TYPING AND CHARACTERIZATION OF FOOT-AND-MOUTH DISEASE VIRUS DETECTED IN NORTHERN PART OF TANZANIA

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ABSTRACT

Foot-and-mouth disease (FMD) is the most economically important viral disease of livestock globally and has been endemic in Tanzania for many years. It is caused by FMD virus (FMDV). The present study was carried out to identify and differentiate serotypes of the virus from FMD outbreaks in the Northern parts of Tanzania. A total of 35 samples (n = 28 cattle epithelial tissues and n = 7 buffalo probang) were tested by reverse-transcription polymerase chain reaction (RT-PCR) to detect viral genome, and 86% of the samples (n = 30) typed by antigen detection ELISA. RT-PCR using serotype specific primers targeting the VP1 region of the FMDV genome detected two serotypes (A, and SAT 2), whereas all four serotypes (A, O, SAT 1 and SAT 2) were detected by antigen detection ELISA (Ag-ELISA). Out of the 35 samples, RT-PCR detected 12 positives (34.3%) and of the 12 positive samples, 5(41.7%) were SAT2 and 4 were serotype A (33.3%), and the other 3(25%) have multiple infections. For Ag-ELISA, 20 out of the 30 samples contained FMDV antigen; 3(15%) were SAT1, 4(20%) SAT2, 2(10%) serotype A, and 1(5%) serotype O. The other 10 had multiple infections. Indeed FMD has been confirmed in Serengeti (SAT2, SAT1, and type A), Simanjiro (SAT1, SAT2, type O and A), Bunda (SAT1, SAT2 and type A) and Same (SAT1, SAT2, type A and O) districts in Northern parts of Tanzania. The findings of this study reveal the existence of at least four serotypes (A, O, SAT1 and SAT 2) in the northern parts of Tanzania. The existence of multiple serotypes and multiple infections complicates control of FMD in the region. Further in-depth studies, including sequencing, are required to clarify the molecular epidemiology of FMDV in the region hence recommendation for appropriate control measures of FMD in Tanzania.

DECLARATION

I, Kelebogile Portia Oratile Mokgele 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.

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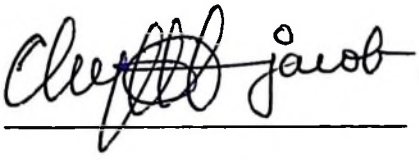

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DEDICATION

This work is dedicated to my nieces Resego, Katlo and Gofiwa; and my nephew Setso.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

°C	degree Celsius
®	Registered trade mark
µl	microliter
Ag	antigen
Ag-ELISA	antigen-enzyme-linked immunosorbent assay
bp	base pairs
BBSRC	Biotechnology and Biological Sciences Research Council
CDC	Centres for Disease Control and Prevention
cDNA	complementary deoxyribonucleic acid
CFT	Compliment Fixation Test
Defra	Department for Environment and Rural Affairs
DNA	deoxyribonucleic acid
DVS	Director of Veterinary Services
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
EMPRES	Emergency Prevention Systems
FAO	Food and Agriculture Organization
FMD	Foot-and-Mouth Disease
FMDV	Foot-and-Mouth Disease Virus
IATA	International Association of Air Transport
IAH	Institute for Animal Health
kb	kilobase
km^2	square kilometers
MAbs	monoclonal antibodies

MEM	Minimum Essential Medium
MLDF	Ministry of Livestock Development and Fisheries
NSP	non-structural protein
nm	nanometers
OD	Optical Density
OIE	World Organization of Animal Health
PAb	polyclonal antibody
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pH	negative log of hydrogen ion concentration
RNA	ribonucleic acid
RT-LAMP	reverse transcription loop-mediated isothermal amplification
RT-PCR	reverse transcription polymerase reaction
SAT	South African Territories
SACIDS	Southern African Centre for Infectious Diseases and Surveillance
TAD	Transboundary Animal Diseases
TBE	Tris-Borate-EDTA
TVLA	Tanzania Veterinary Laboratory Agency
UK	United Kingdom
USA	United States of America
UV	ultraviolet light
VI	virus isolation
VICs	Veterinary Investigation Centres
VNT	virus neutralization test
VP	viral protein segment
WRL	World Reference Laboratory

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Livestock production forms an important part of the African lifestyle but it is affected by the presence of a wide variety of infectious animal diseases, many of which are transboundary animal diseases (TADs). The burden of these infectious diseases is greatest in the developing countries, and the most contagious are found in Africa (Brownlie, 2012). One such disease is foot-and-mouth disease (FMD) which causes economic afflictions of livestock due to the high cost of control and international trade restrictions imposed following outbreaks (Sangré *et al.*, 2004; Li-na *et al,*. 2011). Tanzania is no exception to this; although the country has a huge population of livestock, the sector contributes very little to the economy, due to the presence of these contagious TADs such as FMD, Contagious bovine pleuropneumonia and African swine fever (The Tanzania Five Year Development Plan, 2012).

FMD is the most contagious viral vesicular disease of mammals that mostly affects cloven hoofed livestock such as cattle, sheep, goats, pigs and wildlife including the African buffalo, reindeer, water buffalo, antelope, kudu and wildebeest (Mwiine *et al.*, 2010; OIE 2012; Vosloo, 2013). This disease is caused by foot-and-mouth disease virus (FMDV), a member of the genus *Aphthovirus* in the family *Picornaviridae* which is antigenically and genetically diverse (FAO/OIE, 2013; Vosloo, 2013).

Cattle are the most susceptible animals whereas pigs are the best amplifiers of the virus (FAO/OIE, 2013). Outbreaks of FMD have occurred in most of the world except in New Zealand. The disease is currently endemic in all continents of Africa, Asia, and South

America, except Australia and North America (Grubman and Baxt, 2004). Africa in particular FMD is widely endemic and the existence of unique SAT types especially in sub-Saharan Africa complicates the disease situation (Vosloo *et al.*, 2005). Wildlife also plays an important role in the control of the disease in Africa (Vosloo *et al.*, 2005).

Although mortality is usually low, morbidity can reach 100%, therefore the disease remains a major economic concern for livestock-health in many developing countries and a continued threat to disease-free countries (Maree *et al.*, 2011). FMDV infections in humans are very rare (Armstrong *et al.*, 1967), only about 40 cases diagnosed since 1921; and is characterized by vesicular lesions as well as flu-like symptoms. The disease is however generally mild, short-lived and self timing (OIE, 2012).

Rapid, sensitive and specific laboratory assays are needed for FMD diagnosis and typing of contemporaty circulating FMDV. This is an important requirement to enable prompt and appropriate control measures to restrict the spread of infection and eradicate the disease (Reid *et al.*, 1998; Barlič-Maganja *et al.*, 2004).

FMD is endemic in Tanzania and four serotypes; serotype A, O, South African Territories (SAT 1 and SAT 3), have been circulating in the country for several years (Kasanga *et al.*, 2012). It is considered the most important viral transboundary disease in Tanzania. It has caused great economic losses in the country. The control and eradication of the disease is believed to be complicated by several factors such as uncontrolled animal movements, inactive surveillance systems and even the lack of political will for the control (Vosloo *et al.*, 2002).

1.2 Problem Statement and Justification of the Study

Foot and mouth disease is highly contagious and has a potential of very rapid transmission even across national borders especially if not controlled properly (Lau *et al.*,

2008), thereby causing serious socio-economic and environmental consequences; it is economically the most important livestock viral disease of livestock (Jeirani *et al.*, 2012). The disease has a wide host spectrum such as cattle, sheep, goats, buffalo and other cloven-hoofed animals (Ma *et al.*, 2011). Its presence in a country is a major obstacle to the development of the national livestock industry and on trade of animals and animal products into lucrative export markets (Kivaria, 2003; Chen *et al.*, 2011b). It is a major threat to the food supply, causes severe productivity losses, causes high expenses in control and eradication as well as surveillance and emergency preparedness (Rushton and Knight-Jones, 2012). Besides its socio-economic importance, it causes high mortality in young stock, leads to reduced milk production and loss of weight (Maree *et al.*, 2011). This decrease in productivity significantly affects farmer's livelihoods, food security and rural income generation (Di Nardo *et al.*, 2011).

Since the disease is extremely contagious, and exists in several serotypes and multiple topotypes within each serotype, which do not confer immunity against each other, early and rapid serotyping of suspect FMD is of paramount importance and needs to be accurate (Alamdari *et al.*, 2006). In a bid to control the disease in countries where FMD is endemic, regular vaccination is crucial and the vaccine must contain the representative strains of the serotypes that are in circulation in the region (Suryanarayana *et al.*, 1999; Shao *et al.*, 2010).

Therefore, the differentiation of serotypes is crucial also so that the vaccines used match the circulating strains (Ma *et al.*, 2011). This differentiation of serotypes can be achieved by assays such as serotype-specific antigen detection ELISA and RT-PCR using serotype-specific primers (Morioka *et al.*, 2009).

FMD outbreaks have been encountered in several parts of Tanzania and contributed to significant economic losses primarily to livestock keepers. Of late, many FMD outbreaks

have been reported to occur in the northern parts of Tanzania involving indigenous and exotic cattle farms. The identification and characterization of the serotypes associated exclusively with the recent FMD outbreaks in the Northern parts of Tanzania has not been done. The findings provided by this study will provide information needed for the probable control measures (strategic vaccination programs) in combating the FMD serotypes detected from these outbreaks.

1.3 Research Objectives

1.3.1 Main objective

Identification and serotyping of FMD viruses in cattle and buffalo field samples from the Serengeti, Simanjiro, Bunda, and Ngorongoro district in north Tanzania

1.3.2 Specific objectives

i. To detect the FMDV genome in the Northern parts of Tanzania by serotype-specific RT-PCR.

ii. To compare the performance of RT-PCR and antigen ELISA in detection of FMDV.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Foot-and-Mouth-Disease

The OIE and FAO consider foot-and-mouth-disease as the most contagious vesicular viral disease of a wide variety of economically important domestic animals and wildlife hosts, which occurs in multiple non-cross-protective virus serotypes (Raof *et al.*, 2011; OIE/FAO 2012). It is also considered the most economically important transboundary animal disease worldwide, and outbreaks of the disease require immediate notification to the World Organization for Animal Health (OIE) (Paixáo *et al.*, 2008; EMPRES WATCH-FAO, 2012). The disease is reported to occur worldwide with the exception of a few countries but it is particularly endemic in sub-Saharan Africa (Rweyemamu *et al.*, 2008; Fasina *et al.*, 2012), and has detrimental effects on the economy, prompting the imposition of restrictions of export animal products (Carpenter *et al.*, 2011; Chen *et al.*, 2011b). The potential introduction of the FMD virus, particularly by terrorist groups is also a huge threat to FMD-free countries (Grubman, 2005). FMD also has high mortality rates in infant animals but not in adult animals, as well as high morbidity (Rushton and Knight-Jones, 2012).

The disease was first described in 1514 when Fracastorius described a similar disease of cattle in Italy, but was confirmed to be a viral disease in the nineteenth century (Mahy, 2005).

2.2 Foot-and-Mouth-Disease Etiology

2.2.1 FMD virus classification

The causative agent of foot-and-mouth-disease is a small virus called foot-and-mouth-disease virus (FMDV) and was the first recognized viral pathogen (Longjam *et al.*, 2011).

It belongs to the genus *Aphthovirus* within the family *Picornaviridae* (Bastos *et al.*, 2003; Ma *et al.*, 2011). The virus is antigenically highly variable (Grubman, 2005) and there are seven serologically identified serotypes of FMDV; O, A, C, SAT1, SAT2, SAT3 and Asia 1, as well as multiple subtypes within each of the serotypes - topotypes (Lau *et al.*, 2008; Yang *et al.*, 2011). Immunity to one serotype does not provide any cross-protection against other serotypes (Rweyemamu *et al.*, 2008; Chen *et al.*, 2011a; Raof *et al.*, 2011; OIE, 2012).

2.2.2 Structure

2.2.2.1 Physical structure of virion

The virus particle is a roughly spherical, consists of 70% protein and 30% RNA, and it does not contain a lipid envelope (Hui, 2004). The virion is approximately 30nm in diameter (Longjam *et al.*, 2011). Its capsid consists of a densely-packed icosahedral arrangement of 60 protomers, each consisting of 4 structural proteins, VP1, VP2, VP3 and VP4. VP4 is located on the inside of the capsid, while the others are on the outside (ViralZone, 2008; Hema *et al.*, 2009), with numerous copies of each of the four structural proteins VP1-4 (Grubman, 2005), and ten mature non-structural proteins (NSPs); L, 2A, 2B, 2C, 3A, 3B, 3C, 3D, or some complex such as 3AB or 3ABC (El-Kholy *et al.*, 2006; Fasina *et al.*, 2012). VP1 protein bears the greatest sequence variability amongst these structural proteins. It is the locus of a receptor recognition site and is the major antigenic site of virus attachment, protective immunity and serotype specificity (Hui, 2004; Ma *et al.*, 2011). It is the only structural protein capable of inducing serotype-specific neutralizing antibody response, and when administered alone it can confer protection from challenge by homologous virus (Clavijo and Kitching, 2003). VP1 is the main protein determining antigenic identity and the differences in its sequences are the basis for developing reverse transcriptase polymerase chain reaction tests to identify different serotypes (Stram *et al.*, 1995; El-Bagoury *et al.*, 2011).

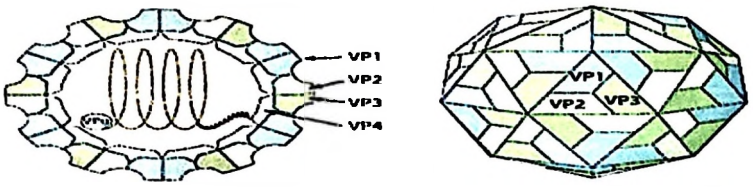

Figure 1: The spherical shaped FMDV virion showing the arrangement of the structural proteins VP1 – VP4. Source: ViralZone (2008).

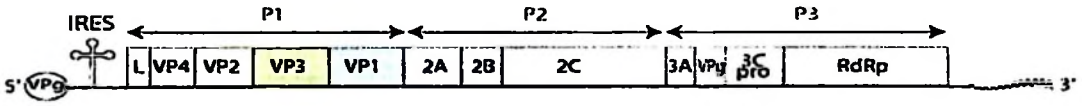

Figure 2: Genome of FMDV. The P1 region encodes the structural polypeptides, while P2 and P3 encode the non-structural polypeptides associated with replication. Source: ViralZone (2008).

