SPATIAL AND TEMPORAL DISTRIBUTION OF FOOT AND MOUTH DISEASE VIRUS IN THE LAKE ZONE OF TANZANIA

JOSEPH MARO GENCHWERE

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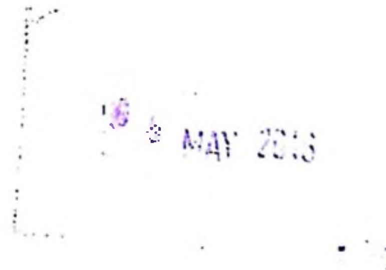

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ABSTRACT

This study was conducted to determine the spatiotemporal distribution of foot-and-mouth disease (FMD) virus (FMDV) serotypes and evaluate the awareness of people on FMD in Tanzania. An observational prospective study involving serological analysis, FMDV antigen detection and questionnaire survey was carried out in the lake zone of Tanzania. Seroprevalence of antibodies to the nonstructural protein (NSP) 3ABC of FMDV and serotype-specific antigen detection were investigated by using SVANOVIR® FMDV 3ABC-Ab ELISA and indirect-sandwich ELISA (sELISA), respectively, while structured questionnaire was used to evaluate the awareness of people on FMD. Both serum and tissue (foot and mouth epithelia) samples were collected from cattle suspected of FMD in 13 districts of the four regions of the Lake zone during the period of 2010 to 2011. A total of 107 (80.5%) out of 133 tested serum samples were seropositive to NSP-3ABC with at least one sample being positive from all 10 districts screened. Fifteen (53.6%) out of 28 tissue epithelial samples collected from FMD cases in 8 districts during the course of this study were positive to serotype O FMDV antigen. Of the 8 screened districts, serotype O FMDV antigens were detected from 7 districts, and no other serotypes were recovered from animal samples screened. Questionnaire survey in 6 districts indicated that farmers in the lake zone were aware on the clinical manifestation 26 (90%) and economic impact 23 (79%) of FMD in the region. The questionnaire data showed that FMD outbreaks had often occurred after rainy seasons 22 (75.9%) predominantly encountered with the highest peaks just after long rains in May-June and at the end of the short rains in November-December of each year. The spatial

distribution of the FMD cases suggested that, FMDV serotype O virus exposure was the only widespread and the cause of 2010-2011 outbreaks in the Lake zone.

DECLARATION

I, Joseph Maro Genchwere, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

07/06/2013

Joseph Maro Genchwere **Date**

(MSc. Applied Microbiology)

The above declaration is confirmed

07-06-2013

Dr. Christopher J. Kasanga **Date**

(Supervisor)

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DEDICATION

This dissertation is dedicated to my wife Beatrice Sui and my beloved children Jennifer and Jessica.

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LIST OF ABBREVIATIONS

$	Dollar
µl	microliter
APHIS	Animal and Plant Health Inspection Service, USA
CBPP	contagious bovine pleuropneumonia
cDNA	complement DNA
CFSPH	Centre for Food Security and Public Health, USA
CFT	complement fixation test
CVL	Central Veterinary Laboratory
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FMD	foot and mouth disease
FMDV	foot and mouth disease virus
HRP	horseradish peroxidase
MCF	malignant catarrh fever
MLD	Ministry of livestock Development
ND	Newcastle disease
NSP	nonstructural protein
OD	optic density
OIE	Office International des Epizooties
ORF	open reading frame
PP	percent positivity

PPR	Peste des Petits Ruminants
RNA	ribonucleic acid
RT- PCR	reverse transcriptase- Polymerase chain reaction
SACIDS	Southern African Center for Infectious Disease Surveillance
SADC	Southern African Development Community
SAT	Southern Africa Territory
SUA	Sokoine University of Agriculture
TADs	Transboundary Animal Diseases
TBDs	Tick borne diseases
UK	United Kingdom
USA	United States of America
VIC	Veterinary Investigation Centre
VP	viral protein

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

Tanzania is endowed with a livestock resource and ranks third in Africa in terms of cattle population after Ethiopia and Sudan. The estimated livestock population comprises 18.5 million cattle, 13.1 million goats, 3.6 million sheep and 30 million indigenous chickens according to National Sample Census of Agriculture 2002/03 - National Report.

Tanzania's economy is mainly based on agriculture, a sector that employs about 85% of its population. Livestock production, which has been increasing in the past years, is limited by disease occurrence (e.g. FMD) and the presence of tsetse flies and wildlife protected zones in large areas of the country (Picado *et al.,* 2010). The country is located in Eastern Africa between Longitude 29° and 41° East and Latitude 1° and 12° South, covers an area of 945 000 km^2. Two thirds of the country land resource is rangelands, suitable for livestock keeping activities (Nyamrunda *et al.,* 2007). Tanzania has a tropical type of climate. In the highlands, temperature range between 10°C and 20°C during cold and hot seasons respectively, the rest of the country has the temperatures which hardly go below 20°C. The hot period spreads between November and February (25°C - 31°C) while the coolest period occurs between May and August (15°C - 20°C). Two rainfall regimes exist; unimodal (December-April) and bimodal (October-December and March-May). In the bimodal regime the March-May rains are referred to as the long rains (*masika*), whereas the October –December rains are generally known as short rains (*vuli*).

In the Lake zone the most prominent rainfall regime is bimodal, the zone comprises of four regions (Mwanza, Mara, Shinyanga and Kagera) which borders all East African countries (Kenya and Uganda in the North and Rwanda and Burundi in the West). The lake zone livestock population is about 7.3 million (39.6%) cattle, 4.5 million (34.2%) goats, 1.3 million (35.6%) sheep and 0.2 million (13.5%) pigs of the total population of animals in Tanzania (MLD, 2006).

Livestock plays an important role in food security for rural population. In additional livestock keeping especially cattle provides labour, indirect contribution to production of food crops by providing draught power for ploughing and transportation and also valued as a source of protein, capital, investment, labour, prestige and respect especially in the Northern and Lake zone of Tanzania.

In the Lake zone and the country as a whole, the main important infectious diseases which potentially hamper the livestock sector are tick borne diseases (TBDs), Contagious bovine pleuropneumonia (CBPP), trypanosomoses, helminths and viral diseases such as Peste des petits ruminants (PPR), Foot and mouth disease (FMD), Malignant catarrh fever (MCF) and Newcastle disease (ND). Foot and mouth disease and Newcastle disease are the most common and frequently observed in the Lake zone. Although FMD is a multi-species disease, cattle, which constitute the main livestock sector, were the only species considered in this study.

Foot and mouth disease (FMD) caused by a variety of FMDV serotypes (and strains) is a highly contagious viral disease of even-toed ungulates (Artiodactyla) and is

considered by Food and Agriculture Organization (FAO), Office International des Epizooties (OIE) as one of the most widely distributed transboundary animal disease (TAD) in the world (FAO, 1999). In Tanzania, the disease is the second most important TAD in cattle after contagious bovine pleuropneumonia (CBPP) (MoWLD, 2003).

The disease impairs meat and milk productivity and limits the use of affected animals for draft purposes. Countries with FMDV are not allowed to export animals or animal products to FMDV-free countries. This contributes further to the economic loss as it impairs exports of products to the international markets and increased costs for controlling the disease (Perez *et al.*, 2008). Consequently, FMD is considered one of the most important diseases of livestock, because it imposes such profound and sustained economic and social hardships to people and the governments of countries where FMDV is endemic (Gallego *et al.*, 2007).

A number of evolutionary and population parameters are believed to be responsible for the emergence and spread of the different virus serotypes (Tully and Fares, 2006). These constraints can be broadly classified as genetic, for example the ability of the virus to evade the host immune response, or ecological such as host mobility and population density (Tully and Fares, 2006).

The highly contagious nature of FMDV and the associated productivity losses make it a primary animal health concern worldwide. Effective vaccines and strictly control measures have enabled FMD eradication in most developed countries, which

maintain unvaccinated, seronegative herds in compliance with strict international trade policies. However, the disease remains enzootic in many regions of the world, posing a serious problem for commercial trade with FMD-free countries (Carrillo *et al.*, 2005).

The distribution of serotypes and FMD-affected countries is uneven across the world. Serotypes A and O are reported in South America, Asia and Africa, Asia1 is found in Asia and the Middle East and SAT serotypes are generally restricted to Africa (Paton *et al.*, 2009). FMDV circulates between countries in endemic regions and periodically causes outbreaks in endemic settings and countries free of disease. The FMD status, given by the presence of disease and the control methods applied, influence the trade between countries according to OIE regulations. The disease is of high economic importance especially to countries that have an intensive animal industry (Mwiine *et al.*, 2010).

According to the office international des epizootics (OIE, 2000), FMD ranks first among the notifiable infectious diseases of animals. The disease is notoriously contagious that it can spread as much as 50 (fifty) miles downwind from one outbreak area to another (Mekonen *et al.*, 2011).

Todate in Africa, only Swaziland, Lesotho and Madagascar are considered completely free of FMD, thus free to trade, and three countries (South Africa, Botswana and Namibia) have FMD-free zones and can therefore export (with restriction) part of their livestock products (OIE, 2010).

1.2 Problem Statement and Justification

Since FMD was first reported in Tanzania in 1927 (Anon, 1927; Chibunda *et al.*, 2006), efforts to eradicate this economically important disease have not been fruitful. Despite of vaccination programmes being imposed in various areas of the country, there are still several FMD outbreaks being encountered each year in many areas of Tanzania. In addition, many areas experience long-standing endemic or periodic epidemics of FMD. Nevertheless, the occurrence and geographical distribution of FMDV serotypes is not clearly known as remains to be fully exploited.

Prediction of FMD occurrence in a region or country and characterization of FMDV serotypes is important in FMD surveillance and control programmes, especially in understanding when and where resources should be directed to prevent or reduce projected increases in disease occurrence. For example, vaccination efforts can be focused in regions of a country where the predicted FMD occurrence exceeds a maximum acceptable threshold. Knowledge of high- and low risk locations and time periods is important in directing surveillance programmes, including increasing both frequency and amount of sampling to high risk areas and/or time periods. The spatiotemporal distribution of FMDV in a country also can be used to validate control programmes, and the information on expected locations of disease may also be used to facilitate trade of animals and animal products among neighbouring regions and thus redirect vaccination efforts to specific areas or regions. The present study aimed at establishing the current spatial and temporal distribution of FMDV serotypes in the lake Zone of Tanzania with the objectives given below.

1.3 Research Objectives

1.3.1 General objective

The aim of this study was to determine the spatial and temporal distribution of FMDV serotypes and to evaluate the awareness of people on FMD in Tanzania.

1.3.2 Specific objectives

i. To identify FMDV serotypes prevailing in the Lake zone of Tanzania.

ii. To establish the current geographic distribution and dynamics of FMDV serotypes in the Lake zone of Tanzania.

iii. To determine the awareness of people on the temporal and spatial occurrence of FMD outbreaks in the Lake zone of Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FMD Definition and Aetiology

Foot-and-mouth disease (FMD) is a highly contagious, vesicular disease of cloven-hoofed animal species (Habiela *et al.,* 2010), and is caused by *Foot-and-mouth disease virus* (FMDV) of the genus *Aphthovirus* and the family *Picornaviridae* (Carrillo *et al.,* 2005; OIE, 2009). There are seven distinct serotypes of FMD virus worldwide, namely O, A, C, South African Territories (SAT) 1, SAT 2, SAT 3, and Asia 1. Within these serotypes, over 60 subtypes have also been described using biochemical and immunological tests. Infection with any one serotype does not confer immunity against another (APHIS, 2007; OIE, 2009; Paton *et al.,* 2009).

