

**STUDY ON THE EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE IN CATTLE
AROUND LAKE MBURO CONSERVATION AREA IN SOUTH-WESTERN
UGANDA**

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DEDICATION

To my wife Jackline Achieng Batwala and our three sons and daughter Daniel, Caleb, Joshua and Deborah for the supportive environment during the course of my studies.

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LIST OF ABBREVIATIONS

Ab	Antibody
AGDP	Agricultural Gross Domestic Product
BHK	Baby hamster kidney
CA	Conservation Area (for wildlife)
cDNA	Complementary deoxyribonucleic acid.
CFSPH	Centre for Food Security and Public Health (of IOWA State, United States of America).
CFT	Complement fixation test
CI	Confidence Interval
CPE	Cytopathic effect
DFZ	Disease Free Zone
DNA	Dioxyribonucleic acid
EITB	Enzyme-linked immunoelectrotransfer blot assay
ELISA	Enzyme-linked immunosorbent assay
EMPRES	Emergence Prevention System for Transboundary Animal and Plant Pests and Diseases (of FAO)
FAO	Food and Agricultural Organization of the United Nations
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GDP	Gross Domestic Product
HRP	Horseradish peroxidase
IETS	International Embryo Transfer Society
IgA	Immunoglobulin A
IgG	Immunoglobulin B
KNP	Kruga National Park (of South Africa)
LFD	Lateral Flow Device test
LMCA	Lake Mburo Conservation Area
LMNP	Lake Mburo National Park (Old name for LMCA)
LPBE	Liquid-phase blocking ELISA
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries of Uganda
MAbs	Monoclonal antibodies

MDA	Maternally derived antibody
MTADM	Master of Transboundary Animal Disease Management
NADDEC	National Animal Disease Diagnostics and Epidemiology Centre
NSPs	Non-Structural Proteins (of the FMDV)
OD	Optical density (readings/values in the ELISA test)
OIE	World Organization for Animal Health (<i>Office International Des Epizooties</i>)
OP	Oesophageal-pharyngeal (fluid sample for FMDV diagnosis)
OR	Odds ratio
ORF	Open reading frame (of the FMDV RNA genome)
PAbs	Polyclonal antibodies
PBS	Phosphate buffered saline
PCP	Progressive control pathway (for global control of FMD)
PKs	Pseudoknot structures (contained in the FMDV RNA genome)
PCR	Polymerase chain reaction
PP	Percent Positivity
RGD	Arginyl-glycyl-aspartic acid tripeptide
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAT	South African Territory (serotype of FMDV)
SP	Structural proteins (of the FMDV)
SPCE	Solid-phase competition ELISA
SPSS	Statistical Package for Social Sciences (computer software)
ssRNA	Single stranded ribonucleic acid
TMB	Tetra-methyl-benzidinel
UBOS	Uganda Bureau of Statistics
UK	United Kingdom
USAHA	United States Animal Health Association of the United States of America
VNT	Virus neutralization test
VP	Viral capsid protein
VPg	Viral genomic protein
WRL	World Reference Laboratory (FAO/OIE Reference Laboratory)
WTTC	World Travel and Tourism Council

ABSTRACT

A cross-sectional survey was carried out from March 2011 to September 2011 with the objectives of determining the prevalence of foot and mouth disease (FMD) in cattle, the risk factors associated with FMD seropositivity and the temporal characteristics of FMD outbreaks in cattle in four districts (Kiruhura, Mbarara, Isingiro and Rakai) around Lake Mburo Conservation Area (LMCA), formerly called Lake Mburo National Park (LMNP), in South-Western Uganda. A total of 404 cattle of 6-12 months of age and never vaccinated against FMD were tested from 41 herds in Kiruhura district. A retrospective assessment of veterinary records to determine the temporal characteristics of the FMD outbreaks was carried out for Kiruhura, Isingiro, Mbarara and Rakai districts for a period of 10 years (2001-2010). The seroprevalence of FMD was 6.2% ($n = 404$) and 36.6% ($n = 41$) at individual and herd levels, respectively. The within herd seropositivity (on-farm seroprevalence) for the seropositive herds ranged from 4.5 to 57.1% with an average of 15%. The location of the herds/farms by sub-county and the husbandry system practiced on the farm/herd were statistically significantly associated with FMD outbreak occurrence on the farms/herds. Within Kiruhura district, herds from Nyakashashara Sub-county were found to be at 2.7 times higher risk (odds ratio) of getting FMD outbreak than those in Sanga Sub-county. The husbandry system was also an important risk factor. Up to 78% ($n = 27$), 45.5% ($n = 11$) and 0.0% ($n = 3$) of the herds under the communal grazing, grazing herd separately and fenced grazing systems, respectively, were found to have got at least one FMD outbreak in the period between 2001-2011. FMD outbreaks were more common during the dry season months (June-July) and shortly after the dry season (March) than in the rainy season months. There is continuous circulation of the FMDV in the cattle population in the study area and control, by vaccination, should consider the seasonal pattern of the outbreaks and collaboration with the neighboring republic of Tanzania.

KEY WORDS: ELISA, Foot and mouth disease, Lake Mburo Conservation Area, Livestock-wildlife interface, Risk factors, South-western Uganda.

1. INTRODUCTION

Wildlife and livestock contribute significantly to the economies of most sub-Saharan African countries. In Uganda, tourism and travel contributes 7.4% to the GDP and employs 5.9% of the total labor force (WTTC, 2010), and most of this is through the visitation of the National parks by foreigners and Ugandan citizens.

Livestock production in Uganda contributes 7.5% to total GDP and 17% to agricultural GDP (AGDP) (Mwebaze, 2006). The major livestock species in Uganda include cattle, sheep, goats, pigs, rabbits, and poultry. It is estimated that mixed farming small holders and pastoralists own over 90% of the cattle herd and all of the small ruminants and non-ruminant stock. Uganda has 11.4 million cattle, 12.4 million goats, 3.4 million sheep, 3.2 million pigs, 37 million chicken, 1.5 million ducks, and 350,000 turkeys (UBOS, 2010).

The major constraints to livestock production in Uganda include the forage and fodder shortage in terms of quantity and quality with resultant poor animal nutrition, inadequate access to permanent water sources, the low genetic potential of the indigenous breeds with the resultant lowered productivity, unreliable rainfall in some areas, the poor infrastructure, facilities and systems for livestock marketing; and animal diseases mainly East coast fever, anaplasmosis, trypanosomosis, helminthosis, Contagious bovine pleural pneumonia and foot and mouth disease (Otim *et al.*, 2004; Mwebaze, 2006; Byenkya, 2007).

Foot and mouth disease (FMD) is an extremely contagious disease of domestic and wild cloven-hoofed animals caused by the foot and mouth disease virus (FMDV). The disease is easily transmitted by direct and indirect contact as well as by aerosol (CFSPH, 2007; USAHA, 2008). It is characterized by fever, vesicular lesions and subsequent erosions of the epithelium of the mouth, tongue, nares, muzzle, feet, or teats (USAHA, 2008). FMD affects domestic ruminants and pigs and more than 70 wildlife species (Mwiine *et al.*, 2010a). The importance of FMD hinges on the fact that it is a highly contagious disease with a wide host range; wide geographical distribution; causes high morbidity in naive adults with resultant decreased milk production and chronic mastitis; permanent hoof damage and loss of draught power; growth retardation; abortion in pregnant animals; and causes high mortality in young (calves and lambs) susceptible animals (Forman *et al.*, 2009; Longjam *et al.*, 2011). The

disease is technically and financially difficult to control due to the ability of the virus to establish carrier status, its high antigenic diversity leading to poor cross-immunity, and the relatively short duration of immunity following vaccination (Forman *et al.*, 2009; Longjam *et al.*, 2011). FMD is a major constraint to international trade in livestock and livestock products in addition to the local trade disruptions when outbreaks occur (James and Rushton, 2002; Longjam *et al.*, 2011).

Information on the epidemiology of FMD in Uganda is limited yet outbreaks are very frequent. Up to 15-57 outbreaks were officially reported per year during the period of 2001-2008 (Ayebazibwe *et al.*, 2010a). Considering that FMD is endemic in Uganda and with the current situation of no policy for FMD active surveillance in place, these figures of the officially reported outbreaks by passive surveillance are by far lower than the true value. Approximately 4.6% of Uganda, equivalent to 11,150 km², is occupied by ten conservation areas, formerly called National Parks, inhabited by a vast number of wild ungulates, including, among others, impalas and buffalos freely roaming across conservation areas and international borders. These wild animals in conservation areas also interact freely with livestock, particularly cattle, both under ranch and extensive communal grazing systems in the adjacent human settlement areas (Ocaido *et al.*, 2006). This free interaction facilitates the sharing of infectious diseases, such as FMD, between the domestic and wild animals (Ocaido *et al.*, 2006). Although the contribution of domestic and wildlife species to the introduction and maintenance of FMD outbreaks in Uganda is not fully known; Ayebazibwe *et al.* (2010b) reported that disease transmission at the livestock-wildlife interface is responsible for the observed relatively higher officially reported FMD outbreak rates from 2001-2008 in areas adjacent to some conservation areas as compared to localities further away from such areas.

The four districts of the study area (Kiruhura, Mbarara, Rakai and Isingiro) lie in the “cattle corridor” of Uganda. The “cattle corridor” is the area believed to have the highest cattle population and extends diagonally across Uganda, from the South-west, touching Rwanda and Tanzania, to the the North-east, Karamoja region, bordering South Sudan and Kenya (Byenkya, 2007; Ayebazibwe *et al.*, 2010a). The “cattle corridor” often experiences lower rainfall and has increasingly become prone to frequent droughts leading to depletion of pasture and water resources, resulting in massive seasonal movements of livestock along the Southwestern–Northeastern stretch to alternative grazing areas hence the increased risk for FMD outbreaks (Ayebazibwe *et al.*, 2010a). Areas in the cattle corridor are the sources of

cattle for slaughter and breeding for other areas in the country which have minimal animal numbers. In effect, the cattle corridor areas also act as a source of animal diseases for areas which have minimal animal numbers.

The study area experiences FMD outbreaks every year, especially at the border with the Republic of Tanzania (Ayebazibwe, personal communication, 2010). Since this area also harbors LMCA, it is not uncommon to suspect wild animals to be the sole source of the outbreaks. This area also lies adjacent to the national border with the republic of Tanzania. The specific risk factors associated with the occurrence of FMD outbreaks in this area have never been fully determined. With exception of the preliminary study carried out on risk factors for FMD outbreaks in Uganda at large, by interviewing District Veterinary Officers by Ayebazibwe *et al.* (2010a), there have been no systematic studies carried out to determine the specific risk factors for the FMD outbreaks in South-western Uganda. So far no attempts have been made to determine the temporal characteristics of the FMD outbreaks in South-western Uganda. The determination of these risk factors and the temporal characteristics of the outbreaks would contribute important information for the development of an early warning system for timely detection and management of future outbreaks. This information would also contribute to development of an effective control strategy of the disease with resultant reduction of the disease pressure in this area. Thus, this study was carried out with the general aim of generating a better understanding of the epidemiology of FMD with emphasis on the risk factors for the occurrence of outbreaks in cattle herds in the areas around LMCA in South-Western Uganda. The specific objectives of the study were:

1. To determine the seroprevalence of FMD in cattle at the livestock-wildlife interface of LMCA, in Kiruhura district, South-Western Uganda.
2. To determine the risk factors associated with FMD seropositivity in cattle and FMD outbreak occurrence on cattle farms/herds at the livestock-wildlife interface of LMCA, in Kiruhura district, South-Western Uganda.
3. To determine the temporal characteristics of the FMD outbreaks in the districts around LMCA.

2. LITERATURE REVIEW

2.1 Definition of foot and mouth disease

FMD is an extremely contagious viral disease of domestic and wild cloven-hoofed animals, easily transmitted by direct and indirect contact as well as by aerosol (CFSPH, 2007; USAHA, 2008). It is characterized by fever, vesicular lesions and subsequent erosions of the epithelium of the mouth, tongue, nares, muzzle, feet, or teats (USAHA, 2008). Although adult animals generally recover, the morbidity rate is very high in naive populations, and significant pain and distress occur in some species (CFSPH, 2007). Sequelae may include decreased milk yield, permanent hoof damage and chronic mastitis (CFSPH, 2007). High mortality rates can be seen in young animals (CFSPH, 2007). This disease is a major constraint to international livestock trade in countries where it is endemic (CFSPH, 2007). FMD was classified by the World Organization for Animal Health (OIE: *Office International des Epizooties*) among the former list A diseases, which were defined as transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products (OIE, 2009b).

2.2. Etiology

2.2.1. Taxonomy

FMD is caused by the foot and mouth disease virus (FMDV) whose genome consists of a single molecule of linear positive sense RNA, 7.2–8.5 kb in size (Murphy *et al.*, 1999). FMDV belongs to the genus *Aphthovirus* in the family *Picornaviridae* (Murphy *et al.*, 1999; USAHA, 2008; Patch *et al.*, 2011). The name, *picornaviridae* is derived from the Latin word ‘Pico’ meaning small and ‘rna’ meaning RNA, which refers to the size and genome type of the virus, while the genus name ‘Aphthovirus’ (Aphtha means mouth ulcer) refers to the vesicular lesions produced in cloven-hoofed animals (OIE, 2009a).

Viruses which are insensitive to ether, chloroform, non-ionic detergents and which have viral ribonucleic acid (RNA) functioning as messenger RNA or as template for progeny RNA are classified as members of the picornavirus family (Sangare, 2005). The family consists of 20 virus species divided into 9 genera (Sangare, 2005) as summarized in the Table 1.

Table 1: The genera and examples of species of each genera of the *picornaviridae* family

No.	Genus	Examples of species
1.	Aphthovirus	Foot and mouth disease virus and Equine rhinovirus.
2.	Enterovirus	Human polioviruses; Human enteroviruses; Bovine enteroviruses; Porcine enteroviruses
3.	Rhinovirus	Human rhinovirus (major cause of human common cold); Bovine rhinoviruses
4.	Hepatovirus	Hepatitis A virus and Simian hepatitis A virus.
5.	Cardiovirus	Encephalomyocarditis virus
6.	Parechovirus	Echovirus
7.	Theiler's murine virus	Encephalomyelitis virus
8.	Kobuvirus	Aichi virus
9.	Teschovirus	Teschen disease virus of pigs.

2.2.2. Physico-chemical properties of the FMD viruses

The FMDV is pH sensitive and is inactivated when exposed to pH below 6.5 or above 9 (Davies, 2002). Residual virus survives in milk and milk products during regular pasteurization, but is inactivated by ultra high-temperature pasteurization (OIE, 2009a). It has been demonstrated that following slaughter, the virus in the carcass is inactivated within 48 hours by the drop in pH during rigor mortis; however, the virus can survive for longer periods of time in lymph nodes and bone marrow that maintain a close to neutral pH at post mortem conditions. The virus survives almost indefinitely in these organs (lymphnodes and bone marrow) if frozen or chilled (FAO, 2002; Davies, 2002; USAHA, 2008). It is therefore believed that matured and deboned meat is free from the virus (Ayebazibwe, 2010). FMDV can withstand different environments including livestock faecal material, organic matter and

straw, especially at low temperatures (Davies, 2002). Organic matter protects FMDV from adverse environmental conditions and enhances its survival and spread on fomites.

The sizes of droplet aerosol also play an important role in the survival or drying out of the virus; droplet aerosol size of 0.5 - 0.7 μm is optimal for longer survival of the virus in the air, while smaller aerosols dry out. In dry conditions the virus also survives longer in proteins e.g. in epithelial fragments (Ayebazibwe, 2010). FMDV survives almost indefinitely at freezing temperatures. Semen and embryos (unless treated according to the International Embryo Transfer Society protocol) can retain the FMDV. Destruction of most strains occurs with heating to 56°C for 30 minutes (CFSPH, 2007).

FMDV is effectively inactivated by some disinfectants including 2% solutions of NaOH or KOH, 4% Na_2CO_3 , citric acid (0.2%) and Virkon-S[®] are effective disinfectants for FMD contaminated objects. However, the virus is resistant to alcohol, phenolic and quaternary ammonium compounds, Iodophores and hypochlorite disinfectants especially in the presence of organic matter (Sahle, 2004; CFSPH, 2007; USAHA, 2008).

2.2.3. Morphology and genomic organization of the FMDV

Picornaviruses, including the FMDV, are small RNA viruses that are enclosed within a non-enveloped protein shell (capsid). The capsid consists of polypeptides, which are devoid of lipo-protein, and hence is stable to lipid solvents like ether and chloroform (Murphy *et al.*, 1999). The virion of picornaviruses is icosahedral and contains one molecule of infectious, positive sense, single stranded RNA (ssRNA), ranging from 7.2 - 8.5 kb in length (Murphy *et al.*, 1999). A viral genomic Protein (VPg), which is enclosed by the 3B genome region, is covalently linked to the 5' end of the genome and a poly (A) tract of variable length is located at the 3' terminus (Murphy *et al.*, 1999). A diameter of 22 - 25 nm capsid is composed of 60 capsomers each consisting of four proteins (VP1-4). VP1- VP3 are exposed on the surface, whilst VP4 is located internally at the pentameric apex of the icosahedrons and contains a myristic acid molecule attached to the amino terminal glycine ((Murphy *et al.*, 1999).

FMDV has a single stranded, positive sense RNA that is approximately 8500 bases (8.5kb) long and consists of a 5' non-coding region (NCR), a single Open Reading Frame (ORF), and a short 3' NCR (Saravan *et al.*, 2011). The RNA is covalently linked to a small protein, VPg,

at its 5'-terminus and translation of the RNA yields a single polypeptide (L-P1-P2-P3) which is subsequently cleaved into the structural (from the P1 region) and non-structural proteins (Grubman and Baxt, 2004), see Figure 1.

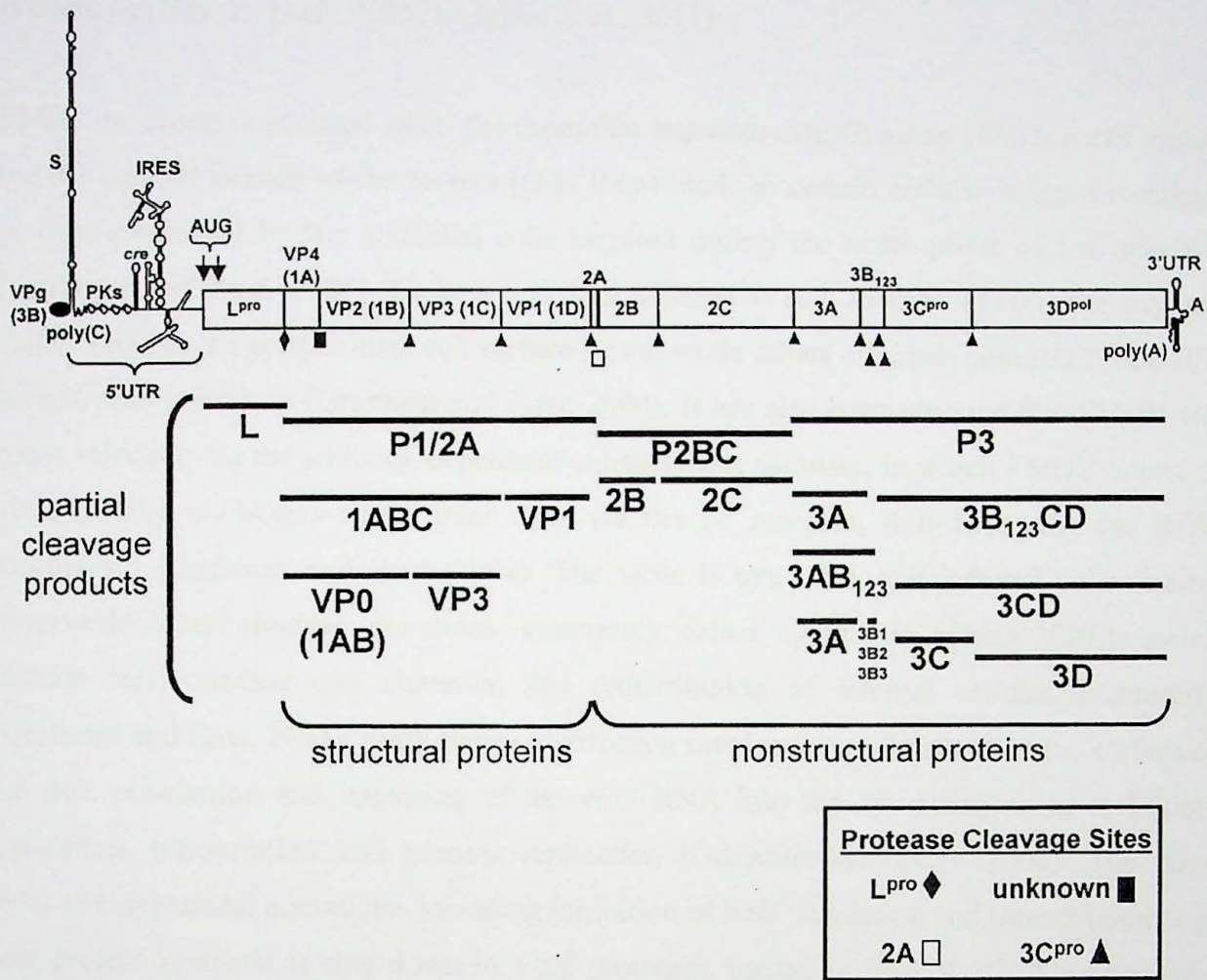


Figure 1: Schematic illustration of the FMDV genome.

The ORF (Open Reading Frame) is shown in the boxed area, with the viral proteins. Also shown are the functional elements of the genome as well as the partial protein cleavage products. The sites of the primary cleavages and the proteases responsible are indicated. (Adapted from Grubman and Baxt, 2004).

The icosahedral capsid of the FMDV consists of 60 copies of each of the four structural proteins VP1, VP2, VP3 and VP4, encoded by genes 1D, 1B, 1C and 1A, respectively. While the first three structural proteins have surface components in the capsid, the fourth (VP4) is

internal. Within the virion there are usually one or two units of the structural protein VP0 (1AB), the precursor of VP2 and VP4 (Grubman and Baxt, 2004; Longjam *et al.*, 2011). The mature virion is produced after the encapsidation of the virion RNA that is accompanied by the cleavage of VP0 to VP2 and VP4. The P2 (2A, 2B, 2C) and P3 (3A, 3B, 3C, 3D) regions encode for non-structural proteins that are involved in viral RNA replication and protein processing (Nayaka *et al.*, 2005; Longjam *et al.*, 2011).

