4 and OIE recognition of ‘FMD free status’ with key outcomes of zero FMD incidence in domestic animals. Here typical activities include carrying our passive and serological surveillance and having systems in place to report and investigate all suspect cases. To leave stage 5 and complete the pathway, the OIE requirements for recognition of “free without vaccination” should be fulfilled and a dossier should be submitted to OIE for recognition of the status. At this stage activities such as surveillance to prove zero disease incidence as specified by the OIE code are carried out.

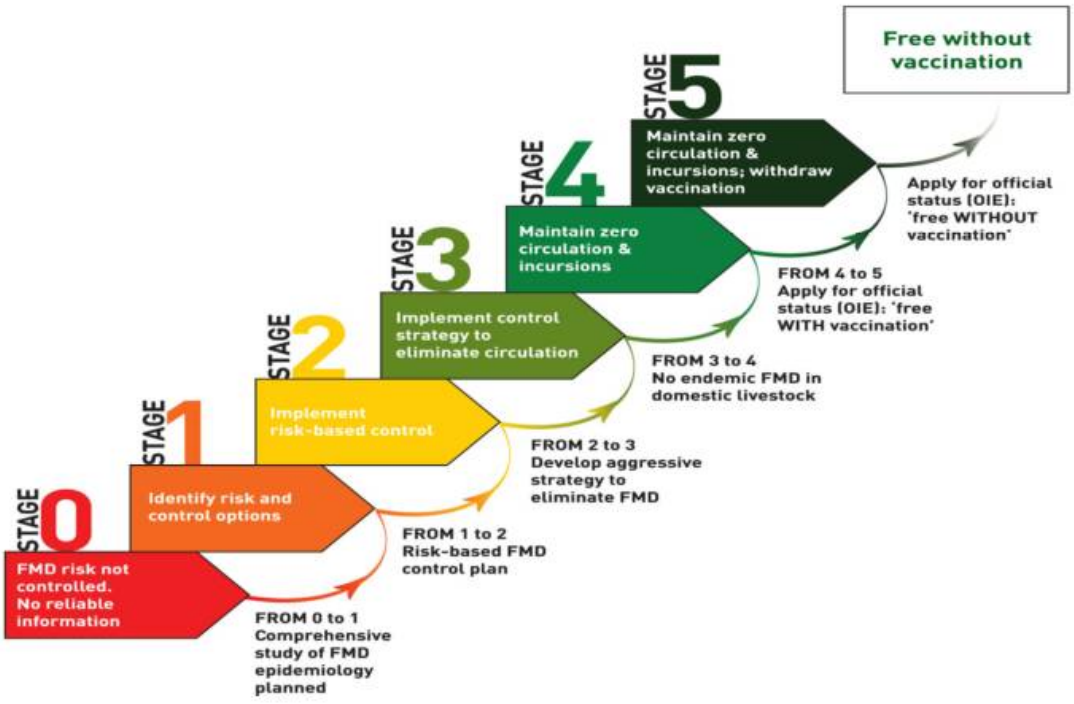



Figure 4: Stages of the Progressive Control Pathway for FMD (FAO, 2011)

### 2.7 International borders and their implications on FMD spread

Rweyemamu *et al*. (2008) and Knowles and Samuel (2003) identified two main FMD epidemiological clusters of FMD. The cluster identifications were based on data on FMD prevalence, distribution of serotypes and topotypes, expert opinions on cross border animal movement, farming systems employed, as well as wild impact. The Great Lakes cluster, also

called the East African Community or Southern-East Africa cluster was identified as one of the two main epidemiological clusters of FMD in Africa. This cluster comprised of Tanzania, Uganda, Kenya, Rwanda and Burundi. The East African region has the highest diversity of FMDV, with six out of seven serotypes having been present in the region over a period of time (Casey-Bryars *et al*., 2018).

Border regions remain central in the epidemiology of trans-boundary animal diseases and often suffer the burden of trans-boundary livestock diseases (Di Nardo *et al*., 2011). The uncontrolled movement of people and animals along borders has been documented as one of the major factors for the introduction and continued circulation of animal diseases, particularly FMD (Fèvre *et al*., 2006; Otte *et al*., 2004). Between 2001 and 2008, sub-counties in Uganda that were adjacent to the Uganda–Tanzanian border registered more outbreaks compared to counties close to other similar borders (Ayebazibwe *et al*., 2010). In addition, a study in Tanzania reported on the increased number of FMD outbreaks around Kagera region (Picado *et al*., 2010), which borders Uganda and Rwanda. While a recent study by Kerfua *et al*. (2018) also showed that more outbreaks were reported in wards/sub-counties that were closer to the Uganda-Tanzanian international border.

According to Lesser and Moisé-Leeman (2009) and EAC (2006), there has been an increase in cross-border trade in the East African region probably because of a shift in economic trends with legal cross-border trade that account for about 10% of the trade that occurs along the country borders (COMESA, 2009). Rweyemamu *et al*. (2008) reported that the poorly regulated movement of animals and animal products is the major risk factors for cross-border spread of TADs such as FMD. Several factors such as prevalence of infection, volume of trade related movements, ability of the virus to survive in animals and their products and potential transmission to susceptible animals, play a substantial role in the epidemiology of FMD along the border areas (Di Nardo *et al*., 2011). The latter study further expounded on the importance of piecing together evidence on livestock systems, animal movement, marketing structures and trade routes in order to establish causes and sources of particular events such as outbreaks of disease. However, available information is very scanty and cannot answer some questions and hence, the exact sources of outbreaks cannot be established. Nevertheless, matching information on data on FMD epidemiology and movement of livestock together with phylogenetic studies can suffice to determine trends of FMDV spread at national and regional level (Knowles & Samuel, 2003). Thus, using

retrospective data on FMD outbreaks can be useful in establishing the epidemiology of FMD in an area such that customised control strategies are developed. Additionally, the use of phylogenetic studies in studying the spread of FMDV gives an accurate depiction of how viruses are spreading in an area and can allow for comparison between the virus and vaccine strains being used.

Several studies have highlighted the role of livestock market systems in the spread of FMD nationally, regionally and globally (Fèvre *et al.*, 2006) and show that FMDV serotype O is responsible for a number of outbreaks in the east African region (Balinda *et al*., 2010a; Knowles *et al.*, 2009). Topotypes EA-3 has been reported to circulate in Ethiopia, Sudan and Somalia while EA-2 found in Tanzania, Kenya and Uganda. Furthermore, topotype EA-4 was found to be restricted to Uganda, Kenya and Ethiopia (Balinda *et al*., 2010a). According to Sangula *et al*. (2010), serotype A-topotype I (G-I) was reported in Kenya and Tanzania whereas, G-VII was reported in Kenya and Ethiopia. These epidemiological patterns illustrate that FMDV circulation within East Africa and the Great Horn of Africa may be attributed to increased trade in live animals and their products (Fèvre *et al*., 2006). The FMDV circulation further points out three main areas at risk namely; the border areas of Kenya, Uganda and Tanzania, the Somalia ecosystem and last but not least the bordering areas between eastern part of Sudan, northern parts of Ethiopia and Eritrea because of high livestock populations in these countries (Di Nardo *et al*., 2011).

Several phylogenetic studies provide evidence of cross border transmission of FMDVs from one country to another. For example, Balinda *et al.* (2010a) reported that East Africa (EA) topotypes 3 and 4 which had never been reported in Kenya and Uganda must have spread into Kenya and Uganda from the neighbouring countries of Sudan and Ethiopia. Another evolutionary study by Sangula *et al*. (2010) showed that SAT 1 serotype which was isolated from Kenya in 1977 could have spread to Tanzania in 2007 through trans-boundary livestock movements. Thus, FMD control strategies, especially vaccination, have to be serotype- and topotype-specific, otherwise vaccine effectiveness may be futile given the lack of cross-protection between serotypes (Kitching *et al*., 2007).

### 2.8 Conclusion from the review and way forward

Knowledge on FMD epidemiology and impact are critical in feeding into the initial stages of the PCP-FMD. Gaps in serotype distribution and epidemiology, phylogenetic studies, risk

factors for FMD, and impact of disease require to be addressed in order to design better controls. According to Upton (2004), more focused studies on animal and veterinary public health and the impact of livestock diseases on farmers and people along the livestock value chain are important in ensuring that farmers can harness profits from keeping livestock. Without strategic disease intervention, success of disease elimination can be very low. Therefore, this study will add to the body of knowledge on the spatial and distribution of FMD in the border areas, provide information on the relatedness of viruses obtained from these areas and the impact of FMD on income and food security. This kind of information provides more insight into trans-border spread of disease and gives an understanding of how to better design control strategies.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Mapping the spatial and temporal distribution of FMD outbreaks

### 3.1.1 Ethical clearance

To conduct research in Tanzania, permission was obtained from the Tanzania Commission for Science and Technology (Permit No: 2016-277-NA-2016-214). While a letter to conduct research at the National Animal Disease Diagnostics and Epidemiology Centre was obtained from the Uganda Ministry of Agriculture Animal Industries and Fisheries with letter number (Letter No: LHE 138/1).

### 3.1.2 Study area

The study was conducted in the districts situated along the international border of Tanzania and Uganda. The districts were Isingiro and Rakai in Uganda and Missenyi and Kyerwa in Tanzania. The districts were purposively selected for inclusion in the study because the study was targeting the districts found along the border of the two countries (Fig. 5).

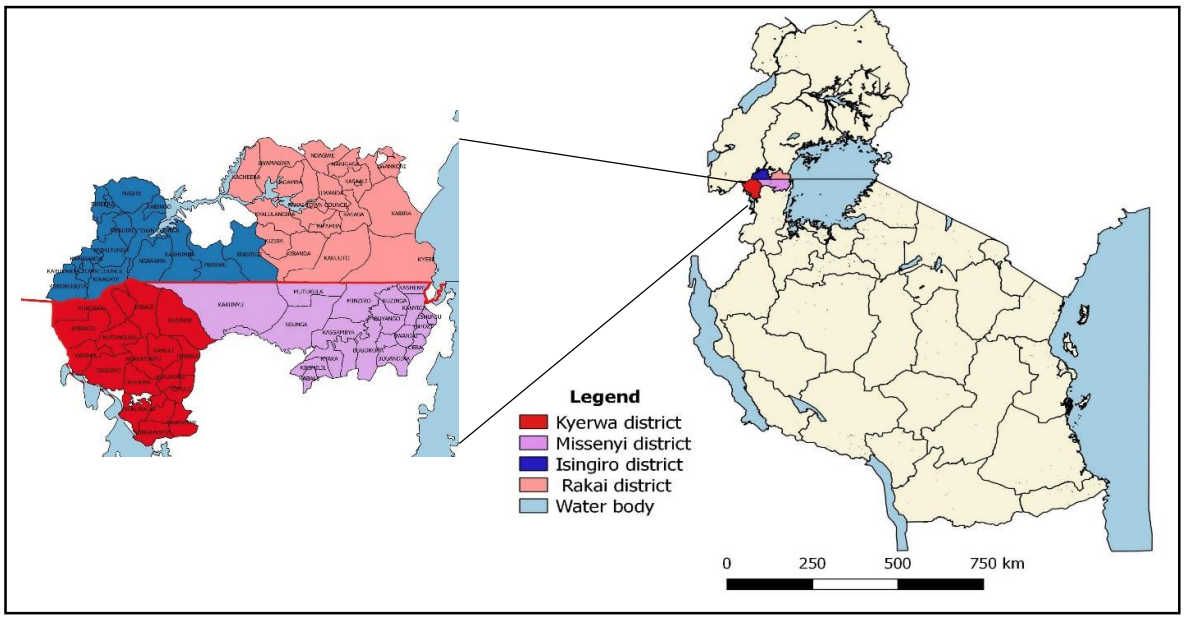



Figure 5: Study districts. Inset map showing border districts of Isingiro and Rakai in Uganda, and Missenyi and Kyerwa in Tanzania (Kerfua *et al.*, 2018)

### 3.1.3 Data compilation

Data on FMD outbreaks from 2011 and 2016 for Tanzanian districts were retrieved from the records of the District Veterinary Officers (DVOs) while that of the Ugandan study districts were retrieved from the archives of the National Animal Disease and Diagnostic Centre (NADDEC) at the Ministry of Agriculture Animal Industry and Fisheries (MAAIF). The NADDEC is mandated to conduct routine national surveillance in response to animal disease outbreaks in Uganda. Published articles on previous FMD outbreaks that occurred in the study area were also included in the study. The articles that were selected for this inclusion were on studies that had been carried out in any of the districts along this border and those that had details on the serotypes that were circulating in these districts. Two articles were retrieved for this purpose.

FMD outbreak according to this study was defined as the presence of FMD clinical signs in at least one herd of cattle in a village within 1 month of the report of an outbreak. The Veterinary Officers (VOs) reported outbreaks based on clinical signs manifested by the affected animals. Cattle were assumed to be FMD-positive if manifested with two or more of the following clinical signs – lesions in the mouth, on the gum, on the tongue, on the hooves and lesions on the mammary glands accompanied by excessive salivation, fever, anorexia and lack of appetite.

A Microsoft Excel data sheet was developed and data was compiled and entered into the sheet. The data compiled included information such as date of outbreak, location where outbreak was reported and Geographic Position System (GPS) (where it was available). Other information such as the livestock species affected, number of animals affected (where available), number of animals at risk (where available) and number of outbreaks that were reported in that ward/sub-county were also included. The data was then cleaned and subjected to analysis.

The month in which the outbreaks were reported were studied in order to identify the possible seasonal variations. In Uganda, the months in which the cases occurred were grouped in to two seasons defined as wet and dry as in accordance to reports from Isingiro District Local Government (IDLG) (2011) and Rakai District Local Government (RDLG) (2013) while in Tanzania, they were grouped as defined in the FAO report on the Kagera region (FAO, n.d.). Thus, the study took into account the wet and dry months which are annually bimodal for all

the districts in both countries. In Uganda, according to this study, the wet period was defined as the rainy months (March to April and September to November) whereas, the dry season associated with high temperatures (27.5 °C – 30 °C) extended from December to February and May to August.

In Kagera region, Tanzania the wet season extended from March to April and from October to December whereas, the dry season was considered to be from December to February and then from May to September.

Data on serotypes that were in circulation during 2011-2016, were retrieved from the DVOs and NADDEC records. Other sources were research articles based on studies that had been previously been carried out in these regions including Genchwere and Kasanga (2014) and Namatovu *et al*. (2015).

### 3.1.4 Data analysis

Analysis of clean data was executed in R software, version 3.3.2 (R Core Team, 2013). Generalised linear mixed effect models (package lme4) using Poisson distribution were used to describe the relationship between the response variable, which were number of outbreaks and the fixed variables (season, wet-dry, border adjacency [yes, no] and year of outbreak). The random variables included the district, sub-county and/or ward in which outbreak was reported. Model selection was carried out and the likelihood ratio tests (LRTs) were used to decide the significance of fixed effects. Quantum Geographic Information Systems (QGIS) 2.16.0 (Open Source Geospatial Foundation 2016) was used to prepare the maps to reflect the sub-counties/wards where FMD outbreaks had been reported in the last 6 years. Since there was no GPS data on the exact location of affected villages, analysis was conducted at the scale of sub-counties/wards.

### 3.1.5 Limitations of the study

Most of the compiled data consisted of reported cases that were based on observed clinical signs of FMD rather than laboratory analyses. The presence or absence of the virus or antibodies to the virus by tests such as real-time or conventional PCR and antigen/antibody ELISAs was not confirmed in most of the cases. Therefore, it cannot be ruled out that the signs could have been exhibited by other diseases that display clinical signs similar to those of FMD. Such differential diagnosis includes bovine papular stomatitis, vesicular stomatitis,

malignant catarrhal fever and bluetongue (Holliman, 2005). However the DVOs said there were no reported incidences of the mentioned diseases. It was also observed that details such as number of cases registered during the outbreaks, GPS location readings, and number of animals at risk were not available and therefore, could not be used for more elaborate analysis such as cluster analysis. Outbreaks only in cattle had been reported and there was no data on other livestock species. This data on outbreaks in these areas may also be subject to underreporting by farmers because of political and social (Dhikusooka *et al*., 2015; Sutmoller *et al*., 2003).

### 3.2 Genetic characterization of FMDVs

#### 3.2.1 Sample collection

Forty three samples were collected from farms that were situated in the study area and had reported FMD outbreaks between 2016 and 2017. Lesion tissues, lesion swabs, oropharygeal fluid/tissue and saliva were obtained from cattle that exhibited clinical signs of the FMD. Protocols for collecting each sample are attached in Appendix 4. Additional information was collected on age, sex and breed for each animal that was sampled. Also, GPS coordinates were collected at household level. Samples were collected in duplicate, half of them in 50% glycerol and 50% phosphate buffered saline (PBS) and then placed in liquid nitrogen and the other half were stored in RNA later (Ambion Inc. Austin, Texas).

#### 3.2.2 Sample processing and diagnosis

**(i) RNA extraction and one step Real Time Polymerase Chain Reaction (RT-PCR)**

Epithelial and probang tissues from the same animal were processed together using the protocol described by OIE (2012). The tissues were cut with sterile scissors and forceps. The sliced tissues were weighed (5 g) and after placed in individual sterile mortars, 10 ml of PBS was added onto each of the mortars. Sterile pestles were used to crush the tissues after which another 5 ml of PBS was added to the mixture. The suspension was centrifuged at 2000 g for 10 minutes, and RNA extraction was performed on the supernatants. Total RNA was obtained from the processed tissue supernatant and the saliva samples. The Invitrogen PureLink RNA Mini Kits (Carlsbad, California) was used for total viral RNA extraction as per the protocol outlined by the manufacturer. The obtained RNA was then subjected to rRT-PCR using Invitrogen™ SuperScript™ III One-Step RT-PCR System with

Platinum™ Taq DNA Polymerase (Carlsbad, California, USA) and the test was run on a Stratagene Mx3000P thermocycler (2009, California). The total reaction volume was 25 µl and composed of 12.5 µl of buffer, 1.5 µl of the probe, 2 µl of each primer with a concentration of 10 pmol, 1.5 µl of nuclease free water, 0.5 µl of SuperScript III RT/Platinum Taq mix and 5 µl of template. The following conditions were used for cDNA synthesis and to run the PCR: 50ºC for 30 minutes, 95ºC for 2 minutes, followed by 40 amplification cycles of 95 ºC for 15 seconds, 56 ºC for 30 seconds, and 72ºC for 30 seconds, and final extension at 72 ºC for 10 minutes. Table 1 describes the sequences of the primers and probe set which targeted the 3D ORF of the FMDV genome.

Table 1: Primer and probe sequences used for rRT-PCR

| **Primers/probe** | **Sequence** | **Gene** |
|---|---|---|
| Forward Primer | 5'- ACT GGG TTT TAC AAA CCT GTG A-3' | 3D |
| Reverse primer | 5'- TCC TTT GCA CGC CGT GGG AC-3' | 3D |
| Probe (FAM-TAMRA) | 5'- GCG AGT CCT GCC ACG GA-3' | 3D |

**(ii) Antigen ELISA**

Antigen ELISA was used to determine the serotype primers to be used for the serotype specific PCR. The ELISA test was carried out on 35 samples that were positive by rRT-PCR. The IZSLER Antigen ELISA kit that was used was obtained from the Pirbright Institute, Pirbright, UK. The protocol used for the antigen ELISA was adapted from that outlined by the kit manufacturers. The polystyrene micro well plates were coated with rabbit sera for the different FMDV serotypes and incubated at 37ºC for an hour. Plates were washed to remove unbound antibodies from the plate. The processed samples were then added to the designated wells and incubation was done at 37ºC for an hour. A wash step was performed in order to remove excess antigen and specific guinea pig anti-FMDV detecting antibodies were after added to the polystyrene plate. Incubation was performed at 37ºC for an hour, after which, a wash step followed. In order to detect the anti-FMDV antibodies, rabbit anti-guinea pig immunoglobulin (Ig) conjugated to horse radish peroxidise was added to the polystyrene plate. After that, a wash step followed and then the substrate was added. After addition of the substrate, a colour product was developed and the reaction was stopped by adding the stop solution. The OD values were measured using the ELISA reader machine at 450 nm

wavelength. A positive control with Optical Density (OD) > 0.1 and a negative control with OD < 0.1 were included in the test and all samples with OD ≥ 0.1 were considered positive while those with OD < 0.1 were considered negative for FMDV.

