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

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ABSTRACT

This study was conducted to establish the spatial and temporal distribution of foot-and-mouth disease (FMD) virus (FMDV) serotypes and evaluate the awareness of people on FMD in the eastern zone of Tanzania. Both observational prospective studies involving serological analysis, FMDV antigen detection and questionnaire survey, and retrospective study on FMDV antigen detection were used in this research. Seroprevalence of antibodies to the nonstructural protein 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 samples were collected from cattle suspected of FMD in six districts of two regions in the eastern zone of Tanzania during the period of 2010 to 2011. A total of 41 (43.6%) out of 94 tested sera in six district were seropositive to non-structural 3ABC protein, with the highest seroprevalence of 81% in Bagamoyo district followed by Kibaha(56.2%), Kinondoni (41.7%), Ilala (34.8%), Kisarawe (16.7%) and Temeke (15.4%) districts. Three FMDV serotypes, namely O, A and SAT 2, were detected in the eastern zone between 2001 and 2011 with type O being the most frequently detected serotype (n = 9; 60%) followed by type SAT 2 (n = 5; 33.3%) and type A (n = 1; 6.7%). Questionnaire survey had revealed high (74.4%) general FMD awareness by farmers, with 75% being knowledgeable on transmission and FMD susceptible animal species in the eastern zone. These findings indicate that the eastern zone of Tanzania is predominantly infected with FMDV serotypes O, A, and SAT 2 with different spatial and temporal distribution, and that FMD outbreaks in the zone could be incriminated to at least these three serotypes. These observations imply that a rational control of FMD by vaccination in the eastern zone of Tanzania should consider incorporation of serotypes O, A and SAT 2 in the relevant vaccine(s). Further studies are

required to elucidate the genetic and antigenic characteristics of circulating FMDV strains in the eastern zone of Tanzania so that an appropriate FMD control measure can be recommended in this region.

DECLARATION

I, Julius Joseph Mwanandota do hereby declare to the Senate of Sokoine University of Agriculture (SUA) that this dissertation is my own original work and has not been submitted for higher degree award at SUA or in any other Institution.

_____ _____

Julius Joseph Mwanandota Date

The above declaration is confirmed

_____ _____

Dr. Christopher J. Kasanga Date

Supervisor

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DEDICATION

This work is dedicated to my grandmother Roza Laurent who laid foundation of my education.

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LIST OF ABBREVIATIONS AND SYMBOLS

BHK	Bovine hamster kidney
Ca	Antigen control
CFT	Complement Fixation Test
CPE	Cytopathic Effect
DIVA	Differentiating infected from vaccinated animals
EDI	ELISA data information
ELISA	Enzyme Linked Immunosorbant Assay
FAO	Food and Agriculture Organization
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
HRP	Horseradish peroxidase
IgA	Immunoglobulin A
IgG.	Immunoglobulin G
IgM	Immunoglobulin M
LPBE	Liquid Phase Blocking ELISA
MoWLD	Ministry of water and livestock development
NASBA	Nucleic acid sequence-based amplification assay
NASBA-ECL	NASBA electrochemiluminescence
NASBA-EOC	NASBA-enzymelinked oligonucleotide capture
NBs	National Bureau of Standard
NCR	Non-coding Region
NSP	Non Structural Protein
OD	Optical Density
OIE	Office International des Epizooties

OP	Oesophageal Pharyngeal
PBS	Phosphate Buffer Saline
PBSTM	PBS containing 0.05% Tween 20, and 5% dried, nonfat skimmed milk
PCR	Polymerase Chain Reaction
PI	Percentage of inhibition
PP	Percentage of Positivity
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAT	Southern African Territories
VP1	Viral Capsid protein one
Vpg	Viral genomic protein
WRL	World Reference laboratory
MAbs	Monoclonal antibodies

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Foot-and-mouth disease (FMD) virus (FMDV) is a highly contagious virus that causes a disease in cloven-hooved livestock and wildlife. Although adult animals generally recover, the morbidity rate is very high in naive populations (Grubman *et al.,* 2004). Sequelae may include decreased milk yield, permanent hoof damage and chronic mastitis. High mortality rates can be seen in young animals. Although foot-and-mouth disease was once found worldwide it has been eradicated from some regions including North America and most of Europe (Valarcher *et al.,* 2008). FMD is endemic in most of the Asian and African countries where it is a major constraint to the international livestock trade. (Sutmoller *et al.,* 2003). Foot-and-mouth disease virus (FMDV) is a member of the genus *Aphthovirus* in the family *Picornaviridae*. There are seven immunologically distinct serotypes of FMDV namely serotype O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1, and over 60 strains within these serotypes (Fiebre Aftosa., 2007). For the past several years there has been several reports describing the occurrence of FMD outbreaks in Tanzania (Swai *et al.,* 2009; Sahle *et al.,* 2008).

Foot and Mouth Disease (FMD) is one of the most important livestock diseases in the world in terms of economic impact. The economic importance of the disease is not only limited to production losses, but also related to the reaction of veterinary services to the presence of the disease and to the restrictions on the trade of animals and animal products both locally and internationally (James and Rushton.,2002). In Tanzania, FMD is the second most important transboundary animal disease in cattle after contagious bovine pleuro pneumonia (MoWLD, 2003).

1.2 Problem statement and justification

FMD control has remained in its latent state of development for many years due to lack of efficiency in early detection of infected animals (Calens & De clercq, 1997). In Tanzania, the main circulating FMD virus serotypes, at least during the period of 1997-2004, were 3 namely serotype O and SAT 1 and 2 (Swai *et al.,* 2009). With the exception NSP tests of the serological test employed in FMD diagnosis are serotype-specific and are highly sensitive, provided that the virus or antigen used in the test is closely matched to the strain circulating in the field (OIE Manual, 2009). However, the serotype, antigenicity and spatial distribution of circulating FMDV field strains in Tanzania have not been extensively studied. Furthermore, it is not clearly known whether the genotypes/topotypes of circulating serotypes undergo genetic changes to several antigenic variants. The known situation of FMD in the eastern zone show predominance of 3 serotypes O, SAT 1 and SAT 2 which occurred between 1997-2004 (Swai *et al.,* 2009). Therefore this study was conducted to establish the seroprevalence of FMD in the Eastern zone of Tanzania The findings of this study will provide information on the type and spatial distribution of FMDV in Tanzania allowing the design for a rational control strategy of FMD by vaccination.

1.3 Objectives

1.3.1 Overall objective

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

1.3.2 Specific objectives

1. To establish the seroprevalence of FMDV infection in the eastern zone of Tanzania

2. To determine the FMDV serotype(s) circulating in the eastern zone of Tanzania.

3. To assess the awareness of livestock keepers on FMD susceptibility and transmission in the eastern zone of Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Foot and mouth disease

Foot and mouth disease (FMD) is the most contagious disease of mammals with a great potential for causing severe economic loss in susceptible cloven-hoofed animals. It is characterized by fever, loss of appetite, salivation and vesicular eruptions on the feet, mouth and teats (Thomson, 1994). It is a list A disease according to OIE disease classifications therefore, Laboratory diagnosis of any suspected FMD case is therefore a matter of urgency (OIE, 2009).

2.2 Taxonomy of Foot and mouth disease virus

Foot and mouth disease (FMDV) belong to the *Aphthovirus* genus of the family picornaviridae in the group of viruses called picornaviruses. Foot-and-mouth disease virus (FMDV) is the prototypic member of the Aphthovirus genus, in which there is also equine rhinitis A virus (ERAV). (Martinez-Salas *et al.,* 2008).The main FMD virus serotypes reported in Tanzania at least during the period of 1997-2004, are 3, namely serotype O and SAT 1 and 2.

Phylogenetic analysis of the viral protein (VP) 1 region of FMD viruses has been used to define genetic relationships between FMDV isolates, geographic distribution of lineages and genotypes. VP 1 sequences have also helped to establish the genetical and geographically linkages of topotypes and trace the source of outbreaks (Sahle., *et al.,* 2004). Topotypes are defined as geographically clustered viruses that form a single genetic lineage generally sharing >85% (O, A, C, and Asia 1) or >80% (SAT 1, SAT 2, and SAT 3) nucleotide identity in the VP1-coding region. (Gelagay *et al.,* 2009)

2.3 Physicochemical properties

Picornaviruses 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 (Cooper *et al.,* 1978). The virus is pH sensitive; and is inactivated when exposed to PH below 6.5 or above 11. However, in milk and milk products, the virion is protected, and can survive at 70^{o}C for 15 seconds and pH 4.6. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes (Mckercher and Callis, 1983). 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 (Sahle, 2004). 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 (Donaldson, 1987).

2.4 FMD Virus morphology

The virus consists of icosahedral protein coat (capsid) and the RNA core has a diameter of 22-25 nm (Robert & Bruce, 1981). The capsid consists of 60 capsomeres each consisting of four proteins (VP1-4). VP1 is the most antigenic protein involved in cell attachment and carries the immunologically important G-H loop which is one of the most important neutralizing sites on the virus (Logan *et al.,* 1993).