2.2.2.2 Genomic structure

The genome is a linear, single-stranded, positive sense RNA of approximately 8.5kb long that lacks proofreading ability thus high genetic and antigenic variability (Vosloo, 2013). It is surrounded by the icosahedral capsid (Grubman and Baxt, 2004; Longjam *et al.*, 2011). The organization of the viral genome is shown in Fig. 2. Generally, FMDV genome can be divided into several regions: 5' untranslated region, protein encoding region and the 3'-untranslated region. The genome has a viral protein (VPg) at the 5' end instead of a methylated nucleotide cap structure. The P1 region encodes the structural proteins (VP4, VP2, VP3 and VP1) that form the capsid of the mature virions (Hui, 2004), while P2 and P3 encode the non-structural proteins associated with replication. L is an additional N-terminal leader protein present in some genera (ViralZone, 2008). The VP1 protein plays an important role in virus attachment, protective immunity and serotype specificity; therefore it is usually targeted for nucleotide sequencing in molecular epidemiology studies on FMD (Yang *et al.*, 2011). Reports by Valdazo-González, (2013)

have also shown that most of the knowledge about the global distribution and molecular epidemiology of the virus is dependent upon the analysis of the VP1 region which comprises approximately 8% of the FMDV genome.

2.2.3 Survival stability and viability of FMDV

The virus is moderately stable under a variety of environmental conditions, being most stable at neutral pH of 7.0 – 8.5 (Bachrach, 1968; Tshering, 1995; Alexandersen *et al.*, 2003), but very sensitive to low pH (OIE, 2012; Schley *et al.*, 2012). FMDV can be easily inactivated by acids and alkali including hydrochloric acid, sulphuric acid and other acids (Curry *et al.*, 1995). It is also remarkably thermolabile, being most stable at 4°C (Mateo *et al.*, 2008); high temperatures such as during pasteurization also inactivate the virus (Tomasula and Konstance, 2004), as well as relative humidity less than 60% (Tshering, 1995; Schley *et al.*, 2012). It can survive in meat and other products for a long time since high concentrations of organic material helps the survival of the virus (Donaldson *et al.*, 1987; Mahy, 2005; Ding *et al.*, 2011). FMDV is resistant to detergents and organic solvents such as ether and chloroform (Alexandersen *et al.*, 2003). Its resistance to harsh environments is attributed to the virus being a naked virus; non-enveloped (Kamolsiripichaiporn *et al.*, 2007).

2.3 Molecular Epidemiology of Foot-and-Mouth-Disease Virus

Foot and mouth disease viruses evolve rapidly due to a high mutation rate during replication, giving rise to intra-serotypic sub-types that may cross-protect incompletely (Paton *et al.*, 2009; Waheed *et al.*, 2009). The virus is antigenically and genetically diverse due to its polymerase lacking a proof-reading mechanism during replication, and based on the VP1 gene which is highly variable and used as the basis for molecular epidemiology (Wadsworth *et al.*, 2006; Waheed *et al.*, 2009). Three continental reservoirs

of FMD exist in Asia, Africa and South America, and these are further divided into 7 major virus pools of infection, recognized by the OIE/FAO FMD network. Each contains three serotypes of virus, which are specific to the region and require specific vaccines (Paton *et al.*, 2009). These pools of infection are shown in Figure 3. Pool 7 viruses almost exclusively occur in South America while some topotypes overlap in multiple pools (Paton *et al.*, 2009). Pool 1 occurs in Eastern Asia, pool 2 in Southern Asia, Pool 3 in Europe-Asia, pool 4 in Eastern Africa, pool 5 in Western Africa, pool 6 in Southern Africa and pool 7 occurs in South America (OIE, 2012).

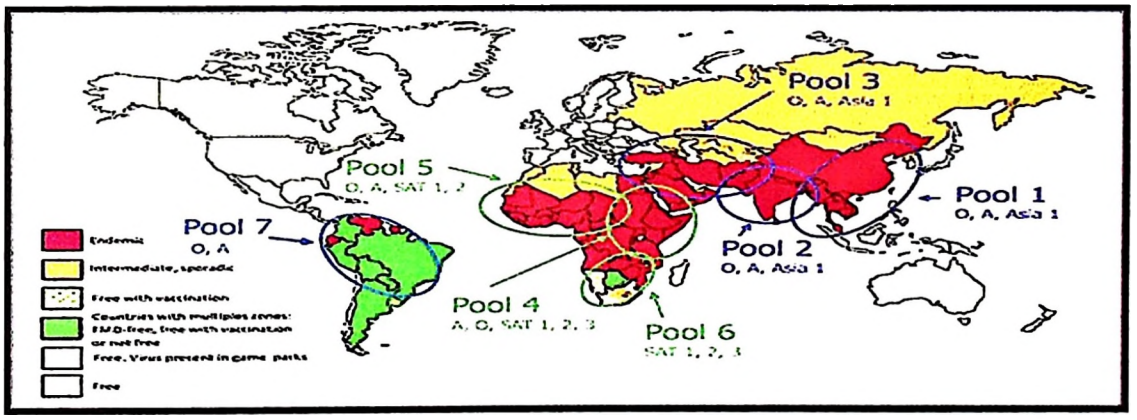

Figure 3: The approximate distribution of regional FMD virus pools according to OIE, 2013. The map shows that currently in Africa there are three FMD virus pools loosely defined as covering East Africa (pool 4), West Africa (pool 5) and Southern Africa (pool 6). Pools 4 and 5 have an overlap. Source: OIE (2013).

2.4 Transmission of Foot-and-Mouth-Disease Virus

FMD is usually spread by the movement of infected animals thereby susceptible ones coming in direct contact with the infected animals (Kitching, 2002; Abubakar *et al.*, 2012). The virus is found in all secretions and excretions from acutely infected animals,

including expired air, saliva, mucus, milk, urine, feces and semen therefore contact with these will lead to infection (Kamolsiripichaiporn *et al.*, 2007; OIE, 2012). Droplets in the respiratory tract of the recipient animals can also cause infections (Kitching, 2002). Peak transmission usually occurs when vesicles rupture (OIE, 2012). Routes of spread of the virus include inhalation of aerosolized virus, ingestion of contaminated feed, and entry of virus through skin abrasions or mucus membrane (OIE, 2012). However, indirect contact via the oral route is possible and causes epidemics in pigs (Kamolsiripichaiporn *et al.*, 2007). Other indirect routes which spread the virus include contaminated personnel, vehicles all classes of fomites or consumption of uncooked meat products (OIE, 2012). In Southern Africa, the major maintenance host of FMD is considered to be the African buffalo (*Syncerus caffer*) and it can act as carriers of the virus for long period and somehow transmit it to cattle (Thomson, 1995; Sangula *et al.*, 2010). Transmission of FMD is usually rapid in an unvaccinated herd (Kitching, 2002).

2.5 Pathogenesis

In natural infections, the primary route of virus entry is the respiratory tract (Kitching, 2002), although the virus can enter through skin abrasions or the gastrointestinal tract. Replication occurs in the pharynx epithelium, producing primary vesicles (Longjman *et al.*, 2011). After this epithelium infection, the virus spreads to different organs and tissues, and production of secondary vesicles mostly in mouth and feet (Longjam *et al.*, 2011). The incubation time varies per species; is between 2 and 14 days in cattle and pigs, 3 to 8 days in sheep and it depends of the infecting dose, the virus strain and the susceptibility of the host (Kitching, 2002). The acute phase of disease lasts for about a week (Longjam *et al.*, 2011), after which adults recover but low levels of the virus may still persist in the oro-pharynx of some ruminants (OIE, 2012).

2.6 Clinical Signs

The severity of the clinical signs differs in animals depending on the strain, infectious dose and susceptibility of the animal (Vosloo, 2013). The clinical signs are more severe in cattle than in small ruminants and young stock may die before showing any vesicles because of virus-induced damage to the heart (Grubman and Baxt, 2004; Vosloo, 2013). Cattle usually have lesions on the lips, dental plate, tongue, feet and teats. They also have a high fever above 40°C (Klein, 2009; Abubakar *et al.*, 2012). Excessive salivation, lameness, depression, loss of weight and decreased milk production are also exhibited (Kitching, 2002; Grubman and Baxt, 2004; Zinnah *et al.*, 2010). Some of these signs are depicted in Figure 4. In pigs the feet are primarily affected by painful vesicles which can lead to severe lameness and purulent arthritis (Klein, 2009). The clinical signs are less severe in goats and sheep, which might show none or only lameness due to inflamed cloves (Klein, 2009).

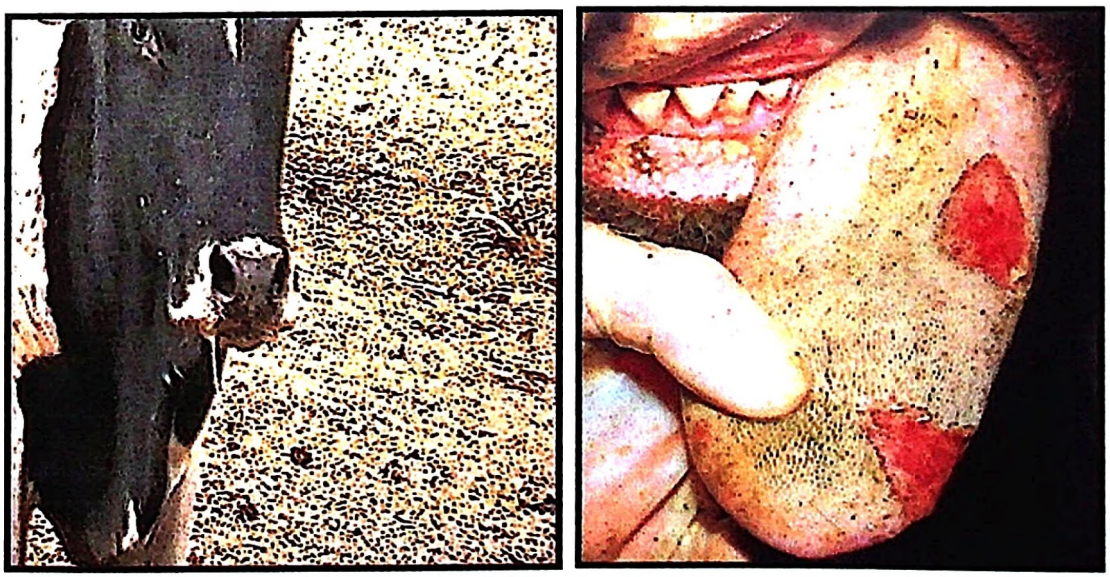

Figure 4: Typical clinical signs of FMD showing a drooling cow (left) and ruptured lesions on tongue of a cow (right). Source: Defra (2005).

2.7 Foot-and-Mouth-Disease Diagnosis

Diagnosis in the field is usually done based on clinical signs. However, FMD cannot be differentiated clinically from other vesicular diseases such as swine vesicular disease, vesicular stomatitis and vesicular exanthema of swine, (Grubman and Baxt, 2004; OIE, 2012). In small animals it can also resemble rinderpest, foot rot, traumatic stomatitis and chemical or thermal burns. In sheep and goats the clinical signs are often mild (Alexandersen *et al.*, 2003; Wernery and Kinne, 2012). Therefore, laboratory diagnosis of any suspected FMD case is a matter of urgency (OIE, 2012), so that quarantine and eradication programs can be implemented as quickly as possible to avoid the spread of the disease (Shaw *et al.*, 2004). Samples of vesicular epithelium or vesicular fluid are preferred in laboratory confirmation and must be kept at a neutral pH of 7.4 to prevent virus and antigen destruction (OIE, 2012). In the absence of tissue samples, diagnosis can be done using serum samples, blood or probang samples (OIE, 2012).

2.7.1 Laboratory diagnosis/confirmation

In the laboratory, FMD diagnosis is usually based on the presence or absence of the virus, the antigen or antibodies against the virus (Shaw *et al.*, 2004; OIE, 2012). The virus isolation, detection of viral antigens or nucleic acids and serology are laboratory methods that can be used to diagnose FMD. These include virus isolation (VI) in cell culture, immunoassays to detect antigen, and assays to detect nucleic acid sequences of the FMDV genome (Lau *et al.*, 2008). In recent years, various molecular methods such as conventional RT-PCR have been used to the universal detection FMDV serotypes and serotype-specific results serve as a source of material for the early detection of FMD virus (Jeirani *et al.*, 2012). ELISA and RT-PCR are standard assays used for detecting FMD (Ding *et al.*, 2011).

Different serological test methods such as complement fixation test (CFT), virus neutralization test (VNT) and enzyme-linked immunosorbent assay (ELISA), are used to

detect antibodies to FMD structural proteins for previous or current infections in unvaccinated animals; they are serotype specific (Longjam *et al.*, 2011; OIE, 2012). Serological tests that detect antibodies to non-structural proteins (NSP) are used for diagnosis in vaccinated animals (OIE, 2012). CFT is one of the oldest test method used for FMD diagnosis but it has been deemed less sensitive and difficult to interpret due to pro- and anti-complementary activity of the samples, therefore it is slowly replaced by more sensitive tests such as antigen-ELISA and molecular techniques, the polymerase chain reaction (Rémond *et al.*, 2002; Longjam *et al.*, 2011).

2.7.1.1 Antigen detection ELISA

Antigen detection ELISA is an immunoassay because it is antibody-based and relies on the antibodies to detect the antigens. It is serotype specific and identifies the viral antigens directly in tissues and vesicle fluids (Van Dessel *et al.*, 2007). The test is used in most routine FMD diagnostic laboratories the FAO/World Reference Laboratory (WRL) for FMD (Perkins *et al.*, 2007; Longjam *et al.*, 2011). The plates are coated with anti-FMD monoclonal antibodies (MAbs) or polyclonal antibodies (PAbs)-, against each of the serotypes, which will eventually trap any FMD virus in the sample incubated with the MAbs on the plate (Ma *et al.*, 2011). Therefore this immunological interaction is able to distinguish serotypes (Morioka *et al.*, 2009). Results can be obtained within 3 - 4 hours.

2.7.1.2 Reverse-transcription polymerase chain reaction (RT-PCR)

PCR has been proven to be a very powerful tool for routine disease diagnosis and molecular epidemiology (King *et al.*, 2006; Waheed *et al.*, 2009). It is currently the most used method for amplifying viral targets (Lau *et al.*, 2008), and more sensitive since it requires only a small quantity of the template and has high specificity as only targeted viral RNA regions are amplified (Rémond *et al.*, 2002).It is used to detect and amplify the

genome fragment of FMDV in diagnostic materials like epithelium, serum, milk and probang samples (King *et al.*, 2006; El-Shehawy *et al.*, 2012; OIE, 2012). When combined with real-time PCR, RT has sensitivity comparable to that of virus isolation and automated procedures enhance sample throughput (Reid *et al.*, 2002; Reid *et al.*, 2003). With the developed serotype specific primers, RT-PCR can also detect the different FMD serotypes (El-Kholy *et al.*, 2006; OIE, 2012), and the differences in VP1 gene sequences are the basis for developing reverse transcriptase polymerase chain reaction (RT-PCR) tests to identify different serotypes of FMD (Stram *et al.*, 1995). The assay also provides an alternative approach for virus detection in oropharyngeal fluids which normally have a low concentration of the virus (Parida *et al.*, 2005; El-Shehawy *et al.*, 2012). It plays an important role in the characterization of FMDV, especially when coupled with nucleic acid sequencing, since it allows for the epidemiological tracing of outbreaks (Saeed *et al.*, 2010).