2.2 FMD Susceptible Species

FMD is primarily a disease of domesticated and wild cloven-hoofed animals. Infection with FMD has been reported in cattle, sheep, goats, swine, antelopes and water buffalo (*Bubalus bubalis*) as well as many wild animal species (Habiela *et al.,* 2010; Mwiine *et al.,* 2010). Many species of cloven-hoofed wildlife may become infected, and the virus has occasionally been recovered from other species as well. Strains of FMDV that infect cattle have been isolated from wild pigs, antelope and deer, while the African buffalo (*Syncerus caffer*) is considered to be the natural definitive host for the FMD serotypes SAT 1, SAT 2 and SAT 3 (OIE, 2009). Spread of FMDV is mostly associated with the legal and illegal movement of infected animals or their products (Knowles *et al.,* 2005). Morbidity is high, but mortality in adult animals is usually low, with deaths due to myocarditis being

reported mainly in young animals. The economic impact of the disease is significant due to both the costs for controlling an outbreak and the resulting loss in trade due to sanctions on export of animals and animal products (Sangare *et al.*, 2001), mortality in young animals, reduced milk production and loss of work efficiency in draught animals (Jamal *et al.*, 2008).

Infection of susceptible animals with FMDV can lead to the development and/or appearance of vesicles on the feet, in and around the oral cavity, and on the mammary glands of females. Infection with FMDV usually predisposes animals to other infectious agents resulting into development of clinical diseases. In dairy cattle mastitis is a common sequel of FMDV pathogenesis. Vesicles can also occur at other sites, such as inside the nostrils and at pressure points on the limbs especially in pigs. The severity of clinical signs varies with the strain of virus, the exposure dose, the age and breed of animal, the host species and the immunity of the animal. Clinical signs of FMD range from a mild or inapparent infection to severe with ultimate death in some cases. Mortality ascribed to multifocal myocarditis is most commonly seen in young animals (OIE, 2009).

2.2 FMDV Classification, Stability and Genome

2.2.1 The virus classification

FMD virus belongs to the genus *Aphthovirus* and the family Picornaviridae (Table 1). The name, Picornaviridae is derived from the Latin word 'Pico' which means small and 'rna' means RNA, which refers to the size and genome type of the virus, while the genus name 'Aphthovirus' refers to the vesicular lesions produced in the mouth of cloven-hoofed animals (OIE, 2004; Tesfaye, 2006).

Table 1: Important picornaviruses of animals and humans

Genus	Virus	Species affected	Disease
Aphthovirus	Foot-and-mouth disease virus type A, O, C, SAT 1, SAT 2, SAT 3 and Asia 1	Cattle, sheep, goats, swine, ruminant wildlife	Foot-and-mouth disease in ruminants and swine
	Equine rhinovirus 1	Horses	Systemic disease, respiratory
Enterovirus	Swine vesicular disease virus	Swine	Swine vesicular disease
	Porcine enterovirus 1	Swine	Polioencephalomyelitis
	Porcine enteroviruses 2-11	Swine	Diarrhea, pericarditis
	Bovine enteroviruses 1-7	Cattle	Usually asymptomatic infection
	Simian enteroviruses 1-18	Monkeys	Usually asymptomatic infection
	Avian enteroviruses	Chickens	Avian encephalomyelitis
	Polioviruses 1, 2, and 3	Ducks	Hepatitis
		Turkeys	Hepatitis
		Humans	Poliomyelitis
	Coxsackieviruses A1-22 and A24 and B1-6	Humans	Aseptic meningitis,poliomyelitis, myocarditis,hand-foot-and-mouth disease
	Human echoviruses 1-7, 9, 11-27, and 29-33	Humans	Aseptic meningitis and other syndromes
	Human enteroviruses 68-71	Humans	Poliomyelitis, keratoconjunctivitis
Cardiovirus	Encephalomyocarditis virus	Swine, elephants rodents	Rarely, encephalomyocarditis
	Theiler's murine encephalomyelitis virus	Mice	Murine poliomyelitis
Rhinovirus	Bovine rhinoviruses 1-3	Cattle	Mild rhinitis
	Human rhinoviruses 1 - 100+,1A, 1B	Humans	Common cold
Hepatovirus	Simian hepatitis A virus	Monkeys	Hepatitis
	Human hepatitis A virus	Humans	Hepatitis
Parechovirus	Human echoviruses 22 and 23	Humans	Aseptic meningitis
Unclassified	Equine rhinovirus 2	Horses	Rhinitis

Source: Murphy *et al.*, (1999)

2.2.2 The virus stability

The FMD virus RNA is enclosed within a non-enveloped protein coat (capsid), which consists of polypeptides devoid of lipo-protein, and hence stable to lipid solvents like ether and chloroform. The virus is very sensitive to pH, and is inactivated when exposed to pH below 6.5 or above 11 (Tesfaye, 2006). Two percent solutions of NaOH or KOH and 4% Na_2CO_3 are effective disinfectants for FMD contaminated objects, but the virus is resistant to alcohol, phenolic and quaternary ammonium disinfectants (Tesfaye, 2006; CFSPH, 2007).

2.2.3 The virus genome

FMDV is a single-stranded, positive-sense RNA virus (Niedbalski and Kesy, 2010). Its genome consists of approximately 8200 nucleotides excluding the poly (C) and (A) tracts and comprises a 5' untranslated region of about 1150 nucleotides, followed by a single open reading frame (ORF) of approximately 6890 nucleotides and a 3' untranslated region of approximately 160 nucleotides. The ORF encodes 12 proteins: the leader protease, four capsid proteins (1A–D) and the non-structural proteins 2A–C and 3A–D (Jackson *et al.*, 2007). During entry into a cell the virion RNA which acts as messenger RNA is translated into a polyprotein which is then cleaved into proteins P1, P2 and P3 by three virus-coded proteases namely; the maturation proteinase, the early 2A proteinase and the 3C protease. The VPO is cleaved into VP4 and VP2 by a third protease during capsid formation so that VP1, VP2, VP3 and VP4 comprise the mature capsid. Structural protein (VP1) is a surface exposed protein and encoded by 1D gene, it contains the major antigenic determinant of the virus and is important for process of cell attachment (Murphy *et al.*, 1999).

2.3 Mode of Transmission

FMD virus can replicate and be excreted from respiratory tract of animals and found in all secretions and excretions from acutely infected animals, including expired air, saliva, milk, urine, feces and semen. Pigs, in particular, produce large quantities of aerosolized virus. Animals can shed FMDV for up to four days before the onset of the clinical signs. The virus is also found in large quantities in vesicle fluid, and peak transmission usually occurs when vesicles rupture. Transmission can occur by direct or indirect contact with infected animals and contaminated fomites; routes of spread include inhalation of aerosolized virus, ingestion of contaminated feed, and entry of the virus through skin abrasions or mucous membranes. The importance of each of these routes varies with the species. For example, pigs are less susceptible to aerosolized virus than cattle or sheep. Sheep may have less obvious symptoms than other species, and have been important in disseminating the virus in some outbreaks (CFSPH, 2007; OIE, 2009).

FMDV can persist for up to nine months in sheep and up to four months in goats. Most cattle carry this virus for six months or less, but some animals remain persistently infected for up to 3.5 years (CFSPH, 2007). Individual African buffalo have been shown to be carriers for at least five years, and the virus can persist in a herd of African buffalo for at least 24 years (Vosloo *et al.*, 2002; CFSPH, 2007; OIE, 2009). In carriers, FMDV is found only in the esophageal-pharyngeal fluid. The amount of virus is small, and it may be found only intermittently. Carriers might be able to transmit FMDV to other animals if they come in close contact; the importance of this route of transmission is controversial. Unequivocal evidence for

transmission from carriers has been reported only from Africa, where African buffalo can spread the disease to cattle. With the exception of African buffalo, wildlife seems to be infected by contact with domesticated animals. FMDV can also be transmitted on fomites including vehicles, as well as mechanically by animals and other living vectors. Airborne transmission can occur under favourable climatic conditions (CFSPH, 2007).

2.4 Pathogenesis

The major route of infection in ruminants is through the inhalation of droplets, but ingestion of infected feed, inoculation with contaminated vaccines, and contact with contaminating clothing, veterinary instruments, and so on can all produce infection. In animals infected via the respiratory tract, initial viral replication occurs in the pre pharyngeal area and the lungs followed by viremic spread to other tissues and organs before the onset of clinical disease. FMD virus is then distributed throughout the body, to reach best sites of multiplication such as the epithelium of oro-pharynx, oral cavity, feet, the udder and heart (Murphy *et al.*, 1999).

2.5 Clinical Signs

Clinical signs usually develop in 3 to 5 days after susceptible animals get in contact with clinically infected animals (Kitching, 2002a), although in natural infection, the incubation period in cattle may range from 2-14 days, while in pigs it is usually two days or more, but can be as short as 18-24 hours and in sheep it is usually 3 to 8 days (CFSPH, 2007).

The severity of clinical signs varies with the strain of virus, the exposure dose, the age and breed of animal, the host species and its degree of immunity. The signs can range from a mild or in apparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2004; OIE, 2009). In cattle, the initial signs are fever of (39.4-40.6^{o} C), dullness, anorexia, and fall in milk production. These signs are followed by excessive salivation, smacking of the lips, grinding of the teeth, saliva drooling, serous nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation (APHIS, 2007). The predilection sites for vesicle formation are areas on the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats (OIE, 2009).

After the formation of vesicles, drooling may be more marked, and nasal discharge, lameness, or both may increase. Pregnant cows may abort, and young calves may die suddenly without developing any vesicle because of inflammation of the heart (Myocarditis) (Blood *et al*, 1994). The course of FMD infection is 2 to 3 weeks although infection may delay recovery of mouth, feet and teat lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight, and breeding problems (CFSPH, 2007). A chronic Panting syndrome characterized by dyspnoea, hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (Radostits *et al.*, 1994).

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

the coronary band or in the interdigital space. Infected animals may abort and lambs may die without showing any clinical sign (Blood *et al.*, 1994).

In swine, the initial signs are fever of (40 - 40.6°C), anorexia and reluctance to move. These signs are followed by vesicles on the coronary band, heels, interdigital space and on the snout. Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle and tend to be a "dry"-type lesion; there is no drooling; sows may abort; and piglets may die without showing any clinical sign (Blood *et al.*, 1994).

Deaths usually occur only in young animals, as the result of multifocal myocarditis; vesicles are not always found. In some outbreaks, the mortality rate in young animals can be high (CFSPH, 2007).

2.6 Pathological Lesions

The characteristic lesions of foot-and-mouth disease are single or multiple, fluid-filled vesicles or bullae from 2mm to 10mm in diameter. The earliest lesions can appear as small pale areas or vesicles. Some vesicles may coalesce to form bullae. Vesicles are generally present for only a short period. Once they rupture, red, eroded areas or ulcers will be seen. These erosions may be covered with a gray fibrinous coating, and a demarcation line of newly developing epithelium may be noted. Loss of vesicular fluid through the epidermis can lead to the development of "dry" lesions, which appear necrotic rather than vesicular. Dry lesions are particularly common in the oral cavity of pigs (Murphy *et al.*, 1999; CFSPH, 2007).