2.5 Genome organization and protein processing

FMDV has single stranded, positive sense RNA that is approximately 8500 bases long and consists of a 5' non-coding region (NCR), a single open reading frame, and a short 3' NCR. It is polyadenylated, on the 3' end and has a small virus encoded protein, viral

protein gene (VPg), covalently attached to the 5' terminus. The major portion of the FMD genome consists of a single large open reading frame of 6996 nucleotides encoding a polyprotein of the 2332 amino acids (type O, (Forss *et al.*, 1984). Four distinct regions are distinguished for the polyprotein namely the L, P1, P2, and P3. Another characteristic, unique to FMDV, is that there are three species of Vpg encoded by protein 3B, termed 3B1, 3B2, and 3B3. All encoded Vpg variants have been shown to be attached to the 5' terminus of viral RNA (King *et al.*, 1982).

The L protein represents the leader protein, where 2 initiation sites (AUG codons) have been identified in FMD virus, namely Lab and Lb (Sangar *et al.*, 1988). The P1 gene product is the precursor of the capsid proteins 1D, 1B, 1C, and 1A. Firstly, the intermediate P1 precursor is processed with the help of viral protease 3C to produce VP0, VP1, and VP3 where the products combine to form empty capsid particles. 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 (Belsham, 1993).

2.5.1 Antigenic variation

There is constant generation of new antigenic variants which occur due to genetic variation through mutation, recombination and selection. Lack of cross protection between FMDV serotypes is also seen in vaccines where vaccination with one antigenic variant of serotype does not necessarily protect an animal when challenged with a different virus of the same serotype (Sangare, 2002). Attempts to characterize the extent of the antigenic variation within the FMD serotype led to the establishment of the techniques whereby viral subtypes could be identified. Initially over 60 different subtypes were identified by

the World Reference Laboratory (WRL), but it quickly became apparent that there is a continuous spectrum of intratypic antigenic variants, making a difficulty to identify specific subtypes (Asseged, 2005). Changes to the genes encoding capsid proteins can result in antigenic variation and evolution of new subtypes (Haydon *et al.,* 2001). This may give rise to immunologically distinct variants that can re-infect individuals that have been previously infected by related viruses. Thus, the degree of cross protection among different subtypes of the same serotype varies. Since there is continual antigenic drift in enzootic situations this is an important factor to consider when selecting vaccine strains (Grubman and Mason, 2002).

2.5.2 Mutation

Lack of replication error checking mechanisms in RNA viruses predisposes them to high rates of mutation as it is true for FMDV. The rate of viral mutation in this type of viruses that exhibit such a deficiency is one nucleotide base change per 10^3 bases per replication cycle (Holland *et al.,* 1982). It is also estimated that a mutation rate of up to 10^{-8} to 10^{-9} nucleotide substitution per year during an epizootiological cycle of FMD viruses can occur. 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, previously described as the quasispecies phenomena (Domingo *et al.,* 1990). This may result in the generation of viral diversity. Changes in the nucleotide compositions of the capsid genes are responsible for the antigenic variability of the virus (Lewis *et al.,* 1991; Meyer *et al.,* 1994).

2.5.3 Natural selection

One of the evolutionary mechanisms employed by RNA viruses is the profile mutant production (Lewis-Rogers *et al.,* 2008). The immune system of an infected animal, which

presumably provides a powerful selective force, is another driving force in viral evolution (Diez *et al.,* 1990).

2.5.4 Recombination

It has been observed that genetic recombination in RNA viruses, 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. (Chibssa, 2006). 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 region of the FMDV. Mutations through recombination could result in the exchange of genetic material that could lead to the generation of new antigenic variants that may escape immune pressure (King *et al.,* 1982).

2.6 Epidemiology

2.6.1 Geographical distribution

FMDV has a global distribution, with the exception of North America, Western Europe, and Australia. Three serotypes, namely O, A, and C, are endemic in most of the countries (Vosloo *et al.,* 2002). Serotype A and O are widespread throughout Sub-Saharan Africa, whilst type C appears to have disappeared from the world as a whole, with the possible exception of Kenya (Kitching, 2002a). However the different serotypes also have subtly different epidemiological behaviors Kitching 2005. The three SAT serotypes, SAT1, SAT2 and SAT3, are generally restricted in distribution to Africa. Occasionally they are found in the Middle East, having spread with the movement of animals exported out of Africa, but they never persist. This is not because of effective intervention, as other serotypes in the Middle East, such as A, O and Asia1, thrive in spite of any control

programme. Even within Africa, the SAT2 virus has a wider distribution and is more frequently found in cattle than the other two. All three are found routinely in the African buffalo. On the other hand, Asia1 virus is never found outside of Asia (except for a brief excursion into Greece in 2000). Type C is characterized by long disappearances from the circulating virus pool, the most recent of which gave optimism that it had completely died out from the globe. However, in 2003, it reappeared in central Brazil after a 10 years absence. There are many enigmas surrounding the behavior of different FMDV serotypes, most of which cannot yet be explained. The tendency by those unfamiliar with the virus is to assume that all strains and serotypes behave in the same fashion, which leads to significant errors of judgment. (Kitching *et al.,* 2007)

Despite the propensity and opportunities for spread of FMDV into new regions, comparisons of VP1 gene sequences of viruses submitted over many years do show a tendency for similar viruses to recur in the same parts of the world (Knowles and Samuel, 2003; Rweyemamu *et al.,* 2008) and this presumably reflects some degree of either ecological isolation or adaptation. Of all mechanisms of transmission of FMD, movements of infected animals are by far the most important, followed by movement of contaminated animal products (Donaldson, 1994).The epidemiological patterns of FMD in endemic area can be defined by eco-system based approach, which was originally described in South America., and can readily be applied to other parts of the world (Rweyemamu *et al.,* 2008).

Table 1**:** Serotypes commonly isolated from certain geographical regions

Continent	**Subcontinent**	**Virus serotypes**
Europe (historically)		A, O, C
Asia	Near East	A, O
	Middle East	A, O, C, Asia 1
	Far East	A, O, C, Asia 1
Africa	Central East to West	A, O
	Northeast Central and South	SAT-1 and -2
	South	SAT-3
South America		A, O, C

Source: Asseged (2005)

2.6.2 The role of carriers in the epidemiology of the disease FMD

The carrier is defined as an animal from which live virus can be recovered after 28 days following infection (Alexander *et al.,* 2002).FMD Carrier animal is one from which FMD virus can be isolated from the oesophageal pharyngeal (OP) area, more than 28 days after infection. This may be a fully susceptible animal which develops clinical disease and in which virus persists following recovery, or a vaccinated animal that has contact with live virus and fails to develop clinical disease, but becomes a carrier. Although it is well established that FMD virus persists in buffalo (up to 5 years), cattle (up to 3 years), Sheep (up to 9 months), and goats (between 3-6 month), the mechanisms underlying persistence and the immunological pathway that eventually leads to viral clearance are not well understood (Rossi *et al.,* 1988; Bastos *et al.,* 2000).

2.6.3 Serotypes and sub types

Up to now there are seven serotypes of (FMDV), namely O, A, C, Southern African Territories (SAT) 1, 2 and 3, and Asia 1. Out of six serotype of FMD virus found in

Africa, four have been isolated and identified in Tanzania (Mlangwa, 1983; Rweyemamu *et al.,* 2008). Identified serotype in Tanzania include Type A, O, SAT 1 and SAT 2 (Swai *et al.,* 2009). Within these serotypes, over 60 subtypes have also been described using biochemical and immunological tests; and new subtypes occasionally arise spontaneously. 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, 2009). At present, a sequencing of FMD virus is increasingly being used to establish intratypic variations of FMD viruses and classifying viruses in to genotypes and lineages (Sahle, 2004).

2.6.4 The role of wild life

FMD can infect other wildlife species apart from African buffalo (Syncerus caffer) these include, Impala (Aepyceros melampus), Kudu (Tragelaphus strepsiceros) species, Warthog (Phacochoerus aethiopicus), and elephants that has a role in epidemiology of the disease. African Buffalo can harbor the infection up 24 years whilst an individual animal can maintain the infection for up to five years. Furthermore, buffalo have unequivocally been shown to be a source of infection for cattle under both natural and experimental conditions (Sangare, 2002).The SAT-type virus transmission is mainly facilitated by close contact between the two species during the acute stage of infection and shedding of virus in large amount. Strong spatial associations between molecular types from outbreaks in cattle and virus recovered from buffalo suggest that any control strategy for FMD in cattle must address control in buffalo (Thompson, *et al.,* 2003). Impala (Aepyceros melampus) is the most frequent infected species and act as intermediaries in disease transmission. Although studies have established that individual impala do not become carriers, it appears that the disease can persist in impala populations for between 6 and 13 months

(Vosloo *et al.,* 2002). Kudu (Tragelaphus strepsiceros) were shown to be gradually infected, with the carrier state of between 106-140 days being demonstrated. Experimental infection of warthog (Phacochoerus aethiopicus) with SAT2 type virus resulted in severe clinical signs of infection, and transmission to in-contact animals. (Thomson, 1994)

2.6.5 Molecular epidemiology

Molecular epidemiologic studies have contributed in planning FMD control strategies by elucidating historical and current disease transmission patterns within and between countries. Additionally, such studies have demonstrated the presence of viral topotypes in both wildlife and domestic animals, information that should be heeded when planning FMD vaccination strategies (Sangare *et al.,* 2004). Molecular techniques can be useful in defining strains, identifying transmission of events, and characterization of biodiversity (Knowles *et al.,* 2001). Phylogenetic analysis of the virus protein 1 (VP1) region of FMD virus has been employed extensively in investigation of molecular epidemiology of the disease worldwide. These techniques have provided useful assistance in studies of the genetic relationships between different isolates FMD virus, geographical distribution of lineages, and genotypes. It was also used for the establishment of 15 genetically and geographically linked topotypes and in tracing the source of virus during outbreaks (Knowles and Samuel, 2003; Sangare *et al.,* 2003).