FMDV infection is initiated when the tripeptide sequence Arg-Gly-Asp (RGD) motif within the β G and β H strands of the protein (G-H loop) binds to certain cellular integrin receptors that are expressed by the epithelial cells targeted during the acute phase of the infection (Grubman and Baxt, 2004). However, certain isolates of cell adapted FMDV use heparan sulfate (HS) as the predominant cell surface ligand while others establish both RGD- and HS-independent infections (Grubman and Baxt, 2004). It has also been reported that FMDV can cause infection *via* the antibody-dependent enhancement pathway, in which FMDV bound to virus-specific antibodies could enter cells via the Fc receptor, thus bypassing the RGD mechanism (Grubman and Baxt, 2004). The virus is cytocidal, and infected cells exhibit observable morphological alterations, commonly called cytopathic effects (CPE), which include cell rounding and alteration and redistribution of internal cellular membranes (Grubman and Baxt, 2004). Early stages of infection involve virus adsorption to the surface of the cell, penetration and uncoating of the viral RNA into the cytoplasm so as to initiate translation, transcription and genome replication (Grubman and Baxt, 2004). The virus induces biochemical alterations, including inhibition of host translation and transcription; e.g. host protein synthesis is shut down by viral proteases, including FMDV 3C, (Grubman and Baxt, 2004).

2.2.4. Genetic Variation

The observed genetic variation in FMD viral genome is the result of a two-step process. Firstly, the replication of viral RNA is error-prone due to the absence of proof reading in the 3D-encoded RNA dependent RNA polymerase (Grubman and Baxt, 2004). Secondly, competitive selection is continuously acting on the genome. Thus, those mutants with a selective advantage in the prevailing environment will be better represented than those with a selective disadvantage (Sahle, 2004).

Mutation

RNA viruses in general, and FMDV in particular, have very high mutation rates, in the range of 10^{-3} to 10^{-5} per nucleotide site per genome replication, due to the lack of error correction mechanisms during RNA replication (Grubman and Baxt, 2004). This high error rate leads to differences of FMDV replicated genomes from the original parental genome of 0.1 to 10 base positions, thus, the quasispecies concept was developed to explain the effects of errors in replication on the evolution of replicating RNA molecules (Grubman and Baxt, 2004). The concept envisions that within any population of virus, all genome sequences are not identical, and that selection occurs at the population level rather than at the individual level. Therefore, new variants of FMD viruses are continuously arising after each replication cycle, which constitute an intratypic population of FMD viruses with different degrees of genetic relationships (Grubman and Baxt, 2004). This may result in the generation of viral diversity. Changes in the nucleotide compositions of the capsid genes are responsible for the genetic or antigenic variability of the virus. Thus, the generation of new variants is considered as one of the major problems in the control of FMD by vaccination since even the best vaccine may induce immunologic pressure within the population that results in the emergence of a new variant (Grubman and Baxt, 2004). Furthermore, the observation that antigenic variation can also occur in tissue culture has implications for vaccine production, since a number of tissue culture passages are required to produce vaccine for a new variant, leading to the possibility that the virus eventually utilized as antigen may not provide the antigenic coverage needed (Grubman and Baxt, 2004).

Recombination

In addition to variation caused by mutation, FMDV has been shown to undergo RNA recombination in tissue culture (Grubman and Baxt, 2004). Recombination involves the exchange of genetic material between two non-segmented RNA genomes resulting from polymerase 'jumping' during RNA synthesis. It has been shown that genetic recombination occurs between viruses of the same serotype as well as between serotypes. Intratypic recombination occurs more frequently than intertypic recombination and it appears that recombination events in FMD occur more readily in the 3' half of the genome, than in the capsid genome of the FMDV. Mutations through recombination may result in the exchange of

genetic material that can lead to the generation of new antigenic variants that may escape immune pressure (Grubman and Baxt, 2004).

2.2.5. Antigenic Variation

One of the consequences of genetic variation through mutation, selection, and recombination is that new antigenic variants are constantly being generated. Not only is there no cross protection between FMDV serotypes, but vaccination with one antigenic variant of a serotype does not necessarily protect an animal when challenged with a different virus of the same serotype (Sangare, 2005). Attempts to characterize the extent of the antigenic variation within the FMD serotypes led to the establishment of the techniques where by viral subtype could be identified. Initially over 60 different subtypes were identified by world Reference laboratory (WRL), but it quickly became apparent that there is a continuous spectrum of intratypic antigenic variants, making it difficult to identify specific subtypes (Grubman and Baxt, 2004). Changes to the genes encoding capsid proteins can result in antigenic variation and evolution of new subtypes (Grubman and Baxt, 2004). This may give rise to immunologically distinct variants that can re-infect individuals that have been previously infected by related viruses. The degree of cross protection among different subtypes of the same serotype thus varies. Since there is continual antigenic drift in enzootic situation this is an important factor to consider when selecting vaccine strains (Grubman and Baxt, 2004; USAHA, 2008).

2.2.6. Serotypes and Subtypes

There are 7 completely immunologically distinct FMDV serotypes: A, O, C (or the so called European types); SAT-1, SAT-2, SAT-3 (South African Territories types) and Asia 1 (USAHA, 2008; OIE, 2009). In addition, within a given serotype there are many immunologically related sub-types (topotypes), particularly among the A, O and C (i.e. A5, A24, C1, O1, etc), totaling more than 60 type-subtype known combinations (USAHA, 2008). New subtypes occasionally arise spontaneously as a combined result of genetic variation of FMDV through mutations, recombinations and host immunological pressure-induced selection (USAHA, 2008). However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used (OIE,

2010). At present, a sequencing of FMD virus is increasingly being used to establish intratypic variations of FMD viruses and classifying viruses into genotypes and lineages (Sahle, 2004).

2.3 Epidemiology

2.3.1. Geographic distribution

FMD is endemic in most parts of Asia, Africa, the Middle East and South America (FAO, 2007; OIE, 2009a). In parts of Africa, virus persistence in wild African buffalo makes eradication unfeasible. North America, New Zealand, Australia, Greenland, Iceland and most of Europe are free of this disease. Sporadic outbreaks have occurred in disease-free countries, with the exception of New Zealand, Greenland, Iceland and the smaller islands of Oceania where FMD has never been reported. The last FMD outbreak in Australia occurred in 1872, USA 1929, Canada 1952, Mexico 1954 (CFSPH, 2007; OIE, 2009a). Western Europe has had recent outbreaks, which have all been successfully controlled. This includes the 2001 and 2007 outbreaks in the UK, which spread to Ireland, France, and the Netherlands, and separate outbreaks in Italy and Greece. Japan has also recently eradicated the 2000 outbreak (FAO, 2007).

The FMD status of any particular country or region can be defined as endemic, epidemic (sporadic), or free. FMD-free regions can be defined by national borders (e.g. Australia, Indonesia), by supranational borders (Europe, North America) or by disease-free zones within non-free areas, which are maintained by movement control, such as those in Namibia and Zimbabwe. Sporadic regions are characterized by repeated incursions of FMD viruses into regions where disease does not usually occur (CFSPH, 2007).

Out of the seven serotypes of the FMDV, six have been isolated in Africa, including serotypes O, A, C, the South African Territory (SAT) serotypes including SAT 1, SAT 2 and SAT 3; thus, only Asia 1 has never been reported on the African continent (Rweyemamu *et al.*, 2008). Out of the six serotypes, serotype C has most likely been eradicated from Africa and the world as it was last reported in Ethiopia in 1983 and in Kenya in 2004 (Mwiine *et al.*, 2010a).

The FMDV Serotypes commonly isolated from certain geographical regions are as shown in table 2.

Table 2: FMDV serotypes commonly isolated from various regions around the world

Continent	Sub-continent	FMDV Serotype
Europe (historically)	-	A, O, C.
	Western Europe, U.K	O.
	South-Eastern Europe	Asia 1
Asia	Near East	A, O
	Middle East	A, O, C
	Far East	A, O, C, Asia 1
	Arabian peninsular	SAT 1 and 2
Africa	Central, East, West Africa	A, O
	Eastern Africa	A, O, C, SAT 1,2 and 3
	North-East, Central and South Africa	SAT 1 and 2
	South Africa	O, SAT 1, 2 and 3
South America	-	A, O, C

Source: (Grubman and Baxt, 2004; FAO, 2007; Sangula, 2010)

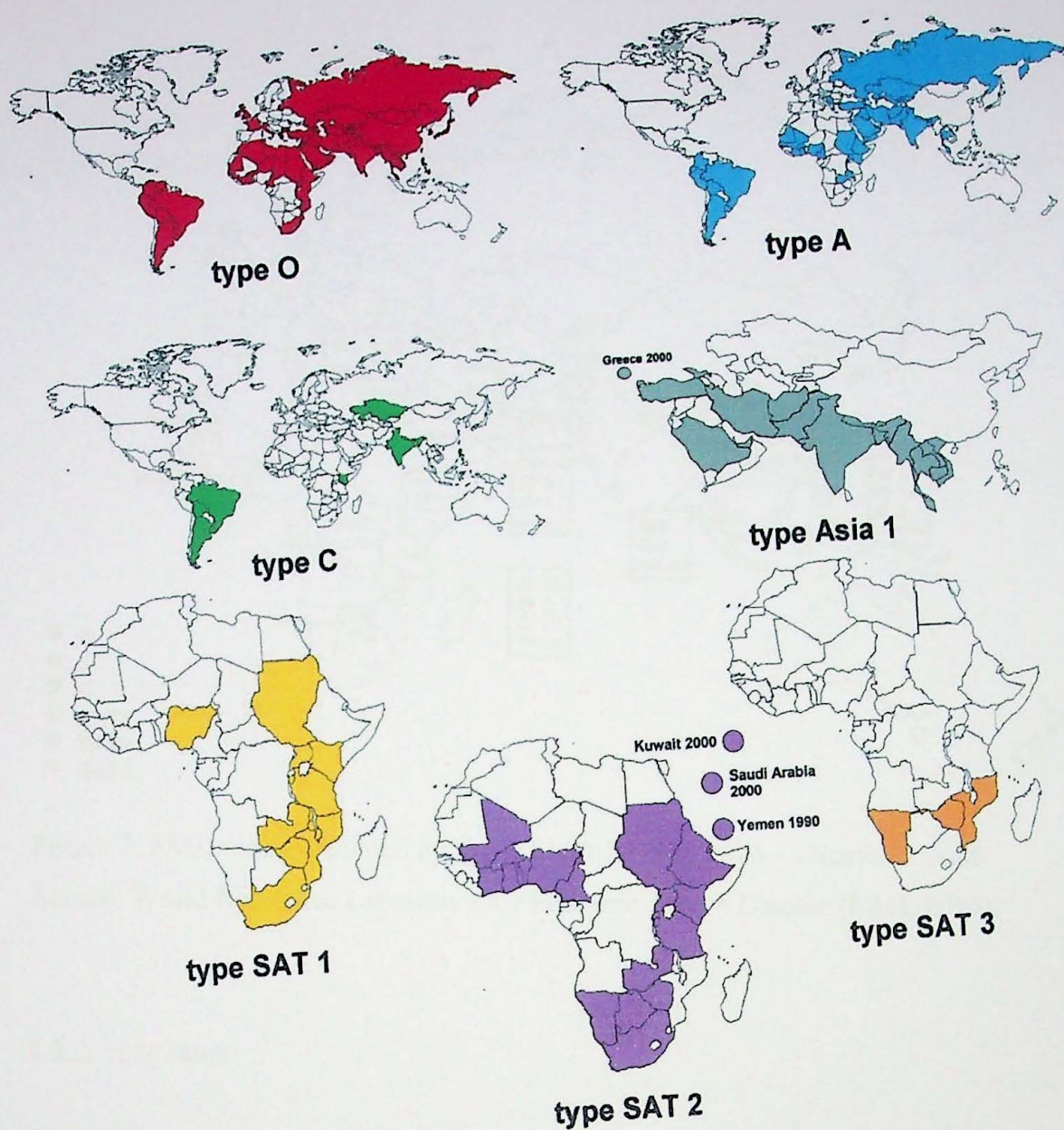


Figure 2: Countries in which FMD was reported to the OIE between 1990 and 2002
Source: (Grubman and Baxt, 2004).

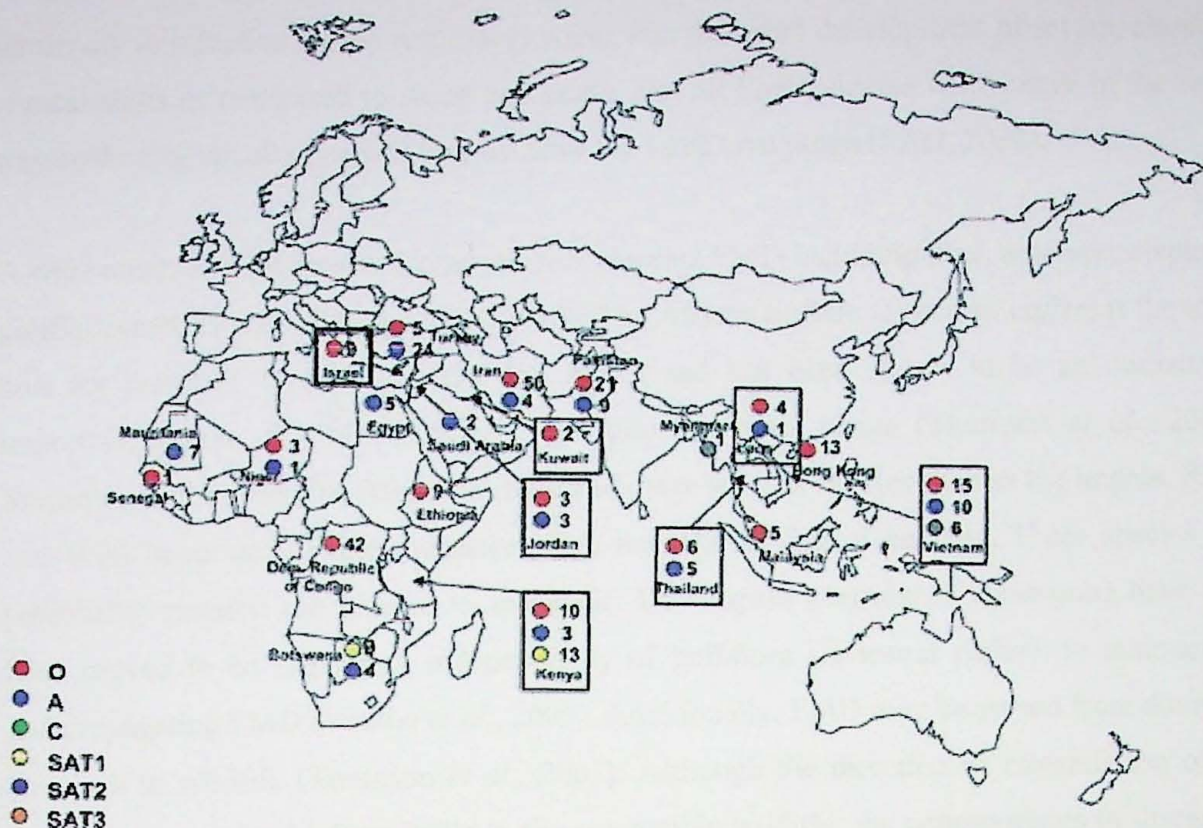


Figure 3: FMD viruses isolated by WRL FMD January 2006 – December 2006
Source: World Reference Laboratory for Foot-and-Mouth Disease (FAO, 2007).

2.3.2. Host range

FMD is highly contagious and affects domestic and over 70 wild *Artiodactyla* species (Davies, 2002). Of the domesticated species, cattle, pigs, sheep, goats and water buffalo (*Bubalus bubalis*) are susceptible to FMD and may also transmit infection to other species (USAHA, 2008 and OIE, 2010). The susceptibility of these animals can vary with breed of animal and strain of virus. The disease is considerably less obvious or sub-clinical in breeds of cattle, sheep, and goats indigenous to Africa and Asia, where FMD is endemic; and these animals are believed to have been the source of infection for countries previously considered disease-free (Davies, 2002). Pigs are regarded as amplifying hosts for the disease because of their ability to be infected orally and their capacity to excrete large quantities (of the order of 3000 times the quantity excreted by cattle) of virus in their exhaled breath (FAO, 2002; Doel, 2003) This is probably most important with pigs which, once infected, generate massive amounts of virus. Cattle are regarded as good indicator hosts because of their extreme

sensitivity to infection by the respiratory route, and the usual development of severe, classical clinical signs as compared to sheep and goats. Not all FMD viruses will behave in the same way epidemiologically nor will they all have the same host range (FAO, 2002).

A wide range of wild cloven-footed animals contract FMD including deer, antelope, elephant, giraffe, camelids and wild pigs (OIE, 2010). The African buffalo (*Syncerus caffer*) is the main host for the SAT types of FMDV in Africa and has been shown to be an occasional, important, source of FMD infection for cattle in South Africa (Thomson *et al.*, 2003). Transmission between the African buffalo and other wildlife species such as the Impala, Kudu and Wild boars occurs and increases when there is sharing of habitats. These species can potentially transmit the disease to livestock. The Impala (*Aepyceros melampus*) have also been proved to be important, independently of buffaloes (*Syncerus caffer*), in maintaining and propagating FMD (Vosloo *et al.*, 2009). Additionally, FMD may be spread from domestic livestock to wildlife (Thomson *et al.*, 2003). Although the direction of transmission of the FMD virus can also be from cattle to the susceptible wildlife, the consequences of disease in wildlife are not as economically significant as in the domestic animals. Infection of wildlife with FMD usually results in a relatively mild disease from which affected animals recover in a week or two, although clinical disease leading to high mortalities has been reported in impala (*Aepyceros melampus*) and mountain gazelles (*Gazella gazella*) as an occasional event (Thomson *et al.*, 2003). Apart from the African buffalo (*Syncerus caffer*), and of recent the Impala, the involvement of other wildlife species in the epidemiology of FMD in the domesticated species is not certain (Vosloo *et al.*, 2009). Although FMD is known as a disease of cloven footed animals it can occur naturally in other animals, e.g., the hedgehog (*Erinaceus spp.*) and infection has been established experimentally in a number of other species. Amongst the camelidae, Bactrian camels and new world camelids have been shown to be susceptible (Davies, 2002).

Elsewhere in the world cattle are usually the main reservoir for FMD viruses, although in some instances the viruses involved appear to be specifically adapted to pigs. The pig-adapted Cathay strain of type O FMDV apparently does not infect large ruminants in the field or experimentally and requires cells of porcine origin for primary isolation. Small ruminants can play an important role in the spread of FMDV, but it is not clear whether the virus can be maintained in these species for long periods in the absence of infection of cattle. Strains of FMDV that infect cattle have been isolated from wild pigs, antelope and deer (Davies, 2002).

2.3.3. Persistence of FMD and the carrier problem

Carrier animals are recovered or vaccinated-and-exposed animals in which FMDV persists in the oropharynx for more than 28 days after infection (Davies, 2002; Grubman and Baxt, 2004; OIE, 2010). The carrier state in FMD is an inapparent infection in which the intermittent isolation of the virus from the oropharynx epithelial scrapings (probang samples) is currently the sole means of detection (Davies, 2002; OIE, 2010). The duration of the carrier state has been demonstrated to continue for up to 3.5 years in cattle, while it seems to be of shorter duration in sheep (9 months) and goats (4 months) while it has not been proven in pigs (Davies, 2002). It has been established that individual African buffalos can harbor the infection for up to 5 years, and that African buffalo herds can maintain the infection for up to 24 years (Gubman and Bax, 2004). Some other wild ruminant species also seem to have the potential to become carriers of FMDV, but have less close contact with domestic ruminants, and will most commonly transmit the infection to cohorts of the same or other species with which they are in close contact (Thomson *et al.* 2003). It is well established that a proportion of animals infected by FMD virus eventually become “carriers” with equal facility irrespective of whether they have been vaccinated, passively immunized or are immunologically naive cattle (Davies, 2002). Although the carrier state in FMD is well documented, the evidence for carrier animals transmitting infection to others is uncertain, except in the African buffalo (Davies, 2002; Grubman and Baxt, 2004).

2.3.4. The role of wildlife in the epidemiology of foot and mouth disease.

In South Africa it has been established that African buffalos are very efficient reservoirs of FMDV, particularly for the Southern African Territory serotypes: SAT 1, SAT 2, and SAT 3 (Thomson *et al.*, 2003; Vosloo *et al.*, 2005; Sahle *et al.*, 2007). Buffalo calves are born free of infection and protected for the first 2-6 months of their life by the colostral maternal antibodies (Vosloo *et al.*, 2007). Thereafter infection is rapid and it has been reported that in Kruger National Park (KNP), South Africa, more than 80% of the buffalo calves develop antibody to SAT1-3 by 12 months of age (Vosloo *et al.*, 2005; Vosloo *et al.*, 2007). Buffalo herds may act as reservoirs for future outbreaks transmitting infection to cattle directly or through other intermediary species like the impala (*Aepyceros melampus*) as a short term transmission hosts (Vosloo *et al.*, 2009). Very few buffalo calves, develop clinical disease following infection. During the acute phase of the infection, which lasts about 1-2 weeks,

there is considerable excretion of virus in all secretions and excretions and during this time the infected buffalo can infect other susceptible species (Sahle *et al.*, 2007; Vosloo *et al.*, 2007). Thereafter, the virus disappears from all secretions and excretions except for the pharynx where low-level viral replication persists in approximately 60% of the buffalo calves (Vosloo *et al.*, 2007). This carrier status may persist for 5 years or longer in a single animal (Sahle *et al.*, 2007) and up to 24 years in an isolated buffalo herd (Vosloo *et al.*, 2005). Other wild ungulates do not become long term carriers of FMD virus, but can spread the disease during acute infection (Sahle *et al.*, 2007).

The mode of transmission between carrier buffaloes and susceptible animals, including cattle, is not known (Vosloo *et al.*, 2005). FMD viruses change during the persistent infection and may give rise to new antigenic variants that could have severe implications on disease control reliant on the use of vaccines (Sahle *et al.*, 2007). It is not known whether buffalo in East Africa are carriers of the O and A type viruses (Vosloo *et al.*, 2005).