In young animals, cardiac degeneration and necrosis can cause gray or yellow streaking in the myocardium; these lesions are sometimes called "tiger heart" lesions (Murphy *et al.*, 1999; CFSPH, 2007).

2.7 Morbidity and Mortality

The morbidity rate varies with the species, immunity and other factors. In regions where FMD is not endemic, the morbidity rate can be as high as 100%. Recovery from infection leads to immunity against the infecting virus, but little or no immunity develops to other serotypes. If several serotypes are endemic in a region, periodic episodes of disease may be seen. If only a single, persistent serotype circulates in a population, clinical disease may be mild and mainly occurs in young animals as they lose their protection from maternal antibodies. Carriers occur in endemic areas. In wild African buffalo populations, 50-70% of the animals may become carriers. Carrier rates from 15% to 50% have been reported in cattle and sheep (CFSPH, 2007).

Mortality rate is low and usually occur only in young animals, as the result of multifocal myocarditis. In some outbreaks, the mortality rate in young animals can be high (CFSPH, 2007).

2.8 FMD World Distribution

FMDV has an essentially global distribution, with the exception of North America, Western Europe, Australia and New Zealand (Schumann *et al.*, 2008). 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 (e.g. Zimbabwe). Sporadic regions are characterized by repeated incursions of FMD viruses into regions where disease does not usually occur. The disease is either eliminated through control program or disappears naturally without intervention.

Some regions have eradicated FMD, often following mass annual prophylactic vaccination campaigns and through the stringent application of zoosanitary measures following outbreaks. Some countries, such as the United Kingdom, have eradicated FMD without resort to vaccination. However, FMD is epizootic in several areas of the world and endemic in much of the developing world, including Africa, Asia, part of South America, and the Middle and Far East. This situation exists despite continued efforts to control the disease and the extensive use of FMD vaccine throughout the affected areas of the world (Asseged, 2005). Western Europe has had recent outbreaks, which have all been successfully controlled. This includes the 2001 outbreak in the UK, which spread to Ireland, France, and the Netherlands, and separate outbreaks in Italy and Greece (Tesfaye, 2006).

In the global distribution of the seven FMDV serotypes (Table 2); Type O is the most commonly encountered serotype worldwide (Niedbalski and Kesy, 2010; Mekonen *et al.*, 2011), and serotypes A and O, have the widest distribution in Africa, Asia and South America. Serotypes SAT 1, SAT 2 and SAT 3 are currently restricted to Africa only and type Asia1 to Asia (Mekonen *et al.*, 2011). Serotype C

appears to have disappeared from the world as a whole with exception of Kenya, historically this is the rarest of the FMDV serotype to have occurred in Africa having been recorded in only three countries namely Ethiopia, Kenya and Angola (Vosloo *et al.*, 2002).

Table 2: Global distribution of FMDV serotypes

Region	FMDV Serotypes
South America	O, A, C
Europe	O, A, C
Africa	O, A, C, SAT 1, SAT 2, SAT 3
Asia	O, A, C, Asia 1
North and Central America	Virus free
Caribbean	Virus free
Oceania	Virus free

Source: Murphy *et al.*, (1999)

The geographical distributions of FMDV serotypes have probably undergone a series of expansions over the last century (Jackson *et al.*, 2007). Current notions of the distribution of FMDV serotypes are subject to under-reporting, particularly in Africa (Kivaria, 2003). SAT serotypes have been and remain almost exclusively restricted to Africa (Vosloo *et al.*, 2002), they are restricted in their distribution mainly to sub-Saharan Africa and they co-exist with the Euro-Asiatic (O, A, C) serotypes in the East African region although serotype C has not been reported since 2004 (Sangula *et al.*, 2010).

The SAT viruses are thought to have originated in sub-Saharan Africa, where they circulate mainly among African buffalo (Vosloo *et al.*, 2002). In southern Africa, the epidemiology of the SAT serotypes is mainly associated with African buffalos

(*Syncerus caffer*) which act as reservoirs and sources of outbreaks (Sangula *et al.*, 2010).

In Eastern Africa, FMD is prevalent in wildlife and within the African buffalo in particular, although their role in the epidemiology of the disease has not been as widely studied as in southern Africa. Most outbreaks of FMD in the region are reported among livestock populations.

The African buffalo has been reported to be a carrier of the SAT serotypes but not the Euro-Asiatic serotypes in East Africa (Bronsvoort *et al.*, 2008; Ayebazibwe *et al.*, 2010). This is similar to the situation in southern Africa. Widespread animal movements in the Eastern Africa region are possibly responsible for long-term circulation and reintroductions of FMDV strains, including SAT 1 (Sahle *et al.*, 2007).

SAT serotypes are also found in North Africa (Vosloo *et al.*, 2002) and SAT 1 and SAT 2 are known to make occasional incursions into the Middle East (Jackson *et al.*, 2007). Serotypes A, O and C were commonly encountered in Europe prior to the introduction of widespread vaccination, and have been reported to circulate in many African countries, the Middle East, Southern Asia, the Far East and South America (Correa Melo *et al.*, 2002; Vosloo *et al.*, 2002). Asia 1 appears to be restricted to Southern and Eastern Asia (Jackson *et al.*, 2007). It has been observed that over the last 40years, particularly genetic subtypes (topotypes) persisted within a limited range of neighbouring countries and only periodically and temporarily spread beyond the borders (Swai *et al.*, 2009).

The existence of geographical and genetic clustering of FMDV suggests ecological adaptation and /or separation, but in many endemically affected areas the temporal and spatial dynamics of infection need to be more accurate. This is determined by analysis of host animal distributions and contact opportunities, serosurveys to estimate weight of infection and use of the latest available techniques in genetic tracing that have been so far applied to tracking FMDV incursions into disease free regions (Cottam *et al.*, 2008).

2.8 FMD Serotypes Distribution in Africa

The epidemiology of FMD in Africa was reviewed by Vosloo *et al.*, (2002) a decade ago. The salient features of this disease in Africa that were highlighted include; the presence of six FMDV serotypes including serotypes O, A, C, SAT 1, SAT 2 and SAT 3 with only Asia 1 serotype reported negative on the continent, with marked differences in the distribution and prevalence of serotypes (Vosloo *et al.*, 2002). FMD is endemic in sub-Saharan African countries, except for Swaziland, Lesotho and Madagascar which are considered completely free of FMD (OIE, 2010).

Serotypes A and O are widespread throughout Sub-Saharan Africa, whilst serotype C appears to have disappeared from the world as a whole, with the exception of Kenya (Kitching, 2002a; Tesfaye, 2006).

Historically, type C is the rarest of the FMDV type to have occurred in Africa, having been recorded only in Ethiopia, Kenya, and Angola. The last outbreaks of serotype C were reported in Kenya in 1996 and 2000 (Kitching, 2002a). Type O is

endemic in some countries of northern Africa, such as Egypt and Libya, while outbreaks due to this serotype have also been reported in Algeria, Morocco, and Tunisia. In central Africa, and West Africa, serotypes O, A, SAT-1 and SAT-2 have been recorded since 1958, while most outbreaks were attributed to serotypes A and SAT-2. The three SAT serotypes are prevalent in Southern and Eastern Africa (Vosloo *et al.*, 2002; Tesfaye, 2006).

2.9 FMD and FMDV Status in Tanzania

FMD is endemic in Tanzania, the first record of the disease was in 1927 in Arusha Region and in Kahama District in Shinyanga region (Anon, 1927; Rweyemamu and Loretu, 1972), and the first virus typed isolation was made in 1954 was attributed to the serotype O virus (Rweyemamu and Loretu, 1972).

Since then the disease has been reported annually in almost every region of the country and is generally endemic (Chibunda *et al.*, 2006; Picado *et al.*, 2011) and four serotypes (O, A, SAT1 and SAT2) have been reported and are the main cause of FMD outbreaks in the country (Vosloo, 2002; Rweyemamu *et al.*, 2008; Picado *et al.*, 2011; Kasanga *et al.*, 2012). However, Swai *et al.*, (2009) reported only three serotypes; Serotype O, SAT 1 and SAT 2. Serotype O was reported in the Southern highland region and in Eastern zone. Serotype SAT 1 in all seven zones and serotype SAT 2 in Northern, Lake and Eastern zones of Tanzania.

Since the disease is endemic in the country, continuous transmission of FMDV causes a number of outbreaks every year (Picado *et al.*, 2011). The persistence of the disease is because of the movement of livestock within and across international

borders (Kivaria, 2003). The presence of four serotypes (O, A, SAT 1 and SAT 2) and large number of wildlife reservoirs with susceptible species, especially African buffalo, complicates the control of FMD. To date, vaccination and movement restrictions have not been able to control the disease and are basically implemented to limit its economic impact (Kivaria, 2003).

The disease has a great impact on livestock sector in Tanzania, its control which should help reducing poverty in Tanzania (Perry and Rich, 2007), requires improving the current knowledge on the disease distribution, dynamics and factors related to its occurrence so that control measures can be implemented more efficiently (Picado *et al.*, 2011).

2.10 The Economic Importance of FMD

FMD is one of the most important livestock diseases in the world in terms of economic impact. Its economic importance is not only due to the ability of the disease to cause loss of production such as decline in milk and beef production as the result of clinical disease, but essentially outbreaks of FMD result in sanitary barriers that prevent the export of bovine and swine products. Furthermore, FMD causes enormous losses to the animal industry due to costs associated with control and eradication measures, including massive vaccination and/or destruction of infected herds (Niedbalski and Kessy, 2010). Therefore, it threatens the livelihoods of simple farmers, large sophisticated farming practices and the national and the international economies of the countries (Asseged, 2005).

The direct production effects in extensive production system include loss of milk, reduced draught animal power and mortality in calves. Restrictions on animal movement and international trade can cause much more serious losses (Tesfaye, 2006). The loss in animal production and international trade restriction imposed following an outbreak makes FMD of a major concern for livestock keepers. 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 cost several million US dollars for a single outbreak (Tesfaye, 2006). A striking example is the recent outbreak of serotype O (the Pan Asian strain) in Great Britain, a country that had been free of FMD since 1981. This devastating epidemic of 2001 spread to Ireland, France and The Netherlands where the United Kingdom alone were forced to slaughter about 4 million infected and in contact animals. The cost of this epidemic in the United Kingdom was estimated to be more than $29 billion (Tesfaye, 2006).

2.11 FMD Diagnosis

Diagnosis of FMD depends mainly on the clinical and pathological examinations, serological and molecular approaches. Laboratory confirmation with tests such as Complement fixation test (CFT) and ELISA, virus isolation technique and RT-PCR and sequencing is necessary, as all vesicular diseases have almost identical clinical signs (CFSPH, 2007).

2.11.1 Clinical diagnosis

The symptoms of FMD vary with the species, but are suggestive. In cattle, FMD suspicion should be raised whenever salivation and lameness occur simultaneously and a when a vesicular lesion and erosions in the oral cavity or on the feet, teats or other areas is seen or suspected. Profuse salivation is uncommon in pigs or sheep, where lameness is more typical. Fever often precedes other clinical signs; therefore, febrile animals should be carefully examined. Early diagnostic lesions may be found before animals start to salivate, have a nasal discharge, or become lame. Clinical diagnosis can present many difficulties due to viral infections of the mucous membrane, which produce similar clinical signs. When sudden death is observed in young cloven-hoofed livestock, older animals should also be examined; young animals that die of heart disease may not have vesicular lesions (Blood *et al.*, 1994).