**(iii) VP1 coding region amplification using conventional PCR**

Conventional PCR was performed using Invitrogen™ SuperScript™ III One-Step PCR System with Platinum™ Taq DNA Polymerase on samples that had turned out positive by rRT-PCR. The ELISA results were used to select the serotype-specific primers targeting VP1 (Table 2) as described by Knowles *et al.* (2016). For each sample, reaction master mix included, 9.2 µl of nuclease free water, 1.6 µl of each primer (reverse and forward), 0.8 µl of dNTPs, 4 µl of buffer and 0.8 µl of Qiagen One –step RT-PCR enzyme. A volume of 2.0 µl of template was added to the master mix to make a 20 µl volume. Nuclease-free water was used as a negative control while known positive controls were supplied by the National Animal Disease Diagnostic Centre as RNA extracts that had previously been stored at -80 ºC. A Techne TC-412 thermocycler (Techne Inc, New Jersey) was used to run the PCR and the following cycle conditions were used; 50 ºC for 30 min and 95 ºC for 15 min, 95 ºC for 10 s., followed by 35 cycles of 30s at 60 ºC for serotype O, 30 s at 55 °C for serotype A and 30 s at 50°C for SAT 1, SAT 2 and SAT 3 and extensions of 72 ºC for 30 s and 72ºC for 10 min as modified from the protocol described by Knowles *et al.* (2016).

Table 2: Serotype primers that were used to perform RT-PCT

| Serotype | Name | Sequence | Direction | Gene | Size |
|---|---|---|---|---|---|
| O | O –1C244F | GCAGCAAAACACATGTCAAACACCTT | + | VP3 | 1165 |
| O/A/C/Asia | EUR–2B52R | GACATGTCCTCCTGCATCTGGTTGAT | - | 2B | |
| A | A–1C562F | TACCAAATTACACACGGGAA | + | VP3 | 866 |
| SAT 1 | SAT1–1C559F | GTGTATCAGATCACAGACACACA | + | VP3 | 1043 |
| SAT 1–3 | SAT–2B208R | ACAGCGGCCATGCACGACAG | - | 2B | |
| SAT2 | SAT2 P1–1223F | TGAACTACCACTTCATGTACACAG | + | VP3 | 1279 |
| SAT3 | SAT3–P1–1222F | AATCTGCATTTCATGTACAC | + | VP3 | 1277 |

**(iv) Gel electrophoresis and cleaning of PCR products**

A 2% agarose Tris borate EDTA gel that was stained with one percent ethidium bromide was used to analyse the PCR products. A 1kb DNA ladder (Gene ruler Fermentas Inc., Burlington, Canada) was run alongside the samples in order to ascertain the correct band size of the amplicons. To prepare the 2% agarose gel, 2 g of agarose (Top Vision, Fisher Scientific. Ottawa, Canada) was added to x1 Tris borate EDTA. A microwave was used to heat the mixture so that the agarose could dissolve and form a gel. A volume of 2 µl of ethidium bromide was added to the gel after it had cooled to about 50$^{o}$C. Appropriate combs were fitted onto a horizontal gel tray and the liquid gel was poured into it. After the gel had set, the combs were removed carefully and the tray was immersed in the electrophoretic tank containing x 1TBE buffer. To prepare the samples to be loaded into the wells, to 2 µl of the amplicons, 2 µl of bromophenol blue dye was added and thoroughly mixed. The gel loading dye was prepared by adding water to the x 6 dye at a ratio of 3:1. The mixture of the PCR products and diluted loading dye was then loaded onto the appropriate wells and run at 120 volts for 45 minutes. After the run, the gel was removed and examined under UV light. The image produced under UV light was captured using the video capture system (Molecular Imager® Gel Dox XR System 170-8170 with Flowgen IS 1000; Bio-Rad, Seoul, Korea).

**(v) Purification and sequencing of PCR products**

Following the instructions by the manufacturer, the PureLink® PCR Purification kit was used to remove excess primers and nucleotides. Elution of the cleaned amplicons was achieved using 50 µl of elution buffer. The cleaned PCR amplicons were sent to Macrogen (Seoul) for sequencing and sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies). Universal reverse primer NK72 were used for all samples whereas specific primers (Table 2) were used for each of the different serotypes.

**3.2.3 Data analysis**

**(i) Sequence analysis**

The nucleotide sequences were received in *abi* file extension format and edited using CLC Work Bench version 9.5.3 (Qiagen, USA) so as to obtain consensus sequences. In order to retrieve related sequences from the Genbank, the generated sequences were run through the Basic Local Alignment Search Tool (BLAST) (Altschul, Gish, Miller, Myers & Lipman,

1990) The serotype O VP1 sequences obtained together with similar sequences were aligned in Molecular Evolutionary Genomic Analysis (MEGA) version 7 (Kumar, Stecher & Tamura, 2016) using ClustalW. The aligned sequences were trimmed down to 639 nucleotides such that same length is achieved for all the sequences in order to allow for subsequent analysis. Serotype A VP1 sequences were also aligned together with similar sequences that were obtained from the BLAST search. The aligned sequences were trimmed down to 640 nucleotides long. Phylogenetic analysis and nucleotide substitution were accomplished using the Neighbour-Joining tree method in MEGA 7 and the Kimura 2-model parameter, respectively (Kimura, 1980). A total of 1000 bootstrap replicates were used to evaluate the robustness of the phylogeny. The obtained sequences from this study were compared with selected virus sequences derived from cattle from Uganda, Tanzania, Malawi, Zambia, Burundi and Kenya (Table A in Appendix 1). Out-group sequences were from the countries of Honk Kong, Vietnam and Turkey.

The MEGA 7 software was used to translate VP1 nucleotide sequences into amino acid sequences, and amino acid sequence comparisons and alignments were carried using Multiple Sequence Alignment with Hierarchical Clustering (Corpet, 1988). Comparison were made between amino acids deduced from sequences of vaccine strains O/KEN/77/78, A/K35/1980 and A/K30/1980 and selected sequences from viruses isolated from Uganda and Tanzania.

### 3.2.4 Limitations of the study

Eleven out of seventeen sequences were obtained for subsequent analysis thus other sequences were missed out on. This could have arisen due to very low yields of the cDNA (amplicons) that could have arisen from low virus load in the initial samples. This limitation could have affected the number of serotypes that could have been obtained in this study.

## 3.3 Impact of FMD on household communities

### 3.3.1 Study area

The study was conducted in Kyaka and Nsunga wards of Missenyi district in Tanzania and in the sub-counties of Endinzi in Isingiro district, Lwamaggwa and Kakuuto in Rakai district, Uganda. Sub-counties and wards that had experienced an outbreak in the last 12 months were purposively selected. Also, the distance of these sub-counties/wards from Mutukula town

which is a major Uganda-Tanzanian border town was considered. Nsunga, Kyaka, Endinzi, Kakuuto and Lwamaggwa are 16, 30, 37, 45 and 102 km from Mutukula, respectively.

### 3.3.2 Sampling and study design

#### (i) Sample size estimation

A total of 288 households (HHs) were estimated for inclusion in the study; 192 households from Uganda and 96 households from Tanzania (96 from Rakai, 96 from Isingiro and 96 from Missenyi). The sample size was based on the formulae by Taherdoost (2018) to determine number of samples required.

$$n = \frac{P\,(100-P)\,Z^2}{E^2}$$

Where,

n is the sample size required

P is the percentage occurrence of state or condition

Z is the value corresponding to the level of confidence required

E is the percentage error

This derivation of sample calculation utilised confidence levels, population percentage or variability, and precision combinations. The percentage error used in this study was 10%, while a 95% confidence level was considered thus a Z value of 1.96. A population percentage or variability of 50% was used since the percentage occurrence of FMD impact in these areas was unknown but also such that the variance and sample size would be maximised (Taherdoost, 2017). Thus based in the above precision, confidence levels and population variability, a sample size of 96 was arrived at for each district.

#### (ii) Sampling strategy

Purposive and random sampling were employed in the sampling strategy. The sub counties/wards were purposively selected based on an outbreak of FMD that had occurred in the ward/sub county in last twelve months. A total of one ward was chosen in Isingiro and three wards were chosen from Rakai and two wards were chosen in Missenyi district. Households were then randomly selected based on randomly generated numbers that were

obtained given a list of cattle owning HHs that were shared by the local area animal health workers.

### 3.3.3 Questionnaire and data collection

A structured questionnaire was used to collect the data and it included questions on income from both livestock and livestock product sales, market prices of animals, milk consumption and sales, commodity market prices, and food consumption (Questionnaire in Appendix 2). Data was collected using a smart phone application called Kobo collect 1.4.8 apk (2017) (ODK Development Team) which is an Open Data Kit system. Before administration of the questionnaire, oral consent was sought from the respondents.

#### (i) Data clean up and analysis

The data collected was downloaded from Kobo collect and cleaned in Microsoft Excel (2008), a total of 255 out of 288 data sets were used for the analysis because some questionnaire had missing data and some errors thus were not included in the analysis. Analysis of data was achieved by using Microsoft Excel (2008) (Microsoft Office Professional Plus). Descriptive statistical analysis included determination of the means, maximum, minimum, and medians of the data set. Comparison of means was performed using the paired t-test to determine the effect of FMD outbreaks on selected variables at $p < 0.05$ (Microsoft Excel, 2008) (Microsoft Office Professional Plus).

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Results

### 4.1.1 Spatial and temporal distribution of FMD outbreaks

#### (i) Spatial distribution

Between 2011 and 2016, a total of 23 and 59 outbreaks were reported in the two Ugandan districts (Table 3) and two Tanzanian districts (Table 4) respectively. The Tanzanian district of Missenyi had the highest number of reported outbreaks (36), this was followed by Kyerwa (23), Isingiro (15) and Rakai (8). Of the 82 outbreaks, 38 (46%) were reported in sub-counties and/or wards that were directly adjacent to the international border with either country (Fig. 6). Overall, the results revealed that sub-counties/wards that were directly adjacent to the Uganda-Tanzania border reported three times more FMD outbreaks compared to those that were not ($x^2 = 5.643$, df = 1, $p < 0.001$).

Table 3: Annual and monthly FMD outbreak distribution in Ugandan districts of Rakai and Isingiro

| **Uganda** | | | | |
|---|---|---|---|---|
| **District** | **Year** | **Sub-county/Ward** | **Number of outbreaks** | **Month** |
| Rakai | 2011 | Kasasa | 01 | February |
| Rakai | 2011 | Kakuuto | 01 | April |
| Isingiro | 2011 | Endinzi | 01 | April |
| Rakai | 2012 | Kakuuto | 01 | June |
| Isingiro | 2012 | Ngarama | 01 | June |
| Isingiro | 2012 | Kabuyanda | 01 | June |
| Rakai | 2013 | Kakuuto | 01 | February |
| Isingiro | 2013 | Kabingo | 01 | February |
| Rakai | 2014 | Kibanda | 01 | September |
| Isingiro | 2014 | Endinzi | 01 | August |
| Rakai | 2015 | Kakuuto | 01 | February |
| Rakai | 2015 | Kakuuto | 01 | May |
| Rakai | 2015 | Kibanda | 01 | May |
| Isingiro | 2015 | Endinzi | 05 | May |
| Isingiro | 2015 | Ngarama | 01 | May |
| Isingiro | 2015 | Endinzi | 02 | August |
| Rakai | 2016 | N/A | 00 | N/A |
| Isingiro | 2016 | Kashumba | 01 | August |
| Isingiro | 2016 | Endinzi | 01 | August |
| **Total** | | | **23** | |

Table 4: Annual and monthly distribution of FMD outbreaks in Tanzanian districts of Missenyi and Kyerwa

| **Tanzania** | | | | |
|---|---|---|---|---|
| **District** | **Year** | **Ward** | **Number of outbreaks** | **Month** |
| Missenyi | 2011 | N/A | No data | N/A |
| Kyerwa | 2011 | N/A | No data | N/A |
| Missenyi | 2012 | Kakunyu | 01 | March |
| Missenyi | 2012 | Mutukula | 02 | July |
| Kyerwa | 2012 | N/A | No data | N/A |
| Missenyi | 2013 | Kakunyu | 03 | October |
| Missenyi | 2013 | Mutukula | 02 | June |
| Missenyi | 2013 | Kitoba | 03 | June |
| Missenyi | 2013 | Bugandika | 02 | June |
| Missenyi | 2013 | Gera | 02 | June |
| Kyerwa | 2013 | Businde | 02 | March |
| Kyerwa | 2013 | Mugaba | 01 | March |
| Kyerwa | 2013 | Kibare | 01 | March |
| Missenyi | 2014 | Kakunyu | 02 | November |
| Missenyi | 2014 | Gera | 02 | July |
| Missenyi | 2014 | Bwanjai | 02 | June |
| Kyerwa | 2014 | Businde | 02 | February |
| Kyerwa | 2014 | Mugabe | 01 | February |
| Kyerwa | 2014 | Kibare | 02 | February |
| Kyerwa | 2014 | Mabira | 01 | February |
| Missenyi | 2015 | Kakunyu | 01 | December |
| Missenyi | 2015 | Kitoba | 03 | July |
| Missenyi | 2015 | Bugandika | 02 | July |
| Missenyi | 2015 | Gera | 02 | July |
| Missenyi | 2015 | Bwanjai | 02 | December |
| Kyerwa | 2015 | Businde | 02 | April |
| Kyerwa | 2015 | Mugaba | 01 | April |
| Kyerwa | 2015 | Kibare | 02 | April |
| Kyerwa | 2015 | Mabira | 02 | April |
| Kyerwa | 2015 | Kamuli | 01 | April |
| Missenyi | 2016 | Kitoba | 02 | June |
| Missenyi | 2016 | Bugandika | 02 | February |
| Missenyi | 2016 | Nsunga | 01 | July |
| Kyerwa | 2016 | Businde | 02 | May |
| Kyerwa | 2016 | Mugaba | 01 | May |
| Kyerwa | 2016 | Kibare | 02 | May |
| **Total** | | | **59** | |

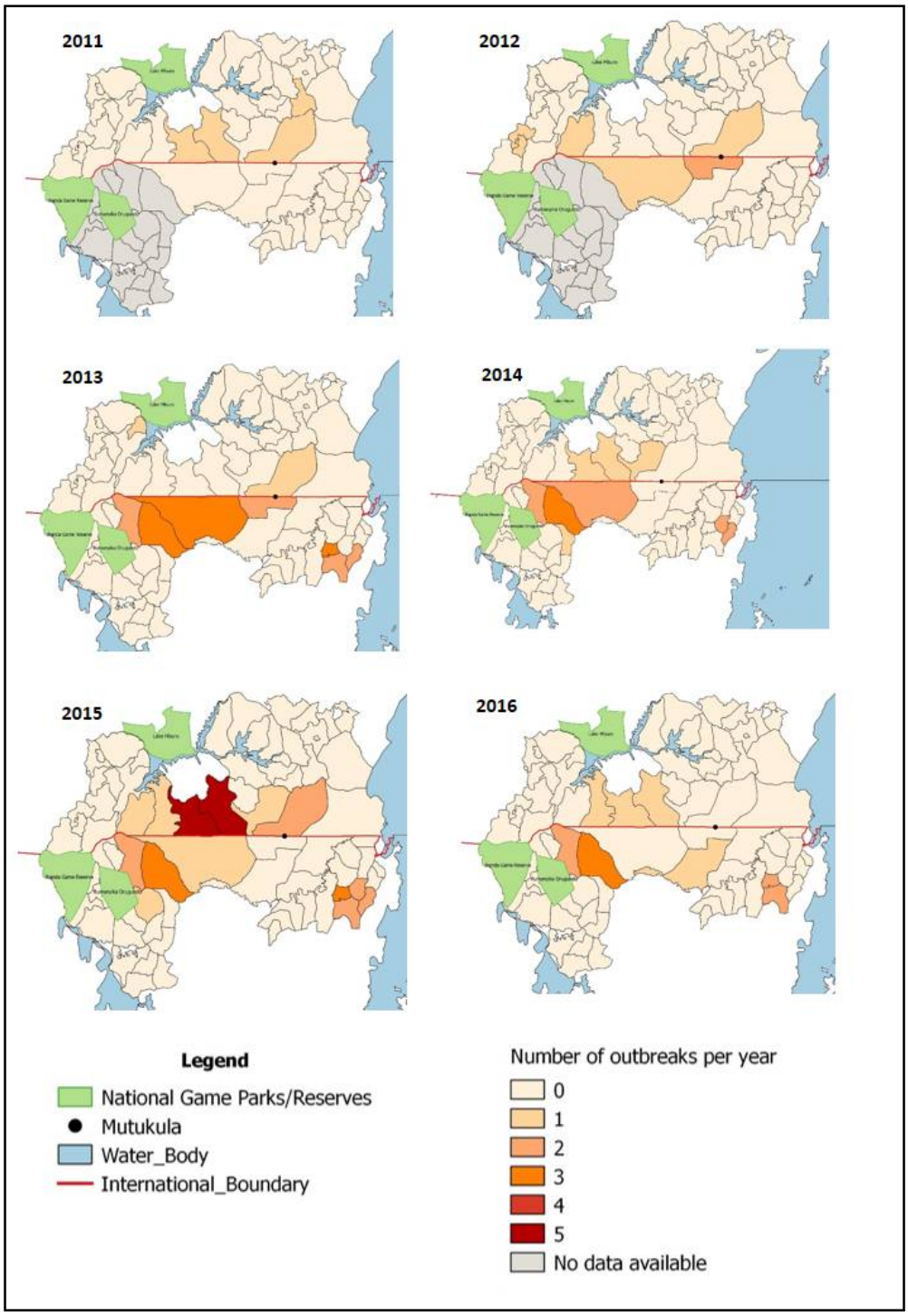



Figure 6: Spatial distribution of FMD in sub-counties/wards in districts located along the Uganda-Tanzania border between 2011 and 2016 (Kerfua *et al.*, 2018)

**(ii) Temporal distribution of FMD**

Temporal data analysis showed that between 2011 and 2016, the months with no outbreaks in Uganda were; January, March, July, October, November and December while, the months with the highest number of reported outbreaks were registered in August (six) and these occurred in Isingiro district. Rakai district recorded February to be the month with highest number of outbreaks (three) (Fig. 7). In the district of Missenyi, most outbreaks reportedly occurred in June and July while in Kyerwa district, the highest number of reported outbreaks was in April (eight). The GLM analysis revealed that FMD outbreaks had occurred 2.7 times more frequently during the dry season than the wet season ($x^2$ = 18.311, df = 1, $p$ < 0.001).

Generally, results showed that there was an average interval of three months apart on the reported FMD outbreaks in the four districts and there was no particular pattern.