Impala (*Aepyceros melampus*) are the most susceptible species in South Africa and are considered an indicator host for the presence of SAT viruses while in Zimbabwe, the kudu have been associated with clinical FMD more frequently than impala (Vosloo *et al.*, 2005). The Impala (*Aepyceros melampus*) have also been proved to be important, independently of buffaloes (*Syncerus caffer*), in maintaining and propagating FMD (Vosloo *et al.*, 2009). FMD infection in impala has frequently spread along water courses in KNP as the virus is transmitted by contact between animals that congregate along the water sources rather than being transmitted via the water itself. The impala in KNP usually become infected with SAT viruses derived from buffalo in the vicinity (Vosloo *et al.*, 2005). Antelopes such as impala and kudu can jump fences up to 2.4m high and this poses a severe problem for disease control where such fences are used to separate wildlife from susceptible domestic animals (Vosloo *et al.*, 2005).

In the Serengeti, where wildebeest (*Connochaetes taurinus*) are the most numerous large mammal species, FMD is infrequently reported, although a severe outbreak caused by SAT-2 was recorded in wildebeest in 1999. Evidence indicated that FMD had spread from domestic animals to the wildebeest (Vosloo *et al.*, 2005).

To assume status as FMD-free country with infected zones, South Africa has made a great effort to eliminate the risk of transmission of FMDV from African buffalos to livestock by fencing of national parks and wildlife reservoirs and vaccinating livestock in buffer zones around these areas (Thomson *et al.*, 2003). In Zimbabwe, some initiatives have been developed involving establishment of disease free buffalo herds, allowing for integration of buffalos into game ranching enterprises in disease free areas (Ayebazibwe, 2010). Such fencing does not exist in Uganda, and considering the frequent and close intermingling of African buffalo herds and domestic ruminants around the conservation areas, it is very compelling to speculate that this large FMDV reservoir would also play a role in the epidemiology of FMDV in this area (Ayebazibwe, 2010).

2.3.5. Molecular epidemiology

Molecular epidemiology of FMD is a study that involves comparison of genetic differences between virus isolates. Phylogenetic analysis of the virus protein 1 (VP1) region of FMD virus has been used extensively to investigate the molecular epidemiology of FMD worldwide. These techniques have assisted in studies aimed at establishing of the genetic relationships between different FMDV isolates, geographical distribution of lineages and genotypes. It is also used for the establishment of genetically and geographically linked topotypes and tracing the source of virus during outbreaks (Sangare *et al.*, 2003). Molecular epidemiology can also be used to show the genomic relationship between vaccine and field strains of the FMDV for all the seven serotypes based on sequences derived from the 1D gene (encoding the VP1 viral protein). Sequence differences of 30-55% of the VP1 gene are obtained among seven serotypes while different subgroups (genotypes, topotypes) are defined by differences of 15-20% (Knowles and Samuel, 2003). Therefore, comparison of whole genome sequences can provide further discrimination between closely related viruses and help to recreate the transmission pathways between farms within outbreaks. The RT-PCR amplification of FMDV RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform these comparisons (OIE, 2010). The evolutionary changes of virus are determined by comparing genomic material from more than one virus with each other. At present, DNA sequencing and phylogenetic trees are widely used to illustrate the genetic relationship between viruses (OIE, 2010).

2.3.6. Mode of transmission

FMD is perhaps the most contagious of animal diseases (FAO, 2002). FMDV can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, milk, urine, feces and semen (FAO, 2002; Sutmoller *et al.*, 2003; OIE, 2010). This virus is also found in large quantities in vesicle fluid, and peak transmission usually occurs when vesicles rupture (OIE, 2010). Pigs, in particular, produce large quantities of aerosolized virus. Animals can shed FMDV for up to 4 days before the onset of symptoms (OIE, 2010). The level of transmission drops precipitously 5 - 7 days after the development of lesions, coinciding with the drop in virus titers and the first development of antibodies (Sutmoller *et al.*, 2003). The disease may be transmitted in many ways, including:

Direct contact

FMD can be readily spread by direct contact between infected and susceptible animals, and this is the most significant mode of transmission. Under this mode, the rate of disease transmission is determined by the herd immunity; stocking density; the level of challenge from both the infected animals and the environment; congregation of animals as occurs at watering points; and animal's movements through risky areas such as livestock markets. In this context, animals that are excreting the virus but have not (yet) developed obvious lesions are particularly significant (FAO, 2002; Sutmoller *et al.*, 2003).

Indirect contact

The FMDV is easily spread mechanically by a variety of fomites such as animal foodstuffs, bedding, equipment, livestock holding areas, vehicles and clothing that have been contaminated with infected secretions and excretions (saliva, milk, faeces, urine and semen). Climatic and environmental factors will determine how long the virus will persist on fomites (Sutmoller *et al.*, 2003; CFSPH, 2007; OIE, 2010).

Swill feeding of pigs and feeding on contaminated milk in calves

Feeding pigs on uncooked swill that contains virus contaminated meat scraps or dairy products has a high potential for spreading infection. Swill originating from aircraft and ships

has been responsible for a number of cases of international spread of the disease (FAO, 2002; Suttmoller *et al.*, 2003; OIE, 2010). Calves are also infected by feeding on contaminated milk from acutely infected dams (OIE, 2010).

Windborne spread

FMDV can be spread by wind up to a distance of 10- 60 km over land and 250-300 km over water (FAO, 2002; OIE, 2010). Under this mode of transmission, spread of the disease is determined by presence of appropriate animal species (cattle) and appropriate climatic conditions including slow and steady wind speed and direction, high relative humidity (optimally > 60 %), weak sunlight and absence of heavy rain. Long-distance windborne transmission has not been observed in Africa, the Middle East, Asia or Latin America (FAO, 2002).

Artificial breeding and sexual transmission

Transmission of the FMDV can occur through artificial insemination using infected semen if protocols described by the International Embryo Transfer Society (IETS) are not followed (FAO, 2002).

Sexual transmission could be a significant route of spread for the SAT type viruses in African buffalo populations (CFSPH, 2007).

Infective humans

People who work with infected animals or materials will carry FMDV on their hair, skin and on clothes. In addition to this, humans can harbor sufficient FMDV in their respiratory tract for 24–48 hours, to serve as a source of infection for susceptible animals (Suttmoller *et al.*, 2003; OIE, 2010), hence, the common practice of 3-5 days of personal quarantine for personnel exposed in research facilities (OIE, 2010). However, during an active outbreak, this may be reduced to an overnight period of time after thorough shower and shampoo, change of clothing, and expectoration (OIE, 2010).

2.4. Pathogenesis

The main route of infection in ruminants is through the inhalation of droplets, but ingestion of infected feed or contaminated milk (calves), inoculation with contaminated vaccines, insemination with contaminated semen, and contact with contaminated fomites can all produce infection (Grubman and Baxt, 2004). In animals infected via the respiratory tract, initial viral replication occurs in the pre-pharyngeal area and the lungs followed by viraemic spread to other tissues and organs before the onset of clinical disease. FMDV is then distributed throughout the body, to reach best sites of multiplication such as the epithelium of Oro-pharynx, oral cavity, Feet, the udder and heart. Vesicles develop at multiple sites, generally on the feet and tongue, and are usually preceded by fever. Severe lesions often occur in areas subjected to trauma or physical stress, and most animals develop viremia (Grubman and Baxt, 2004). The incubation period can be between 2 and 14 days, depending on the infecting dose and route of infection (OIE, 2010). The FMDV also, probably, replicates in the mammary gland of susceptible cow and in the pituitary gland. Viral excretion commences about 24 hours prior to the onset of clinical disease and continues for several days. The acute phase of the disease lasts about one week and viremia usually declines gradually coinciding with the appearance of strong humoral responses (Grubman and Baxt, 2004).

2.5. Immune response

Immunity to one serotype, following vaccination or natural infection, does not cross-protect against other serotypes, but intra-serotypic subtypes may cross-protect incompletely (Paton *et al.*, 2009). The virus elicits a rapid humoral response in either infected or vaccinated animals. Virus-specific antibodies protect animals in a serotype-specific manner against re-infection, or against infection in the case of vaccination, and protection is generally correlated with high levels of neutralizing antibodies (Grubman and Baxt, 2004; USAHA, 2008). The response is directed to epitopes on the three external structural proteins (SP) and good protective immunity is apparent between 7 and 14 days after either infection or vaccination (Grubman and Baxt, 2004; USAHA, 2008). Animals infected with FMDV produce antibodies to SP and non-structural proteins (NSP) and these circulating antibodies can be detected as early as 3–5 days after the appearance of the first clinical signs and are known to peak at around 14 days

post infection (Dukpa *et al.*, 2011). Antibodies to the FMDV 3ABC NSP have been found to be still detectable at 1–2 years after infection of experimental animals (Paton *et al.*, 2009). In cattle, the immunoglobulin G1 (IgG1) response predominates over IgG2 and antibody, including IgA, can be detected in upper respiratory tract secretions early in infection. There is also a possibility of macrophages clearing the virus from the infected animal by phagocytosis of opsonized virus. The role of cell-mediated immunity against FMDV in the infected animal is still largely unknown (USAHA, 2008).

Immunity after natural infection lasts for 1-4 years in cattle (3-6 months in pigs) with closely related virus of the same serotype, but remain fully susceptible to other serotypes (Doel, 2003; Radostits *et al.*, 2006). In endemic areas, periodic outbreaks occur which sweep through the animal populations and then subside. A 6-year epidemic cycle has been demonstrated in India. This is probably due to the disappearance of immunity which develops during an epidemic and the sudden flaring up from small foci of infection when the population becomes susceptible again. When outbreaks follow each other in quick succession, the presence of more than one strain of virus should be suspected. In countries where general vaccination is practiced every year, outbreaks are usually associated with different strains imported in carrier animals or infected meat (Radostits *et al.*, 2006).

Vaccines provide only partial immunity against antigenic variants of the same serotype. Potent vaccines confer immunity as early as 4 days after injection. However, vaccinal immunity is relatively shorter, being 6-8 months in cattle, 6 months in pigs and 5-6 months in sheep (FAO, 2002; Radostits *et al.*, 2006). A proportion of vaccinated animals, although protected against the clinical disease, may become sub-clinically infected after natural challenge and excrete virus. It is important to note that animals incubating the disease when vaccinated may still develop the disease, sometimes in a milder form, and that vaccinated, exposed animals may still transmit infection for 7-14 days after vaccination and exposure (FAO, 2002).

2.6. Clinical signs

The incubation period is 2–14 days (Andrews *et al.*, 2004; OIE, 2009a). The severity of clinical signs varies with the strain of virus, exposure dose, age and breed of animal, host species, and degree of host immunity. The signs can range from a mild or inapparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2009a). Morbidity may approach 100%. Mortality in general is low in adult animals (1–5%) but higher in young calves, lambs and piglets (20% or higher). Recovery in uncomplicated cases is usually after about two weeks (OIE, 2009a).

In cattle, the initial signs are fever of 39.4 - 40.6°C, dullness, anorexia, and fall in milk production (CFSPH, 2007; Radostits *et al.*, 2006). These signs are followed by excessive drooling of saliva. The saliva may look thicker with long strands, and more foaming due to the dehydration of the febrile animal (CFSPH, 2007). This is followed by smacking of the lips, grinding of the teeth, serous nasal discharge; stamping, shaking or kicking of the feet or lameness; and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the tongue, dental pad, gums, soft palate, nostrils, muzzle, inter-digital space, coronary band, and teats (Andrews *et al.*, 2004; Sahle, 2004; Radostits *et al.*, 2006). After vesicle formation, drooling may be more marked, and nasal discharge, lameness, or both may increase. Vesicles often rupture within 24 hours, becoming erosions. Lesions on the coronary band may cause growth arrest lines on the hoof (USAHA, 2008). In severe cases, the hooves may be sloughed. Pregnant cows may abort. Calves may die suddenly without developing any vesicle due to heart failure as a result of multifocal myocarditis (USAHA, 2008). Morbidity can approach 100% in all age groups, but Mortality in adult animals is rare. In young animals mortality can exceed 50% as a result of the myocarditis (CFSPH, 2007; USAHA, 2008). Most adults recover in 2-3 weeks, although secondary infections may lead to a longer recovery time. Long term Sequelae may include hoof deformation, chronic mastitis, low milk production, unthriftiness, failure to gain weight and chronic breeding problems. A lactating animal may not recover to pre-infection production level because of damage to the mammary secretory tissue. (CFSPH, 2007; USAHA, 2008). A chronic Panting syndrome characterized by dyspnoea, anaemia, hair overgrowth and heat intolerance has also been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (Andrews *et al.*, 2004; Tesfaye, 2006).

In sheep and goats, if the clinical signs occur, it tends to be very mild, and may include dullness, fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mouth lesions are often not noticeable or severe, and generally appear as shallow erosions (CFSPH, 2007; USAHA, 2008). Vesicles may also be noted on the teats, and rarely on the vulva or prepuce. Milk production may drop, and rams can be reluctant to mate. Mild lameness may be the only sign. In lame animals, there may be vesicles or erosion on the coronary band, heel bulbs or in the inter-digital space but they may rupture and be hidden by foot lesions from other causes. Infected animals may abort and nursing lambs and kids may die without showing any clinical sign due to heart failure (CFSPH, 2007; USAHA, 2008). Up to 25% of infected sheep remain asymptomatic, and 20% have lesions only at one site (CFSPH, 2007; USAHA, 2008).

In swine, the initial signs are fever of 40 - 40.6°C, anorexia, reluctance to move, and squeal when forced to move (USAHA, 2008). These signs are followed by vesicles on the coronary band, the heels, and the inter-digital space with resultant painful lameness and reluctance to move (USAHA, 2008). There is no drooling in pigs. Vesicles are sometimes found on the snout, muzzle, gums or udder (teats), as well as on the hock or elbows if the pigs are housed on rough concrete floors. Mouth lesions are usually small and less apparent than in cattle. Pregnant gilts or sows may abort. Young pigs up to 14 weeks may die suddenly due to heart failure, without showing any clinical sign (CFSPH, 2007; USAHA, 2008).

The symptoms in wildlife resemble those seen in domesticated livestock. Vesicles and erosions may be found at various sites, particularly on the feet and in the mouth. More severe lesions occur where there is frequent mechanical trauma, such as, on the feet and snout of suids or the carpal joints of warthogs. In some species the disease is subclinical while others develop a severe acute syndrome. Infections with SAT-type viruses in African buffalo are often subclinical. Severe disease has been documented in mountain gazelles, impala, blackbuck, white tailed-deer, warthogs and a kangaroo. Young animals of any species can die suddenly of myocarditis (CFSPH, 2007; USAHA, 2008).

2.7. Pathology

In cattle, the diagnostic lesions are single or multiple fluid filled vesicles ranging from 2 mm to 10 cm in diameter (CFSPH, 2007; USAHA, 2008). These can occur at all sites of predilection. Usually gross lesions on the tongue progress in the following manner; a small-blanching whitish area develops in the epithelium; fluid fills the area and a vesicle (blister) is formed; vesicle enlarges and may coalesce with adjacent ones and then rupture, leaving an eroded (red) area or ulcer. Gray fibrinous coating forms over the eroded area that becomes yellow, brown or green till the epithelium is restored but a demarcation line of newly developing epithelium may be noted. The line then gradually fades with time (CFSPH, 2007; USAHA, 2008). Vesicles are generally present for only a short period. Occasionally “dry” FMD lesions develop in the tongue and appear necrotic rather than vesicular. The vesicle in the interdigital space is usually large because of the stress on the epithelium caused by movement and weight. The lesion at the coronary band at first appears blanching; then there is separation of the skin and horn. When healing occurs, new horn is formed, but a line resulting from the coronitis is seen on the wall of the hoof. Animals that die may have grayish or yellowish streaking in the myocardium with degeneration and necrosis. These findings are known as “tiger heart”. Skeletal muscle lesions occur but are rare (CFSPH, 2007; USAHA, 2008).

In sheep and goats lesions in the mouth and vesicles on the coronary band may be few, small and difficult to find. Young animals that die may have a “tiger heart” appearance of the myocardium (CFSPH, 2007; USAHA, 2008).

In swine vesicles on the snout can be large and filled with clear or bloody fluid. Mouth lesions are usually the “dry” type and appear as necrotic epithelium. Feet lesions are usually severe and the hoof can become detached. Animals that die may have a “tiger heart” appearance of the myocardium (CFSPH, 2007; USAHA, 2008).

2.8. Economic importance

FMD is globally the most economically important infectious disease of livestock (Shirley *et al.*, 2010). The economic importance of the disease is not only due to the ability of the disease

to cause losses of production, but also related to the reaction of veterinary services to the presence of the disease and to the restrictions on the trade of animals both locally and internationally (James and Rushton, 2002; Forman *et al.*, 2009). FMD, therefore, threatens the livelihoods of simple farmers, large sophisticated farming practices and the national and the international economies of the countries (Rutagwenda, 2003).

The direct production effects in extensive production system include loss of milk due to chronic mastitis; reduced draught animal power from lesions on the feet which reduce the animals' ability to work in terms of cultivation and transporting goods (Rutagwenda, 2003); lower rates of live-weight gain in growing animals due to reduced feed intake; and reduction in reproductive capacity due to the increased abortion rates of up to 10% and delayed conception in breeding stock (James and Rushton, 2002). The disease also causes up to 6% mortality in calves. FMD may lead to premature culling of recovered animals in intensive production systems as a result of permanent foot, udder or thyroid damage.

Restrictions on animal movement and international trade in animals and animal products can cause much more serious losses (James and Rushton, 2002). In addition, the presence of FMD in an exporting country can affect the export of other products, such as fresh fruit, flowers, tobacco and vegetables, to FMD-free countries (James and Rushton, 2002). The control of outbreak (slaughter of infected and in contact, disposal of carcass in disease free zones) and the loss due to the ban on livestock exports costs several million US dollars for a single outbreak (Junker *et al.*, 2009). A striking example is the 2001 outbreak of serotype O (the Pan Asian strain) in Great Britain, a country that had been free of FMD since 1981. This epidemic spread to Ireland, France and The Netherlands. The United Kingdom alone was forced to slaughter a total of 4.1 million infected and in contact animals (cattle, sheep, goats and swine combined). The cost of this epidemic in the United Kingdom was estimated to be £3.1 billion (Perry and Rich, 2007; Junker *et al.*, 2009).

In Uganda, where FMD is endemic, studies have shown that FMD outbreaks severely affect the food production capacity and income of households (Rutagwenda, 2003). In non-pastoral communities, the ox ploughs are substituted with the less efficient hand hoes for cultivation as most oxen are affected during outbreaks. In the pastoral communities, farmers cannot sell their livestock and livestock products due to the official closure of the markets in addition to the livestock movement and quarantine restrictions imposed during outbreaks as veterinary

measures taken to curb the outbreak spread as vaccination goes on (Rutagwenda, 2003). Other economic effects of FMD in Uganda include: Reluctance of some farmers to keep the high yielding exotic cattle due to their high susceptibility to FMD as compared to the local breeds; environmental degradation as pastoralists resort to charcoal burning during outbreaks; disruption in social lives, for example, social gatherings such as weddings are reduced during outbreak periods as they involve animal slaughtering which is banned during the outbreak periods (Rutagwenda, 2003). The disease also causes severe revenue loss. In a study carried out in 6 neighboring districts in Uganda (out of the 112 districts) it was estimated that a total revenue loss of US\$ 8,010,000, for all the six districts combined, is incurred if one FMD outbreak occurred in these districts (Rutagwenda, 2003). This loss is as a result of the dampening of businesses of all the stake holders involved along the livestock and livestock products value chains.

2.9. Diagnosis

In the early stage of infection, clinical diagnosis is based on clinical signs and lesion identification. In addition, there are several techniques for detection of the FMDV or viral antigens and antibodies against the FMDV as an indication that infection has taken place.

2.9.1. Field diagnosis

FMD should be suspected whenever salivation and lameness occur simultaneously or when a vesicular lesion is seen or suspected in cloven-hoofed domestic or wild animals (USAHA, 2008). Fever often precedes other clinical signs, therefore, febrile animals should be carefully examined. Vesicular lesions may be found before animals start to salivate, have a nasal discharge, or become lame. Clinical diagnosis can present many difficulties due to other viral infections of the mucous membrane which produce similar clinical signs. FMD is clinically indistinguishable from Vesicular stomatitis (occurs in cattle, horses and pigs), Swine vesicular disease and Vesicular exanthema of swine (Radostits *et al.*, 2006; OIE, 2009a). Other differential diagnoses for FMD include rinderpest (but was eradicated); bovine viral diarrhoea and mucosal disease; infectious bovine rhinotracheitis, bluetongue, epizootic haemorrhagic disease; bovine mammillitis; bovine papular stomatitis; contagious ecthyma; and malignant catarrhal fever (Radostits *et al.*, 2006; OIE, 2009a).

2.9.2. Laboratory diagnosis

Laboratory diagnosis of FMD is by virus isolation, demonstration of FMD viral antigen or nucleic acid in samples of tissue or fluid or by detection of antibodies against the FMDV. Antibodies to viral nonstructural proteins (NSPs) can be used as indicators of infection, irrespective of the vaccination status (OIE, 2010).

Specimens

The preferred tissue for diagnosis is the epithelium from unruptured or freshly ruptured vesicles or the vesicular fluid. Where collecting this is not possible, blood and/or oesophageal-pharyngeal (OP) fluid samples taken by a probang cup in ruminants or throat swabs from pigs provide an alternative source of virus. Other samples, such as, myocardial tissue, blood with anticoagulant, Serum, lymph nodes, thyroid gland, adrenal gland, kidney, and heart are good sources of specimens from postmortem but vesicles are preferable if present (OIE, 2010).

Epithelial samples should be placed in a transport medium of pH 7.2 – 7.6. The medium can be glycerol and 0.04M phosphate buffer or glycerol and Phosphate buffered saline (PBS). In either case, it is preferable to add antibiotics such as penicillin 1000 IU, neomycin sulphate 100 IU, polymixin B sulphate 50 IU, and mycostatin 100 IU. The samples should be kept refrigerated or on ice until received by the laboratory for virus isolation or nucleic acid identification (OIE, 2010).