2.11.2 Differential diagnosis

FMD has the clinical signs that resembles with other vesicular diseases; it is clinically indistinguishable with vesicular stomatitis, swine vesicular disease and swine vesicular exanthema (Jullu, 2004; APHIS, 2007). In cattle, oral lesions can resemble rinderpest, infectious bovine rhinotracheitis, bovine viral diarrhea /mucosal disease, malignant catarrhal fever, Bovine mammillitis and epizootic hemorrhagic disease. In small ruminants the lesions can be confused with bluetongue, Peste des petits ruminant (PPR) and contagious ecthyma (Jullu, 2004; CFSPH, 2007; APHIS, 2007). However in humans, the disease can be confused by "hand, foot and mouth disease" caused by *Coxsackie A virus* which is a different virus but with the same family "picornaviridae" (Murphy *et al.*, 1999) as summarized in Table 1.

2.11.3 Laboratory diagnosis

Due to the highly contagious nature and economic importance of FMD, the laboratory diagnosis and serotype identification of the virus should be done in a virus-secure laboratory (OIE, 2009).

a) Virus isolation

The isolation and characterization of the virus is the "golden standard" for the diagnosis of viral diseases. The suspensions of field samples suspected to contain FMD virus are inoculated into cell cultures and examined for cytopathic effect (OIE, 2009).

b) Complement fixation test (CFT)

Several foot-and-mouth disease virus strains were examined by complement-fixation tests in microplates and in tubes. It was established that the two systems are comparable, although greater reproducibility is obtained with tube tests, but the CFT has been replaced by ELISA (OIE, 2009). Although CFT is a fast method it needs high virus load and results are sometimes affected by pro-and anticomplementary activities of the test sample (Neeta Longjam *et al.*, 2011).

c) Enzyme-linked immunosorbent assays (ELISAs)

Liquid-phase blocking ELISA: It detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals; the test is based upon specific blocking of the FMDV (Hamblin *et al.*, 1986 a).

Indirect sandwich ELISA: The test detects FMD viral antigen and is used for identification of viral serotypes (OIE, 2009).

d) Nonstructural protein (NSP) antibody test

This is an antibody detection test directed to recombinant FMDV NSPs (for example 3A, 3B, 2B, 2C, 3ABC). The detection of antibody to the NSPs of FMDV can be used to identify past or present infection with any of the seven serotypes of the virus. Therefore the 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 (OIE, 2009). Compared to the liquid phase blocking ELISA, 3ABC ELISA allows differentiation between samples from infected (3ABC positive) and vaccinated (3ABC negative) animals (Hamblin *et al.*, 1986a).

The 3ABC ELISA is also rapid test for screening of large number of sera. In areas where more than one serotypes exist, the test is also 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, 2002).

e) Nucleic acid recognition methods

This refers to methods such as Agarose gel-based RT-PCR assay, Real-time RT-PCR assay and Sequencing (OIE, 2009). The polymerase chain reaction (PCR) can be used to amplify the genome fragments of FMD virus in diagnostic material. 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 (Tesfaye, 2006).

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 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).

2.12 FMD Control

Control of FMD relies mainly on prophylactic vaccination using polyvalent vaccines, animal movement restrictions, surveillance and quarantine measures (Murphy *et al.*, 1999; OIE, 2002). The official attitude of a country regarding control of a disease depends on how seriously the disease affects the country, the financial and technical ability of the country (Tesfaye, 2006). The degree of control of FMD thus varies as follows: Routine vaccination is used where the disease is endemic; in contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use of strict movement controls and slaughter of infected and contract animals when outbreaks occur (OIE, 2004).

2.12.1 The disease control in endemic areas

In endemic areas, the disease is generally controlled by vaccination and movement restriction of animals. Vaccination against FMD virus is achieved with inactivated vaccines that should induce protective immunity against each type of antigens incorporated in the vaccine (Asseged, 2005). Therefore, when vaccinating animals, it is important that the vaccine contain the same subtype of virus as in the area. This necessitates frequent checking of the serotype and subtype during an outbreak because FMD virus frequently changes during natural passage through various species (Tesfaye, 2006).

Immunity to one serotype provides protection only against the homologous viruses. Therefore, active disease surveillance must be effective which needs a strong field service as well as proper laboratory facilities with efficient methods of detection and characterization of the virus. The extent of vaccination coverage is of considerable importance to the protection of livestock populations since for FMD, it is estimated that 80 to 85% of individuals must have virus-neutralizing antibody to achieve herd immunity (Asseged, 2005).

2.12.2 Control in disease-free areas

Stamping out

The more affluent FMD free nations, those with an economically significant live animal and animal product export trade, and those whose livestock are highly susceptible to FMD, have contingency plans to deal rapidly with confirmed FMD outbreaks (Sangare, 2002; Tesfaye, 2006).

In the first instance, 'stamping out' policy, consisting of the slaughter of all affected and in-contact susceptible animals would be instigated, together with associated zoosanitory measures including, the imposition of movements restriction, to control the outbreaks. The stamping out is done with full compensation paid for animals slaughtered. The success of 'stamping out' is recognized by the OIE in its guidelines on re- establishing trade following an outbreak (Asseged, 2005).

Emergency vaccination

The use of emergency FMD vaccines, first provide protective immunity as rapidly as possible to susceptible herd, and secondly, to reduce the amount of virus released and thereby limit the amount of virus circulating and spreading beyond the restricted area (OIE, 2004; Asseged, 2005).

Protective vaccination

This is used effectively in animals not already exposed to FMD virus. It would therefore be employed outside the 3km protection zone and outside any predicted aerosol spread of virus from the infected premise. All vaccinates would be naive to FMD antigen, and would require a minimum of 3 to 4 days to develop protective immunity. This protective vaccination would thus form a ring around the infected area, preventing diseases spread, and allows the outbreak to expire within the protection zone, where infected herds would quickly be identified and slaughtered (Asseged, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Research Design and Study Area

An observational prospective study was conducted between October 2010 and July 2011, purposively visiting the reported FMD suspected outbreaks from different parts of the Lake zone. This study was conducted in the Lake zone which is located in the northern part of Tanzania (Longitudes 031° 81' to 034° 15'E and Latitudes 01° 36'S to 01° 78'S) and comprises Mwanza, Mara, Kagera and Shinyanga regions. The first three regions border Lake Victoria. The Lake zone borders all East African Countries including Uganda and Kenya in the North and Rwanda and Burundi in the West.

The Lake Zone can be divided into three major agroecological zones: an intermediate zone (950–1500 mean above sea level) (Mwanza, Shinyanga, part of Kagera and Mara region) receiving low rainfall (850–1100 mm); an intermediate zone (950–1500 masl) with high rainfall (>1100 mm) (parts of Shinyanga [Kahama District] and Mara [Tarime and Serengeti Districts]); and a highland zone (>1500 masl) with high rainfall (>1500mm) (parts of Tarime District and Kagera Region). The zone has a bimodal rainfall pattern. The short rains (*vuli*) start in October and peak in December, whereas the long rains (*masika*) start in March and peak in April. The dry season starts in June and ends in September (Mafuru *et al.,* 1999).

The study was conducted in all four regions of the Lake Zone and involving 13 districts. The regions and districts (in the parenthesis) where this study was carried

out included Mara (Rorya, Serengeti and Musoma Rural), Mwanza (Ilemela, Kwimba and Misungwi), Shinyanga (Bariadi, Maswa, Kahama and Shinyanga Rural) and Kagera (Ngara, Muleba and Missenyi).

3.2 Sample Size Determination

Samples were collected randomly in the household and pulled as one sample from the suspected FMD cattle. The sample size (household) used was 161. The Formula used for calculating sample size $\mathbf{n = Z^2\ P\ Q\ /L^2}$ Calculated sample size = 96, which is the minimum sample size.

Where, n= estimated sample size, P=50%, L=10%. Z= 1.96 is student t value for an expected confidence level, P=expected population proportion, Q=1-P and L= the accepted absolute error or precision (Cochran, 1977).

3.3 Sample Collection, Type and Source

All samples were collected following suspected FMD outbreak from different geographical locations of the Lake zone of Tanzania during the period of November 2010 to February 2011. In addition, samples from Veterinary Investigation center (VIC)-Mwanza collected during April to July 2010 were also included in the study. The samples included epithelial tissues (28) and sera from whole blood (133).

3.3.1 Epithelial tissue sample collection

The epithelial tissue samples were collected from suspected FMD cattle. Twenty eight (28) epithelial samples were collected from eight (8) districts of the Lake zone after suspected FMD outbreaks. The collected samples included 2 epithelial tissues

in Ngara, 10 in Misungwi, 5 in Serengeti, 2 in Musoma Rural, 2 in Rorya, 3 in Missenyi, 2 in Muleba and 2 in Kahama districts during April 2010 to February 2011. All the epithelial samples were collected during the course of field outbreaks. Intact and /or ruptured vesicular flaps of epithelial tissues from tongue, gums and buccal cavity surroundings of FMD suspected animals were collected and placed in transport media (prepared at VIC-Mwanza laboratory) containing sterile glycerol (50% v/v), antibiotics and 0.04 M phosphate buffer at pH 7.4. The samples in transport media, kept on ice, were transported to VIC-Mwanza and then to Central Veterinary Laboratory (CVL), Temeke, Dar-es-Salaam and afterwards stored at −20°C.

3.3.2 Preparation of serum samples

A total of 133 sera were prepared from blood collected from cattle in 10 districts of the Lake zone during the course of field outbreak during November 2010 to February 2011. The collected samples included 19 sera in Ngara, 27 sera in Misungwi, 17 in Serengeti, 7 in Musoma Rural, 13 in Rorya, 13 in Kwimba, 5 in Maswa, 15 in Bariadi, 12 in Shinyanga Rural and 4 in Ilemela districts. Whole blood was collected from a jugular vein of clinically FMD suspected cattle into plain sterile vacutainer tubes (BD-Vacutainer® CAT, BD-Plymouth, PL6 7BP, UK) and stored overnight at room temperature for serum separation. The serum was then transferred into a single sterile cryovial already labeled and transported in an icebox to VIC-Mwanza laboratory for temporary storage, and then to CVL-Temeke for laboratory analysis. In the laboratory, the sera were stored at −20 ^{0}C until laboratory analysis.

3.3.3 Data collection using structured questionnaires

A face to face interview with 29 simple structured questionnaires was conducted to people to evaluate their awareness on FMD outbreaks and for gathering information on the current descriptive spatiotemporal distribution of the disease from various areas of the Lake zone. Questionnaire survey form is as shown in Appendix 1.

This study involved 29 respondents in six districts. The number of respondents interviewed in each district is as indicated in Table 3.

Table 3: Agro ecological zone distribution of questionnaire respondents

District	Number of respondents
Ngara	2
Serengeti	7
Rorya	5
Kwimba	5
Bariadi	5
Shinyanga Rural	5
Total	**29**

3.4 FMD Laboratory Analysis

3.4.1 Detection of antibodies to the FMDV nonstructural protein 3ABC using 3ABC-Ab ELISA

The SVANOVIR® FMDV 3ABC-Ab ELISA (Centro Virologia Animal, COREPRO Buenos Aires, Argentina) is designed to detect antibodies to the nonstructural protein 3ABC in bovine serum samples and to differentiate between naturally infected and vaccinated animals. It detects all serotypes of the FMD virus. It also detects animals being in the carrier status. The kit procedure was based on a solid phase indirect ELISA.