2.8 Control of Foot-and Mouth-Disease

The control of FMD is complicated because a high proportion of infected cattle and other species are persistently infected and that FMDV can persist in the oro-pharyngeal region of cattle up to two and half to three and a half years post infection (Mwiine *et al.*, 2010), while in buffaloes it can last up to 5 years (Mwiine *et al.*, 2010). The ability of FMDV to infect different hosts and to exist as multiple types and variants also make it difficult to control the disease (Valarcher *et al.*, 2006).

There is no treatment for FMD (Wernery and Kinne, 2012), the animals recover after a few days. Vaccination is one of the principle methods for control and eradication for the disease and the vaccines must match the serotype and strain of the infecting strain (Paton *et al.*, 2009). The timing of vaccination should also be uniform and regular to attain best results (Pattnaik *et al.*, 2012). Movement restrictions, quarantine of affected premises and

destruction of infected animals are also used in an attempt to eradicate the virus during outbreaks in countries where FMD is endemic (Grubman, 2005; OIE, 2012). Other complementary measures such as cleaning and disinfection of affected areas are also essential. FMD-free countries do not vaccinate their stock but prefer to use strict movement controls and slaughter of infected and contact animals when outbreaks have occurred (Doel *et al.*, 1994; Paton *et al.*, 2009). The control of outbreaks is however dependent upon a system of monitoring and early detection, which requires basic familiarity with clinical signs and the ability to characterize the strain of virus responsible, by laboratory tests (Shaw *et al.*, 2004; El-Shehawy *et al.*, 2011).

2.9 Geographical Distribution of Foot-and-Mouth-Disease

2.9.1 World distribution

The geographical distribution of FMDV serotypes has probably undergone a series of expansions and contractions over the last century; however, the current distribution is believed to be under-reported, especially in Africa (Namatovu *et al.*, 2013). FMD is still endemic in Africa, Asia, the Middle East and South America (Figures 5 and 6) (Pineda-Krch *et al.*, 2010). Its persistence in wild Africa buffalo in most parts of Africa makes it impossible to eradicate the disease (Vosloo *et al.*, 2005; OIE, 2012). Regions of the world which are considered FMD free include Europe, North America, and parts of South America, Australia, Greenland, Iceland and some island regions of Asia (Figures 5 and 6) and have stringent regulations preventing the introduction of the virus (Carpenter *et al.*, 2011; Wernery and Kinne, 2012). Sporadic outbreaks have occurred in disease-free countries with the exception of New Zealand, Greenland, Iceland and the smaller Islands of Oceania (Carpenter *et al.*, 2011; OIE, 2012).

The serotypes of FMDV are not evenly distributed across the world where the disease occurs (Rweyemamu *et al.*, 2008; Raof *et al.*, 2011). All the serotypes except Asia 1 have been isolated in Africa or Europe (Vosloo *et al.*, 2005; Raof *et al.*, 2011; Namatovu *et al.*,

2013), but this serotype dominates the Middle East and Asia (Bronsvoort *et al.*, 2004). SAT (South African Territories) serotypes remain almost exclusively restricted to Africa (Jackson *et al.*, 2007; Picado *et al.*, 2010). In sub-Saharan Africa the predominant serotypes are O, A, SAT 1 and SAT 2 (Habiela *et al.*, 2010), but the most widely distributed is SAT 2 (Bastos *et al.*, 2003). Serotype SAT3 rarely occurs in species other than the Cape buffalo in southern Africa (Bronsvoort *et al.*, 2004). Periodically there have been incursions of SAT1 and SAT2 from Africa into the Middle East (Bastos *et al.*, 2003; Rweyemamu *et al.*, 2008).

Serotypes O and A are widely distributed worldwide (Chen and Liu, 2013). Asia has type O, A, C, and Asia1, while South America contents with only type O, A and C (Rweyemamu *et al.*, 2008; Raof *et al.*, 2011). Serotype O is the most common type and widely distributed worldwide compared to other serotypes; it has affected many countries throughout the world, especially the Middle-East-South Asia region (Hemadri *et al.*, 2002; Bai *et al.*, 2011). Type A and O are reported in South America, Asia and Africa, Asia 1 occurs in Asia and the Middle East (Picado *et al.*, 2010). Type C on the other hand has limited distribution compared to the other serotypes, being limited to the Indian sub-continent (Hui, 2004; Chen and Liu, 2013), and has not been observed for many years, since 2004 (Sangula *et al.*, 2010; Chen and Liu, 2013; Paprocka and Fitzner, 2013). It may also now be confined to Africa since it has not been reported elsewhere recently (Vosloo *et al.*, 2002).

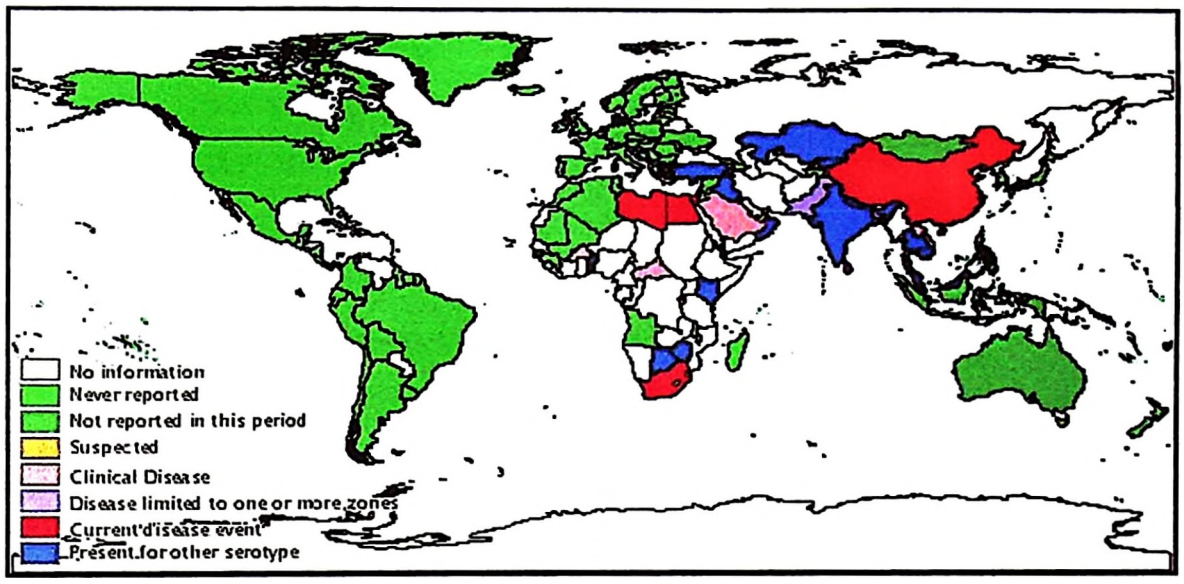

Figure 5: A map of the world, showing the world distribution of FMD according to OIE, indicating the disease status in each country across the world during the period of January and June 2012. Source: OIE (2013).

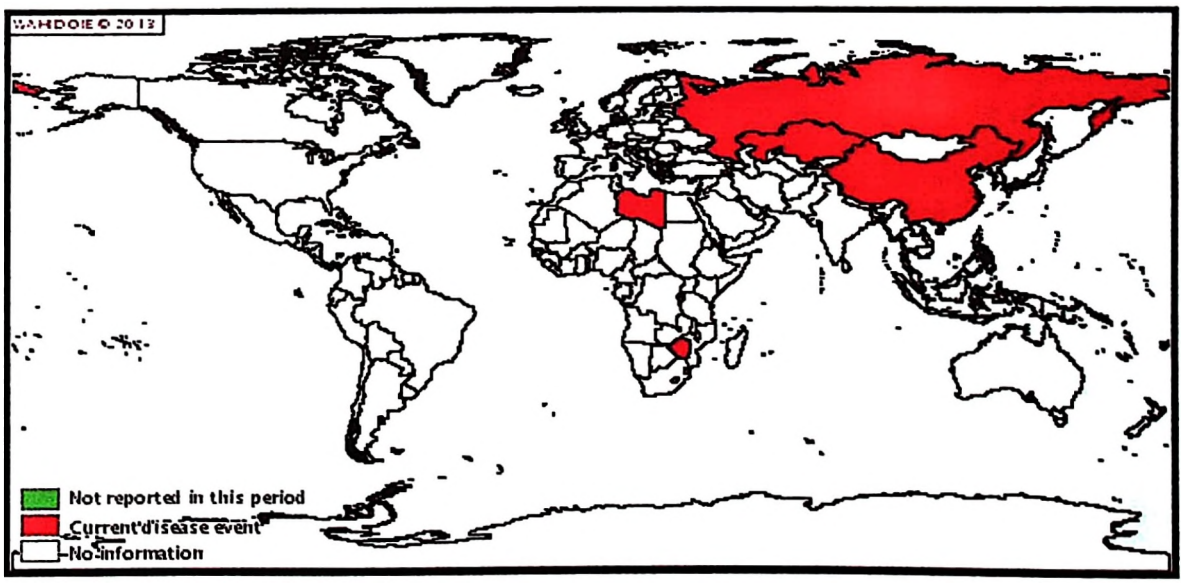

Figure 6: A map of the world showing the current FMD situation worldwide according to OIE, between January and June 2013. Source: OIE (2013).

2.9.2 FMD situation in Tanzania

Foot-and-mouth disease is endemic in Tanzania and it affects an extensive area in the country (Kivaria, 2003; Catley *et al.*, 2004), and has especially detrimental effects on pastoral and agro-pastoralists who almost entirely depend on livestock products for food and economic security (Cleaveland *et al.*, 2014). It was first documented in 1927 in Kahama District, Arusha, and was attributed to type O virus (Catley *et al.*, 2004), but first isolated in 1954 (Kasanga *et al.*, 2012), and the continuous transmission FMDV causes a number of outbreaks almost each year in different parts of the country (Catley *et al.*, 2004; Picado *et al.*, 2010; Cleaveland *et al.*, 2014). This persistent occurrence can be attributed to the uncontrolled movement of livestock within and across international borders (Kivaria, 2003; Catley *et al.*, 2004). It is also difficult to determine the exact source of outbreaks or trace the transmission of the disease over time due to the inconsistent vaccination programmes in the country (Kasanga *et al.*, 2012). Kivaria, (2003) has reported that the presence of this disease in the country is a major obstacle to the development of the national livestock industry because of its adverse effect on livestock production and the trade of animals and animal products to the lucrative export market.

Serotypes O, A, SAT-1 and SAT2 were noted in the late 1960s and SAT3 was reported in the Arusha region in 1996 (Catley *et al.*, 2004). The control of the disease therein is complicated further by the occurrence of four serotypes (SAT1, 2, O and A), and the large number of wildlife reserves with susceptible species, especially the African buffalo which is present in high numbers in the country (Catley *et al.*, 2004; Picado *et al.*, 2010). SAT 2 also occurs across the whole country while serotype A is exclusively in the Eastern coastal zone. Serotype O on the other hand is found in the Southern and Northern highlands and the lake areas (Kasanga *et al.*, 2012).

However, the predominat serotype in the eastern zone has been found to be type O, followed by SAT2 then type A (Joseph *et al.*, 2014).

Quarantine and restriction of animal movements, particularly in areas with well-defined farming systems are the control measures implemented during outbreaks in Tanzania, in order to control the spread and economic impacts of the disease (Kivaria, 2003).

Due to the occurrence of many different serotypes of FMDV circulating in Tanzania, uncertainties about which vaccines to use, their effectiveness, the role of wildlife as sources of infection, vaccination and movement control have not successfully controlled the disease (Cleaveland *et al.*, 2014). FMD surveillance is based on passive case detection and reports from the field veterinarians, and diagnosis is based on clinical signs, few samples are submitted to the laboratory for confirmation (Picado *et al.*, 2010). The government used to support vaccinations by providing vaccine but lately it is done by individual farmers but since it is relatively expensive, not all farmers afford to do it (TADs Tanzania); this leaves the FMD situation into a dire state in the country.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Tanzania is a sub-Saharan country located in East Africa, bordered by the Indian Ocean on the East, Burundi, Rwanda and the Democratic Republic of Congo on the West, Kenya and Uganda in the North, then Mozambique, Zambia and Malawi in the South. The country covers around 945 000km^2 and its climate varies from tropical to temperate (Picado *et al.*, 2010).

Tanzania has the third largest cattle population in Africa after Ethiopia and Sudan, and the livestock industry plays an important role in the economy of the country but it is limited by the occurrence of diseases such as foot-and-mouth disease (Picado *et al.*, 2010).

The tissue samples were collected from cattle in several villages of Serengeti, Simanjiro, Bunda and Ngorongoro districts as indicated in Fig 7 and 8. The areas were selected based on the reports of previous FMD outbreaks in cattle and buffalo herds as reported by veterinary investigation centres (VICs) to Tanzania Veterinary Laboratory Agency (TVLA) and Director of Veterinary Services (DVS), of the Ministry of Livestock and Fisheries Development.

Probang samples were collected from buffaloes in Mkomazi National Park, which is a livestock-wildlife interface area; therefore there is a likelihood of livestock getting in contact with wild animals in this area (Fig. 8). FMD outbreaks often occur in these areas and the samples were collected 7 months after an outbreak occurred.

Mkomazi National Park is located in North Eastern Tanzania on the Kenyan border. It is in Same District, Kilimanjaro Region. It lies 75km from Kilimanjaro and 550km from Dar es Salaam; in the northern part of Tanzania (Fig. 8).