Sequence differences of 30% to 55% of the VP1 gene were obtained between seven serotypes of FMD while different subgroups (genotypes, topotypes) were defined by differences of 15% to 20% (Knowles and Samuel, 2003). Since 1987, the analysis of the genetic distance and phylogenetic resolution of the sequence of VP1 encoding gene have provided crucial epidemiological information covering different degree of genetic relationships between field isolates (Samuel *et al.,* 1999). 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 (Sahle, 2004).

2.6.6 Mode of transmission

FMD is a highly transmissible disease in which limited number of infective particles can initiate host infection (Sellers, 1971). Contaminated animal products, non-susceptible animals, agricultural tools, people, vehicles and airborne transmission (Donaldson *et al.*, 1987) can contribute to the mechanical dissemination of the disease. FMDV multiplication and spread can also depend on the host species, nutritional and immunological status, population density, animal movements and contacts between different domestic and wild host species and animals capable of mechanical dissemination of the virus (Nishiura *et al.*, 2010). FMD virus can replicate and be excreted from respiratory tract of animals leading to airborne excretion of virus during the acute phase of infection, although, FMD virus may occur in all the secretions and excretions of acutely infected animals, including the expired air. Therefore, after an animal becomes infected by any means, the primary mode of spread is via respiratory aerosols from infected animals (requires proper humidity and temperature). When proper humidity and temperature are maintained, FMD virus can be carried up to 250 km across the sea and up to 60 km across the land. The prior condition has been held responsible for the FMD outbreak that occurred in France and then spread to UK in 1981 (Kitching, 1992) emphasizing the possible windborne spread of the virus under prevailing environmental conditions. At present, there are Computer models that can predict the most likely wind-borne spread of the virus from infected herds and allow the examination of a variety of control strategies (Sahle, 2004). Other important means of spread are by direct contact between infected and susceptible animals and indirectly by exposure of susceptible animals to the excretion and secretion of acutely infected animals.

A person in contact with infected animals can have sufficient FMD virus in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals (Asseged, 2005).

2.7 Pathogenesis

Susceptible livestock may be infected with FMDV as a result of direct or indirect contact with infected animals or with an infected environment. When infected and susceptible animals are in close proximity, the aerial transfer of droplets and droplet nuclei is probably the most common mode of transmission. Gener**a**lly the main route of infection in ruminants is through the inhalation of droplets, but ingestion of infected feed, inoculation with contaminated vaccines, insemination with contaminated semen, and contact with contaminated clothing, veterinary instruments, can also be the source of 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 sites such as the epithelium of Oro-pharynx, oral cavity, feet, the udder and heart. Virus probably replicate in the mammary gland of susceptible cow, 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 (Murphy *et al.,* 1999) Recovered cattle produce neutralizing antibodies and can resist re infection by the same subtype of virus for up to one year. It was suggested that heat intolerance was a sequel to FMD and was caused by damage to the endocrine system (Radostits *et al.*, 1994)

2.8 Immune response

FMDV, like most other members of the Picornaviridae, shuts off host transcription and cap-dependent translation and is able to replicate very efficiently in tissue culture. Similarly, in infected animals, the virus rapidly replicates at the initial site of infection in the respiratory system and disseminates to its natural sites of predilection in oral and pedal epithelial regions. To accomplish this task the virus has developed the ability to counteract the host innate immune response, the first arm of the host's defense system. Studies have demonstrated that FMDV infection also subverts the development of the host adaptive immune response (Grubman *et al.,* 2008)

The protection of a susceptible host against FMD virus correlates with the neutralizing antibodies level. Infection with one-serotype produces complete protection against homologous virus, but little or no protection against heterologous viruses (Samina *et al.,* 1998). Serotype specific immunity is based on the presence of neutralizing antibodies to the viral capsid proteins, VP1, VP2 and VP3 which express neutralizing epitopes, these antibodies normally develop 7 to 21 days after exposure to the virus. The immunoglobulin M (IgM) is most prevalent in the early convalescent serum and is less specific to the different serotypes than Immunoglobulin G (IgG). IgG is produced in the later stage during the FMD infection and the reaction between the serotype and the homologous antibodies is highly specific. It has been reported that healing of lesions and clinical recovery in infected animals would not occur until a few days after the IgG1 antibodies have developed. The localized antibody response, specific to anti-FMD IgM and IgA antibodies in the pharyngeal fluid of cattle develops 7 days after exposure to the virus, while IgG activity reaches a peak in serum only 14-21 days after infection (Mulcahy, *et al.,* 1990).

The age of individuals has also been shown to influence the antibody response against FMD virus. Calves (age one week to six months) deprived of maternal antibodies responded as well as, or better than 18 months old cattle to initial vaccination against FMD. Although serum antibody levels play an important role in host protection against FMD virus infection, the cellular responses mediated by T-helper and T- cytotoxic cells also play a role in the immune response to FMD virus infection (Sanz-Parra., *et al.*, 1998)

2.9 Diagnosis of FMD

A presumptive clinical diagnosis associated with laboratory tests such as serology, virus isolation, and antigen detection are the basis for the diagnosis at the herd level. Clinical diagnosis based on lesion identification, in the early stage of infection, laboratory diagnosis of FMD virus or viral antigens can be done by using several techniques like virus isolation,(conventional and real time): DNA sequencing targeting viral genome RT-PCR, ELISA and electron microscopy. However, different serological methods are used to detect antibody against FMD virus and is the main indication that infection has taken place. Either full diagnosis of FMD can be detailed explained in OIE manual (2009)

2.9.1 Field diagnosis

In cattle, FMD should be considered whenever salivation and lameness occur simultaneously and when a vesicular lesion is seen or suspected. 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 other viral infections of the mucous membrane, which produce similar clinical signs. Differential diagnosis for FMD should include vesicular stomatitis, rinderpest, malignant catarrhal fever, bovine

herpes 1 infections, swine vesicular disease, vesicular exanthema of swine and bluetongue (Blood *et al.,* 1994).

2.9.2 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, 2004).

2.9.2.1 Specimens

Appropriate samples for FMD laboratory diagnosis are; Vesicular fluid usually contains the highest quantity of virus. Epithelium from early vesicles and from recently ruptured vesicles is a tissue of choice for virus isolation (OIE, 2009). When epithelium tissue is not available from ruminant animals e.g. in advance or convalescent cases and infection is suspected in the absence of clinical sign, samples of oesophageal-pharyngeal fluids(OP) is collected by means of a probang and used for virus isolation (Asseged, 2005). Other samples such as, blood with anticoagulant, Serum, and lymph nodes, thyroid gland, adrenal gland, kidney, and heart are good specimens from postmortem.

2.9.2.2 Virus isolation

Virus isolation (VI) remains the ultimate proof of the presence of live FMDV. Primary cells like bovine thyroid cells (Snowdon, 1966) or lamb kidney cells (House and House, 1989) are very sensitive but laborious to maintain. Cell lines are easier to cultivate but less sensitive. Mostly pig cell lines are used such as IB-RS-2, PK15 or SK6 or a baby hamster kidney cell line (BHK-21). The cell line used must be sensitive enough to isolate FMDV from samples coming from different species. Pig cell lines were not always suitable for isolation of FMDV coming from goats or sheep excreting sometimes very low amounts of

virus (Bouma *et al.,* 2001). However most sensitive cell culture system is the primary bovine thyroid (BTY) cell but it is difficult and expensive to maintain it for diagnostic work (Ferris, N. P *et al.,* 2006). The situation above lead to the introduction of Fetal Goat Tongue Cell Line (Brehm *et al.,* 2009).The suspensions of field samples suspected to contain FMD virus are inoculated into cell cultures, incubated at 37 0C and examined for cytopathic effect (CPE) after 24 to 48 hours post infection. No CPE confirms the absence of FMDV in the samples. 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.,* 1989).