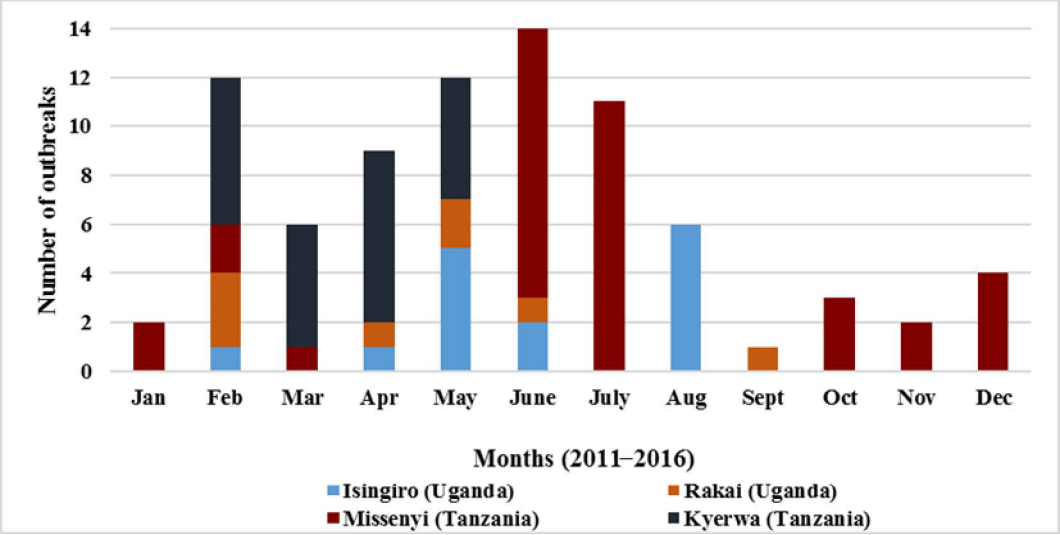



Figure 7: Temporal distribution of the numbers of FMD outbreaks that occurred in districts located at the Uganda-Tanzania border (Kerfua *et al.*, 2018)

**(iii) Serotype distribution**

Serotypes O, SAT 1 and SAT 2 were reported to have occurred in some of the study districts between 2011 and 2016 (Table 5).

Table 5: Foot-and-mouth disease serotypes detected between 2011 and 2016 in the study districts located at the border of Uganda and Tanzania

| Year | Country | District | SC/ward | Serotype | Source |
|---|---|---|---|---|---|
| 2011 | Uganda | Rakai | Kasasa | O | Namatovu *et al.* (2015) |
| 2011 | Tanzania | Missenyi | Mutukula | O | Genchwere and Kasanga, (2014) |
| 2013 | Uganda | Isingiro | Kabingo | SAT 2 | Namatovu *et al*. (2015) |
| 2015 | Uganda | Isingiro | Endinzi | SAT 1 | NADDEC |
| 2015 | Uganda | Isingiro | Kashumba | SAT 1 | NADDEC |
| 2015 | Uganda | Isingiro | Ngarama | SAT 1 | NADDEC |

### 4.1.2 Genetic characterization of FMDVs obtained from study districts

A total of 35 out of 43 samples analysed turned out positive using rRT-PCR having cycle threshold (CT) values of less than 35 (Fig. 8). Only 17 samples turned out positive by convention PCR using serotype specific primers. The obtained band sizes were as expected; approximately1065 base pairs (bp) for serotype O and approximately 866 bp for serotype A (Fig. 9). Out of the 17 samples that were positive by conventional PCR, only eleven sequences of good quality were obtained for subsequent analysis. The BLAST analysis of the 11 VP1 sequences showed that eight sequences belonged to serotype O whereas three belonged to serotype A. Two of the obtained sequences were from samples obtained in 2016 while nine were from 2017 samples. Of the eight serotype O sequences, four were from the Isingiro district (Uganda) and four from Tanzania (Kyerwa [one] and Missenyi [three]). All serotype A sequences were obtained from samples collected in 2017. One sequence was from the Rakai district and two were from Missenyi district. Results showed that one epidemiological unit (cattle farm) in Tanzania had both O and A FMDVs (TAN/08/O/2017 and TAN/10/A/2017) detected. These were detected in samples that were obtained from a single outbreak that occurred in July 2017. The antigen ELISA results revealed that processed epithelial samples had serotypes O, A and SAT 2 detected.

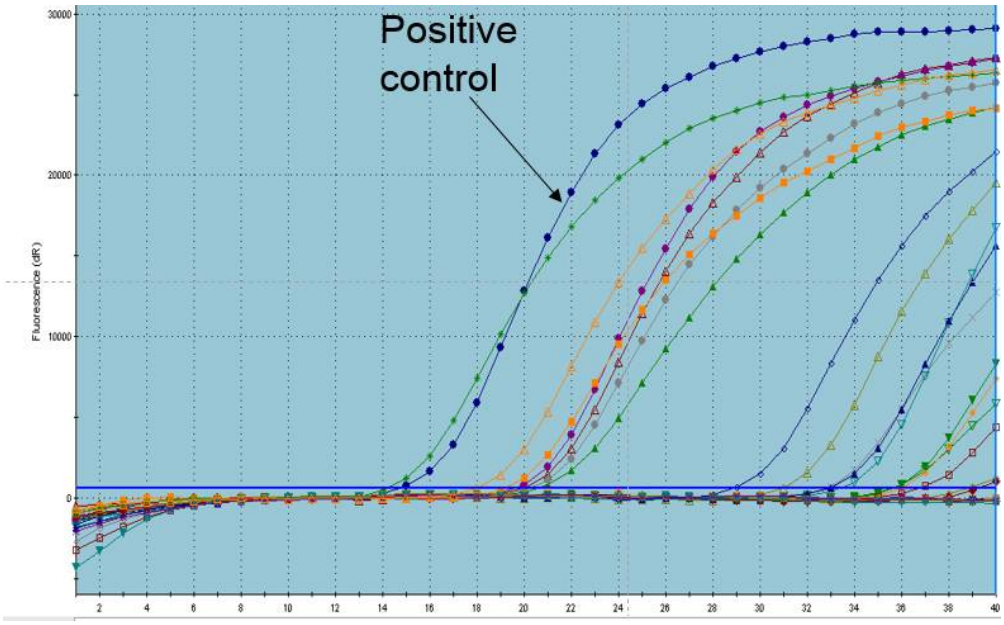
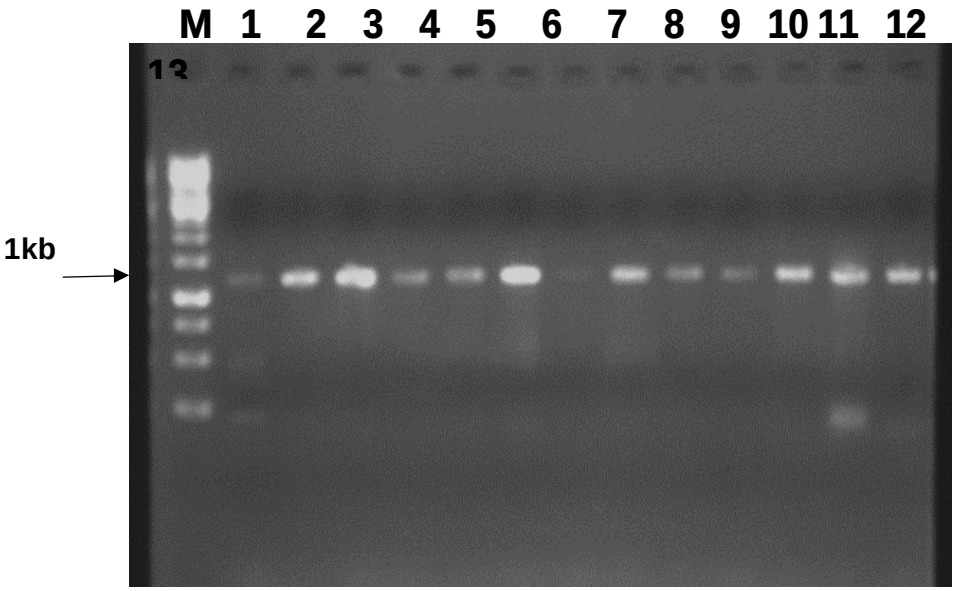



Figure 8: Amplification plots for rRT-PCR, showing samples with ct values less than 40 Samples with Ct values below 35 were considered positive (Susan Diana Kerfua)




**M**- 1kb marker, **1**-T363, **2**-T56, **3**- TM11, **4**- M25, **5**-U15, **6**-M02, **7**-NC, **8**-M20, **9**-M07, **10**-T51, **11**-U147, **13**-PC

Figure 9: Electrophoretic gel image showing the samples that were positive for FMD by serotype O specific primers (Susan Diana Kerfua)

### (i) Analysis of the serotype O VP1 nucleotide sequences

Further analysis of the serotype O VP1 sequences showed there was average nucleotide divergence of 4.9% between the viruses obtained from this study. The phylogenetic analysis showed that the viruses grouped together into one clade (as shown in the neighbour-joining tree in Fig. 10). The four sequences from Isingiro district had a 100% nucleotide pairwise similarity, thus only one of the sequences (UG/13/O/217) was used to represent them in the analyses that followed. Further analysis of O-type sequences showed that they were closely similar with Tanzania sequence (O/TAN/10/2014) and Zambian sequence (O/ZAM14/2010) and had average nucleotide divergence of 7%. The O-type sequences generated from this study belonged to topotype East Africa-2 (EA-2). The vaccine strain O/KEN/77/78, was observed to group differently from the study sequences and belonged to topotype EA-1.

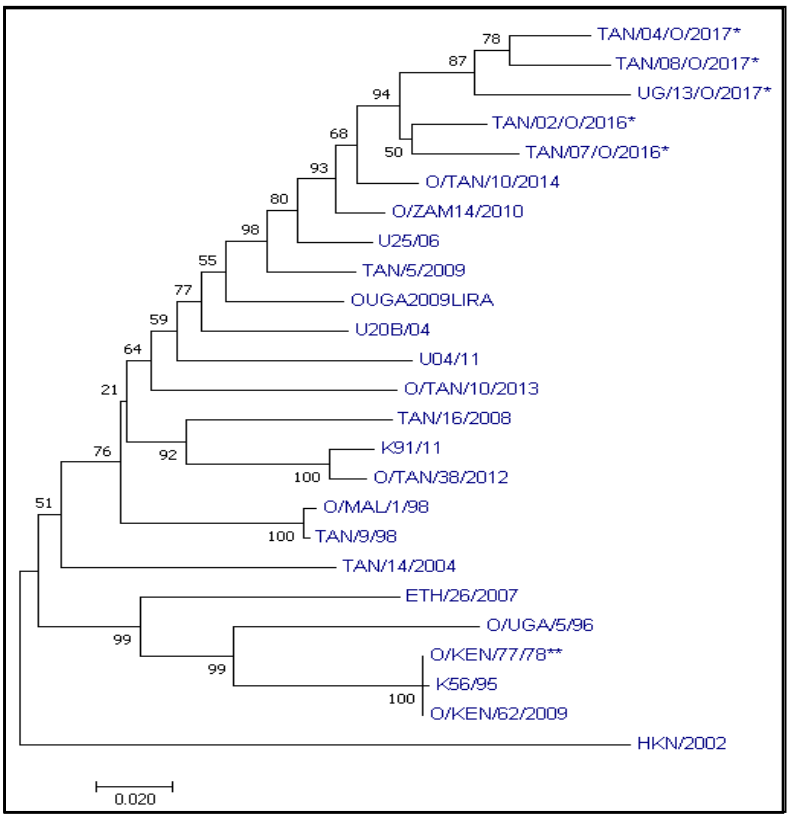



Figure 10: Neighbour-joining tree showing serotype O phylogeny. Bootstrap values of 1000 were used in analysis and the percentage of trees in which the associated taxa clustered together is shown next to the branches. Vaccine strains are marked with double astericks while study sequences are marked with single astericks (Kerfua *et al.*, 2019)

**(ii) Serotype O VP1 amino acid sequence alignment and analysis**

Alignment and comparison of the twenty 200-amino-acid-long sequences to the vaccine strain, O/KEN/77/78, currently incorporated in the trivalent FMD vaccine (used in East Africa) showed that Arginine (R) Glycine (G) Aspartic acid (D) (RGD) motif at positions 139-142 was preserved. However, it was observed that there was high variability between the O/KEN/77/78 and obtained study strains in the flanking region upstream of the RGD (positions 129-137) (Fig. 11). Upstream at the -10 RGD motif, up to seven amino acid changes were observed. Analysis between the study viruses revealed less variation was exhibited -10 downstream of the RDG motif, whereas there was significant variation +10 up stream of the motif. The TAN/07/O/2016, TAN/02/O/2016 and UG/13/O/2017 virus sequences showed closer alignment in their amino acid sequences compared to the other virus sequences obtained. Non-synonymous mutations were seen at position 131 in TAN/08/O/2017 where G was altered to V and position 135 in sequences TAN/04/O/2017 and TAN/08/O/2017, where A was converted to V. Other non-synonymous changes were seen in sequences TAN/04/O/2017 and TAN/08/O/2017, where P was altered to A and V was changed to A, respectively. Non-synonymous changes were also observed in positions 129 (R to L), 131 (G to S), 132 (R to G), 133 (A to T), 134 (P to S), 138 (V to T) and 136 (T to A). The amino acid alignments between UG/13/O/2017 and TAN/02//2016 showed a close match whereas average amino acid difference was 22.04% between sequences obtained from this study and vaccine strain O/KEN/77/78.

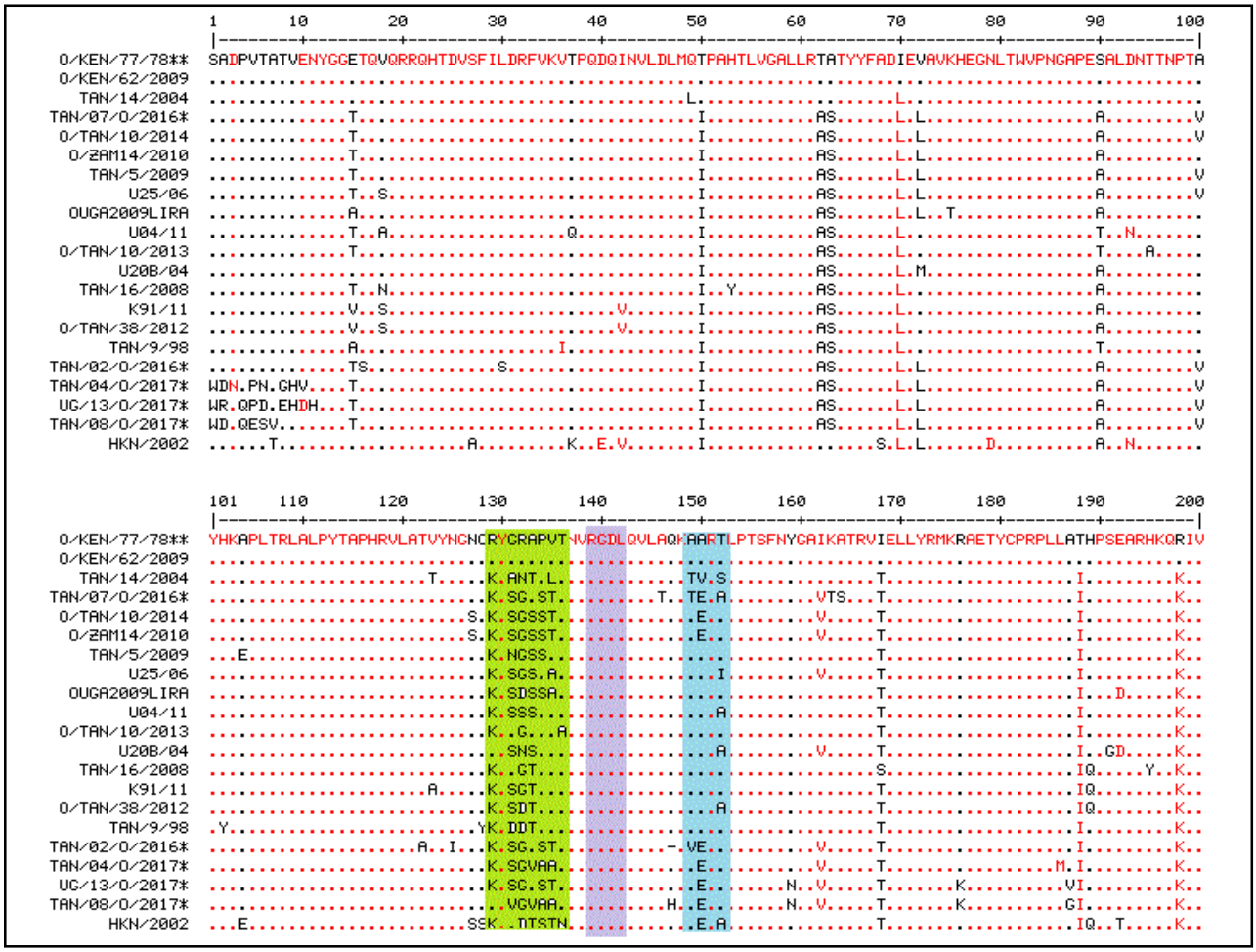


Figure 11: Amino acid sequence alignment for serotype O sequences. Sequences were compared with reference strain KP202877 (KEN/77/78). The conserved RGD motif is in the purple. Identical regions between the rest of the sequences and the reference strain KP202877** are represented with dots. The green and blue triangles show the changes upstream and downstream of the RGDL motif. Study sequences are shown in single asterick at the end of the sequence name (Kerfua *et al*., 2019)

**(iii) Analysis of the serotype A VP1 nucleotide sequences**

All three serotype A sequences belonged to topotype Africa G-1 with an overall 7.4% pairwise sequence divergence between them. The study sequences were observed to be closely similar and grouped together with the 2013 Ugandan sequence that was obtained from Isingiro (Namatovu *et al*., 2015) (Fig. 12). The vaccine strain, K5/1980 similarly belonged to the Africa G-I topotype and had a 16.4% pairwise nucleotide difference from the obtained study strains while vaccine strain, K35/1980 belonged to topotype, Africa G-VII.

**(iii) Serotype A VP1 amino acid sequence alignments and analysis**

The vaccine strain, K5/1980 was used as reference strain in the amino acid alignment. Although it belonged to the same topotype as the study strains (Africa G-I), it was observed that out of the 196 positions, there were 50 variable positions and the obtained strains showed dissimilarity from vaccine strain, K5/1980 at positions 124-130 (Fig. 13). The receptor-binding proteins motif RGD (144-145) was shown to be preserved across all viruses that were included in the alignment. Nevertheless, the flanking regions upstream of the RGD motif (from positions -1 to -6) were significantly variable, with major changes observed in positions 138-143. From the downstream +2 from the RDG motif two amino acid changes were seen and non-synonymous mutations were seen at position 138 where K was altered to T and position 140 where A changed to T. The rest of non-synonymous mutations were observed at position 141 (G to T and G to R). The average amino acid diversity between vaccine strain K5/1980 and obtained study sequences was 15.9%.