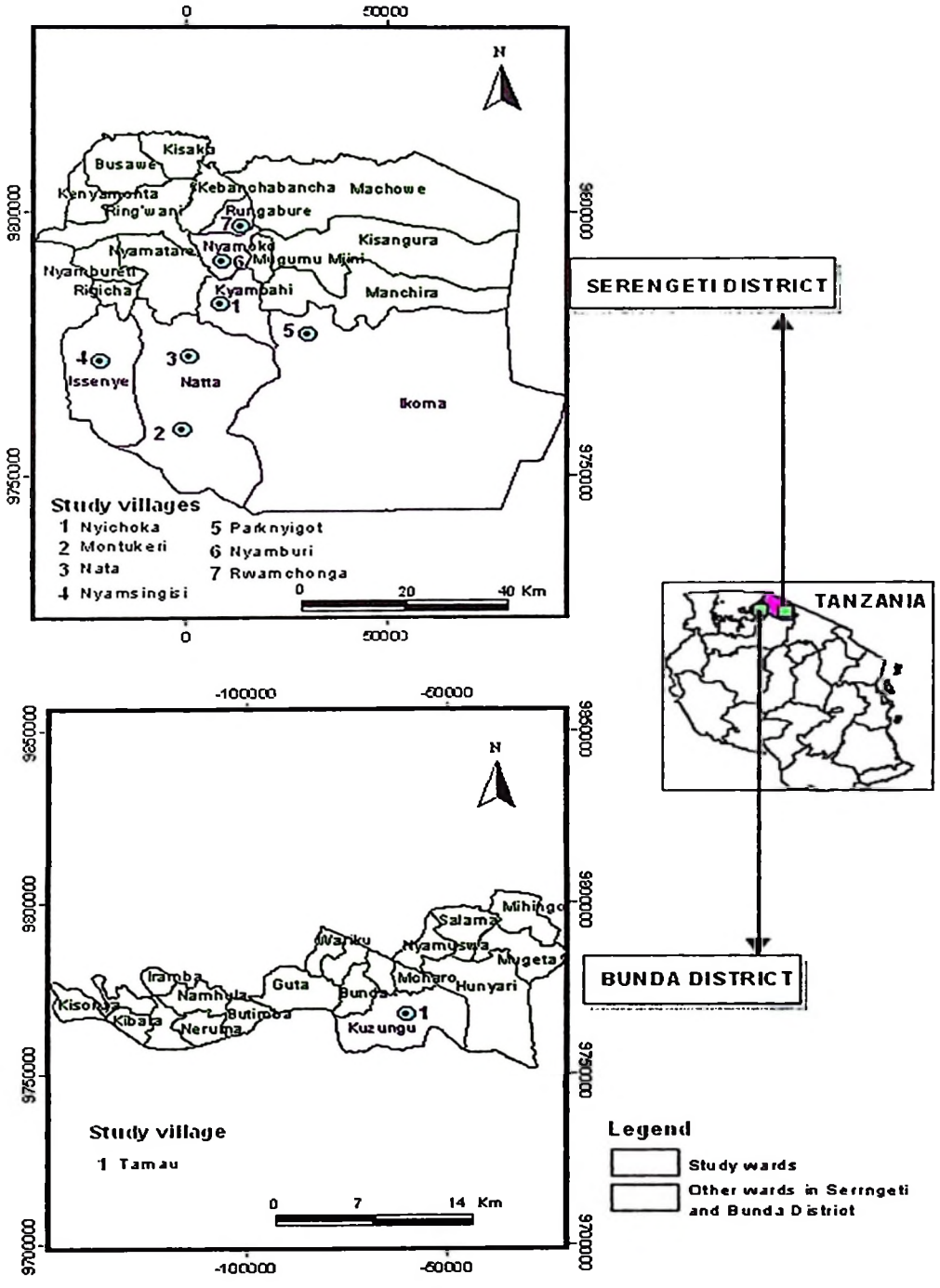

Figure 7: Maps of Serengeti district (top) and Bunda district, where epithelial tissue samples were collected.

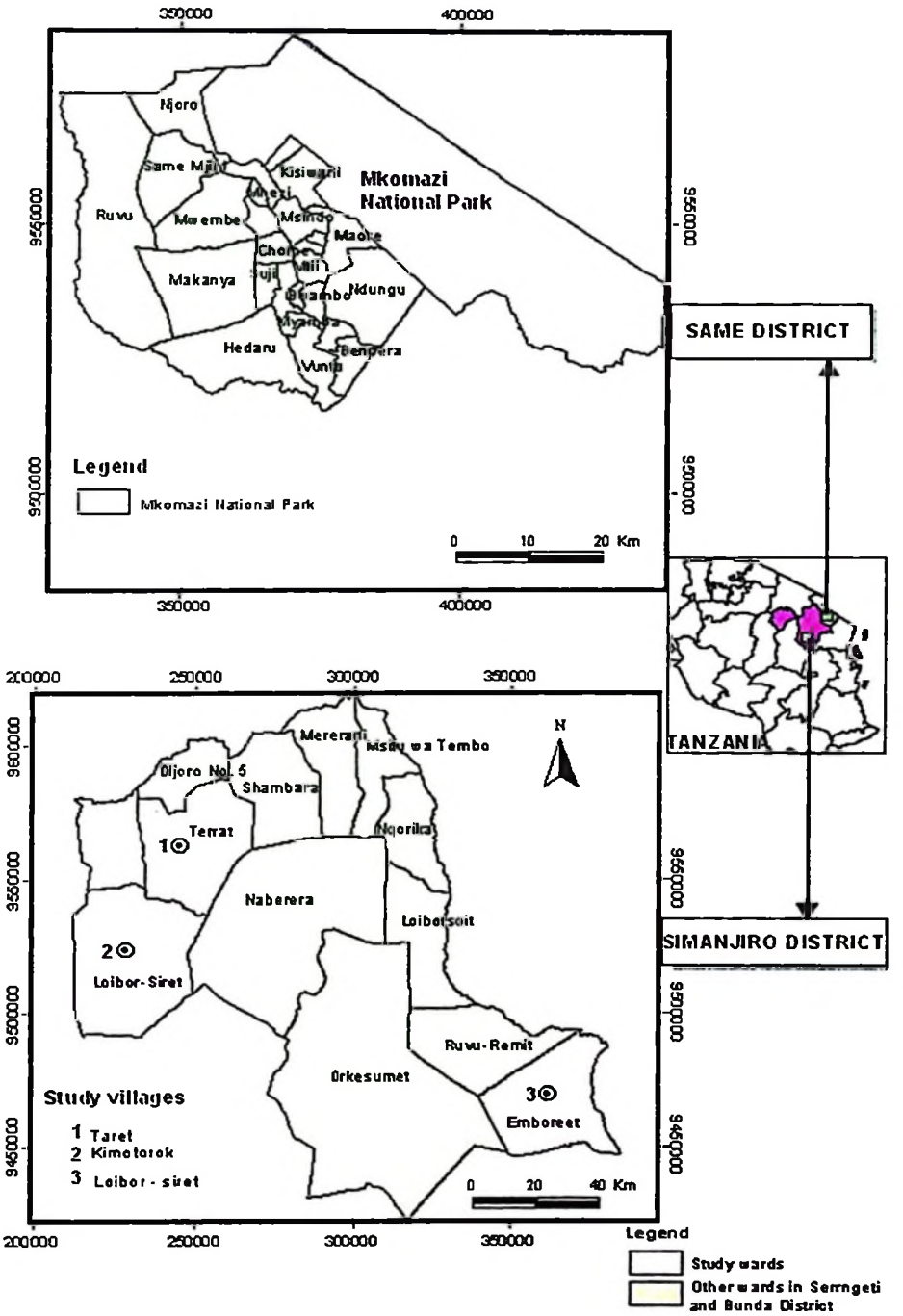

Figure 8: Maps of Same (top) and Simanjiro districts (bottom), where probang and epithelial tissue samples were collected respectively.

3.2 Study Design

This was a cross-sectional study design with purposive sampling method whereby samples were collected from areas reported to have FMD outbreaks reported by District Veterinary Officers as well as livestock field officers and farmers from districts adjacent to wildlife-protected ecosystems in the northern part of Tanzania. Lesions from the gum and foot area of cattle (n = 30), and probang samples (n = 7) were collected from buffaloes exhibiting clinical signs in the villages of interest. Tissue samples were collected between July 2011 and May 2013; while probing samples had been collected in August 2011.

3.3 Specimen Collection and Handling

3.3.1 Collection of epithelial tissues from cattle

Suspect FMD infected cattle (n = 30) were physically examined for lesions on the foot and on the mouths then restrained by ropes and nose tongs for sampling. Approximately $2cm^2$ of epithelium from unruptured or freshly ruptures lesions were cut with the aid of scissors or scapel blade and forceps. The samples were then put into cryovials which were in turn placed in liquid nitrogen immediately. Each tube was labeled using codes describing the village where it was collected, type of lesion, date collected, and the ear tag number of the animal. The samples were transported to the laboratory, where they were kept at -80°C before RNA extraction was performed.

3.3.2 Collection of probang samples from buffaloes

The buffaloes (n = 7) were located with help of a chatter plane and separated them from other dangerous animals such as lions. The buffaloes were then sedated by darts with Etorphine (M99) to immobilize them for easy sampling without injury and for animal welfare. The mouth of the animal was opened and placing a hand over the pharyngeal

area, then the probang cup inserted until it reached the esophagus. The probang cup was shoved back and forth several times until there was slight resistance when pushed forward, indicating that it had reached the cranial esophagus. The probang cup was carefully withdrawn to ensure that the oropharyngeal fluid does not get spilled. The fluid was then checked visually to ensure that there were visible cellular materials then directly poured into a sterile cryovial. If no cellular materials were seen then re-sampling was done after the throat area was washed by water. Between collections from each animal, the probangs were washed with water then dipped into a bucket containing 0.2% citric acid to disinfect them. The probangs were then washed three times with tap water before use in another animal. The cryovials were closed tightly and labeled with the biodata of the animal and the date of sampling. The vials were placed in a liquid nitrogen tank immediately after sampling.

3.3.3 Transportation of samples to the laboratory

All the samples were then transported to the Tanzania Veterinary Laboratory Agency (TVLA) following International Association of Air transport (IATA) regulations. In TVLA they were kept in a -80°C freezer until they were tested.

3.4 Tissue Preparation Prior to Testing

Epithelial tissues from feet and gum lesions were ground individually with the aid of sterile sand and pestle and mortars. The ground tissues were resuspended in minimum essential medium (MEM) with L-Glutamine and phenol red (Wako Pure Chemical Industries Ltd.), to make a 10% tissue suspension. The homogenate was then centrifuged at 8000 rpm for 5 minutes at room temperature then the supernatant was immediately used for RNA extraction, or stored at -80°C until extraction or antigen ELISA was done.

3.5 Laboratory Testing

3.5.1 Serotyping of FMDV by RT-PCR

3.5.1.1 Total RNA extraction

RNA was extracted from the samples (homogenized tissue and probang samples) and controls (the positive controls were samples previously confirmed to be positive for FMD while the negative control was nuclease free water). All samples were subjected to four treatments with serotype-specific RT-PCR using four primers, using QIAamp Viral RNA Mini extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (Appendix 2). Briefly, lysis was done using a lysis buffer followed by protein precipitation using absolute ethanol. The lysate was then passed through a Qiagen column followed by washing and cleaning of bound RNA. Afterwards, the RNA was eluted with an elution buffer, then the extracted viral RNA was stored at -80°C until amplification.

3.5.1.2 Reverse transcription polymerase chain reaction (RT-PCR)

The master mix of the reagents used per reaction was prepared using a modified protocol of AgPath-ID one-step RT-PCR kit (Applied Biosystems, Courtaboef, France) (Table 1), in one tube, then 22.5µl of the mix distributed to each PCR tube and 2.5µl of RNA added to each respective tube. The PCR was done in a GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, USA). All reactions were run along with a positive and negative control. The positive controls were RNA obtained from samples previously confirmed to be positive for FMD while the negative control was nuclease free water.

All samples were subjected to four treatments with serotype-specific RT-PCR using four primers sets for SAT1, SAT2, serotype A and O. Degenerate primers SAT-B-208R were used as reverse primers for SAT1 & 2, whereas EUR 2B52R were used for serotype A & O, as indicated in Table 2. The complete VP1 region of the genome was amplified in this PCR. The primers were supplied by Applied Biosystems, UK.

Table 1: PCR reaction mix per sample

Reagent	Volume (µl) per reaction
Nuclease free water	9
2X reaction buffer	5
10 pmol Forward primer	2.5
10 pmol Reverse primer	5
25X RT-PCR enzyme	1
RNA template	2.5
Final volume	25

3.5.1.3 Cycling conditions for the different serotypes used in the study

The PCR reaction was done at the following temperature cycle: RT reaction, 42°C for 30 minutes (1 cycle); initial denaturation, 94°C, 5 minutes (1 cycle); 35 PCR cycles for denaturation at 94°C for 60 seconds; annealing at 50°C for 60°C (for SAT 1 & 2);then a final extension at 72°C for 5 minutes. The annealing temperature for serotype A and O is 55°C and 60°C respectively.

Table 2: List of oligonucleotide primers used for RT-PCR in this study

Serotype	Name		Sequence	Amplicon size
SAT 1	SAT1-1C559F	(forward)	5'-GTGTATCAGATCACAGACACACA -3'	731bp
	SAT2-B-208R	(reverse)	5'-ACAGCGGCCATGCACGACAG-3'	
SAT 2	SAT2-1D209CF	(forward)	5'-CCACATACTACTTTTGTGACCTGGA-3'	715bp
	SAT-B-208R	(reverse)	5'-ACAGCGGCCATGCACGACAG-3'	
Serotype A	A-1C612F	(forward)	5'-TAGCGCCGGCAAAGACTTTGA-3'	800bp
	EUR-2B52R	(reverse)	5'-GACATGTCCTGCATCTGGTTGAT-3'	
Serotype O	O-1C272F	(forward)	5'-TBGCRGGNCTYGCCCAGTACTAC-3'	1132bp
	EUR-2B52R	(reverse)	5'-GACATGTCCTGCATCTGGTTGAT-3'	

Key: nucleotide B- C, G or T; N– any nucleotide C, G, T or G; Y- C or T; R – G or A.

3.5.1.4 Agarose gel electrophoresis

The PCR products were separated by agarose gel electrophoresis using 1.5% agarose gel in 1X TBE buffer (Ambion® 10X Tris-Borate-EDTA buffer, USA), stained with ethidium bromide. A 5µl PCR product was mixed with 2µl of 6X loading dye (Promega, Madison, USA), before being loaded in respective wells as well as a 1kb DNA marker (Promega, Madison, USA). Electrophoresis was run at 100 volts for 45 minutes before visualization under UV light (Sigma-Alderich, St. Louis, USA).

3.5.2 Antigen detection ELISA

A total of 30 samples (24 tissues and 6 probang) were analysed by antigen detection ELISA (IAH, Pirbright, UK), as described previously by Hamblin *et al.*, (1984), to detect and type the antigens into different serotypes (Table 4 and Appendix 3). Only thirty (30) samples were analysed by antigen ELISA due to the limitation of reagents or kits for antigen ELISA, whereas 35 samples were tested by PCR. In brief, microplates pre-coated with catching monoclonal antibodies (MAbs) and with positive and negative controls already incorporated were used. The tissue homogenate or probang samples were added to the plate and incubated at room temperature, after which pan-FMD MAb and pan-SATs MAbs pools were added, and then a substrate/chromogen was added. After incubation for colour development, the plates were read at a wavelength of 450nm in a Labsystems multiskan plate reader (MTX Lab Systems, Vienna, USA).

3.6 Data Analysis

Microsoft Excel 2007 was used to calculate the percentage positive samples per assay and the OD values were calculated as per the instructions on the kit provided by the manufacturer (Appendix 3, Table 6). Basically, the samples were assumed to be positive if the OD values were equal or greater than 0.1.

CHAPTER FOUR

4.0 RESULTS

4.1 Clinical Signs and Gross Pathology

Suspect animals showed clinical signs such as drooling saliva, lameness and lack of appetite. FMD-suspected animals revealed lesions on the mouth, gums and even on the feet. Some lesions were so severe that the animals were having difficulty walking. These are typical signs of FMD.