2.9.2.3 Enzyme linked immunosorbent assay (ELISA)

ELISA came into use as diagnostic methods for many infectious diseases around the year 1975 since when it has been used as one of the most accepted serological technique. The first report of the use of an indirect ELISA in screening cattle for antibodies against FMDV was reported by Abu Elzein and Crowther (1978). Subsequently, a sandwich ELISA using convalescent bovine immunoglobulin (Igs) as capture and anti-146S guinea pig sera as tracing sera was found suitable for detection and quantification of FMD virus in infected tissue culture fluid and epithelial tissue samples (Crowther and Abu Elzein, 1979). In the antibody detection ELISA test sera are pre-mixed with standard FMD virus before addition to an ELISA plate coated with anti-FMD antibody. If antibody is present in the test sera this will block the standard virus, which will be unable to bind to the coating antibody on the plate. If there is no virus-specific antibody in the test sera then the standard virus will be available to be trapped on the plate, and this will be detected by a positive colour reaction indicating a negative test result. (Hamblin *et al.,* 1986a, Hamblin *et al.,* 1986b).

2.9.2.4 Solid-phase competition enzyme-linked immunosorbent assay (SPCE)

The method described (Paiba. *et al.,* 2004) can be used for the detection of antibodies against each of the seven serotypes of FMDV. As an alternative to guinea-pig or rabbit antisera, suitable MAbs can be used coated to the ELISA plates as capture antibody or peroxidase-conjugated as detecting antibody (Brocchi. *et al.,* 1990). A commercial kit is available for serotype O with a different format but similar performance characteristics (Chenard *et al.,* 2003).

The solid-phase competitive ELISA is more specific but as sensitive as the liquid-phase blocking ELISA (Mackay *et al.,* 2001,). Methods have been described for the development of secondary and working standard sera (Goris & De Clercq 2005a) and for charting assay performance (Goris & De Clercq, 2005b). SPCE has replaced the Liquid Phase Blocking ELISA (LPBE) as prescribed test for screening, due to the evidence of a higher specificity combined with an equivalent sensitivity and a better robustness (Mackay *et al.,* 2001).

2.9.2.5 Antibody detection by liquid phase blocking ELISA (LPBE)

The LPBE detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals (Hamblin *et al.,* 1986 a). The test is based upon specific blocking of the FMDV antigen in liquid phase by antibodies in the serum sample. Rabbit antisera specific for the different serotypes of FMDV are passively adsorbed to polystyrene micro wells. After the test serum is allowed to mix with the specific FMDV antigen; the test serum/antigen mixture is then transferred to an ELISA plate coated with FMDV trapping antiserum (rabbit FMD antisera). The presence of antibodies to FMDV in the serum sample will result in the formation of immune complex and consequently reduce the amount of free antigen trapped by the immobilized rabbit antiserum. In turn, fewer guinea pigs anti FMDV detecting antibodies will react in the next incubation step after the

addition of enzyme labeled (HRP) anti-guinea pig Ig conjugate. Following incubation, the substrate/chromogen solution, containing H_2O_2 is added to each well, before being stopped after 15 minutes by addition of sulfuric acid. A change in colour development is read with spectrophotometer at 492 nm filters, in comparison to antigen Control (Ca), containing free antigen only. The diagnostic threshold for this assay is set at 50% inhibition (50PI). If either or both replicate PI values of test serum fall above 50 PI, then that test serum fall above 50 PI, and then that test serum is tentatively considered to be positive. If both replicate PI value of a test serum fall below 50 PI then the test serum is considered as negative (Ferris, 2004).

2.9.2.6 Non structural protein (NSP) ELISA test and DIVA based diagnostic approach

The detection of antibodies to the NSP 3ABC of FMDV has been shown to be a sensitive and specific method to differentiate between infection and vaccination (Clavijo *et al.,* 2004). In areas where conventional vaccines, with traces of NSP are used repeatedly, some may develop specific NSP antibody (Mackay *et al.,* 1998b). Adjustment of Vaccine manufacturing methods have been reported where by the NSP component can be reduced to a level that will not cause detectable seroconversion following vaccination (Doel, 2001). DIVA Technique arise when the ability to identify and selectively delete genes from a pathogen has allowed the development of "marker vaccines" that, combined with suitable diagnostic assays, consequently allowing differentiation of infected from vaccinated animals by differentiation of antibody responses induced by the vaccine (no antibodies generated to deleted genes) from those induced during infection with the wild-type virus (Uttenchal *et al.,* 2010).

A number of antigenic non-structural proteins (NSP) of FMD were identified, out of which 3ABC gene appears to be the most reliable marker of FMD virus replication (Grubman, 2005, Handerson, 2005). The deletion of NSP (3ABC) gene has been used for enabling DIVA approach for FMD. Indirect ELISA test for the detection of antibodies against non-structural proteins will play an essential role in the serological survey of livestock herd's in future post-outbreak situations.

The SVANOVIR® Foot and Mouth Virus 3ABC-Ab ELISA Kit is designed to detect FMDV specific antibodies in bovine serum samples. The kit procedure is based on a solid phase indirect Enzyme Linked Immunosorbent Assay (ELISA). In this procedure, samples are exposed to non-infectious FMDV antigen (NSP 3ABC) coated wells on microtitre plates. FMDV antibodies (if present in the test sample) bind to the antigen in the well. HRP conjugate added subsequently forms a complex with the FMDV antibodies. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue-green colour develops which is due to the conversion of the substrate by the conjugate. The reaction is stopped by addition of the stop solution. The result can be read by a microplate photometer, where the optical density (OD) is measured at 405 nm.

2.9.2.7 Antigen detection by indirect sandwich ELISA

The kit is based on a standard indirect sandwich ELISA technique to determine the presence of FMDV antigens in tissue samples as described previously (Roeder & Le Blanc Smith 1987; Ferris & Dawson, 1988). Rabbit antisera specific for the different serotypes of FMDV are passively adsorbed to polystyrene microwells. With the addition of test sample, antigen (if present) is trapped by the immobilized antibodies. Specific guinea pig anti-FMDV detecting antibodies are then added which react with the trapped antigen. The

bound guinea pig antibodies are detected by means of the rabbit anti-guinea pig 1g conjugated to horse radish peroxidase. With the addition of substrate/chromogen solution, a colour product develops which may be measured and interpreted with respect to the antigen content of the test sample.

2.9.2.8 Nucleic acid recognition methods

RT-PCR is used as diagnostic tool for FMD virus where Specific primers are designed to distinguish seven serotypes. In-situ hybridization techniques have also been developed for investigating the presence of FMD virus RNA in tissue samples (Woodbury, *et al.,* 1995). With hereditary information enclosed within its 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). A nucleic acid sequence-based amplification (NASBA) assay for the detection of foot and mouth disease virus (FMDV) use two detection methods: NASBA electrochemiluminescence (NASBA-ECL) and a newly developed NASBA-enzyme linked oligonucleotide capture (NASBA-EOC). Those two techniques were evaluated and compared with other laboratory-based methods. Data analysis supports the use of NASBA as a rapid and sensitive diagnostic method for the detection and surveillance of FMDV (Lau *et al.,* 2008).

2.9.2.9 Loop mediated isothermal amplification (LAMP)

LAMP is a method used to amplify RNA and DNA in which the test primer pairs amplify the template which gives a long stem loop product under isothermal conditions. RT-LAMP products are usually analyzed by 0.5-2.5% agarose gel electrophoresis depending on the size of the amplicon to produce sensitive and rapid detection of FMD virus. (Chen *et al.,* 2011).

2.10 Control of FMD

FMD was mainly controlled by the stamping-out approach supported with emergency ring vaccination carried out on territories being under a direct risk of the infection (Paprocka, 2004). Vaccination is one of the main options of FMD control in endemic areas where movement restriction is used with little enforcement. Vaccines used are those which induce protective immunity against each type of antigens incorporated in the vaccine. In some cases immunity to one of the serotype fails to protect against other members of the same serotype. Live attenuated or in activated bi-, tri- or polyvalent vaccine which contains the representative strains of the serotypes that are in circulation in the region must be used (Gonzalez *et al.,* 1992). In Tanzania the common vaccine used is multivalent vaccine supplied by KEVEVAPI from Kenya which consists of serotype O A SAT-1 and SAT-2.

2.11 Importance of FMD

The importance of FMD is observed in terms its sequelae and its contagious nature post outbreak. It causes decreased milk yield, permanent hoof damage and chronic mastitis. High mortality rates can be seen in young animals. Where it is endemic, this disease is a major constraint to the international livestock trade. In addition to disruption of animal trade, FMD outbreaks have widespread economic and social impacts both in the short and

long term, including disruptions of animal feed, veterinary pharmaceutical and tourism associated industries. (Kitching *et al.*, 2007)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Duration and study location

This study was carried out between September 2010 and October 2011 in six districts of Dar es salaam and Pwani regions as shown in map below (Figure 1). The study consists of 3 three different studies which were performed to meet the specific objectives.