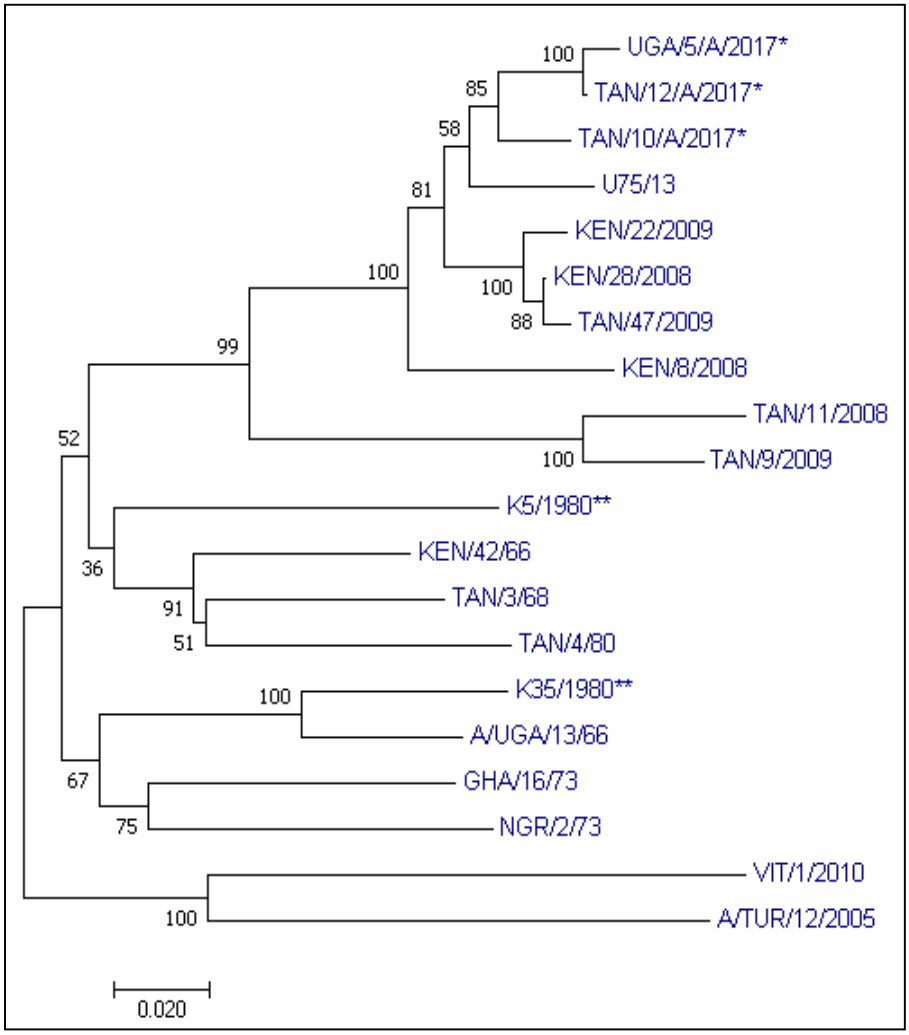



Figure 12: Neighbour-joining tree showing serotype A phylogeny of 20 nucleotide sequences. Bootstrap values of 1000 were used in analysis and percentage of trees in which the associated taxa clustered together is shown next to the branches. The vaccine strain that has been in use is shown with double astericks while the study sequences are with single astericks (Kerfua *et al*., 2018)

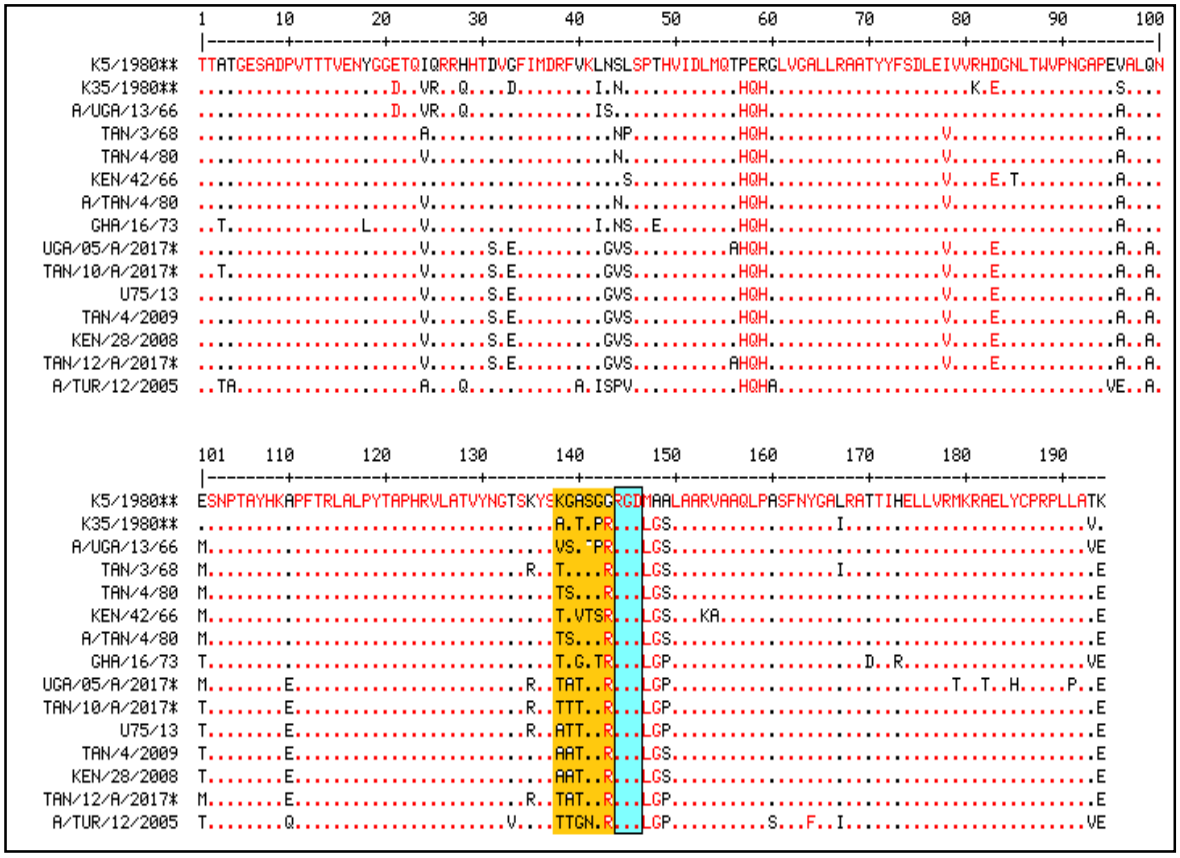



Figure 13: Amino acid sequence alignment for serotype A viruses. Sequences were compared with reference strain K35/1980. The conserved RGD motif is in the light blue rectangle The dots represent identical regions with the reference strain K5/1980** while the orange rectangle shows the changes downstream of the RGD region. Study sequences are shown with a single asterisk at the end of the sequence name. (Kerfua *et al*., 2019)

### 4.1.3 Impact of FMD on household income and food security

#### (i) Household demographics

A total of 255 data sets out of 288 that were collected in February 2018 were analysed. Table 6 illustrates the mean household demographics for the HHs in selected border districts in Ugandan and Tanzanian. Households in Rakai and Isingiro had an average of five (5) children with an average of two (2) children being less than 5 years old while three (3) were between 5-17 years old. Average number of adult males and adult females per HH was two (2) for each category. In Missenyi, each HH had an average of one (1) child below 5 years old and three (3) children between 5-17 years. The average number of adult males in Missenyi HH was one (1) whereas number of adult females was two (2). The livestock

numbers in Rakai and Isingiro were approximately twice that of Missenyi HHs as reflected in Table 6. Results also showed that 42% of HHs in Rakai and Isingiro relied solely on sales of livestock and their products while only 11% of the HHs in Missenyi did.

Table 6: Mean household demographic data for Rakai, Isingiro and Missenyi districts

| Children (<5) | Children (5-17) | Males | Females | Cattle | Goats | Sheep | HHs that solely relied on livestock |
|---|---|---|---|---|---|---|---|
| **Uganda (Rakai and Isingiro)** | | | | | | | |
| 2 | 3 | 2 | 2 | 43 | 15 | 5 | 42% |
| **Tanzania (Missenyi)** | | | | | | | |
| 1 | 3 | 1 | 2 | 25 | 6 | 2 | 11% |

**(ii) Household income from livestock and livestock sales**

The results showed that 56% (n=170) of the households from Rakai and Isingiro and 34% (n=85) of the Missenyi farmers had experienced at least one FMD outbreak on their farm within the last 12 months before the time of study. Of the farmers that had experienced an outbreak, 97% of the Rakai and Isingiro farmers and 76% of the farmers in Missenyi said the outbreak had affected cattle, with an average of 49% of farmers having reported calf mortality with an average of 11 calves that had died. Eighty-eight percent (88%) of the Rakai and Isingiro farmers compared to 54% of Missenyi farmers said they had experienced losses due to reduced livestock prices. There was a loss in income from sales of livestock and livestock products by over 60% while livestock market prices reduced by nearly half (Table 7, 8 and 9).

Monthly household income from sales of livestock and livestock products during FMD outbreak declined by over 50% (Table 7). Before outbreaks, in the Ugandan districts of Isingiro and Rakai farmers earned three times more from both livestock and livestock product sales than farmers in Missenyi, Tanzania before outbreaks. During the outbreak, farmers in the selected Ugandan district earned two and four times more from livestock sales and livestock product sales, respectively, in contrast to the farmers in Missenyi. Table 7 elucidates the decrease in income from livestock sales and sales of livestock income.

Table 7: Mean monthly household income from livestock and livestock product sales before and during FMD outbreaks in selected Ugandan and Tanzanian districts

| Item | Before | During | Difference | % Difference |
|---|---|---|---|---|
| | | **Isingiro and Rakai in Uganda** | | |
| Livestock sales (UgX) | 1 249 460 | 476 962 | -772 498 (-US$214.58) | -62% |
| Livestock product sales (UgX) | 711 466 | 198 929 | -512 537 (-US$142.37) | -72% |
| | | **Missenyi in Tanzania** | | |
| Livestock sales (TZS) | 213 166 | 63 690 | -149 476 (US$ -71.18) | -70% |
| Livestock product sales (TZS) | 117 892 | 58 939 | -58 939 (US$- 29.07) | -50% |

UgX = Uganda Shillings US$ 1=UgX 3600, US$ 1= 2100 TZS (Rate in 2018)

**(iii) Livestock market prices**

In both countries, livestock market prices decreased by over 40%. Figure 14 illustrates the differences observed in the impact of FMD on different livestock species. The price of cattle in Rakai and Isingiro reduced by over 44.8% where as the price in Missenyi reduced by slightly over 50.6%. Further observations highlight a decrease in price of goats by 41.3% in Rakai and Isingiro and 40% in Missenyi, while sheep prices decreased by about 41.7% in Rakai and Isingiro and slightly higher than 50% in Missenyi. Tables 8 and 9 reflect the average price values that were realized.

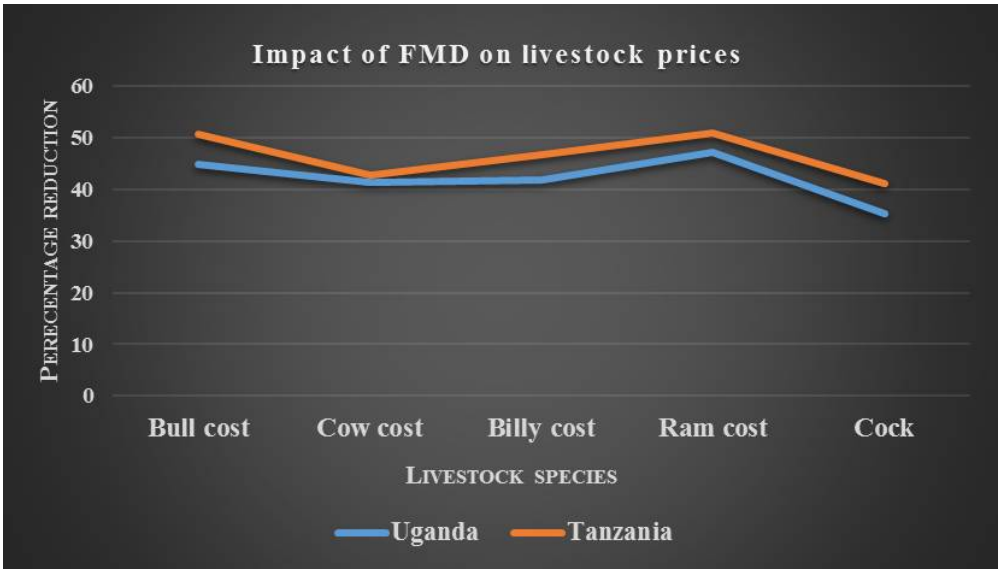



Figure 14: Percentage reduction of livestock market prices in the districts of Rakai and Isingiro in Uganda and the district of Missenyi in Tanzania

Table 8: Mean livestock market prices in Rakai and Isingiro districts before and during an FMD outbreak

| Item | Cost before (UgX) | Cost during (UgX) | Cost Difference (UgX) | Cost Difference US$ | % difference |
|---|---|---|---|---|---|
| Bull | 1 590 949 | 877 948 | -713 001 | -198.10 | -44.8 |
| Cow | 1 312 704 | 770 961 | -541 743 | -150.48 | -41.3 |
| Male goat (Billy) | 174 046 | 101 480 | -72 566 | -20.15 | -41.7 |
| Male sheep (Ram) | 124 000 | 65 495 | -58 505 | -16.25 | -47.2 |
| Cock | 21 422 | 13 852 | -7 570 | -2.10 | -35.3 |

US$1=UgX 3 600, US$ 1= 2 100 TZS

Table 9: Mean livestock market prices in Missenyi district before and after an FMD outbreak

| Livestock | Cost before (TZS) | Cost during (TZS) | Cost Difference (TZS) | Cost Difference (US$) | % difference |
|---|---|---|---|---|---|
| Bull | 864 638 | 427 029 | -437 609 | -208.39 | -50.6 |
| Cow | 553 116 | 316 912 | -203 116 | -112.48 | -42.7 |
| Male goat (Billy) | 73 143 | 39 000 | -34 143 | -16.26 | -46.7 |
| Male sheep (Ram) | 54 519 | 26 826 | -27 693 | -13.19 | -50.8 |
| Cock | 14 917 | 8 500 | -6 147 | -3.06 | -41.2 |

US$1=UgX 3 600, US$ 1= 2 100 TZS (Rate in 2018)

**(iv) Food commodity prices and food availability**

Milk consumption decreased in households in Rakai and Isingiro =by 48% while in households in Missenyi it decreased by 57%. There was a significant ($p < 0.05$) decrease in milk price in Rakai and Isingiro whereas milk price in Missenyi HHs increased during the outbreak as shown in Table 10.

Table 10: Mean milk sales and consumption in the districts of Rakai and Isingiro and Missenyi districts

| Item per HH | Before outbreak | | During outbreak | | % change (Rakai and Isingiro) | % change (Missenyi) |
|---|---|---|---|---|---|---|
| | Rakai and Isingiro | Missenyi | Rakai and Isingiro | Missenyi | | |
| Milk price per litre (US$) | 0.29 | 0.44 | 0.22 | 0.49 | -24 | 11 |
| Milk consumption (litres) | 4.85 | 1.57 | 2.53 | 0.67 | -48 | -57 |

**(v) Impact of FMD on other food commodity prices**

The obtained results reflected a decline in the prices of selected food commodities in HHs in both Rakai and Isingiro districts of Ugandan and Missenyi district in Tanzanian The commodities whose prices significantly reduced ($p < 0.05$) in Uganda were: chicken, beef, eggs, milk and goat meat as illustrated in Table 11. Consumption of beef reduced in households in Rakai and Isingiro and increased in Missenyi HHs while fish and poultry consumption reduced throughout the households (Table 12).

Table 11: The mean commodity prices for Rakai and Isingiro districts before and during FMD outbreaks

| **Item** | **Price Before (UgX)** | **Price During (UgX)** | **Price Difference (UgX)** | **Price difference (US$)** |
|---|---|---|---|---|
| Egg | 380.8 | 278.3 | 102.5 | 0.03*** |
| Beef (kg) | 9 407 | 6 212 | 3 195 | 0.89*** |
| Goat meat(kg) | 11 361 | 8319 | 3042 | 0.85*** |
| Milk (1 litre) | 518 | 397 | -121 | -0.03*** |
| Beans(kg) | 2051 | 1943 | 108 | 0.03* |
| Maize flour (kg) | 2 063 | 2233 | 170 | 0.047 |
| Fish (1 piece) | 4094 | 4761 | 667 | 0.19 |

*** Significant at $p < 0.001$ **significant at $p < 0.01$ *significant at $p < 0.05$

US$1=UgX 3 600, US$ 1= 2 100 TZS

Table 12: The mean commodity prices in Missenyi district before and during an FMD outbreak

| Item | Cost Before (TZS) | Cost During (TZS) | Cost Difference (TZS) | Cost Difference (US$) |
|---|---|---|---|---|
| Egg | 284.3 | 253.7 | -30.6 | 0.01* |
| Beef | 4 823 | 2 573 | -2 250 | 1.08*** |
| Goat's meat | 5 677 | 2 974 | -2 703 | 1.29*** |
| Milk(1litre) | 471 | 512 | 41 | 0.02** |
| Beans | 1 237 | 1 109 | -128 | 0.06 |
| Maize flour | 1 146 | 1 081 | -65 | 0.03 |
| Fish | 2 900 | 2 145 | -755 | 0.36** |

*** Significant at $p < 0.001$ **significant at $p < 0.01$ *significant at $p < 0.05$

**(vi) Household food consumption**

There was a significant decrease ($p = 0.03$) in beef intake in Ugandan HHs (Table 13). Although there was an increase in beef intake in Missenyi district HHs, the difference was not significant ($p < 0.05$). In both countries fish and chicken consumption also decreased during an outbreak ($p < 0.05$) period although not significantly.

Table 13: Mean consumption of beef, chicken and fish in study districts before and during FMD outbreaks

| Item | Rakai and Isingiro | | | Missenyi | | |
|---|---|---|---|---|---|---|
| | Before | During | p value | Before | During | p value |
| Beef consumption (kg) | 2.28 | 1.6 | 0.03* | 4.16 | 5.64 | 0.15 |
| Chicken consumption (whole) | 0.7 | 0.69 | 0.32 | 1.4 | 1.15 | 0.25 |
| Fish consumption (pieces) | 0.68 | 0.62 | 0.61 | 2.56 | 2.27 | 0.46 |

*** Significant at $p<0.001$ **significant at $p < 0.01$ *significant at $p < 0.05$

**(vii) Meals per day and food variety**

The study showed that in both countries, an average household had two major meals per day before the outbreak period. When the outbreak occurred, 35% of Ugandan households prepared more meals, 39% had less meals and 26% had the same number of meals as they did before the outbreak, while in Tanzania, 30% had more meals, 66% had similar number of meals and 4% had less meals per day. The average number of dishes per meal for both countries were two dishes which consisted of a starchy food [mostly maize meal *("ugali'),* bananas or sweet potatoes] and a sauce [either beans, beef, vegetables, or a milk sauce called "*eshabwe"* (in Isingiro and some parts of Rakai)]. In Uganda, 27% of the households reported a decrease in the variety of dishes, 35% had more variety and 35% had no change in variety during the outbreak period. The results for Tanzanian households showed that 63% had similar variety of dishes, 27% had more variety and 0% had less variety during the outbreak as shown in Table 14.

Table 14: Average number of meals per day and food variety before and during an FMD outbreak

| District | Number of meals during an outbreak | | | Food variety during an outbreak | | |
|---|---|---|---|---|---|---|
| | Same | Fewer | More | Similar | Less | More |
| Rakai and Isingiro | 26% | 39% | 35% | 38% | 24% | 38% |
| Missenyi | 66% | 4% | 30% | 77% | 0% | 23% |

**(viii) Expenditure on treatments and vaccinations**

Table 15 reflects the treatment and vaccination costs incurred by farmers for the different livestock species. Results showed that Ugandan farmers spent an average of approximately US$ 7 on treatment and US$ 0.5 as vaccination cost per head of cattle whereas, Tanzanian farmer spend about US$ 12 and US$ 1.5 for treatment and vaccination cost, respectively. The observations further showed that farmers spent more on cattle vaccinations and treatments than they did on the other livestock species.