4.2 Detection of Foot-and-Mouth-Disease Virus by Serotype-Specific RT-PCR

A total of 35 samples (28 cattle epithelial tissues and 7 buffalo probang) from the Serengeti, Bunda, Simayirooro and Same districts in the northern part of Tanzania, were tested for FMDV by RT-PCR. Out of the 35 samples, FMDV genome was detected in 12 samples (34.3%) using 4 sets of serotype specific primers targeting the VP1 gene. Of the 12 samples, 9 (75%) tested positive for at least one of the four serotypes (SAT2 = 5 and serotype A = 4), while 3(25%) had cross reactions, reacting positive to both serotypes (SAT2 and type A), therefore not classified. No samples tested positive for SAT 1 and serotype O (0%) with this assay. RT-PCR did not detect FMDV genome in any of the probang samples. This is all evident in Tables 4, 5 and appendix 4.

Samples which tested positive gave an amplicon size of approximately 800bp and 715bp for serotype A, and SAT2 respectively (Fig. 9 and 10). All the negative control wells were clean, without any evidence of cross-contamination in the RT-PCR reactions.

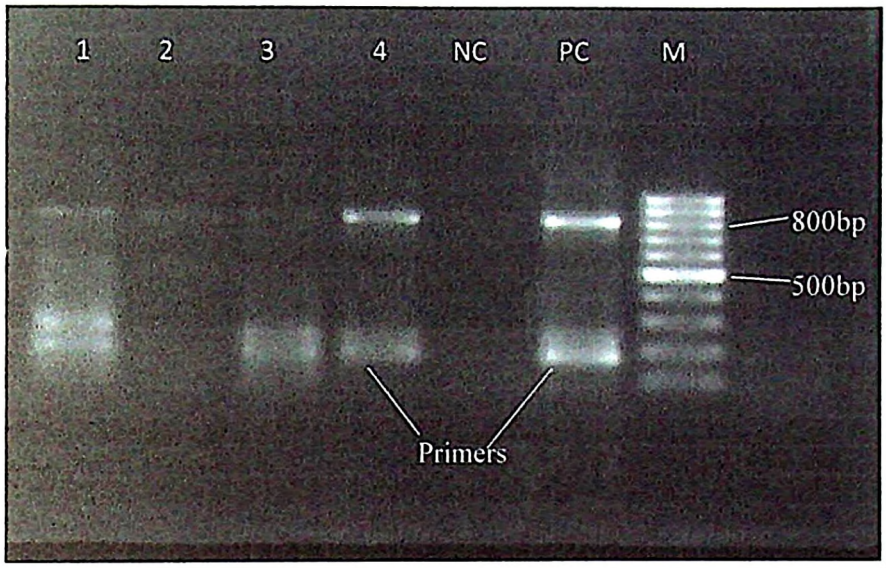

Figure 9: Serotype A RT-PCR products visualized under the UV light, where well 1-4 are samples, NC is negative control (nuclease free water) and PC is positive control (A/TAN/4/2009) and M is the 100bp DNA marker.

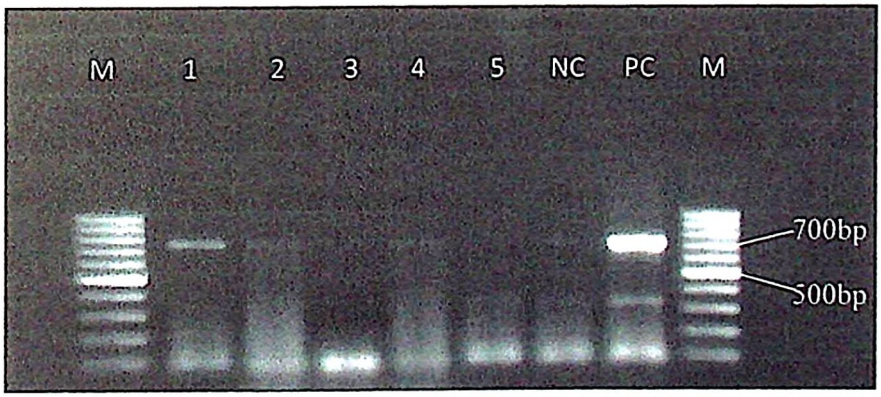

Figure 10: SAT2 RT-PCR products visualized under the UV light, where well 1-5 are samples, NC is negative control (Nuclease free water) and PC is positive control (SAT2/TAN/8/2011) and M is the 100bp DNA marker.

4.3 Detection of Antigens by Antigen ELISA

Thirty samples (24 cattle tissues and 6 buffalo probang samples) were screened for FMD antigens by Ag-ELISA. Out of these 30 samples, 20 (66.7%) samples were positive for FMDV antigens while 10 (33.3%) were negative. Out of the 20 positive samples, 10

(50%) were positive without any cross reactions (SAT1 = 3, SAT2 = 4, serotype A = 2 and serotype O = 1). This is depicted in Table 6. The other 10 samples (50%) reacted with more than one serotype. Only 1 tissue sample reacted to all the four serotypes, with serotype A having the strongest OD value above 1.5. In the 6 probang samples, only 2 samples were positive for FMD antigens: 1 reacted to all the four serotypes and another to three of the serotypes. Appendix 5 shows the details of the results obtained, while appendix 6 details all the samples in the study.

Table 3: Distribution of OD values per serotype. OD values greater than 0.1 indicate a positive result

	SAT1	SAT2`	TYPE A	TYPE O	OD Range	Mixed infections
Positive	18	17	23	26	<0.1	N/A
Negative	3	4	2	1	≥ 0.1	10

Table 4: The serotypes detected by RT-PCR and antigen ELISA in different districts

					SEROTYPE	
Lab ID	Village	District	species	sample type	RT-PCR	Ag-ELISA
1	Rwamchanga	Serengeti	Cattle	Epithelial tissue	SAT 2	SAT1, A
2	Kimotorok	Simayiro	Cattle	Epithelial tissue	SAT 2	SAT 1
3	Nyichoka	Serengeti	Cattle	Epithelial tissue	SAT 2	None
4	Parknyigot	Serengeti	Cattle	Epithelial tissue	NEG	None
5	Nyichoka	Serengeti	Cattle	Epithelial tissue	SAT 2, A	SAT2
6	Nyamburi	Serengeti	Cattle	Epithelial tissue	None	A
7	Terat	Simayiro	Cattle	Epithelial tissue	None	Not tested
8	Nata	Serengeti	Cattle	Epithelial tissue	None	SAT1, 2
9	Tamau	Bunda	Cattle	Epithelial tissue	SAT2, A	SAT1, 2
10	Nyamisingisi	Serengeti	Cattle	Epithelial tissue	None	SAT1, 2
11	Montukeri	Serengeti	Cattle	Epithelial tissue	None	None
12	Terat	Simayiro	Cattle	Epithelial tissue	SAT2, A	SAT2
13	Tamau	Bunda	Cattle	Epithelial tissue	None	None
14	Nata	Serengeti	Cattle	Epithelial tissue	None	None
15	Rwamchanga	Serengeti	Cattle	Epithelial tissue	SAT2	SAT 1, 2
16	Terat	Simayiro	Cattle	Epithelial tissue	A	A
17	Terat	Simayiro	Cattle	Epithelial tissue	None	SAT 2
18	Terat	Simayiro	Cattle	Epithelial tissue	A	A, O
19	Nyichoka	Serengeti	Cattle	Epithelial tissue	None	SAT2
20	Terat	Simayiro	Cattle	Epithelial tissue	None	Not tested
21	Loiborsire	Simayiro	Cattle	Epithelial tissue	SAT2	SAT2
22	Terat	Simayiro	Cattle	Epithelial tissue	A	SAT1,2
23	Parknyigot	Serengeti	Cattle	Epithelial tissue	None	SAT1
24	Nyichoka	Serengeti	Cattle	Epithelial tissue	None	None
25	Nata	Serengeti	Cattle	Epithelial tissue	None	None
26	Terat	Simayiro	Cattle	Epithelial tissue	A	SAT1,2
27	Terat	Simayiro	Cattle	Epithelial tissue	A	SAT1,2,A,O
28	Nyamburi	Serengeti	Cattle	Epithelial tissue	None	Not tested
7	Mkomazi	Same	buffalo	Probing	None	SAT1,2,A,O
13	Mkomazi	Same	buffalo	Probing	None	SAT1,2,A,O
23	Mkomazi	Same	buffalo	Probing	None	None
31	Mkomazi	Same	buffalo	Probing	None	Not tested
34	Mkomazi	Same	buffalo	Probing	None	None
35	Mkomazi	Same	buffalo	Probing	None	None
36	Mkomazi	Same	buffalo	Probing	None	None

It is evident also that all the serotypes SAT1, SAT2, type A and O were detected in Simanyiro and Same district; three (SAT1, SAT2, and type A) detected in Serengeti but only SAT1, SAT 2 and type A were found in Bunda district.

In Serengeti and Simayiro districts the most popular serotype was found to be SAT 2 having been detected in 8 and 6 samples respectively, in these two districts. In same district only two samples tested positive for FMDV and all the serotypes were present in the two samples. Bunda district on the other hand had one sample testing positive for serotype A, SAT1 and SAT2. This is evident in Table 5.

Table 5: Serotypes per district

District	Detected serotypes	Number of samples per serotype
Serengeti	SAT1, SAT2, type A	SAT1: 8
		SAT2: 5
		Type A: 3
Simayiro	SAT1, SAT2, type A, O	SAT1: 8
		SAT2: 5
		Type A: 3
		O: 2
Bunda	SAT1, SAT2, type A	All detected in 1 sample
Same	SAT1, SAT2, A,O	all serotypes in 2 animals

Table 6: A comparison of the results generated by RT-PCR and antigen ELISA

Assay	Sample size	Total positive	SAT 1	SAT 2	Type A	Type O	cross reactions	positive probang
ELISA	30	20	3(15 %)	4(20%)	2(10%)	1(5%)	10(50%)	2
RT-PCR	35	12	0(0%)	5 (41.7%)	4(33.3%)	0(0%)	3(15%)	0

CHAPTER FIVE

5.0 DISCUSSION

FMD is highly contagious and causes devastating economic losses in a country because of the negative effects on livestock production and international restrictions on animals and animal products (Bai *et al.*, 2011). It is endemic in Tanzania and has been causing recurrent disease outbreaks throughout the country over several years (Picado *et al.*, 2011; Kasanga *et al.*, 2012). The disease has a great impact on the economy of the country's livestock sector, which is one of the main economic activities in the country (Picado *et al.*, 2010). Therefore, rapid and accurate diagnosis is of paramount importance in the implementation of effective control measures to curb the spread of the disease (Saeed *et al.*, 2009; Jeirani *et al.*, 2012). Not only does the confirmation of infection in an area provide scientific basis for first hand information for appropriate control measures, but also for disease eradication and attempting FMD free status following an outbreak (Ma *et al.*, 2011). For the effective control of the disease, outbreaks should also be detected at an early stage and persistent infections should also be recognized to prevent further transmittance (Alamdari *et al.*, 2006; Longjam *et al.*, 2011). However, the epidemiology of the disease in this country is rather complicated due to the occurrence of multiple serotypes (Picado *et al.*, 2010), the presence of a large number of wildlife serving as reservoirs of susceptible species, especially the African buffalo, movement of animals within and across international borders.

The early detection of the circulating serotypes could shed light into the type of vaccines which should be administered in the shortest time, to the animals in the areas affected by those serotypes FMD. This is also important because early and decisive reaction is required in order to contain and eventually eliminate the disease without serious socio-economic consequences (Hassanein *et al.*, 2011).

The clinical signs which were observed in cattle in this study are highly suggestive of FMD, characterized by lesions on the feet and tongue, excessive drooling of saliva and inability to walk. These are common features often observed for FMD (Pharo, 2002; Remond *et al.*, 2002; Nakker and Sharma, 2008). However, since diseases such as vesicular stomatitis and swine vesicular disease produce lesions similar to FMD, there was a need to confirm the diagnosis by laboratory methods (Kasanga *et al.*, 2014). In this case it was done by RT-PCR and antigen ELISA as supported by Nakker and Sharma (2008), who have reported that the detection of FMD antigen and nucleic acids in affected tissues of suspect animals is sufficient for its diagnosis.

In this study, four serotypes (A, O, SAT 1 and SAT 2) were detected in the northern parts of Tanzania. Owing to the multiple serotypes of the disease however, the serotypes affecting any one region must be identified in order to select the most appropriate antigens to include in the vaccine preparation (Reid *et al.*, 1999; Cooke and Westover, 2008; Soleimanjahi *et al.*, 2013). The identification of the four serotypes in northern parts of Tanzania provides valuable information required for rational control of FMD in the region.

Although RT-PCR is considered highly sensitive and specific, and able to detect the virus at low concentrations in tissue samples without the need for virus isolation in tissue culture (Suryanarayana *et al.*, 1999; Waheed *et al.*, 2009), in this case the assay was not able to confirm SAT 1 and serotype O in any of the samples yet the antigens to the virus were detected by Ag-ELISA. This could have been associated with the primer design for this particular serotype, hence there is need to use alternative primers in future.

Some discrepancies were found in the results generated by the two assays. RT-PCR did not detect any FMDV genome in any probang (oesophageal-pharyngeal scraping) sample,

whereas antigen ELISA test detected all the serotypes (A, O, SAT 1 and SAT 2) in only two probang samples tested (Table 5). This is in agreement with previous findings which support that the virus is intermittently detectable in probang samples and the titre is usually low and also decline over time (Moonen *et al.*, 2004). Therefore detecting the virus in probang samples is not always an easy task to accomplish. The cross-reactions among serotypes (SATs) could have possibly contributed to the discrepancies observed since in ELISA these detected serotypes indicate possible multiple infections in the two samples. Another possibility is that the test assays have different sensitivity.

It is not surprising that antigen ELISA was able to detect all serotypes in the clinical samples, yet only two were confirmed by RT-PCR (Table 5). A similar study by Knowles *et al.*, (2007), also found that initial testing of clinical samples suggested multiple FMDV serotypes may have been involved in an outbreak, although only serotype A was later confirmed. RT-PCR does not always detect viral RNA especially in cases where the RNA has undergone degradation since RNA viruses have no nucleocapsid protein to protect the RNA from putrefaction in a poor quality sample, therefore if the RNA is degraded it is difficult to detect the genome by PCR but can be detected by ELISA since ELISA only detects the antigen (Reid *et al.*, 1998). Therefore it is possible that the RNA in the samples could have undergone degradation hence inability for RT-PCR to detect the FMDV genome.