The SVANOVIR® FMDV 3ABC-Ab ELISA is developed in collaboration with CEVAN (Centro Virologia Animal), COREPRO Buenos Aires, Argentina. The kit was provided by the SADC-TADs Program and the Ministry of Livestock and Fisheries Development, and obtained from the Institute for Animal Health, Pirbright Laboratory, UK. A total of 133 serum samples were tested for antibodies against 3ABC non-structural protein of FMDV from the suspected FMDV-infected animals.

ELISA was performed following manufacturer's instructions; Briefly, all reagents were equilibrated to room temperature ranging from 18 to 25°C before use. Serum samples and controls were pre-diluted 1/40 in sample dilution buffer. In duplicates, 50µl of pre-diluted positive control serum and negative control serum were added respectively onto selected wells, then 50µl of pre-diluted sample were added into selected wells, the plates were sealed and incubated at 37°C for 30 minutes, and rinsed 3 times with PBS Tween buffer. Afterwards, 50µl of HRP conjugate was added to each well and the plates were sealed and incubated at 37°C for 30 minutes and then rinsed 3 times with PBS Tween buffer.

At each rinse cycle the wells were filled up, plates were emptied and tapped hard to remove all remains of fluid. After tapping, 50µl substrate solution was added to each well and incubated for 30 minutes at room temperature in dark. The reaction was stopped by adding 50 µl of stop solution to each well and mixed thoroughly, and immediately the optical density (OD) of the controls and samples were measured at 405nm in a microplate photometer and air was used as blank. The laboratory plate layout results are shown in Appendix 4.

Calculations of results were done in two steps as described below.

1. Corrected OD Values (ODCorr)

The optical density (OD) values in wells coated with NSP 3ABC were corrected by subtracting the OD values of the corresponding wells containing the control antigen.

$OD_{NSP\ 3ABC} - OD_{Control} = OD_{Corr}$ .... (1)

2. Percent Positivity Values (PP)

All Corrected OD Values for the test samples as well as the Negative Control (Neg C) were related to the corrected OD value of the positive control as follows:

$$PP = \frac{\text{Test Sample or Neg C } (OD_{Corr})}{\text{Positive Control } (OD_{Corr})} \times 100 \quad \text{.................. ....(2)}$$

Interpretation of the results:

Criteria for test validity

To ensure validity the Positive Controls should have a corrected OD value greater than 0.8 and the Negative Controls should have a corrected OD value of less than 0.3. If the respective PP value for the Negative Control results in a positive interpretation according to criteria below, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of test sample results

Serum PP	Interpretation
< 48	Negative
≥ 48	Positive

3.4.2 FMDV serotyping using indirect sandwich ELISA

A total of 28 epithelial tissue samples from the clinically infected animals were assayed for FMD virus antigens and serotyped with indirect sandwich ELISA as described by Roeder & Le Blanc Smith, (1987); Ferris and Dawson, (1988). The kit was provided by the SADC- TADs Program and the Ministry of Livestock Development and Fisheries, and supplied by the Institute for Animal Health, Pirbright Laboratory, UK.

Procedure: Indirect sandwich ELISA was performed following manufacturer's instructions;

Briefly, plates were coated with 50µl trapping rabbit antibody stock (Rabbit anti-FMDV serotypes O, A, C, SAT1, SAT2, SAT3 and Asia1) diluted 1:1000 in coating buffer (carbonate/bicarbonate) into 96 wells of NUNC Maxisorp microplate. The eight compartments from top to bottom received respectively antisera to FMDV serotypes O, A, C, SAT1, SAT2, SAT3 and Asia1 and Normal non immune serum, and incubated at 37 ^{0}C for one hour, with continuous shaking. Then, microplates were washed with Phosphate buffered saline (PBS), on microplate 1 wells of columns 1 to 6 were loaded with 50µl of Diluent buffer A (PBS + Tween 20). To well 1 of row A, 12.5µl of control antigen type O were added. To well 1 of row B, 12.5µl of control antigen type A were added. To well 1 of row C, 12.5µl of control antigen type C were added. To well 1 of row D, 12.5µl of control antigen type SAT1 were added. To well 1 of row E, 12.5µl of control antigen type SAT2 were added. To well 1 of row F, 12.5µl of control antigen type SAT3 were added. To well 1 of row G, 12.5µl of control antigen type Asia1 were added. To well 1 of row H, 12.5µl of the negative control antigen were added.

Thereafter, serial dilutions were performed from well 1 to 4 to make a fivefold dilution series of each control antigen. In column 5 and 6 of each plate were filled with 50μl diluent buffer A in rows A to H. The remainder of the plate and other plates were loaded with 50μl test samples in duplicates and incubated at 37^0C for one hour with continuous shaking.

After microplates were washed, 50μl detecting antibody (Guinea pig anti-FMDV serotypes O, A, C, SAT1, SAT2, SAT3 and Asia1 and normal, non-immune serum), diluted 1:100 in diluent buffer B (PBS, Tween 20 and skimmed milk powder) was added into each plate wells in appropriate order (rows A to H receive respectively antisera, and incubated at 37^0C for one hour with continuous shaking. After washing the plates, 50μl of conjugate (Horseradish peroxides (HRP) conjugated rabbit anti-guinea pig immunoglobulin) diluted 1:200 in diluent buffer B was added into all wells from A to H of each microplate and incubated at 37^0C for 45 minutes with continuous shaking.

Finally, the plates were washed and 50μl of substrate/chromogen (Hydrogen peroxide (H_2O_2)/Ortho-Phenylenediamine (OPD) solution was added and incubated at ambient Temperature in the dark for 15 minutes before 50μl of stopping solution (Sulphuric acid (H_2SO_4) was added into all 96 wells of the microplates. The ELISA reader was connected to the computer loaded with ELISA Data Information (EDI) Software, which is used to automate the reading of optic density (OD) value and calculate the mean corrected OD value for control and test samples according to the protocol and the plates read with spectrophotometer at 492nm filter. A mean correct OD value of >0.1 above background was considered to be positive.

3.5 Statistical Data Analysis

Descriptive statistics for laboratory data of both sandwich and non-structural protein 3ABC ELISA were used and frequency distributions calculated (Bertola and Thrusfield 2005). Prevalence of positive animals was determined by dividing the number of positive serum samples by the total number of samples tested. Microsoft Excel software was used for the calculations. For questionnaire survey, data were coded in such a way that variables (awareness, species affected, clinical signs, season and economic impacts) represented 1 and 0 represented negative awareness. The data were analyzed manually with the aid of Microsoft Excel® software in graphics presentation for results interpretation since the information collected did not involve any normal distribution assumption and sample size was less than 30.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Farmers' knowledge, awareness, socio-economic impact and practices related to the temporal and spatial occurrence of FMD outbreak in the Lake Zone

A total of 29 open-ended questionnaires were face to face administered to livestock keepers in 20 villages of the six districts of the Lake zone. The number of questionnaires in each district is as indicated in the brackets: Ngara district (02), Serengeti (7), Rorya (5), Kwimba (5), Bariadi (5) and Shinyanga (5) questionnaires.

The data collected showed that FMD, which is locally known as *amarenge* (foot disease) in Kurya tribe and *achany* in Luo tribe both in Mara region, *isuna* in Hangaza tribe in Kagera region and *malonda* in Sukuma tribe in Mwanza and Shinyanga, was well known to farmers and they are well acquainted with the awareness of the disease. Out of 29 respondents, 26 (90%) were aware of the disease, species affected (Fig.1) and its clinical signs, 21 (72%) morbidity, 10 (35%) mortality and 23 (79%) socio-economic impacts (Table 4). The husbandry systems practiced in the investigated herds were agro pastoral with free animal movements.

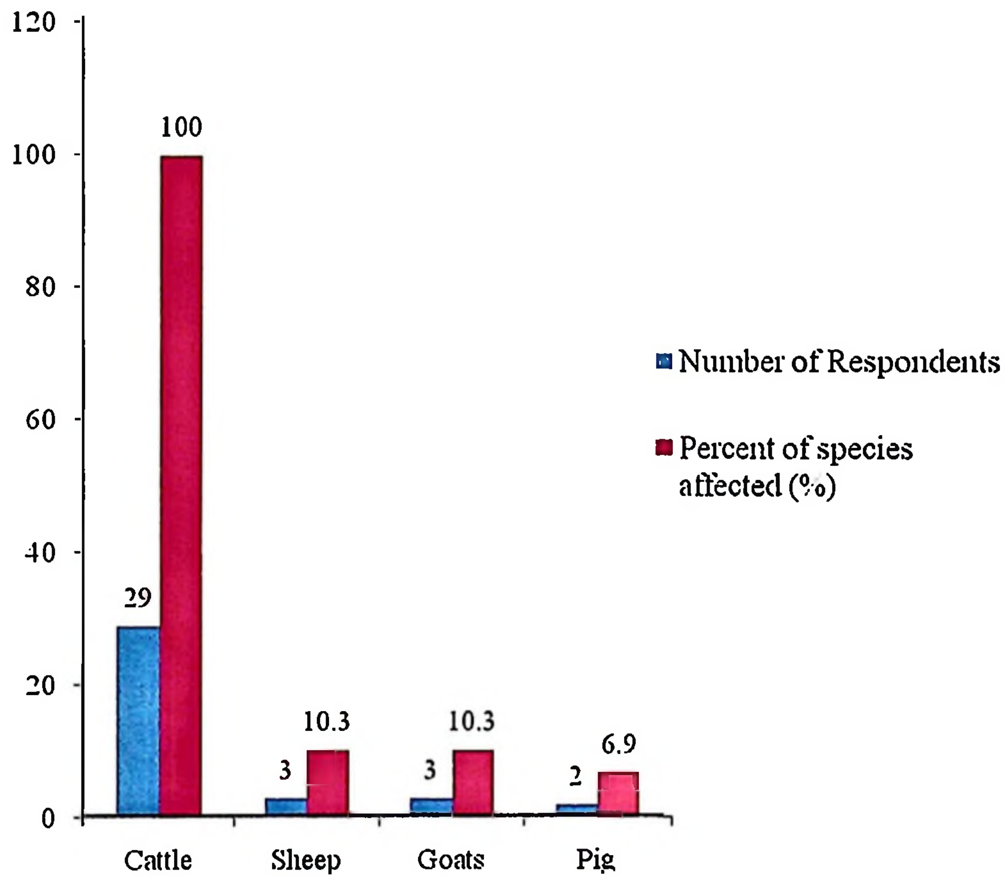


Figure 1: Species affected by FMD as scored by the respondents.

The data showed that FMDV affects mostly cattle (100%) with less extent to sheep (10.3%), goats (10.3%) and pigs (6.9%) (Fig.1). the prominent clinical signs mentioned by most of the farmers interviewed were: presence of vesicles in and around the buccal cavity, anorexia, excessive salivation and lameness.