Table 15: Average treatment and vaccination costs due to the outbreak in selected districts located at the Uganda-Tanzania border

| Item (per head) | Respondent Proportion | Amount | Amount (US$) |
|---|---|---|---|
| | Rakai and Isingiro | (UgX) | |
| Treatment of cattle | 85% | 24 788 | 6.89 |
| Treatment of goats | 31% | 7 473 | 2.08 |
| Treatment of sheep | 0% | - | - |
| Vaccination of cattle | 95% | 2 045 | 0.57 |
| Vaccination of goats | 38% | 1 268 | 0.35 |
| Vaccination of sheep | 26% | 1 218 | 0.34 |
| | **Missenyi** | **(TZS)** | |
| Treatment of cattle | 64% | 25 012 | 11.90 |
| Treatment of goats | 0% | - | - |
| Treatment of sheep | 0% | - | - |
| Vaccination of cattle | 57% | 3 095 | 1.47 |
| Vaccination of goats | 38% | 1 972 | 0.94 |
| Vaccination of sheep | 12% | 2 000 | 0.95 |

## 4.2 Discussion

### 4.2.1 General discussion

The role of border areas in FMD epidemiology remains pertinent in the spread, maintenance and control of the disease particularly in endemic countries. This study provides insight into the trans-boundary dynamics of FMD epidemiology and its impact in a border context. The main aim of the study was to obtain a better understanding of FMD dynamics along the Uganda-Tanzanian border area with the hope that the findings from this study can provide insightful information that both countries can incorporate into their control strategies. The overall goal of the study was to enhance food security and household income through reduction of FMD outbreaks in the cross-border settings.

In the first objective of the study regarding spatial and temporal distribution of FMD outbreaks that occurred between 2011 and 2016, the results clearly align with other similar studies (Amaral, Gond & Tran, 2016; Dukpa, Robertson & Ellis, 2011; Picado *et al*., 2010; Ayebazibwe *et al*., 2010). Domenech *et al*. (2016), which argued that although border areas benefit from trade and take advantage of differences in commodity prices, there is a constant state of disease transmission from one country to the other. Thus, because of the legal and illegal trade in these areas, border areas are constantly plagued with diseases (Domenech *et al*., 2016). Studies in Bhutan, Uganda, Tanzania and Paraguay have elucidated that areas that were closer to international border areas had significantly higher numbers of FMD outbreaks than those that were not (Dukpa *et al*., 2011; Picado *et al*., 2010; Ayebazibwe *et al*., 2010; Munsey *et al*., 2019). Previous epidemiological studies reported that the dry season usually coincided with high number of FMD outbreaks (Kivaria, 2003; Ayebazibwe *et al*., 2010). During dry months, farmers trek long distances looking for pastures and water, and animals usually converge around watering points and grazing grounds and thus, the transmission of the virus is easily facilitated between and within herds (VanderWaal, Gilbertson, Okanga, Allan & Craft, 2017). The difference in number of FMD outbreaks in both countries was very significant ($p < 0.001$), with Tanzania having almost twice the number of outbreaks compared to Uganda. This could be attributed to the different livestock policies affecting FMD control in either country or even general management systems. Vaccination subsidy in Uganda most probably motivated farmers to vaccinate their animals more regularly than their counterparts in Tanzania (MLD, 2006; Muleme *et al*., 2012). In addition, vigilant reporting by animal health personnel at the Tanzanian side may have resulted into more outbreaks

being reported than on the Ugandan side of the border. Issues that pertain to underreporting of FMD in some areas cannot be disregarded because studies have shown that farmers are reluctant to report due to political or economic consequences that may arise (Gunarathne, Kubota, Kumarawadu, Karunagoda & Kon, 2015). Further observations from this study revealed that for all the six years, outbreaks were reported consistently in some sub-counties/wards probably due to livestock congregational points such as in livestock markets, watering dams and communal grazing grounds. Such areas where livestock congregate for one reason or another easily facilitate the spread of the FMDV (Boklund, Halasa, Christiansen & Enoe, 2013).

This objective had a number of limitations that could have affected the quality of the results obtained. One of the limitations was that most of the reported outbreaks were not confirmed in the laboratory but were based on clinical diagnosis by Veterinary Officers. Without laboratory confirmation, reported cases maybe be doubted as to whether they were truly FMD cases. Nonetheless, a study showed that Tanzanian livestock farmers knew how to identify FMD signs and their descriptions of FMD cases had a highly positive association with positive ELISA results got in the study (Genchwere & Kasanga, 2014). Additionally none of the diseases that share signs with FMD were reported to have occurred in the area of study during the period that was being studied according to the DVOs. Nevertheless, the limitations cited created an opportunity for the authors to recommend to the Ministries in charge of Livestock in both countries to ensure that all suspected FMD cases are confirmed in the laboratory before control options are rolled out. The limited information on serotype circulation in this area stemmed from the few suspected cases that were confirmed in the laboratory. The issue of few cases being diagnosed was highlighted by Muleme *et al*. (2012) who observed that only 7.4% of FMD cases reported in Uganda between 2001 and 2010 were confirmed in the laboratory. The limited serotype information obscured the true depiction of the serotype distribution in the border area thus could not make useful inferences for the study. Additionally, the lack of information on serotypes in circulation makes it difficult to inform policy on which vaccine serotypes to use for vaccination (Kitching *et al*., 2007). Thus limited knowledge on serotypes/topotypes in circulation in an area makes it difficult to ascertain which vaccine strains can be used for preventing outbreaks.

In the initial stages of the PCP-FMD, comprehensive understanding of FMD epidemiology facilitates the right selection of vaccine strains to be used. Therefore, the second objective on the genetic characterisation of obtained viruses, sought not only to determine the serotypes in circulation but also to ascertain how they were genetically related to the vaccine strains. The results offered new perceptions into relationships between viruses obtained from trans-border outbreaks and vaccine strains in use. Circulating virus strains were further compared against the vaccine strains in use by insilico methods. Although recently, whole genomes sequences are being used to study relationships between viruses in much detail, using VP1 coding sequences to infer genetic variability makes it a cost effective and quick while still establishing genetic relationships (Jamal & Belsham, 2013). Previous studies have shown that serotype O EA-2 viruses have been dominant in most outbreaks in East Africa (Kasambula *et al*., 2012; Kasanga *et al*., 2011; Balinda *et al*., 2010a) while serotype O EA-1 topotype seems to be getting into extinction. The epidemiological factors that could explain the dominance of serotype O EA-2 topotype in most outbreaks remain elusive. The detection of serotype A has been rarely reported (Namatovu *et al*., 2013) and has been attributed to infrequent surveillance systems (Namatovu *et al*., 2013). Notwithstanding this, other studies have observed that the serotype A is generally rare, even as was reported in Asia by Kitching *et al*. (2007). However, Casey-Bryars *et al*. (2018) showed that the circulation of particular serotypes may occur in a region or country in waves, disappearing for some years before they reoccur. Nevertheless, the need to determine the epidemiological factors that attributed to the rare occurrence of serotype A remains critical so as to inform strategic control.

The low diversity observed between these viruses implies that the viruses obtained belonged to the same lineage. The work furthermore supports the view that back and forth human-livestock movement may be critical in trans-boundary disease spread (Fèvre *et al*., 2006; Di Nardo *et al*., 2011). At the time of the study, there were two outbreaks in the study area within a span of eight months highlighting how endemic FMD is in both countries. Within this time, one farm had two different serotypes O and A circulating, demonstrating that there can be multiple serotypes circulating within the same epidemiological unit at the same time. This kind of scenario not only advocates for quick laboratory diagnosis but also for the use of effective multivalent vaccines. The serotype O viruses obtained belonged to EA-2 which has been the case for most recently circulating FMDVs in Uganda (Namatovu *et al*., 2015; Mwiine *et al*., 2019). The results from the alignment of translated amino acid sequences between the vaccine strains and the circulating viruses showed that currently used vaccines

had high variations in the alignments with the viruses obtained. Particularly, the field vaccine strain K5/1980 which belonged to the same topotype as the circulating strains showed significant variation upstream of the conserved RGD motif which could affect the performance of the vaccines being used. The RGD motif is critical in the binding of the virus to the intergrin on the mucosal surface of the host. Therefore, while the RGD motif was conserved across all virus amino acid derivatives, amino acid functions upstream and downstream the motif sequence could be altered (Carrillo *et al*., 2005). The study hypothesizes that these amino acid functions could be contextual in that non-synonymous changes upstream and downstream of this motif could alter protein functionality. This could then mean that the vaccine strains may not elicit antibodies that are protective against circulating strains. Thus, field evaluation studies for this vaccine for effective control of the disease would be relevant to avoid wastage of resources on such vaccines.

The importance of budg*et* allocation to disease control was one of the important contributing factors for most developed countries in achieving success in FMD eradication. Although disease impact studies have seldom been carried in African countries, these help to determine the allocation of defined resources to the control of a disease such as to avoid wastage or under allocation of resources. The PCP-FMD stresses the importance of impact studies among other studies in order to move from stage 0 to stage 1 of the pathway (FAO, 2011). The findings from objective three attested to what has been found in other countries where FMD impact studies have been carried out (Bayissa *et al*., 2011; Rushton & Knight-Jones, 2012; Baluka *et al*., 2014; Mdetele, Kasanga, Seth & Kayunze, 2015). Foot-and-mouth disease has been associated with low productivity in endemic countries (Rushton & Knight-Jones, 2012) and presence of FMD has been highlighted as a major factor in hindering livestock markets and making farmers unable to access fair markets when they need them. The decline in income from livestock sales is probably because farmers are unable to sell their livestock during outbreaks and if they did, they sold at very low costs. Most farmers reported reduced livestock prices by nearly 50% during the outbreak in both countries (Tables 8 and 9) as was also observed by Rutagwenda (2003) in Ugandan districts of Kumi and Isingiro. There was no apparent difference in market prices in all the districts despite the fact that official quarantines had only been imposed in the Ugandan districts (Fig. 14). However, compared to 65% of the farmers from Uganda, 39% of the Tanzanian farmers said they experienced losses due to reduced livestock prices. This could be because only 11% of Tanzanian households relied solely on livestock as a major source of income compared to

42% of Ugandan households. The results showed that income from livestock and livestock product sales in Ugandan HHs was higher than that in Tanzanian HHs. This could probably be so because the western and central Uganda has been involved in livestock improvement programmes such as improved cattle breeds and dairy technologies which could have led to increased milk production (Kabuga, 2014) thus better income from sales of livestock products. The significant decrease in sales of livestock products in Uganda could be possibly due to the combination of both decline in milk production and milk prices which happened during the outbreak. The milk price in Ugandan districts decreased significantly after the outbreak whereas in Tanzania the milk price increased. The price of milk could have increased in Tanzania due to decreased milk supply as observed in other impact studies (Baluka, 2016; Mdetele *et al.*, 2015). However, in the Ugandan districts, the milk price increased despite the low supply due to the outbreak. The presence of milk processing plants in western Uganda could have possibly allowed for the dictation of the milk price in the Ugandan districts by the processing plants. In the Tanzanian study districts, however, because there are no milk processing plants in or close to the study areas, milk was sold directly to those who required it thus, perhaps the farmer was able to dictate his/her own price.

Generally, the results showed that presence of FMD affected the price of other commodities like meat, eggs and fish in some places (Tables 10 and 11). Similar observation was reported by Perry and Grace (2009) and Knight-Jones and Rushton (2013) who showed that FMD affects other commodity prices thus changing the market trends. The study revealed that most farmers spent money on treatment of cattle than any other livestock species. This could be most likely because cattle are the most affected by FMD and tend to show signs and lesions more readily than the other species (Kitching & Hughes, 2002). Also in many communities, cattle are more valued than other species of livestock (Bettencourt, Tilman, Narciso, Carvalho & Henriques, 2015) and this could perhaps also explain why farmers spent readily on the cattle than other species. The average percentage of farmers who treated their livestock was higher in Uganda than in Tanzania (Table 14). Most likely because Uganda, compared to Tanzania had a higher percentage of farmers who solely depended on livestock for livelihood as discussed above. The average cost of treatment of cattle was at US$ 6.89 in Uganda while the cost was higher in Tanzania at US$ 11. The differences in the costs could be attributed to the different economies in both countries but also due to the differences in livestock disease control policies. The involvement of the government in the control of FMD in Uganda may have contributed to the high vaccination rate of 95% among the Ugandan farmers. In

Tanzania, the vaccination rate was at 57% which was very high compared to previous studies that have observed 2% rates of vaccination (Mdetele *et al.*, 2015; Railey *et al.*, 2018). The reasons for the high vaccination rate deduced from this study cannot be fully explained, however, it may probable that there could be a border effect, and Tanzanian farmers may be influenced by Ugandan farmers who have high vaccination rates.

In this study, the consumption and prices of items like poultry (chicken and eggs) and fish were significantly affected in some villages. Increase in beef supply possibly explains why the other commodity prices were significantly affected. Decrease in beef prices due to increased supply of beef could have led to the low demand of poultry and fish which were unable to compete fairly in the market. Decrease in price of poultry impacts on poultry keeping households having a direct negative impact on their income. Some of the farmers explained that nobody bought chicken nor fish during an FMD outbreak due to increased beef supply in the village (Farmer group Endinzi sub-county, personal communication, 18th November 2017). Although the price of chicken decreased significantly, there was a decrease in chicken and fish consumption in both countries probably because of the meat surplus in the villages.

Implications of FMD on food security in households in Mbarara and Kumi were made apparent by Rutagwenda (2003). In this study, households had an average of 2.2 meals in Tanzania and 2.5 meals in Uganda as was similarly reflected in another study (The National Food Security Division - Ministry of Agriculture Livestock and Fisheries, 2017). However, when the FMD outbreak occurred, most Ugandan households had less meals than their Tanzanian counterparts. In both countries, about 30% of the households admitted to having more meals. Overall, the number of meals was more stable in Tanzania than in Uganda. This food stability could be explained by the fact that majority of Tanzanian households visited relied also on crop farming (37%) compared to 0.08% of the Ugandan farmers. It is more likely that households that cultivated crops and those with diversified income had more stable sources of food and income during FMD outbreaks (Otte *et al.*, 2004; Omondi *et al.*, 2017) than the households that solely relied on livestock income. The Ugandan farmers of whom most were *Ankole* tribe said when there was an FMD outbreak, they were required to have more meals a day because they could no longer take milk regularly. The Ankole are known for their high milk consumption and can rely on milk all day long. Outbreaks of FMD impact on household milk productivity and consequently its consumption (Otte *et al.*, 2004; Kabuga,

2014). According to this study, at least each household had a child that was below the age of 5 years who would require a constant supply of milk to meet their nutritional needs. The average milk consumption of about half a litre in Missenyi raises questions as to if children's nutritional requirements were being met. The average number of 3 school going children per household (6-17 years) also meant that there was hardly any milk for children to carry to school during FMD outbreaks. During the interview, farmers disclosed that during outbreaks children packed porridge made from maize flour as a substitute for milk, thus implications on the dietary needs of the children. The low circulation of money in the villages after an outbreak may that mean households are unable to afford certain commodities and foods and this, has implications on food security and nutrition of the people especially children within the households (Rutagwenda, 2003).

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

From the findings of the present work, it can be concluded that: the temporal and spatial distribution of FMD outbreaks established that more outbreaks between 2011 and 2015, were clustered around the sub-counties/wards adjacent to the Uganda-Tanzania border. The study results also established that certain villages and sub-counties/wards were persistently affected throughout the years of study. The observations showed that most FMD outbreaks occurred during the dry months of the year.

The phylogenetic study revealed that serotype O and A were prevalent in outbreaks that occurred in the districts at this border area between 2016 and 2017. The study further showed that there was low genetic diversity between the viruses from both countries, meaning that they were closely related and belonged to the same lineages. The study further showed that serotype O viruses from both countries belonged to topotype EA-2 while the serotype A viruses belonged to Africa-GI topotype. Insilico analysis of the vaccine strain and circulating serotypes showed that the serotype O vaccine strain was antigenically different from the circulating strains by 22.04% and belonged to a different topotype EA-1. Although the serotype A vaccine belonged to the same topotype as the strains obtained from this study, antigenic differences upstream and downstream of the RGD motif could affect vaccine efficacy.

The impact studies showed that income from sales from livestock and livestock products from both countries reduced by over half. Market prices of livestock from both countries reduced with livestock prices having reduced by over 40%. Outbreaks of FMD affected the availability and prices of certain food items such as beef, milk, poultry and fish. The study also showed that outbreaks of FMD affected the consumption of certain foods such as milk, eggs and fish thus having some implications on nutrition of the study population and lastly outbreaks of FMD increased costs of treatment of livestock with cattle being the most treated and vaccinated species.

## 5.2 Recommendations

The following recommendations can be derived from this study:

(i) Since border areas are crucial in FMD epidemiology, the governments of Uganda and Tanzania should create platforms that encourage collaboration between the animal health personnel at the border areas. Collaboration in terms of disease control in these areas is crucial because neighbouring countries may differ in terms of control strategies which may impact the circulation of a disease.

(ii) There is need for the reports submitted on FMD outbreaks by the District Veterinary Officers to be more comprehensive detailing information on the GPS location where the outbreak occurred, serotype responsible for outbreak, number of animals that were infected and number of animals at risk. The study recommends that for each outbreak that is reported officially, it should be accompanied with the laboratory findings.

(iii) There is need for more epidemiological studies to be carried out in border areas. These studies should be able to establish the livestock movement's patterns and ascertain reasons as to why certain serotypes are prominent in specific locations.

(iv) The study recommends vaccine evaluation studies, both at laboratory and field levels. Such studies can be able to provide important information on the efficacy and effectiveness of the current vaccines in use.

(v) More studies on cost benefit analysis for FMD are essential in order for the two governments to know the worth on investing in FMD control. This would provide important information for the PCP-FMD.

## REFERENCES


African Union - Inter African Bureau for Animal Resources. (2009). *Strategic Plan*. Retrieved on 3 January, 2019, from http://20100419_ibarstrategicplan_en.pdf.

Alexandersen, S., Zhang, Z., Reid, S. M., Hutchings, G. H., & Donaldson, A. I. (2002a). Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001. *Journal of General Virology*, *83*, 1915–1923. https://doi.org/10.1099/0022-1317-83-8-1915

Alexandersen, S., Zhang, Z., & Donaldson, A. (2002b). Aspects of the persistence of foot-and-mouth disease virus in animals – the carrier problem. *Microbes and Infection*, *4*, 1099–1110. https://doi.org/10.1016/s1286-4579(02)01634-9

Alexandersen, S., Zhang, Z., Donaldson, A., & Garland, A. J. (2003). The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology*, *129*, 1–36. https://doi.org/10.1016/s0021-9975(03)00041-0

Altschul, S., Gish, W., Miller, W., Myers, E. W., & Lipman, D. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amaral, T. B, Gond, V., & Tran, A. (2016). Mapping the likelihood of foot-and-mouth disease introduction along the border between Brazil and Paraguay. *Pesquisa Agropecuária Brasileira*, *51*, 661. https://doi.org/10.1590/S0100-204X2016000500029

Anderson, E. C., Doughty, W. J., Anderson, J., & Paling, R. (1979). The pathogenesis of foot-and-mouth disease in the African buffalo (Syncerus caffer) and the role of this species in the epidemiology of the disease in Kenya. *Journal of Comparative Pathology*, *89*, 541–549. https://doi.org/10.1016/0021-9975(79)90045-8

Ayebazibwe, C., Tjørnehøj, K., Mwiine, F. N., Muwanika, V. B., Ademun Okurut, A. R., Siegismund, H. R., & Alexadersen, S. (2010). Patterns, risk factors and characteristics of reported and perceived foot-and-mouth disease (FMD) in Uganda. *Tropical Animal Health and Production*, *42(7)*, 1547–1559. https://doi.org/10.1007/s11250-010-9605-3

Ayelet, G., Mahapatra, M., Gelaye, E., Egziabher, B. G., Rufeal, T., Sahle, M., … Knowles, N. J. (2009). Genetic characterization of foot-and-mouth disease viruses, Ethiopia, 1981-2007. *Emerging and Infectious Diseases, 15,* 1409–1417. https://doi.org/10.3201/eid1509.090091

Bachrach, H. L. (1968). Foot and mouth disease. *Annual Reviews in Microbiology*, *22,* 201–44. https://doi.org/ 10.1146/annurev.mi.22.100168.001221

Balinda, S. N., Belsham, G. J., Masembe, C., Sangula, A. K., Siegismund, H. R., & Muwanika, V. B. (2010). Diversity and transboundary mobility of serotype O foot-and-mouth disease virus in East Africa: Implications for vaccination policies. *Infection, Genetics and Evolution, 10(7),* 1058–1065. https://doi.org/ 10.1016/j.meegid.2010.06.017

Baluka, S. A. (2016). Economic effects of food and mouth disease outbreaks along the cattle marketing chain in Uganda. *Veterinary World, 9,* 544–553. https://doi.org/10.14202/vetworld.2016.544-553

Baluka, S. A., Ocaido, M., & Mugisha, A. (2014). Prevalence and economic importance of foot-and-mouth disease, and contagious bovine pleuropneumonia outbreaks in cattle in Isingiro and Nakasongola districts of Uganda. *Discourse Journal of Agriculture and Food Sciences, 2 (4),* 107–117.