Geographically the study area lies between latitudes 37 and 39 east and longitude 5 and 7 south. According to meteorological statistics the average temperature for the two regions is about 28^0C. The average annual rainfall of 800 mm as minimum and 1000 mm as maximum per year. The heavy rainfall covers 120 days between March and June every year and spreads throughout the two regions (National Bureu of Statistics (Nbs), 2007).

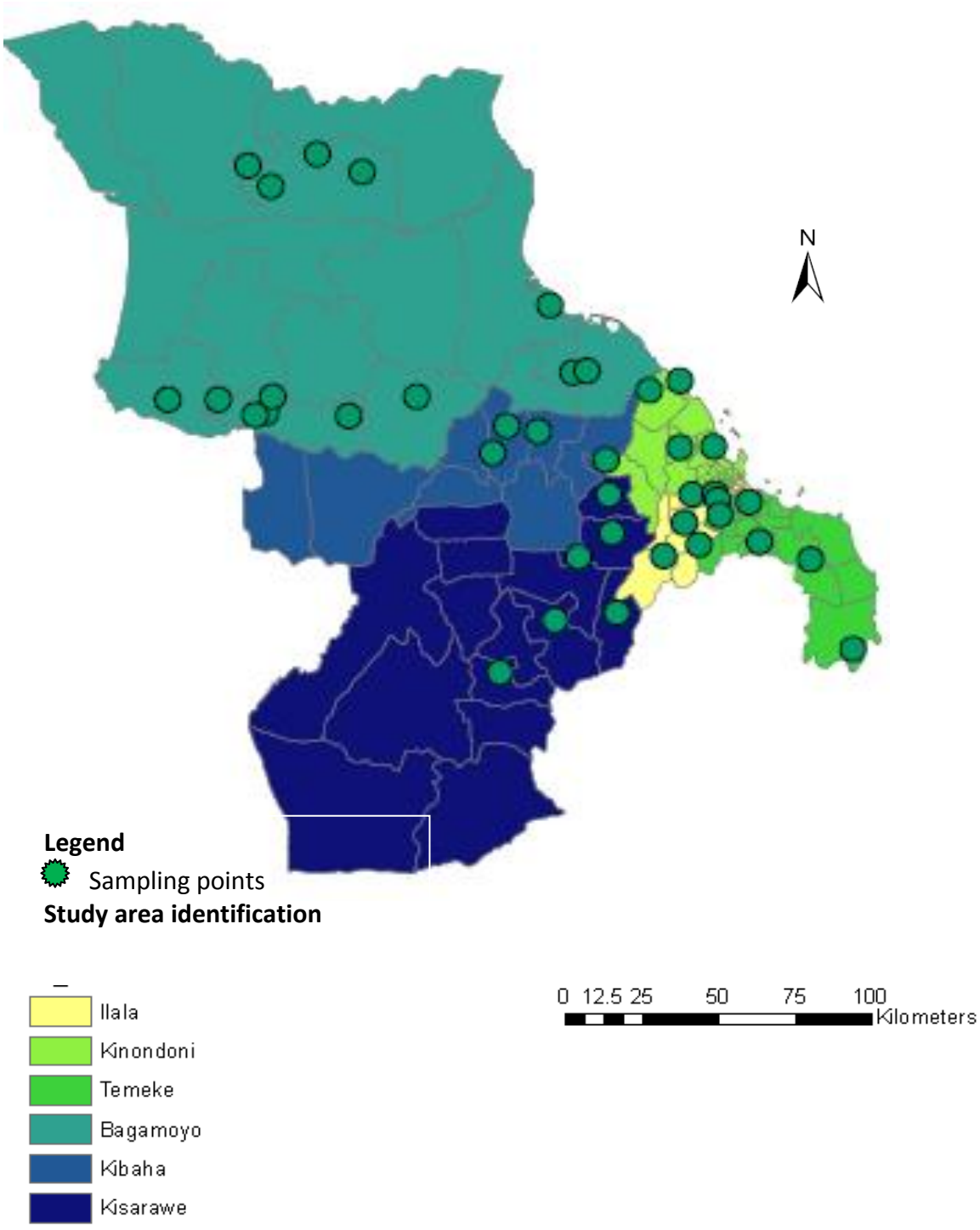

Figure 1. Map of Dar es salaam and Pwani Region showing specific areas where samples were obtained**.**

3.2 Study design

This study involved both prospective and retrospective approaches in data collection. Prospective study was conducted by serosurvey and questionnaire administration to generate information on spatial distribution of FMD in the eastern zone of Tanzania. Laboratory analysis of samples involved conventional veterinary investigation methods where 3ABC ELISA was employed to generate information on seroprevalence of FMD and standard indirect sandwich ELISA to identify the serotypes of the virus in the study area. The second part was structured questionnaire which generate data on the awareness of FMD in the eastern zone of Tanzania. Retrospective study was conducted by reviewing CVL records to generate information on temporal distribution of FMD in the eastern zone of Tanzania for the period of ten years (from 2001 to 2011). Retrospective study generated information on FMDV serotypes observed in the eastern zone of Tanzania for the last 10 years from 2001 to 2011.

3.3 Sampling design

Study animals involved were from six districts namely Kibaha, Kisarawe, Bagamoyo Kinodoni Temeke and Ilala. In each district a maximum of 25 herds and minimum of 12 herds were sampled. Sample collection within the herd was at the maximum of five for herd with greater or equal to 5 animals and minimum of one for a herd with one animal. The serum was pooled to get representative sample of the herd. The sampled herds were selected purposefully from different village/street. The sample collected were blood, and tissue in case of clinical FMD. A total of 94 herds were sampled from 94 villages/street, in which the number of herds was selected base on sample size formula below.

Formula used $n = Z^2 P Q / L^{2,}$

n= required sample size

Estimated prevalence P=50%,

Marginal Error L=10%,

Confidence level at 95% (standard value of 1.96) Z= 1.96

Q=1-P

$$\frac{1.96^2 \times 0.5\ (1- 0.5)}{0.01^2} = 96 \text{ herds}$$

3.4 Sample collection

3.4.1 Serum sample collection

Whole blood was collected from a jugular vein of purposefully selected cattle into 5 ml sterile vacutainer tubes and stored overnight at room temperature for serum separation. Serum was then transferred into a single sterile cryovial, bearing the names of the herd owner and transported in an icebox, to central veterinary laboratory, Temeke, for the laboratory analysis. In the laboratory, the sera were stored at -20^0C until laboratory investigation.

3.4.2 Tissue sample collection

Intact or ruptured vesicular flaps of epithelial tissue from tongue, gums buccal cavity surroundings and foot were collected from FMD suspected cases. Epithelia tissues were collected into Transport media prepared by mixing glycerol, antibiotics and PBS at the PH of 7.04. The sample collected in transport media were transported CVL in cool box with ice packs.

3.5 Serum sample analysis

Serum sample analysis have been done by detecting viral antibody where Non-structural protein (NSP) ELISA was used according to OIE Manual 2009. In the study sera from herds of cattle were used.

3.5.1 Non-structural protein (NSP) ELISA

All reagents were left to equilibrate to room temperature 18 to 25°C before use. Then in duplicates, 50 µl of pre-diluted sample, positive control serum and negative control serum, were added into selected wells. The plates were sealed and incubated at 37°C for 30 minutes. After incubation plates were rinsed for 3 times with PBS Tween Buffer followed by addition of 50 µl of HRP conjugate to each well. Rinsing and incubation was done as above. Later, 50µl of substrate was added on to each well and incubated for 30 minutes at room temperature (18 to 25°C) in dark. Finally the reaction terminated by adding 50 µl of stop solution to each well and mixed thoroughly. The results were read using a spectrophotometer at 405nm wavelength.

Calculations

Deductions of results were done in two steps.

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.

ODNSP 3ABC-ODControl= ODCorr

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:

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

3.6 Tissue sample analysis

3.6.1 Tissue sample preparation

Tissue sample was prepared by grinding the suspension of the sample in sterile sand in a sterile pestle and mortar with a small volume of tissue culture medium and antibiotics (MEM-1). Further medium was added until a final volume of nine times that of the epithelial sample has been added, giving a 10% suspension. Later it was clarified on a bench centrifuge at 2000 g for 10 minutes. Once clarified, such suspensions of field samples suspected to contain FMDV was ready for FMDV detection using sandwich FMD antigen ELISA

3.6.2 Antigen detection by Sandwich ELISA

Briefly, rabbit antisera specific for the different types and subtypes of FMDV were adsorbed to polystyrene plates. Following the addition of the test sample, the antigen is trapped by the immobilized antibodies. Specific guinea pig antisera were added to react with the trapped antigen. The reaction was detected by the addition of anti-guinea pig antibody conjugated to horseradish peroxidase (HRP). The development of coloured reaction after the addition of the substrate/chromogen mixture allowed identification of the antigen.