RT-PCR had fewer cross-reactions (3) as compared to Ag-ELISA, which had 10 cross-reactions (Table 5). These are considered as unidentified FMDV strains or a possibility of multiple infections with several FMDV serotype in some animals. It is possible that an animal had been infected by one strain of FMD, and then it recovered but remained a carrier of that particular serotype before it got infected by another strain or serotype since

recovery from infection, or protective vaccination with one serotype will not protect against subsequent infection with another (Alexandersen *et al.*, 2003). The high diversity of FMDV due to high mutation leads to quasi-species and recombination demonstrated by Waheed *et al.*, (2003), probably contribute to the inability to identify the viruses serotypically in this case. This phenomenon of mixed serotype FMDV infections is not new since previous studies by Hedger (1972) demonstrated the possibility of isolating SAT1, SAT2 and SAT3 from African buffalo in Botswana. However, this is a rare occurrence and it has never been documented in Tanzania but it has been reported in the neighbor country Kenya (Sangula *et al.*, 2005). Reports by Woodbury *et al*, (1994), also indicate the presence of mixed serotype infections in cattle, in Saudi Arabia.

The presence of SAT2 in cattle in the region (Same, Serengeti and Simanjiro districts) of study could possibly be attributed to the presence of buffaloes in the Serengeti National Park, which are believed to be the reservoirs of this particular serotype. Buffaloes are often associated with transmitting FMDV serotype 2 to domestic animal because the national parks are not fenced therefore livestock can graze in close proximity with the wild animals. Even so, persistently infected cattle and sheep can still become sources of new outbreaks even though African buffaloes have been proved to be able to transmit FMDV (Sun *et al.*, 2004).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

According to the findings of this study, it can be concluded that four serotypes (SAT1, SAT2, type A and type O), were present and co-circulated in four districts namely Serengeti, Bunda, Simanjiro and Same in the Northern part of Tanzania. The outbreaks could be due to mixed or multiple infections in some animals since both assays (RT-PCR and Ag-ELISA) have detected multiple cross-reactions of serotypes in some samples. Antigens to SAT2 and serotype A have been detected by Ag-ELISA and confirmed by RT-PCR but SAT 1 and type O were detected by ELISA. Therefore both methods can be used for the primary diagnosis of FMD.

6.2 Recommendations

i. Confirmation of infections using more sensitive methods like PCR should be done after ELISA screening in order to confirm true FMD serotypes in any suspect outbreaks, but alternative primers must be considered especially for detecting SAT1.

ii. These tests however are only used to confirm the clinical diagnosis and identify the FMDV serotypes but they cannot determine the possible source of infection in an outbreak therefore further studies need to be carried out on a larger sample size in future, and the RT-PCR can be coupled with sequence analysis to determine genetic correlations between field isolates and help molecular epidemiology. Phylogenetic analysis of the virus isolates obtained study area need to be done to ascertain the relatedness and possible source of infection.

iii. Frequent serological screening of the animals in the FMD prone areas is essential to determine the carriers of the virus this should be continually conducted in the region(s).

iv. Future studies can concentrate on studying the possibility of mixed infections in animals from this region.

v. Vaccines used in this area should contain inactivated antigens corresponding to the detected serotypes in this study. There is also need to conduct vaccine matching studies with circulating FMDV in the areas of concern.

REFERENCES

Abubakar, M., Kanwal, S. and Saeed, A. (2012). Persistence, emergence and distribution of foot and mouth disease virus (FMDV); global and Pakistan perspectives. *Pakistan Journal of Life and Social Sciences* 10(2): 84 – 90.

Alamdari, M., Ghorashi, S. A., Ahmadi, M. and Salehi-Tabar, R. (2006). Detection of foot-and-mouth disease virus and identification of serotypes in East Azerbaijan province of Iran. *Veterinarski Archiv* 76(5): 413 – 419.

Alexandersen, S., Zhang, Z., Donaldson, A. I. and Garland, A. J. M. (2003). The pathogenesis of foot-and-mouth disease. *Journal of Comparative Pathology* 129: 1 – 36.

Armstrong, R., Davie, J. and Hedger, R. S. (1967). Foot-and-mouth disease in man. *British Medical Journal* 4(78): 529 – 530.

Bachrach, H. L. (1968). Foot-and-mouth disease. *Annual Review of Microbiology* 22: 201 – 244.

Bai, X., Li, P., Bao, H., Liu, Z., Li, D., Lu, Z., Cao, Y., Shang, Y., Shao, J., Chang, H., Luo, J. and Liu, X. (2011). Evolution and molecular epidemiology of foot-and-mouth disease virus in China. *Chinese Science Bulletin* 56(21): 2191 – 2201.

Barlič-Maganja, D., Grom, J., Toplak, I. and Hostnik, P. (2004). Detection of foot-and-mouth disease virus by RT-PCR and microplate hybridization assay using inactivated viral pathogens. *Veterinary Research Communications* 28(2): 149 – 158.

Bastos, A. D. S., Haydon, D. T., Sangaré, O., Boshoff, C. I., Edrich, J. L. and Thomson, G. R. (2003). The implications of virus diversity within the SAT2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. *Journal of General Virology* 84: 1595 – 1606.

Bronsvoort, B. M. de C., Radford, A. D., Tanya, V. N., Nfon, C., Kitching, R. P. and Morgan, K. L. (2004). Molecular epidemiology of foot-and-mouth disease virus in the Adamawa Province of Cameroon. *Journal of Clinical Microbiology* 42(5): 2186 – 2196.

Brownlie, J. (2012). A foresight vision for infectious diseases in Africa. *Ondestepoort Journal of Veterinary Research* 79(2): 8 – 61.

Carpenter, T. E., O'Brien, J. M., Hagerman, A. D. and McCarl, B. A. (2011). Epidemic and economic impacts of delayed detection of foot-and-mouth disease: a case study of a simulated outbreak in California. *Journal of Veterinary Diagnostic Investigation* 23: 26 – 33.

Catley, A., Chibunda, R. T., Ranga, E., Makungu, S., Magayane, F. T., Magoma, G., Madege, M. J. and Vosloo, W. (2004). Participatory diagnosis of a heat-intolerance syndrome in cattle in Tanzania and association with foot-and-mouth disease. *Preventive Veterinary Medicine* 65: 17 – 30.

Chen, H., Zhang, J., Liu, Y. and Liu, X. (2011a). Rapid typing of foot-and-mouth disease serotype Asia 1 by reverse transcription loop-mediated isothermal amplification. *Virology Journal* 8: 489, 13pp.

Chen, H., Zhang, J., Liu, Y. and Liu, X. (2011b). Detection of foot-and-mouth disease virus RNA by reverse transcription loop-mediated isothermal amplification. *Virology Journal* 8: 510, 3pp.

Chen, H. and Liu, Y. (2013). Immunity of foot-and-mouth disease serotype Asia I by sublingual vaccination. *PLoS ONE* 8(5).

Clavijo, A. and Kitching, P. (2003). The nature and diagnosis of foot-and-mouth disease. *Clinical Microbiology Newsletter* 25(11): 81 – 88.

Cleaveland, S., Fyumagwa, R., Haydon, D., Kazwala, R., King, D., Perry, B. D., Reeve, R., and Yongolo, M. (2014). Towards the strategic control of endemic foot-and-mouth disease in Africa: new techniques for a neglected problem. [http://www.bbsrc.ac.uk] site visited on 15/09/2014.

Cooke J. N. and Westover, K. M. (2008). Serotype-specific differences in antigenic regions of foot-and-mouth disease virus (FMDV): A comprehensive statistical analysis. *Infection, Genetics and Evolution* 8: 855 – 863.

Curry, S., Abrams, C. C., Fry, E., Crowther, J. C., Belsham, G. J., Stuart, D. I. and King, A. M. (1995). Viral RNA modulates the acid sensitivity of foot-and-mouth disease virus capsids. *Journal of Virology* 69(1): 430 – 438.

Defra (Department for Environment, Food and Rural Affairs). (2005). Archive: Foot-and-mouth disease. [http://archive.defra.gov.uk/foodfarm/farmanim al/diseases/atoz/ fmd/about/clinical-signs/pics-cattle.htm] site visited on 23/07/2013.

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

Ding, Y., Liu, Y., Zhou, J., Chen, H., Wei, G, Ma, L. and Zhang, J. (2011). A highly sensitive detection of foot-and-mouth disease virus by gold nanopariticle improved immuno-PCR. *Virology Journal* 8: 148, 5pp.

Doel, T. R., Williams, L. and Barnett, P. V. (1994). Emergency vaccination against foot-and-mouth disease: rate of development of immunity and its implications for the carrier state. *Vaccine* 12(7): 592 – 600.

Donaldson, A. I., Gibson, C. F., Oliver, R., Hamblin, C. and Kitching, R. P. (1987). Infection of cattle by airborne foot-and-mouth disease virus: minimal doses with O1 and SAT 2 strains. *Research in Veterinary Science* 43: 339 – 346.

El-Bagoury, G. F., El-Sayed, W. M., El-Nahas, E. M., El-Habbaa, A. S. and Azab, A. H. (2011). Sequence analysis of VP1 gene foot-and-mouth disease virus serotype A and isolates from different governorates in Egypt. *Benha Veterinary Medical Journal* I: 122 – 128.

El-Kholy, A. A., Soliman, H. M. T., Helmy, N. A. and Rahman, A. O. (2006). Genetic identification of the foot-and-mouth disease virus caused 2006 outbreak in Egypt. *Arab Journal of Biotechnology* 10(1): 193 – 206.

El-Shchawy, L., Abu-Elnaga, H., Abdel, A. M., Fawzy, H., Al-Watany, H. and Azab, A. (2011). Laboratory diagnosis of Foot and Mouth Disease using real-time RT-PCR in Egypt. *Life Science Journal* 8(3): 384 – 387.

El-Shehawy, L., Azab, A. M. H., Mossad, W., El-Sayed, E., Ismail, A. and Deghady, W. (2012). Real-time RT-PCR assay for detection of different serotypes of FMDN in Egypt. *Veterinary World* 5(12): 732- 737.

EMPRES WATCH-FAO (2012). Foot-and-mouth disease caused by serotype SAT2 in Egypt and Lybia: A regional concern for animal health in North Africa and the Middle East. [http://www.fao.org/ag/againfo/programmes/en/empres/diseases.asp] site visitcd on 14/06/2013.

FAO/OIE, Impact of foot-and-mouth disease, supporting document 1. (2013) [http://www.oie.int/doc/ged/D11888.PDF] site visited on 17/07/2013.

Fasina, F. O., Connell, D. R., Talabi, O. A., Lazarus, D. D., Adeleke, G. A., Olusanya, T. P. and Hernandez, J. A. (2012). Foot-and-mouth-disease (FMD) virus strains and examination of exposure factors associated with FMD seropositivity of cattle herds in Nigeria during 2007-2009. *Preventive Veterinary Medicine* 109: 334 - 342.

Grubman, M. J. and Baxt, B. (2004). Foot-and-mouth disease. *Clinical Microbiology Reviews* 17: 465 - 493.

Grubman, M. J. (2005). Development of novel strategies to control foot-and-mouth disease: Marker vaccines and antivirals. *Biologicals* 33: 227 - 234.

Habiela, M., Alamin, M. A. G., Raouf, Y. A. and Ali, Y. H. (2010). Epizootiological study of foot-and-mouth disease in the Sudan: the situation after two decades. *Veterinarski Archives* 80(1): 11 - 26.

Hamblin, C., Armstrong, R. M. and Hedger, R. S. (1984). A rapid enzyme-linked immunosorbent assay for the detection of foot-and-mouth disease viruses in epithelial tissues. *Veterinary Microbiology* 9: 435 - 443.

Hassanein, S. A., El-Wahab, W. A., Eweis, M. and Mahmoud, M. M. (2011). Serodiagnosis of foot-and-mouth disease (FMD) virus for differentiation between naturally infected and vaccinated cattle and buffaloes. *International Journal of Virology* 7(4): 198 – 203.

Hedger, R. S. (1972). Foot-and-mouth disease and the African buffalo (Syncerus cafer). *Journal of Comparative Pathology* 82: 19 – 28.

Hema, M., Chandran, D., Nagendrakumar, S. B., Madhanmohan, M. and Srinivasan, V. A. (2009). Construction of an infectious cDNA of foot-and-mouth disease virus type O_1BFS 1860 and its use in the preparation of candidate vaccine. *Journal of Biosciences* 34(1): 45 - 58.

Hemadri, D., Tosh, C., Sanyal, A. and Venkataramanan, R. (2002). Emergence of a new type O foot-and-mouth disease virus: its phylogenetic and evolutionary relationship with the PanAsia pandemic strain. *Virus genes* 25(1): 23 – 34.

Hui, R. K. (2004). Molecular epidemiology of and vaccine development against foot-and-mouth disease virus in Hong Kong, Hong Kong. Thesis for Award of PhD at University of Hong Kong, 140pp.

Jackson, A. L., O'Neill, H., Maree, F., Blignaut, B., Carrillo, C., Rodriguez, L. and Haydon, D. T. (2007). Mosaic structure of foot-and-mouth disease virus genomes. *Journal of General Virology* 88: 487 - 492.

Jeirani, F., Ahmadi, A., Azimi, M. and Mahravani, H. (2012). Rapid and accurate diagnosis of foot-and-mouth disease virus by real-time PCR in field samples. *Archives of Razi Institute* 67(2): 101 - 107.

Joseph, J., Kasanga, C.J., Yongolo, M., Mpelumbe-Ngeleja, C., Sallu, R., Mkama, M., and Masambu, J. (2014). Spatial and temporal distribution of foot-and-mouth disease virus in the eastern zone of Tanzania, *Onderstepoort Journal of Veterinary Research* 81(2), Art. #738, 1 page. [http:// dx.doi.org/10.4102/ojvr. v81i2.738] site visited on 2/09/2014.

Kamolsiripichaiporn, S., Subharat, S., Udon, R., Thongtha, P. and Nuanualsuwan, S. (2007). Thermal inactivation of foot-and-mouth disease viruses in suspension. *Applied and Environmental Microbiology* 73(22): 7177 - 7184.

Kasanga, C. J., Sallu, R., Kivaria, F., Mkama, M., Masambu, J., Yongolo, M., Das, S., Mpelumbe-Ngeleja, C., Wambura, P. N., King, D. P. and Rweyemamu, M. M. (2012). Foot-and-mouth disease virus serotypes detected in Tanzania from 2003 to 2010: Conjecture status and future prospects. *Journal of Veterinary Research* 79(2), Article no 462, 4 pp. [http://dx.doi.org/10.4102/ojvr.v79i2.462.] site visited on 15/07/2013.

Kasanga, C. J., Yamazaki, W., Mioulet, V., King, D. P., Mulumba, M., Ranga, E. Deve, J., Mundia, C., Chikungwa, P., Joao, L., Wambura, P. N., and Rweyemamu, M. M. (2014). Rapid, sensitive and effective diagnostic tools for foot-and-mouth disease virus in Africa, *Onderstepoort Journal of Veterinary Research* 81(2) Article no 727, 5pp. [http://dx.doi.org/10.4102/ojvr.v81i2.727] site visited on 12/09/2014.

King, D. P., Ferris, N. P., Shaw, A. E., Reid, S. M., Hutchings, G. H., Giuffre, A. C., Robida, J. M., Callaham, J. D., Nelson, W. M. and Beckham, T. R. (2006). Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two indcpendent real-time reverse transcription-polymerase chain reaction assays. *Journal of Veterinary Diagnostic Investigation* 18: 93 – 97.