Barasa M., Catley, A., Machuchu, D., Laqua, H., Puot E., Tap Kot, D., & Ikiror, D. (2008). Foot-and-mouth disease vaccination in South Sudan: Benefit–cost analysis and livelihoods impact. *Transboundary and Emerging Diseases, 55,* 339–351. https://doi.org / 10.1111/j.1865-1682.2008.01042.x.

Bari, F. D., Parida, S., Tekleghiorghis, T., Dekker, A., Sangula, A., Reeve, R., … Mahapatra, M. (2014). Genetic and antigenic characterization of serotype A FMD viruses from East Africa to select new vaccine strains. *Vaccine, 32,* 5794–800. https://doi.org/10.1016/j.vaccine.2014.08.033

Bayissa, B., Ayelet, G., Kyule, M., Jibril, Y., & Gelaye, E. (2011). Study on seroprevalence, risk factors, and economic impact of foot-and-mouth disease in Borena pastoral and agro-pastoral system, southern Ethiopia. *Tropical Animal Health and Production*, *43*, 759 –766. https://doi.org/ 10.1007/s11250-010-9728-6

Bedard, K. M., & Semler, B. L. (2004). Regulation of Picornavirus gene expression. *Microbes and Infection*, *6*, 702–713. https://doi.org/10.1016/j.micinf.2004.03.001

Bengis, R., (2005). In S. A, Osofsky; S. Cleaveland, W. Karesh, M. Kock, P. Nyhus., & A. Yang (Eds.), *Transfrontier conservation area initiatives in Sub-Saharan Africa: some animal health challenges. Conservation and Development Interventions at the Wildlife/Livestock Interface: Implications for Wildlife, Livestock and Human Health*. (pp. 15–19). Gland, Switzerland/Cambridge, UK.

Bettencourt, E. M. V., Tilman, M., Narciso, V., Carvalho, M. L. S., & Henriques, P. D. S. (2015). The livestock roles in the wellbeing of rural communities of Timor-Leste. *Revista de Economia e Sociologia Rural*, *53*, 63–80. https://doi.org/10.1590/1234-56781806-94790053s01005

Beyi, A. (2012). *Costs and benefits of foot and mouth disease vaccination practice in commercial dairy farms in central Ethiopia*. MSc Thesis. Wageningen University, Wageningen, Netherlands.

Boklund, A., Halasa, T., Christiansen, L. E., & Enoe, C. (2013). Comparing control strategies against foot-and-mouth disease: will vaccination be cost-effective in Denmark? *Preventive and Veterinary. Medicine*, *111*, 206–219. https://doi.org/10.1016/j. prevetmed.2013.05.008

Callens, M., De Clercq, K., Gruia, M., & Danes, M. (1998). Detection of foot-and-mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot-and-mouth disease. *Veterinary Quarterly*, *20,* 37–40. https://doi.org/10.1080/01652176.1998.9694964

Carrillo, C., Tulman, E. R., Delhon, G., Lu, Z., Carreno, A., Vagnozzi, A., … Rock, D. L. (2005). Comparative genomics of foot-and-mouth disease virus. *Journal of Virology*, *79,* 6487–6504. https://doi.org/10.1128/JVI.79.10.6487-6504.2005

Casey-Bryars, M., Reeve, R., Bastola, U., Knowles, N. J., Auty, H., Bachanek-Bankowska, K., … Lembo, T. (2018). Waves of endemic foot-and-mouth disease in eastern Africa suggest feasibility of proactive vaccination approaches. *Nature Ecology and Evolution*, *2*, 1449 –1457. https://doi.org/: 10.1038/s41559-018-0636-x

Chen, H. T., Peng, Y. H., Zhang, Y. G., & Liu, X. T. (2012). Detection of foot-and-mouth disease serotype O by ELISA using a monoclonal antibody. *Hybridoma (Larchmt), 31,* 462- 464. https://doi.org/10.1089/mab.2012.0081

Chitray, M., de Beer T. A. P., Vosloo, W., & Maree, F. F. (2014). Genetic heterogeneity in the leader and P1-coding regions of foot-and-mouth disease virus serotypes A and O in Africa. *Archives of Virology*, *159,* 947–961. https://doi.org/10.1007/s00705-013-1838-9

CLC Genomics Workbench 9.5.3. Retrieved on 09 January, 2018, from www.qiagenbioinformatics.com.

Coetzer, J. A. W., Thomson, G. R., & Tustin, R. C. (1994). *Infectious Diseases of Livestock with Special Reference to Southern Africa*. Cape Town: Oxford University Press.

Common Market for East and Southern Africa (COMESA). (2009). Annual Report, 2009. Retrieved 15 February, 2018, from https://www.comesa.int/wp-content/uploads/2019/02/2009-Comesa-Annual-Repport.pdf.

Conroy, K., Goodman, A. R., & Kenward, S. (2010). Lessons from the Chars Livelihoods Programme, Bangladesh (2004-2010). Paper presented at the CPRC International Conference, University of Manchester, United Kingdom, 1-22. Retrieved on 13 December, 2016, from http://www.chronicpoverty.org/uploads/publication_files/conroy_goodman_kenward_chars.pdf

Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research, 25, 16,* 10881–10889. https://doi.org/10.1093/nar/16.22.10881

Devendra, C., Swanepoel, F. J. C., Stroebel, A., & van Rooyen, C. J. (2010). Implications and innovative strategies for enhancing the future contribution of livestock. In: F. Swanepoel., A. Stroebel., & S. Moyo (Eds.), *The role of livestock in developing communities: Enhancing multifunctionality. (pp.* 171–190). Wageningen, The Netherlands: The Technical Centre for Agriculture and Rural Cooperation.

Dhikusooka, M. T., Tjørnehøj, K., Ayebazibwe, C., Namatovu, A., Ruhweza, S., Siegismund, H. R., & Belsham, G. J. (2015). Foot-and-mouth disease virus serotype SAT 3 in long-horned Ankole calf, Uganda. *Emerging Infectious Diseases, 21,* 111–114. http://doi.org/ 10.3201/eid2101.140995

Di Nardo, A., Knowles, N. J., & Paton, D. J. (2011). Combining livestock trade patterns with phylogenetics to help understand the spread of foot-and-mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. *Revue Scientifique et Technique de l'Office International des Epizooties, 30 (1),* 63–85. https://doi.org/10.20506/rst.30.1.2022

Domenech, J., Lubroth, J., Eddi, C., Martin, V., & Roger, F. (2016). Regional and international approaches on prevention and control of animal trans-boundary and emerging diseases. *Annals of the New York Academy of Sciences, 1081,* 90 –107. https://doi.org/ 10.1196/annals.1373.010

Donaldson, A. I. (1972). Influence of relative humidity on the aerosol stability of different strains of foot-and-mouth disease virus suspended in saliva. *Journal of General Virology, 15,* 25–33. https://doi.org/10.1099/0022-1317-15-1-25

Dukpa, K., Robertson, I. D., & Ellis, T. M. (2011). The seroprevalence of foot-and-mouth disease in the sedentary livestock herds in four districts of Bhutan. *Preventive Veterinary Medicine, 100,* 231–236. https://doi.org/10.1016/j.prevetmed.2011.04.013

East African Community (EAC). (2006). East African Community Development Strategy 2006-2010. East African Secretariate.

Ferris, N. P., Grazioli, S., Hutchings, G. H., & Brocchi, E. (2011). Validation of a recombinant integrin αvβ6 monoclonal antibody based antigen ELISA for the diagnosis of foot-and-mouth disease. *Journal of Virology Methods*, *175*, 253–260. https://doi.org/10.1016/j.jviromet.2011.05.026

Fèvre, E. M., Bronsvoort, B. M., Hamilton, K. A., & Cleaveland, S. (2006). Animal movements and the spread of infectious diseases. *Trends in Microbiology*, *14(3*), 125. https://doi.org/10.1016/j.tim.2006.01.004

Follett, E. A., Pringle, C. R., & Pennington, T. H. (1975).Virus development in enucleate cells: echovirus, poliovirus, pseudorabies virus, reovirus, respiratory syncytial virus and Semliki Forest virus. *Journal of General Virology*, *26*, 183–196. https://doi.org/10.1099/0022-1317-26-2-183

Food and Agriculture Organisation. (2004). Livestock sector report horn of Africa review of the livestock sector. Retrieved 12 Decemeber, 2017 from, http://www.fao.org/ag /againfo/resources/en/publications/sector_reports/lsr_IGAD.pdf

Food and Agriculture Organization. (2009). The State of food and agricutue: livetsock in the balance. Retrieved 10 February, 2016 from, http://www.fao.org/3/a-i0680e.pdf.

Food and Agriculture Organization. (2011). Progressive control pathway for FMD control. Retrieved on 04 December, 2017, from http://www.fao.org/ag/againfo/commissions/ docs/pcp/pcp-26012011.pdf.

Food and Agriculture Organization. (2012). Development of a Long Term Roadmap for the Progressive Control of FMD in Eastern Africa 2012-2022. Workshop report. Nairobi, Kenya .31pp. Retrieved on 12 December, 2017, from http://www.fao.org/fileadmin/ user_upload/eufmd/docs/PCP_nairobi/Report.pdf.

Food and Agriculture Organization. (n.d). Background information on natural resources in the Kagera river basin. Retrieved 25 November, 2017, from http://www.fao.org/ fileadmin/templates/nr/kagera/Documents/Suggested_readings/nr_info_kagera.pdf.

Forman, S., Le Gall, F., Belton, D., Evans, B., François, J. L., Murray, G., … Yoshimura, S. (2009). Moving towards the global control of foot and mouth disease: an opportunity for donors. *Revue Scientifique et Technique de l'Office International des Epizooties, 28,* 883–896. https://doi.org/10.20506/rst.28.3.1935

Gainaru M. D., Thomson G. R., Bengis R. G., Esterhuysen J. J., Bruce W., & Pini, A. (1986). Foot and mouth disease and the African buffalo (Syncerus caffer). II: Virus transmission and excretion during acute infection. *Onderstepoort Journal of Veterinary Research, 53,* 75–85.

Gall, F. L., & Leboucq, N. (2004). The role of animal disease control in poverty reduction, food safety, market access and food security in Africa. *L'Office International des Epizooties (OIE).*

Genchwere, J. M., & Kasanga, C. J. (2014). Spatial and temporal distribution of foot-and-mouth disease virus in the Lake zone of Tanzania. *Onderstepoort Journal of Veterinary Research*, *81*, 2–5. Retrieved on March 16, 2017, from http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0030-24652014000200015&lng=en&tlng=es.

Gunarathne, A., Kubota, S., Kumarawadu, P., Karunagoda, K., & Kon, H. (2015). Is hiding foot and mouth disease sensitive behavior for farmers? A survey study in Sri Lanka. *Asian Australasian Journal of Animal Science, 29,* 280–287. https://doi.org/10.5713/ajas.15.0241

Haydon, D. T., Samuel, A. R., & Knowles, N. J. (2001). The generation and persistence of genetic variation in foot-and-mouth disease virus. *Preventive Veterinary Medicine, 51,* 111–124. https://doi.org/10.1016/S0167-5877(01)00210-0

Herrero, M., Thornton, P. K., Gerber, P., & Reid, R. S. (2009). Livestock, livelihoods and the environment: understanding the trade-offs. *Current Opinion in Environmental Sustainability, 1(2),* 111–120. https://doi.org/10.1016/j.cosust.2009.10.003

Herrero, M., Havlík P., Valin, H., Notenbaert, A. M., Rufino, M., Thornton, P. K., …Obersteiner, M. (2013). Global livestock systems: biomass use, production, feed efficiencies and greenhouse gas emissions. *PNAS, 110 (52),* 20888–20893. https://doi.org/ 10.1073/pnas.1308149110

Holliman, A. (2005). Differential dignosis of diseases causing oral lesions in cattle. *In Practise, 27,* 2–13. https://.doi.org/10.1136/inpract.27.1.2

Holland, J. J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S., & Vanderpol, S. (1982). Rapid evolution of RNA genomes. *Science, 215,* 1577–1585. https://doi.org/10.1126/science.7041255

Hughes, G. J., Mioulet, V., Kitching, R. P., Alexandersen, S., Donaldson, A. I., & Woolhouse, M. E. J. (2002). Foot-and-mouth disease virus infection of sheep: implications for diagnosis and control. *Veterinary Records, 150,* 724–727. https://doi.org/10. 1136/vr. 150.23.724

IGAD Center for Pastoral Areas and Livestock Development. (2013). *The contribution of Livestock to the Ugandan economy*. Policy Brief Series, Policy Brief No: ICPALD 7/CLE/8/2013. Retrieved on 15 October, 2017, from https://igad.int/attachments/ 714_The%20Contribution%20of%20Livestock%20to%20the%20Ugandan%20Economy. pdf.

Isingiro District Local Government (IDLG). (2011). Five Year District Development Plan 2011/2012–2015/2016, District Council- Minute, 2016, FC/5/2010/2011/36. Retrieved on 18 May, 2017, from npa.ug/wp-content/themes/npatheme/documents/ West/Isingiro%20DDP.pdf.

Jackson, T., Sheppard, D., Denyer, M., Blakemore, W., & King, A. M. (2000). The epithelial integrin αvβ6 is a receptor for foot and-mouth disease virus. *Journal of Virology, 74,* 4949–4956. https://doi.Org/10.1128/ jvi.74.11.4949-4956.2000

Jackson, T., King, A. M., Stuart, D., & Fry, E. (2003). Structure and receptor binding. *Virus Research, 91,* 33–46. https://doi.org/10.1016/s0168-1702(02)00258-7

Jamal, S. M., & Belsham, G. J. (2013). Foot-and-mouth disease: past, present and future. *Veterinary Research, 5*, 116. https://doi.org/10.1186/1297-9716-44-116.

James, A. D., & Ellis, P. R. (1978). Benefit-cost analysis in foot-and-mouth disease control programmes. *British Veterinary Journal, 134,* 47–52. https://doi.org/10.1016/S0007-1935(17)33589-3

James, A. D., & Rushton, J. (2002). The economics of foot-and-mouth disease. *Revue Scientifique et Technique de l'Office International des Epizooties, 21,* 637–644. https://doi.org/10.20506/rst.21.3.1356

Jemberu, W. T., Mourits, M. C., Woldehanna, T., & Hogeveen, H. (2014). Economic impact of foot and mouth disease outbreaks on smallholder farmers in Ethiopia. *Preventive Veterinary Medicine, 116,* 26–36. https://doi.org/ 10.1016/j.prevetmed.2014.06.004

Kabuga, N. (2014). Adoption and impact of improved cow breeds on household welfare and child nutrition outcomes :Empirical evidence from Uganda. *Annual Conference of the Agricultural Economics Society*. Retrieved on 15 April, 2017, from https://ageconsearch.umn.edu/bitstream/170517/2/Nassul_Kabunga_Kabunga_AES_Impact of Dairy Cows.pd.

Kasambula, L., Belsham, G. J., Siegismund, H. R., Muwanika, V. B., Ademun-Okurut, A. R., & Masembe, C. (2012). Serotype identification and VP1 coding sequence analysis of foot-and-mouth disease viruses from outbreaks in eastern and northern Uganda in 2008/9. *Transboundary and Emerging Diseases, 59,* 323–330. https://doi.org/10.1111/j.1865-1682.2011.01276.x

Kasanga, C. J., Sallu, R., Mpelumbe-Ngeleja, C. A. R., Wadsworth, J., Ferris, N. P., & Hutchings, G. H. (2011). Infectious diseases of economic importance: Molecular biological characteristics of foot-and-mouth disease viruses collected in Tanzania from 1967 to 2009. *Onderstepoort Journal of Veterinary Research, 79,* Art. #474, 1.

Kerfua, S. D., Shirima, G., Kusiluka, L., Ayebazibwe, C., Mwebe, R., Cleaveland, S., & Haydon, D. (2018). Spatial and temporal distribution of foot-and-mouth disease in four districts situated along the Uganda -Tanzania border: Implications for cross-border efforts in disease control. *The Onderstepoort Journal of Veterinary Research, 85(1),* e1–e8. https://doi.org/ 10.4102/ojvr.v85i1.1528

Kitching, R. P. (2002). Clinical variation in foot- and-mouth disease: cattle. *Revue Scientifique et Technique de l'Office International des Epizooties, 21,* 499–504. https://doi.org/10.20506/rst.21.3.1343

Kitching, R. P., & Hughes, G. J. (2002). Clinical variation in foot-and-mouth disease: sheep and goats. *Revue Scientifique et Technique de l'Office International des Epizooties, 21,*505–512. https://doi.org/10.20506/rst.21.3.1342

Kitching, R. P., & Alexandersen, S. (2002). Clinical variation in foot-and-mouth disease: pigs. *Revue Scientifique et Technique de l'Office International des Epizooties, 21,*513–518. https://doi.org/10.20506/rst.21.3.1367

Kitching, P., Hammond, J., Jeggo, M., Charleston, B., Paton, D., Rodriguez, L., & Heckert, R. (2007). Global FMD control–Is it an option? *Vaccine, 25,* 5660 –5664. https://doi.org/10.1016/j.vaccine.2006.10.052

Kivaria, F. M. (2003). Foot-and-mouth disease in Tanzania: An overview of its national status. *Veterinary Quarterly, 25(2),*72–78. https://doi.org/ 10.1080/01652176.2003. 9695147

Knight-Jones, T. J. D., & Rushton, J. (2013). The economic impacts of foot and mouth disease: What are they, how big are they and where do they occur ? *Preventive Veterinary Medicine, 112,* 161–173. https://doi.org/10.1016/j.prevetmed.2013.07.013

Knight-Jones, T. J. D., McLaws, M., & Rushton, J. (2017). Foot-and-mouth disease impact on smallholders: what do we know, what don't we know and how can we find out more? *Transboundary and Emerging Diseases, 64,* 1079–1094. https:doi.org/ 10.1111/tbed.12507

Knowles, N. J., & Samuel, A. R. (2003). Molecular epidemiology of foot-and-mouth disease virus. *Virus Research, 91,* 65–80. https://doi.org/10.1016/s0168-1702(02)00260-5

Knowles, N. J., Nazem Shirazi, M. H., Wadsworth, J., Swabey, K. G., Stirling, J. M., Statham, R. J., … Paton, D. J. (2009). Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. *Transboundary and Emerging Diseases, 56,* 157-169. https://doi.org/10.1111/j.1865-1682.2009.01074.x

Knowles, N. J., He, J., Shang, Y., Wadsworth, J., Valdazo-González, B., Onosato, H., … King, D. P. (2012). Southeast Asian foot-and-mouth disease viruses in Eastern Asia. *Emerging Infectious Diseases, 18*, 499–501. https://doi.org/10.3201/eid1803.110908

Knowles, N. J., Wadsworth, J., & King, D. P. (2016). VP1 sequencing protocol for foot and mouth disease virus molecular epidemiology. *Revue Scientifique et Technique de l'Office International des Epizooties, 35*, 1–28. https://doi.org/10.20506/rst.35.3.2565

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Lesser, C., & Moise-Leeman, F. (2009). Informal cross-border trade and trade facilitation reforms in sub-Saharan Africa. OECD Trade *Policy Working Paper* No. 86. Paris: OECD

Manju, G., Ramamurthy, V., Bramhadev, P., Aniket, S., Gurumurthy, C. B., Divakar, H., & Chakradhar T. (2001). Sequence Analysis of the RNA Polymerase Gene of Foot-and-Mouth Disease Virus Serotype Asia 1. *Virus Genes, 22*, 21–26. https://doi.org/10.1023/A:1008174100886

Mdetele, D., Kasanga, C., Seth, M., & Kayunze, K. (2015). Socio-economic impact of foot and mouth disease in wildlife-livestock interface and non-interface of Tanzania. *World's Veterinary Journal, 5(3),* 31-35.