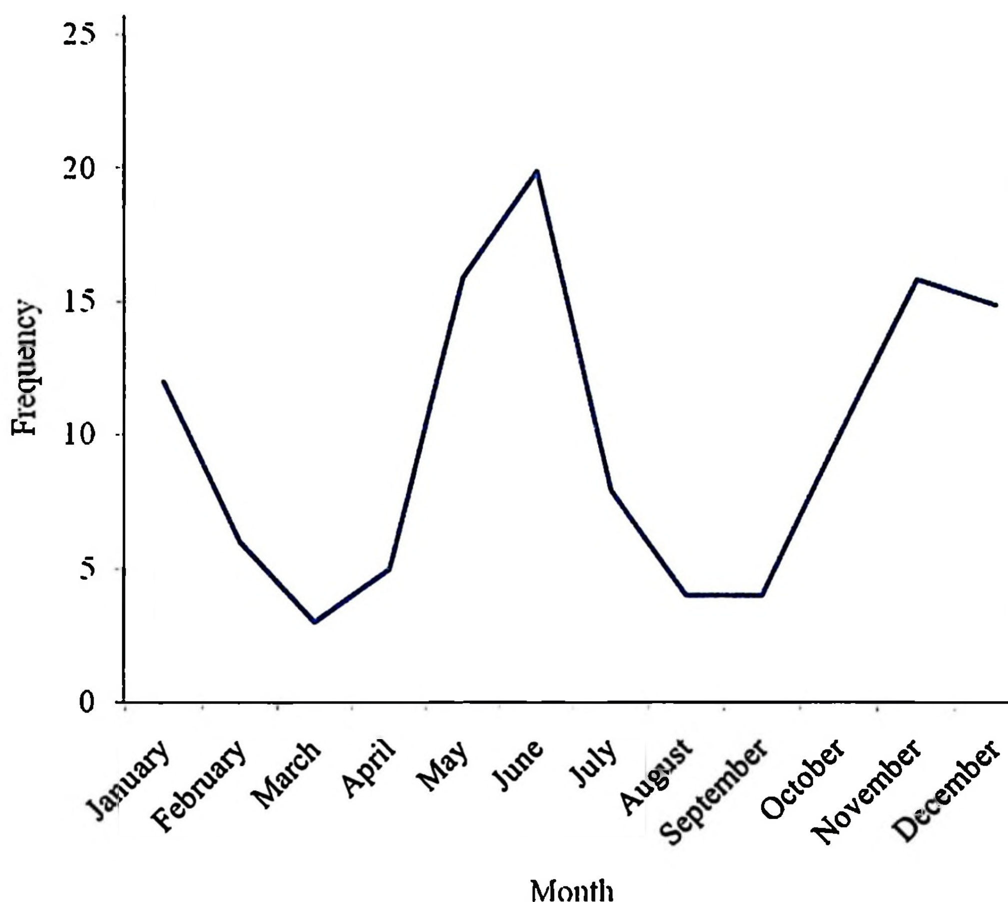


Figure 2: Temporal trends of FMD outbreak as scored by the respondents.

Table 4: Farmers' knowledge and awareness on FMD

	Respondents (n/N)	Percentage
FMD Awareness	26/29	90
Species affected	26/29	90
Clinical signs	26/29	90
Morbidity	21/29	72
Mortality	10/29	35
Economic impact	23/29	79

The questionnaire data showed that FMD outbreaks often occur after rainy seasons (dry seasons) 22 (75.9%), with less extent to rainy season 5 (17.2%) and rare

occurrence in all the year 2 (6.9%) despite variation of climate. It was predominantly encountered with the highest peaks just after long rains (*masika*) in May-June and at the end of the short rains (*vuli*) in November-December (Fig. 2). Higher frequency of the disease occurrence was noted during the months of June 20 (69%) and May/November 16 (55%) and relatively low during the month of March 3 (10%) to April 5 (17%) and August-September 4 (14%) (Fig. 2).

The Disease declines in the rainy seasons in March-April and September-October, and in dry months of January 12 (41%) to February 6 (21%) and July 8 (28%) to August 4 (14%) (Fig.2). However, from this study, temporal pattern of FMD occurrences field experience suggested that the disease often occurs after rainy seasons (dry season), despite variation of climate.

According to the respondents, 21 (72%) mentioned that the morbidity rate was high, While the mortality rate was low in young animals and extremely low in both young and adult animals 10 (35%). The economic losses mentioned were largely due to the decrease in draught power and loss of market, death of newborn and suckling calves, long hair coat and heat intolerance locally known in Sukuma tribe as *luzwiga* and regarded as a sequela to FMD were 23 (79%) respondents. The Clinical signs observed by the farmers 26 (90%) were mouth and foot lesions, hypersalivation, anorexia and lameness.

Farmers were using salt, crushed sisals mixed with ashes, bitter tree leaves locally known as *mravumba* in Hangaza tribe to cure mouth ulcers as a local treatment.

Modern treatments were also practiced by farmers by applying antibiotics (*Penstreptomycin* and *Oxytetracyclin*) to protect infected animals from secondary bacterial infection.

4.1.2 Seroprevalence of FMDV using 3ABC- Antibody ELISA

From 133 serum samples examined for the presence of antibodies to the 3ABC non-structural protein of FMD virus, 107 (80.5%) were positive animals (Table 6). The highest prevalence was recorded in Musoma district 7 (100%) followed by Serengeti 16 (94.1%), Kwimba 12 (92.3%), Misungwi 24 (88.9%), Bariadi 13 (86.7%), Rorya 11 (84.6%), Maswa 4 (80%), Ngara 12 (63.2%), Shinyanga rural 7 (53.8%) and Ilemela District 1 (25%) being the lowest. All 29 villages sampled, at least one or all samples were shown positive to 3ABC-FMDV antibodies as shown in Table 5.

Table 5: Results of serum samples for NSP-FMDV antibodies in Lake Zone

District	Village	Sample positive	Total sample	Percentage Positive (%)
Ngara	Nyakiziba	6	10	60.0
	Kumtana	6	9	66.7
Misungwi	Zarambi farm	15	16	93.8
	Njelenje farm	9	11	81.8
Kwimba	Malya	10	10	100
	Kitunga	2	3	66.7
Ilemela	Bwiru ziwani	1	4	25.0
Serengeti	Park Nyigoti	2	2	100.0
	Nyinchoka	5	5	100.0
	Nyiberekera	3	3	100.0
	Maburi	3	4	75.0
	Gusuhi	3	3	100.0
Musoma (R)	Singu	3	3	100.0
	Kiabakari	4	4	100.0
Rorya	Kowak	4	4	100.0
	Orio	3	5	60.0
	Rabor	3	3	100.0
	Makongoro	1	1	100.0
Maswa	Binza	2	3	66.7
	Shanwa	2	2	100.0
Bariadi	Imalilo	2	3	66.7
	Mahaha	3	3	100.0
	Lugulu	6	6	100.0
	Bubale	2	3	66.7
Shinyanga (R)	Iselamagazi	1	3	33.3
	Nhomango	4	4	100
	Solwa	2	6	33.3
	Total	**107**	**133**	**80.5**

Table 6: Results of NSP-FMDV antibodies per districts of Lake Zone

District	Sample positive	Sample tested	Percentage of Positive
Bariadi	13	15	86.7
Ilemela	1	4	25.0
Kwimba	12	13	92.3
Maswa	4	5	80.0
Musoma	7	7	100
Misungwi	24	27	88.9
Ngara	12	19	63.2
Rorya	11	13	84.6
Serengeti	16	17	94.1
Shinyanga	7	13	53.8
Grand Total	**107**	**133**	**80.5**

4.1.3 Detection of FMDV serotypes prevailing in the Lake Zone using Sandwich ELISA (sELISA)

Table 7 shows overall results on 28 bovine epithelial tissue samples tested against seven FMDV antigen using sELISA and Table 8 shows summarized results obtained per each serotype. Out of 28 samples tested by sELISA, 15 samples (53.6%) contained FMDV antigen specific for serotype O. The rest 13 samples (46.4%) were tested negative for any of the seven serotypes. All samples from Ngara district were negative to all serotypes (Table 7). Samples tested positive to FMDV serotype O were collected from Misungwi 2 (20%), Musoma 2 (100%), Serengeti 3 (60%), Rorya 2(100%), Missenyi 3 (100%), Muleba 2 (100%) and Kahama 1 (50%) as shown in Table 7.

Table 7: FMDV antigen detection using sandwich ELISA in Lake Zone

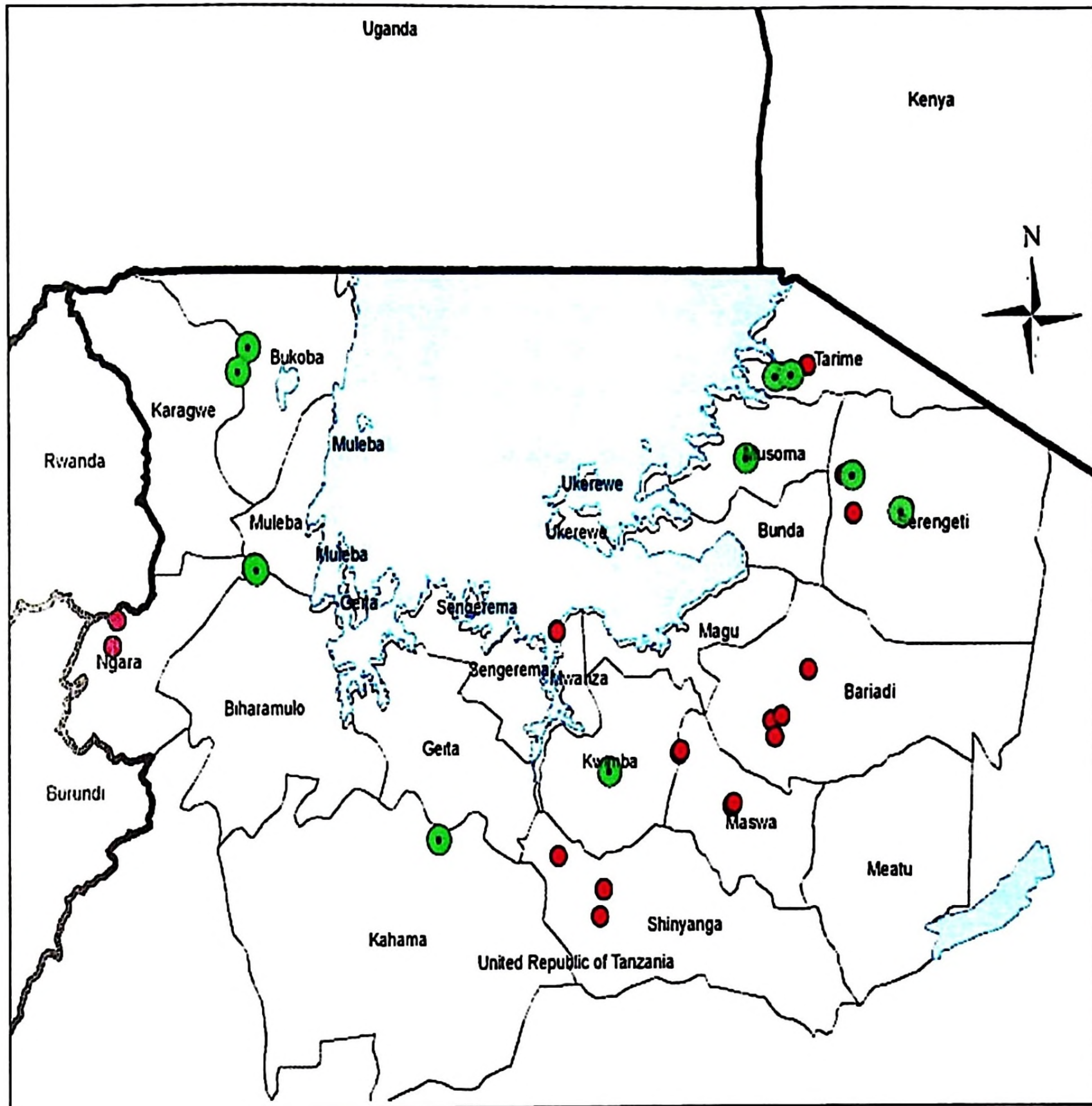
District	Sample positive	Sample tested	Percent positive (%)	Serotype
Ngara	-	2	-	-
Misungwi	2	10	20	Type O
Musoma	2	2	100	Type O
Serengeti	3	5	60	Type O
Rorya	2	2	100	Type O
Misenyi	3	3	100	Type O
Mulcba	2	2	100	Type O
Kahama	1	2	50	Type O
Total	**15**	**28**	**53.6**	**Type O**