Kitching, R. P. (2002). Clinical variation in foot and mouth disease: cattle. *Revue Scientifique et Technique de l'office international des Epizooties* 21(3): 499 - 504.

Kiravia, F. M. (2003). Foot-and-mouth disease in Tanzania: an overview of its national status. *Veterinary Quarterly* 25(2): 72 - 78.

Klein, J. (2009). Understanding the molecular epidemiology of foot-and-mouth disease virus. *Infection, Genetics and Evolution* 9: 153 - 161.

Knowles, N. J., Wadsworth, J., Reid, S. M., Swabey, K. G., El-Kholy, A. A., El-Rahman, A. O. A., Soliman, H. M., Ebert, K., Ferris, N. P., Hutchings, G. H., Statham, R. J., King, D. P. and Paton, D. J. (2007). Foot-and-mouth disease virus serotype A in Egypt. *Emergency Infectious Disease* 13(10): 1593 - 1596.

Lau, L-T., Reid, S., King, D. P., Lau, A. M-F., Shaw, A. E., Ferris, N. P. and Yu, A. C-H. (2008). Detection of foot-and-mouth disease by nucleic acid sequence-based amplification (NASBA). *Veterinary Microbiology* 126: 101 - 110.

Li-na, M., Jie, Z., Hao-tai, C., Jian-hua, Z., Yao-zhong, D. & Yong-sheng, L. (2011). 'An overview on ELISA techniques for FMD', *Virology Journal* 8, 419. [http://dx.doi.org/10.1186/1743-422X-8-419] site visited on 5/09/2013.

Longjam, N., Deb, R., Sarmah, A. K., Tayo, T., Awachat, V. B. and Saxena, V. K. (2011). A brief review on diagnosis of foot-and-mouth disease of livestock: conventional to molecular tools. *Veterinary Medicine International* 2011, article ID905768, 17 pp.

Ma, L., Zhang, J., Chen, H., Zhou, J., Ding, Y. and Liu, Y. (2011). An overview on ELISA techniques for FMD. *Virology Journal* 8(419), 9pp.

Mahy, B. W. J. (2005). Introduction and history of foot-and-mouth disease virus. *Current Topics in Microbiology and Immunology* 288: 1 - 8.

Maree, F. F., Blignaut, B., Aschenbrenner, L., Burrage, T. and Rieder, E. (2011). Analysis of SAT1 type foot-and-mouth disease virus capsid proteins: influence of receptor usage on the properties of virus particles. *Virus Research* 155: 462 - 472.

Mateo, R., Luna, E., Rincón, V. and Mateo, M. G. (2008). Engineering viable foot-and-mouth diasease viruses with increased thermostability as a step in the development of improved vaccines. *Journal of Virology* 82(24): 12232 - 12240.

Mooncn, P., Jacobs, L., Crienen, A. and Dekker, A. (2004). Detection of foot-and-mouth disease virus among vaccinated cattle. *Veterinary Microbiology* 103: 151 - 160.

Morioka, K., Fukai, K., Yoshida, K., Yamazoe, R., Onozato, H., Ohashi, S., Tsuda, T. and Sakamoto, K. (2009). Foot-and-mouth disease virus antigen detection enzyme-linked immunosorbent assay using multiserotype-reactive monoclonal antibodies. *Journal of Clinical Microbiology* 47(11): 3663 - 3668.

Mwiine, F. N., Ayebazibwe, C., Alexandersen, S., Olaho-Mukani, W., Ademun, A. R. and Tjornehoj, K. (2010). Sero-epidemiological investigation of foot-and-mouth disease virus serotypes in cattle around Lake Mburo National Parkin South-Western Uganda. *Journal of Veterinary Medicine and Animal Health* 2(4): 46 - 54.

Nakker, N, K. and Sharma, R. (2008). Retrospective diagnosis of foot-and-mouth disease outbreaks by liquid phase blocking enzyme linked immunosorbent assay. *Haryana Veterinarian* 47: 28 – 31.

Namatovu, A., Wekesa, S. N., Tjørnehøj, K., Dhikusooka, M. T., Muwanika, V., Siegsmund, H. R. and Ayebazibwe, C. (2013). Laboratory capacity for diagnosis of foot-and-mouth disease in Eastern Africa: implications for the progressive control pathway. *BMC Veterinary Research* 9(19), 11pp.

OIE/FAO (2012). Foot-and-mouth disease situation Monthly report, October 2012. [www.fao.org/fileadmin/user_upload/eufmd/docs/FMD_monthly_reports/October_2012] site visited on 13/06/2013.

OIE. (2012). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, (2012). Foot and Mouth Disease, Chapter 2.1.5. [http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/] Site visited on 7/05/2013.

Paixáo, T. A., Neta, A. V. C., Paiva, N. O., Reis, J. R., Barbosa, M. S., Serra, C. V., Silva, R. R., Beckham, T. R., Martin, B. M., Clarke, N. P., Adams, L. G. and Santos, R. L. (2008). Diagnosis of foot-and-mouth disease by real time reverse transcription polymerase chain reaction under field conditions in Brazil. *BMC Veterinary Research* 4:53, 6pp.

Paprocka, G. and Fitzner, A. (2013). Global occurrence of foot-and-mouth disease in 2010, 2011 and 2012. *Medyciyna Weterynaryjna* 69(1): 16 - 20.

Parida, S., Cox, S. J., Reid, S. M., Hamblin, P., Barnett, P. V., Inoue, T., Anderson, J. and Paton, D. J. (2005). The application of new techniques to the improved detection of persistently infected cattle after vaccination and contact exposure to foot-and-mouth disease. *Vaccine* 23: 5186 - 5195.

Paton, D. J., Sumption, K. J. and Charleston, B. (2009). Options for control of foot-and-mouth disease: knowledge, capacity and policy. *Philosophical Transactions of The Royal Society B* 364: 2657 - 2667.

Pattnaik, B., Subramaniam, S., Sanyal, A., Mohapatra, J. K., Dash, B., Ranjan, R. and Rout, M. (2012). Foot-and-mouth disease: Global status and future road map for control and prevention in India. *Agricultural Research* 1 (2): 132 – 147.

Perkins, J., Clavijo, A., Ortiz, J. I., Salo, T. J., Holland, H. J., Hindson, B. J. and McBride, M. T. (2007). Toward a multiplex serotyping immunoassay for foot-and-mouth disease virus. *Journal of Veterinary Diagnostic Investigation* 19: 180 - 184.

Pharo, H. J. (2002). Foot-and-mouth disease: an assessment of the risks facing New Zealand. *New Zealand Veterinary Journal* 50(2): 46 – 55.

Picado, A., Speybroeck, N., Kivaria, F., Mosha, R. M., Sumaye, R. D., Casal, J. and Bekvens, D. (2010). Foot-and-mouth disease in Tanzania from 2001 to 2006.In: *Transboundary and Emerging Diseases* 58: 44 - 52.

Pineda-Krch, M., O'Brien, J. M., Thunes, C. and Carpetnter, T. E. (2010). Potential impact of introduction of foot-and-mouth disease from wild pigs into commercial livestock premises in California. *American Journal of Veterinary Research* 71(1), 7pp.

Raof, A. M. A., Haleem, I. Y., Aly, N. M., Garhy, M. M. and Hosny, G. A. (2011). Epidemiological diagnosis of foot-and-mouth disease among cattle in Sharkia and Kafr El Sheikh Governorates. *International Journal of Virology* 7(4): 191 - 197.

Reid, S. M., Forsyth, M. A., Hutchings, G. H. and Ferris, N. P. (1998). Comparison of reverse transcription polymerase chain reaction, enzyme linked immunosorbent assay and virus isolation for the routine diagnosis of foot-and-mouth disease. *Journal of Virological Methods* 70: 213 - 217.

Reid, S. M., Hutchings, G. H., Ferris, N. P. and De Clercq, K. (1999). Diagnosis of foot-and-mouth disease by RT-PCR: evaluation of primers for serotypic characterization of viral RNA in clinical samples. *Journal of Virological Methods* 83: 113 – 123.

Reid, S. M., Ferris, N. P., Hutchings, G. H., Samuel, A. R. and Knowles, N. J. (2000). Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. *Journal of Virological Methods* 89: 167 - 176.

Reid, S. M., Ferris, N. P., Hutchings, G. H., Zhang, Z., Belsham, G.J. and Alexandersen, A. (2002). Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcriptase chain reaction assay. *Journal of Virological Methods* 105(1): 67 – 80.

Reid, S. M., Grierson, S. S., Ferris, N. P., Hutchings, G. H., and Alexandersen, S. (2003). Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *Journal of Virological Methods* 107(2): 129 – 139.

Rémond, M., Kaiser, C. and Lebreton, F. (2002). Diagnosis and screening of foot-and-mouth disease. *Comparative Immunology, Microbiology & Infectious Diseases* 25: 309 - 320.

Rushton, J. and Knight-Jones, T. (2012). The impact of foot-and-mouth disease. In: *The global foot-and-mouth disease control strategy*, FAO/OIE Global Conference on foot and mouth disease Control, 27 - 29 June 2012, Bangkok, Thailand. 27pp.

Rweyemamu, M., Roeder, P., MacKay, D., Sumption, K., Brownlie, J., Leforban, Y., Valarcher, J-F., Knowles, N. J. and Saraiva, V. (2008). Epidemiological patterns of foot-and-mouth disease worldwide. *Transboundary and Emerging Diseases* 55(1): 52 - 72.

Saeed, A., Khan, Q. M., Waheed, U., Arshad, M., Asif, M. and Farooq, M. (2010). RT-PCR evaluation for identification and sequence analysis of foot-and-mouth disease serotype O from 2006 to 2007 in Punjab, Pakistan. *Comparative Immunology, Microbiology and Infectious Disease,* 730, 6pp.

Sangaré, O., Bastos, A. D. S., Venter, E. H. and Vosloo, W. (2004). A first molecular epidemiological study of SAT-2 type foot-and-mouth disease viruses in West Africa. *Epidemiology and Infection* 132(3): 525 - 532.

Sangula, A. K., Wekesa, S. N., Ng'ang'a, Z. W., and Wamwayi, H. M. (2005). Detection of multiple serotypes of foot-and-mouth disease virus in stored isolates and the implication for control of the disease in Kenya. *Kenya Veterinarian* 28: 20 – 23.

Sangula, A. K., Belsham, G. J., Muwanika, V. B., Heller, R., Balinda, S. N., Masembe, C. and Siegismund, H. R. (2010). Evolutionary analysis of foot-and-mouth disease virus serotype SAT1 isolates from east Africa suggests two independent introductions from Southern Africa. *BMC Evolutionary Biology* 10: 371, 8pp.

Schley, D., Tanaka, R. J., Leungchavaphongse, K., Shahrezaei, V., Ward, J., Grant, C., Charleston, B. and Rhodes, C. J. (2012). Modelling the influence of foot-and-mouth disease vaccine antigen stability and dose on the bovine immune response. *PLoS ONE* 7(2): e30435. doi:10.1371/journal.pone.0030435.

Shao, J-J., Chang, H-Y., Zhou, G. Q., Cong, G-Z., Du, J-Z., Lin, T., Gao, S-D., He, J-J., Liu, X-T., Liu, J-X. and Gao, J-L. (2010). Rapid detection of foot-and-mouth disease virus by reverse transcription loop-mediated isothermal amplification (RT-LAMP). *International Journal of Applied Research in Veterinary Medicine* 8(2): 133 - 142.

Shaw, A. E., Reid, S. M., King, D. P., Hutchings, G. H. and Ferris, N.P. (2004). Enhanced laboratory diagnosis of foot-and-mouth disease by real-time polymerase chain reaction. *Revue Scientifique et Technique de l'office international des Epizooties* 23(3): 1003-1009.

Soleimanjahi, H., Sedeh, F. M., Jalilian, A. R. and Mahravani, H. (2013). Sequence analysis of the foot-and-mouth disease virus type O/IRN/2007 *VP1* Gene from Iranian isolate. *Folia Biologica (Praha)* 59: 93 – 98.

Stram, Y., Molad, T., Chai, D., Gelman, B. and Yadin, H. (1995). Detection and subtyping of foot-and-mouth disease virus in infected cattle by polymerase chain reaction and amplified VP1 sequencing. *Journal of Veterinary Diagnostic Investigation* 7: 52 - 55.

Sun, T., Lu, P. and Wang, X. (2004). Localization of infection-related epitopes on the non-structural protein 3ABC of foot-and-mouth disease virus and the application of tandem epitopes. *Journal of Virological Methods* 119: 79 - 86.

Suryanarayana, V., Madanamohan, B., Bist, P., Natarajan, C. and Tratschin, J. D. (1999). Serotyping of foot and mouth disease virus by antigen capture reverse transcriptase/polymerase chain reaction. *Journal of Virological Methods* 80: 45 - 52.

The Tanzania Five, year Development Plan 2011/2012 – 201/2016. [www.tanzania.go.tz] Site visited on 18/07/2013.

Thomson, G.R. (1995). Overview of foot-and-mouth disease in Southern Africa. *Revue Scientifique et Technique de l'office international des Epizooties* 14(3): 503 - 520.

Tomasula, P. M. and Konstance, R. P. (2004). The survival of foot-and-mouth disease virus in raw and pasteurized milk and milk products. *Journal of Diary Science* 87: 1115 - 1121.

Tshering, P. (1995). An economic evaluation of the impact of foot-and-mouth disease and its control in Bhutan. Dissertation for Award of MSc. Degree in Development of animal and Production Programmes at University of Reading, Berkshire, United Kingdom, 149pp.

Valarcher, J-F., Knowles, N. J., Xakharov, V., Scherbakov, A., Zhang, Z., Shang, Y-J., Liu, Z-X., Liu, X-T., Sanyal, A., Hemadri, D., Tosh, C., Rasool, T. J., Pattnaik, B., Schumann, K. R., Beckam, T. R., Linchongsubongkoch, W., Ferris, N. P., Roeder, P. L. and Paton, D. J. (2006). Multiple origins of foot-and-mouth disease virus serotype Asia 1 outbreaks, 2003 – 2007. *Emerging Infectious Diseases* 15 (7): 1046 – 1051.

Valdazo-González, B., Timina, A., Scherbakov, A., Abdul-Hamid, N. F., Knowles, N. K. and King D. P. (2013). Multiple introductions of serotype O foot-and-mouth disease viruses into East Asia in 2010-2011. *Veterinary Research* 44(76), 12 pp.

Van Dessel, W., Vandenbussche, F., Staes, M., Goris, N. and De Clercq, K. (2008). Assessment of domestic potential of immune-RCA in 96-well ELISA plates for foot-and-mouth disease virus. *Journal of Virological Methods*, 147: 151 - 156.

ViralZone, (2008). Aphthovirus [http://viralzone.expasy.org/all_by_species/98.html] site visited on 18/07/2013.