Where epithelial tissue is not available, OP fluid samples can be collected and submitted to the laboratory for virus isolation or nucleic acid identification. The same laboratory procedures may be used to detect viraemia by examining serum samples. OP fluid samples should be placed in 2ml transport medium of pH 7.2 in a 5ml capacity container capable of withstanding freezing above dry ice (solid carbon dioxide) or liquid nitrogen. The transport medium is composed of 0.008M phosphate buffer containing 0.01% bovine serum albumin, 0.002% phenol red and antibiotics (OIE, 2010).

Identification of the agent

Virus isolation

The isolation and characterization of the virus is the "golden standard" for the diagnosis of viral diseases. Suspensions of field samples suspected to contain FMD virus are prepared and inoculated into cell cultures or into 2-7 days old unweaned mice of selected inbred strains. Sensitive cell culture systems include the primary bovine (calf) thyroid cells and primary pig, calf or lamb kidney cells (OIE, 2010). The cell cultures are incubated at 37°C and examined for cytopathic effect (CPE), 24 to 48 hours post infection. If no CPE is detected, the cells are frozen and thawed, used to inoculate fresh cultures and examined for CPE for another 48 hours. No CPE confirms absence of FMDV in the samples. Once CPE is complete, culture fluids (or musculo-skeletal tissues from dying mice) can be used in antigen detection or nucleic acid recognition tests. Virus isolation is a very sensitive method, but laborious and expensive and there is a risk of disseminating the virus into the environment (Kitching *et al.*, 2005).

Immunological methods (Antigen detection)

Three tests used for antigen detection are described below.

The Antigen Enzyme-linked immunosorbent assay (ELISA) is an OIE recommended test for international trade because of its high sensitivity. It is an indirect sandwich ELISA used to detect FMD viral antigen and differentiating all the seven serotypes (OIE, 2010).

The complement fixation test (CFT) is recommended by the OIE as an alternative test for international trade but it is less sensitive than the Antigen ELISA. The CFT can be used for detection of all the serotypes, differentiating the subtypes and for establishing serological relationships (r values). However, although CFT is a fast method it needs a high virus load and results are sometimes affected by pro-and anti-complementary activities of the test sample (OIE, 2010).

The pen side tests such as the lateral flow device test (LFD) are commercially available but not yet registered by the OIE as manufacturers have not yet submitted the respective

validation dossiers for these tests (OIE, 2010). These tests are rapid and easy-to-perform. They allow on-site diagnosis to be made on farms under field conditions. They can be used for serotype identification for all the seven serotypes (Longjam *et al.*, 2011)

Nucleic acid recognition methods

The reverse transcription polymerase chain reaction (RT-PCR) can be used to amplify the genome fragments of FMDV in diagnostic materials including epithelium, milk, serum and OP samples. Specific primers have been designed to distinguish between each of the seven serotypes and in-situ hybridization techniques have been developed for investigating the presence of FMD virus RNA in tissue samples (OIE, 2010; Longjam *et al.*, 2011). RT-PCR tests are rapid and sensitive. The tests in use are of two types, including, the agarose gel-based RT-PCR and the real-time RT-PCR (OIE, 2010).

Unlike many living organisms where the hereditary information is enclosed within a DNA genome, FMD virus has an RNA genome that can be sequenced directly, but RNA is unstable and is usually first transcribed into complementary DNA (cDNA) prior to performing the nucleotide sequence. Reverse transcriptase (RT) when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes. In epidemiological studies of FMD virus, nucleotide sequencing of the VP1 gene has been used extensively to determine the relationships between the field isolates. The technique is also routinely used to investigate genetic variation, molecular evolution in carrier animals, and to identify the source of infection in outbreak conditions (Vosloo *et al.*, 2002).

Serological tests for FMD (Antibody detection)

Serological tests for FMD are performed in support of four main purposes namely: to certify individual animals prior to import or export; to confirm suspected cases of FMD; to substantiate absence of infection; and to demonstrate the efficacy of vaccination (OIE, 2010). These tests are of two types: those that detect antibodies to viral structural proteins (SP) and those that detect antibodies to viral nonstructural proteins (NSPs).

The SP tests are serotype-specific and detect antibodies elicited by vaccination and infection, therefore, they do not differentiate between infected and vaccinated animals. They include the

virus neutralization test (VNT), the solid-phase competition ELISA (SPCE) and the liquid-phase blocking ELISA (LPBE). These tests are highly sensitive, provided the virus or antigen used in the test is closely matched to the strain circulating in the field (OIE, 2010). They are OIE prescribed tests for trade and are appropriate for confirming previous or ongoing infection in non-vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field (OIE, 2010). The VNT requires cell culture facilities, the use of live virus and takes 2–3 days to provide results. The ELISAs are blocking- or competition-based assays that use serotype-specific polyclonal antibodies (PABs) or monoclonal antibodies (MABs), are quicker to perform and are not dependent on tissue culture systems and the use of live viruses. However, low titre false-positive reactions can be expected in a small proportion of the sera in either ELISA. Screening animals by ELISA and confirming the positives by the VNT minimizes the occurrence of false-positive results (OIE, 2010).

NSPs (3A, 3B, 2B, 2C, 3ABC) can be measured by different ELISA formats or by immunoblotting. These ELISAs either use purified antigens absorbed directly to microplates or use PABs or MABs to trap specific antigens from semi-purified preparations. The NSP antibody tests include the Indirect enzyme-linked immunosorbent assay (ELISA) and the Enzyme-linked immunoelectrotransfer blot assay (EITB) (OIE, 2010). Antibody to the polyproteins 3AB or 3ABC are generally considered to be the most reliable indicators of infection (Kitching *et al.*, 2005; OIE, 2010).

The NSPs antibody tests can be used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated, but without specifying the serotype (OIE, 2010). Therefore, these tests can be used to confirm suspected cases of FMD and to detect viral activity or to substantiate freedom from infection on a population basis. For certifying animals for trade, the tests have the advantage over SP methods that the serotype of the virus does not have to be known (OIE, 2010). However, there is experimental evidence that some cattle, vaccinated and subsequently challenged with live virus and confirmed persistently infected, may not be detected in some anti-NSP tests, causing false-negative results. This is because the immune status of some of these animals, following vaccination, does not allow sufficient viral replication to induce detectable antibodies to NSP (Kitching *et al.*, 2005; OIE, 2010). Lack of vaccine purity may also affect diagnostic specificity as the presence of NSPs in some vaccine preparations may result in misclassification (false positives) in animals that have been repeatedly vaccinated using

vaccines which are not purified of NSPs. An additional problem of NSP tests is that they do not distinguish between infected animals that have eliminated virus and those that are carriers (Kitching *et al.*, 2007).

Antibody detection by the FMDV 3 ABC-Ab ELISA

The FMDV 3ABC-Ab ELISA is one of the NSP tests. The detection of antibody to the polyprotein 3ABC proteins is a useful indicator of FMDV infection with any of the seven serotypes of the virus (Kitching *et al.*, 2007). Antibody to the 3ABC is only found in virus-infected animals where the FMDV has replicated but not in vaccinated animals where vaccines purified of NSPs have been used (Kitching *et al.*, 2007).

Briefly, the test is carried out as follows: Microtiter plates are supplied pre-coated with recombinant FMDV 3ABC viral antigen; dilutions of the samples to be tested are incubated in the well of these plates. Any antibody specific for 3ABC binds to the antigen in the wells and forms antigen-antibody complex on the plate well surface. Unbound material is removed from the wells by washing. Peroxidase labeled anti-IgG conjugate is added, which binds to the antibodies of the sample complex with the 3ABC antigen. Unbound conjugate is removed by washing, and the Tetra-methylbenzidine (TMB) containing substrate is added to the wells. The degree of colour which develops (optical density measured at 450nm), is directly proportional to the amount of antibody specific to 3ABC present in the sample. The diagnostic relevance of the result is obtained by comparing the optical density (OD) which develops in wells containing the samples with the OD from the wells containing the positive control. The FMDV 3ABC-Ab ELISA is a rapid test for screening of a large number of sera. In areas where more than one serotype exist, the test is cheaper compared to the conventional liquid phase blocking ELISA, which has the disadvantage that each serum sample must be tested against all existing serotypes (Sangare, 2005).

2.10. Control of foot and mouth disease

Disease control is the continued implementation of measures to reduce morbidity and mortality from disease, while eradication refers to the regional extinction of an infectious agent (Thrusfield, 2007). Options and requirements for the control of FMD differ between

FMD-free countries and those with endemic infection (Andrews *et al.*, 2004). The procedures commonly used are (a) control by eradication and (b) control by vaccination, or a combination of the two. In countries where the disease is endemic, or where there are wildlife reservoirs, eradication is seldom practicable. In areas with only occasional epidemics, slaughter of all infected and in-contact animals is usually carried out. For countries in large continents, regional and international cooperation is required for effective control and eradication (Radostitis *et al.*, 2006), thus, the initiation of the Progressive Control Pathway for global FMD control (PCP-FMD), with emphasis on FMD-endemic geographical regions such as Africa, by FAO and OIE (FAO, 2009).

2.10.1. Control in endemic areas

Mass prophylactic vaccination against FMD, usually practiced only in the cattle population, is the first step towards controlling FMD in endemic areas. The aim of this policy is that, over a period of years the of FMDV load in the environment as well as the number of outbreaks and individual animal clinical cases will be reduced if a greater than 75% level of immunity is achieved (Singh, 2011). This is because vaccination of cattle, sheep or pigs (including infected ones) with high potency vaccines induces protection and reduces the potential (amount) and duration of virus shedding and transmission to susceptible animals following natural infection of the vaccinated animal (Golde *et al.*, 2005; Orsel *et al.*, 2005; USAHA, 2008). In addition, some vaccinated animals, when infected by a heterologous strain, tend to remain subclinical (Radostits *et al.*, 2006), hence, the reduction in number of clinical cases following vaccination.

FMD vaccination is by use of inactivated (killed) mono-, bi- or tri-valent vaccines which are most effective against infection with the homologous strain from which the vaccine was prepared, but with reduced protection against closely related strains of FMDV (Andrews *et al.*, 2004; CFSPH, 2007). It is therefore necessary to identify the field serotypes and strains of FMDV that pose the most significant threat in a given area, since there is no universal FMD vaccine that covers all serotypes and strains, currently (Andrews *et al.*, 2004; CFSPH, 2007). Thus, because of the increasing occurrence of antigenically dissimilar substrains, the production of vaccines from locally isolated virus is becoming a more common practice. The virus is obtained from infected tongue tissue, a cell culture of bovine tongue epithelium or other cell culture. Baby hamster kidney (BHK) is a favored viral cultural medium and BHK

vaccine is now in general use (Radostits *et al.*, 2006). If endemic FMD co-exists with a productive dairy industry, such as found in the Arabian Peninsula, it may be necessary to include up to eight strains of FMDV in the vaccine to protect against all the potential field strains (Kitching *et al.*, 2007).

Following the first dose of vaccine, immunity develops 4-5 days post vaccination depending on the potency of the vaccine (Kitching *et al.*, 2007). Serviceable immunity after a single vaccination can be relied on for only 6-8 months in cattle (Kitching *et al.*, 2007). Vaccines produced from 'natural' virus give longer immunity than those produced from 'culture' virus. Vaccines produced in oil-adjuvant offer promise of providing longer immunity, and require only annual and bi-annual revaccination in adult cattle and young stock, respectively, or every 4-6 months in pigs (Radostits *et al.*, 2006).

Livestock from non-vaccinated dams are fully immunocompetent and respond to FMD vaccination from an early age (Doel, 2003). Excellent immune responses have been observed within the first 2 weeks of life with cattle, sheep and pigs. Calves as young as 1 week respond to vaccination as effectively as adult cattle under experimental and field conditions, provided they are free of maternally derived antibody (Doel, 2003; Radostits *et al.*, 2006).

In the case of routine vaccination programmes, the presence of maternally derived antibody (MDA) suppresses the response of young animals to FMD vaccine and vaccination programmes need to be developed to take account of this limitation (Doel, 2003). In cattle and pigs, the influence of MDA on FMD vaccine efficacy can extend for a number of months particularly if the colostral MDA has been elevated by repeated vaccination of the dam with high quality vaccine. Less commonly, 5 or 6 months may elapse before an animal responds to vaccine and there may be no evidence of an anamnestic response, i.e. previous vaccinations in the face of MDA do not necessarily prime the immune system (Doel, 2003). A theoretical concern is the possibility that vaccination, in the presence of MDA, might influence or prevent the development of immunological memory, with obvious consequences for the immune status of long-lived animals. There have been reports that oil based FMD vaccines circumvent the problem of MDA. However, the evidence for this is not conclusive (Doel, 2003). In practical terms under endemic conditions, it is not realistic to hold young animals until all of the individual titres of MDA have completely waned otherwise the virus is presented with a 'window of susceptibility' (Doel, 2003).

Calves from unvaccinated dams should be vaccinated at 4 months and revaccinated at 8 months of age, but calves from vaccinated cows should be vaccinated twice, the first at 6 months and the second at 10 months of age. The important considerations in calves are to avoid vaccination while the calf is still carrying maternal antibodies derived from colostrum and to avoid infection before they can develop active immunity. Immunity is present 7-20 days after vaccination, depending on the antigenicity of the vaccine (Radostits *et al.*, 2006).

It is not usual to include sheep, goats, and pigs in a general vaccination program unless they are also affected during outbreaks (Radostits *et al.*, 2006). However, it is recommended that piglets be vaccinated at 8-12 weeks followed by a boost 4 weeks later, and that sows be vaccinated 3-4 weeks before farrowing or every 4-6 months (Radostits *et al.*, 2006). In pigs a bi or trivalent, inactivated, adjuvant vaccine gives strong immunity for 6 months and some resistance for 12 months. Severe local reactions (abscesses and granulomas) at vaccination sites can be reduced by the inclusion of an oil-adjuvant. However, vaccination of pregnant sows leads to a high rate of abortions and stillbirths. In sheep, monovalent or trivalent vaccines give immunity for 5-6 months but the sheep may act as inapparent carriers (Radostits *et al.*, 2006).

The duration of immunity in the vaccinated pigs following a typical programme of two doses, a month apart, is not greatly different from that of pigs recovered from infection, 3-6 months (Doel, 2003). Cattle recovered from infection are able to resist reinfection with the same strain for up to 4.5 years although considerably shorter periods have been observed (Doel, 2003). In contrast, vaccines confer about 6 months immunity in cattle following the initial two dose regime. However, the situation with cattle is more complex because of the prevalence of the carrier state in animals recovered from clinical disease (Doel, 2003). The presence of continuous or sporadic presence of virus in carrier animals obviously gives an opportunity for repeated stimulation of the immune response and hence maintenance of protective antibody titres (Doel, 2003).

Routine mass vaccination programmes may lead to long-term immunity in individuals within a cattle population (Doel, 2003). It was reported that Dutch calves which received their first (single) dose of vaccine lost protective antibody titres within a few months of vaccination. However, annual booster vaccinations of young and older animals showed that antibody titres were fully sustained throughout the following 12 months until the next round of vaccination

(Doel, 2003). In Europe, cattle were found to have significant titres of neutralizing antibodies directly attributed to vaccination 6-7 years after cessation of the vaccination programme (Doel, 2003). Well organized and extensive vaccination programmes, such as those used in Europe before 1991, not only confer high levels of protection to the individual animal and the herd but prevent establishment of the disease in the country as a whole and set the scene for eventual eradication (Doel, 2003).

Alternatives to general vaccination are modified programs including 'ring' vaccination to contain outbreaks; 'frontier' vaccination to produce a buffer area between infected and free countries; and vaccination of selected herds on a voluntary basis when an outbreak is threatened. Such emergency vaccinations can reduce the risk of spreading infection by reducing the rate of virus excretion (Radostits *et al.*, 2006). Barrier vaccination can reduce the danger of FMD entering a country or area and has been successful in preventing exotic strains of FMD entering Europe through Turkey and Greece, and in allowing South Africa and Zimbabwe to restrict vaccination to their borders and around game reserves (Andrews *et al.*, 2004). Countries which do not vaccinate or vaccinate against two or three strains may use emergency vaccination to control an outbreak due to a new strain of FMD by slaughtering affected animals and ring vaccinating around the infected area with a monovalent vaccine specifically against the exotic strain (Andrews *et al.*, 2004).

While many countries do bring FMD under control using vaccination every 6 months together with other zoo-sanitary control methods, where such additional control strategies are not applied, and the level of circulating virus is high, even vaccination every 10 weeks is not sufficient to stop clinical disease. FMD vaccines, because of their production methods, are expensive. These factors together ensure that current vaccines will not be effective or consistently used to control FMD in many of the poorer countries in which FMD is endemic (Kitching, 2007). In addition, chronic trypanosomosis, prevalent in Sub-Saharan Africa, causes immune suppression in cattle leading to poor immune response to vaccination. Seasonal malnutrition, as occurs during droughts, also contributes to sub-optimal response to vaccines (Plum *et al.*, 2007).

Other control measures practiced in some countries include: Livestock movement control based on permit systems; control of contact between infected/reservoir wild animals and domestic livestock by fencing and zoning; strict quarantine measures; and frequent inspection

in high-risk areas so as to detect disease and institute counter measures as soon as outbreaks occur (Sahle, 2004). However, the movement of nomadic people with their animals, and of wild animals across international borders in Africa, the Middle and Far East, makes disease regulations impossible to enforce. The airborne spread of FMD virus cannot be controlled by legislation (Andrews *et al.*, 2004). In Africa, the occurrence of six out of the seven FMDV serotypes; the presence of the FMDV carrier and reservoir (the African buffalo); and the lack of controlled national borders and animal movement makes FMD control very difficult (Vosloo *et al.*, 2007).

In southern Africa the problem of the African buffalo has been managed through the use of game fencing to keep livestock and wildlife separate. In eastern and central Africa, where there are still major buffalo populations mixed with cattle and other livestock, the problem is much more difficult. However, one possibility, as in South Africa, is for the development of FMD-free buffalo herds by rearing disease free buffalo calves (Vosloo *et al.*, 2002; and Bronsvoort *et al.*, 2008).

Some countries in southern Africa, such as, Namibia, Botswana and South Africa, have created OIE recognized FMD disease free zones (DFZs) without vaccination. These DFZs are essentially dependent on exclusion of buffalo and are separated from the “infected zones” by fencing and creating buffer zones between the “infected zones”, the “buffalo zone”, and the DFZ. Cattle within the buffer zones are vaccinated every six months with a vaccine containing all the three SAT serotypes. As far as possible, the vaccines used contain virus strains that cover the topotypes of FMDV prevalent in the buffalo populations in the vicinity. In addition, surveillance zones are maintained between the “buffer” and “free” zones. Movement control within and between these zones is enforced. In most countries, vaccinated animals are branded to ensure that they cannot be moved into the free zones where vaccination is not allowed. The animals may only be sold within the endemic and buffer zones, and movement of animal products is also restricted (Vosloo *et al.*, 2002).

However, despite these measures (fencing and vaccination) FMD outbreaks still occur in this area though at a relatively lower frequency. Between the years 2000-2007, five FMD outbreaks occurred in cattle in the area adjacent to the Kruger National Park in South Africa (Jori *et al.*, 2009). Conservationists/environmentalists are critical of the fences as they sometimes block migration routes of the wild animals and prevent access of wildlife to water,

resulting in ecological disturbance and wildlife mortality as was evidenced in Botswana (Mbaiwa and Mbaiwa, 2006). This has necessitated the need for alternative and ecologically sensitive strategies of controlling FMD. The double fencing control strategy for FMD at the livestock-wildlife interface is also limited by the extremely high costs involved in fence erection and maintenance and the leakage of small ungulates (such as the Impala) through the fence which poses a risk to the surrounding livestock if the ungulates are infected (Vosloo *et al.*, 2002).

2.10.2. Control in disease free areas

The majority of outbreaks (>68 %) in FMD free countries are caused by the legal or illegal importation of infected animals or infected animal products including contaminated feed. Therefore, preventive measures include: adequate import controls and quarantine procedures for live animals; establishing proper risk analysis of the hazards associated with the importation of animal products from FMD affected countries; education and training of veterinarians and stake holders on the recognition and diagnosis of FMD; and maintenance of competent laboratories that use validated and controlled testing procedures (CFSPH, 2007; USAHA, 2008). Waste food (swill) fed to swine is a particular concern. Heat-treatment of all swill fed to pigs reduces the risk of an outbreak. Some countries have banned swill feeding altogether, due to difficulties in ensuring that adequate heat-treatment protocols are followed (CFSPH, 2007).

When an FMD outbreak occurs in a “free” country (area) the first requirement is making a prompt and accurate diagnosis, followed by prompt quarantines of affected index farms and aggressive trace back and trace forward of animal movements within the last 3-4 weeks of the event (Kitching *et al.*, 2005; USAHA, 2008). By estimating the age of clinical lesions observed on the infected animals, and inspecting the movement records of animals coming onto and off the farm, the veterinary epidemiological team may be able to establish the possible origin and potential spread of the outbreak (Kitching *et al.*, 2005). Efforts should be made to find the oldest lesions in the herd, and backdate the time of introduction. Aging of lesions is undertaken to determine the date of entry of the virus into the herd. As a guide, unruptured vesicles are 0 to 2 days old; newly ruptured vesicles with epithelial remnants are 1 to 3 days; ruptured vesicles without epithelium or fibrous healing are 3 to 10 days and open lesions with marked fibrous tissues are 7 days or more.

Quarantines and movement restrictions are instituted in the infected area with simultaneous stamping-out of the infected and in-contact animals, and cleansing and disinfection of affected premises, equipment and vehicles. Vaccination, as an additional and effective tool to contain the outbreak, may be used to reduce the spread of FMDV or protect specific animals, such as, those in zoological collections (Sutmoller *et al.*, 2003; CFSPH, 2007). The decision to use vaccination is complex, but, the selection of the optimal strategy to minimize disruption of the local and national socio-economic structure must be weighted against the expected duration of restriction and loss of export trade and markets, basing on technical considerations (Sutmoller *et al.*, 2003). In cases where stamping-out is done, infected carcasses must be disposed of safely by incineration, rendering, burial or other techniques. FMDV in milk from infected cows can be inactivated by heating to 100°C for more than 20 minutes. Slurry can be heated to 67°C for 3 minutes. Rodents and other vectors may be killed to prevent them from mechanically disseminating the virus. Wild birds, rodents, and invertebrates such as flies and ticks may all carry virus from one place to another. Rats may play a larger role in spread of the disease as they can become infected naturally and shed virus in their faeces and urine for some time; dust contaminated with infected rat urine or faeces might result in infection by inhalation. Good biosecurity measures should be practiced on uninfected farms to prevent entry of the virus. Humans who have been exposed to infected animals should avoid susceptible livestock for an overnight period of time after thorough shower and shampoo, change of clothing, and expectoration (CFSPH, 2007; OIE, 2010).