Table 8: Summary of FMDV serotypes detection using sandwich ELISA

	Reactions of clinical samples to seven FMDV antigens						
Serotype	**O**	**A**	**C**	**SAT1**	**SAT2**	**SAT3**	**Asia1**
Positive/Total	15/28	0/28	0/28	0/28	0/28	0/28	0/28

4.1.4 Spatial distribution of FMD and FMDV serotypes in the Lake Zone

Spatial occurrence of FMD and FMDV serotypes in the Lake Zone was evenly distributed in all villages and districts sampled with only serotype O virus detected in all four regions as shown in Fig. 3. Epithelial samples collected from 8 districts for FMD serotyping, only one district (Ngara) were tested negative to all the seven serotypes, but the seven districts most of the samples were positive only to FMD serotype O virus. Seroprevalence of FMDV based on detection of antibodies to non structural protein 3ABC from serum samples taken in 28 villages was positive with variation in percentage of positive samples (Table 5). The spatial distributions of FMDV seroprevalence to nonstructural protein 3ABC indicated that all villages were tested positive (Table 5 and Fig. 3).



Legend

Serotype O.csv Events

FMD_ Positive

Figure 3: Spatial distribution of FMD and FMDV serotypes in Lake Zone.

4.2 Discussion

The study shows that FMD is endemic and widely spread throughout all the four regions of the Lake zone of Tanzania (Table 5 and Fig.3). The disease is of high economic importance especially to countries that have an intensive animal industry (Mwiine *et al.,* 2010). The study of the disease on questionnaire survey indicated that the group of informants were aware of the disease, and they described well most of the clinical presentations of the disease; indeed most of the signs listed for FMD 26(90%) such as; mouth and foot lesions, hypersalivation, anorexia and lameness were consistent with what has been described by other researchers (Radostits *et al.,* 1994; Jullu, 2004; Tesfaye, 2006; CFSPH, 2007 and Swai *et al.,* 2009).

The findings of this study indicate high correlation between livestock keepers' awareness of FMD and serological diagnosis of FMDV infection by NSP 3ABC-Ab ELISA in the diagnosis of FMD. These results also conforms to the positive results obtained by sELISA test which indicated 53.6% to be positive for FMDV serotype O in 7 districts (Table 7). This observation indicates correlation of laboratory results to the findings of questionnaire survey on the awareness of farmers on the clinical manifestation of FMD under the field condition.

Seasonal incidence of FMD was found to be high at the end of long rain season (May-June) and at short rain season *"vuli"* (November-December) as shown in (Fig. 2). The lowest incidence of FMD outbreak being reported during long rainy season *"masika"* (March-April) and in August-September (Fig.2). The reasons for this observation could be ascribed to movement of animals for search of pastures and

water whereby many herds of cattle meet thereby hastening the spread of infection from one herd to another.

The FMD study on NSP 3ABC antibody detection revealed the evidence of infection and spatial distribution of the disease in the lake zone by 80.5% (Table 6). The study using sELISA revealed that the spatial distributions of FMD outbreaks in the year 2010/11 in the Lake zone were caused mainly by serotype O virus (Fig. 3). These findings are in agreement with Rweyemamu *et al.*, (2008), who reported that serotype O was the most widely prevalent serotype in most parts of the world including Tanzania.

The detection of serotype O virus antigens in all four regions of the Lake zone (Mara, Mwanza, Shinyanga and Kagera) in the current study, which was reported negative by Swai *et al.* (2009) and Jullu, (2004) in this zone, but reported to be present in some years ago and in other zones, depicts the possibility of re-introduction of serotype O that causes the acute epidemic (sporadic) outbreaks of FMD in almost all the districts of the Lake zone. The re-introduction of type O virus could be due to loose and free border movement of animals (livestock and wildlife) to and from neighbouring countries such as Kenya and Uganda in the Eastern Africa. Furthermore, the spread of serotype O virus within the districts of the Lake zone could be associated with movement of animals from one district/region to another during trade and marketing, and also long distance grazing movements practiced by agro pastoralists with large herd of cattle for search of pastures and water.

This study has indicated the evidence for presence of FMD as an endemic situation in almost all the districts and regions of the Lake zone. Serotype O has been detected as the most and probably the only prevailing serotype in the Lake zone of Tanzania during the 2010/11 outbreaks.

Serotype O virus has not been detected in the Lake zone some years back, the research conducted by Jullu (2004) by using both sELISA and real time PCR reported only serotype SAT 1 in Musoma urban and SAT2 in Musoma Urban and Tarime from samples collected in 1999, samples collected in 2003 in Ukerewe and Mwanza city detected serotypes SAT 2. Jullu (2004) used the same protocol and kit for sELISA as I used to the current study.

Type O was detected in the Northern Zone, Eastern zone (Dar es salaam) and Southern Highland (Iringa and Mbeya) from samples collected for the period 1997 to 2003 (Jullu, 2004). Swai *et al.* (2009) detected only serotypes SAT 1 and SAT 2 in the Lake zone from samples collected between 1997 to 2004. Recently, findings by Kasanga *et al.* (2011) from the samples collected from 1967 to 2009 could not detect the presence of serotype O in the Lake zone.

The detection of only serotype O in samples collected in the Lake zone during 2010 and 2011, which was not detected by other researchers (Jullu, 2004; Swai *et al.*, 2009 and Kasanga *et al.*, 2011), gives a great challenge of the routine disease surveillance. The absence could also be due to small sample size submitted for investigation, restricted areas of surveillance or absence of surveillance system.

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The recent occurrence of epidemic (sporadic) outbreak with the classical clinical signs signifies the naïve immunity status of the animals and hence probably introduction of either the new strain of serotype O virus or re-introduction of serotype O virus with variant pathogenicity which was not present for some years back. In regarding to the location of the Lake Zone and the widespread of the FMDV serotype O in all the four regions of the Lake zone gives the probability of the infection either from the vaccines or from the neighbouring countries, since some farmers especially from Kagera region do vaccinate their animals by using FMD polyvalent vaccine from Kenya, the rest of the zone have no recent history of FMD vaccination.

The current study has paved the way to the FMDV serotype (serotype O) prevailing in the Lake zone and also the extent of spatial distribution of FMD in the zone. This is the most important step as the information detailed therein would be useful in vaccine matching and selection of vaccine candidate strains for the Region. This will help in the recommendation for an appropriate approach and proper control programme of FMD in Tanzania.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMENDATION

5.1 Conclusion

The results indicated that FMD was detected in all thirteen districts investigated in the Lake zone. The observed agreement between livestock keepers in the current study and previous veterinary literatures regarding most of exhibited signs of important cattle diseases has proven that in the Lake Zone, farmers/ livestock keepers have great knowledge on FMD with disease outbreak, period of occurrence and diagnosis of the disease. The analysis of questionnaire data demonstrated that FMD is more prevalent during dry seasons (after rain season): December-February and June-July, consistent with seasonal cattle movement, which intensifies during long dry period (December -February). The level of seropositivity, loss of market and death due to morbidity and mortality, as investigated through questionnaire data and nonstructural protein 3ABC test, justify a need for control program.

5.2 Recommendations

This finding suggests further that animals should be protected through vaccination before they are subjected to stress of movement and mixing. This study further elucidated that FMD serotype O is circulating in the Lake zone, signifying to periodically assess the efficacy of the vaccinal strains in the field.

Therefore, based on these findings, the following are the immediate key recommendations: Questionnaire administration method should be used during animal health research, so as to share knowledge and experience from the livestock keepers or pastoral community.

Cross-protection and vaccine-matching of the field isolates to available vaccines is required. An extensive and regular sero-surveillance, virus isolation, and characterizations of the field FMDV isolates need to be conducted for a possible development of relevant vaccine from the local circulating field serotype or strains. Progressive control of FMD is the most pragmatic approach for Lake Zone. The critical components of such an approach should include a national FMD control initiatives, continuous surveillance and monitoring of the FMD situation, facility for FMD virus serotyping, isolation and sub-typing, and availability of an effective and relevant vaccine from a local prevailing virus serotype or strains and also a quick response to the disease occurrence and disease information network.

Meanwhile, with the critical need for improved diagnostic tests to detect viral infection, effort need to be concentrated on the development of simple, rapid, noninvasive tests that can be performed without expensive laboratory equipments. In this context although various molecular tools are very promising but to make it a successful tool search for more rapid and accurate tests as well as an earlier detection system in preclinical state in the field are needed for the future.

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APPENDICES

Appendix 1: Checklist for questionnaires

Checklist for evaluation of farmers' awareness, perception, socio-economic impact and practices related to Foot and Mouth disease (FMD) outbreak.

A. Background information:

Respondent name...............................Sex.......Age......... Date.............

Respondent occupation...

Respondent position in household..................... Level of education......

Region............District...............Ward...................Village...............

Livestock herd composition: Cattle.........Sheep.......Goats.........Pigs.........

B. FMD occurrence

Question: Have you seen or heard this disease in your herd or elsewhere? When does this disease occur?

Local name		
Swahili name		
Occurrence:	**Season (s)**	
	Month (s)	

C. Clinical signs and Socio economic impact

Question: What types of animal species are affected? Any apparent clinical signs associated with the disease? and rate of morbidity and mortality? How do you rank severity? (Ranking in numerical number in ascending order).

Type of animal species affected (Tick where appropriate)

Cattle	**Goats**	**Sheep**	**Pigs**	**Donkey**	**others**

Clinical signs

1.	
2.	
3.	

Level of morbidity, mortality and severity

Animal spp	Morbidity (High/Low)	Mortality (High/Low)	Number of mortality	Ranking (severity) vs species
Cattle				
Sheep				
Goats				
Pigs				

D. Morbidity rate distribution with animal classes

Question: Which class of animal are more susceptible and affected? (Tick where appropriate)

Young	
Adult	
All age	

E. Mortality rate distribution with animal classes

Question: Which class of animal are more likely to die when they are infected?

(Tick where appropriate)

Young	
Adult	

F: Socio-economic impact

Question: What loss do you get during the outbreak?

Losses

1.	
2.	
3.	

G: How farmers do diagnose and treat the disease

Question: How do you diagnose the disease? And how do you respond to or treat the disease?

Diagnosis

1.	
2.	
3.	

Treatment

Treatment (Yes/No)	Local Treatment	Modern Treatment

H: Local knowledge on how to treat FMD

Question: How do you locally treat the disease?