Interpretation of results

1. Color development occurs in the control wells of the microtitre plates (rows A to H, columns 1 to 4 on plate 1) this indicates that the wells have been correctly coated with reagents readings

2. Mean background reactions for each plate was calculated by adding the OD values of wells 5 and 6 for each row (serotype) and dividing by two. Corrected OD values were obtained by subtracting each mean background OD for each serotype from the recorded OD for that serotype. A mean of each group of two wells for each test sample were obtained to give a mean 'corrected' OD value for each sample against each antiserum serotype

3. Plates accepted were those with the mean corrected OD of the strong and weak positive controls greater than a value of 0.1 above background. For test samples a mean corrected OD of > 0.1 above background were considered as positive result and the serotype was read

3.7 Retrospective antigenicity study

The study was conducted by gathering FMD information recorded at CVL Temeke in the last 10 years. The type of records used was sample registration books which keeps all types of samples submitted at the laboratory from different parts of the country and their results generated within the laboratory or from other laboratory. Focus of investigation was on FMD tissue samples from eastern zone of Tanzania tested positive in the last 10 years (from 2001 to201.

3.8 Questionnaire survey

A cross-sectional study was undertaken in 2 regions of the eastern zone of Tanzania namely Dar es salaam and Pwani, which involved 6 districts. A purposive sample of 90 herds from six district 15 herds from each district (Temeke, Ilala, Kinondoni Kibaha, Kisarawe and Bagamoyo) was selected.

Criteria for enrolment of herds were the willingness of farmers to co-operate, history of participation to other programme(s), and physical accessibility of the villages/streets during the study period.

Interviews were conducted in the 90 selected herds in 2011, by using a structured questionnaire that was administered to farmers (respondents). In connection with FMD awareness evaluation the following responses were obtained and analyzed: Types of Farming system and herd production, Maintenance of the same animals in a herd to assess the involvement of animal movement to FMD outbreaks. FMD knowledge, (clues were checked among farmers to evaluate respondent exposure to the disease by hearing, listening or experiencing it after its occurrence in his/her herd for the last 10 years), types of animal species affected, knowledge on major FMD clinical signs, Awareness of the disease in terms of Morbidity, Mortality, and case fatality rate distribution between age groups, vaccination and treatment/management practices FMD cases.

3.9 Data collection and methods of analysis.

Laboratory investigation and questionnaire results were analyzed using Epi-info programme version 3.5.1 (Coulombier *et al.,* 2001). Retrospective study data which was number of FMDV positive cases were analyzed by using Microsoft excel. From Laboratory investigation optical density (OD) was converted into percentage of positivity for each sample to obtain data that were analyzed by epi-info programme. In questionnaire study number of respondents in each question was analyzed. The data were further analyzed by excel to produced pie, bar charts and histogram. In all the analyses, statistical confidence level was set at 95% and $P \leq 0.05$ was set for significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Seroprevalence of FMDV using 3ABC ELISA

From 94 Herds examined for the presence of antibodies to the 3ABC non-structural protein of FMD virus, 39 (41%) contained, at least, one positive animal (Table 4). The highest prevalence recorded in Bagamoyo district (81%) was significantly different ($p < 0.05$) from that obtained in other districts (Table 2).

Table 2: Seroprevalence of FMDV infection in 94 herds tested in six Districts

S/No.	Districts	No of herd	No of Animals	No of positive Herds	No of negative Herds	No of positive Samples	Seropreval ence (%)
1.	Bagamoyo	16	76	13	3	60	81
2.	Kibaha	16	74	9	7	42	56
3.	Kisarawe	12	47	2	10	5	17
4.	Ilala	25	108	8	17	40	32
5.	Kinondoni	12	103	5	7	23	42
6.	Temeke	13	53	2	11	10	15
	Total	**94**	**461**	**39**	**53**	**180**	**41**

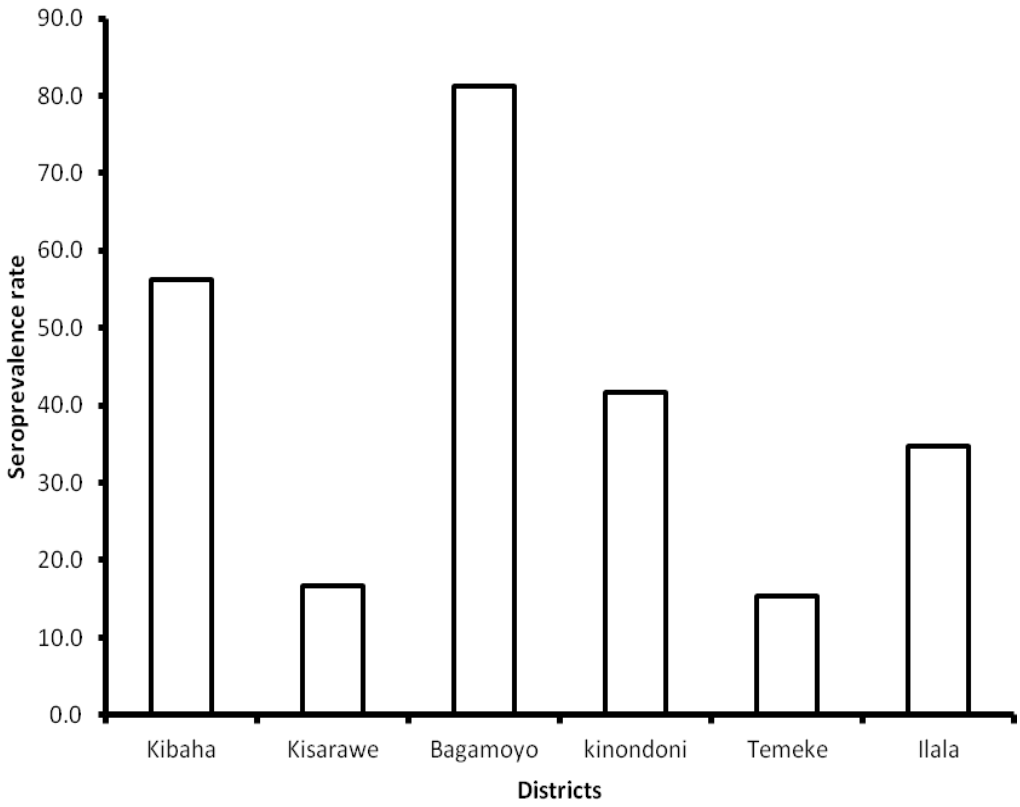

Figure 2. Bar charts of FMDV seroprevalance in six districts of the eastern zone of Tanzania

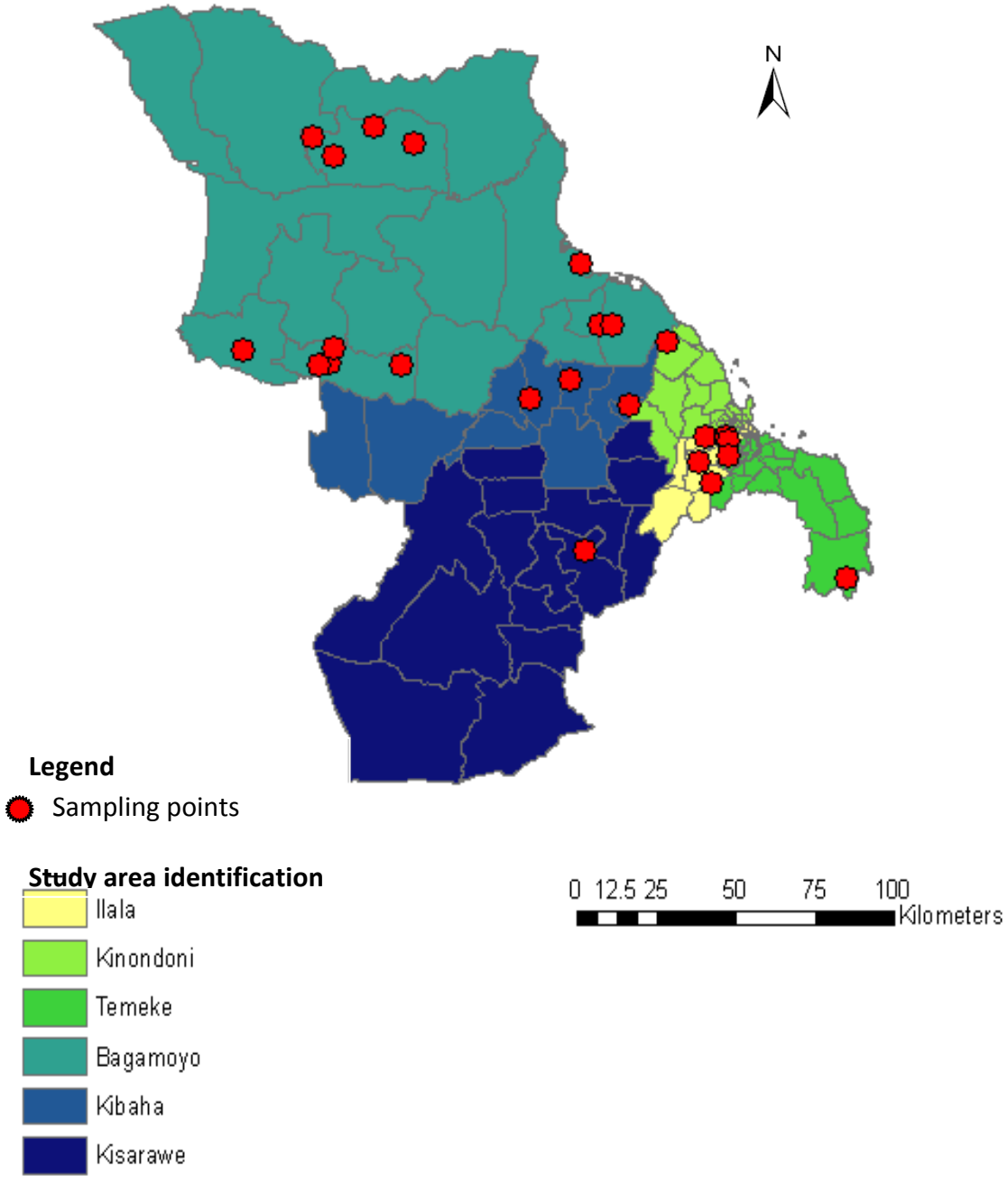

Figure 3. Map showing the spatial distribution FMDV seroprevalence in six districts of the eastern zone of Tanzania