In general, depending on the nature of the outbreak, any of the following control strategies can be implemented: stamping-out of infected farms and direct potential contagious contacts; stamping-out of infected farms plus ring (circle) culling; stamping-out of infected farms plus ring or area vaccination followed by slaughter of all vaccinated animals ('suppressive' vaccination); stamping-out of infected farms plus ring or area vaccination ('protective' vaccination); and ring vaccination only without stamping-out of infected farms and slaughter of vaccinated animals (Sutmoller *et al.*, 2003).

The steps used in the UK and other European countries to control an FMD outbreak in 2001 through massive depopulation of affected, contact, and healthy non-exposed at risk animals (pre-emptive slaughter) are (for the most part) neither socially nor politically acceptable. The procedures taken by the Netherlands, Uruguay and Argentina, also in 2001, to control FMD outbreaks through massive vaccination demonstrated that it is possible to control an FMD

outbreak faster and with lower costs through the use of strategic vaccination campaigns, even with the current vaccines which are less than optimal (USAHA, 2008).

2.11. Foot and mouth disease in Uganda

FMD is endemic in Uganda and outbreaks occur annually (Mwiine *et al.*, 2010b). According to the OIE Code for Terrestrial Animal Diseases (OIE Code, 2010), the occurrence of FMDV infection is confirmed by detection of the virus in samples from one or more animals with or without clinical signs consistent with FMD or by detection of antibodies in samples from non-vaccinated animals showing clinical signs consistent with FMD. However, in Uganda since FMD is endemic, an outbreak is suspected when clinical signs consistent with FMD are observed in one or more susceptible animals and a suspected FMD outbreak is confirmed by detection of the FMDV in samples from one or more susceptible animals showing clinical signs consistent with FMD (Rutebarika C., personal comm., 2011). The FMDV was first isolated in Uganda in 1953 and since then, outbreaks occur frequently in livestock (Ayebazibwe, 2010). Up to 15-57 outbreaks were officially reported per year during the period 2001-2008 (Ayebazibwe *et al.*, 2010). The most important risk factor for consideration in FMD transmission in Uganda is the animal movements, such as, movements during Christmas period, and dry season periods (Ayebazibwe, 2010). Other reported risk factors include: proximity to wildlife, livestock husbandry systems and proximity to national borders (Ayebazibwe, 2010).

Approximately 4.6% of Uganda equivalent to 11,150 km² is occupied by ten National parks (wildlife conservation areas) inhabited by a vast number of wild ungulates, including, among others, impalas and buffalos freely roaming across National parks and international borders (Ayebazibwe *et al.*, 2010b). There is also free interaction between wild animals in the Conservation areas and the livestock, particularly cattle, both under ranch and extensive communal grazing systems in the adjacent human settlement areas (Ocaido *et al.*, 2006). This free interaction facilitates the sharing of infectious diseases, such as FMD, between the domestic and wild animals (Ocaido *et al.*, 2006).

Estimates from 73 FMD outbreaks in cattle and a few samples from apparently healthy Ugandan African buffalos collected between 1958 and 2000, indicated that 31% of the

infections were attributed to serotype O, 26% to A, 25% to SAT 2, 14% to SAT 1, 3% to C and 1% to SAT 3. Serotype C was last recorded in Ugandan cattle in 1971; while SAT 3 has never been isolated from Ugandan livestock (SAT 3 was isolated from the Ugandan African buffalo only). Serotype Asia 1 has never been recorded on the African continent (Ayebazibwe, 2010). The serological and molecular studies carried out in East Africa, Uganda inclusive, for the period 1932-2010 show that FMDV serotype O is currently the predominant serotype responsible for most FMD outbreaks in livestock in East Africa (Sangula, 2010) and particularly in Uganda (Mwiine *et al.*, 2010a). Serotype A has the highest genetic diversity with a wide distribution across East Africa, Uganda inclusive. All the SAT serotypes (SAT 1, SAT 2, SAT 3) do exist in circulation in East Africa, Uganda inclusive, however, SAT 2 is the most wide spread among the SAT serotypes (Sangula, 2010). Serotype C has most likely been eradicated from Africa and was last reported in Ethiopia in 1983 and in Kenya in 2004 (Mwiine *et al.*, 2010a).

The strategies for control of FMD in Ugandan livestock include a combination of vaccination, quarantine measures, public education, farm-level precautionary measures and in rare cases, slaughter of the affected cases (Ayebazibwe, 2010). However, the success of the vaccination efforts and animal movement restrictions to control outbreaks is severely impeded by lack of proper infrastructure and appropriate FMD vaccines matching the circulating FMDV field strains (Mwiine *et al.*, 2010a). The current outbreak strains for serotype O in East Africa, Uganda inclusive, are genetically distant from the vaccine strain (Sangula, 2010). Uganda mainly imports Kenyan and Botswana FMDV trivalent vaccines composed of serotypes O, SAT 1 and SAT 2. These FMD vaccines used in Uganda are not purified of non-structural proteins (NSP) (Mwiine *et al.*, 2010b).

Uganda has a passive disease surveillance system based on reporting of cases of outbreaks of FMD by the livestock owners to the local veterinarians who then report to the national disease control department. This information is maintained in a database at the national veterinary epidemiology unit. Due to various reasons including the remoteness of the villages, poor telecommunications in remote areas, lack of incentives for disease reporting both for the farmer and the extension staff, the endemic nature of the disease, and low mortality, many outbreaks may go unreported (Ayebazibwe *et al.*, 2010a).

3. MATERIALS AND METHODS

3.1 General description of the study area

The study was conducted in four districts (Kiruhura, Mbarara, Rakai, and Isingiro) surrounding Lake Mburo Conservation Area (LMCA), formerly called Lake Mburo National Park (LMNP), in the South-western region of Uganda. This area lies in the “cattle corridor” of Uganda. The cattle corridor is the area believed to have the highest livestock numbers and extending diagonally across Uganda, from the pastoralist Ankole area in the Southwest, touching Rwanda and Tanzania, to the Karamoja region bordering South Sudan and Kenya in the Northeast (Ayebazibwe, 2010). The study area is bordered by Kyenjojo, Mubende and part of Kamwenge districts to the North, Sembabule district to the North-east, Lyantonde and Masaka districts to the East, Ibanda district to the North-west, Ntungamo district to the West and the Republic of Tanzania to the South as shown in Figure 4.

The area, topographically, consists of undulating hills and valleys. The hills rise about 100 – 200m above the flat valley bottoms which are between 1250 – 1525m above sea level (Byenkya, 2007). The annual rainfall is between 760 – 1270mm. The rainfall is bimodal with peaks in the months of March-to-May and September-to-November. The major dry season is in the months of June, July and August (Byenkya, 2007).

Agriculture is the mainstay of the economy of the people in the area, mainly practicing subsistence crop and livestock keeping, but with several commercial farms and ranches. The total cattle population in the area, in the year 2008, was estimated at 952,426 (UBOS, 2009). This population is distributed in the area as 149,992 cattle in Mbarara, 342,315 cattle in Kiruhura, 180,345 cattle in Isingiro, and 279,594 cattle in Rakai district (UBOS, 2009). The Ankole is the predominant cattle breed in the area, however, exotic breeds, cross breeds and other local breeds are also kept (Mwebaze, 2006). The communal/pastoral grazing system predominates in the area, however, other grazing systems such as fenced dairy farms, ranching and mixed farming systems with food crops also exist (Mwebaze, 2006).

LMCA, which occupies an area of about 260km², is a home to a number of game animals including Impala (*Aepeyceros melampus*), Zebra (*Equus bruchelli*), Warthog (*Phacochoerus*

aethiopicus), Eland (*Taurotragus oryx*), Hippopotamus (*Hippopotamus amphibius*), Buffalo (*Syncerus caffer*), Bushbuck (*Tragelaphus scriptus*) and Waterbuck (*Kobus ellipsiprymnus*) and Topi (*Damaliscus lunatus jimella*) (Ocaido *et al.*, 2006). There is no buffer zone between LMCA and the surrounding communal grazing areas and ranches, thus, it is common to see numerous wild animals freely grazing and browsing together with livestock (Ocaido *et al.*, 2006; Rannestad *et al.*, 2006).

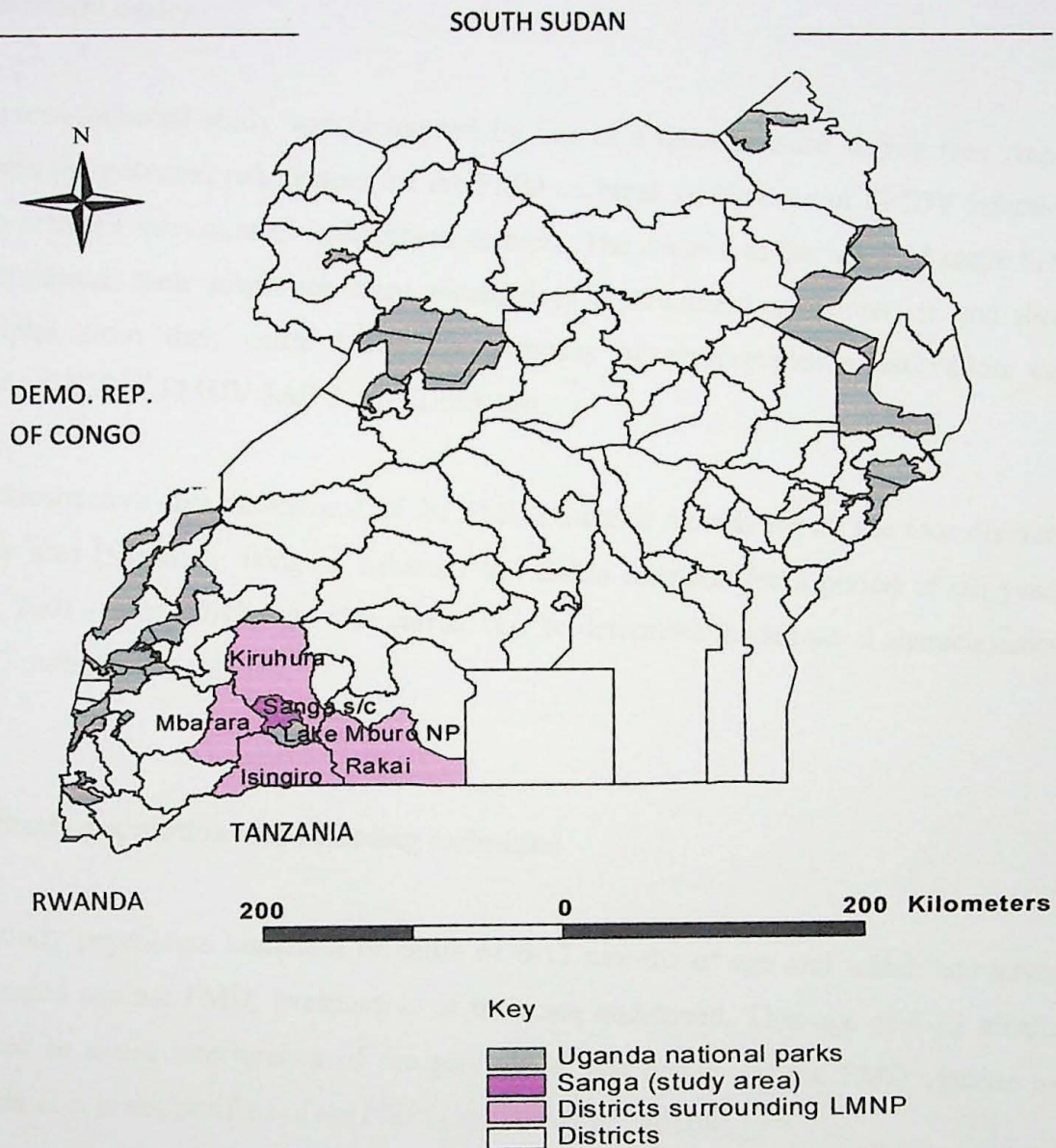


Figure 4: Map of Uganda showing the study area.

The sampling of animals for seroprevalence and associated risk factor determination was conducted only in Kiruhura district in Nyakashashara and Sanga Sub-counties. Assessment of the FMD outbreak records to determine the temporal characteristics of the FMD outbreaks was done for all the four districts bordering the LMCA (Kiruhura, Mbarara, Isingiro and Rakai).

3.2. Study design

A cross-sectional study was conducted by use of a questionnaire survey (see Annex 1) to assess the potential risk factors for the FMD outbreak occurrence or FMDV infection in the two selected sub-counties in Kiruhura district. The owners of the selected cattle herds were interviewed, their responses were recorded in a structured questionnaire and then blood samples from their cattle taken for purposes of seroprevalence estimation using the SVANOVIR™ FMDV 3ABC-Ab ELISA test.

A retrospective data assessment of the FMD outbreak records for all the four districts in the study area (Kiruhura, Isingiro, Mbarara and Rakai districts) for a period of ten years, from Jan., 2001 – Dec., 2010, was also carried out to determine the temporal characteristics of the FMD outbreaks in the area.

3.3. Study population and sampling technique

The study population consisted of cattle of 6-12 months of age and which had never been vaccinated against FMD, irrespective of their sex and breed. This age of 6-12 months was selected to avoid interference of the serological test results by the FMD vaccine used in Uganda as it is not purified of the NSPs (Mwiine *et al.*, 2010b).

A multistage sampling method was employed. Animals for blood sampling were selected only from Kiruhura district, Nyakashashara and Sanga sub-counties, due to the resource limitations of this study. Kiruhura district was purposively selected basing on the fact that it harbors most of the LMCA. Within Kiruhura district, two sub-counties were selected purposively basing on their geographical location adjacently to LMCA. Within each of the selected two sub-

counties, about 20% of the parishes were randomly selected to be included in the study (total of 3 parishes). From each of the selected parishes, 20% of the villages were randomly selected (total of 6 villages). From each of the selected villages 10% of the cattle herds were randomly selected. From each of the selected herds an attempt was made to randomly select 10% of the number of eligible animals in each herd. However, some herd owners with high numbers of eligible animal populations did not allow all the 10% of their eligible animals to be sampled. In addition to this, it was not possible, in some cases, to employ random selection of individual animals in the herd as some herds were sampled while grazing on open pastures. In summary, 2 sub-counties, 3 parishes, 6 villages, 41 herds (and 41 herd owners) and 404 animals were included in this study. FMD seroprevalence of 61% in Kasese district and 4% in Bushenyi district was reported in cattle (Mwiine *et al.*, 2010). Both Kasese and Bushenyi districts are located in the South-western region of Uganda and farmers in Kasese predominantly practice communal grazing as is the case for those in Kiruhura (study area) (Mwiine *et al.*, 2010b). Basing on this, the sample size was calculated by assuming an estimated FMD prevalence of 50% with a 95% confidence interval and a 5% desired precision using Thrusfield (2007). The formula below was applied (Thrusfield, 2007):

$$n = \frac{1.96^2 \times P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}$$

Where,

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

$$n = \frac{1.96^2 \times 0.5 (1 - 0.5)}{0.05^2} = 384 \text{ cattle}$$

3.4. Questionnaire survey

A total of 41 questionnaire copies (see annex 1) were administered to 41 herd owners to assess the potential risk factors for FMD seroprevalence and outbreak occurrence in the two selected sub-counties of Kiruhura district. The questionnaire was pre-tested before administering. The questionnaire was designed to assess the most important factors that could

be associated with FMD, including, interaction of livestock with wild animals; cattle breed; cattle herd migration; grazing herds within the conservation area; locality by sub-county; distance of farm from the conservation area; species composition of the herds; cattle herd size; husbandry system; FMD vaccination; and livestock movement to other villages/parishes in search of water or pasture. These factors were then assessed for their association with the occurrence of FMD infections/seroprevalence and FMD outbreak occurrence on the herds/farms.

During questionnaire survey, additional epidemiological information was recorded including: the farmers' perception on the sources of FMD outbreaks for their livestock; the wildlife species which commonly interact with their livestock and the reasons for the interaction; the seasonal period when wild animals interact with the farmers' livestock; the wildlife species which have ever been seen sick on or near the farmers' farms and the observed symptoms in the sick wildlife species sighted on or near the farmers' farms.

Following administration of the questionnaire to the herd owner, with his/her consent, blood samples were taken from his/her animals.

3.5. Blood sample collection and testing

3.5.1. Sample collection

Blood samples were collected from the two selected sub-counties of Kiruhura district. Whole blood was collected from either the jugular or the medial coccygial (tail) vein of randomly selected cattle into 10 ml sterile vacutainer tubes and stored overnight at room temperature (18-25°C) for serum separation. Each blood sample was then transferred into a sterile cryovial, bearing the initials of the names of the owner, village, herd code, breed and sex of the sampled animal. The samples were then transported in an icebox, to the serology laboratory at the National Animal Disease Diagnostics and Epidemiology Center (NADDEC), Entebbe, Uganda, for the laboratory investigation. In the laboratory, the serum was stored at -20°C until laboratory investigation.

3.5.2. Laboratory testing

The SVANOVIR™ FMDV 3ABC-Ab ELISA test was used to differentiate serological positive and negative animals. Controls and test sera were first diluted in 1: 40 concentrations using sample dilution buffer. The odd columns of the wells of the Microplate were pre-coated with FMDV 3ABC viral antigens, while the even columns of the wells were pre-coated with control antigens. Duplicates of 50µl of diluted controls and test sera were dispensed into the wells. The plates were sealed with lids and incubated for 30 min at 37°C. The incubated Microplates were rinsed three times by filling the wells with about 300µl of diluted Phosphate Buffered Saline (PBS) Tween buffer at 1: 20 concentration using distilled water. A bottle of lyophilized horseradish peroxidase (HRP) conjugate was reconstituted with 6 ml PBS-Tween buffer and after 1 min, 50µl of the reconstituted HRPconjugated anti-bovine IgG was added into the wells of the Microtitre plates. The plates were then covered and incubated for 30 min at 37°C. After washing the plates, 50µl of substrate solution was dispensed into wells of the Microtitre plates and incubated for 30 min at room temperature (18-25°C) in a dark place. The reaction was stopped by adding 50µl of stop solution to each well of the plate, and the content was mixed thoroughly. Finally, the optical densities (OD) were measured using air as blank and read at 405 nm filter in a Microplate photometer connected to a computer with appropriate software packages to automate the reading of the optical densities. The OD were measured within 15 minutes after the addition of stop solution. Calculations of the results were carried out in two steps as follows:

Step 1: The OD values in wells coated with FMDV NSP 3ABC viral antigens were corrected by subtracting the OD values of the corresponding wells containing the control antigen.

$$OD_{\text{NSP 3ABC}} - OD_{\text{control}} = OD_{\text{Corr}}$$

Step 2: All corrected OD values for the test sera as well as the negative control (Neg C) were related to the corrected OD value of the positive control as follows:

$$PP = \frac{\text{Test sera or Neg C } (OD_{\text{Corr}})}{\text{Positive Control } (OD_{\text{Corr}})} \times 100$$

Where PP is the percent positivity.

Test interpretation was conducted by cut-off value provided by the manufacturer (Svanova Biotech AB, Uppsala Science Park, SE-753 83 Uppsala, Sweden). Based on this,

$PP_{\text{Test sera}} < 48$ were taken as seronegative and $PP_{\text{Test sera}} \geq 48$ were seropositive.

3.6. Retrospective assessment of the FMD outbreak records

Veterinary record of officially reported FMD outbreaks in the four districts (Kiruhura, Mbarara, Isingiro and Rakai) surrounding LMCA was assessed for a period of 10 years from January 2001 to December 2010. The records were accessed from the Epidemiology Unit of the disease control section of the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Entebbe, Uganda. The data captured from these records included: the month and year of outbreak occurrence and the location of the outbreak by parish, sub-county and district. These data were used to determine the temporal characteristics of the FMD outbreaks in comparison with the long term average monthly rainfall patterns in the study area (the four districts). This was done to assess whether the occurrence of FMD outbreaks in the study area was related to the seasons of the year.

3.7 Data management and analysis.

The selected areas were first coded, and animals from each herd were given the same herd code, but different animal codes. All possible answers in the questionnaire were coded. Then, taking into account the different risk factors for the occurrence of FMD, data from the questionnaires was entered and stored in Microsoft Excel. Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 18 software. Descriptive statistics were computed. Prevalence was determined by dividing the number of positive serum samples (animals) by the total number of samples (animals) tested. A herd was considered positive if one or more serum samples from that herd tested positive. The within herd seropositivity was calculated by dividing the number of positive animals in an affected herd by the total number of animals tested in that herd.

The Chi-square test and odds ratio were used to analyze whether potential risk factors were significantly associated, statistically, with seropositivity of the individual animals/herds. The same statistical tests were also used to analyze whether potential risk factors were statistically significantly associated with outbreak occurrence on farms/herds. In all the analyses, confidence level was at 95% and $p \leq 0.05$ was set for significance. An on-line Bayesian calculator (www.causascientia.org/math_stat/ProportionCI.html) was used to calculate the confidence intervals.

The Chi-square test was run independently at two intervals: In the first case, seropositivity was considered as the dependent variable and the risk factors that would likely predict the outcome variable in this case were considered as independent variables. In the second case, FMD outbreak occurrence on the herd/farm (a herd having got an FMD outbreak in the last 10 years) was considered as the dependent variable and the risk factors as independent variables. The potential risk factors considered included: locality by Sub-county; distance of the farm from the Lake Mbuho Conservation Area (LMCA); species composition of the herds (only cattle or mixed with goats, sheep or pigs); herd size; husbandry system practiced on the farm; vaccination of the herds against FMD; movement of herds to other villages or parishes; herd migration to other districts; interaction with wildlife; grazing the herd within the conservation area (CA); and the cattle breeds kept on the herd.

Herd migration to other districts and interaction with wild animals, as potential risk factors, were dropped as all the 41 sampled herds did not have this practice of migrating to other districts and only one herd was reported not to have interaction with wild animals.

The retrospective data on FMD outbreaks in the study area was analyzed in Microsoft Excel, 2007, by plotting of line graphs of number of outbreaks against time period in relation to the rainfall patterns in the study areas.