Medina, M., Domingo, E., Brangwyn, J. K., & Belsham, G. J. (1993).The two species of the foot-and-mouth disease virus leader protein, expressed individually, exhibit the same activities. *Virology, 194*, 355–359. https://doi.org/10.1006/viro.1993.1267

Miguel, E., Grosbois, V., Caron, A., Boulinier, T., Fritz, H., Cornelis, D., Foggin, C., … Garine-Wichatitsky, M. (2013). Contacts and foot and mouth disease transmission from wild to domestic bovines in Africa. *Ecosphere*. *4*, 51. https://doi.org/10.1890/ES12-00239.1

Ministry of Agriculture Animal Industry and Fisheries. (2012). *Annual Performance Report for FY 2011/2012*. 66 pp. Retrieved 19 January, 2017, from http://www.resakss.org/sites/default/files/Uganda%202012_%20JSR%20Report.pdf.

Ministry of Livestock and Fisheries Development. (2006). *National Livestock Policy*, pp 1–55. Retrieved on 31 January, 2016, from https://www.tnrf.org/files/EINFO_National_Livetock_Policy_Final_as_per_Cabinet_Dec-2006.pdf.

Ministry of Livestock Fisheries Development. (2015). Tanzanian Livestock Modernisation Initiative. Retrieved 5 July, 2018 from, https://livestocklivelihoodsandhealth.org/wp-content/uploads/2015/07/Tanzania_Livestock_Modernization_Initiative_July_2015.pdf.

Moffat, K., Howell, G., Knox, C., Belsham, G. J., Monaghan, P., Ryan, M. D., & Wileman. T. (2005). Effects of foot-and-mouth disease virus nonstructural proteins on the structure and function of the early secretory pathway: 2BC but not 3A blocks endoplasmic reticulum-to-Golgi transport. *Journal of Virology, 79,* 4382–4395. https://doi.org/ 10.1128/JVI.79.7.4382-4395.2005

Monaghan, P., Gold, S., Simpsom, J., Zhang, Z., Weinreb, P. H., Violette, S. M., Alexandersen, S., & Jackson, T. (2005). The αvβ6 integrin receptor for foot-and-mouth disease virus is expressed constitutively on the epithelial cells targeted in cattle. *Journal of General Virology, 86,* 2769–2780. https://doi.org/10.1099/vir.0.81172-0.

Morioka, K., Fukai, K., Yoshida, K., Yamazoe, R., Onozato, H., Ohashi, S., ... Sakamoto, K. (2009). Foot-and-mouth disease virus antigen detection enzyme-linked immunosorbent assay using multiserotype-reactive monoclonal antibodies. *Journal of Clinical Microbiology*, **47**, 3663-3668.

Msibi, S. S. (2015). *Potential for domestic biogas as household energy supply in South Africa*. Published Dissertation for Award of Applied Science MSc Degree at University of Pretoria. South Africa. pp 59-70.

Muleme, M., Barigye, R., Khaitsa, M., Berry, E., Wamono, A., & Ayebazibwe, C. (2012). Effectiveness of vaccines and vaccination programs for the control of foot-and-mouth disease in Uganda, 2001–2010. *Tropical Animal Health and Production, 45(1),* 35–43. https://doi.org /10.1007/s11250-012-0254-6

Munsey, A., Mwiine, F. N., Ochwo, S., Velazquez-Salinas, L., Ahmed, Z., Maree, F., … VanderWaal, K. (2019). Spatial distribution and risk factors for foot and mouth disease virus in Uganda: Opportunities for strategic surveillance. *Preventive Veterinary Medicine, 171,* 104766. https://doi.org/10.1016/j.prevetmed.2019.104766

Murphy, S. P., & Allen, L. H. (2009). Nutritional importance of animal source foods. *Journal of Nutrition*, *133,* 3932S–3935S. https://doi.org/10.1093/jn/133.11.3932S.

Mwiine, F. N., Velazquez-Salinas, L ., Ahmed, Z., Ochwo, S., Munsey, A., Kenney, M., Lutwama, J. J., … Rieder, E. (2019). Serological and phylogenetic characterization of foot and mouth disease viruses from Uganda during cross-sectional surveillance study in cattle between 2014 and 2017. *Transboundary and Emerging Diseases*, *66,* 2011–2024. https://doi.org/10.1111/tbed.13249

Namatovu, A., Tjørnehøj, K., Belsham, G. J., Dhikusooka, M. T., Wekesa, S. N., Muwanika, V. B., … Ayebazibwe, C. (2015). Characterization of foot-and-mouth disease viruses (FMDVs) from Ugandan cattle outbreaks during 2012-2013: Evidence for circulation of multiple serotypes. *Plos One*, *10*, e0114811. https://doi.org/10.1371/journal.pone.0114811

Namatovu, A., Wekesa, S. N., Tjørnehøj, K., Dhikusooka, M. T., Muwanika, V. B., Seigsmund, H. R., & Ayebazibwe, C. (2013). Laboratory capacity for foot-and-mouth disease in Eastern Africa: Implications for progressive control pathway. *BioMedCentre, Veterinary Research, 9,* 13. https://doi.org/10.1186/1746-6148-9-19

Novak, J. E., & Kirkegaard, K. (1991). Improved method for detecting poliovirus negative strands used to demonstrate specificity of positive strand encapsidation and the ratio of positive to negative strands in infected cells. *Journal of Virology, 65*, 3384–3387.

Office International des Epizooties. (2012). Foot and mouth disease, In OIE Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France. pp. 190–216.

Otte, M. J., Nugent, R., & McLeod, A. (2004). *Transboundary animal diseases: Assessment of socio-economic impacts. Livestock Policy*. *Discussion, 9*, 1–46. Rome, Italy: Food and Agriculture Organisation.

Pearson, R. A., & Vall, E. (1998). Performance and management of draught animals in sub-Saharan Africa: a review. *Tropical Animal Health Production*. *30,* 309–324. https://doi.org/10.1023/A:1005059308088

Pendell, D., Leatherman, J., Schroeder, T., & Alward. G. (2007). Regional economic impacts of a FMD outbreak. *Journal of Agricultural and Applied Economics*. *39,* 19–33. https://doi.org/10.1017/S1074070800028911

Perry, P. B., Kalpravidh, W., Coleman, P. G., Horst, H. S, McDermott, J. J., Randolph, T. F., & Gleeson, L. J. (1999). The economic impact of foot-and-mouth disease and its control in South-East Asia: a preliminary assessment with special reference to Thailand. *Revue Scientifique et Technique de l'Office International des Epizooties*, *18,* 478–497.

Perry, B., & Grace, D. (2009). The impacts of livestock diseases and their control on growth and development processes that are pro-poor. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, *364*, 2643–2655. https://doi.org/: 10.1098/rstb.2009.0097.

Pica-Ciamarra, U., Baker, D., Morgan, N., & Zezza, A. (2011). *Measuring the contribution of livestock to household livelihoods: a livestock module for multi-topic household surveys (English)*. Washington DC, United States of America: World Bank Group. Retrieved on 14 March, 2008, from http://documents.worldbank.org/curated/en/ 305651468180570400/Measuring-the-contribution-of-livestock-to-household-livelihoods-a-livestock-module-for-multi-topic-household-surveys.

Picado, A., Speybroeck, N., Kivaria, F., Mosha, R. M., Sumaye, R. D., Casal, J., & Berkvens, D. (2010). Foot-and-mouth disease in Tanzania from 2001 to 2006, *Transboundary and Emerging Diseases, 58,* 44–52. https://doi.org/10.1111/j.1865-1682.2010.01180.x

QGIS Geographic Informations System, version 2.16. (2016). Computer software, QGIS Development Team, Open Source Geospatial Foundation. Retrieved on 23 January,2017, from http://qgis.osgeo.org.

Railey, A. F., Lembo, T., Palmer, G. H., Shirima, G. M., & Marsh, T. L. (2018). Spatial and temporal risk as drivers for adoption of foot-and-mouth disease vaccination. *Vaccine*, *36*, 5077–5083. https://doi.org/10.1016/j.vaccine.2018.06.069

Rakai District Local Government (RDGL). (2013). Production and marketing department. Retrieved on 25, November, 2017, from http://www.umb.no/statisk/ebok/ document/ Rakai_development_plan.pdf.

R Core Team. (2013). R: A language and environment for statistical computing, version 3.3.2 R foundation for Statistical Comupting, Vienna, Austria, Computer software. Retrieved on 03 January, 2016 from http://www.R-project.org/.

Reid, S. M., Ferris, N. P., Brüning, A., Hutchings, G. H., Kowalska, Z., & Åkerbolm, L. **(**2001). Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen. *Journal of Virology Methods, 96,* 189–202. https://doi.org/10.1016/S0166-0934(01)00334-2

Rich, K., & Wanyioke, F. (2010). An assessment of the regional and national socio-economic impacts of the 2007 Rift Valley fever outbreak in Kenya. *American Journal Tropical Medical Hygiene, 83*, 52–57. https://doi.org/ 10.4269/ajtmh.2010.09-0291

Robertson, B. H., Grubman, M. J., Weddell, G. N., Moore, D. M., Welsh, J. D., Fisher, T.,… Kleid, G. (1985). Nucleotide and amino acid sequence coding for polypeptides of foot-and-mouth disease virus type A12. *Journal of General Virology, 54,* 651660.

Rodriguez, A., Martínez-Salas, E., Dopazo, J., Dávila, M., Sáiz, J. C., & Sobrino, F. (1992). Primer design for specific diagnosis by PCR of highly variable RNA viruses: typing of foot-and-mouth disease virus. *Virology, 189,* 363–367. https://doi.org/10.1016/ 0042-6822(92)90717-4

Rushton, J. (2009) *The Economics of animal health and production*. Wallingford, UK: CABI Publishing.

Rushton, J., & Knight-Jones, T. (2012). *The impact of foot and mouth disease,* (Supporting document N ° 1). OIE/FAO.

Rutagwenda, T. (2003). *The implications and consequences of foot and mouth disease outbreak on food security and household incomes in selected districts in Uganda. A report to Food and Agriculture Organization, TCP/UGA/O168 (E) 1 May 2003*. Rome, Italy: FAO

Rweyemamu, M. M., & Astudillo, V. M. (2002). Global perspective for foot and mouth disease control. In Foot and mouth disease: facing the new dilemmas. *Revue Scientifique et Technique de l'Office International des Epizooties, 21* (3), 765–773. https://doi.org/ 10.20506/rst.21.3.1378

Rweyemamu, M., Roeder, P., Mackay, D., Sumpton, K., Brownlie, J., Leforban, Y., Valarcher, J. F., … Saraiva, V. (2008). Epidemiological patterns of foot-and-mouth disease worldwide. *Transboundary and Emerging Diseases*, 55, 57–72. https://doi.org/10.1111/j.1865-1682.2007.01013.x

Sangula, A. K., Belsham, G. J., Muwanika, V. B., Heller, R., Balinda, S. N., & Siegismund, H.R. (2010). Co-circulation of two extremely divergent serotype SAT 2 lineages in Kenya highlights challenges to foot-and-mouth disease control. *Archives of Virology, 155*, 1625–1630. https://doi.org/: 10.1007/s00705-010-0742-9

Serrano, P., Rodriguez-pulido, M., & Sáiz, M., Martínez-Salas, E. (2006). The 3 ' end of the foot-and-mouth disease virus genome establishes two distinct long-range RNA-RNA interactions with the 5' end region. *Journal of General Virology, 87*, 3013–22. https://doi.org/ 10.1099/vir.0.82059-0

Shankar, B., Morzaria, S., Fiorucci, A., & Hak, M. (2012). Animal disease and livestock-keeper livelihoods in Southern Cambodia. *International Development Planning Review, 34*, 39–63. https://doi.org/10.3828/idpr.2012.3

Sobrino, F., Davila, M., Ortin, J., & Domingo, E. (1983). Multiple genetic variants arise in the course of replication of foot-and-mouth disease virus in cell culture. *Virology, 128,* 310–318. https://doi.org/10.1016/0042-6822(83)90258-1

Sutmoller, P., & Gaggers, C, A. (1965). Foot and Mouth disease carriers. *Journal of Veterinary Records, 77*, 968–969.

Sutmoller, P., Barteling, S. S., Olascoaga, R. C., & Sumption, K. J. (2003).Control and eradication of foot-and-mouth disease. *Virus Research, 91*,101–44. https://doi.org/ 10.1016/s0168-1702(02)00262-9

Taherdoost, H. (2018). Determining sample size: How to calculate survey sample size. *International Journal of Economics and Management Systems, 2*, 2017.

The National Food Security Division - Ministry of Agriculture Livestock and Fisheries. (2017). *Comprehensive Food Security and Nutrition Assessment Report*.

Thompson, D., Muriel, P., Russell, D., Osborne, P., Bromley, A., Rowland, M., … Brown, C. (2002). Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Revue Scientifique et Technique de l'Office International des Epizooties, 21,* 675–687. https://doi.org/ 10.20506/rst.21.3.1353

Thomson, G. R., & Bastos, A. D. S. (2004). Foot-and-mouth disease. In: Coetzer, J. A. W. & Tustin, C (Eds). *Infectious Diseases of Livestock,* (pp. 1324–1365). Oxford, England: Oxford University Press.

Uganda Bureau of Statistics. (2014).*Statistical Abstract.* Retrieved on 30 June, 2016, from https://www.ubos.org/onlinefiles/uploads/ubos/statistical_abstracts/Statistical_Abstract_2014.pdf.

Upton, M. (2004). *The role of livestock in economic development and poverty reduction*. Rome, Italy: Food and Agriculture Organisation. Retrieved on 15 December, 2017, from http://www.fao.org/3/i2744e/i2744e00.pdf

Van Bekkum, J., Frenkel, H., Frederiks, H., & Frenkel, S. (1959). Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Tijdschrift voor Diergeneeskunde, 84,* 1159–1164.

VanderWaal, K., Gilbertson, M., Okanga, S., Allan, B. F., & Craft, M. E. (2017). Seasonality and pathogen transmission in pastoral cattle contact networks. *Royal Society Open Science, 4,* 170808. https://doi.org/10.1098/rsos.170808

Vangrysperre, W., & De Clercq, K. (1996). Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. *Archives of Virology, 141*, 331–344. https://doi.org/10.1007/BF01718403

Vosloo, W., Bastos, A. D. S., Sangare, O., Hargreaves, S. K., & Thomson, G. R. (2002). Review of the status and control of foot-and-mouth disease in sub-Saharan Africa. *Revue Scientifique et Technique de l'Office International des Epizooties, 21*, 437–449. https://doi.org/10.20506/rst.21.3.1349

Vosloo, W., Bastos, A. D. S., Sahle, M., Sangare, O., & Dwarka, R. M. (2005). In Karesh, W. B., Kock, M. D., Nyhus, P. J., & Yang, A. (Eds.), Virus topotypes and the role of wildlife in foot and mouth disease in Africa, *Conservation and development interventions at the wildlifelivestock interface: Implications for wildlife, livestock and human health,* (pp. 67–73).Gland, Switzerland: International Union for Conservation of Nature.

Wekesa, S. N., Sangula, A. K., Belsham, G. J., Muwanika, V. B., Heller, R., Balinda, S. N., … Siegismund, H. R. (2015). Genetic diversity of serotype A foot-and-mouth disease viruses in Kenya from 1964 to 2013; implications for control strategies in eastern Africa. *Infectious Genetic Evolution, 21*, 408–417. https://doi.org/10.1016/j.meegid.2013.12.006