Local treatment

1.	
2.	
3.	

I: Type of commercial available medicament used for FMD

Question: What type of commercially available medicament used?

Modern Treatment

1.	
2.	
3.	

J: Complication as a sequela to the FMD

Question: What are the complications as a sequela to the disease?

Complications

1.	
2.	
3.	

K: Any comment (s) from the farmer

Appendix 2: Plate layout used for FMDV 3ABC-Ab ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	C+	C+	5	5	13	13	21	21	29	29	37	37
B	C+	C+										
C	C-	C-										
D	C-	C-										
E	1	1										
F	2	2										
G	3	3									43	43
H	4	4									44	44

Key: C+: Positive serum control C^{-} : Negative serum control

1, 2, 3.......44: Test samples

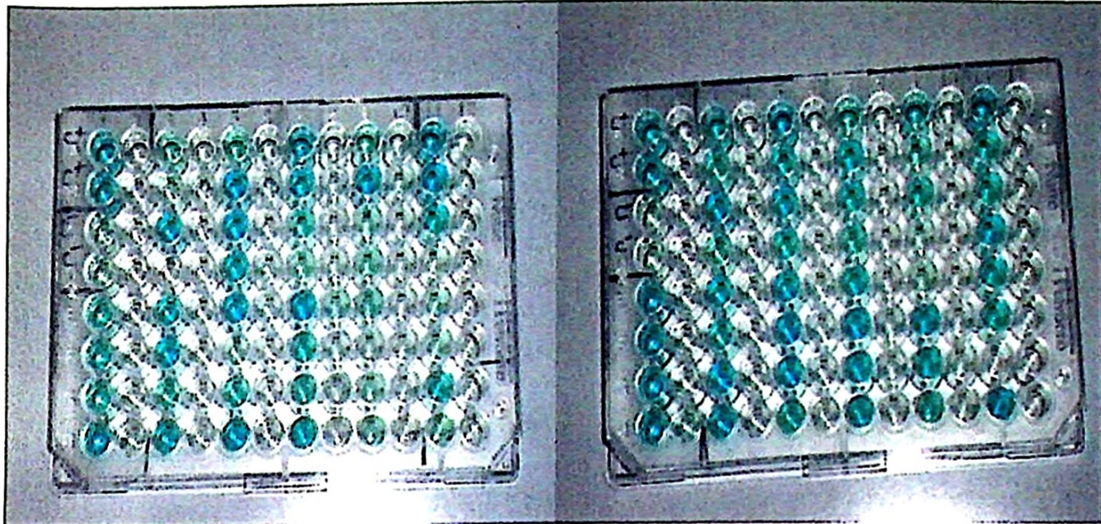
Appendix 3: Plate layout used for Indirect sandwich ELISA

		Control Antigens											
		1/5	1/25	1/125	1/625			Sample1		Sample2		Sample3	
Serotype		1	2	3	4	5	6	7	8	9	10	11	12
O	A												
A	B												
C	C												
SAT1	D												
SAT2	E												
SAT3	F												
Asia1	G												
Normal	H												

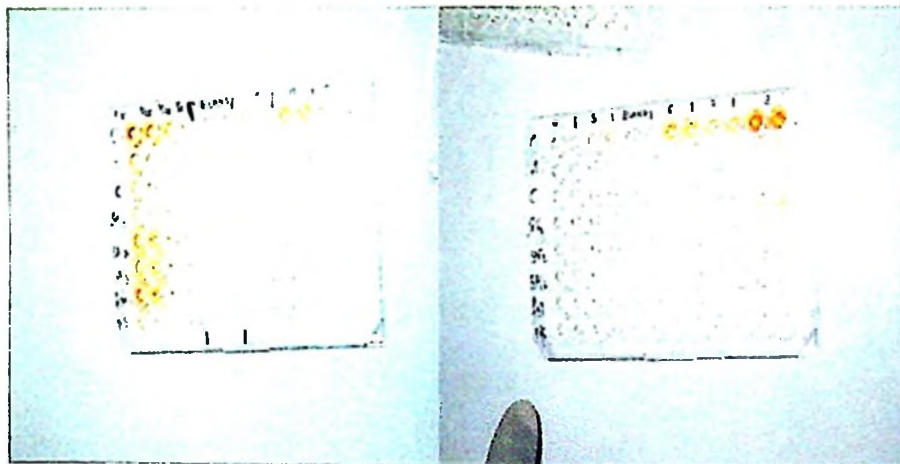
Appendix 4: Plate layout used for Indirect sandwich ELISA

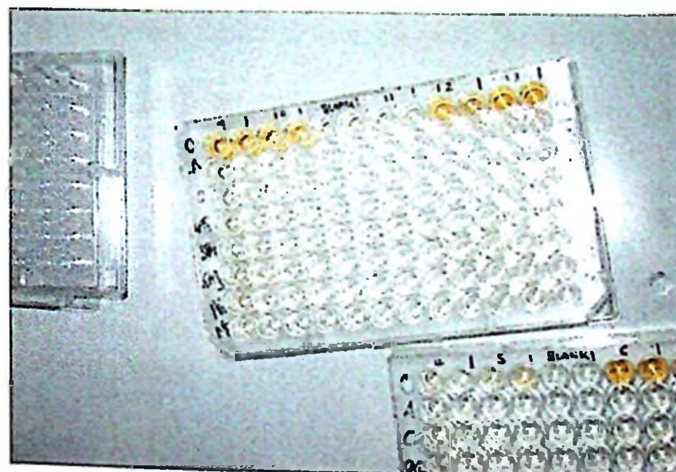
Serotype		Sample 4		Sample 5				Sample 6		Sample 7		Sample 8	
O	A												
A	B												
C	C												
SAT1	D												
SAT2	E												
SAT3	F												
Asia1	G												
Normal	H												

Appendix 5: Microplates layout for laboratory results of FMDV 3ABC- Ab ELISA



Appendix 6: Microplates layout laboratory results of FMDV sandwich ELISA





Appendix 7: Standard operating procedures for NSP FMDV 3ABC-Ab ELISA

All reagents should equilibrate to room temperature 18-25°C before use.

Handle all materials and obey all rules according to the Good Laboratory Practice.

1. Add 50 µl of pre-diluted Positive Control Serum and Negative Control Serum respectively, to selected wells coated with FMDV viral antigen and to corresponding wells coated with control antigen.

For confirmation purposes it is recommended to run the control sera in duplicates.

2. Add 50 µl pre-diluted serum sample to selected wells coated with FMDV viral antigen and to a corresponding well coated with control antigen.

The samples can be tested in singlicates or in duplicates. However, for confirmation purposes it is recommended to run the samples in duplicates.

3. Seal the plate and incubate at 37°C (98.6°F) for 30 minutes.
4. Rinse the plate 3 times with PBS-Tween Buffer. At each rinse cycle fill up the wells, empty the plate and tap hard to remove all remains of fluid.
5. Add 50 µl of HRP Conjugate to each well containing controls and bovine samples, seal the plate and incubate at 37°C (98.6°F) for 30 minutes.
6. Repeat step #5.
7. Add 50 µl Substrate Solution to each well. Incubate for 30 minutes at room temperature 18-25°C (64 -77°F) in dark. Begin timing when the first well is filled.
8. Stop the reaction by adding 50 µl of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #8

9. Measure the optical density (OD) of the controls and samples at 405 nm in a microplate photometer (use air as blank) within 15 minutes after the addition of Stop solution to prevent fluctuation in OD values.
10. Calculations of results are done in two steps as described below.

1. Corrected OD Values (OD_{Corr})

The optical density (OD) values in wells coated with NSP 3ABC were corrected by subtracting the OD values of the corresponding wells containing the control antigen.

$$OD_{NSP\ 3ABC} - OD_{Control} = OD_{Corr}$$

2. Percent Positivity Values (PP)

All Corrected OD Values for the test samples as well as the Negative Control (Neg C) were related to the corrected OD value of the positive control as follows:

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

Interpretation of the results:

Criteria for test validity: To ensure validity the Positive Controls should have a corrected OD value greater than 0.8 and the Negative Controls should have a corrected OD value of less than 0.3. If the respective PP value for the Negative Control results in a positive interpretation according to criteria below, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of test sample results

Serum PP	Interpretation
< 48	Negative
≥ 48	Positive

Appendix 8: Standard operating procedures for FMD indirect sELISA

i. ELISA plates are coated with 50 µl/well rabbit antiviral sera in 0.05M carbonate/bicarbonate buffer, pH 9.6. Rows A to H receive, respectively, antisera to serotypes O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1.

ii. Leave overnight at 4°C in a stationary position or place on an orbital shaker set at 100–120 revolutions per minute in a 37°C incubator for 1 hour.

iii. Prepare test sample suspension (10% original sample suspension).

iv. The ELISA plates are washed five times in PBS.

v. On each plate, load wells of columns 4, 8 and 12 with 50 µl PBST. Additionally, add 50 µl of PBST to wells 1, 2 and 3 of rows A to H on plate 1.

To well 1 of row A of plate 1 add 12.5 µl of control antigen type O, to well 1 of row B add 12.5 µl of control antigen type A; continue in this manner for control antigen of types C, SAT 1, SAT 2, SAT 3 and Asia 1 in order to well 1, rows C to H.

Mix diluent in well 1 of rows A to H and transfer 12.5 µl from well 1 to 2 (rows A to H), mix and transfer 12.5 µl from well 2 to 3, mix and discard 12.5 µl from well 3 (rows A to H) (this gives a five-fold dilution series of each control antigen).

It is only necessary to change pipette tips on the micropipette between antigens. The remainder of the plate can be loaded with the test sample(s).

Add 50 µl of sample one to wells 5, 6 and 7 of rows A to H, the second sample is placed similarly in columns 9, 10 and 11, rows A to H.

If more than two samples are to be tested at the same time, the other ELISA plates should be used as follows: Dispense 50 µl of the PBST to the wells (rows A to H) of columns 4, 8 and 12 (buffer control columns).

Note that the control antigens are not required on these plates. These test samples may be added in 50 μl volumes in rows A to H to columns 1, 2, 3; 5, 6, 7; 9, 10, 11, respectively.

vi. Cover with lids and place on an orbital shaker at 37°C for 1 hour.

vii. Wash the plates by flooding with PBS – wash three times as before and empty residual wash fluid. Blot the plates dry.

viii. Transfer 50 μl volumes of each guinea-pig serum dilution to each plate well in the appropriate order, e.g. rows A to H receive, respectively, antisera to serotypes O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1.

ix. Cover the plates with lids and replace on the orbital shaker. Incubate at 37°C for 1 hour.

x. The plates are washed again three times, and 50 μl of rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase is added to each well. The plates are incubated at 37°C for 1 hour on a rotary shaker.

xi. The plates are washed again three times, and 50μl of substrate solution, containing 0.05% H2O2 plus orthophenylene diamine or a suitable alternative chromogen, is added to each well and kept in dark.

xii. The reaction is stopped after 15 minutes by the addition of 50 μl of 1.25 M sulphuric acid. The plates are read at 492 nm on a spectrophotometer linked to a computer loaded with ELISA Data Information (EDI) Software, which is used to automate the reading of optic density (OD) value and calculate the mean corrected OD value for control and test samples according to the protocol. A mean correct OD value of >0.1 above background was considered to be positive.