4. RESULTS

4.1 Seroprevalence

The overall seroprevalence at individual animal level and herd level was 6.2% (n = 404) and 36.6 (n = 41), respectively. Tables 3 and 4 show the overall herd level seroprevalence and individual animal level seroprevalence, respectively, in the two selected sub-counties, three parishes and six villages in Kiruhura district.

The herd level seroprevalence was 32.0% (n = 25) in Nyakashashara and 43.8% (n = 16) in Sanga sub-county. The highest herd level seroprevalence among villages was observed in Nshara (100%, n = 1), followed by Rwabarata (62.5%, n = 8), Nyanga (40.0%, 5), Kachera (28.6%, n = 7), and the lowest in Akashenshero (25%, n = 12) and Rwamuhuku village (25%, n = 8). The highest herd level seroprevalence among parishes was observed in Nyakahija parish (100%, n = 1), followed by Rwabarata (43.8%, n = 16) and the lowest was in Rurambira parish (29.2%, n = 24) as shown in Table 3.

Table 3: FMD herd level seroprevalence distribution by the selected sub-counties, parishes and villages (Kiruhura district)

Sub-county	Parish	Village	No. of Herds Tested	No. of Herds +ve	Prev (%)	95% CI
Nyakash-ashara	Rurambira	Akashenshero	12	3	25.0	7.4 – 51.4
		Kachera	7	2	28.6	6.5 – 62.1
		Nyanga	5	2	40.0	10.5 – 76.1
	Nyakahija	Nshara	1	1	100	22.4 – 100
Sanga	Rwabarata	Rwabarata	8	5	62.5	31.5 – 87.6
		Rwamuhuku	8	2	25.0	5.4 – 56.8
Overall			41	15	36.6	23.2 – 51.6

The individual level seroprevalence in Nyakashashara and Sanga sub-counties was 7.2% (n = 207) and 5.1% (n = 197), respectively. Among villages, the highest individual animal level seroprevalence was observed in Nyanga village (14.3%, n = 35), followed by Nshara (8.0%, n = 25), Rwabarata (7.4%, n = 94), Kachera (6.1%, n = 66), Akashenshero (4.9%, n = 81) and the lowest was in Rwamuhuku village (2.9%, n = 103). Among the parishes, the highest individual animal level seroprevalence was observed in Nyakahija parish (8.0%, n = 25), followed by Rurambira (7.1%, n = 182) and the lowest was in Rwabarata parish (5.1%, n = 197) as shown in Table 4.

In general, the differences in the seroprevalence of FMD among the different administrative divisions (sub-counties, parishes and villages) were not statistically significant (p = 0.446) as shown in Tables 6 and 7.

Table 4: FMD individual animal level seroprevalence distribution by the selected sub-counties, parishes and villages (Kiruhura district)

Sub-county	Parish	Village	No. of Cattle Tested	No. of cattle+ve	Prev (%)	95% CI
Nyakashashara	Rurambira	Akashenshero	81	4	4.9	1.5 – 11.2
		Kachera	66	4	6.1	1.9 – 13.6
		Nyanga	35	5	14.3	5.4 – 28.1
	Nyakahija	Nshara	25	2	8.0	1.4 – 22.8
Sanga	Rwabarata	Rwabarata	94	7	7.4	3.3 – 13.9
		Rwamuhuku	103	3	2.9	0.7 – 7.5
Overall			404	25	6.2	4.1 – 8.8

The overall within herd seropositivity (on-farm seroprevalence) for the herds which tested positive ranged from 4.5 to 57.1%, with an average of 15.9%. The within herd seropositivity in Nyakashashara sub-county ranged from 5.0 to 57.1% while that in Sanga sub-county was from 4.5 to 20% as shown in Table 5.

Table 5: Within herd seropositivity (on-farm seroprevalence) for the herds which tested positive

Sub-county	Herd code	No. of Cattle tested	No. of Cattle +ve	Seropositivity (%)	95% CI
Nyakashashara	025	20	1	5.0	0.3 – 20.8
	024	25	2	8.0	1.4 – 22.8
	012	11	1	9.1	0.6 – 34.1
	019	10	1	10.0	0.6 – 36.8
	007	6	1	16.7	1.3 – 52.7
	016	11	2	18.2	3.6 – 45.0
	014	10	3	30.0	9.3 – 58.8
	022	7	4	57.1	25.4 – 85.1
Sanga	034	22	1	4.5	0.2 – 19.1
	026	15	1	6.7	0.3 – 26.6
	027	11	1	9.1	0.6 – 34.1
	039	17	2	11.8	2.1 – 31.8
	031	8	1	12.5	0.9 – 43.3
	028	10	2	20.0	4.1 – 48.4
	029	10	2	20.0	4.1 – 48.4

4.2. Association of potential risk factors with FMD seropositivity.

The results of the statistical analyses made for the association of seropositivity with potential risk factors at herd and individual levels are summarized in Tables 6 and 7, respectively.

Table 6: Association of potential risk factors with seropositivity at herd level

Risk factor	No. of Herds tested	No. of Herds +ve	Prev (%)	95% CI	χ^2	p-value
Locality of herd by Sub-county						
Nyakashashara	25	8	32.0	16.5 – 50.9	0.581	0.446
Sanga	16	7	43.8	22.6 – 66.6		
Distance of farm from the CA						
Less than 10 km	32	11	34.4	19.9 – 51.2	0.307	0.580
Between 10 and 50 km	9	4	44.4	18.2 – 73.2		
Species comp. of the herd						
Cattle only	20	6	30.0	13.6 – 50.9	0.730	0.393
Cattle, goats/sheep	21	9	42.9	24.0 – 63.2		
Cattle herd size						
Between 10 and 20	1	0	0.0	0.0 – 77.6	3.759	0.153
Between 21 and 50	15	3	20.0	5.7 – 43.1		
Greater than 50	25	12	48.0	29.8 – 66.5		
FMD vaccination practice on the herd.						
Vaccination not practiced on the herd.	2	2	100	36.8 – 100	3.644	0.058
Vaccination practiced on the herd	39	13	33.3	20.1 – 48.6		
Movement of herds to other villages/ parishes.						
Herds do not move	31	12	38.7	23.3 – 55.9	0.247	0.619
Herds move	10	3	30.0	9.3 – 58.8		
Grazing of herds within the CA.						
Not grazed within the CA	33	12	36.4	21.7 – 53.0	0.004	0.952
Herds grazed within CA	8	3	37.5	12.4 – 68.5		
Cattle breeds Kept on the farm/herd						
Local breeds only	28	9	32.1	17.3 – 50.0	2.921	0.232
Pure exotic breeds only	2	0	0.0	0.0 – 63.2		
Locals and crosses	11	6	54.5	28.1 – 79.3		

Table 7: Association of potential risk factors with seropositivity at individual animal level

Risk factor	No. of Cattle tested	No. of Cattle +ve	Prev (%)	95% CI	χ^2	P-Value
Locality by Sub-county						
Nyakashashara	207	15	7.2	4.2 – 11.3	0.819	0.365
Sanga	197	10	5.1	2.6 – 8.7		
Distance of farm from CA						
Less than 10 km	311	19	6.1	3.8 – 9.1	0.014	0.904
Between 10 and 50 km	93	6	6.5	2.6 – 12.7		
Species comp. of the herd						
Cattle only	174	8	4.6	2.1 – 8.4	1.332	0.249
Cattle, goats/sheep	230	17	7.4	4.5 – 11.2		
Cattle herd size						
Between 10 and 20	5	0	0.0	0.0 – 39.3	1.943	0.379
Between 21 and 50	107	4	3.7	1.2 – 8.5		
Greater than 50	292	21	7.2	4.6 – 10.5		
Cattle breed						
Local breed	325	20	6.2	3.9 – 9.1	1.498	0.473
Cross breed	62	5	8.1	3.0 – 16.5		
Exotic breed	17	0	0.0	0.0 – 15.3		
Husbandry system						
Communal grazing	249	15	6.0	3.5 – 9.4	0.197	0.906
Fenced/Paddocked	53	4	7.5	2.4 – 16.7		
Grazing separately	102	6	5.9	2.4 – 11.6		
FMD vaccination practice on the herd.						
Vaccination not practiced on the herd	19	2	10.5	1.9 – 28.9	0.646	0.421
Vaccination practiced on the herd	385	23	6.0	3.9 – 8.6		
Herds moving to other villages/parishes						
Herds do not move	289	18	6.2	3.8 – 9.4	0.003	0.958
Herds move	115	7	6.1	2.7 – 11.5		
Herd grazing within the CA.						
Not grazed within CA	304	18	5.9	3.6 – 9.0	0.151	0.698
Grazed within CA	100	7	7.0	3.1 – 13.1		

Analysis of the potential risk factors considered under this study showed that there was no statistically significant difference ($p > 0.05$) in FMD seropositivity at both herd and individual animal levels, between herds located in Nyakashashara sub-county and those in Sanga; herds located $< 10\text{km}$ from LMCA and those $10 - 50\text{km}$ away; herds composed of cattle only and those of a mixture of cattle and goats/sheep; herds with 10- 20, 21-50 and >50 cattle; herds which move to other villages/parishes in search of pasture and those which do not; herds grazed within and those not grazed within LMCA; and herds consisting of local breeds only, pure exotics and a mixture of locals and crosses. There was no statistically significant difference in FMD seropositivity in herds/farms and individual animals between herds on which FMD vaccination was practiced and those on which vaccination was not practiced. There was no statistically significant difference in FMD seropositivity between individual animals of the local, cross and pure exotic breeds; and individual animals under communal, fenced/paddocked and separately grazed herds as husbandry practices.

Association of potential risk factors with FMD outbreak occurrence in herds in the two selected sub-counties was also similarly analyzed and the results were as summarized in Table 8. In this case, the factors considered included: the locality by sub-county; distance of the farm from LMCA; species composition of the herd; herd size; husbandry system; and FMD vaccination practice in the herd. There was no statistically significant difference ($p > 0.05$) in FMD outbreak occurrence on farms/in herds between cattle herds located $< 10\text{km}$ and those $10 - 50\text{km}$ away from LMCA; herds composed of cattle only and those composed of a mixture of cattle and goats/sheep; herds with 10-20, 21-50 and > 50 cattle; and herds/farms on which vaccination was practiced and those on which vaccination was not practiced.

However, there was a statistically significant difference ($p = 0.001$) in the occurrence of FMD outbreaks between the farms/herds located in Sanga sub-county and those in Nyakashashara sub-county. There was also a statistically significant difference ($p = 0.010$) in the occurrence of FMD outbreaks among herds/farms under communal, fenced/paddocked and separately grazed household herds as husbandry systems practiced on the farm as shown in Table 8. Herds located in Nyakashashara sub-county were more likely to experience FMD outbreaks than those in Sanga sub-county. Up to 84% ($n = 25$) and 31% ($n = 16$) of the herds in Nyakashashara and Sanga Sub-counties, respectively, had experienced at least one FMD outbreak in the period between January, 2001 and June, 2011 as shown in Table 8.

Table 8: Association of potential risk factors with FMD outbreak occurrence (experience) in herds/on farms for the period January 2001 to June 2011

Risk factor	No. of Herds sampled	Herds with FMD out- break Experience			χ^2	P-value
		No.	Percent (%)	95% CI		
Locality by Sub-county						
Nyakashashara	25	21	84.0	67.0 – 94.6	11.701	0.001
Sanga	16	5	31.3	13.1 – 54.5		
Distance from CA						
Less than 10 km	32	20	62.5	45.6 – 77.5	0.053	0.819
Between 10 -50 km	9	6	66.7	36.7 – 89.3		
Species comp. of the herd						
Cattle only	20	13	65.0	43.9 – 82.7	0.042	0.837
Cattle, goats/sheep	21	12	57.1	36.8 – 76.0		
Cattle herd size						
Between 10 and 20	1	1	100	22.4 – 100	1.461	0.482
Between 21 and 50	15	8	53.3	30.1 – 75.6		
Greater than 50	25	17	68.0	49.1 – 83.5		
Husbandry system						
Communal grazing	27	21	77.8	60.4 – 90.3	9.130	0.010
Fenced/Paddocked	3	0	0.0	0.0 – 52.7		
Grazing separately	11	5	45.5	20.7 – 71.9		
Vaccination against FMD						
Not practiced	2	0	0.0	0.0 – 63.2	3.644	0.058
practiced	39	26	66.7	51.4 – 79.9		

Determination of the odds ratio showed that herds in Nyakashashara sub-county were at 2.7 times higher risk of getting an FMD outbreak than those in Sanga sub-county as summarized in table 9. Basing on the husbandry system practiced on the farm, it was observed that 77.8% (n = 27), 45.5% (n = 11) and 0.0% (n = 3) of the herds under the communal grazing, grazing household herds separately and fenced/paddocked grazing systems, respectively, had experienced at least one FMD outbreak in the period between January, 2001 and June, 2011 as shown in Table 9. Determination of the odds ratio showed that herds in the communal grazing husbandry system were at 4.2 times higher risk of having FMD outbreak than those under the “grazing household herd separately”.

Table 9: Risk estimate for Sub-county locality

	Value	95% CI	P-value
Odds Ratio for sub-county (Nyakashashara/ Sanga)	0.087	0.019 – 0.389	0.001
For cohort FMD OB. = No	0.233	0.089 – 0.606	
For cohort FMD OB. = Yes	2.688	1.274 – 5.671	
N of Valid Cases	41		

4.3. Additional epidemiological information captured during the questionnaire survey

In order to generate more information about the epidemiology of FMD in the study area, farmers were asked additional questions during the questionnaire survey (Annex 1).

Farmers' perceptions on the sources of FMD outbreaks on their farms

Farmers were asked to mention the sources of FMD outbreaks on their farms. About 51% (n = 41) of the farmers mentioned infected wild animals and infected cattle; 29.3% mentioned contaminated drinking water for livestock; 19.5% wind; 4.9% infective pastures and 2.4% mentioned people, as the source of FMD outbreaks on their farms. The responses were as summarised in Table 10.

Table 10: Farmers' perceptions on the sources of FMD outbreaks on their farms

FMD infection source	No. of farmers	% of farmers
Infected Wildlife	21	51.2
Infected cattle	21	51.2
Contaminated drinking water for livestock	12	29.3
Wind	8	19.5
Infective pastures	2	4.9
People	1	2.4

Wild animal species which commonly come in close contact with the farmers' livestock

All farmers (100%) mentioned the impala and zebra as the major species which commonly come in close contact with their livestock. Other species included the African buffalo, Eland, topi, warthog, hippopotamus, bushbuck and waterbuck as shown in Table 11.

Table 11: Farmers' responses on the wild animal species which commonly come in close contact with their livestock

Species	No. of farmers	% of farmers
Impala	41	100.0
Zebra	41	100.0
African buffalo	26	63.4
Eland	15	36.6
Topi	13	31.7
Warthog	11	26.8
Hippopotamus	9	22.0
Bushbuck	6	14.6
Water buck	4	9.8

Seasonal period during which livestock come into close contact with wild animals

All the 41 farmers interviewed mentioned that wild animals come into close contact with their livestock any time. 97.6 % said that this contact occurs outside the conservation area and 19.5% said it occurs within the conservation area. The percentages overlap because other farmers mentioned that the contact occurs anytime both outside and within the conservation area. The responses were as summarised in Table 12.

Table 12: The seasonal period during which livestock come into close contact with wild animals

Period	No. of farmers	% of farmers
Wet season within the CA	0	0.00
Wet season outside the CA	0	0.00
Dry season when livestock go to the CA	0	0.00
Dry season when wildlife leave the CA to the farm land.	1	2.40
Any time within the CA	8	19.50
Any time outside the CA	40	97.60

Reasons why livestock get into close contact with wild animals

About 95.1% (n = 41) of the farmers mentioned the close proximity and lack of a barrier as the major reason for their livestock coming in close contact with wild animals. Other reasons included: wild animals searching for water or pasture, wild animals looking for the commercially provided salt licks at farmers' kraals, wild animals looking for the naturally existing salt licks in the ranches adjacent to the conservation area, farmers grazing their cattle within the conservation area and wild animals straying out of the conservation area due to fire outbreaks in the conservation area as shown in Table 13.

Table 13: Reasons why livestock get into close contact with wild animals

Reason	No. of farmers	% of farmers
Close proximity and lack of a barrier between the farm land and the CA.	39	95.1
Wild animals searching for water or pasture	28	68.3
Wild animals look for the commercially provided salt licks at farmers' kraals.	6	14.6
Wild animals look for the naturally existing salt licks in the ranches adjacent to the CA.	3	7.3
Farmers grazing their cattle within the CA.	2	4.9
Wild animals straying out of the CA due to fire outbreaks in the CA.	1	2.4

Wild animal species which had ever been seen sick and the symptoms observed

Thirty one farmers (75.6%; n = 41) had ever sighted and 10 farmers (24.4%, n =41) had never sighted sick wild animals on or near their farms. The 31 farmers who had ever seen, the species they had ever sighted sick on/near their farms included the impala, zebra, buffalo, eland, bushbuck and warthog as shown in Table 14.

Table 14: Wild animal species which had ever been seen sick on or near the farmers' farms

Species seen sick	No. of farmers	% of farmers
Impala	18	58.1
Zebra	16	51.6
Buffalo	15	48.4
Eland	6	19.4
Bush buck	2	6.5
Warthog	2	6.5

The symptoms observed by the 31 farmers among the wild animal species were grouped into four, including: limping of impala; weakness/inappetance of Impala, Eland, Buffalo or Zebra; emaciation of impala, bushbuck, eland or warthog; and salivation in eland or impala as summarized in Table 15.

Table 15: Symptoms observed by farmers in the sick wild animals seen on or near their farms

Symptom	No. of farmers	% of farmers
Limping of Impala	18	58.1
Weakness/Inappetance of Impala, Eland, Buffalo or Zebra.	10	32.3
Emaciation of Impala, Bushbuck, Eland or Warthog.	2	6.5
Salivation in Eland or Impala	1	3.2

Vaccination frequency against FMD

Most farmers (82%, n = 41) were vaccinating their cattle only in response to occurrence of outbreaks in the area. Only one farmer, a Government ranch, was vaccinating twice a year and had never experienced FMD outbreak on his farm/ranch since 1991, despite its being directly adjacent to the conservation area. Other farmers (4.9%) never vaccinated their cattle at all while others (9.8%) vaccinated once every year as shown in Table 16.

Table 16: Vaccination frequency against FMD

Vaccination frequency	Number of farmers	% of farmers
In response to outbreaks	34	82.9
Once a year	4	9.8
Never at all	2	4.9
Twice a year	1	2.4

4.4. Temporal characteristics of the FMD outbreaks in districts around LMCA.

The temporal characteristics of the FMD outbreaks in the four districts of the study area (Mbarara, Kiruhura, Isingiro and Rakai district) were assessed for a period of ten years, from 2001- 2010. Kiruhura and Isingiro districts were originally part of Mbarara district, but were curved out to form independent districts in 2003. The data source for this study was the information generated and contained in the annual reports from the districts to MAAIF, Epidemiology unit, Entebbe. The analysis is therefore based upon the officially reported cases of FMD outbreaks for the study period.

In all, a total of 44 outbreaks were reported from the four districts over the 10 years period. The annual occurrence of the reported outbreaks in the study areas was as shown in Figure 5, while Table 17 shows the distribution of the outbreaks per month per year. The FMD outbreak pattern in the study area was marked by regular fluctuations in the frequency of the disease outbreaks both within and over the years. The number of outbreaks per year increased steadily from 2001 to peak level in 2004, it then dropped almost steadily from 2004 to 2007

(only 1 outbreak in 2007), it again went to peak level in 2008 and dropped steadily from 2008 to 2010.

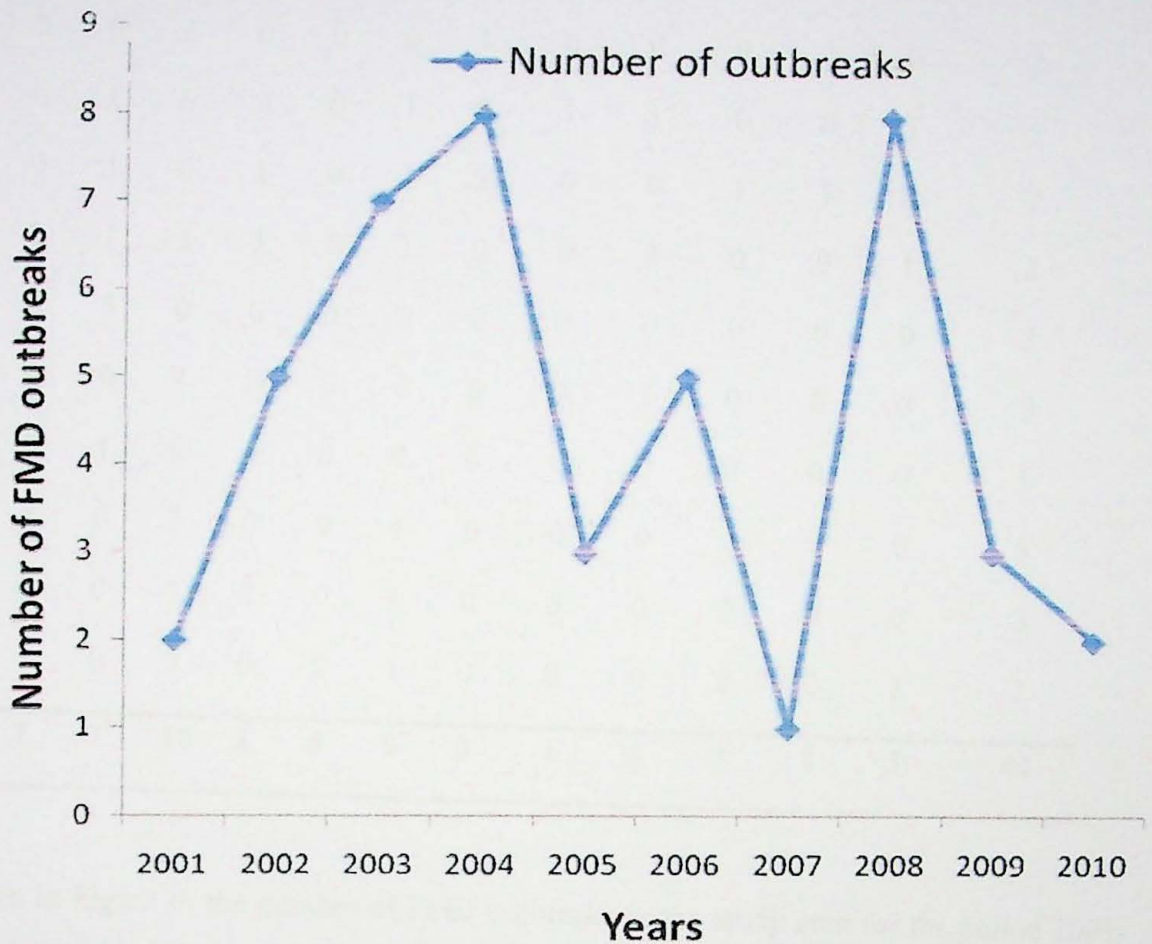


Figure 5: Distribution of FMD outbreaks in the different years, 2001 - 2010

The highest number of outbreaks was reported in 2004 and 2008, whereby 8 outbreaks occurred in each year. The 8 outbreaks in 2004 occurred in the months of January, February, March, April, June, September, and December; while those of 2008 occurred in the months of January, March and June. The lowest number of outbreaks was in 2007 where only one outbreak occurred in February for the whole year in all the four study area districts as shown in Figure 5 and Table 17.