# APPENDICES

## Appendix 1: List of FMDV serotype O and A VP1 sequences used in the study

Table 16: List of FMDVs used in the VP1 analysis

| SN | Country | Year | Serotype | Accession Number | Sequence name | Reference |
|---|---|---|---|---|---|---|
| 1 | Tanzania | 2016 | O | Not yet available | TAN /02/O/2016 | This study |
| 2 | Tanzania | 2016 | O | Not yet available | TAN/07/O/2016 | This study |
| 3 | Tanzania | 2017 | O | Not yet available | TAN/04/O/2017 | This study |
| 4 | Tanzania | 2017 | O | Not yet available | TAN/08/O/2017 | This study |
| 5 | Uganda | 2017 | O | Not yet available | UG/13/O/2017 | This study |
| 6 | Uganda | 2017 | O | Not yet available | UG/03/O/2017 | This study |
| 7 | Uganda | 2017 | O | Not yet available | UG/09/O/2017 | This study |
| 8 | Uganda | 2017 | O | Not yet available | UG/11/O/2017 | This study |
| 9 | Uganda | 2005 | O | HM756628 | U25/06 | Balinda *et al*., 2010a |
| 10 | Tanzania | 2008 | O | KF561684 | TAN/16/2008 | Kasanga *et al.*, 2014 |
| 11 | Uganda | 2004 | O | HM756621 | U20B/04 | Balinda *et al*., 2010a |
| 12 | Tanzania | 2002 | O | MF592671 | O/TAN/10/2014 | Casey-Bryars *et al*.,2018 |
| 13 | Zambia | 2006 | O | KU821591 | O/ZAM/14/2010 | Van Borm *et al*., 2016 |
| 14 | Uganda | 2009 | O | JN974308 | OUGA2009LIRA | Kasambula *et al*., 2012 |
| 15 | Uganda | 2011 | O | KF478938 | U04/11 | Namatovu *et al*., 2015 |
| 16 | Tanzania | 2009 | O | KF561685 | TAN/5/2009 | Kasanga *et al*., 2015 |
| 17 | Tanzania | 2013 | O | MF592650 | O/TAN/10/2013 | Casey-Bryars *et al*., 2018 |
| 18 | Tanzania | 2012 | O | MF592623 | O/TAN/38/2012 | Casey-Bryars *et al*., 2018 |
| 19 | Malawi | 1998 | O | DQ165074 | O/MAL/1/98 | Unpublished |
| 20 | Ethiopia | 2007 | O | FJ798138 | ETH/26/2007 | Ayelet *et al*., 2009 |
| 21 | Tanzania | 1998 | O | KF561677 | TAN/9/98 | Kasanga *et al.*, 2015 |
| 22 | Tanzania | 2004 | O | KF561682 | TAN/14/2004 | Kasanga *et al.*, 2015 |
| 23 | Uganda | 1996 | O | EU919247 | O/UGA/5/96 | Chitray *et al*., 2014 |
| 24 | Kenya | 1978 | O | HM756588 | O/KEN/77/78 | Balinda *et al*., 2010 |
| 25 | Kenya | 1995 | O | HM756601 | K56/95 | Balinda *et al*., 2010 |
| 26 | Kenya | 2009 | O | KR149720 | KEN/62/2009 | Wekesa *et al*., 2015 |
| 27 | Kenya | 2011 | O | KF135292 | K91/11 | Wekesa *et al*., 2015 |
| 28 | Hong Kong | 2002 | O | AY317098 | HKN/2002 | Feng *et al*., 2004 |
| 29 | Tanzania | 2017 | A | Not yet available | TAN/10/A/2017 | This study |
| 30 | Tanzania | 2017 | A | Not yet available | TAN/12/A/2017 | This study |
| 31 | Uganda | 2017 | A | Not yet available | UGA/5/A/2017 | This study |
| 32 | Uganda | 2013 | A | KP089985 | U75/13 | Namatovu *et al*., 2015 |
| 33 | Kenya | 2009 | A | KF561703 | KEN/22/2009 | Kasanga *et al.*, 2015 |
| 34 | Tanzania | 2009 | A | KF561697 | TAN/47/2009 | Kasanga *et al.*, 2015 |
| 35 | Kenya | 2008 | A | KF561702 | KEN/28/2008 | Kasanga *et al.*, 2015 |
| 36 | Tanzania | 2009 | A | KF561693 | TAN/9/2009 | Kasanga *et al.*, 2015 |
| 37 | Tanzania | 2008 | A | KF561690 | TAN/11/2008 | Kasanga *et al.*, 2015 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 38 | Kenya | 2008 | A | KF561701 | KEN/8/2008 | Kasanga *et al*., 2015 |
| 39 | Kenya | 1966 | A | KF561699 | KEN/42/66 | Kasanga *et al*., 2015 |
| 40 | Tanzania | 1968 | A | KF561688 | TAN/3/68 | Kasanga *et al*., 2015 |
| 41 | Tanzania | 1980 | A | KF561689 | TAN/4/80 | Kasanga *et al*., 2015 |
| 42 | Kenya | 1980 | A | KJ440846 | K35/1980 | Wekesa *et al*., 2015 |
| 43 | Kenya | 1980 | A | KJ440848 | K5/1980 | Wekesa *et al*., 2015 |
| 44 | Uganda | 1966 | A | KF112925 | A/UGA/13/66 | Ludi *et al*., 2016 |
| 45 | Ghana | 1973 | A | KF561698 | GHA/16/73 | Kasanga *et al*., 2015 |
| 46 | Nigeria | 1973 | A | KF561704 | NGR/2/73 | Kasanga *et al*., 2015 |
| 47 | Turkey | 2005 | A | FJ755100 | A/TUR/12/2005 | Knowles *et al*., 2009 |
| 48 | Vietnam | 2010 | A | JQ070332 | VIT/1/2010 | Knowles *et al*., 2012 |

## Appendix 2: Questionnaire used in study

**NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY, ARUSHA TANZANIA**

**Impact of foot-and-mouth disease outbreaks on household income and food security in communities found along the Uganda- Tanzania border**

**Date:** ……

**Interviewer's Name**:…….....

**1.0 Geographic data:**

District:……

Sub-county/Ward:…….Village:……
……

**GPS readings:** ……

**Date:** dd/mm/yyyy

**Household ID** ……

**2.0 Respondent's and household details:**

| | | | |
|---|---|---|---|
| **Name of respondent** | **Sex**:<br>Male☐<br>Female ☐ | **Relationship of respondent to HHH** | 1. Spouse 2. Sister/brother<br>3. Parent 4. Child<br>5. In-law 6. Other (specify |
| | **Sex of HHH** :<br>Male ☐ Female ☐ | **Level of education of respondent** | 1. Never been to school<br>2. Enrolled/completed Primary education<br>3. Enrolled/completed Secondary education<br>4. Enrolled/completed Tertiary education<br>5. Enrolled/completed University<br>6. Enrolled/completed Post graduate education<br>7. Don't know |
| | | **Level of education of** | Codes as above |

| | | | | |
|---|---|---|---|---|
| | | | **HHH** | |
| No of adults in HH (> 18 years) | Female ☐ | Male☐ | **Residence** | 1. Urban 2. Rural 3. Periurban |
| No of children (5 years-18 years) | Female ☐ | Male☐ | **Tribe of HHH** | (((enter))) |
| No of children in HH (< 1 day-5 years) | Female ☐ | Male☐ | | |

**3.0 FMD outbreaks in the past twelve months**

| | | | | |
|---|---|---|---|---|
| 3.1 | Was there an FMD outbreak in this village in the past twelve months? | Yes | 1 | **If no skip to 3.3** |
| | | No | 2 | |
| | | Don't know | 3 | |
| 3.2 | When did the outbreak occur (month and year) | ***Link to calendar (pick middle of the month if not sure of exact date)*** | | |
| 3.3 | Was there an FMD outbreak on your farm in the past twelve months? | Yes | 1 | **If No to both 3.1 and 3.3, skip to 4.1** |
| | | No | 2 | |
| | | Don't know | 3 | |
| 3.4 | In which year and month(s) did the outbreak(s) occur)? | ***Link to calendar( pick middle of the month if not sure of exact date)*** | | |
| 3.5 | When the outbreaks occurred on your farm, which livestock species were affected? | Cattle | 1 | **Multiple** |
| | | Goats | 2 | |
| | | Sheep | 3 | |
| | | Pigs | 4 | |
| | | Others (specify) | 5 | |
| 3.6 | If yes to either 3.1. 3.3 or both, did you buy any livestock during the outbreak(s)? | Yes | 1 | **Single** |
| | | No | 2 | |
| | | Don't know | 3 | |
| 3.7 | If yes to either 3.1. 3.3 or both, did you buy any livestock products during the outbreak? | Yes | 1 | **Single** |
| | | No | 2 | |
| | | Don't know | 3 | |
| 3.8 | If yes, to either 3.1. 3.3 or both, did you sell any livestock during the outbreak? | Yes | 1 | **Single** |
| | | No | 2 | |
| | | Don't Know | 3 | |
| 3.9 | If yes to either 3.1. 3.3 or both, did you sell any livestock products during the outbreak | Yes | 1 | **Single** |
| | | No | 2 | |
| | | Don't know | 3 | |

**4.0 Quarantines**

| | | | | |
|---|---|---|---|---|
| 4.1 | Was an FMD quarantine imposed in this area in the last twelve months? | Yes | 1 | **If No skip to 5.1** |
| | | No | 2 | |
| | | I don't know | 3 | |
| 4.2 | For how long was the FMD quarantine imposed? | One month | 1 | |
| | | Two months | 2 | |
| | | Three months | 3 | |
| | | More than three months | 4 | |
| | | I don't know/remember | 5 | |
| 6.3 | Did imposed FMD quarantine affect your HH in any way? | Yes | 1 | |
| | | No | 2 | |
| | | I don't know | 3 | |

**4.4 How would you rank the effect of quarantine restrictions on your household? Rank each of**

| **Restrictions** | **Rank** |
|---|---|
| Human movement restrictions | |
| Local livestock movement restrictions | |
| International border movement restrictions for livestock | |
| Disinfection of compound | |
| Livestock grazing restrictions | |
| Sales of live animals | |
| Sales of milk | |
| Sales of meat | |
| Other (please fill in)_____? | |

**the restrictions from (1-3) 1= Not very important, 2= Important, 3= Very important**

**5.0 Losses during FMD outbreaks**

| | | | |
|---|---|---|---|
| 5.1 | Did your HH incur any losses during the last FMD outbreak(s) on your farm? | Yes | 1 |
| | | No | 2 |
| | | Don't know | 3 |
| 5.2 | Which losses did your HH incur during the last FMD outbreak on your farm? | Reduction in milk consumption<br>Yes ☐ No ☐ Don't know ☐ | If yes, by how many *'tumpecos'* (cups) per day? |
| | | Reduction in milk sales<br>Yes ☐ No☐ Don't know☐ | If yes, by how many 'tumpecos' (cups) per day? |
| | | Death of young animals (>1 year)<br>Yes ☐ No☐ Don't know☐ | If yes, how many young animals (<1 year) died during the outbreak? |
| | | Death of animals (adult females > 1 year)<br>Yes ☐ No☐ Don't know☐ | If yes, how many adult females (>1 year) died during the outbreak? |
| | | Death of animals (adult males > 1 year)<br>Yes ☐ No ☐ Don't know ☐ | If yes, how many adult males (>1 year) died during the outbreak? |
| | | Expenses on FMD vaccination per dose of cattle | If yes, how much did you spend per dose per cattle vaccinated? |

| | | | |
|---|---|---|---|
| | | Yes ☐ No☐ Don't know ☐ | |
| | | Expenses on vaccination per dose of goats<br>Yes ☐ No ☐ Don't know ☐ | If yes, how much did you spend per dose per goat vaccinated? |
| | | Expenses on vaccination per dose of sheep<br>Yes ☐ No ☐ Don't know ☐ | If yes, how much did you spend per dose per sheep vaccinated? |
| | | Expense per treatment of cattle<br>Yes ☐ No☐ Don't know☐ | If yes, how much did you spend per cattle head? |
| | | Expense per treatment of goats<br>Yes ☐ No☐ Don't know ☐ | If yes, how much did you spend per goat? |
| | | Reduced bull cost<br>Yes ☐ No☐ Don't know☐ | If yes, by how much was the cost of a bull reduced? |
| | | Reduced cow cost<br>Yes ☐ No ☐ Don't know ☐ | If yes, by how much was the cost of a cow reduced? |
| | | Reduced adult male goat cost<br>Yes ☐ No☐ Don't know ☐ | By how much was the cost of an adult male goat reduced? |
| | | Reduced adult female goat cost<br>Yes☐ No☐ Don't know ☐ | By how much was the cost of an adult female goat reduced? |
| | | Reduced adult male sheep cost<br>Yes ☐ No☐ Don't know☐ | By how much was the cost of an adult male sheep reduced? |
| | | Reduced adult female sheep cost<br>Yes ☐ No☐ Don't know ☐ | By how much was the cost of an adult female sheep reduced? |

### 6.3 Effect of FMD outbreaks on income and price commodity

#### 6.3.1 Before FMD outbreak and during an outbreak

| | | | |
|---|---|---|---|
| 6.3.1.1a | How much money (on average) do you get per month for all livestock sales? | 6.3.1.1b | What was your income for all livestock sales in the month (s) when there was an outbreak of FMD on your farm/in the village? |
| 6.3.1.2a | How much money (on average) do you get per month from sales of all livestock products? | 6.3.1.2b | What was your income per month from sales of all livestock products when there was an outbreak on your farm/village? |
| 6.3.1.3a | What is the current price of a bull in this village? | 6.3.1.3b | What was the price of a bull in the village the last time there was an outbreak? |
| 6.3.1.4a | What is the current price of a cow in this village? | 6.3.1.4b | What was the price of a cow in the village the last time there was an outbreak? |
| 6.3.1.5a | What is the current price of a male sheep in this village? | 6.3.1.5b | What was the price of a male sheep in the village the last time there was an outbreak? |
| 6.3.1.6a | What is the current price of a goat in this village? | 6.3.1.6b | What was the price of a male goat in the village the last time there was an outbreak? |
| 6.3.1.7a | What is the current price of a cock in this village? | 6.3.1.7b | What was the price of a cock in the village the last time there was an outbreak? |
| 6.3.1.8a | What is the current price of an egg in this village | 6.3.1.8b | What was the price of an egg in the village the last time there was an outbreak? |

| | | | |
|---|---|---|---|
| 6.3.1.9a | What is the price of a kg of beef in this village? | 6.3.1.9b | What was the price of a kg of beef in the village the last time there was an outbreak? |
| 6.3.2.0a | What is the price of a kg of goat meat in this village? | 6.3.2.0b | What was the price of a kg of goat meat in the village the last time there was an outbreak? |
| 6.3.2.1a | What is the price of a kg of beans in this village? | 6.3.2.1b | What was the price of a kg of beans in this village the last time there was an outbreak? |
| 6.3.2.2a | What is the price of one medium sized fish in this village? | 6.3.2.2b | What was the price of one medium sized fish in this village the last time there was an outbreak? |
| 6.3.2.3a | What is the price of maize flour in this village? | 6.3.2.3b | What was the price of a kg of maize flour in the village the last time there was an outbreak? |
| 6.3.2.4a | How much do you pay for vaccination per dose per cattle? | 6.3.2.4b | What was the vaccination cost per dose of one cattle in the village the last time there was an outbreak? |
| 6.3.2.5a | What is the vaccination cost per dose per goat? | 6.3.2.5b | What was the vaccination cost per dose per goat in the village the last time there was an outbreak? |
| 6.3.2.6a | What is the vaccination cost per dose per head of sheep? | 6.3.2.6b | What was the vaccination cost per dose per head of sheep in the village the last time there was an outbreak? |
| 6.3.2.7a | What is the price of a‘tumpeco’ (cup) of milk in this village? | 6.3.2.7b | What was the cost of a ‘tumpeco’ (cup) of milk in the village the last time there was an outbreak? |

**7.0 Household food consumption**

| | | | |
|---|---|---|---|
| 7.1.1a | How many ‘tumpecos’ (cups) of milk does the HH consume per day? | 7.1.1b | How many ‘tumpecos’ (cups) of milk did the HH consume per day the last time there was an outbreak on the farm/village? |
| 7.1.2a | How many ‘tumpecos’ (cups) of mtindi/fermented milk does the HH consume per day? | 7.1.2b | How many ‘tumpecos’ (cups) of mtindi/fermented milk did the HH consume per day during the last FMD outbreak on your farm/village? |
| 7.1.3a | How many kilogrammes of beef does the HH consume per month? | 7.1.3b | How many kilogrammes of beef did the HH consume per month the last time there was an FMD outbreak on your farm/village? |
| 7.1.4a | How many chicken does the HH consume per month? | 7.1.4b | How many chicken did the HH consume per month the last time there was an outbreak? |
| 7.1.5a | How many fish does the HH consume per month? | 7.1.5b | How many fish did the HH consume per month the last time there was an outbreak on your farm/village? |
| 7.1.6a | How many meals does the HH have per day? | 7.1.6b | Did the HH have less or more or similar number of meals per day the last time there was an outbreak on your far/village? |
| 7.1.7a | How many dishes does the HH have per major meal? | 7.1.7b | Did the HH have less or more or similar number of dishes per major meal the last time there was an FMD outbreak on your |

| | | | |
|---|---|---|---|
| | | | farm/village? |

**8.0 What is the major sources of income in your HH?**

| | |
|---|---|
| 1 | Crop sales |
| 2 | Sale of livestock and livestock products |
| 3 | Sale of both crop and livestock |
| 4 | Non-farm employment |
| 5 | Sale of natural products |
| 6 | Other income sources ( Specify) |

**8.1 How many livestock do you own?**

**Cattle ……..**

**Goats ……**

**Sheep ……...**

**Others (specify)…….**

**9.0 Any comments/questions?**

**……**
**……**
**……**

**END-Thank You**

**Appendix 3: Consent form**

**NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY, ARUSHA TANZANIA**

**Impact of foot-and-mouth disease outbreaks on household income and food security in communities found along the Uganda- Tanzania border**

**INTRODUCTION**

I am involved with a project with the **Nelson Mandela African Institution of Science and Technology (NM-AIST)** under the Program for Enhancing Health and Productivity in Livestock.

We are investigating the economic impact of foot-and-mouth disease on communities along the border between Uganda and Tanzania. There is very limited information available on the household income, losses and nutritional impact of FMD.

The research is being supported through an award from **Nelson Mandela African Institution of Science and Technology (NM-AIST)**, supported by the Bill and Melinda Gates Foundation.

**WHY YOUR HOUSEHOLD WAS CHOSEN**

Because this village has been having cases of FMD outbreaks, we believe that the information me get from your household can help us understand the impact of the disease on livelihoods.

**WHAT ACTIVITIES ARE INVOLVED**

We would like to collect information on income from livestock and livestock product sales, effect of quarantines, the losses associated with FMD outbreaks in the communities. We would like to collect information also on the nutritional impact of FMD on households.

**HOW SHALL WE USE THIS INFORMATION**

The questionnaire data that we collect will be stored securely at NMAIST, and will be shared with researchers involved in the project. The information we collect will not be shared with any other persons and will not be connected with any of your personal data.

**OF WHAT USE WILL THE RESULTS BE?**

The results from this study will contribute to the PhD of a Ugandan student called Susan Diana Kerfua who is registered at the Nelson Mandela African Institution of Science and Technology. The results of the study will also be published in scientific articles and the data

that you have shared with us will not be traced back to you. This information will also be made available in report form to the Ministry of Agriculture, Livestock and Fisheries in Tanzania and the Ministry of Agriculture Animal Industries and Fisheries in Uganda.

**IF YOU MAY, YOU CAN REFUSE OR WITHDRAW FROM THE STUDY**

You can choose not to take in the study. At any stage if you want to withdraw you can do so and no penalty will be elicited. If you would like to talk to someone about withdrawal after we have left your household, please call Susan Kerfua on +255753035188/+256772895904.

**HOW SHALL I GAIN FROM TAKING PART IN THE STUDY?**

The information will inform the government of some of the impact of FMD on communities and this may in turn be important when designing control and prevention strategies for FMD in this area/country.

**IS THERE ANY HARM IN BEING INVOLVED IN THIS STUDY?**

No there is none.

**CONFIDENTIALITY**

All the information you share with us will not be shared with other persons who are outside the project.

**THANK YOU!**

**On behalf of all the project investigators, we would like to thank you for your time today.**

**STATEMENT OF CONSENT**

"I acknowledge that this research survey has been clearly explained to me and I have clearly understood what it entails which includes its purpose, my rights, risks and future benefits. In confirm that the information above has been read to me and I have understood it. I do agree to take part in this study voluntarily."

**Participant has agreed:** Yes ☐ No ☐

Name of participant:_____

| Name of Interviewer | Date | Signature |
|---|---|---|
| _____ | _____ | _____ |

**Appendix 4: Sample collection, transportation and biosecurity mini protocols**

**Samples that were collected for the study of FMDV**

Epithelium or vesicular fluid.

At least 2 g of epithelial tissue was collected from unruptured vesicles or those that were recently raptured. Epithelial tissue was mainly collected from the tongue, the feet and the buccal cavity of the infected animal. Immediately after collection, the sample was placed in mixture of 2ml 50% phosphate buffer saline and 50% glycerol with pH 7.2. The duplicate sample was place in 2ml of RNAlater.

Oro-pharyngeal fluid

Where epithelial tissue was not available, from some cattle, oro-pharyngeal fluid samples were collected by means of a probang cup.

Saliva

Saliva was collected and mixed at a ratio of 1:10 with phosphate-buffered saline and stored at 4-10 ℃ for transport to the laboratory. Where saliva was unavailable, the mouth of an affected animal was be rinsed with phosphate buffered saline and collected.

**Transportation and storage**

After samples were collected, they were immediately place in an icebox with temperature between 4-10$^{o}$C after for a couple of hours. They were after placed in liquid nitrogen and transferred to the laboratory for analysis.

**Biosecurity**

All team members disinfected their shoes, hands and tryvek suits after visiting each farm. Tyres were also disinfected before the team dispatched to another farm. Disinfectant used was Virkon ®S at dilution of 1:100.