Vosloo, W., Bastos, A. D. S., Sangare, O., Hargreaves, S. K. and Thomson, G. R. (2002). Review of the status and control of foot-and-mouth disease in sub-Saharan Africa. *Revue Scientifique et Technique de l'office international des Epizooties*, 21(3): 437 - 449.

Vosloo, W., Bastos, A. D. S., Sahle, M., Sangare, O. and Dwarka, R. M. (2005). Virus topotypes and the role of wildlife in foot-and-mouth disease in Africa. In: *Wildlife Interface; implications for wildlife, livestock and human health (Edited by Cleaveland, S., Kavesh, W. B., Kock, M. D., Nyhus, P. J. and Yang, A.). International Union for Conservation of Nature*, Gland, Switzerland. pp. 67 - 73.

Vosloo, W. (2013). Foot-and-mouth disease: persistent threat. *Microbiology Australia* 34(1): 18 - 21.

Waheed, U., Saeed, A., Mobeen, A. and Khan, Q. M. (2009). The VP1 (Capsid Protein) gene based DNA sequencing for epidemiological analysis of FMDV isolated from buffaloes in Pakistan. *Pakistan Journal of Zoology Supplementary Series* 9: 333 - 339.

Wadsworth, J., Knowles, N. K., Swabey, K. G., Stirlin, J. M., Statham, R. J., Li, Y., Hutchings, G. H., Ferris, N. P. and Paton, D. J. (2006). Recent spread of new strains of foot-and-mouth disease type A in the Middle East and North Africa. European Commission For The Control of foot-and-mouth disease (EUFMD), 17-20 October 2006, Paphos, Cyprus. 105-112 pp.

Wernery, U. and Kinne, J. (2012). Foot and mouth disease and similar virus infections in camelids: a review. *Revue Scientifique et Technique de l'office international des Epizooties* 31(3): 907 – 918.

Woodbury, E. L., Samuel, A. R., Knowles, N. J., Hafez, S. M. and Kitching, R. P. (1994). Analysis of mixed foot-and-mouth disease virus infections in Saudi Arabia: prolonged circulation of an exotic serotype. *Epidemiology and Infection* 112: 201 – 211.

Yang, X., Zhou, Y-S., Wang, H-N., Zhang, Y., Wei, K. and Wang, T. (2011). Isolation, identification and complete genome sequence analysis of a strain of foot-and-mouth disease virus serotype Asia1 from pigs in southwest of China. *Virology Journal*, 8:175, 9pp.

Zinnah, M. A., Islam, M. T., Rahman, M. M., Hossain, M. T., Zinnah, M. A., Bari, M. R., Haque, M H., Khan, M. S. R. and Islam, M. A. (2010). Standardization of multiplex reverse transcription-polymerse chain reaction and typing of foot-and-mouth disease virus prevalent in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 8(2): 149 – 155.

APPENDICES

Appendix 1: SAMPLING OF PROBANG FROM A BUFFALO

Appendix 2: RNA EXTRACTION USING THE QIAGEN RNA MINI KIT

In a 1.5ml eppendorf tube, 560µl of prepared buffer AVL containing carrier RNA and 140µl of either homogenized tissue or probang fluid was added. The contents were pulse vortexed for 15 seconds to ensure efficient lysis and incubated at room temperature for 10 minutes for complete viral particle lysis. After incubation, the tube was briefly centrifuged at 8000rpm to remove drops from the inside of the lid. Five hundred and sixty microlitres of absolute ethanol (96–100%) was added to the sample mixture, then mixed by pulse-vortexing for 15 seconds then centrifuged at 8000rpm to remove drops from inside the lid.

After centrifugation, 630µl of the sample mixture was transferred to the QIAamp mini column placed in a 2ml collection tube. The column was centrifuged at 8000rpm for 1 minute. The collection tube with the filtrate was discarded and the spin column replaced into a clean 2ml collection tube. If the solution has not completely passed through the membrane, centrifugation was repeated at a higher speed until all of the solution had passed through. The remaining volume of sample mixture was transferred to the column, then centrifugation done at 8000rpm for 1 minute, after which the collection tube with filtrate was discarded and replaced with a clean 2ml collection tube.

The bound nucleic acid was washed by adding 500µl of buffer AW1 to the column and centrifuged at 8000rpm for 1 minute. The collection tube with the filtrate was discarded and replaced with a clean collection tube. The second wash was achieved by adding 500µl of buffer AW2 and centrifuged at 13000rpm for 3 minutes. The collection tube with the filtrate was discarded and replaced with a clean 1.5ml eppendorf tube.

The elution of RNA was achieved by adding 60µl of buffer AVE equilibrated to room temperature, to the column. Column contents were incubated at room temperature for one minute, then centrifuged at 8000rpm for 1 minute. The viral RNA, was immediately stored at -80°C until amplification.

Appendix 3: FMDV ANTIGEN DETECTION ELISA TEST FOR SEROTYPING OF FMDV O, A, SAT1 AND SAT2

Six samples were tested in one ELISA microplate (rows A-F), including one positive control (row G) for each of the serotypes A, O, SAT1, SAT2 and negative controls (row H) which are already pre-coated in each plate. Each sample was diluted 1:2 in diluent buffer and 50µl of the diluted samples were distributed in duplicates in each well of the respective row. A further 50µl of the diluent buffer was added in rows G and H, and then the plates were incubated for 1 hour at room temperature. The wells were emptied and the plates were washed three times with 200µl of washing solution (10X PBS-Tween 1:10 with distilled water). At each wash, the plates were incubated at room temperature for 3 minutes. After emptying the residual fluid, the plates were tapped firmly on absorbent tissue paper then 50µl conjugate A (1:10 in diluent buffer) was added to columns 1 to 8, and conjugate B (1:10 in diluent buffer) was added column to 9 to 12. The plates were covered and incubated at room temperature for another hour. The plates were washed four times, incubating the last one for 5minutes instead of 3minutes. 50µl of Substrate/chromogen solution equilibrated to room temperature was added to all wells and the plates were incubated in a dark place for 20 minutes. The reaction was stopped by adding 50µl of the stop solution to all wells. The plates were tapped gently on the side to mix the contents before immediately reading the Optical Density (OD) values of each well, at 450nm wavelength using a microplate reader.

Criteria for test validity

The positive controls were expected to give OD values of 1.0 units or higher and the negative controls below 0.1 in wells H1 to H8, and slightly higher in wells H9 to H12.

Interpretation of the results

The results for the different serotypes were interpreted with different criteria as indicated in below.

Interpretation of ELISA test results per serotype

Negative for FMDV	OD < 0.1 with all catching MAbs, after subtracting the OD of the respective negative control
FMDV positive type O	OD ≥ 0.1 with the type O MAb and with the pan-FMDV O,A,C,Asia1 MAb
FMDV positive type A	OD ≥ 0.1 with at least one of the two type A MAbs and with the pan-FMDV O,A,C,Asia1 MAb
FMDV positive type SAT1	OD ≥ 0.1 with the type SAT1 catching MAbs after subtracting the OD of the respective negative control
FMDV positive type SAT2	OD ≥ 0.1 with the type SAT2 catching MAbs after subtracting the OD of the respective negative control
FMDV positive (untyped)	OD ≥ 0.1 with the type O catching MAbs after subtracting the OD values of the respective negative control

Appendix 4: RAW DATA OF FMDV SEROTYPE SPECIFIC RT-PCR

RT-PCR results for cattle epithelial tissues

Sample no.	District	SAT 1	SAT 2	TYPE A	TYPE O
1	Simanjiro	NEG	POS	NEG	NEG
2	Serengeti	NEG	POS	NEG	NEG
3	Serengeti	NEG	POS	NEG	NEG
4	Serengeti	NEG	NEG	NEG	NEG
5	Serengeti	NEG	POS	POS	NEG
6	Simanjiro	NEG	NEG	NEG	NEG
7	Serengeti	NEG	NEG	NEG	NEG
8	Bunda	NEG	NEG	NEG	NEG
9	Serengeti	NEG	POS	POS	NEG
10	Serengeti	NEG	NEG	NEG	NEG
11	Simanjiro	NEG	NEG	NEG	NEG
12	Bunda	NEG	POS	POS	NEG
13	Serengeti	NEG	NEG	NEG	NEG
14	Serengeti	NEG	NEG	NEG	NEG
15	Simanjiro	NEG	POS	NEG	NEG
16	Simanjiro	NEG	NEG	POS	NEG
17	Simanjiro	NEG	NEG	NEG	NEG
18	Serengeti	NEG	NEG	POS	NEG
19	Simanjiro	NEG	NEG	NEG	NEG
20	Simanjiro	NEG	NEG	NEG	NEG
21	Simanjiro	NEG	POS	NEG	NEG
22	Serengeti	NEG	NEG	POS	NEG
23	Serengeti	NEG	NEG	NEG	NEG
24	Serengeti	NEG	NEG	NEG	NEG
25	Simanjiro	NEG	NEG	NEG	NEG
26	Simanjiro	NEG	NEG	POS	NEG
27	Serengeti	NEG	NEG	POS	NEG
28	Same	NEG	NEG	NEG	NEG

RT-PCR results for probang samples

Sample no.	District	SAT 1	SAT 2	TYPE A	TYPE O
7	Same	NEG	NEG	NEG	NEG
13	Same	NEG	NEG	NEG	NEG
23	Same	NEG	NEG	NEG	NEG
31	Same	NEG	NEG	NEG	NEG
34	Same	NEG	NEG	NEG	NEG
35	Same	NEG	NEG	NEG	NEG
36	Same	NEG	NEG	NEG	NEG

Appendix 5: Ag-ELISA RAW DATA RESULTS

	SAT1		SAT2			SEROTYPE A		SEROTYPE O	
SAMPLE	Mean OD	Result	Mean OD	Result	Mean OD1	Mean OD2	Result	Mean OD	Result
1	0.699	POS	-0.06	NEG	0.097	0.1075	POS	0.098	NEG
2	0.175	POS	-0.0785	NEG	0.086	0.0915	NEG	0.091	NEG
3	0.0055	NEG	-0.06785	NEG	0.069	0.077	NEG	0.082	NEG
4	-0.035	NEG	-0.144	NEG	1.3195	1.294	NEG	0.1125	POS
5	-0.022	NEG	0.7525	POS	0.0705	0.0765	NEG	0.0765	NEG
6	-0.0835	NEG	-0.065	NEG	1.581	1.601	POS	0.0805	NEG
8	0.706	POS	0.373	POS	0.512	0.84	NEG	0.0925	NEG
9	0.4636	POS	0.908	POS	0.0905	131	NEG	0.0865	NEG
10	0.238	POS	0.838	POS	0.1065	0.176	NEG	0.099	NEG
11	-0.0055	NEG	-0.007	NEG	1.136	1.304	NEG	0.0835	NEG
1_	-0.003	NEG	0.984	POS	0.0825	0.0825	NEG	0.0785	NEG
13	-0.0015	NEG	-0.051	NEG	0.0915	0.0845	NEG	0.085	NEG
15	0.265	POS	0.195	POS	0.095	0.096	NEG	0.0845	NEG
16	-0.065	NEG	0.011	NEG	1.3625	1.4225	POS	0.084	NEG
17	-0.2655	NEG	4475	POS	0.0705	0.067	NEG	0.072	NEG
18	-0.2625	NEG	-0.2005	NEG	0.107	0.093	POS	1.29	POS
19	-0.047	NEG	-0.0255	NEG	0.063	0.0695	NEG	0.0625	NEG
21	0.009	NEG	0.3125	POS	0.0735	0.076	NEG	0.0715	NEG
22	0.6805	POS	0.1115	POS	0.1176	0.2545	NEG	0.0915	NEG
23	0.9435	POS	-0.0645	NEG	0.0825	0.0815	NEG	0.0805	NEG
24	-0.087	NEG	-0.265	NEG	0.0785	0.0875	NEG	0.093	NEG
25	-0.0131	NEG	-0.2255	NEG	0.069	0.233	NEG	0.093	NEG
26	1.495	POS	0.6835	POS	0.08	0.0935	NEG	0.079	NEG
27	0.2745	POS	0.259	POS	1.57	1.564	POS	0.113	POS
7	0.525	POS	0.2905	POS	0.141	0.132	POS	0.1005	POS
13	0.3135	POS	1945	POS	0.1025	0.11	POS	0.0935	NEG
23	-0.01795	NEG	-0.1845	NEG	0.068	0.0695	NEG	0.069	NEG
34	-0.054	NEG	-0.239	NEG	0.07	0.0775	NEG	0.0795	NEG
35	-0.1435	NEG	-0.114	NEG	0.0645	0.065	NEG	0.0765	NEG
36	-0.0405	NEG	-0.2895	NEG	0.0745	0.071	NEG	0.074	NEG

KEY

Samples 1 – 27: cattle epithelial tissues

Buffalo probang samples

cross reactions

Appendix 6: LIST OF SAMPLES USED IN THE STUDY AND THEIR ORIGIN

Sample ID	Eartag no.	Date collected	Village	District
1	7495	28-05-12	Rwamchanga	Serengeti
2	7255	02-05-12	Kimotorok	Simanjiro
3	no tag	22-07-11	Nyichoka	Serengeti
4	7054	18-04-12	Parknyigot	Serengeti
5	8494	03-10-12	Nyichoka	Serengeti
6	8426	15-09-12	Nyamburi	Serengeti
7	8234	17-08-12	Terat	Simanjiro
8	6808	29-07-11	Nata	Serengeti
9	6670	02-03-12	Tamau	Bunda
10	6630	01-03-12	Nyamisingisi	Serengeti
11	6517	03-02-12	Montukeri	Serengeti
12	8270	18-08-12	Terat	Simanjiro
13	8191	15-08-12	Tamau	Bunda
14	7215	01-05-12	Nata	Serengeti
15	6544	04-02-12	Rwamchanga	Serengeti
16	6653	14-08-12	Terat	Simanjiro
17	7456	25-05-12	Terat	Simanjiro
18	7302	31-05-13	Terat	Simanjiro
19	7347	01-06-12	Nyichoka	Serengeti
20	7137	29-04-12	Terat	Simanjiro
21	7061	17-04-12	Loiborsire	Simanjiro
22	7934	18-01-13	Terat	Simanjiro
23	7189	30-04-12	Parknyigot	Serengeti
24	6974	25-03-12	Nyichoka	Serengeti
25	6592	29-02-12	Nata	Serengeti
26	7012	17-04-12	Terat	Simanjiro
27	8108	28-08-12	Terat	Simanjiro
28	7423	05-05-12	Nyamburi	Serengeti
7	TAN 10	no date	Mkomazi	Same
13	TAN25	no date	Mkomazi	Same
23	TAN7	no date	Mkomazi	Same
31	TAN11	no date	Mkomazi	Same
34	TAN32	no date	Mkomazi	Same
35	TAN19	no date	Mkomazi	Same
36	TAN16	no date	Mkomazi	Same

Samples 1 – 28 are cattle epithelial tissues and 7 – 36 are buffalo probing samples