Table 17: Distribution of FMD outbreaks per month per year, 2001 – 2010

Year	Number of outbreaks per month												Total
	Ja	Fe	Ma	Ap	Ma	Ju	Ju	Au	Se	Oc	No	De	
2001	0	0	1	0	0	0	1	0	0	0	0	0	2
2002	0	1	0	0	0	1	0	1	1	0	0	1	5
2003	0	1	0	1	0	1	2	0	0	1	1	0	7
2004	1	1	1	1	0	1	0	0	2	0	0	1	8
2005	0	3	0	0	0	0	0	0	0	0	0	0	3
2006	0	0	4	0	0	0	0	0	1	0	0	0	5
2007	0	1	0	0	0	0	0	0	0	0	0	0	1
2008	1	0	3	0	0	4	0	0	0	0	0	0	8
2009	1	0	1	0	0	1	0	0	0	0	0	0	3
2010	0	0	0	0	0	1	0	0	0	0	0	1	2
Total	3	7	10	2	0	9	3	1	4	1	1	3	44

As shown in Figure 6, the number of FMD outbreaks in the study area for the period 2001-2010 raised steadily from the month of January to reach peak level in March. In this area, the average rainfall also increases almost steadily from the month of January to reach its first peak in April. As the rainfall reaches peak level in April, the number of FMD outbreaks drops drastically from the March peak to zero in May (rainy season) when the average rainfall is still high. As the average rainfall drastically decreases to minimum levels (dry season) in the area in the months of June and July, the number of outbreaks rapidly increases to reach a second peak in the months of June-July. The number of outbreaks then decreases from the peak in July to very minimum levels in the months of October and November, as the rainfall also reaches its second peak in October and November. From the month of November to December, the number of outbreaks slowly starts rising as the rainfall also drops. Therefore, the FMD outbreaks are related to the seasons in the study area.

The highest numbers (peaks) of FMD outbreaks happened in the months of March (following the dry season of January - February) and June –July (the major dry period). The outbreaks decreased from the dry season periods to the rainy season months with the lowest numbers observed in the months of October – November (peak rainy months) and no outbreak occurred in the month of May (rainy month) for the whole 10 year period. The average monthly rainfall estimates for South-western Uganda (Byenkya, 2007), where the study area falls, were analysed in relation to the monthly variation of the FMD outbreaks in the study area as shown in Figure 6.

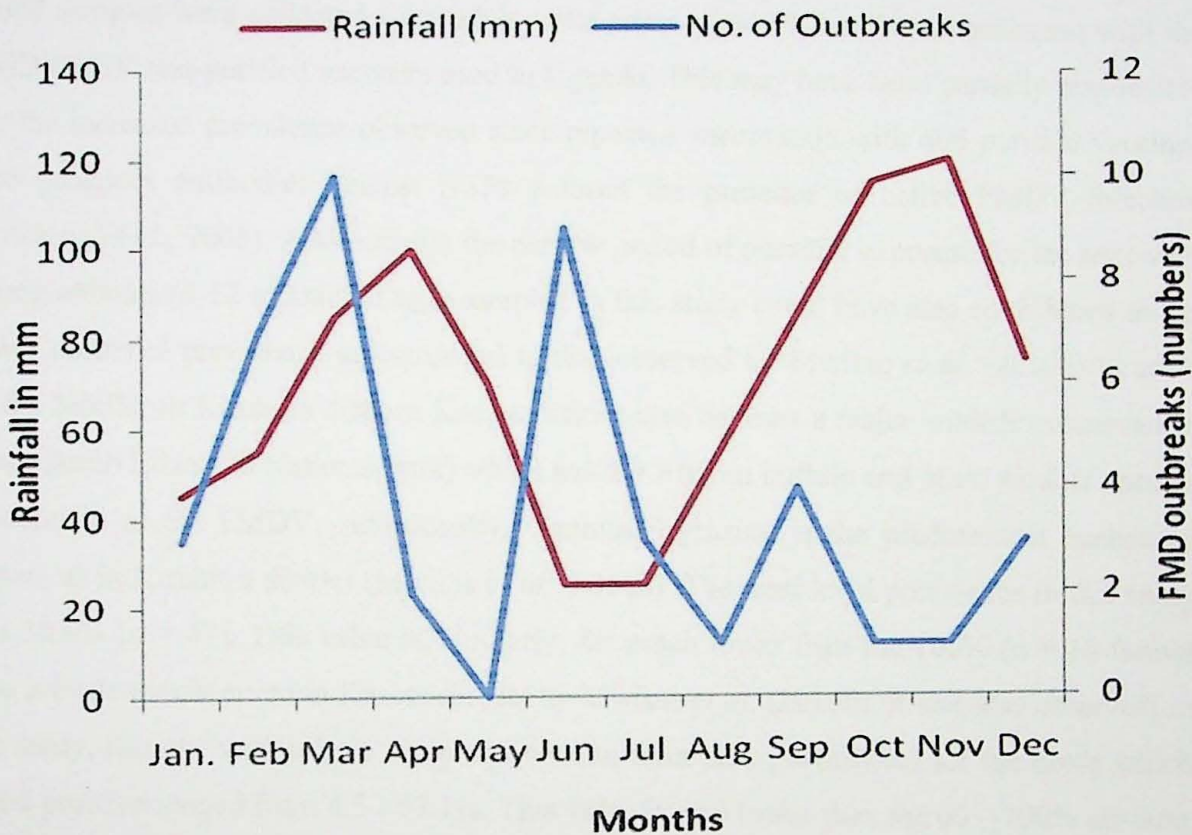


Figure 5: Monthly reported FMD outbreaks in the four districts, 2001-2010 and long term average monthly rainfall estimates for South-western Uganda

5. DISCUSSION

All the herds sampled were free from clinical FMD and there was no report of FMD outbreak in the two sampled sub-counties of Kiruhura district during the sampling period. In this study, the overall individual animal level seroprevalence of antibodies against FMDV NSPs in the 6-12 months old non-vaccinated cattle was 6.2% ($n = 404$). This value is much lower than that observed by Mwiine *et al.* (2010b) of 61% ($n = 193$) for Kasese district in South-western Uganda. This difference might be due to the fact that, in the study by Mwiine *et al.* (2010b), blood samples were collected from adult cattle some of which had been vaccinated with the FMDV NSP non-purified vaccines used in Uganda. This may have been partially responsible for the increased prevalence observed since repeated vaccination with non-purified vaccines also produces antibodies against NSPs without the presence of active FMDV infection (Kitching *et al.*, 2005). Additionally, the narrow period of possible exposure for the relatively young animals (6-12 months of age) sampled in this study could have also contributed to the lower observed prevalence as compared to that observed by Mwiine *et al.* (2010b) in adult cattle. Similar to Kiruhura district, Kasese district also harbours a major wildlife conservation area (Queen Elizabeth National Park) which has the African buffalo and other wildlife species susceptible to the FMDV. Additionally, communal grazing is the predominant husbandry system as in Kiruhura district (Mwiine *et al.*, 2010b). The herd level prevalence in this study was 36.6% ($n = 41$). This value is, similarly, far much lower than the 100% ($n = 16$ farms) herd prevalence observed in Kasese district by Mwiine *et al.* (2010b). It was also observed, in this study, that the within herd seropositivity (on-farm sero-prevalence) for the herds which tested positive ranged from 4.5 - 57.1%. This value is also lower than the 60 – 100% on-farm level prevalences for antibodies against FMDV NSPs reported by Mwiine *et al.*, (2010b).

The detection of antibodies against FMDV NSPs in 36.6% herds and 6.2% individual animals of 6-12 months of age is an indication of a recent-past or present infection with any of the six serotypes of FMDV, except Asia 1, that have been reported to occur in Uganda. The existence of recent-past and/or present infections by the FMDV in the sampled cattle herds, without having expressed clinical signs of FMD, could be due to continuous circulation of FMDV in the cattle population of the study area even during periods when outbreaks (clinical disease) are absent. The SVANOVIRTM FMDV 3ABC-Ab ELISA test employed in this study is one of the FMDV NSP antibody tests used to detect past or present infection by any of the seven

FMDV serotypes (Bronsvort *et al.*, 2008 and OIE, 2010). Antibodies to the FMDV NSPs are expressed in the host animal only when virus replication occurs (Kitching *et al.*, 2005). These neutralizing antibodies against FMDV NSPs can be detected in cattle and pigs for up to 1-2 years after experimental infection (Parton *et al.*, 2009 and Molla *et al.*, 2010). Maternal antibodies in calves from vaccinated cows wane at the age of 5 – 6 months, thus, by 6 months these calves are free of maternal antibodies against FMDV (Doel, 2003 and Radostits *et al.*, 2006). Additionally, none of the 41 herds tested showed clinical signs of FMD yet 6.2% of the individual tested animals were positive for antibodies against NSPs of FMDV. This may have been due to innate resistance of the indigenous breeds to FMDV as a result of continuous exposure to small doses of the FMDV, whereby, depending on the serotype and strain of the infecting virus, the infected animal may not develop overt clinical disease, but remain asymptomatic and eventually eliminate the virus completely from its body or may become persistently infected with no clinical signs (Davies, 2002; Grubman and baxt, 2004). FMDV NSP antibody tests cannot differentiate between the following three groups of animals: Animals without clinical signs but newly (≤ 28 days) infected, persistently infected animals and animals which have been recently infected but eliminated the virus (Kitching *et al.*, 2007). A persistently infected (carrier) animal is one in which the FMDV persists in the oropharynx for more than 28 days after infection (Grubman and baxt, 2004; OIE, 2010).

It has been reported that in Kruger National Park (KNP), South Africa, more than 80% of the African buffalo calves develop antibody to SAT1-3 by 12 months of age (Vosloo *et al.*, 2005; Vosloo *et al.*, 2007). In comparison with the cattle in the sampled sub-counties of Sanga and Nyakashashara, Kiruhura district, Uganda, which have direct or indirect contact with the African buffalos in LMCA, the antibody prevalence in the sampled cattle of 6-12 months of age, in this study, was lower (6.2%).

Antibodies against FMDV sero-types: SAT 1, SAT 2 and SAT 3 were identified in the African buffalo in the Ugandan National Parks (Ayebazibwe *et al.*, 2010b) as well as SAT 1 and SAT 3 in cattle in Uganda (Mwiine *et al.*, 2010a) suggesting that African buffalos in the different National Parks in Uganda may play an important role in the epidemiology of SAT serotypes of FMDV. However, determining the FMDV serotypes involved in the detected seropositive reactor cattle was beyond the scope of this study. Further studies in this locality should involve serotyping and phylogenetic analysis of isolated viruses from the African

buffalos and cattle to assess the possible sharing of FMD infections between cattle and wildlife, especially the African buffalo.

This study showed that, although the herd level seroprevalence for herds from Sanga sub-county (43.8%; $n = 16$) was higher than that of herds from Nyakashashara sub-county (32%; $n = 25$), the difference was not statistically significant ($p > 0.05$). It was similarly observed that there was no statistically significant difference ($p > 0.05$) in seropositivity between individual animals from Sanga and those from Nyakashashara sub-county. This could be due to the fact that both sub-counties are adjacent to LMCA with similar husbandry systems and livestock interact with wild animals in both, giving almost a similar exposure risk to FMDV at herd and individual animal levels in both sub-counties. The presence of frequent close contact between the impala and livestock in all the farms/herds sampled could be responsible for the observed non significant difference in the herd level seroprevalence between herds located in Nyakashashara sub-county and those in Sanga; herds located less than 10 km from LMCA and those 10 -50 km away; herds which move to other villages/parishes in search of pasture and those which do not; herds grazed within and those not grazed within LMCA; and herds/individual animals under the communal, fenced/paddocked and separately grazed herds as husbandry practices. This is because the impala can jump over the fences and interact with livestock in the fenced farms in the area as reported by the farmers during the questionnaire survey. In this study, all herd owners (100%; $n = 41$) of the herds sampled mentioned that their livestock commonly came in close contact with the Impala and Zebra; and 63% said that close contact also occurred with the African buffalo. Rannestad *et al.* (2006) observed that Impala, zebra, bushbuck and waterbuck had significantly higher densities on the dairy ranch adjacent to LMCA, Uganda, compared to the conservation area itself. Impala, bushbuck and waterbuck are susceptible to FMD, with the impala being the most susceptible, and the direction of transmission between these wild ungulates and livestock is possible either way (Vosloo *et al.*, 2005). Furthermore, in this study, 58% of the farmers had ever sighted sick impala on their farms with the FMD suggestive symptom of limping. The impala, in addition to the African buffalo, are important in maintaining and propagating FMD (Vosloo *et al.*, 2009). However, in a similar study, Molla *et al.* (2010) observed significant differences in FMD herd level seroprevalence in South Omo Zone, Ethiopia, between herds located < 10 km, 10–200 km and those herds located beyond 200 km from the wildlife conservation areas (National parks/wildlife sanctuaries).

The herd level seroprevalence for herds composed of only one species, cattle, (30.0%; n = 20 herds) was lower than that of herds composed of multiple species, cattle and goats/sheep (42.9%; n = 21 herds). This difference was, however, not statistically significant ($p > 0.05$). It is known that, unlike carrier animals, acutely infected animals that do not develop clinical disease (sub-clinically infected) may be highly contagious and able to disseminate infection (Sutmoller and Olascoaga, 2002). Although sheep and goats tend to be sub-clinically infected (Sutmoller and Olascoaga, 2002), infection of these species in this area cannot exist without infection of cattle since cattle are the predominant species kept in the area and are known to be more extremely sensitive to FMDV infection by the respiratory route than the sheep/goats (FAO, 2002; Sellers and Gloster, 2008). Sheep and goats can develop persistent infection (carrier status) for 5-12 months but evidence of FMDV transmission from carrier sheep or goats has never been demonstrated (Sutmoller and Olascoaga, 2002). Therefore, in this study area, the presence of goats and/or sheep on the same farm/herd with cattle plays a negligible role in FMDV infection of cattle on the same herd/ farm. A similar observation was made in Bench Maji zone, South-western Ethiopia whereby a lower prevalence (6.06%, n = 99 cattle) in herds composed of cattle only was observed compared to the prevalence in herds composed of cattle and goats/sheep (15.52%, n = 174 cattle) but the difference was also not statistically significant (Gelaye *et al.*, 2009). However, in another study, carried out in southern Ethiopia, herds composed of cattle and goats/sheep had higher odds (5.1 times), of being FMD seropositive, than those composed of cattle alone (Megersa *et al.*, 2009).

Herd level seroprevalence of 0.0% (n = 1), 20% (n = 15) and 48% (n = 25) was observed, in this study, for the small (1- 20 cattle), medium (21-50 cattle) and large (> 50 cattle) herds, respectively. The differences in seroprevalence were not statistically significant ($p > 0.05$). FMD is readily spread by direct contact between infected and susceptible animals and this direct contact is promoted by high stocking densities and animal crowding and congregation (Sutmoller *et al.*, 2003). About 77.8% of the farmers in the study area, including those with small herds, practice communal grazing which involves watering their cattle at communal water dams where various herds meet and congregate. Farmers with small and medium herds are reluctant to construct privately owned water dams as do those with large herds. The small, medium and large herds are, thus, subjected to a similar risk of acquiring the infection during the daily communal grazing and watering. This could be the reason for the non-significant difference in the herd level seroprevalence between the small, medium and large herds. A similar study carried out in Bench Maji zone, Southern Ethiopia, observed no significant

difference in seroprevalence rates between the small (1-15 cattle), and medium (16-30 cattle) herds, but significant differences were observed between the large (> 30 cattle) and small herds; and between the large and medium herds (Gelaye *et al.*, 2009).

The herd level seroprevalence for herds in which vaccination was not practiced was higher (100%, n = 2 herds) than that for herds in which vaccination against FMD was practiced (33.3%, n = 39). Also at the individual animal level, the seroprevalence for cattle from herds in which vaccination was not practiced was higher (10.5%, n = 19 cattle) than that for cattle from herds in which vaccination was practiced (6%, n = 385 cattle). The difference in seroprevalence between herds in which vaccination was practiced and those in which it was not practiced, both at herd and individual animal level, was not statistically significant ($p > 0.05$). Vaccination of cattle, sheep or pigs (including infected ones) is known to induce protection and reduce the amount and duration of virus shedding and transmission to susceptible animals following natural infection of the vaccinated animals (Golde *et al.*, 2005; Orsel *et al.*, 2005 and USAHA, 2008). Thus, since only non-vaccinated animals were sampled, those from herds in which vaccination is practiced would be expected to give a significantly lower seroprevalence. However, in this area, animals are predominantly communally grazed. In addition to this, the impala and other wild susceptible ungulates known to be involved in FMD transmission commonly have close contact with livestock. Thus, un-vaccinated susceptible animals from herds which practice vaccination end up having close contact with un-vaccinated animals from other herds which do not practice vaccination in addition to the wild ungulates. This phenomenon gives almost similar exposure risk for animals from herds practicing and those not practicing vaccination.

Since vaccination is the major control measure in Uganda, this calls for a compulsory annual vaccination of all cattle in the area for a predetermined radius of about 40km from LMCA, as this study shows that individual farmers who practice vaccination still remain with a similar risk, for their young un-vaccinated animals, as those who do not vaccinate.

Herd and individual animal level seroprevalence of 0.0% (n = 2 herds) and 0.0% (n = 17 cattle); 32.1% (n = 28 herds) and 6.2% (n = 325 cattle); and 54.5% (n = 11 herds) and 8.1% (n = 62 cattle) were observed for herds and individuals of the pure exotic breeds only; herds and individuals of the local breeds only; and herds of a mixture of local and cross breeds and individuals of the cross breeds, respectively. The differences in seroprevalence among the

different breeds at herd and individual levels were found to be statistically not significant ($p > 0.05$). Though non-significant statistically, the results showed that animals from herds with pure exotic cattle had not been exposed to live FMDV. It is possible that the less number of herds with pure exotics sampled (2 herds) contributed to the 0.0% seroprevalence. However, a study conducted by Negussie *et al.* (2011) in three regional states of Ethiopia: Amhara, Oromia, and Addis Ababa, observed significant differences among the individual cattle seroprevalence for the cross breeds (28.3%, $n = 46$), indigenous breeds (42.9%, $n = 415$) and exotic breeds (80.0%, $n = 35$).

Analysis of the potential risk factors revealed that there was no statistically significant difference ($p > 0.05$) in FMD outbreak occurrence on farms/in herds between cattle herds located < 10 km and those 10 – 50 km away from LMCA. This could be due to presence of frequent close contact between livestock and wild ungulates, such as the impala, in all the farms/herds sampled irrespective of their location in relation to the distance from LMCA.

It was also observed that there was no statistically significant difference ($p > 0.05$) in FMD outbreak occurrence on farms/in herds between herds composed of cattle only and those composed of a mixture of cattle and goats/sheep. This could be due to the fact that, although sheep and goats tend to be sub-clinically infected (Sutmoller and Olascoaga, 2002), infection of sheep/goats in this area cannot exist without infection of cattle since cattle are the predominant species kept in the area and are known to be more extremely sensitive to FMDV infection by the respiratory route than the sheep/goats (FAO, 2002; Sellers and Gloster, 2008). Sheep and goats can develop persistent infection (carrier status) for 5-12 months but evidence of FMDV transmission from carrier sheep or goats has never been demonstrated (Sutmoller and Olascoaga, 2002). Therefore, in this study area, the presence of goats and/or sheep on the same farm/herd with cattle plays a negligible role in FMD outbreak occurrence on the farm/herd.

There was no statistically significant difference ($p > 0.05$) in FMD outbreak occurrence on farms/in herds between the small (10-20 cattle), medium (21-50 cattle) and large (> 50 cattle) herds. This could be due to the fact that the small, medium and large herds are, in this area, are subjected to similar risks of acquiring the infection during the daily communal grazing and watering. About 77.8% of the farmers in the study area, including those with small herds, practice communal grazing which involves watering their cattle at communal water dams

where various herds meet and congregate. Farmers with small and medium herds are reluctant to construct privately owned water dams as do those with large herds.

There was no statistically significant difference ($p > 0.05$) in FMD outbreak occurrence on farms/in herds between herds/farms on which vaccination was practiced and those on which vaccination was not practiced. Outbreaks in this area were reported by farmers to be associated with the influx of cattle from Tanzania. These outbreaks could, therefore, be as a result of incursions of new FMDV strains, mainly from Tanzania by infected cattle, since FMDV is continuously circulating in the area. Sangula (2010) reported that the current FMDV serotype O strains involved in FMD outbreaks in East Africa, Uganda inclusive, were genetically distant from the vaccine strain. FMDV serotype O is currently the predominant serotype responsible for most FMD outbreaks in livestock in East Africa (Sangula, 2010) and particularly in Uganda (Mwiine *et al.*, 2010a). Since vaccination only protects, fully, against genetically closely related FMDV strains within a serotype (USAHA, 2008), the absence of a statistically significant difference in FMD outbreak occurrence on farms/in herds between herds/farms on which vaccination was practiced and those on which vaccination was not practiced could be due to a possible mismatch between the FMDV strains incorporated in the vaccines in use and the serotype O wild strains introduced from Tanzania. There is, therefore, need to carry out further studies to determine the genetic relationship between the FMDV vaccine strains in use and the circulating wild strains across the Uganda-Tanzania border.