4.1.2 Retrospective study of FMDV in the eastern zone of Tanzania

The retrospective data on serotyping of FMDV antigens were obtained by Ag detection ELISA. Serotypes detected between 2001 and 2011 were mainly serotype O, A and SAT-2. Serotype O dominated than other serotypes in terms of occurrence in the eastern zone of Tanzania

Table 3: Results of retrospective study on tissue sample submitted at CVL for 10 years

Year	Serotypes detected		
	SAT 2	O	A
2001	0	0	0
2002	0	1	0
2003	0	0	0
2004	3	2	0
2005	0	0	0
2006	1	1	0
2007	0	0	0
2008	0	0	0
2009	0	0	1
2010	1	1	0
2011	0	1	0
Total	**5**	**6**	**1**

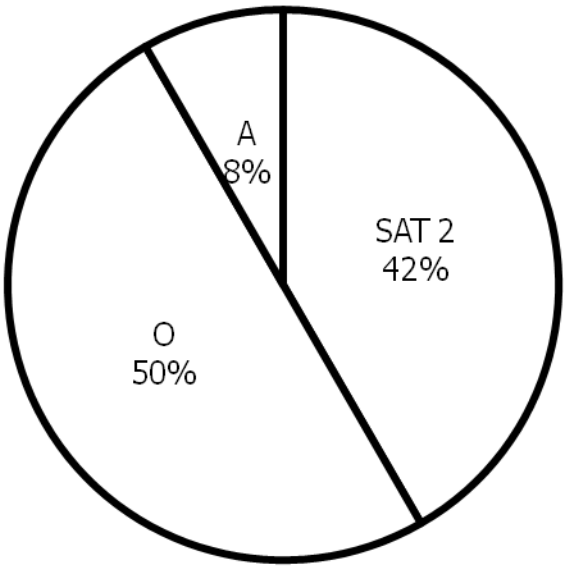

Figure 4. Occurence of FMDV serotypes in perccentage in ten years

Serotype O with 50% predominated in the eastern zone followed by SAT 2 (42%) while serotype A had scanty occurrence in the region with 8%.seroprevalence.

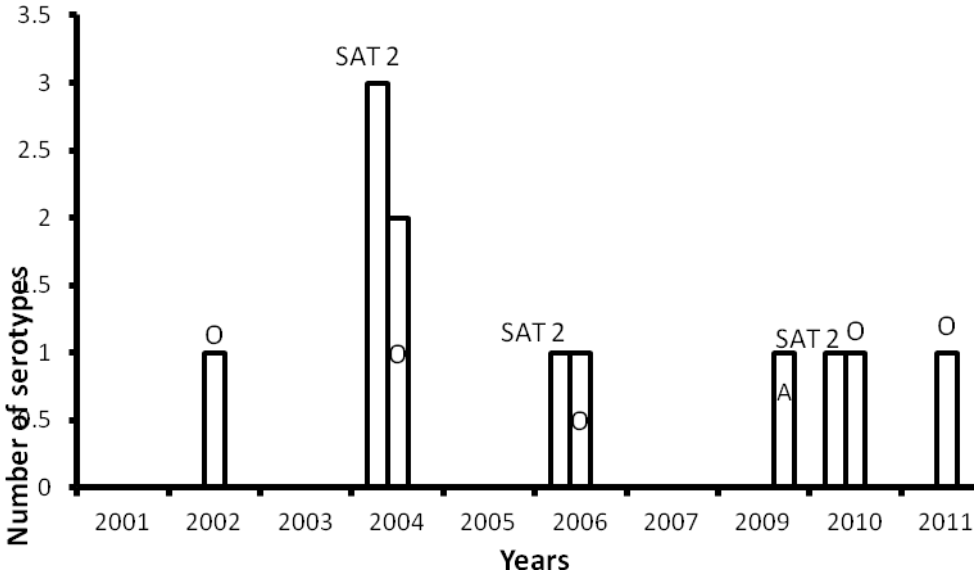

Figure 5. The histogram showing the number of cases and serotypes occurred in different years

Figure 6. Percentages of combined positive cases serotype SAT 2, O and A, and their temporal distribution in ten years from (2001 to 2011).

The outbreak of FMD positive cases was very high in 2004 about 42% of all the positive cases in 10 years. In the year 2006 and 2010 positive cases detected was 17% in each year. There were few outbreaks of (8%) in the year 2002, 2009 and 2011,while in the year 2001, 2003, 2005, 2007 and 2008 there was no positive cases of FMD outbreaks.

4.1.3 Detection of FMDV serotypes antigen using sELISA assay

The 3 tissue samples collected for FMDV test in 2011 during this study one was positive and was typed as serotype O.

4.1.4 Questionnaire survey

Frequency distributions of FMD awareness evaluation in terms of percentage were as shown in Table 4 (Appendix 2). Farming system in the study area consists of majority modern system at the rate of 83.3% with minority pastoral system at the rate of 16.7%. Herd production distributed in its categories by dairy system with higher rate of 84.4% compared to dual purpose and meat with 1.1%and 14.4% respectively. At district level farming system is as shown in Table 5 (Appendix 2). Animals were maintained in the same area for a long time as shown at the rate of 98.9% except one farmer who was nomadic.

General awareness of the disease was observed to be present where FMD knowledge assessment revealed 74.4% of respondents shown to have heard the disease elsewhere. This was further substantiated with farmer’s knowledge on species affected by FMD where 75% of respondents show awareness on species affected by FMD. Knowledge on

clinical signs of the disease was 55% of respondents signifying FMD awareness in terms of clinical signs at that rate. Awareness in terms of distribution of disease morbidity mortality and case fatality between age groups indicated was insignificant. Susceptibility of the disease between age groups indicated that all age groups were susceptible at the rate of 45% as opposed to adult age group (15.6%) and young age group (16.7%). Vaccination awareness was significantly very poor where 87.8% did not vaccinate their animals against FMD while only 11.1% perform vaccination. At district level vaccination status is as shown in Table 5. The vaccine used for FMD control in the eastern zone was multivalent vaccine manufactured by KEVEVAPI (a Kenya state owned Corporation). The vaccine composed of four serotypes (serotype O, A, SAT-1 and SAT-2).

4.2 Discussion

The overall seroprevalence rates of 43.6% in this study represent the extent of FMD spread in the Eastern zone of Tanzania. The highest district level seroprevalence (81.1%) recorded in Bagamoyo (Figure 4) as Compared to Kibaha (56.3%) Kinondoni (41.7%), Temeke (15.4 %), Ilala (34.8% and Kisarawe (16.7%) probably reflects farming system of Bagamoyo and Kibaha district which consist mixed type of farming as shown in Table:5. This type of farming allows mixing of animals from different location which could account for high transmission rate of FMDV and hence FMD outbreaks. Moreover, Bagomoyo and Kibaha are located along the high way where is a center for cattle movement that facilitates contact among cattle from different locations. Along this highway cattle transported to Dar es Salaam are at some point offloaded from the trucks for feeding and drinking. This type of practice is more serious when animals are tracked by foot. The above information has also been depicted in the map (figure 1) where spatial distributions of FMD positive cases have been observed. The highest densities of FMD positive cases were found mainly in Bagamoyo and Kibaha district which correspond to

the locations of mixed type of farming systems. Mixed type of animal production systems typically rely on frequent movement of animals thus facilitating spread of FMD. Location of highway across Bagamoyo and Kibaha districts has significant impact on spatial distribution of FMD in the study area because highway is used for transportation of cattle from up country to Dar es salaam. Ilala district has higher seroprevalence (34.8%) because it has cattle offloading point (Pugu rail station) and secondary market (Pugu cattle secondary market) for animals coming from mainland via central rail line. The scanty positive cases of FMD observed in Temeke and Kisarawe districts in this study could be ascribed to little geographical evidence of FMD risk factors like cattle movement and mixed animal production systems. Similar study has been done in north eastern part of Ethiopia to determine seroprevalence and associated risk factors for seropositivity of FMD by using 3ABC ELISA. Differences in geographical locations, age groups and herd sizes were risk factors found statistically ($p<0.05$) associated with the occurrence of FMD (Jenbere T. S *et al.*, 2011)

Farming system, herd production and maintenance of animals in a herd describe the status of farmers in relation to the animal health and production. FMD affect dairy farmers where modern farmers are severely affected because of international trade restrictions. Questionnaire survey had revealed high (74.4%) general FMD awareness by farmers, with 75% being knowledgeable on transmission and FMD susceptible animal species in the eastern zone. This survey also shows that FMD is very important in the study area where 83.30% and 84.40 % are modern and dairy farmers respectively.