About 51% ($n = 41$) of the farmers mentioned infected wild animals and infected cattle as the source of FMD outbreaks on their farms. They explained that FMD outbreaks in their area were associated with cattle movement from the neighbouring Tanzania. Farmers reported that this cattle movement is effected either by the (illegal) cattle traders who buy cattle from Tanzania and sell to farmers in Isingiro district or the movement is effected by the Ugandan migratory herders from Isingiro district who migrate to Tanzania during the dry season (to look for pasture and water) and come back with infected cattle at the end of the dry season (start of the rainy season). Animals from Kiruhura district then get the infection from those in Isingiro during the communal grazing. The Manager of Nshara ranch added that the ranch had been able to avoid FMD outbreaks for the last 20 years (last outbreak was in 1991) by increasing vigilance around the ranch whenever outbreaks occur in the neighbouring areas to block out other people's livestock, in addition to the bi-annual vaccination.

However, there was significant difference ($p = 0.001$) in the occurrence of FMD outbreaks between the farms/herds located in Sanga sub-county and those in Nyakashashara sub-county. Up to 84% ($n = 25$) and 31% ($n = 16$) of the herds in Nyakashashara and Sanga sub-counties, respectively, had experienced at least one FMD outbreak in the period between January, 2001 and June, 2011. This could be because the parishes sampled in Sanga are more easier-to-reach than those sampled in Nyakashashara where there are no roads. The presence of a good road to the LMCA headquarters favors faster movement of veterinarians to vaccinate the animals in case of an outbreak and faster mobilization of the farmers to increase vigilance. Most outbreaks are caused by infected cattle from Tanzania, therefore, the easier means of communication provided by the road may explain why less farms in Sanga experience outbreaks than those in Nyakashashara sub-county.

There was also a significant difference ($p = 0.010$) in the occurrence of FMD outbreaks among herds/farms under communal, fenced/paddocked and separately grazed household herds as the husbandry systems practiced on the farm. It was observed that 77.8% ($n = 27$), 45.5% ($n = 11$) and 0.0% ($n = 3$) of the herds under the communal grazing, grazing household herds separately and fenced/paddocked grazing systems, respectively, had experienced at least one FMD outbreak in the period between January, 2001 and June, 2011. All herd owners (100%; $n = 41$) of the herds sampled mentioned that their livestock commonly came in close contact with the Impala and Zebra. Outbreaks in this area are thought to be as a result of incursions of new FMDV strains, mainly from Tanzania by infected cattle, since FMDV is continuously circulating. The ease of vigilance to avoid infection during an outbreak reduces in the order of fenced grazing- grazing household herd separately- communal grazing, with the communally grazed herds being at the highest risk of acquiring infection during grazing and congregation at water points.

In this study, it was observed that the number of outbreaks per year increased steadily from 2001 to peak level in 2004 (a period of 4 years), it then dropped almost steadily from 2004 to 2007 (only 1 outbreak in 2007), it again went to peak level in 2008 (another period of 4 years) and dropped steadily from 2008 to 2010. The number of FMD outbreaks reached peak level at intervals of about 4 years (2001-2004 and 2005-2008). This may probably be due to the disappearance of natural immunity which develops during the period of high number of outbreaks and the sudden flaring up from small foci of infection when the population becomes susceptible again. Immunity after natural infection lasts for 1– 4 years in cattle with closely

related virus of the same serotype, but remains fully susceptible to other serotypes (Radostits *et al.*, 2006). It has been observed that in endemic areas periodic FMD outbreaks occur which sweep through the animal populations and then subside, thus, a 6-year epidemic cycle has been demonstrated in India (Radostits *et al.*, 2006). Furthermore, it was observed that in this study area, 82.9% (n = 41) of the farmers vaccinate their cattle against FMD only in response to outbreak occurrence. This implies that vaccination is more intensified (covering more animals in a wider area) when the number of outbreaks is at its maximum. Although vaccination with the only available inactivated vaccines on the market gives a shorter immune protection of about 6-8 months (Kitching *et al.*, 2007), it reduces the FMDV load in the environment. This is because vaccination of cattle, sheep or pigs (including infected ones) with high potency vaccines induces protection and reduces the amount and duration of virus shedding and transmission to susceptible animals following natural infection of the vaccinated animals (Golde *et al.*, 2005; Orsel *et al.*, 2005 and USAHA, 2008). This, in effect, will contribute to the reduction in the number of outbreaks following the intensive vaccination. But as time goes on, with the reduction in the immunity, the FMD viral load in the environment increases contributing to the occurrence of outbreaks. However, these observed fluctuations in the FMD outbreak frequency over the years could have also been contributed by under reporting by the farmers and/or veterinary extension agents in some years. Uganda has a passive disease surveillance system based on reporting of cases of outbreaks of FMD by the livestock owners to the local veterinarians who then report to the national disease control department. This information is maintained in a database at the national veterinary epidemiology unit. Due to various reasons including the remoteness of the villages, poor telecommunications, lack of incentives for disease reporting both for the farmer and the extension staff, the endemic nature of the disease, and low mortality, many outbreaks could go unreported (Ayebazibwe *et al.*, 2010a). Therefore information obtained from such sources may not reflect the actual disease situation due to the underreporting. To substantiate on the observed outbreak trend in this study, there is need to assess the outbreak records for a wider time period of at least 20 years.

Although the number of outbreaks decreased drastically in some years, it did not reach zero. This could have been due to the fact that animals become naturally immune to the locally existing FMDV serotypes or strains during the peak outbreak period, but new strains are brought in by newly introduced cattle or by persistently infected wild animals such as the African buffalo. These new serotypes or strains are responsible for maintaining some number

of outbreaks during the low outbreak period. Immunity after natural infection or vaccination gives protection to the animal against closely related virus of the same serotype, but remains fully susceptible to other serotypes (Radostits *et al.*, 2006). In this study, farmers incriminated infected wild animals and the introduction of infected cattle from the neighboring republic of Tanzania, by fellow farmers and (illegal) cattle traders, as the two major sources of FMD infection for their cattle.

Analysis of the monthly FMD outbreak pattern in relation to the monthly average rainfall pattern in the study area within the years, 2001-2010, showed that outbreaks were more common shortly after the first dry season of January-February (March) and during the major dry season (June-July) compared to the parts of the year with average and above average rainfall (April-May and September-October-November). This is consistent with the seasonal cattle movements, mixing of cattle herds and crowding at water points, and the livestock nutritional stress (seasonal malnutrition) which intensify during the dry period months of January-February and June-July. Similar observations of increased outbreak numbers in dry periods of the year, due to increased cattle movements and mixing of herds, were also reported in the Borana Pastoral area, South Ethiopia (Tesfaye, 2006). In the West Bengal state of India, it was observed that FMD outbreaks were very high in the winter months due to the favorable climatic conditions for FMDV transmission and increased cattle movements; low in the rainy season months due to the unfavorable climatic conditions for FMDV transmission and reduced cattle movements; and very low in the month of May due to the extremely hot weather of 38°C - 42°C (Bhattacharya *et al.*, 2005). Dry seasons in this study area are characterized by pasture and water scarcity. Most of the privately owned water dams at the individually owned farms dry up in dry seasons necessitating the farmers to walk their cattle long distances to the bigger-capacity communal dams constructed by Government or to the scarce natural water sources in the area. At the water sources several herds mix and cattle crowd in strife for water. This daily congregation of cattle at the water points favors FMD transmission by contact, the most significant mode of transmission in cattle (Sutmoller *et al.*, 2003).

In Isingiro district, which neighbors Tanzania, some farmers migrate their herds to Tanzania to look for water and pasture during the dry seasons. Some farmers near the national border even have permanent homes both in Uganda and in Tanzania, thus, they keep on shifting their herds either way depending on the prevailing conditions. This phenomenon may be

responsible for the increase in the number of FMD outbreaks in the month of March, which is the beginning of the rainy season in the study area. Herds usually come back from Tanzania at the beginning of the rainy season (March), most likely, with some infected with FMD strains which are new to the non-migratory herds in Uganda. These then flare-up the outbreaks of FMD. In this study, farmers incriminated the introduction of infected cattle from the neighboring republic of Tanzania, by fellow farmers and (illegal) cattle traders, as one of the major sources of FMD infection for their cattle. In a study which involved analysis of FMD outbreaks in sub-counties adjacent to the five international borders of the landlocked Uganda (Southern Sudan, Democratic republic of Congo, Kenya, Rwanda and Tanzania), it was observed that the highest number of FMD outbreaks, between the years 2001– 2008, occurred in the sub-counties bordering Tanzania (Ayebazibwe *et al.*, 2010a). Plum *et al.*(2007) reported that the seasonal malnutrition due to pasture scarcity and the stress due to walking long distances in search of water, as occurs during dry seasons, may contribute to reduced resistance of the animals to infection due to immuno-suppression.

6. CONCLUSION AND RECOMMENDATIONS

In this study, there was continuous circulation of FMDV in the cattle population in the districts around LMCA even in the absence of outbreaks (clinical disease). Individual farmers who practice vaccination still remain with a similar risk to the endemically circulating FMDV strains, for their young un-vaccinated animals, as those who do not vaccinate. Since vaccination is the major control measure in Uganda, a compulsory annual vaccination of all cattle in the area would be a better way out.

There could be a mismatch between the FMDV strains incorporated in the vaccines in use and the wild strains introduced from Tanzania. This calls for a need to carry out further studies to determine the genetic relationship between the FMDV vaccine strains in use and the circulating wild strains across the Uganda-Tanzania border.

The risk of FMD outbreaks occurring on individual farms/herds varies with the husbandry practice, with the communally grazed having the highest risk, followed by the separately grazed household herds and the fenced farms having the lowest risk. Therefore, fencing of the farms and ranches in the area should be encouraged.

The number of FMD outbreaks in the districts around LMCA (Kiruhura, Mbarara, Isingiro and Rakai) reaches peak level at intervals of about 4 years. However, there is need to assess the outbreak records for a wider time period of at least 20 years to substantiate on the observed outbreak trend in this study.

This study also shows that FMD outbreaks in the districts around LMCA are related to the annual rainfall pattern (seasons) in the area, with more outbreaks occurring in the month of March just after the dry season of January – February and during the dry season of June – July. The existing major control measure, vaccination, should consider this seasonal phenomenon in timing of the vaccination campaigns to ensure that cattle are fully protected during the high risk periods of the year.

It is highly likely that the outbreaks in these areas are mostly contributed by the incursions of new FMD serotypes/strains by infected cattle from the neighbouring republic of Tanzania.

Cattle movement control is impractical (at least at present) at the Uganda-Tanzania border, yet the number of outbreaks per year in this area, which lies within the “cattle corridor” of Uganda, continues to be at a relatively high level with the associated loss due to morbidity, vaccination and quarantine measures instituted during outbreaks. A collaborative control programme between the two neighbouring countries, involving collaborative vaccination, animal movement control, public education and monitoring of FMD strains circulating between the Uganda and Tanzania would significantly reduce the disease in the area.

Therefore, based on the above conclusion the following recommendations are forwarded

- There is need to assess the outbreak records for a wider time period of at least 20 years to substantiate on the observed outbreak trend in this study.
- A collaborative FMD control programme between the two neighbouring countries, involving collaborative vaccination, animal movement control, public education, FMD diagnosis and monitoring of FMDV strains circulating between Uganda and Tanzania should be practiced.
- Fencing of the farms and ranches in the area should be encouraged to reduce the occurrence of outbreaks at least at individual farm level.
- The existing major FMD control measure, vaccination, should consider the seasonal phenomenon in timing of the vaccination campaigns to ensure that cattle are fully protected during the high risk periods of the year. The months of November (end of long rainy season) and May (end of short rainy season), two times per year, are suggested as the most suitable time for vaccination against FMD in South-western Uganda.
- Vaccination of cattle should be compulsory for all herds in the area.
- Further studies should be carried out to determine the genetic relationship between the FMDV vaccine strains in use and the circulating wild strains across the Uganda-Tanzania border.
- Research should be done to establish the role played by the different ungulates, especially the impala, in the epidemiology of FMD in the area.

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8. ANNEXES

Annex 1. Questionnaire used to collect data on risk factors for FMD seropositivity in cattle and risk factors for FMD outbreak occurrence on farms/herds.

Questionnaire No:

QUESTIONNAIRE FOR FARMERS ON THE RISK FACTORS FOR FOOT AND MOUTH DISEASE (FMD) OUTBREAKS AROUND LAKE MBURO CONSERVATION AREA

This questionnaire is aimed at determining the risk factors for Foot and Mouth Disease outbreaks in cattle in the areas around Lake Mburo National Park. This data will contribute some of the information required for future designing of control strategies for the disease in the area. All the information given will be kept confidential. You are kindly requested to co-operate in completing this study.

Instructions: Please fill in the appropriate answers in the space provided for each question.

Where there is no space provided, please tick the appropriate answer(s) as applicable.

1.0 General Information

1.1 Date of interview

1.2 Name of the interviewer.....

1.3 Name of the farmer.....1.4 Gender1.5 Village.....

1.6 Farm code..... 1.7 Parish.....1.8 Sub-county.....

1.9 District..... 1.10 GPS reading.....

1.11 What is the distance of your farm from National Park?

(i) Less than 10km (ii) Between 10 and 50km, inclusive (iii) Between 50 and 100km

3.0 Risk factors and sources of FMD outbreaks.

3.1 What are the risk factors for the occurrence of FMD outbreaks on your farm? *(Tick in the space provided under Yes or No)*

Risk Factor	Yes	No	Comments
Do your livestock move to other villages/parishes in search of water or pasture?			
Does your herd migrate to other areas?			
Do your livestock interact with wildlife?			
Do you graze your animals communally?			
Is your farm proximal to a livestock market or slaughter slab?			
Does your herd interact with other unvaccinated herds?			

3.2. What are the possible sources of the FMD outbreaks on your farm? *(Tick in the space provided under Yes or No)*

Source	Yes	No	Comments
Have infected wildlife ever been the source of FMD outbreak on your farm?			
Have other infected cattle ever served as a source of FMD outbreak on your farm?			
Have other infected goats ever been the source of FMD outbreak on your farm?			
Have other infected sheep ever been the source of FMD outbreak on your farm?			
Have other infected pigs ever been the source of FMD outbreak on your farm?			
Have infective pastures ever been the source of FMD outbreak on your farm?			
Has drinking water for livestock ever been the source of FMD outbreak on your farm?			
Have Livestock feeds for feeding your livestock ever been the source of FMD outbreak on your farm?			
Has wind ever been the source of FMD outbreak on your farm?			
Have people ever been the source of FMD outbreak on your farm?			
Has milk ever been the source of FMD outbreak on your farm?			

4.0 Livestock Management System

4.1. (a) When do you often encounter FMD outbreaks on your farm?

- (i) Dry Season (ii) Rainy Season (iii) Any Season

4.1. (b) Name the calendar months when this happens.....

4.2. (a) Do your livestock come in contact with Wild animals?

- (i) Yes (ii) No

4.2. (b) If yes to 4.2 (a) above, specify the wild animal species.....

4.3. When do your livestock get into close contact with wildlife?

- (i) During wet season within the protected area?
(ii) During wet season outside the protected area?
(iii) During the dry season when livestock go to the protected area?
(iv) During the dry season when wild animals leave the protected area to the farmland?
(v) Any time within the protected area?
(vi) Any time outside the protected area?
(vii) Others (*Specify*)

4.4. Why do your livestock get into close contact with wild animals?

- (i) Search for water or pasture (ii) To mate among each other.
(iii) Close proximity and lack of a barrier
(iv) Others (*Specify*).....

4.5. (a) Have you ever seen a sick wild animal on or near your farm?

- (i) Yes (ii) No

4.5. (b) If yes to 4.5 (a) above, specify the species.....

4.6. (a) Do you vaccinate your cattle against FMD?

- (i) Yes (ii) No

4.6 (b) If yes to 4.6 (a) above, who vaccinates your cattle against FMD?.....

4.6 (c) If yes to 4.6 (a) above, how often do you vaccinate your cattle against FMD?

- (i) Once a year (ii) Twice a year (iii) Every two years
(iv) In response to outbreaks (v) Never at all
(vi) Others (Specify).....

4.7. What is the husbandry system practiced on your farm?

- (i) Communal grazing..... (ii) Fenced grazing/Paddocked.....
- (iii) Zero grazing..... (iv) Tethering.....
- (v) Grazing household herd separately.....

Annex 2. Microtitre plate layout used for the SVANOVIR™ FMDV 3ABC-Ab ELISA test.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N	N	7	7							39	39
B	P	P	8	8								
C	1	1	9	9								
D	2	2	10	10								
E	3	3	11	11							43	43
F	4	4	12	12							44	44
G	5	5	13	13							45	45
H	6	6	14	14							46	46

N = Negative control sample

P = Positive control sample

1, 2, 3, etc. = Test sample number

9. CURRICULUM VITAE

PERSONAL DATA

Name : Batwala Stephen

Address (Permanent) : Namayingo District Local Government, P. O. Box 64, Bugiri, Uganda.

Current Address : Addis Ababa University, School of Veterinary Medicine, Debre zeit, Ethiopia.

Date of Birth : 28th/10/1974

Place of Birth : Bugiri, Uganda.

Gender : Male

Age : 38 Years

Nationality : Ugandan

Marital Status : Married, with four Children

Profession : Veterinarian

Telephone : +256 772 696310 (Uganda); +251 912 832808 (Ethiopia).

E-mail : batwalastephen@gmail.com

Alternative E-mail : stephenbatwala@yahoo.com

Languages: English : Excellent user in Listening, Reading, Speaking and Writing.
Kiswahili : Basic user in Listening, Reading and Speaking.
German : Very basic user in Listening, Reading, Speaking and Writing.
Lusoga : Mother tongue

EDUCATIONAL BACKGROUND AND QUALIFICATION

- Bachelor degree in Veterinary Medicine (BVM) from Makerere University, Faculty of Veterinary Medicine, Kampala, Uganda, 1993 - 1998.

- Achieved the Uganda Advanced Certificate of Education (UACE) from the Uganda National Examinations Board (UNEB), at Bukoyo Secondary School, Iganga, Uganda, 1991 – 1993.
- Achieved the Uganda Certificate of Education (UCE) from UNEB, at Bukooli College, Bugiri, Uganda, 1987- 1990.
- Achieved the Uganda Primary Leaving Certificate of Education from UNEB, at Bugiri Primary School, Bugiri, Uganda, 1980 – 1986.

SPECIAL COURSES AND TRAINING

- Certificate in Epidemiology and control of infectious diseases: A training organized by MAAIF through the Department of Livestock Health and Entomology under the auspices of the Avian and Human Influenza project (AHIP), and conducted by Makerere University in conjunction with MAAIF, 31st July- 9th August, 2011, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.
- Certificate in Uganda Biosecurity, Surveillance and outbreak response for Highly Pathogenic Avian Influenza (HPAI): A training organized and conducted by MAAIF under the AHIP project, 19th -23rd January, 2009, Tororo, Uganda.
- Training in Animal disease surveillance and reporting: A training organized and conducted by MAAIF, 14th -18th September, 2004, Kampala, Uganda.
- Participatory Rural Appraisal (PRA) Tools and Techniques: a training organized by MAAIF under the Farming in Tsetse Controlled Areas of Eastern Africa (FITCA) Project, 3rd – 7th May 2004, Tororo, Uganda.
- Certificate in Artificial Insemination: A training conducted by the National Animal Genetic Resources center and Data Bank, Entebbe, Uganda, 24th July-14th August, 2003, Entebbe, Uganda.

WORK EXPERIENCE

June 2011- to date

- District Veterinary Officer, Namayingo District Local Government Administration, P. O. Box 64, Bugiri, Uganda.

1999 – May 2011

- Veterinary Officer (in Charge of 4 Sub-counties), under Bugiri District Local Government Administration, P. O. Box 37, Bugiri, Uganda.

Special duties assigned during service as Veterinary Officer

2005 - 2009

- Sub-county Coordinator for the National Agricultural Advisory Services (NAADS) Programme, Buyinja Sub-county, Bugiri District Local Government Administration.

2005-2007

- Field Extensionist for Heifer Project International (HPI) under South Bugiri Development Association (SOBDEA), Buyinja Sub-county, Bugiri District Local Government Administration.

2002- 2005

- District Coordinator, Bugiri District Local Government Administration, for the Pan African Control of Epizootics (PACE) project.

PROFESSIONAL AFFILIATIONS

Member of the Uganda Veterinary Association.

Registered by the Uganda Veterinary Board as a Veterinary Surgeon.

SKILLS AND COMPETENCES

Social skills and competences

Good ability to adapt to multicultural environments, gained through my BVM field research project implementation in South-Western Uganda, my work as a field Veterinarian and my study in Ethiopia and Germany.

Good Listening and Communication skills, gained through my work as a field Veterinarian.

Organisational skills and competences

Acted as a Sub-county Chief for one month during my service as a Veterinary Officer. I was the PMA (Programme for Modernization of Agriculture) focal point person for Buyinja Sub-County, Bugiri District Local Government Administration, from 2002-2008 during my service as a Veterinary Officer.

I was a Sub-County NAADS Coordinator for Buyinja Sub-County from 2005-2009.

Technical skills and competences

Good experience in laboratory diagnostic techniques such as ELISA, gained during my Master thesis laboratory research work.

Good experience in application of Participatory Rural Appraisal (PRA) techniques in studying disease impacts and disease dynamics in communities gained from the implementation of the FITCA (Farming in Tsetse Controlled Areas of eastern Africa) Project in Bugiri District, Uganda; and from the Socio-economics, Community mobilization and participation, Project planning and Management module covered at Addis Ababa University, Ethiopia under the MTADM course.

Computer skills and competences

Good command of Microsoft Office tools (Word, Excel, and PowerPoint), gained through training.

Good Internet user, gained through training.

RESEARCH WORK DONE

Ecto- and Haemo-parasites of Small mammals in and around Lake Mburo National Park in South-Western Uganda, a research carried out from 1997-1998 as part of the requirements for the award of the Bachelor of Veterinary Medicine of Makerere University.

REFEREES

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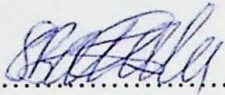
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10. SIGNED DECLARATION

I, BATWALA STEPHEN hereby declare that this thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Signed..........

Date: 1st/12/2011