About 76% of respondents were knowledgeable on the type of species affected by FMD either cattle were thought by respondents to be severely affected than other species because of the urban farming which is dominated by only cattle. Poor vaccination cover

observed across the study area show that FMD awareness in the study area has not been depicted in the control of the disease. A good reason for most farmers not vaccinating their animals is the optional priorities in disease management where FMD being not a killer disease among other diseases. Vaccination history which was collected during sampling as well as during questionnaire administration also suggest that seroprevalence obtained in this study may be largely attributed to natural FMDV infection rather than vaccination. Similar study has been done on FMD awareness assessment in India where the results of the study shows the availability vaccination services and the FMD public awareness created resulted into negligible cases of FMD in linkage villages (Singh B,P *et al.,* 2007)

According to the retrospective study and antigenic typing using few samples collected in the study period FMD Serotypes circulating in eastern zone of Tanzania were O, A, and SAT2. This is in agreement with previous findings from CVL where the serotypes identified from 1997 to 2004 from eastern Zone were O, A, and SAT2 (Swai *et al.,* 2009). Serotype O predominates in the study area as compared to other serotypes because eastern zone have been the reservoir of serotype O even before 2001 (Swai *et al.,.,* 2009). Temporal distribution of FMD in the study has been in erratic trends where high prevalence of 42% in 2004 followed by 17% in 2006 and 2010, 8% in other year 2002, 2009 and 2011while in the year 2001, 2003, 2005, 2007 and 2008 there was no positive cases of FMD outbreaks. The inconsistency level of the temporal occurrence of FMDV serotypes in eastern zone of Tanzania could be ascribed to under reporting of outbreaks and logistical reasons rather than actual trends of disease occurrence in the area. Similar study has been done in Bhutan where the study highlights the incursion of the PanAsia strain of the O serotype into the country, possibly, through the transboundary movement of animals (Dukpa, K., *et al.,* 2011) The spatial distribution of high seroprevalence in area with mixed type of farming and its location along the high way and the decrease in

seroprevalence in area which are far from highly seropositive area will provide suggestion to the type of control strategy to be used for eastern zone and nearby zones.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The spatial distribution of FMDV in this study has been determined by location of FMD risk factors which was movement of animals and farming systems. Movement of animals made Bagamoyo, and Kibaha kinondoni and Ilala districts to have higher seroprevalence than other districts in the study area. Mixed type of farming system made Bagamoyo and Kibaha to have higher seroprevalence as observed in this study. Temporal distribution of FMDV shows predominance of serotype O in erratic trend probably due to lack of good surveillance infrastructure for the period considered in retrospective study.

5.2 Recommendations

1. More effort should be put on evaluating the current situation of FMD disease status and control in the country, by up scaling of serosurveillance infrastructure.
2. Antigenic study should be done to produce a good vaccination strategy which base on vaccine matching.
3. Extensions services must emphasize on the benefits which farmers are likely to accrue in the future if FMD is successfully controlled.
4. There is a need for undertaking systematic study which will involve a wider scope of livestock species, areas, and seasonality in order to come up with a wider informed status of FMDV in Tanzania.

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APPENDICES

Appendix 1: Checklist for evaluation of foot and mouth disease (FMD) awareness in eastern zone of Tanzania

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. Specific information

1. Question: What types of Farming system and herd production?

Farming system

1	Modern	
2	Pastoral	

Type of herd production

1	Dairy	
2	Meat	
3	Dual purpose	

2. Question: Are you maintaining the same animal all the time?

1	Settled	
2	Nomadic	
3	Trader	

3. Question: Have you seen or heard this disease in your herd or elsewhere?

1	Yes	
2	No	

4. Question: What types of animal species are affected?

1	Cattle, Goats, Sheep, Pigs,	
2	others	
3	I don't know	

5. Question: choose the more severely affected species?

1	Cattle,	
2	Sheep	
3	Goats	
4	Pigs	
5	All	
6	I don't know	

6. Question: Any apparent clinical signs associated with the disease?

1	1.salivation lameness blisters/wound in mouth/feet	
2	2. lameness, swelling of hind quarter	
3	3.swelling of lymph nodes fever	
4	I don't know	

7. Question: What is the Morbidity rate distribution with animal classes?

1	Young	
2	Adult	
3	All age	
4	none	

8. Question: What is the Mortality rate distribution with animal classes?

1	Young	
2	Adult	
3	All age	
4	none	

9. Question: Which classes of animal are more likely to die when they are infected? (Tick where appropriate)

1	Young	
2	Adult	
3	All age	
4	none	

10. Question: Which class of animal is more susceptible to the FMD? (Tick where appropriate)

1	Young	
2	Adult	
3	All age	
4	none	

11. Question: Do you vaccinate your animals?

1	Yes	
2	No	

12. Question: if yes at what rate do you vaccinate your animals?

1	Once per year	
2	Twice per year	
3	No vaccination	

13. Question: Is there any type of available medicament used for FMD?

1	Yes	
2	No	
3	I don’t know	

14. Question: What type of available medicament used?

1	Modern	
2	local	
3	none	

K: Any comment (s) from the farmer

Appendix 2 Frequency distributions tables for evaluation of FMD awareness in eastern zone of Tanzania

Table 4: Frequency distributions of FMD awareness evaluation in terms of percentages

Question	Answer	No. of respondents	Percent
	Modern	75	83.30
Farming system	Pastoral	15	16.70
	Dairy	76	84.40
	Meat	13	14.40
Herd production	Dual purpose	1	1.10
Maintenance of animal in the	Settled	89	98.90
herd	Nomadic	1	1.10
	Yes	67	74.40
FMD Knowledge	No	23	25.60
Type of species affected	Cattle, Goats, Sheep, Pigs,	76	84.40
	I don't know	14	15.60
Specie most severely affected	Cattle,	61	67.80
	Sheep	1	1.10
	Pigs	5	5.60
	All	13	14.40
	I don't know	10	11.10
Clinical signs associated with	salivation lameness		
the disease	blisters/wound in mouth/feet	55	61.10
	lameness, swelling of hind quarter	14	15.60
	swelling of lymph nodes fever	2	2.20
	I don't know	19	21.10
Morbidity rate distribution	Young	1	1.10
between age group	All age	33	36.70
	none	56	62.20
Mortality rate distribution	Young	6	6.70
between age group	none	84	93.30
Case fatality rate distribution	Young	36	40.00
between age group	Adult	9	10.00
	All age	7	7.80
	none	38	42.20
The susceptibility distribution	Young	15	16.70
within age group	Adult	14	15.60
	All age	39	43.30
	none	22	24.40
Vaccination status	Yes	10	11.11
	No	80	88.89
Treatment after infection	Yes	41	45.60
	No	31	34.40
	No	18	20.00
Type of Treatment	Modern	41	45.6
	Local	4	4.40
	none	45	50.00

Table 5: Frequency distributions of farming system FMD vaccination status and FMD knowledge evaluation in terms of percentages

Question	District	Answer		
		Modern	Pastoral	Dairy
Farming system	Bagamoyo	33.3%	33.3%	33.3%
	KIBAHA	73.3%	0.0%	26.7%
	kisarawe	100.0%	0.0%	0.0%
	KINONDONI	100.0%	0.0%	0.0%
	Ilala	100.0%	0.0%	0.0%
	Temeke	93.3%	6.7%	0.0%
		Yes	No	I don’t know
Vaccination status	Bagamoyo	0.0%	100.0%	0.0%
	KIBAHA	6.7%	93.3%	0.0%
	kisarawe	6.7%	93.3%	0.0%
	KINONDONI	14.3%	78.6%	7.1%
	Ilala	13.3%	86.7%	0.0%
	Temeke	20.0%	80.0%	0.0%
FMD Knowledge	Bagamoyo	93.3%	6.7%	0.0%
	KIBAHA	80.0%	20.0%	0.0%
	kisarawe	66.7%	33.3%	0.0%
	KINONDONI	93.3%	6.7%	0.0%
	Ilala	53.3%	46.7%	0.0%
	Temeke	60.0%	33.3%	6.7%